Mims' Pathogenesis of Infectious Disease

Sixth Edition

Anthony A. Nash
Robert G. Dalziel
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Preface

It has been 12 years since the fifth edition of this book was published. In that time, we have witnessed a revolution in the technologies underpinning studies of the pathogenesis of infectious disease. In the fifth edition, 30 bacterial genomes had been completed. We now have access to thousands of completed pathogen genomes and those of many mammalian, avian and piscine 'host' species. This has led to new ways of exploring how pathogen and host interact, and how they evolve. In turn the new insights gained into pathogenesis are leading to improvements in diagnostics, vaccines and therapeutics.

The sixth edition maintains the standards and unique style of earlier editions despite the absence of Professor Mims whose vision was paramount in bringing pathogenesis to a wider audience. In particular, we have endeavoured to use accessible language and simple but colourful diagrams to convey the mechanisms of pathogenesis to students of infectious disease.

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CHAPTER

1

General Principles

When writing a book about infectious diseases, it is important to define exactly what we mean in using the term in order to provide a context for the information to come. In general biological terms, the relationship between two distinct but associated organisms can be classified as parasitic, where one benefits at the expense of the other, or symbiotic (mutualistic), where both benefit. There is another commonly used category called commensalism, whereby the organisms co-exist without damage to either organism. It is often difficult to use this category with confidence, because an apparently commensal association often proves on closer examination to be really parasitic or symbiotic.

The classification system can be applied to the association between microorganisms and vertebrates. Generalised infections such as measles, tuberculosis or typhoid are clearly examples of parasitism. On the other hand, the microbiota inhabiting the rumen of cows or the caecum of rabbits, enjoying food and shelter and at the same time supplying the host with food derived from the utilisation of cellulose, are clearly symbiotic. In addition, recent and on-going research is revealing the great variety of ways by which resident bacteria are contributing to the normal function of their host species. For example, the bacteria that live on human skin may at first be considered as commensals. They enjoy shelter and food (sebum, sweat, etc.) but are normally harmless. If the skin surface is examined by the scanning electron microscope, the bacteria, such as *Staphylococcus epidermidis* and *Proprionibacterium acnes*, are seen in small colonies scattered over a moon-like landscape. The colonies contain several hundred individuals[1] and the bacteria adhere to the epithelial squames that form the cornified skin surface, and extend between the squames and down the mouths of the hair follicles and glands onto the skin surface. They can be reduced in numbers, but never eliminated, by scrubbing and washing, and are most numerous in more moist regions such as the armpit, groyne and perineum. The dryness of the stratum corneum makes the skin an unsuitable environment for most bacteria, and merely occluding and thus hydrating an area with polythene sheeting leads to a large increase in the number of bacteria. The secretions of apocrine sweat glands are metabolised by skin bacteria, and odoriferous amines and other substances such as 16-androstene steroids are

[1]The average size of these colonies is determined by counting the total number of bacteria recovered by scrubbing and comparing this with the number of foci of bacterial growth obtained from velvet pad replicas. The sterile pad is applied firmly to the skin, then removed and applied to the bacterial growth plate.

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produced, giving the body a characteristic smell that modern man, at least, finds unpleasant.[2] Deodorants, containing aluminium salts to inhibit sweating, and often antiseptics to inhibit bacterial growth, are therefore often applied to the apocrine gland areas in the axillae. However, body smells have been of great significance in the social and sexual life of humans and mammals in general. Not all body smells are produced by bacteria, and skin glands may secrete substances known as pheromones that are themselves odoriferous but some skin bacteria do contribute to body smells and could for this reason be classified as symbiotic rather than parasitic. There is also evidence that harmless skin bacteria inhibit the colonisation and growth of more pathogenic bacteria, again indicating benefit to the host and a symbiotic classification for these bacteria.

A microbe's ability to multiply is obviously of paramount importance; indeed, we call a microbe dead or nonviable if it cannot replicate.[3] The ability to spread from host to host is of equal importance. Spread can be horizontal in a species, whereby one individual infects another by contact, or via insect vectors (Figure 1.1). Alternatively, spread can be 'vertical' in a species, with parents infecting offspring via sperm, ovum, the placenta, the milk, or by contact. Clearly if a microbe does not spread from one individual to another it will die with the individual and cannot persist in nature. The crucial significance of the ability of a

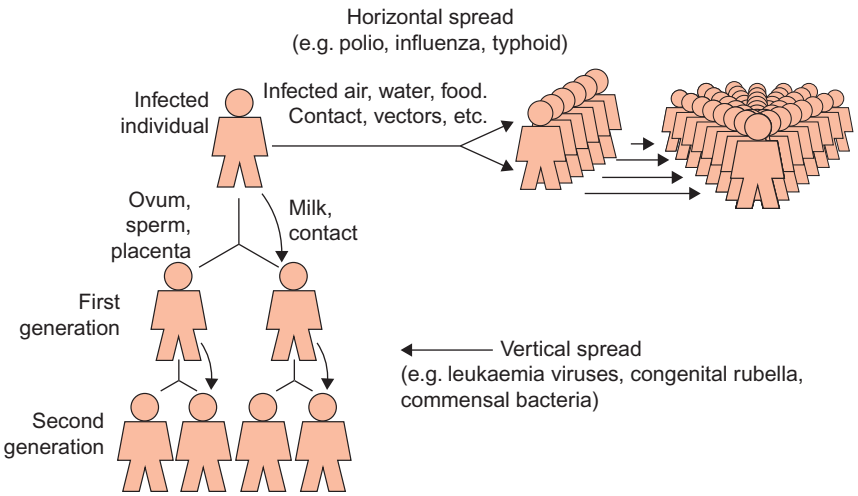

FIGURE 1.1 Vertical and horizontal transmission of infection.

[2]The smell of feet encased in shoes and socks is characteristic, and in many European languages it is referred to as cheese-like. Between the toes lives *Brevibacterium epidermidis*, which converts L-methionine to methane thiol, a gas that contributes to the smell. A very similar bacterium is added to cheeses such as Brie to enhance odour and flavour.

[3]Sterilisation is the killing of all forms of microbial life, and appropriately the word means making barren, or devoid of offspring.

microbe to spread can be illustrated by comparing the horizontal spread of respiratory and sexually transmitted infections (STIs). An infected individual can transmit influenza or the common cold to a score of others in the course of an innocent hour in a crowded room. An STI also must spread progressively from person to person if it is to maintain itself in nature, but even the most energetic lover could not transmit a venereal infection on such a scale. A chain of horizontal infection in this case, however, requires a chain of sexual contact between individuals. If those infected at a given time never had sexual contact with more than one member of the opposite sex, the total incidence could double in a lifetime, and when the infected people died the causative microbe would be eliminated. In other words, STIs must be transmitted to more than one member of the opposite sex if they are to persist and flourish. The greater the degree of sexual promiscuity, the greater the number of sex partners, the more successful such infections can be. Further discussion of STIs are included in the next chapter.

Only a tiny proportion of the microorganisms associated with humans have the potential to give rise to pathological changes or cause disease. Vast numbers of bacteria live harmlessly in the mouth and intestines, on the teeth and skin, and most of the 150 or so viruses that infect humans cause no detectable illness in most infected individuals, in spite of cell and tissue invasion. This is to be expected because, from an evolutionary point of view, successful microbes must survive, multiply, and leave viable descendants. A successful parasitic microbe lives on or in the individual host, multiplies, spreads to fresh individuals, and thus maintains itself in nature (Table 1.1).

A successful parasitic microbe, like all successful parasites, will obtain what it requires for proliferation from the infected host without causing much damage. If an infection is debilitating or even lethal, there will be a reduction in numbers of the host species and thus in the numbers of the microorganism. Thus, although a small number of microbial pathogens cause disease in a majority of those infected (so-called true pathogens), most are comparatively

TABLE 1.1 Obligatory Steps for Infectious Microorganisms

Step	Phenomenon	Requirement	Chapter
1. Attachment ± entry into body	Infection (entry)	Evade host's natural protective and cleansing mechanisms	2
2. Local or general spread in the body	Local events, spread	Evade immediate local defences and the natural barriers to spread	3, 5
3. Multiplication	Multiplication	Multiply; many offspring will die in host or *en route* to fresh host	
4. Evasion of host defences	Microbial answer to host defences	Evade phagocytic and immune defences long enough for full cycle in host to be completed	4, 6, 7
5. Shedding (exit) from body	Transmission	Leave body at site and on a scale that ensures spread to fresh host	2
6. Cause damage in host	Pathology, disease	Not strictly necessary but often occurs[a]	8

[a]Some damage may be inevitable if efficient shedding is to occur (e.g. common cold, diarrhoea, skin vesicles).

harmless, causing either no disease, or disease in only a small proportion of those infected. Polioviruses, for instance, are transmitted by the faecal–oral route and cause a subclinical intestinal infection under normal circumstances. But in an occasional host the virus invades the central nervous system and causes meningitis, sometimes paralysis, and very occasionally death. This particular site of multiplication is irrelevant from the virus point of view, because growth in the central nervous system is quite unnecessary for transmission to the next host. Well-established infectious agents have therefore generally reached a state of balanced pathogenicity in the host and cause the smallest amount of damage compatible with the need to enter, multiply, and be discharged from the body.

The importance of balanced pathogenicity is strikingly illustrated in the case of the natural evolution of myxomatosis in the Australian rabbit. After the first successful introduction of the virus in 1950 more than 99% of infected rabbits died, but subsequently new strains of virus appeared that were less lethal. The less lethal strains of virus were therefore selected during the evolution of the virus in the rabbit population, because they persisted longer and were therefore more successful parasites. The genetics of the rabbit population also changed, because those that were genetically more susceptible to the infection were eliminated. Rabies, a virus infection of the central nervous system, seems to contradict, but in fact exemplifies, this principle. Infection is classically acquired from the bite of a rabid animal and the disease in man is almost always fatal, but the virus has shown no signs of becoming less virulent. Man, however, is an unnatural host for rabies virus, and it is maintained in a less pathogenic fashion in animals such as vampire bats and skunks. In these animals, there is a relatively harmless infection and the virus is shed for long periods in the saliva, which is the vehicle of transmission from individual to individual. Rabies is thus maintained in the natural host species without serious consequences. But bites can infect the individuals of other species, 'accidentally' from the virus point of view, and the infection is a serious and lethal one in these unnatural hosts.

Although successful parasites cannot afford to become too pathogenic, some degree of tissue damage may be necessary for the effective shedding of microorganisms to the exterior, as for instance in the flow of infected fluids from the nose in the common cold or from the alimentary canal in infectious diarrhoea. Otherwise there is ideally very little tissue damage, a minimal inflammatory or immune response, and a few microbial parasites achieve the supreme success of causing zero damage and failing to be recognised as parasites by the host. Different microbes show varying degrees of attainment of this ideal state of parasitism.

The concept of balanced pathogenicity is helpful in understanding infectious diseases, but many infections have not yet had time to reach this ideal state. In the first place, as each microorganism evolves, occasional virulent variants emerge and cause extensive disease and death before disappearing after all susceptible individuals have been infected, or before settling down to a more balanced pathogenicity. Secondly, a microbe recently introduced into a host (e.g. human immunodeficiency virus (HIV) in humans) may not have had time to settle down into this ideal state. Thirdly, some of the microbes responsible for serious human diseases had appeared originally in one part of the world, where there had been a weeding out of genetically susceptible individuals and a move in the direction of a more balanced pathogenicity. Subsequent spread of the microorganism to a new continent has resulted in the infection of a different human population in whom the disease is much

more severe because of greater genetic susceptibility. Examples include tuberculosis spreading from resistant Europeans to susceptible Africans or North American Indians, and yellow fever spreading from Africans to Europeans. Finally, there are a number of microorganisms that have not evolved towards a less pathogenic form in man because the human host is clearly irrelevant for the survival of the microorganism. Microorganisms of this sort, such as those causing rabies (see above), scrub typhus, plague, leptospirosis and psittacosis, have some other regular host species which is responsible, often together with an arthropod vector, for their maintenance in nature.[4] The pathogenicity for man is of no consequence to the microorganism. Several human infections that are spillovers from animals domesticated by man also come into this category, including brucellosis, Q fever, anthrax, and livestock-associated meticillin-resistant *Staphylococcus aureus* (MRSA) infections. As humans colonise every corner of the earth, they encounter an occasional microbe from an exotic animal that causes, quite 'accidentally' from the point of view of the microorganisms, a serious or lethal human disease. Examples include Lassa fever and Marburg disease from African rodents and monkeys, respectively.[5]

On the other hand, a microorganism from one animal can adapt to a new species. Advances in DNA sequencing and phylogenetic analyses are revealing much about the evolutionary history of pathogens. Measles, which could not have existed and maintained itself in humans in the Palaeolithic era, probably arose at a later stage from the closely related rinderpest virus that infects cattle. New human influenza viruses continue to arise from birds, and the virus of the acquired immunodeficiency syndrome (AIDS), the modern pestilence, seems to have arisen from a very similar virus infecting monkeys and chimpanzees in Africa. In addition, livestock strains of *S. aureus* most likely originated in humans but jumped into animal hosts since domestication occurred several thousand years ago.

Microorganisms multiply exceedingly rapidly in comparison to their vertebrate hosts. The generation time of an average bacterium is an hour or less, as compared with about 20 years for the human host. Consequently, microorganisms evolve with extraordinary speed in comparison with their vertebrate hosts. Vertebrates, throughout their hundreds of millions of years of evolution, have been continuously exposed to microbial infections. They have developed highly efficient recognition (early warning) systems for foreign invaders, and effective inflammatory and immune responses to restrain their growth and spread, and to eliminate them from the body. If these responses were completely effective, microbial infections would be few in number and all would be terminated rapidly;

[4]These infections are called *zoonoses*.

[5]Lassa fever is a sometimes lethal infection of man caused by an arenavirus. The virus is maintained in certain rodents in West Africa as a harmless persistent infection, and man is only occasionally infected. Another serious infectious disease occurred in 1967 in a small number of laboratory workers in Marburg, Germany, who had handled tissues from vervet monkeys recently imported from Africa. The Marburg agent is a virus and has since reappeared to cause fatal infections in Zaire and the Sudan, but nothing is known of its natural history. Monkeys are not natural hosts and are probably accidentally infected, like man. Since 1976, Ebola virus, related to Marburg, has caused dramatic local outbreaks in Zaire and Sudan. In 2014 a major outbreak of Ebola resulted in the deaths of thousands of people in West Africa. Like Lassa fever, it can spread from person to person via infected blood, but its natural host is unknown. However, bats are a likely reservoir.

microorganisms would not be allowed to persist in the body for long periods. But microorganisms, faced with the antimicrobial defences of the host species, have evolved and developed a variety of characteristics that enable them to by-pass or overcome these defences. In any case, the normal commensal microbiota is tolerated because it performs critical functions required for the general health and well-being of the host. The defences are not infallible, and the rapid rate of evolution of microorganisms ensures that they are always many steps ahead. If there are possible ways round the established defences, microorganisms are likely to have discovered and taken advantage of them. Successful microorganisms, indeed, owe their success to this ability to adapt and evolve, exploiting weak points in the host defences. The ways in which the phagocytic and immune defences are overcome are described in Chapters 4 and 7.

It is the virulence and pathogenicity of microorganisms, their ability to kill and damage the host, that makes them important to the physician or veterinarian. If none of the microorganisms associated with man did any damage, and none was notably beneficial, they would be interesting but relatively unimportant objects. In fact, they have been responsible for the great pestilences of history, have at times determined the course of history, and continue today, in spite of vaccines and antibiotics, as major causes of disease (see Table A.1). Also, because of their rapid rate of evolution and the constantly changing circumstances of human life, they continue to present threats of future pestilences. Importantly, pathogens constantly 're-invent' themselves through evolution in order to counteract human efforts at control such as antibiotic treatment. In fact the emergence of bacterial resistance to virtually all classes of antibiotics is one of the greatest current threats to man's capacity to treat infectious diseases. Overall, it is the purpose of this book to describe and discuss the mechanisms of infection and the characteristics that make microorganisms pathogenic. In addition to understanding the role of commensal microbiota in the health host, this is the central significant core of microbiology as applied to medicine.

In the last 12 years since the previous edition of this book, dramatic advances in molecular biological techniques have been made resulting in broad new insights into our understanding of the biology of microbes and how they impact on our lives. We now have a vastly more detailed understanding of host pathogen interactions at the cellular, genetic and biochemical levels based on our ability to manipulate microbial and host genetics in order to understand the critical interactions and responses involved. By such means a great deal of biochemical information can be obtained about the microbial determinants involved in mediating different aspects of the complex infection process.

But the most dramatic developments in recent years have come in DNA sequencing technologies. New and emerging methods can produce vast amounts of sequence information rapidly and relatively inexpensively. This has resulted in many thousands of whole genome sequences for bacterial and parasite pathogens and several hundred higher order organisms (vertebrates and invertebrates) becoming available in the public DNA sequence databases. In addition, metagenomic studies which qualitatively and quantitatively examine the microbial content within biological samples are informing our understanding of microbial diversity in different ecological niches. In the current 'post-genomic' era as it has come to be known, generating sequence information is relatively facile. It is the mining of the data and the assignment of functional relevance which is the bottle-neck in terms of biological understanding. New sequencing

technologies also allow examination of genome-wide gene expression, building on previous transcriptomic approaches, such as microarrays.

The recent advances made in DNA sequencing technology have resulted in large complex genomes such as the human genome requiring only a matter of days to complete. Now that all the genomes of the major domestic livestock species have been completed, the opportunity exists to study the genome-wide interactions between pathogen and host genomes.

By extracting mRNAs from bacteria grown in culture and from the same organism from an infection site (or grown in conditions which mimic infection conditions), it is possible to identify which gene(s) are expressed or repressed in the two situations. This may point to factors which are essential for survival during infection and which thus may represent novel therapeutic targets. Similar studies can also be carried out in cells infected by viruses, allowing the host cell response to these pathogens to be dissected. In addition to sequencing technologies, major advancements in microscopy and imaging, particularly for *in vivo* analysis, are resulting in very enhanced views of the way by which microbes cause disease. Overall, we are in a very exciting time with tremendous potential for understanding the biology of infectious diseases. Considering that we are also in an age when the options for treating bacterial infections are fast reducing due to the increase in antibiotic resistance, and that the threat of emerging viral pathogens is very apparent, we must utilise our improved understanding of infectious disease to design rational ways for their control.

In order to facilitate an understanding of infectious disease it is possible to distinguish different phases of the 'pathogenic cycle', including an appreciation for the bacterial, host and environmental factors which contribute to infectious disease and the outcome of infection. In addition to the bacterial virulence determinants involved, an understanding of the host's phagocytic and immune defences is important, and these are briefly set out in Chapters 4, 6 and 9. There are additional chapters on resistance and recovery from infection, persistent infection, and the prevention of infection by vaccines.

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CHAPTER

2

Attachment to and Entry of Microorganisms into the Body

OUTLINE

INTRODUCTION

Figure 2.1 shows a simplified diagram of the mammalian host. In essence, the body is traversed by a tube, the alimentary canal, with the respiratory and urinogenital tracts as blind pouches from the alimentary canal or from the region near the anus. The body surface is covered by skin, with a relatively impermeable dry outer layer, and usually fur. This gives a degree of insulation, and the structure of skin illustrates the compromise between the need to protect the body, yet at the same time maintain sensory communication with the outside world, give mechanical mobility, and, especially in man, act as an important thermoregulatory organ. It is the largest 'organ' in the body, with a weight of 5 kg in humans.

The dry, protective skin cannot cover all body surfaces. At the site of the eye it must be replaced by a transparent layer of living cells, the conjunctiva. Food must be digested and

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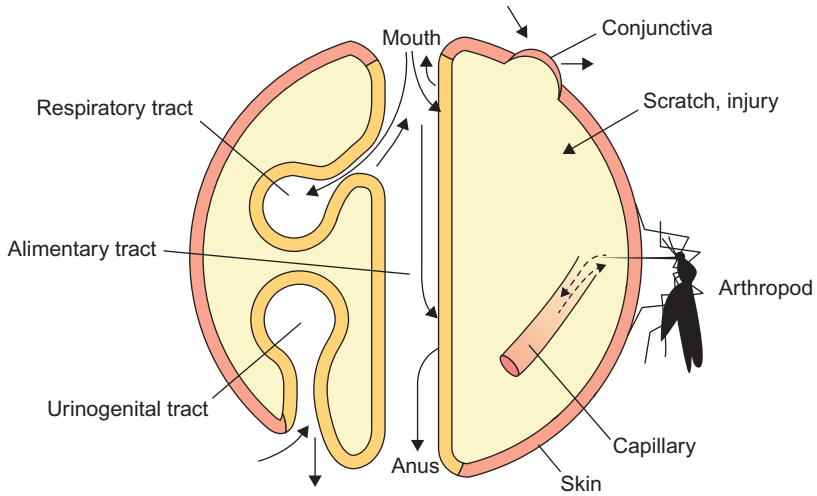

FIGURE 2.1 Body surfaces as sites of microbial infection and shedding.

the products of digestion absorbed, and in the alimentary canal therefore, where contact with the outside world must be facilitated, the lining consists of one or more layers of living cells. Also in the lungs the gaseous exchanges that take place require contact with the outside world across a layer of living cells. There must be yet another discontinuity in the insulating outer layer of skin in the urinogenital tract, where urine and sexual products are secreted and released to the exterior. The cells on all these surfaces are covered by a fluid film containing mucin, a complex hydrated gel that waterproofs and lubricates. In the alimentary canal, the lining cells are inevitably exposed to mechanical damage by food and they are continuously shed and replaced. Shedding and replacement is less pronounced in respiratory and urinogenital tracts, but it is an important phenomenon in the skin, the average person shedding about 5×10^8 skin squames per day.

The conjunctiva and the alimentary, respiratory and urinogenital tracts offer pathways for infection by microorganisms and the penetration of these surfaces is more easily accomplished than for intact outer skin. A number of antimicrobial systems have been developed in evolution to deal with this danger, and also special cleansing systems to keep the conjunctiva and respiratory tract clean enough to carry out their particular function. In order to colonise or penetrate these body surfaces, microorganisms must first become attached, and there are many examples of specific attachments that will be referred to (see Table 2.1 where they are listed in some detail). One striking feature of acute infectious illnesses all over the world is that most of them are either respiratory or diarrhoea-like in nature. They are not necessarily severe infections, but they are the most abundant. In other words, infectious agents are for much of the time restricted to the respiratory and intestinal tracts.

It is possible to divide all infections into three groups (Figure 2.2). First, those in which the microorganisms have specific mechanisms for attaching to and sometimes penetrating the body surfaces of the normal, healthy host. This includes the infections listed in Figure 2.3. In the second group, the microorganism is introduced into the body of the

TABLE 2.1 Examples of Attachments of Microorganisms to Host Cell/Body Surface With Information on Ligand Receptor System Derived from *in vitro* Studies on Cultured Cells

Microorganism/Disease	Target Site or Cell	Microbial Ligand(s)	Receptor
VIRUSES			
Influenza virus/flu	Respiratory epithelium	Viral haemagglutinin	Neuraminic acid
Rhinovirus/common cold	Respiratory epithelium	Viral capsid protein	Intercellular adhesion molecules (ICAM-1)
HIV-1/AIDS	$CD4^+$ T cell	Viral envelope gpl20 proteins	CD4 proteins
Epstein–Barr virus/ glandular fever	B cell	Viral envelope protein	CD21
Herpes simplex virus/ cold sore/genital herpes	Most cells	Glycoprotein	Heparan sulphate
Measles virus/measles	Most primate cells	Viral haemagglutinin	CD46 (membrane cofactor protein)
Foot and mouth disease	Tissue culture cell	VP1	Vitronectin integrin
Virus			Receptor
Coxsackie virus A9	Tissue culture cell	VP1	Integrins
BACTERIA			
Chlamydia/conjunctivitis/ Urethritis	Conjunctivial/urethral epithelia	GAG; MOMP (major outer membrane protein; nonspecific, and specific attachment)	GAG receptors
Mycoplasma pneumoniae/ atypical pneumonia	Respiratory epithelium	'Foot' on *Mycoplasma* surface	Neuraminic acid
Neisseria meningitides/ carrier state	Nasopharyngeal epithelium	Type IV Pili; Opa (opacity associated) proteins	Heparin sulphate proteoglycan. Opa proteins also bind to vitronectin/integrins in HeLa and Hep-2 cells, and CD66 in neutrophils
Vibrio cholerae/cholera	Intestinal epithelium	Tcp (demonstrably important in humans); others	
Escherichia coli			
ETEC/diarrhoea	Intestinal epithelium	K88 (pigs); K99 (calves, lambs); Colonisation factors (humans)	Neu5Glc(α2–3) Gal(β1–4) Glc(β1–1) ceramide
EPEC/diarrhoea	Intestinal epithelium	Bfp, Intimin (an OMP)	Tir (a bacterial protein; translocated intimin receptor), host cell co-factor

(*Continued*)

TABLE 2.1 (Continued)

Microorganism/Disease	Target Site or Cell	Microbial Ligand(s)	Receptor
EHEC/haemorrhagic colitis; haemolytic uraemic syndrome	Colonie epithelium	Intimin	Tir
UPEC/pyelonephritis	Urinary tract	P fimbriae	Gal(αl−4) Gal
NMEC/neonata/meningitis	Endothelial and epithelial cells	S fimbriae	α-Sialyl-(2−3)-β-galactose-containing receptor molecules
Shigella spp./dysentery	Tissue culture cell	Ipa (invasion plasmid antigens) BCD	Integrin
Streptococcus mutans/caries	Teeth	Glycosyl transferase, glucan (glue)	
Streptococcus pyogenes/throat infections; other more serious infections	Pharyngeal epithelium	M protein	Fibrinogen/fibronectin
Staphylococcus aureus	Extracellular matrix, epithelial cells	Clumping factor A, B, Fibronectin, binding proteins A, B	Fibrinogen Cytokeratin, loricrin, fibrinogen, integrins
Listeria monocytogenes	Range of clinical disease	Internalins A, B	E-cahedrin (A)
Legionella pneumophila/Legionnaires' disease	Macrophage	Adsorbed C3bi	Integrin (CR (complement receptor) 3)
Mycobacteria tuberculosis	Macrophage	Adsorbed C3bi	CR3
Mycobacteria leprae	Schwann cells	ML-LBP21	α-Dystroglycan
Treponema pallidum/syphilis	Tissue culture cell	Adsorbed fibronectin	Fibronectin receptor
Bordetella pertussis/whooping cough	Respiratory epithelium, macrophage	Several adhesins (fimD, pertussis toxin, FHA, pertactin; others)	Several integrins
Yersinia enterocolitica/diarrhoea	Intestinal epithelium	Invasin (OMP)	Integrins
PROTOZOA			
Leishmania mexicana	Macrophage	Surface glycoprotein (Gp63)	CR3
Leishmania major	Macrophage	Adsorbed C3bi	CR3
Histoplasma capsulatum	Macrophage	HSP60	CD18
Plasmodium vivax/malaria	Erythrocyte of susceptible human	Merozoite	'Duffy' antigen

(*Continued*)

TABLE 2.1 (Continued)

Microorganism/Disease	Target Site or Cell	Microbial Ligand(s)	Receptor
Plasmodium falciparum/ malaria	Erythrocyte of susceptible human	Merozoite	Glycophorin A, B
Trypanosoma cruzi	Tissue culture cell	Adsorbed fibronectin	Fibronectin receptor
Babesia/babesiasis in cattle	Erythrocyte	Complement-mediated attachment	CR3
Giardia lamblia/diarrhoea	Duodenal, jejunal epithelia	Taglin GLAM-1 on disc	Manose-6-phosphate
Entamoeba histolytica/ amoebic dysentery	Colonie epithelium	170 kDa Gal/ GalNAcLectin	Mucins
Trypanosome cruzi/ trypanosomiasis		gp85/TS, Tc85 Penetrin	Fibronectin, laminin, heparin

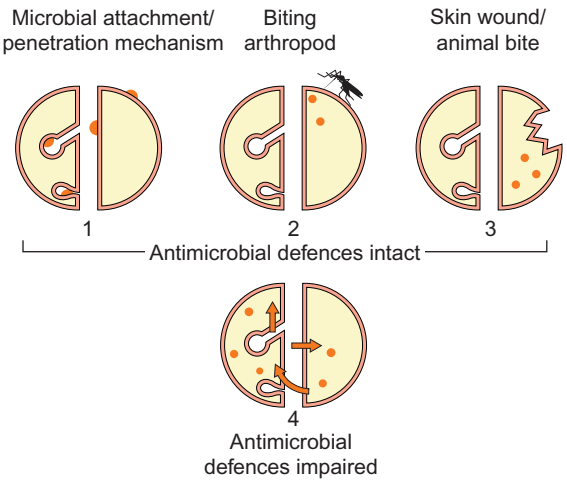

FIGURE 2.2 Four types of microbial infection can be distinguished. *Adapted from Mims et al., 1998.*

normal healthy host by a biting arthropod, as with malaria, plague, typhus or yellow fever. Here the microorganism possesses specific mechanisms for infection of the arthropod and depends on the arthropod for introduction into the body of the normal healthy host. The third group includes infections in which the microorganism is not by itself capable of infecting the normal healthy host. There must be some preliminary damage and impairment of defences at the body surface, such as a skin wound, damage to the respiratory tract initiated by a microbe from the first group or an abnormality of the urinary tract interfering with the flushing, cleansing action of urine. Alternatively, there may be a more general defect in body defences leading to a compromised immune system. The opportunistic infections described later in this chapter come into this third group, including a large proportion of the infections seen in hospitals.

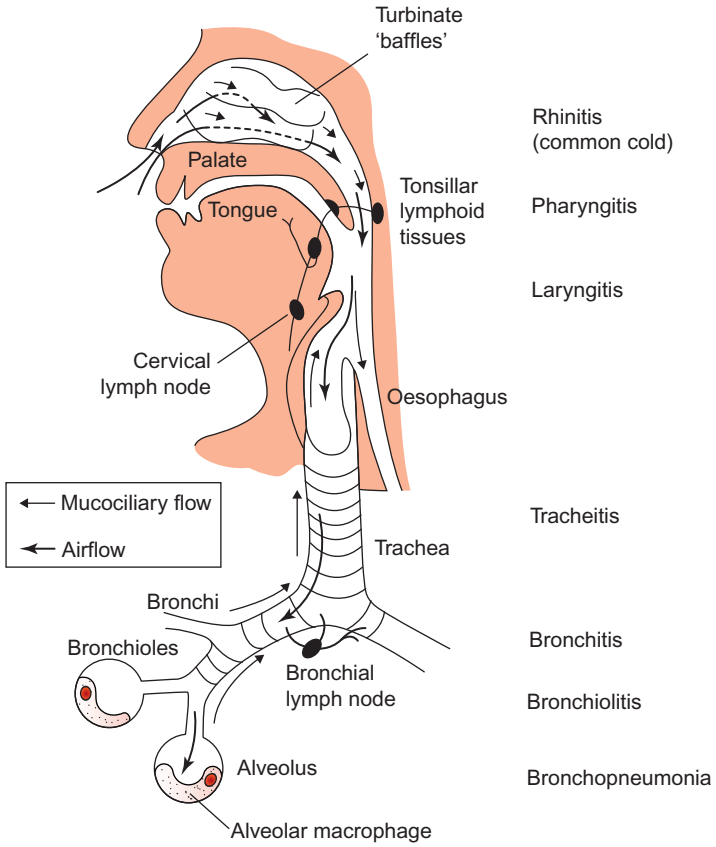

FIGURE 2.3 Mechanisms of infection in the respiratory tract.

ADHESION/ENTRY: SOME GENERAL CONSIDERATIONS

Adhesins mediate binding of microbes to their hosts and belong to numerous classes of surface structure present on microorganisms (Table 2.1). Adhesins are more than simply the determinants of pathogen location: they are effectors of important aspects of the biology of infection. The receptors on the eukaryotic cell surface, which confer specificity to the initial binding, comprise an array of oligosaccharides of transmembrane glycoproteins or extracellular matrix proteins (see Table 2.1 for examples), which normally play important roles in mediating cell–cell and cell–extracellular matrix interactions. They also play a key role in some cell signalling processes, such as those involving actin rearrangements, by virtue of their contact with the cytoskeleton.

Since most pathogens possess more than one adhesin system, the fate of the interaction between the pathogen and the host will be determined by which receptor or sequential combination of receptors is engaged. For example, pertussus toxin is an important toxin produced by *Bordetella pertussis* (see Chapter 8). The S2 and S3 subunits of the toxin B

oligomer (see Figure 8.5) bind to the surface of macrophages, resulting in the upregulation of integrin CR3. The activated CR3 in turn binds with the filamentous haemagglutinin (FHA) adhesin of *B. pertussis*, leading to the uptake of the organism. Viruses may also bind to more than one receptor. These may be used in invading different types of cell, or one receptor is for binding to the cell and another for penetration. HIV gp120 binds to CD4 on susceptible cells (Table 2.1) but the gp41 component of the envelope glycoprotein complex needs to bind to a chemokine receptor in order to allow penetration.

THE SKIN

The skin is a natural barrier to microorganisms and is penetrated at the site of breaks in its continuity, whether macroscopic or microscopic (Table 2.2).

Microorganisms other than commensals (residents) are soon inactivated, probably by fatty acids (skin pH is about 5.5) and other materials produced from sebum by the commensals. In the perianal region, for instance, where billions of faecal bacteria are not only deposited daily, but then, in man at least, rubbed into the area, there is evidently an astonishing resistance to infection. Faecal bacteria are rapidly inactivated here, but the exact mechanism, and the possible role of perianal gland secretions, is unknown.

TABLE 2.2 Microorganisms That Infect the Skin or Enter the Body via the Skin

Microorganisms	Disease	Comments
Arthropod-borne viruses	Various fevers	150 distinct viruses, transmitted by infected arthropod bite
Rabies virus	Rabies	Bite from infected animals
Wart viruses	Warts	Infection restricted to epidermis
Pox viruses	Smallpox, myxomatosis	Entry via abrasions
Herpes simplex virus	Herpes gladiatorum or Scrumpox/Wrestler's pox	Associated with contact sports
Staphylococci	Boils, etc.	Commonest skin invaders
Rickettsia	Typhus, spotted fevers	Infestation with infected arthropod
Leptospira	Leptospirosis	Contact with water containing infected animals' urine
Streptococci	Impetigo, erysipelas	
Bacillus anthracis	Cutaneous anthrax	Systemic disease following local lesion at inoculation site
Treponema pallidum and *pertenue*	Syphilis, yaws	Warm, moist skin is more susceptible
Yersinia pestis	Plague	Bite from infected rodent flea
Plasmodia	Malaria	Bite from infected mosquito
Trichophyton spp. and other fungi	Ringworm, athlete's foot	Infection restricted to skin, nails, hairs

Bacteria on the skin, as well as entering hair follicles and causing lesions (boils, styes), can also cause trouble after entering other orifices. Staphylococcal mastitis occurs in many mammals, but is of major importance in the dairy industry, and is thought to arise when the bacteria are pushed up and past the teat canal of the cow as a result of vacuum fluctuations during milking.

Large or small breaks in the skin due to wounds are obvious routes for infection. The hepatitis B or C viruses can be introduced into the body if the needle of the doctor, tattooist, drug addict, acupuncturist or ear-piercer is contaminated with infected blood. Shaving upsets the antimicrobial defences in the skin and can lead to staphylococcal infection of the shaved area on the male face (sycosis barbae) or female axilla. Pre-operative shaving, although a well-established ritual, seems to enhance rather than prevent infection in surgical wounds. Various sports in which there is rough skin-to-skin contact can result in infections (streptococci, staphylococci, skin fungi) being transmitted at the site of minor breaks in the skin. It is commonly referred to as scrumpox, but is seen in judo and in wrestling as well as in rugby football. In the last 15 years, an epidemic clone of methicillin-resistant *Staphylococcus aureus* (USA300) has emerged as a common cause of outbreaks of skin infections among sports teams, prison inmates and sauna dwellers.

Bites are also important sites for the entry of microorganisms.

Small Bites

Biting arthropods such as mosquitoes, mites, ticks, fleas and sandflies penetrate the skin during feeding and can thus introduce pathogenic agents into the body. Some infections are transmitted mechanically, the mouthparts of the arthropod being contaminated with the infectious agent, and there is no multiplication in the arthropod. This is what happens in the case of myxomatosis. Fleas or mosquitoes carry myxoma virus on their contaminated mouthparts from one rabbit to another. When transmission is said to be biological, as in yellow fever or malaria, this means that the infectious agent multiplies in the arthropod, and, after an incubation period, appears in the saliva and is transmitted to the susceptible host during a blood feed. Mosquitoes or ticks, in the act of feeding, probe in the dermal tissues, secreting saliva as they do so. The mosquito proboscis may enter a blood capillary and is then threaded along the vessel, with further injections of saliva occurring during the ingestion of blood. Infected saliva is thus introduced directly into the dermis and often into the vascular system, the counterpart of a minute intradermal or intravenous injection of microorganisms. Other diseases transmitted biologically by arthropods include typhus and plague, and in these cases the microorganisms multiply in the alimentary canal of the arthropod. Plague bacteria (*Yersinia pestis*) from the infected flea are regurgitated into the skin during feeding, and the human body louse infected with typhus rickettsiae defecates during feeding, the rickettsiae subsequently entering the body through the bite wound.

Large Bites

The classical infectious disease transmitted by a biting mammal is rabies. The rabies virus is shed in the saliva of infected foxes, dogs, wolves, vampire bats, etc. and

introduced into bite wounds. Human bites of humans are uncommon, most people having neither the temperament nor the teeth for it. When they do occur, human bites can cause troublesome sepsis because of the fusiform and spirochaetal bacteria normally present in the mouth that are introduced into the wound. Teeth often make an involuntary inoculation of bacteria into skin during fist fights. The hero's decisive punch can then bring him knuckle sepsis as well as victory! Most cats carry *Pasteurella multocida* in their mouths, and cat bites, although less common than dog bites, are likely to cause infection. Bites from tigers or cougars and particularly Komodo dragons can lead to potentially lethal *P. multocida* infections.

RESPIRATORY TRACT

Air contains a variety of suspended particles, and the total quantity seems large if one says that there are more than 1000 million tonnes of suspended particulate matter in the earth's atmosphere. Most of this is smoke, soot and dust, but microorganisms are inevitably present. Inside buildings there are 400–900 microorganisms per cubic metre, nearly all of them nonpathogenic bacteria or moulds. Therefore with a ventilation rate of 6 l/min at rest, the average man would inhale at least eight microorganisms per minute or about 10,000 per day. Efficient cleansing mechanisms remove inhaled particles and keep the respiratory tract clean, and infection of the respiratory tract has to be thought of in relation to these mechanisms, which are designed to remove and dispose of inhaled particles, whatever their nature.

A mucociliary blanket covers most of the surface of the lower respiratory tract. It consists of ciliated cells together with single mucus-secreting cells (goblet cells) and subepithelial mucus-secreting glands. Foreign particles deposited on this surface are entrapped in mucus and borne upwards from the lungs to the back of the throat by ciliary action (Figure 2.3). This has been called the mucociliary escalator. The nasal cavity (upper respiratory tract) has a similar mucociliary lining, and particles deposited here are also carried to the back of the throat and swallowed. The average person produces 10–100 ml mucus from the nasal cavity each day and a similar amount from the lung. The terminal air spaces of the lower respiratory tract are the alveoli, and these have no cilia or mucus but are lined by macrophages. IgG and secretory IgA are the predominant antibodies in the lower and upper respiratory tracts respectively, and afford specific defence once the immune system has been stimulated.

A great deal of experimental work has been carried out on the fate of inhaled particles, and particle size is of paramount importance. The larger the particle, the less likely it is to reach the terminal portions of the lung. All particles, whether viral, bacterial, fungal or inert, are dealt with in the same way. Larger visible particles are filtered off by the hairs lining the nostrils, and particles 10 μm or so in diameter tend to be deposited on the 'baffle plates' in the nasal cavity, consisting of the turbinate bones covered by nasal mucosa. Smaller particles are likely to reach the lungs, those 5 μm or less in diameter reaching the alveoli. Inhaled particles of soot and other particulates are taken up by alveolar macrophages, some of which later migrate to the pulmonary lymph nodes. Town dwellers can

be recognised in the postmortem room because of the grey colour of their pulmonary lymph nodes.[1]

If a microorganism is to initiate infection in the respiratory tract, the initial requirements are simple. First, the microorganism must avoid being caught up in mucus, carried to the back of the throat and swallowed. Second, if it is deposited in alveoli it must either resist phagocytosis by the alveolar macrophage, or if it is phagocytosed it must survive or multiply rather than be killed and digested.

It would seem inevitable that a microorganism has little chance of avoiding the first fate unless the mucociliary mechanisms are defective, or unless it has some special device for attaching firmly if it is lucky enough to encounter an epithelial cell. The highly successful myxoviruses, for instance, of which influenza is an example, have an attachment protein (the haemagglutinin) on their surface which specifically attaches to a receptor molecule (neuraminic acid of a glycoprotein) on the epithelial cells. A firm union is established and the virus now has an opportunity to infect the cell. Depending on the strain of virus, the common cold rhinoviruses use either the cell adhesion molecule ICAM-1 or the low-density lipoprotein receptor as cellular receptors.[2] *Mycoplasma pneumoniae* has a special projection on its surface by which it attaches to neuraminic acid receptors on the epithelial cell surface. The bacterium responsible for whooping cough (*B. pertussis*) has a similar mechanism for attachment to respiratory epithelium, and this undoubtedly contributes to its ability to infect the normal lung; attachment is mediated via an FHA, pili and an outer membrane protein. The most abundant protein on the surface of *S. aureus*, known as staphylococcal protein A (SpA), mediates binding to the Tumour Necrosis Factor Receptor 1 (TNFR1) on lung epithelium, leading to induction of a severe inflammatory response. This interaction may be important in the pathogenesis of severe pneumonia in humans. Bacteria that lack such devices will only establish infection when the mucociliary cleansing mechanism is damaged. *Streptococcus pneumoniae* has the opportunity to invade the lungs and cause pneumonia when mucociliary mechanisms are damaged or there is some other weakening of natural host defences. A virus infection is a common source of mucociliary damage. Destructive lesions of the respiratory tract are induced by viruses such as parainfluenza virus 3 or influenza, and various bacteria, especially streptococci, then have the opportunity to grow in the lung and produce a secondary pneumonia. People with chronic bronchitis show disturbed

[1]There is also a movement of macrophages from the lower respiratory tract up to the back of the throat on the mucociliary escalator. At least 10^7 macrophages a day are recoverable in normal rats or cats, a similar quantity in normal people, and more than this in patients with chronic bronchitis. This is a route to the exterior for macrophages laden with indigestible materials.

[2]Although made use of by invading microorganisms, receptors are clearly not there for this purpose, and serve other functions such as hormone binding and cell–cell recognition. Sometimes virus receptors are present only on certain types of cell, which can account for cell tropisms and other features of the disease. For instance, the receptor for Epstein–Barr virus is the C3d receptor (CD21) on B cells, which are thus infected and undergo polyclonal activation, and the main receptor for HIV is the CD4 molecule on T-helper cells, whose infection and depletion contributes to the serious immune deficit in AIDS.

mucociliary function, and this contributes to the low-grade bacterial infection in the lung which may be a semipermanent feature of the disease. People with cystic fibrosis have a mutation in the cystic fibrosis transmembrane conductance regulator (CFTR1) leading to prevention of the movement of salt and water in and out of cells. As a result, cells in the respiratory epithelium produce abnormally thick mucus which cannot be effectively transported by cilia leading to the sequestration of potentially pathogenic microorganisms. Also there is suggestive evidence that cigarette smoking and atmospheric pollutants lead to temporary or permanent impairment of the mucociliary defences (see Chapter 11). Finally, there are many ways in which natural host defences are compromised in hospital patients. Patients with indwelling tracheal tubes, for instance, are particularly susceptible to respiratory infection because the air entering the tracheal tube has been neither filtered nor humidified in the nose. Dry air impairs ciliary activity and the indwelling tube causes further epithelial damage. General anaesthesia decreases lung resistance in a similar way, and in addition depresses the cough reflex.

Certain microorganisms that infect the respiratory tract directly depress ciliary activity, thus inhibiting their removal from the lung and promoting infection. *B. pertussis* attaches to respiratory epithelial cells and in some way interferes with ciliary activity. *Haemophilus influenzae* produces a factor that could be important *in vivo*. The factor slows the ciliary beat, interferes with its coordination and finally causes loss of cilia. At least seven ciliostatic substances are produced by *Pseudomonas aeruginosa*, which causes a devastating respiratory infection in cystic fibrosis patients. Ciliary activity is also inhibited by *M. pneumoniae*. The mycoplasma multiply while attached to the surface of respiratory epithelial cells, and the ciliostatic effect is possibly due to hydrogen peroxide produced locally by the mycoplasma. Cilia are defective in certain inherited conditions. In Kartagener's syndrome, for instance, impaired ciliary movement leads to chronic infections in lungs and sinuses. Spermatozoa are also affected, and males with this condition are infertile.

The question of survival of airborne microorganisms after phagocytosis by alveolar macrophages is part of the general problem of microbial survival in phagocytic cells, and this is dealt with more fully in Chapter 4. Tubercle bacilli survive in the alveolar macrophages of the susceptible host, and respiratory tuberculosis is thought to be initiated in this way. The common cold viruses, in contrast, which are very commonly phagocytosed by these cells, fail to survive and multiply, and therefore cause no perceptible infection in the lower respiratory tract. Growth of many of these viruses is in any case restricted at 37°C, being optimal at about 33°C, the temperature of nasal mucosa. Under certain circumstances, the antimicrobial activity of alveolar macrophages is depressed. This occurs, for instance, following the inhalation of toxic asbestos particles and their phagocytosis by alveolar macrophages. Patients with asbestosis have increased susceptibility to respiratory tuberculosis. Alveolar macrophages infected by respiratory viruses sometimes show decreased ability to deal with inhaled bacteria, even those that are normally nonpathogenic, and this can be a factor in secondary bacterial pneumonias (see Chapter 8).

Normally the lungs are almost sterile, because the microorganisms that are continually being inhaled are also continually being phagocytosed and destroyed or removed by mucociliary action.

GASTROINTESTINAL TRACT

The intestinal tract must take what is given during eating and drinking, and also various other swallowed materials originating from the mouth, nasopharynx and lungs. Apart from the general flow of intestinal contents, there are no particular cleansing mechanisms, unless diarrhoea and vomiting are included in this category. The lower intestinal tract is a seething cauldron of microbial activity, as can readily be appreciated from the microscopic examination of fresh faeces. Multiplication of bacteria is counterbalanced by their continuous passage to the exterior with the rest of the intestinal contents. A single *Escherichia coli*, multiplying under favourable conditions, might well increase its numbers to about 10^8 within 12–18 h, the normal intestinal transit time. The faster the rate of flow of intestinal contents, the less the opportunity for microbial growth, so that there is a much smaller total number of bacteria in diarrhoea than in normal faeces. On the other hand, a reduced flow rate leads to increased growth of intestinal bacteria. This is not known to be harmful in individuals on a low fibre diet, but is a more serious matter in the blind-loop syndrome. Here, surgical excision of a piece of intestine results in a blind length in which the flow rate is greatly reduced. The resulting bacterial overgrowth, especially in the small intestine, is associated with symptoms of malabsorption, because the excess bacteria metabolise bile acids needed for absorption of fats and also compete for vitamin B_{12} and other nutrients.

The commensal intestinal bacteria are often associated with the intestinal wall, either in the layers of mucus or attached to the epithelium itself. If a mouse's stomach or intestine is frozen with the contents intact and sections are then cut and stained, the various commensal bacteria can be seen in large numbers, intimately associated with the epithelial cells. This makes it easier for them to maintain themselves as permanent residents.

Helicobacter pylori are Gram-negative microaerophilic spiral bacteria which reside in the stomachs of humans and other primates. They can persist for years and possibly for life. They live in the mucus overlay of the gastric epithelium. They do not appear to invade the tissue, but the underlying mucosa is invariably inflamed, a condition termed chronic superficial gastritis. To prove that *H. pylori* was the cause of the gastritis, Dr Barry Marshall, an Australian gastroenterologist and his colleague infected themselves by ingesting a culture of *H. pylori* and both developed gastritis, one lasting 14 days and the other nearly three years. Marshall subsequently won the Nobel prize for medicine for his troubles! The chronic inflammatory process is linked with peptic ulceration and gastric cancer, two of the most important diseases of the upper gastrointestinal tract. *H. pylori* infection precedes ulceration, is nearly always present, and eradication of the organism by antibiotic therapy results in healing of the ulcer and a very low rate of ulcer recurrence. However, since many more people carry the organism than have ulcers, there must be other as yet unidentified predisposing factors which play a role in disease. There is also an association between *H. pylori* infection and gastric cancer. Urease is produced in abundance by this organism. Presumably it acts on urea, present in low concentrations, to form ammonia which locally neutralises acid and thus enables the bacteria to survive in this hostile environment. In addition, the presence of a 'pathogenicity island' (PAI) known as the cag PAI, which encodes for several virulence factors, is associated with an increased inflammatory response.

Pathogenic intestinal bacteria must establish infection and increase in numbers, and they too often have mechanisms for attachment to the epithelial lining so that they can avoid being carried straight down the alimentary canal with the rest of the intestinal contents. Indeed, their pathogenicity is likely to depend on this capacity for attachment or penetration. The pathogenicity of cholera, for instance, depends on the adhesion of bacteria to specific receptors on the surface of intestinal epithelial cells, and other examples are included in Table 2.1. Clearly, the concentration and thus the adsorption of bacterial toxins will also be affected by the balance between production and removal of bacteria in the intestine. Certain protozoa cause intestinal infections without invading tissues, and they too depend on adherence to the epithelial surface. *Giardia lamblia* attaches to the upper small intestine of man by means of a sucking disc, assisted by more specific binding (Table 2.1).

The likelihood of infection via the intestinal tract is certainly affected by the presence of mucus, acid, enzymes and bile. Mucus protects epithelial cells, perhaps acting as a mechanical barrier to infection, and contains secretory IgA antibodies that protect the immune individual against infection. Motile microorganisms (*Vibrio cholerae*, certain strains of *E. coli*) can propel themselves through the mucus layer and are thus more likely to reach epithelial cells to make specific attachments.[3] *V. cholerae* also produces a mucinase that probably helps its passage through the mucus. Microorganisms infecting by the intestinal route are often capable of surviving in the presence of acid, proteolytic enzymes and bile. This also applies to microorganisms shed from the body by this route. The streptococci that are normal human intestinal inhabitants (*Streptococcus faecalis*) grow in the presence of bile, unlike other streptococci. This is also true of other normal (*E. coli*, *Proteus*, *Pseudomonas*) and pathogenic (*Salmonella*, *Shigella*) intestinal bacteria. It is noteworthy that the gut picornaviruses (hepatitis A, coxsackie-, echo- and polioviruses) are resistant to bile salts and to acid. The fact that tubercle bacilli resist acid conditions in the stomach favours the establishment of intestinal tuberculosis. Most bacteria, however, are acid sensitive and prefer slightly alkaline conditions.[4] Intestinal pathogens such as *Salmonella* or *V. cholerae* are more likely to establish infection when they are sheltered inside food particles or when acid production in the host is impaired (achlorhydria). Volunteers who drank different doses of *V. cholerae* contained in 60 ml saline showed a 10^4-fold increase in susceptibility to cholera when 2 g of sodium bicarbonate were given with the bacteria. Classical strains of cholera were used, and the minimal disease-producing dose without bicarbonate was 10^8 bacteria. Similar experiments have been done in volunteers with *Salmonella typhi*. The minimal oral infectious dose was 10^3–10^4 bacteria, and this was significantly reduced by the ingestion of sodium bicarbonate.

The intestinal tract differs from the respiratory tract in that it is always in motion, with constantly changing surface contours. The surface is made up of villi, crypts and other

[3]Nonmotile microorganisms, in contrast, rely on random and passive transport in the mucus layer. How important is mucus as a physical barrier? Gonococci and *Chlamydia* are known to attach to spermatozoa, and they could be carried through the cervical mucus as 'hitch-hikers' so that spermatozoa could help transmit gonorrhoea and nonspecific urethritis.

[4]The standard Sabouraud's medium for the isolation of yeasts and moulds has an acid pH (5.4) in order that bacterial growth should be generally inhibited.

irregularities, and the villi themselves contract and expand. Particles in the lumen are moved about a great deal and have good opportunities for encounters with living cells; this is what the alimentary canal is designed for, if food is to be mixed, digested and absorbed. Viruses, by definition, multiply only in living cells; thus enteric viruses must make the most of what are primarily chance encounters with epithelial cells. Polio-, coxsackie- and echoviruses, and the human diarrhoea viruses (rotaviruses, certain adenoviruses, etc.) form firm unions with receptor molecules on the surface of intestinal epithelial cells, thus giving time for the penetration of the virus into the cell. On the other hand, enteric bacteria that enter the mucosa are able to increase their numbers by growth in the lumen before entry, but it is not surprising that there are also mechanisms for bacterial attachment to epithelial cells (Table 2.1). Penetration of viruses into cells is discussed at a later stage, but it takes place either by endocytosis (phagocytosis into virus-sized vesicles) of the virus particle, or by fusion of the membrane of enveloped viruses with the cell membrane so that the contents of the virus particle enter the cell. These alternatives are not so distinct, because the virus particle in an endocytic vesicle is still in a sense outside the cell and still has to penetrate the cell membrane for its contents to be released into the cytoplasm, and enveloped viruses achieve this by fusion.

Most epithelial cells, whether epidermal, respiratory or intestinal, are capable of phagocytosis, but this is on a small scale compared with those specialist phagocytes, the macrophages and polymorphonuclear (PMN) leucocytes. Certain pathogenic bacteria in the alimentary canal are taken into intestinal epithelial cells by a process that looks like phagocytosis. As seen by electron microscopy in experimental animals, *Salmonella typhimurium* (Figure 2.4) attach to microvilli forming the brush border of intestinal epithelial cells. The microvilli degenerate locally at the site of attachment, enabling the bacterium to enter the cell, and the breach in the cell surface is then repaired. A zone of degeneration precedes the bacterium as it advances into the apical cytoplasm. In general, commensal

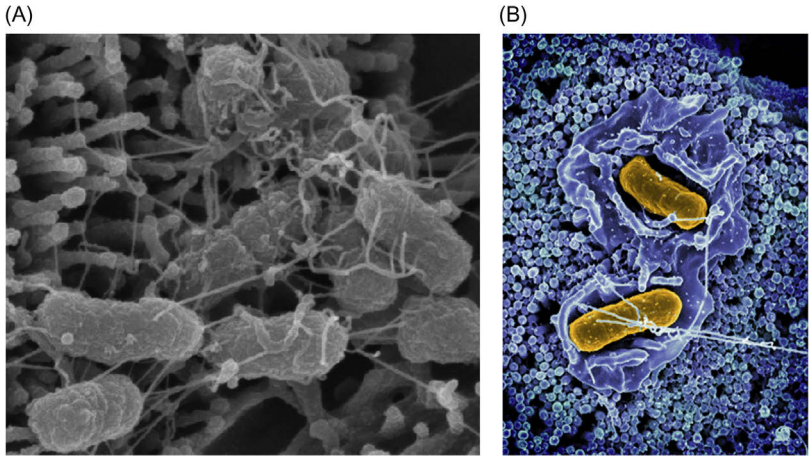

FIGURE 2.4 Attachment and entry of *S. typhimurium*. Scanning electron microscopy of *Salmonella* adherence after innoculation of a bovine-ligated distal ileal loop. (A) and invasion of human intestinal epithelial cells (B). Note the fimbrial and flagellae apparatus in (A) and the characteristic ruffling of cells induced by Salmonella in (B). *Provided by J. Elvidge (A) and O. Steele-Mortimer, Rocky Mountain labs, NIAID, NIH, USA (B).*

intestinal bacteria do not appear to be taken up when they are attached to the intestinal epithelium. We are beginning to understand intestinal invasion at the molecular level. The sequelae to penetration of the epithelium will depend on bacterial multiplication and spread on toxin production, cell damage and inflammatory responses.

Microbial toxins, endotoxins and proteins can certainly be absorbed from the intestine on a small scale, and immune responses may be induced. Antibodies to materials such as milk, eggs and black beans can be detected when they form a significant part of the diet, and insulin is absorbed after ingestion as shown by the occurrence of hypoglycaemia. Diarrhoea promotes the uptake of proteins, and absorption of protein also takes place more readily in the infant, especially in species such as the pig or horse that need to absorb maternal antibodies from milk. As well as large molecules, particles the size of viruses can be taken up from the intestinal lumen,[5] and this occurs in Peyer's patches. Peyer's patches are isolated collections of lymphoid tissue lying immediately below the intestinal epithelium. The epithelial cells here are highly specialised (so-called M (microfold) cells) and take up particles and foreign proteins, delivering them to underlying immune cells with which they are intimately associated by means of cytoplasmic processes. When large amounts of a reovirus for instance (see Table A.5) are introduced into the intestine of a mouse, the uptake of virus particles by M cells and delivery to immune cells, from whence they reach local lymph nodes, can be followed by electron microscopy. It seems appropriate that microorganisms in the intestine are sometimes 'focused' into immune defence strongholds. In addition, some bacterial pathogens such as Salmonella have the remarkable capacity to induce the transformation of gut cells into M cells in order to promote their own cellular uptake.

The normal intestinal microorganisms of man are specifically adapted to life in this situation, and most of them are anaerobes of the *Bacteroides* group, although *E. coli*, enterococci, lactobacilli and diphtheroids are common. Because of the acid pH, the stomach harbours small numbers of organisms, but the total numbers increase as the intestinal contents move from the small to the large intestine, and there are about 10^8–10^{10} bacteria g^{-1} in the terminal ileum, increasing to 10^{11} g^{-1} in the colon and rectum. Bacteria normally compose about a quarter of the total faecal mass. Recently, developments in sequencing technologies have facilitated extensive research into the diversity of commensal bacterial populations (microbiota) and their role in the host. The normal flora are in a balanced state and tend to resist colonisation with other bacteria. Possible mechanisms include killing other bacteria by bacteriocins (see Glossary), competition for food substances or attachment sites, and the production of bacterial inhibitors. For instance, in mice the resident coliforms and *Bacteroides* produce acetic and propionic acids which are inhibitory for *Shigella* (dysentery) bacteria. Patients treated with broad-spectrum antibiotics show changes in normal intestinal flora and this may allow an abnormal overgrowth of microorganisms, such as the fungus *Candida albicans* and *Clostridium difficile*. In breast-fed infants, the predominant bacteria in the large bowel are lactobacilli and their metabolic activity produces acid and other factors that inhibit other microorganisms. As a result of this, and perhaps also because of antibacterial components present in human milk,

[5]When rats drink water containing very large amounts of bacteriophage T7 (diameter 30 nm), intact infectious phages are recoverable from thoracic duct lymph within 20 min.

breast-fed infants resist colonisation with other bacteria, such as the pathogenic strains of *E. coli*. Bottle-fed infants, on the other hand, lacking the protective lactobacilli, are susceptible to pathogenic strains of *E. coli*, and these may cause serious gastroenteritis.

Intestinal microorganisms that utilise ingested cellulose serve as important sources of food in herbivorous animals. In the rabbit, for instance, volatile fatty acids produced by microorganisms in the caecum yield 20% of the daily energy requirements of the animal. The rumen of a 500 kg cow is a complex fermentation chamber whose contents amount to 70 l. In this vast vat many different species of bacteria multiply continuously, utilising cellulose and other plant materials, and protozoa live on the bacteria. As the microbial mass increases, the surplus passes into the intestine to be killed, digested and absorbed. Large volumes of CO_2 and methane are formed and expelled from both ends of the cow. The passage of methane represents a loss of about 10% of the total energy derived from food. In man, intestinal bacteria do not normally have a nutritive function but they break down and recycle the components of desquamated epithelial cells, and may be involved in the synthesis of vitamins.

Mechanisms of Attachment to and Invasion of the Gastrointestinal Tract

Pursuit of the mechanisms and the microbial determinants responsible for invasion and replication within intestinal mucosae by enteric pathogens is one of the most actively researched areas in bacterial pathogenicity. Diarrhoeal disease is caused by invasive species (e.g. Salmonellae, Shigellae and enteroinvasive *E. coli*) as well as noninvasive species (e.g. *V. cholerae*, enteropathogenic and enterotoxigenic *E. coli*) and is still responsible for a huge proportion of the total morbidity and mortality in developing countries; a separate section is devoted to this topic in Chapter 8. Also, in recent years the incidence in the United Kingdom and other developed countries of *Salmonella* and *Campylobacter* infections has risen dramatically and remains high. Intense research is being conducted to elucidate the mechanisms of invasion which has led to detailed knowledge of how bacteria invade the gut. What follows is an overview of the biological significance of this fast-moving field.

General Considerations

Before dealing with the molecular mechanistic work, it is important to record a note of caution regarding experimental systems. Several attachment/invasin systems, operative in cultured cells, have been described in *Yersinia* spp., yet only the chromosomally encoded 'invasin' appears to be important in interaction with M cells in Peyer's patches through which *Yersinia* penetrate the gut. Moreover, once they have negotiated the M cell barrier, *Yersinia* are essentially extracellular pathogens, yet for years they have been studied as paradigms of intracellular pathogens! The most widely used system for modelling human typhoid-like infections caused by *S. typhi* is infection of the mouse with *S. typhimurium* in which the ratios of oral to intraperitoneal LD_{50} values obtained for parent and mutant strains are compared. By this means one can deduce whether a mutation has affected the ability of the pathogen to negotiate the gut mucosa or to survive some later stage in the complex host pathogen interaction as, for example, an encounter with macrophages. However, the gut mucosa is a complex, highly organised tissue and increases in oral LD_{50} values do not *per se* indicate whether this is due to failure to negotiate the epithelial layer

of enterocytes, or to handle the hypertonic conditions at the tips of villi, or to spread across the deeper submucosal layers. In any case, if one is interested in the mechanisms of diarrhoeal disease induced by *S. typhimurium*, one cannot use mice since *S. typhimurium* in mice (as its name implies) is equivalent to *S. typhi* in man and causes a systemic infection rather than a localised mucosal infection. For the latter the best small laboratory model is the rabbit.

'Pathogenicity islands' (PAIs, PaIs, PIs; the acronyms still vary) and other mobile virulence elements encode one or more virulence factor (e.g. adhesins, invasins, iron uptake systems, toxins and secretion systems, and doubtless others), and are present in the genome (chromosome or plasmid) of pathogenic bacteria but absent from the genome of related nonpathogens. They range in size from 10 to 200 kb and often have different G + C content, suggesting their acquisition by horizontal transfer of DNA into new hosts. PAIs are often flanked by direct repeat sequences with tRNA genes, a common target for insertion into the chromosome. The *Shigella* virulence plasmid has been called an 'archipelago' of PAIs and smaller elements (1−10 kb) 'islets'. The second development concerns the recognition of at least eight secretion systems in Gram-negative bacteria. Type I is sec (secretory system) independent and exemplified by the secretion of α-haemolysin of *E. coli*; it secretes proteins from the cytoplasm across the inner and outer membranes in one step facilitated by a small number of ancillary genes. Type II secretion is sec-dependent and secretes effector proteins using the general secretory pathway (GSP). Type III systems are complex, sec-independent systems closely related to the flagella assembly system; they are also activated on contact with host cells. They are known to be extremely important in a growing number of pathogens (as we shall see) and facilitate translocation of bacterial effector molecules directly into the host cell membrane or cytoplasm. Type IV involves secretion of proteins where all the necessary information for transmembrane negotiation inheres in the secreted protein itself. More recently, type V and VI modes of protein secretion have also been identified. While type VI involves direct transport of toxins into other bacteria and into eukaryotic cells, type V include the autotransporters which have been shown to be involved in a myriad of functions associated with virulence. Type VII are characteristic of *Mycobacteria* spp. and also play a central role in virulence, whereas type VIII is responsible for the secretion and assembly of prepillins for Gram-negative fimbriae.

Enteropathogenic E. coli *(EPEC)*

EPEC was the first serotype of *E. coli* to be incriminated as a pathogen. Its designation as EPEC is unfortunate as all pathogenic *E. coli* are in a real sense enteropathogenic, but the nomenclature is rigidly embedded. It is essentially a noninvasive pathogen with only rare reports of its presence inside human gut epithelial cells; it can, however, be internalised by cultured cells. The pathognomonic lesion of EPEC is the pedestal type 'attaching and effacing' (A/E) lesion induced on microvilli-bearing enterocytes resulting in an 'intimate' type of adherence (Figure 2.5). The adherence is different to the nonintimate adherence exhibited by *V. cholerae* and enterotoxigenic *E. coli*, which both attach via adhesins that project from the organism to the host cell. There are two main genetic elements which confer virulence on EPEC: (i) *bfp* genes (bundle-forming pili (BFP) encoded in the EPEC adherence factor (EAF) plasmid) and (ii) the genes encoding the determinants of A/E

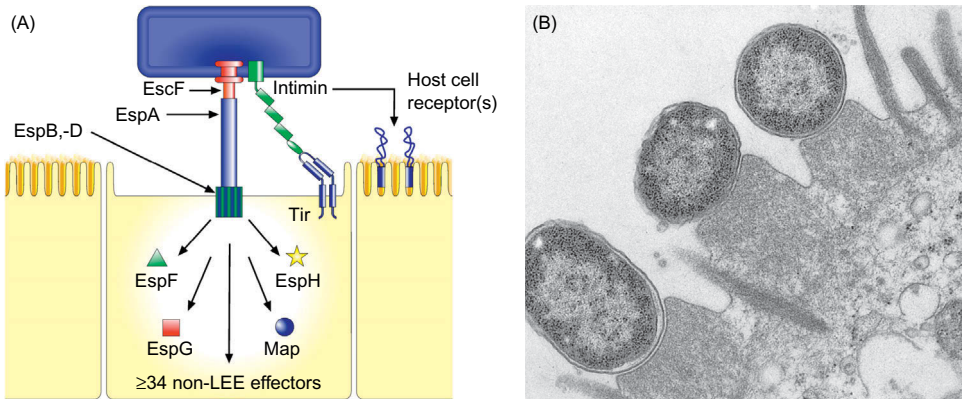

FIGURE 2.5 Classical EPEC-induced A/E pedestal formation. (A) Schematic representation of pedestal formation. Cell contact stimulates the expression of LEE-encoded proteins and the assembly of a protein translocation apparatus (translocon). The translocon consists of pores in the bacterial envelope (EscC-generated pore) and in the host membrane (EspB/D-generated pore) with the pores connected by a hollow EspA filament, thereby providing a continuous channel from the bacterial envelope to the host cell cytosol. The translocon is used to translocate Tir into the host cell where it becomes inserted into the host cell membrane; EPEC Tir (but not EHEC O157:H7 Tir) becomes phosphorylated on tyrosine residues following translocation. Translocated Tir and other effector proteins transduce signals that induce breakdown of the brush-border microvillous actin cytoskeleton with consequent vesiculation of the microvillous membrane. Intimate adhesion and pedestal formation results from the interaction of intimin and Tir and the accumulation of actin (and other cytoskeletal proteins) beneath intimately attached bacteria following microvillous effacement. Effector proteins may be LEE or non-LEE encoded. (B) Electron micrograph of EHEC-induced pedestals. *(A) Stevens and Wallis, 2005 and (B) provided by M. Stevens. Roslin Institute, University of Edinburgh.*

encoded in the chromosomally located LEE (locus of enterocyte effacement) pathogenicity island. BFP have been shown in human volunteer studies to be important, although not absolutely necessary, in the colonisation of EPEC; their expression is regulated by the *per* (plasmid-encoded regulator) genes located in the EAF plasmid. The *per* regulator also controls expression of other membrane proteins and thus acts as a global regulator, a feature now increasingly recognised in pathogenic bacteria. Upon contact with epithelial cells, expression of LEE is triggered, the sequelae to which is summarised in Figure 2.5. The incredible fact is that the organism expresses and inserts its own receptor Tir (translocated intimin receptor), which after phosphorylation and interaction with intimin triggers a signalling cascade which results in diarrhoea.

Enterohaemorrhagic E. coli *(EHEC)*

Unlike EPEC which colonises predominantly the small intestine, EHEC colonises the lymphoid follicle-dense mucosa at the terminal rectum. The mechanism of initial attachment is not clear, but sequential attachment is via a Tir–intimin interaction. After initial attachment, the clinical outcome of infection is quite different from that induced by EPEC.

SHIGELLA

Initial entry of *Shigella dysenteriae* into the colonic mucosa is via M cells in follicle-associated epithelia (FAE) through which they migrate without killing the M cell.

Shigellae are then able to infect intestinal epithelial cells via their basolateral membranes: they do not penetrate via brush borders. The genetic system encoding this invasive phenotype is a PAI in the virulence plasmid, which includes the Mxi/Spa secretory apparatus and the Ipa (invasion plasmid antigen B) proteins which are the major effectors of entry (Figure 2.7). Infected epithelial cells are induced to release the inflammatory cytokines interleukin-8 (IL-8) and tumour necrosis factor-α (TNFα). In addition, *Shigella* infects macrophages, inducing IpaB-mediated apoptosis and IpaB-mediated release from those macrophages of IL-1β, another potent inflammatory cytokine. The inflammatory response destabilises epithelial integrity and permeability by the extrusion of PMN cells, thereby allowing direct access of more *Shigella* to the basolateral membranes of epithelial cells. Interaction with PMN cells results in killing of organisms with concomitant release of tissue-damaging granules. Within minutes after entry, *S. flexneri* escapes from its vacuole by virtue of IpaB which, in addition to triggering entry, is responsible for lysis of the vacuolar membrane and escape of the organisms into the cytoplasm. *Shigella* then express the *olm* (organelle-like movement) phenotype, which allows the spread of organisms throughout the cytoplasm along actin stress cables which run between anchorage sites of cells adhering to substrata. The details of the mechanism responsible for this movement are not known but cytoskeletal actin is involved in one of several ways, possibly by myosin-like proteins giving rise to an ATP-powered movement. In addition they express the *ics* (intra−inter-cellular spread) phenotype which allows colonisation of the adjacent cells. This second type of movement, also seen with intracellular *Listeria monocytogenes*, is accompanied by the appearance of 'comet'-like rearrangements of actin in the cell. Rapid polymerisation of actin filaments occurs localised at one end of the bacterium, resulting in a forward movement of the bacterium. Nucleation and polymerisation of actin is mediated by *virG* (*icsA*). The organism is now propelled towards the cell membrane and into a protrusion of the adjacent cell membrane now surrounded by a double membrane. The latter is ruptured by the product of *icsB* gene, thereby releasing the organism into adjacent cells where rapid intracytoplasmic multiplication can again take place.

SALMONELLA

Much of the existing information on *Salmonella* invasion relates to studies in mice from which the near dogma has developed that entry via M cells in the FAE is a *sine qua non* for intestinal invasion. However, this is clearly not the case in rabbits, calves and pigs where concurrent entry of *Salmonella* into M cells and enterocytes can be seen. There are at least two invasive biotypes of *Salmonella* which cross conventional serotypic boundaries.

Histotoxic *Salmonella*

The main feature of the early damage to epithelia caused by histotoxic strains of *Salmonella* serotype Typhimurium is a toxin-mediated detachment of enterocytes from rabbit terminal ileum which is preceded by cleavage of tight junctions. This leads to the release of microvilli-bearing cells which degenerate rapidly into spherical highly vacuolated entities. Similar lesions can be produced in rabbit tissues challenged *in vivo* and *in vitro* with live histotoxic Dublin strains. Sterile supernates from rabbit gut challenged

in vitro with a histotoxic Typhimurium strain induce an almost identical picture of epithelial disintegration when added to fresh tissue from the same animal. In calves and pigs, histotoxic strains of Dublin cause extensive tissue damage to both absorptive epithelium (AE) and FAE. *Salmonella* serotype Choleraesuis is not histotoxic. These observations are of crucial importance in attempting to understand the pathogenesis of *Salmonella* infection. By virtue of their ability to denude epithelia, these organisms open up new routes of invasion and tissue transmigration.

Nonhistotoxic *Salmonella*

Nonhistotoxic *Salmonella* cause shortening of whole villi. Here the picture, as observed in the rabbit ileal loop model, is totally different from the one described for histotoxic *Salmonella*. Bacteria enter via brush borders (and bacteria-laden cells are shed). There is no evidence of a rapid initial cleavage of tight junctions. The time scale of events is quite different with maximum cell shedding leading to truncation of villi occurring at 12–14 h post-challenge. Behind the extrusion of bacteria-laden cells, the epithelium is resealed. The significance of these observations is discussed in Chapter 8. Virulent strains induce a massive influx of PMN cells.

In recent years, there has been a huge effort, still actively ongoing, to discover the molecular genetic basis of virulence of *Salmonella*. As a result, at least five 'pathogenicity islands' (PIs) have been recognised in the *Salmonella* chromosome. It is premature to give a detailed coverage of this complex field in relation to *Salmonella*, as the field is rapidly developing, but a few emerging points are summarised. First, the genes recognised in these *Salmonella* PI (SPI) clusters are mainly to do with invasion of eukaryotic cells, intracellular survival and systemic infection. Second, at least SPI-1 and SPI-2 are known to encode type III secretion systems. Some of the secreted proteins have been recognised and are involved in the translocation of effector molecules into eukaryotic target cells, thereby promoting invasion. Appendage structures have been observed whereby Typhimurium attaches to gut epithelia (Figure 2.5) which are remarkably similar to those described for EPEC.

YERSINIA

As indicated above, *Yersinia* has long been regarded as an intracellular pathogen, but histopathological examination shows clearly that after penetration of the intestinal epithelium through M cells, and destruction of Peyer's patches, *Y. enterocolitica* is found in lymphoid follicles where it is potently anti-phagocytic by virtue of its virulence plasmid PAI (see Chapter 4).

CAMPYLOBACTER JEJUNI

Campylobacter jejuni is a major global cause of food poisoning which is derived principally from poultry. Two distinct flagellin genes are carried and *C. jejuni* is highly motile. The organism also produces a cytolethal distending toxin which assists in immune avoidance. Whole genome sequence of hundreds of isolates of *Campylobacter* spp. from different animal hosts has revealed the key role of gene recombination during its evolution and genome-wide association studies have started to reveal the determinants involved in survival in different host species.

GIARDIA LAMBLIA

Giardia lamblia colonises the human small bowel and causes diarrhoea. There are two candidate adhesins: taglin (trypsin-activated *G. lamblia* lectin) and GLAM-1 (*G. lamblia* adherence molecule-1). The current perception is that initial contact of the parasite with the gut wall is via taglin, which is distributed round the surface of the parasite, and that the disc-specific GLAM-1 present on this organelle is responsible for the avid attachment of the disc to the target cell surface.

ENTAMOEBA HISTOLYTICA

The trophozoite form of *Entamoeba histolytica* lives in the lumen of the large bowel, the only known reservoir for this parasite. The trigger mechanisms which convert this organism into the pathogen, causing serious invasive amoebiasis, are not known. The ability to adhere to colonocytes *in vivo* seems to be the exception rather than the rule. More is known about the putative determinants of gut damage and the factors important in the spread of the organism formation of lesions in the liver.

OROPHARYNX

The throat (including tonsils, fauces, etc.) is a common site of residence of microorganisms as well as of their entry into the body.

The microbial inhabitants of the normal mouth and throat are varied, exceedingly numerous, and are specifically adapted to life in this environment. Bacteria are the most numerous, but yeasts (*C. albicans*) and protozoa (*Entamoeba gingivalis*, *Trichomonas tenax*) occur in many individuals. Oral bacteria include streptococci, micrococci and diphtheroids, together with *Actinomyces israeli* and other anaerobic bacteria. Some of these are able to make very firm attachments to mucosal surfaces, and others to teeth which provide a long-term, nondesquamating surface. *Streptococcus mutans*, for instance, uses the enzyme glycosyl transferase to synthesise glucan (a high molecular weight polysaccharide) from sucrose. The glucan forms an adhesive layer, attaching bacteria to the surface of teeth and to other bacteria (Table 2.1). If there are no teeth, as in the very young or the very old, *S. mutans* has nothing to 'hold on to' and cannot maintain itself in the mouth. The dextran-containing secretions constitute a matrix in which various other bacteria are present, many of them anaerobic. It forms a thin film attached to the surface of the tooth which is called dental plaque and is visible as a red layer when a dye such as erythrosine is taken into the mouth (Figure 2.6). Dental plaque is a complex microbial mass containing about 10^9 bacteria g^{-1}. Certain areas of the tooth are readily colonised, especially surface fissures and pits, areas next to the gum, and contact points between neighbouring teeth. The film is largely removed by thorough brushing, but re-establishes itself within a few hours. When teeth are not cleaned for several days the plaque becomes quite thick, a tangled forest of microorganisms. Dietary sugar is utilised by bacteria in the plaque and the acid that is formed decalcifies the tooth and is responsible for dental caries. The pH in an active caries lesion may be as low as 4.0. Unless the bacteria, the sugar (and the teeth) are present, dental caries does not develop. When monkeys are fed on a caries-producing diet, the extent

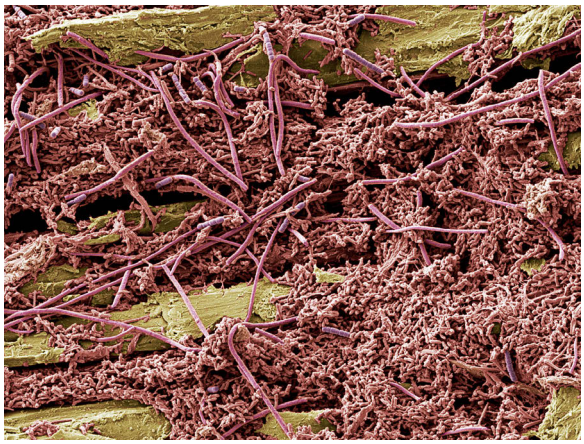

FIGURE 2.6 Dental plaque on a used toothpick, coloured scanning electron micrograph (SEM). Magnification: x650 when printed at 10 centimetres wide. *Reproduced with permission: STEVE GSCHMEISSNER/SCIENCE PHOTO LIBRARY*

of the disease can be greatly reduced by vaccination against *S. mutans*,[6] and vaccines are being developed for use against caries in man. Caries are already becoming less common, but if the vaccines are effective, caries (and many dentists) could one day be eliminated. Western individuals with their tightly packed, bacteria-coated teeth and their sugary, often fluoride-deficient diet, have been badly affected, and it is legitimate to regard dental caries as one of their most prevalent infectious diseases.

Periodontal disease is another important dental condition that affects nearly everyone (and most animals) to a greater or lesser extent. The space between the tooth and gum margin has no natural cleansing mechanism and it readily becomes infected. This results in inflammation with accumulation of polymorphs and a serum exudate. The inflamed gum bleeds readily and later recedes, while the multiplying bacteria can cause halitosis. Eventually the structures that support the teeth are affected and teeth become loose as bone is resorbed and ligaments weakened. Bacteria such as *Actinomyces viscosis*, *Actinobacillus actinomycetemcomitans*, and especially *Porphyromonas gingivalis* are commonly associated with periodontal disease. Some members of the oral microbiota such as viridans Streptococci may gain access to the blood stream and can cause life-threatening infective endocarditis (IE), an infection of the heart valves in which Streptococcal IE commonly affects individuals with prior valvular damage.

Certain strains of streptococci adhere strongly to the tongue and cheek of man but not to teeth, and can be shown to adhere to the epithelial cells in cheek scrapings. Pharyngeal cells can be obtained by wiping the posterior pharyngeal wall with a wooden applicator stick, and experiments show that virulent strains of *Streptococcus pyogenes* and other bacteria such as Corynebacteria make attachments to these cells via pili-like structures extended

[6]Antibodies are protective, as shown when orally administered monoclonal antibody to outer components of *S. mutans* prevented colonization. It is noteworthy that in one study of 11 agammaglobulinaemic patients, all were badly affected by caries, and four lost all their teeth quite rapidly between the age of 20 and 30 years. Local antibody to the relevant bacteria would be protective, and additional antimicrobial forces are present in crevicular spaces. The crevicular space is a small fluid-filled cleft between the edge of the gum and the tooth, containing antibodies (IgG, IgM), complement and phagocytic cells derived from plasma.

from the bacterial surface. As in the intestines, the presence of the regular microbial residents makes it more difficult for other microorganisms to become established. Possible mechanisms for this interference were mentioned in the preceding section. Changes in oral flora upset the balance. For instance, the yeast-like fungus *C. albicans* is normally a harmless inhabitant of the mouth, but after prolonged administration of broad-spectrum antibiotics, changes in the normal bacteria flora enable the pseudomycelia of *C. albicans* to penetrate the oral epithelium, grow and cause thrush.

Saliva is secreted in volumes of a litre or so a day, and has a flushing action in the mouth, mechanically removing microorganisms as well as providing antimicrobial materials such as lysozyme (see Glossary) and secretory antibodies. It contains leucocytes, desquamated mucosal cells and bacteria from sites of growth on the cheek, tongue, gingiva, etc. When salivary flow is decreased for 3–4 h, as between meals,[7] there is a fourfold increase in the number of bacteria in saliva. Disturbances in oral antimicrobial and cleansing mechanisms may upset the normal balance. In dehydrated patients, or those ill with typhus, typhoid, pneumonia, etc., the salivary flow is greatly reduced, and the mouth becomes foul as a result of microbial overgrowth, often with some tissue invasion. Vitamin C deficiency reduces mucosal resistance and allows the normal resident bacteria to cause gum infections. As on all bodily surfaces, there is a shifting boundary between harmless coexistence of the resident microbes and invasion of host tissues, according to changes in host resistance.

During mouth breathing, the throat acts as a baffle on which larger inhaled particles can be deposited, and microorganisms in saliva and nasal secretions are borne backwards to the pharynx. Microorganisms in the mouth and throat need to be attached to the squamous epithelial surface or find their way into crevices if they are to avoid being washed away and are to have an opportunity to establish infection. One of the earliest and most regular symptoms of upper respiratory virus infections is a sore throat, suggesting early viral growth in this area, with an inflammatory response in the underlying tissues. It may also signify inflammation of submucosal lymphoid tissues in the tonsils, back of the tongue, and throat, which form a defensive ring guarding the entrance to alimentary and respiratory tracts.

URINOGENITAL TRACT

Urine is normally sterile, and since the urinary tract is flushed with urine every hour or two, invading microorganisms have problems in gaining access and becoming established. The urethra in the male is sterile, except for the terminal third of its length, and microorganisms that progress above this point must first and foremost avoid being washed out during urination. That highly successful urethral parasite, the gonococcus, owes much of its success to its special ability to attach very firmly to the surface of urethral epithelial cells, partly by means of pili projecting from its surface.[8] Similarly, uropathogenic *E. coli*

[7]Salivary flow continues between meals, the average person swallowing about 30 times an hour.

[8]The gonococcus is soon killed in urines that are acid (<pH 5.5), and this helps explain why the bladder and kidneys are not invaded. The prostate is at times affected and the gonococcus accordingly grows in the presence of spermine and zinc, materials that are present in prostatic secretions and that would inhibit many other bacteria.

(UPEC) adhere to uroepithelial cells by means of a well-characterised pilus. The bladder is not easily infected in the male; the urethra is 20 cm long, and generally bacteria need to be introduced via an instrument such as a catheter to reach the bladder. The female urethra is much shorter, only about 5 cm long, and more readily traversed by microorganisms; it also suffers from a dangerous proximity to the anus, the source of intestinal bacteria. Urinary infections are about 14 times as common in women, and most women have urinary tract infections at some time. Bacteruria,[9] however, often occurs without frequency, dysuria, or other symptoms. Even the urethral deformations taking place during sexual intercourse may introduce infection into the female bladder.[10] Spread of infection to the kidney is promoted by the refluxing of urine from bladder to ureter that occurs in some young females.

Urine, as long as it is not too acid, provides a fine growth medium for many bacteria and the entire urinary tract is more prone to infections when there is interference with the free flow and flushing action of urine, or when a 'sump' of urine remains in the bladder after urination. Incomplete emptying also leads to urinary infection in pregnant women, and this is partly due to the sluggish action of muscles in the bladder wall. But the bladder is more than an inert receptacle for infected urine and responds with inflammation and secretory antibody production. The normal bladder wall, moreover, appears to have some intrinsic but poorly understood antibacterial activity. Uropathogenic strains of *E. coli* bind to epithelial cells lining the bladder, which respond by exfoliating. As a host defence this is not enough because the bacteria then invade deeper tissues.

The vagina has no particular cleansing mechanism and would appear to present an ideal site for colonisation by commensal microorganisms. During the reproductive lifetime of the woman, the vaginal epithelium contains glycogen because of the action of circulating oestrogens. Doderlein's bacillus (a lactobacillus) colonises the vagina, metabolising the glycogen to produce lactic acid. The lactic acid gives a vaginal pH of about 5.0, and together with other products of metabolism inhibits colonisation by all except Doderlein's bacillus and a select number of bacteria, including various nonpyogenic streptococci and diphtheroids. Normal vaginal secretions contain up to 10^8 bacteria ml^{-1}. Other microorganisms are unable to establish infections, except the specialised ones that are therefore responsible for venereal diseases. Oestrogens thus generate an antimicrobial defence mechanism just at the period of life when contaminated objects are being introduced into the vagina. Before puberty and after the menopause, the vaginal epithelium lacks glycogen, the secretion is alkaline, and bacteria from the vulva, including staphylococci and streptococci, can become established.

The ascent of microorganisms from vagina to uterus is blocked at the cervix because of the downward flow of mucus and the action of cilia, together with local production of lysozyme. Once the cervical barrier has been interfered with, after abortion, miscarriage,

[9]By the time it has been voided and tested in the laboratory, urine always contains bacteria. For routine purposes it is not regarded as significant unless there are more than 10^5 bacteria (ml urine)$^{-1}$. But many women have frequency and dysuria with smaller numbers of bacteria in urine and in some cases, perhaps, the infection has spread no further than the urethra.

[10]The importance of sexual activity is often assessed by comparing nuns or prostitutes with 'ordinary' women. Bacteruria is 14 times commoner in ordinary women than in nuns, and in one study, sexual intercourse was the commonest precipitating factor for dysuria and frequency in young women. On the other hand, an innocent bubble bath may facilitate spread of faecal organisms into the urethra.

childbirth or the presence of an intrauterine contraceptive device, invasion of the uterus, fallopian tubes, etc., becomes easier. Gram-negative intestinal bacteria, group B streptococci or anaerobes are likely causes of infection. The cervix is less of a barrier to more expert invaders *Neisseria gonorrhoeae* and *Chlamydia trachomatis*.

CONJUNCTIVA

The conjunctiva is kept moist and healthy by the continuous flow of secretions from lachrymal and other glands. Every few seconds the lids pass over the conjunctival surface with a gentle but firm windscreen wiper action. Although the secretions (tears) contain lysozyme (see Glossary) and other antimicrobial substances such as defensins (see Glossary), their principal protective action is the mechanical washing away of foreign particles. Microorganisms alighting on the conjunctiva are treated like inanimate particles of dirt or dust and swept away via the tear ducts into the nasal cavity. Clearly, there is little or no opportunity for initiation of infection in the normal conjunctiva unless microorganisms have some special ability to attach to the conjunctival surface. The conjunctiva, however, suffers minor injuries whenever we get 'something in the eye', and these give opportunities for infection, as would defects in the cleansing mechanisms due to lachrymal gland or lid disease. The *Chlamydia* responsible for inclusion conjunctivitis and for that greatest eye infection in history, trachoma,[11] are masters in the art of conjunctival infection. They attach to heparan sulphate-type receptors on cell surfaces, doubtless also taking advantage of breaches in the defence mechanisms. The conjunctiva is also infected from the 'inside' during the course of measles, when the virus spreads via the circulation and is somehow seeded out to conjunctival blood vessels.

The conjunctiva is infected by mechanically deposited rather than by airborne microorganisms. Flies, fingers and towels play an important role in diseases such as trachoma, and it is significant that the types of *C. trachomatis* that cause urethritis also often infect the eye, presumably being borne from one to the other by contaminated fingers. Certain enteroviruses (enterovirus 70, coxsackie virus A24) cause conjunctivitis, and conjunctivitis due to adenovirus 8 is one of the many diseases that can be caused by the physician (iatrogenic diseases). It is transmitted from one patient to the next by the instruments used in extracting foreign bodies from the eye. Microorganisms present in swimming baths have a good opportunity to infect the conjunctiva, water flowing over the conjunctiva depositing microorganisms and at the same time causing slight mechanical and chemical damage. Both *Chlamydia* and adenovirus 8 have been transmitted in this way. During the birth of an infant, gonococci or *Chlamydia* from an infected cervix can be deposited in the eye to cause severe neonatal conjunctivitis. Certain free-living protozoa (*Acanthamoeba*) present in soil and sometimes in water supplies can infect the cornea to cause keratitis. This occurs in India, perhaps because of foreign bodies or other infections in the eye, and also in those wearing contact lenses.

In the two preceding sections, several references have been made to *Chlamydia* as an important agent of occulogenital disease. *Chlamydia* are obligate intracellular parasites,

[11]Worldwide, 500 million people are infected, and five million blinded by it.

which can only replicate within host cells. Not much is known about the details of the infection process *in vivo* but a great deal has been learnt about the biology of infection using cultured epithelial cells *in vitro* as model systems and through comparative whole genome sequence analysis. Both of the two major species (*C. trachomatis* and *C. psittaci*) attach to host cells, enter by endocytosis, avoid lysosomes, and initiate their complex replication cycle, leading to development of characteristic inclusion bodies within infected cells. Attachment and entry into cells are obviously critical steps in pathogenesis, but are still not well understood. A number of bacterial determinants, including the major outer membrane protein, glycosaminoglycans, heat shock protein, and OmcB, have been implicated. It has been shown that protein disulfide isomerase is necessary for attachment and invasion of CHO6 cells *in vitro*. Elementary bodies (EBs) enter and differentiate into reticulate bodies (RBs), the replicative form of the organism, which then differentiate into more infectious EBs. The replication of RBs is controlled in a highly complex manner by the availability of nutrients – energy components (ATP) and in particular amino acids.

THE NORMAL MICROBIOTA

The commensal microorganisms that live in association with the body surfaces of man have repeatedly been referred to in this chapter. It has been calculated that the normal individual houses about 10^{12} bacteria on the skin, 10^{10} in the mouth and nearly 10^{14} in the alimentary canal. For comparison there are about 10^{13} cells in the body. Most of these are highly specialised bacteria,[12] utilising available foods, often with mechanisms for attachment to body surfaces, and with evolutionary adaptation to a specific host. In recent years, an increased appreciation for the critical roles of the human microbiota in various biological functions in the host has led to an explosion of research investigating the diversity and function of microbes symbiotically colonising the host. The fundamental role of the microbiota in maintaining the healthy status quo of humans and animals is now widely appreciated but there is much regarding the complex interactions with the host which is yet to be understood. Much of the recent research has focused on examining the diversity and stability of microbial populations in the host with major initiatives such as the NIH-funded Human Microbiome project and the International MetaHIT initiative. One study has examined faecal samples from a number of healthy individuals and identified three major community groups referred

[12]The specialised secretion of the genital mucosa of both sexes (smegma) has its own resident bacterium, *Mycobacterium smegma*, which often contaminates urine. Skin residents include certain yeasts, *Pityrosporum ovale* and *Pityrosporum orbiculare. P. ovale* appears to be responsible for that widespread but humble human condition, dandruff. It is a good parasite, present on most male scalps, feeding on dead skin scales with minimal inconvenience to the host. Fascinating mites *(Demodex folliculorum* and *brevis)* reside unobtrusively in hair follicles or sebaceous glands, feeding on epithelial cells and on sebum. These mites are present in all human beings, and their spectacular success as parasites is reflected by a healthy person's astonishment when shown an adult mite attached to the base of his plucked eyelash. Other mites of the same genus parasitise horses, cattle, dogs, squirrels, etc.

to as 'enterotypes' which were not specific to particular global geographic regions but contained enterotype-specific combinations of species.

Importance of the Normal Microbiota to the Host

There are numerous ways by which the microbiota contribute to the well-being of the host. For example, it is now well established that bacteria present in the gut contribute important metabolic functions. The gut microbiota contain pathways for amino acid, carbohydrate and central metabolism, and many genes are expressed in specific conditions. Accordingly, transcriptomic studies are likely to be very informative in identifying functional variation underlying diseases that straightforward genetic information may miss. Because of their numbers, the intestinal bacteria have considerable metabolic potential (said to be equal to that of the liver) and products of metabolism can be absorbed. For instance, intestinal bacteria are important in the degradation of bile acids, and glycosides such as cascara or senna taken orally are converted by bacteria into active forms (aglycones) with pharmacological activity. Metabolic products occasionally cause trouble. Substances like ammonia are normally absorbed into the portal circulation and dealt with by the liver, but when this organ is badly damaged (severe hepatitis) they are able to enter the general circulation and contribute to hepatic coma. Adult Bantus, Australian aborigines, Chinese, etc. differ from Anglo-Saxons in that the small intestinal mucosa fails to produce the enzyme lactase. This is presumably related to the fact that these people do not normally drink milk as adults. If lactose is ingested, it is metabolised by the bacteria of the caecum and colon, with the production of fatty acids, carbon dioxide, hydrogen, etc., giving rise to flatulence and diarrhoea.

The resident bacteria are highly adapted to the commensal life, and under normal circumstances cause minimal damage. They are present throughout life, and avoid inducing the inflammatory or immune responses that might expel them. In the normal individual, the only other microorganisms that can establish themselves are by definition 'infectious'. These sometimes cause disease and are eventually eliminated. In other words, if it is inevitable that the body surfaces are colonised by microorganisms, it can be regarded as an advantage that colonisation should be by specialised nonpathogenic commensals.

The health benefits of diets rich in soya associated with improved osteoporosis and cardiovascular disease have been attributed to (S)-equol produced from the soya isoflavone daidzen by bacterial rather than human enzymes. Of note, only about 30% of Western adults produce (S)-equol with a diet of soya-rich foods compared with ~60% of adults from Asia.

Studies have shown that samples taken over time from an individual are relatively homogenous compared to samples taken from other individuals indicating that most people are stably colonised with relatively distinct bacterial communities. The human microbiome has been described to consist of three major states (enterotypes) determined by the collection of bacterial species in each. Overall, the enterotypes are characterised by a relatively high representation of Bacteroides, Prevotella or Ruminococcus. However, these observations have not been replicated in some study cohorts from different global regions. Some differences in the microbiota are known to contribute directly to disease states. The traditional way to obtain evidence about the function of something is to see what happens when it is removed. There have been many studies on germ-free animals,

including mice, rats, cats, dogs and monkeys. The mother is anaesthetised shortly before delivery, and infants are delivered by caesarean section into a germ-free environment or 'isolator' and supplied with sterile air, food and water. Germ-free individuals, not unexpectedly, have a less well-developed immune system, because of the absence of microorganisms. Antigens are present in food, but the intestinal wall is thinner, and immunoglobulin synthesis occurs at about 1/50th of the rate seen in ordinary individuals. Germ-free animals that are coprophagous (rabbits, mice) also show a great enlargement of the caecum, which may constitute a quarter of the total body weight. It can cause death when it undergoes torsion. The caecum rapidly diminishes to normal size when bacteria are fed to the germ-free individual. Otherwise, the germ-free individual seems better off and generally has a longer life span. Even caries are not seen, because this requires bacteria. Gnotobiotic (bacteria-free) mice which were then colonised with the microbiota from an obese mouse gained fat quicker than mice colonised with the microbiota from a lean mouse. Importantly, differences in gut microbiota have demonstrated an association with Crohn's disease, ulcerative colitis, irritable bowel disease and *C. difficile*-associated diseases (CDAD). However, studies are often confounded by the use of variable treatments that may hide clear disease-associated traits of the microbiota.

As an example, *C. difficile* is normally a resident of the healthy gut flora, although virulent epidemic clones have emerged and disseminated widely. Typically, *C. difficile* causes disease when antibiotics compromise the gut microbiota leading to potential lethal infections, particularly in the elderly. Antibiotics can have a profound effect on the microbiota and can lead to a switch to an alternative but stable microbiota. In recent years, the use of faecal transplants has been shown to be remarkably successful in treating *C. difficile* infections. Importantly, we are starting to understand the identity of the key bacterial species present in the faecal samples which are sufficient to restore a more 'normal' microbiota and clear the infection. This should hopefully lead to effective therapeutics which are more appetising than the current unprocessed option.

Another major role for the microbiota is to protect against invasive bacteria by colonisation resistance. Intestinal bacteria such as *E. coli*, for instance, fail to establish themselves in the normal mouth and throat, and disturbances in the normal flora induced by long courses of broad-spectrum antibiotics may permit the overgrowth of *C. albicans* in the mouth or staphylococci in the intestine. In one unusual experiment, none of 14 volunteers given 1000 *S. typhi* by mouth developed disease, but one of four did so when the antibiotic streptomycin was given at the same time. Streptomycin probably promoted infection by its bacteriostatic action on commensal intestinal microorganisms. It is known that other *Salmonella* infections of the intestine persist for longer when antibiotics are given.

It must be remembered that pathogenic as well as commensal microorganisms are absent from the germ-free animal, and in experimental animals it is possible to eliminate only the specific microbial pathogens, leaving the normal flora intact. This can be done by obtaining animals (mice, pigs, etc.) by caesarean section and rearing them without contact with others of the same species, but not in a germ-free environment. Alternatively germ-free animals can be selectively contaminated with commensal microorganisms. These specific pathogen-free (SPF) animals have increased body weight, longer life span and more

successful reproductive performance, with more litters, larger litters and reduced infant mortality. Furthermore, it has long been known that chickens, pigs, etc. grow larger when they receive broad-spectrum antibiotics in their food, presumably because certain unidentified microorganisms are eliminated. This forms the basis for the use of antibiotics as growth promoters. The elimination of specific pathogenic microorganisms, however, is a less theoretical matter. SPF mice are routinely maintained in laboratories and are much superior to non-SPF animals, as mentioned above. The population of the developed countries of the world (United States, Canada and northern Europe) can be likened to SPF mice, most of the serious microbial pathogens having been eliminated by vaccines, quarantine and other public health measures, or kept in check by good medical care and antibiotics. The peoples of the developing countries of the world, on the other hand, are comparable to the conventionally reared, non-SPF mice, who are exposed to all the usual murine pathogens. The comparison is complicated by the often inadequate diet of those in the developing world. A World Health Organisation (WHO) survey of 23 countries showed that in developing countries the common pathogenic infections such as diphtheria, whooping cough, measles and typhoid have respectively 100, 300, 55 and 160 times the case mortality seen in developed countries. Compared with those in the developed countries, people in developing countries often tend to be smaller, with a shorter life span, and poorer reproductive performance (abortions, neonatal and infantile mortality). They are the non-SPF people.

Opportunistic Infection

There is one important consequence of the existence of the normal microbial flora. These microorganisms are present as harmless commensals and are normally well behaved. If, in a given individual, this balance is upset by a decrease in the normal level of resistance, then the commensal bacteria are generally the first to take advantage of it. Thus, damage to the respiratory tract upsets the balance and enables normally harmless resident bacteria to grow and cause sinusitis or pneumonia. Minor wounds in the skin enable skin staphylococci to establish small septic foci, and skin sepsis is particularly common in poorly controlled diabetes. This is probably due to defective chemotaxis and phagocytosis in polymorphs, which show impaired energy metabolism. High concentrations of blood sugar and the presence of ketone bodies may play a part, but a more direct effect of diabetes is suggested by the observation that adding insulin to diabetic polymorphs *in vitro* rapidly restores their bactericidal properties. Commensal faecal bacteria infect the urinary tract when introduced by catheters, and commensal streptococci entering the blood from the mouth can cause endocarditis if there are abnormalities in the heart valves or endocardium. The tendency of commensal bacteria to take opportunities when they arise and invade the host is universal. These infections are therefore called opportunistic infections.

Opportunistic infections are common nowadays. This is partly because many specific microbial pathogens have been eliminated, leaving the opportunistic infections relatively more numerous than they were. Also, modern medical care keeps alive many people who have impaired resistance to microbial infections. This includes those with congenital immunological or other deficiencies, those with lymphoreticular neoplasms, and a great

many patients in intensive care units or in the terminal stages of various illnesses. Modern medical treatment also often requires that host immune defences are suppressed, as after organ transplants or in the treatment of neoplastic and other conditions with immunosuppressive drugs. Also, certain virus infections (e.g. feline leukaemia, AIDS in man) can cause a catastrophic depression of immune responses (see Chapter 7). In each case, opportunistic microorganisms tend to give trouble.

There are other opportunistic pathogens in addition to the regular commensal bacteria. *C. albicans*, a common commensal, readily causes troublesome oropharyngeal or genital ulceration. *P. aeruginosa* is essentially a free-living species of bacteria, sometimes present in the intestinal tract. In hospitals it is now a major source of opportunistic infection. This is because it is resistant to many of the standard antibiotics and disinfectants, because its growth requirements are very simple, and because it is so widely present in the hospital environment. It multiplies in eyedrops, weak disinfectants, corks, in the small reservoirs of water round taps and sinks, and even in vases of flowers. *P. aeruginosa* causes infection especially of burns, wounds, ulcers and the urinary tract after instrumentation.[13] It is a common cause of respiratory illness in patients with cystic fibrosis.[14] When resistance is very low, it can spread systematically through the body, and nowadays this is a frequent harbinger of immunological collapse. Viruses also act as opportunistic pathogens. Most people are persistently (latently) infected with cytomegalovirus, herpes simplex virus, varicella zoster virus, etc., and these commonly cause disease in immunologically depressed individuals, although the level of 'immunosuppression' required to induce a reactivation of these viruses varies markedly. Cytomegalovirus, for instance, reactivates within the first six months after most renal transplant operations, due to the immunosuppressive drugs and may cause hepatitis and pneumonia. HSV reactivates much more frequently, and in response to more natural immunosuppressive stimuli such as stress. The fungal parasite *Pneumocystis carinii* is an extremely common human resident, normally of almost zero pathogenicity, but can contribute to pneumonia in immunosuppressed individuals. *C. difficile* is another example of an opportunistic pathogen which causes a spectrum of disease (ranging from antibiotic-associated diarrhoea to fatal pseudomembranous colitis) sometimes after a course of antibiotics. Resident spores do not normally germinate in the presence of a normal microflora; antibiotic-induced imbalance in the latter creates the conditions for rapid vegetative growth of *C. difficile* and release of toxins.

[13]*Pseudomonas* demonstrated its versatility by causing a profuse rash in users of a hotel jacuzzi (whirlpool). The bacteria multiplied in the hot, recirculated, inadequately treated water, and probably entered the skin via the orifices of dilated hair follicles.

[14]Cystic fibrosis, the most common fatal hereditary disease in Caucasians (about 1 in 20 carry the gene), involves defects in mucus-producing cells. The lung with its viscid mucus becomes infected with *S. aureus* and *H. influenzae*, but the presence of *P. aeruginosa* is especially ominous. *Pseudomonas* strains from cystic fibrosis patients often produce a jelly-like alginate rather than the regular 'slimy' type of polysaccharide (see Table 4.1), and this may physically interfere with the action of phagocytes. Lung damage is largely due to the action of bacterial and phagocytic proteases.

EXIT OF MICROORGANISMS FROM THE BODY

After an account of the entry of microorganisms into the body, it seems appropriate to mention their exit. General principles were discussed in Chapter 1. Nearly all microorganisms are shed from the body surfaces (Figure 2.1). The transmissibility of a microorganism from one host to another (Figure 2.7) depends to some extent on the degree of shedding, on its stability, and also on its infectiousness, or the dose required to initiate infection (see Table 11.1). For instance, when 10 bacteria are enough to cause oral infection (*S. dysenteriae*), the disease will tend to spread from person to person more readily than when 10^3 bacteria are required (salmonellosis). The properties that give increased transmissibility are not the same as those causing pathogenicity. There are strains of influenza virus that are virulent for mice, but which are transmitted rather ineffectively to other mice, transmissibility behaving as a separate genetic attribute of the virus. For other microorganisms also, such as staphylococci and streptococci, transmissibility may vary independently of pathogenicity.

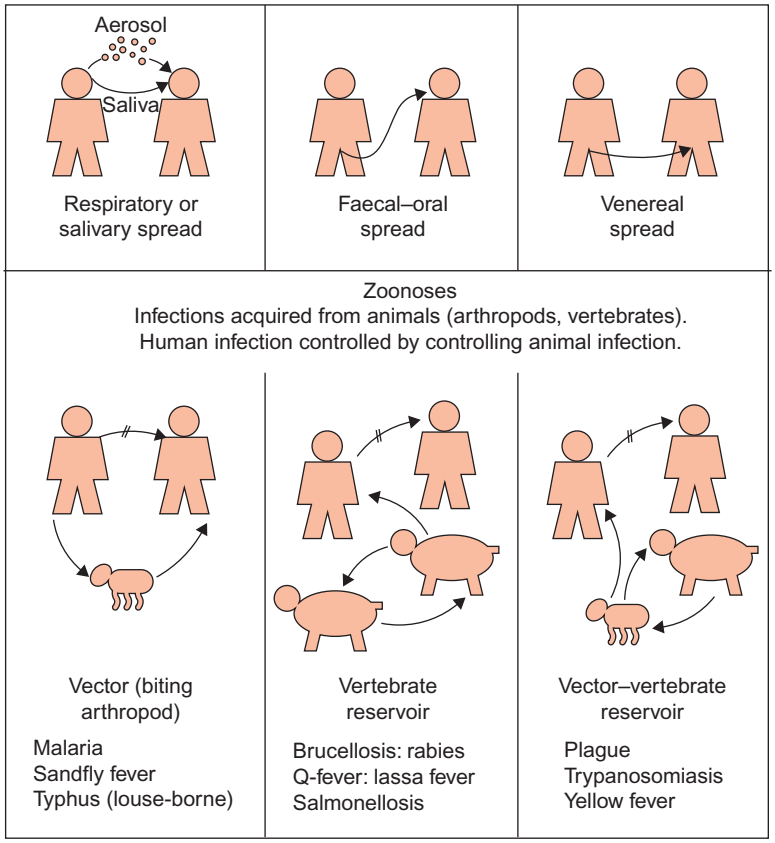

FIGURE 2.7 Types of transmission of infectious agents. Respiratory or salivary spread – not readily controllable. Faecal–oral spread – controllable by public health measures. Venereal spread – control is difficult because it concerns social factors. Zoonoses – human infection controlled by controlling vectors or by controlling animal infection.

Respiratory Tract

In infections transmitted by the respiratory route, shedding depends on the production of airborne particles (aerosols) containing microorganisms. These are produced to some extent in the larynx, mouth and throat during speech and normal breathing. Harmless commensal bacteria are thus shed, and more pathogenic streptococci, meningococci and other microorganisms are also spread in this way, especially when people are crowded together inside buildings or vehicles. There is particularly good aerosol formation during singing and it is always dangerous to sing in a choir with patients suffering from pulmonary tuberculosis. Microorganisms in the mouth, throat, larynx and lungs are expelled to the exterior with much greater efficiency during coughing; shedding to the exterior is assured when there are increased mucus secretions and the cough reflex is induced. Tubercle bacilli in the lungs that are carried up to the back of the throat are mostly swallowed and can be detected in stomach washings, but a cough will project bacteria into the air.[15]

Efficient shedding from the nasal cavity depends on an increase in nasal secretions and on the induction of sneezing. In a sneeze (Figure 2.8) up to 20,000 droplets are produced[16] and during a common cold, for instance, many of them will contain virus particles. The largest droplets (1 μm diameter) fall to the ground after travelling 4 m or so, and the

FIGURE 2.8 Jets of droplets erupt from a man's nose as he sneezes. The sneeze reflex is involuntary and is usually triggered by irritation of the nasal lining. *Reproduced with permission: DR. JOHN BRACKENBURY/SCIENCE PHOTO LIBRARY*

[15]*Mycobacterium leprae* multiplies in nasal mucosa and 10^8 bacilli a day can be shed from the nose of patients with lepromatous leprosy. The bacteria are shed as plentifully as from patients with open pulmonary tuberculosis, and also survive in the dried state.

[16]Most of the droplets in fact originate from the mouth, but larger masses of material (streamers) as well as droplets are expelled from the nose when there is excess nasal secretion. A cough, in contrast, produces no more than a few hundred particles. Talking is also a source of airborne particles, especially when the consonants, f, p, t and s are used. It is perhaps no accident that the most powerfully abusive words in the English language begin with these letters, so that a spray of droplets (possibly infectious) is delivered with the abuse.

smaller ones evaporate rapidly, depending on their velocity, water content and on the relative humidity. Many have disappeared within a few feet and the rest, including those containing microorganisms, then settle according to size. The smallest (1–4 μm diameter), although they fall theoretically at 0.3–1.0 m/h, in fact stay suspended indefinitely because air is never quite still. Particles of this size are likely to pass the turbinate baffles and reach the lower respiratory tract. If the microorganisms are hardy, as in the case of the tubercle bacillus, people coming into the room later on can be infected. Many other microorganisms are soon inactivated by drying of the suspended droplet or by light, and for transmission of measles, influenza, the common cold or the meningococcus, fairly close physical proximity is needed. Conversely, foot and mouth disease virus spreads by air and wind over surprisingly long distances.[17]

Shedding from the nasal cavity is much more effective when fluid is produced and, among the viruses that are shed from this site, evolution has favoured those that induce a good nasal discharge.[18] In the crowded conditions of modern life, with unprecedented numbers of susceptible individuals in close physical proximity and with only temporary nasal immunity (see Chapter 6), there is rapid selection for the virus strains that spread most effectively. There are more than 100 antigenically different common cold viruses, and there is currently little prospect of control by vaccination or chemoprophylaxis.

Saliva

Microorganisms reach the saliva during upper or lower respiratory tract infections, and may be shed during talking and other mouth movements as discussed earlier. Certain viruses such as mumps, Epstein–Barr virus (EBV), and cytomegalovirus in man infect the salivary glands. The virus is present in the saliva, and shedding to the exterior takes place in infants and young children by the contamination of fingers and other objects with saliva. Adolescents and adults who have escaped infection earlier in life exchange a good deal of saliva in the process of kissing, particularly 'deep' kissing. In developing countries, EBV infects mainly infants and children, and at this age causes little or no illness. In developed countries, however, infection is often avoided during childhood, and primary infection with EBV occurs at a time of life when sexual activity

[17]Pigs infected with foot and mouth disease virus excrete in their breath 100 million infectious units each day. With relative humidity of more than 65% the airborne virus survives quite well, and can be carried in the wind across the sea from France to the Channel Islands or England where cattle, who inhale 150 m^3 air a day, become infected. Outbreaks of this disease are often explained by studying air trajectories and other meteorological factors. In humans, legionellosis (see Glossary) can spread by air over shorter distances. An outbreak in Glasgow affected 33 people and had its source in a contaminated industrial cooling tower, cases occurring downwind up to a distance of 1700 m.

[18]Nasal secretions are inevitably deposited (directly or via handkerchiefs) onto hands, which can then be a source of infection. Contamination of other people's fingers, and thus of their nose and conjunctiva, might be as important as aerosols in the transmission of these infections.

is beginning. At this age, it gives rise to the more serious clinical conditions included under the heading of glandular fever. In animals also, saliva is often an important vehicle of transmission, depending on social and sexual activities such as licking, nibbling, grooming and fighting. Rabies and the foot and mouth disease virus may be present in large amounts in saliva.[19]

Spitting is an activity practised only by man and a few animals including camels, chameleons and certain snakes. Microorganisms resistant to drying, such as the tubercle bacillus, can be transmitted in this way. The expectorated material contains saliva together with secretions from the lower respiratory tract. In the days when pulmonary tuberculosis was commoner, spitting in public places came to be frowned upon and there were laws against it. It is perhaps better for the chronic bronchitic to discharge his voluminous secretions discreetly into a receptacle rather than swallow them, but the expectoration of mere saliva in public places, now becoming commoner again, is a regrettable reversion to the unaesthetic days of the spittoon.

Skin

Shedding of commensal skin bacteria takes place very effectively. Skin bacteria are mostly shed attached to desquamated skin scales, and an average of about 5×10^8 scales, 10^7 of them carrying bacteria, are shed per person per day, the rate depending very much on physical activity. The fine white dust that collects on surfaces in hospital wards consists to a large extent of skin scales. The potentially pathogenic *S. aureus* colonises especially the nose (nose-picking area), fingers and perineum. Shedding takes place from the nose and notably from the perineal area. Males tend to be more effective perineal shedders than females, and this is partly hormonal and partly because of friction in this area; shedding can be prevented by wearing occlusive underpants. A good staphylococcal shedder can raise the staphylococcal count in the air from $<36\ m^{-3}$ to $360\ m^{-3}$. Although people with eczema or psoriasis shed more bacteria from the skin, it is not known why some normal individuals are profuse shedders; the phenomenon is important for cross infection in hospitals.

For microorganisms that cause skin lesions (see Table 5.2), however, shedding to the environment is not necessarily very important. Shedding takes place only if the skin lesion breaks down, as when a vesicle ruptures or if the microorganism penetrates through to the outer layers of the epidermis (wart virus). Even then, spread of infection is often by direct bodily contact, as with herpes simplex, syphilis or yaws, rather than by shedding into the environment.

Intestinal Tract

All microorganisms that infect the intestinal tract are shed in faeces. Those shed into the bile, such as hepatitis A (a picornavirus) and typhoid bacilli in the typhoid carrier,

[19]Rabies virus, for instance, enhances its own transmission by invading the limbic system of the brain. This alters the behaviour of the infected animal, making it more aggressive, more likely to roam, and thus more likely to bite another individual.

also appear in the faeces. Microorganisms swallowed after growth in the mouth, throat or respiratory tract can also appear in the faeces, but most of them are not resistant to acid, bile and other intestinal substances and are inactivated. Faeces are the body's largest solid contribution to the environment,[20] and although the microorganisms in faeces are nearly all harmless commensals, it is an important source of more harmful microorganisms. During an intestinal infection, intestinal contents are often hurried along and the faeces become fluid. There is no exact equivalent to the sneeze, but diarrhoea certainly leads to increased faecal contamination of the environment and spread to other individuals. In animal communities and in primitive human communities, there is a large-scale recycling of faecal material back into the mouth. Contamination of food, water and living areas ensures that this is so, and the efficiency of this faecal–oral movement is attested to by the great variety of microbes and parasites that spread from one individual to another by this route. If microorganisms shed into the faeces are resistant to drying and other environmental conditions, they remain infectious for long periods. Protozoa such as *E. histolytica* produce an especially resistant cyst which is the effective vehicle of transmission, and *Clostridia* spp. and *B. anthracis* form resistant spores that contaminate the environment and remain infectious for many years. The soils of Europe are heavily seeded with tetanus spores from the faeces of domestic animals, and these spores can infect the battlefield wound or the gardening abrasion to give tetanus. Viruses have no special resistant form for the hazardous journey to the next host, but they show variable resistance to thermal inactivation and drying. Poliovirus, for instance, is soon inactivated on drying.

Many microorganisms are effectively transmitted from faeces to mouth after contamination of water used for drinking. The great water-borne epidemics of cholera are classical examples,[21] and any faecal pathogen can be so transmitted if it survives for at least a few days in water. In densely inhabited regions, faecal contamination of water is inevitable unless there is adequate sewage disposal and a supply of purified water. Two hundred years ago in England, there were no water closets and no sewage disposal, and human excrement was deposited in the streets. There was nowhere else to put it,

[20] Herbivorous animals make a bigger and less well-controlled contribution than do human beings. The output of a pig is about three times and a cow 10 times that of a man. We are less fussy about the disposal of animal sewage and this can be important for instance in transfer of salmonellosis. The amount from an individual animal seems less important than its quality and site of deposition when we consider the appalling canine contribution to public parks and paths in dog-ridden cities.

Gas is another intestinal product, and a few hundred millilitres depart from the anus and mouth of the normal person each day. About half is nitrogen from swallowed air, the rest being mostly methane (CH_4). Microbial fermentation in the gut forms H_2 and CO_2, which methanogenic bacteria convert to CH_4. This is particularly prominent after ingestion of beans, which have a polysaccharide not handled by digestive enzymes of humans.

[21] Dr John Snow, a London physician, charted the cases of cholera on a street map during an outbreak in 1854. After observing that all cases had used water from the same pump in Broad Street, Soho, he removed the handle of this pump. The outbreak terminated dramatically, and the mode of transmission was thus demonstrated nearly 40 years before Koch identified the causative organism.

although one enterprising Londoner in 1359 was fined 12 pence for running his sewage by a pipe into a neighbour's cellar. Water supplies came from rivers and from wells, of which there were more than 1000 in London. Efficient sewage disposal and piped water supplies are a comparatively recent (nineteenth century) development. Nowadays, the map of the London sewage system resembles that of the London Underground (subway) system. Water for domestic use is collected into vast reservoirs before being shared out to tens of thousands of individuals. This would give great opportunities for spread once pathogens entered the water supply, but water purification and chlorination ensures that this spread remains at almost zero level. Life in present-day urban society depends on the large-scale supply of pure water and the large-scale disposal of sewage. Both are complex and vital public services of which the average citizen or physician is profoundly ignorant. Largely as a result of these developments the steady flow of faecal materials into the mouth that has characterised much of human history has been interrupted.

Urinogenital Tract

Urine can contaminate food, drink and living space, and the same things can be said as have been said about faeces. Urine in the bladder is normally sterile and is only contaminated with skin bacteria as it is discharged to the exterior. The pathogens present in urine include a specialised group that are able to spread through the body and infect the kidney or bladder. The leptospiral infections of rats and other animals are spread in this way, sometimes to man. *Leptospira*[22] survive in water, can penetrate the skin, and people are infected following contact with contaminated canals, rivers, sewage, farmyard puddles and other damp objects. Polyomavirus spreads naturally in colonies of mice after infecting tubular epithelial cells in the kidney and being discharged to the exterior in urine. Mice carrying lymphocytic choriomeningitis (LCM) virus (an arenavirus related to Lassa fever) or hantaviruses shed the virus in urine and can thus infect people in mouse-infested dwellings. Humans infected with polyomavirus, or with cytomegalovirus, excrete the virus in urine. Urinary carriers of typhoid have a persistent infection in the bladder, especially when the bladder is scarred by *Schistosoma* parasites, and typhoid bacilli are shed in the urine.

Microorganisms shed from the urethra and genital tract generally depend for transmission on mucosal contacts with susceptible individuals. Herpes simplex type 2 can infect the infant as it passes along an infected birth canal during delivery, and gonococci or *Chlamydia* infect the infant's eye in the same way. Venery, however, gives far greater opportunities for spread, as was discussed in Chapter 1. If there is a discharge, organisms are carried over the epithelial surface and transmission is more likely to take place.

[22]There are more than 20 different serotypes carried by mice, rats, swine, dogs, cattle and leptospirosis is the most widespread zoonosis in the world. In the United Kingdom nowadays, cases of rat-borne leptospirosis occur in the bathers, canoeists, etc. who use canals and rivers, rather than sewer workers or miners, and leptospirosis from cattle continues to cause a mild disease in farmers and cowmen.

The transmission of microorganisms by mucosal contact is determined by social and sexual activity. In animals, licking, nuzzling, grooming and biting can be responsible for the transmission of microorganisms such as rabies and herpes viruses. In recent years, there have been major changes in man's social and sexual customs, and this has had an interesting influence on certain infectious diseases. Generally speaking, there has been less mucosal contact in the course of regular social life. In modern societies, saliva is exchanged less freely between children or within a family, and children are more likely to escape infections that are spread via saliva such as those due to EBV. Some of the papillomaviruses (e.g. HPV 16) which cause so-called 'genital' warts (and cervical carcinoma) can be found in saliva, possibly indicating a more ancient method of spread.

Things are different when we consider sexual activity. For adolescents and adults, mucosal contacts are possibly increasing in frequency, but more importantly they are being made with a greater number of different partners. Sexual activity is now considered less 'sinful', and the fact that it is safer (pregnancy is avoidable and disease is treatable) means that multiple partners are commoner than they used to be. Furthermore, infectious agents are transmitted with much greater efficiency now that many couples use oral rather than mechanical contraceptives. All these things have led to a great flowering of sexually transmitted diseases, which with respiratory infections are now the commonest communicable diseases in the world. Their incidence is rising. The four most frequent sexually transmitted diseases in England today are *Chlamydia*, gonorrhoea, anogenital herpes (HSV-1 and -2) and genital warts.[23] AIDS has had an impact on sexual promiscuity. HIV originated in central (sub-Saharan) Africa where it is spread by (vaginal) heterosexual intercourse. In developed countries, it is still mostly a disease of male homosexuals, drug addicts, and haemophiliacs. In these countries, promiscuity has already been curtailed, as indicated by falling gonorrhoea infection rates. In 2012, it was estimated that 35 million people were living with HIV/AIDs, with an estimated 2.3 million new cases that year. HIV is most prevalent in Africa, Asia and Latin America, where it continues to spread heterosexually.

A list of sexually transmitted diseases is given in Table 2.3. Even the more serious diseases such as syphilis and gonorrhoea have been difficult to control. A small number of sexually active individuals can be relied upon to infect many others, if they evade the public health network.

Because almost all mucosal surfaces in the body can be involved in sexual activity, microorganisms encounter a number of interesting opportunities to infect new bodily sites. Thus, *Neisseria meningitidis*, a resident of the nasopharynx, is occasionally recovered from

[23]This is not to say that promiscuity is a new thing. The well-charted sexual adventures of Casanova (1725–1798) brought him four attacks of gonorrhoea, five of chancroid, and one of syphilis, while Boswell (1740–1795) experienced 19 episodes of (mainly gonococcal) urethritis. Of course, these activities were not restricted to those who became famous or wrote books. But the extraordinary increase in man's mobility has transformed social life and, together with the factors mentioned above, has had a major impact on the sexual transmission of infectious diseases.

TABLE 2.3 Principal Sexually Transmitted Diseases in Man[a]

	Microorganism	Disease	Comments
Viruses	Herpes simplex type 2	Genital herpes	Very common – reactivates
	Human papillomavirus	Genital warts	Very common – involvement in cervical and penile cancer makes them more than ornamental appendages
	HIV-1[b]	AIDS	Most cases are in the Third World and are spread by heterosexual (vaginal) intercourse. In the First World most common in male homosexuals and transmitted by anal intercourse
	Hepatitis B	Hepatitis	Spread mainly in male homosexuals
Bacteria	*Chlamydia trachomatis* (types D–K)	Nonspecific urethritis	Responsible for more than half of cases; causes eye infection in newborn
	C. trachomatis (types L1–L3)	Lymphogranuloma inguinale	Ulcerating papule plus lymph node suppuration. Commoner in tropics and subtropics
	Ureoplasma spp.	Nonspecific urethritis	Importance not clear. Require 10% urea for growth, which would direct them to urogenital tract
	Neisseria gonorrhoeas	Gonorrhoea	Acute and more severe urethritis in male; chronic pelvic infection in female; eye infection in newborn
	Treponema pallidum	Syphilis	Syphilis was name of infected shepherd in Frascator's poem (1530) describing disease
	Haemophilus ducreyi	Chancroid	Genital sore, lymph node suppuration, commoner in subtropics
	Calymmato-bacterium granulomatis	Granuloma inguinale	Commoner in subtropics. Ulcerative lesions
Fungi	*Candida albicans*	Vulvovaginitis (balanoposthitis in male)	Asymptomatic vaginal carriage common
Protozoa	*Trichomonas vaginalis*	Vulvovaginitis (urethritis in male)	Disease worse in female (compare gonorrhoea)

[a]Also common are pediculosis pubis (caused by the crab louse *Phthirus pubis*) and genital scabies (caused by the scabies mite *Sarcoptes scabiei*). Many infections occur in people under the age of 24 years, although increased numbers of infection occur in 'middle-aged' individuals. In addition, there are special 'at risk' groups, such as tourists, long-distance lorry drivers, seamen and homosexuals.
[b]Human immunodeficiency virus.

the cervix, the male urethra and the anal canal. *N. gonorrhoeae* infects the throat and the anal region. *Chlamydia* can at times be recovered from the rectum and pharynx as well as the urethra. Genito–oro–anal contacts in sexually promiscuous communities give chances for intestinal microorganisms to spread between individuals in spite of good sanitation

and sewage disposal.[24] For example, there have been examples of sexual transmission of *Salmonella*, *G. lamblia*, hepatitis A, pathogenic amoebae and *Shigella*.

Blood

Most of the microorganisms that are transmitted by blood-sucking arthropods such as mosquitoes, fleas, ticks, sandflies or mites, have to be present in blood. This is true for arthropod-borne viruses, rickettsiae, malaria, trypanosomes and many other infectious agents. In these diseases transmission is biological. The microorganism is ingested with the blood meal, multiplies in the arthropod and then is discharged from the salivary gland or intestinal tract of the arthropod to infect a fresh host. To infect the arthropod vector, the blood of the vertebrate host must contain adequate amounts of the infectious agent. Microorganisms can be said to have been shed into the blood. A few microbes that are shed into blood (hepatitis B, hepatitis C) are transmitted not by biting arthropods but by modern devices such as needles, syringes, and blood transfusions. Presumably, other routes such as saliva and mucosal contact are also significant. Could these viruses have arisen from ancestors that were spread by biting arthropods?

Miscellaneous

Microorganisms rarely occur in semen, which is not designed by nature for shedding to the environment. Perhaps it is because of the superb opportunities for direct mucosal spread during sexual intercourse that only an occasional microorganism, such as cytomegalovirus in man, has made use of semen as a vehicle for transmission. Milk, in contrast, is a fairly common vehicle for transmission. Mumps virus and cytomegalovirus are shed in human milk, although perhaps not very often transmitted in this way, but the mammary tumour viruses of mice are certainly partly transmitted via milk. Cows' milk containing *Brucella abortus*, tubercle bacilli or Q fever rickettsia is a source of human infection.

No Shedding

In a very few instances, transmission takes place without any specific shedding of microorganisms to the exterior. Anthrax, for instance, infects and kills susceptible animals, and the corpse as a whole then contaminates the environment. Spores are formed aerobically, where blood leaks from body orifices, and they remain infectious in the soil for very long periods. It seems that spores are only formed during the terminal stages of the illness or after death, so that death of the host can be said to be necessary for the transmission

[24]In Western societies, intestinal pathogens can also spread by more innocent pathways, as when amoebiasis was transmitted to 15 patients who received colonic irrigation in a clinic in Colorado.

of this unusual microorganism. Again, kuru is only transmitted after death when the infectious agent in the brain is introduced into the body via mouth, intestine or fingers during cannibalistic consumption of the carcass.

Finally, certain microorganisms such as rubella spread from parent to offspring directly by infecting the developing embryo. If sections from mice congenitally infected with LCM virus are examined after fluorescent antibody staining, infected ova can be seen in the ovary. Also, ovum transplant experiments show that similar infection occurs with murine leukaemia virus, and the embryos of most strains of mice have leukaemia virus antigens present in their cells. All progeny from the originally infected individuals are infected and there is no need for shedding to the exterior. Some other mode of spread would be necessary if there were to be infection of a fresh lineage of susceptible hosts.

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CHAPTER

3

Early Stages of Infection After Pathogen Entry

OUTLINE

GROWTH IN EPITHELIAL CELLS

Some of the most successful microorganisms multiply in the epithelial surface at the site of entry into the body, produce a spreading infection in the epithelium and are shed directly to the exterior (Table 3.1). This is the simplest, most straightforward type of microbial parasitism. If the infection progresses rapidly and microbial progeny are shed to the exterior within a few days, the whole process may have been completed before the immune response has had a chance to influence the course of events. It takes at least a few days for antibodies or immune cells to be formed in appreciable amounts and delivered to the site of infection. However, we may underestimate the time of appearance of antibodies, as the first antibodies formed are immediately complexed with the microorganism and no free antibody appears until antibody is present in excess. With a variety of respiratory virus infections, especially those caused by rhinoviruses, coronaviruses, parainfluenza viruses and influenza viruses, epithelial cells are destroyed, and inflammatory responses induced, but there is little or no virus invasion of underlying tissues. The infection is terminated partly by the innate immune response, and partly because most locally available cells have been infected. Interferons are important resistance factors. They are low

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TABLE 3.1 Microbial Infections that are Generally Associated with Epithelial Surfaces of the Body

Microbe	Respiratory Tract and Conjunctiva	Urinogenital Tract	Skin	Intestinal Tract
Viruses	Influenza Parainfluenza 1–4 Rhinoviruses Coronaviruses	Certain papilloma viruses	Papilloma viruses (warts) Molluscum contagiosum	Rotaviruses of man, mouse, etc.
Chlamydias	Trachoma inclusion conjunctivitis	Nonspecific urethritis	–	–
Mycoplasma	*Mycoplasma pneumoniae* (atypical pneumonia)	T strains (nonspecific urethritis)	–	–
Bacteria	*Bordetella pertussis* *Corynebacterium diphtheriae* *Streptococci*	Gonococcus	Staphylococci *Corynebacterium minutissimum*[a]	Most Salmonellae; Shigellae *Campylobacter* sp.
Rickettsias	–	–	–	–
Fungi	*Candida albicans* (thrush)	*Candida albicans*	*Trichophyton* spp. (athlete's foot, ringworm, etc.)	–
Protozoa	–	*Trichomonas vaginalis*	–	*Entamoeba coli* *Giardia lamblia*

[a]This bacterium commonly infects the stratum corneum and causes erythrasma, a scaly condition of the axilla, groyne and between toes.

molecular weight proteins, coded for by the cell, and formed in response to infection with nearly all viruses (see Chapter 9). The interferon formed by the infected cell is released and can act on neighbouring or distant cells, protecting them from infection. Freshly formed virus particles from the first infected epithelial cell enter the fluids bathing the epithelial surface and are borne away to initiate fresh foci of infection at more distant sites. Interferons too can reach these sites, and as more and more interferon is formed on the epithelial sheet, more and more cells are protected, so that the infectious process is slowed and finally halted. Other antiviral factors probably play a part, and the adaptive immune response itself comes into action in the final stages. Interferons are produced a few hours after infection of the first epithelial cell at a site where they are needed and without the delay characteristic of the immune response. The immune response provides resistance to subsequent re-infection, but it does not appear to be of primary importance in recovery from respiratory infections of this type.

The spread of infection is very rapid on epithelial surfaces that are covered with a layer of liquid because of the ease with which the microorganism in the fluid film encounters cells and is disseminated over the surface. This is true for the respiratory infections mentioned above, and also for infections of intestinal epithelium, such as those caused by the human diarrhoea viruses, e.g. rotavirus. The argument does not apply, however, to local infections of the skin. In this case, where the microorganism is not carried across the epithelial surface in a liquid film to establish fresh foci of infection, the whole process takes a

much longer time. Papilloma (wart) viruses, for instance, cause infection in a discrete focus of epidermal cells; indeed a wart consists of a clone of cells produced by the division of a single initially infected cell. The inevitably slow evolution of single virus-rich lesions should mean that immune responses have the opportunity to respond to and limit the infection. Papillomaviruses, however, escape the attention of the immune system. In the basal layer of the epidermis, adjacent to the antibodies and immune cells that arrive from dermal blood vessels, the virus infection is incomplete; in this layer of the epidermis, only a subset of virus genes are transcribed, no virus structural proteins are produced and no virus DNA replication occurs, therefore no virus particles are produced. Some of the proteins which are produced are involved in driving the cell into proliferation or differentiation, others act to downregulate the IFN system and MHC presentation. The infected basal cell is therefore not recognised and not a target for the immune response. As the cells move further away from these immune forces, approaching the epidermal surface and becoming keratinised, more and more virus is produced for liberation to the exterior. Neither antibodies nor immune cells are present on this dry surface to influence virus multiplication and shedding.

The respiratory viruses described above have a hit and run type of infection of epithelial cells, and are very successful parasites. A number of other viruses, including measles and chickenpox, infect inconspicuously via the respiratory tract, then spread systemically through the body and only emerge again to cause widespread respiratory infection and shedding to the exterior after a prolonged incubation period. The limitation of rhinoviruses and human coronaviruses to the surface of the upper respiratory tract is at least partly determined by their optimum growth temperature. Many of them replicate successfully at 33°C, the temperature of nasal epithelium, but not very well at the general body temperature, 37°C. Thus, they do not spread systemically nor to the lung. It is also likely that a lack of expression of the virus receptor involved in entry of the virus to the cell plays a major role in determining spread or lack thereof. Viruses of the influenza and parainfluenza groups can infect the lung as well as the nasal mucosa, but they are generally limited to the epithelial surfaces. The limitation is not absolute. Occasionally in adults and more often in infants, influenza and parainfluenza viruses spread to infect the heart, striated muscle or the central nervous system.

The spread of infection from epithelial surfaces is also controlled by the site of virus maturation from cells. Influenza and parainfluenza viruses are liberated (by budding) only from the free (external) surface of epithelial cells, as is appropriate for infection limited to surface epithelium. A similar restriction in the topography of budding is seen with rabies virus in the infected salivary gland. However, vesicular stomatitis virus is released only from the basal surface of the epithelial cell, from whence it can spread to subepithelial tissues and then through the body; topographical restriction in the site of virus release from epithelial cells reflects the polarisation of function in these cells, which in turn depends on the maintenance of tight junctions between them.

Many bacterial infections are largely confined to epithelial surfaces (Table 3.1). This is a feature, for instance, in diphtheria and streptococcal infections of the throat, gonococcal infections of the conjunctiva or urethra and most *Salmonella* infections of the intestine. To a large extent this is because host antibacterial forces, to be described at a later stage, do not permit further invasion of tissues. Under most circumstances, these bacteria are not able to overcome the host defences, but gonococci and streptococci, at least, often spread locally

through tissues and occasionally systemically through the body. For example, Group A Streptococcus occasionally causes necrotizing fasciitis or 'flesh-eating disease'. Gonococci cause a patchy infection of the columnar epithelium of the male urethra, reaching subepithelial tissues 3–4 days after infection; the yellow discharge consists of desquamated epithelial cells, inflammatory exudate, leucocytes and gonococci. Subepithelial spread probably takes the infection to other parts of the urethra and to local glands.

Most Gram-negative bacteria have only a very limited ability to invade a given host. In man, *E. coli*, *Proteus* spp. and *Pseudomonas aeruginosa* are only capable of invasion when defences are impaired or when bacteria are inadvertently introduced into a suitable site in the body (see Chapter 2). They cause systemic infection in debilitated, malnourished, or immunosuppressed patients; they produce sepsis in the uterus after abortion, and when they are introduced into the body by intravascular devices or catheters. Certain Gram-negative bacteria penetrate the intestinal epithelium but get no further, as in *Shigella* dysentery and salmonellosis. One or two highly specialised Gram-negative bacteria penetrate intestinal epithelium, enter lymphatics and spread systemically through the body to cause enteric or typhoid fever (*Salmonella typhi* and *paratyphi*).

A few bacteria show a temperature restriction similar to that described above for rhinoviruses, which prevents anything more than local spread. For instance, the lesions in leprosy (*Mycobacterium leprae*) are confined to cooler parts of the body (skin, superficial nerves, nasal mucosa, testicles, etc.). Other mycobacteria (*Mycobacterium ulcerans* and *M. marinum*) occur in water and enter human skin through superficial abrasions, especially in warm countries, and cause chronic skin ulcers. These bacteria, which also infect fish, have an optimum growth temperature of 30–33°C and remain restricted to the skin.

Fungi of the dermatophyte group (ringworm, athlete's foot[1]) infect skin, nails and hair, but are restricted to the dead keratinised layers of epithelium. Fungal antigens are absorbed from the site of infection and immune (including allergic) responses are generated, which at least partly account for the failure to invade deeper tissues.

INTRACELLULAR MICROORGANISMS AND SPREAD THROUGH THE BODY

Some of the important microorganisms that regularly establish systemic infections after traversing epithelial surfaces are listed in Table 3.2.

There is one important distinction between intracellular and extracellular microorganisms. If an obligate intracellular microbe is to spread systemically from the body surface, it must first enter the blood or lymph. This means gaining access to the lumen of a subepithelial lymphatic or blood vessel, either as a free microorganism, or alternatively after entering a mobile cell (leucocyte) that will carry it to other parts of the body. The microorganism cannot replicate until it reaches a susceptible cell, and the absence or shortage of such cells except at the body surface would prevent or seriously hinder its spread through

[1]Fungi causing this condition flourish in a moist environment, and athlete's foot is restricted to those who encase their feet in shoes. However, those who do not wear shoes (e.g. tropical Africa) are vulnerable to other fungi that enter skin at sites of injury and cause deeper lesions called mycetomas.

TABLE 3.2 Examples of Infections in Which Microorganisms Enter Across Epithelial Surfaces and Subsequently Spread Through the Body

Microbe	Respiratory Tract and Conjunctiva	Urinogenital Tract	Skin	Intestinal Tract
Viruses	Measles	Herpes simplex 2	Arboviruses	Enteroviruses
	Rubella			Certain adenoviruses
	Varicella			
Bacteria	Psittacosis	Lympho-granuloma venereum	–	–
	Mycobacterium tuberculosis	*Treponema pallidum*	*Bacillus anthracis*	*Salmonella typhi*
	Yersimia pestis			
	Q fever	–	Typhus	Q fever?
Fungi	Cryptococcosis Histoplasmosis	–	Maduromycosis	Blastomycosis
Protozoa	Toxoplasmosis	–	Malaria Trypanosomiasis	*Entamoeba histolytica*

the body. Thus, rotaviruses and rhinoviruses replicate at the epithelial surface but cannot infect leucocytes, and in any case would be unlikely to find susceptible cells elsewhere in the body if they entered blood or lymphatic vessels. Certain viruses (yellow fever, poliovirus) spread through the body to reach susceptible target organs (liver, central nervous system) after free virus particles have entered vessels below the skin or intestinal epithelium. Measles virus and tubercle bacilli infect leucocytes, which carry them through the body to organs such as the liver, spleen, skin and lung. A remarkable example of an intracellular bacterium which can manipulate host cells to assist in dissemination is *M. leprae*. *M. leprae* targets Schwann cells which are their primary niche and re-programmes them so that they return to a stem cell-like state. The properties of these cells include plasticity and migration and this facilitates the dissemination of *M. leprae* within those cells by differentiation and via macrophage release. These findings demonstrate how at least one intracellular bacteria can hijack host cell programming in order to promote bacterial spread within the host.

If, on the other hand, the microbe is able to replicate outside cells and does not have to find a susceptible cell, it can in principle multiply locally, in the blood and lymph, and in whatever part of the body it gets to. Extracellular replication itself, however, conveys a serious disadvantage, because the microorganism is exposed to all the antimicrobial forces that the body can summon up. Indeed, bacteria and other microorganisms that are capable of extracellular replication generally advertise their presence by releasing a variety of products into surrounding fluids, many of which cause inflammation and thus bring antibacterial agents such as immunoglobulins, complement and leucocytes to the site of the infection. Lymphatics are also dilated and carry the infecting organisms to lymph nodes for further exposure to antibacterial and immune forces. Intracellular microorganisms in contrast, although exposed to the infected cell's own defence mechanisms, are directly

TABLE 3.3 Early Defences[a]

Acute Phase Proteins	Current Chapter
Lysozyme	See Glossary
Lactoferrin	Current chapter
Interferons and other cytokines	See Chapter 9
Complement activation (by the alternative pathway)	See Chapter 6
Phagocytosis	See Chapter 4
Natural killer (NK) cells	See Chapter 6
Apoptosis	See Glossary and Chapter 8
Collectins	See end of Chapter 6

[a]These early or 'innate' defences operate immediately after a microbe has penetrated the body, during that critical period before immune responses have had time to come into action.

exposed to the general bodily defences only during transit from one infected cell to another. However, if the infected cell is recognised as such by the immune defences, it can be destroyed (see Chapters 6 and 9). A number of bacteria and protozoa, such as *Mycobacterium tuberculosis*, *Legionella pneumophila*, *Brucella abortus* or *Leishmania donovani*, carry out much of their multiplication in macrophages that have ingested them. Although they are not obligate intracellular parasites, this shifts the host–microbe battlefield into the cell. The battle is then waged in the infected macrophage, whose antimicrobial powers (see Chapter 4) and participation in immune defences (see Chapters 6 and 9) become of critical importance.

The infected host has a variety of defences that operate without delay, before the immune response comes into action (Table 3.3). These 'early' defences are referred to in this and in subsequent chapters, and they are the type of defences that mattered before the immune system had evolved. Many microbes have strategies for interfering with these defences. For example, cells infected with a virus can commit suicide before the virus has completed its growth cycle in the cell. This is called apoptosis and occurs after reovirus, HIV, and other infections. The fact that viruses (e.g. adenoviruses) have developed mechanisms for inhibiting apoptosis indicates that it plays an important part in defence. Apoptosis occurs also in bacterial infections. When uropathogenic strains of *E. coli* infect bladder epithelium, the host responds by apoptosis of the infected cells. The actual value of this response is not clear.

SUBEPITHELIAL INVASION

After traversing the epithelial cell layer, a microorganism encounters the basement membrane. The basement membrane acts as a filter and can to some extent hold up the infection, but its functional integrity is soon broken by inflammation or epithelial cell damage. The invading microorganism has now reached the subepithelial tissues (Figure 3.1),

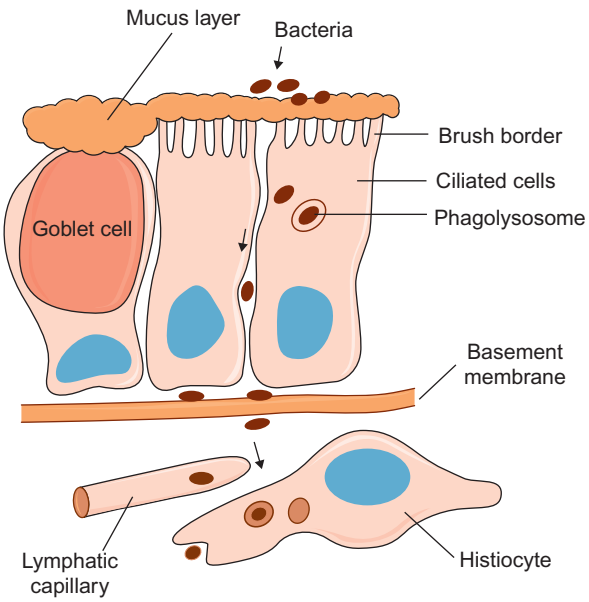

FIGURE 3.1 Microbial invasion across an epithelial surface.

and here it is exposed to three important host defence systems. These are (i) the tissue fluids, (ii) the lymphatic system leading to the lymph nodes and (iii) phagocytic cells.

These three host defence mechanisms are of supreme importance and come into play whatever part of the body is infected, whether the nasal mucosa, meninges, urethra, cardiac muscle or liver lobule. Each depends for its action on the inflammatory response, because this response brings the phagocytes and serum factors to the site of infection and promotes drainage from the site by the lymphatic system. Therefore a short account of the inflammatory response will be given, and after this, each of the three antimicrobial factors will be considered separately. Table 3.2 shows some of the important microorganisms that regularly spread through the body in spite of these antimicrobial factors.

The Inflammatory Response

The capillary blood vessels supplying a tissue bring oxygen and low molecular weight materials to the cells, taking away carbon dioxide and metabolic or secretory products. There is also a constant passage of plasma proteins and leucocytes from capillaries into normal tissues, and these are returned to the blood via the lymphatic system after entering lymphatic capillaries. Indeed, their presence in tissues is inferred from their presence in lymphatics draining these tissues. The cells are nearly all T lymphocytes, which leave blood capillaries by actively passing through endothelial cells. After moving about and performing any necessary tasks in the tissues, the lymphocytes penetrate lymphatic capillaries and thus enter the lymph. The lymph, with its content of proteins and cells, then passes through the local lymph nodes and generally at least one more lymph node before entering the thoracic lymph duct and being discharged into the great veins in the

thorax or abdomen. Blood lymphocytes also enter lymph nodes directly and in larger numbers through post-capillary venules. The constant movement of lymphocytes from blood to tissues or lymph nodes, and back via lymphatics to the blood again, is called *lymphocyte recirculation.* Circulating lymphocytes are mostly T cells, and in the course of their continued entries into tissues and lymph nodes they have regular opportunities to encounter any microbial antigens that may be present. There is in fact a regular monitoring of tissues by T lymphocytes, and this is referred to as *immune surveillance.*

The various plasma proteins occur in the tissues in much the same proportion as in plasma, the actual concentrations depending on the structure of the capillary bed. As determined by concentrations in local lymphatics, the leaky sinusoids of the liver let through 80–90% of the plasma proteins into liver tissue, the less leaky capillaries of the intestine admit 40–60% into intestinal tissues and capillaries of skeletal muscle with their continuous lining only 10–30% (Figure 3.2). Thus, immunoglobulins, complement components, etc. occur regularly in normal tissues, but in lesser concentrations than in blood. There is some discrimination against very large molecules because the largest immunoglobulins (IgM) do not leave the blood vessels and are not detectable in afferent lymph.

There is a prompt and vigorous change in the microcirculation when tissues are damaged or infected. Capillaries and post-capillary vessels are dilated, gaps appear between endothelial cells, and the permeability of these vessels increases, allowing leakage from the blood of a protein-rich fluid. Increased amounts of immunoglobulins, complement components and other proteins are then present in tissues, and fibrinogen, for instance, may be converted into fibrin so that a diffuse network of fibrils is laid down. Circulating leucocytes (especially neutrophils and monocytes) adhere to endothelial cells, and this is followed by active passage (diapedesis) of leucocytes between endothelial cells and out

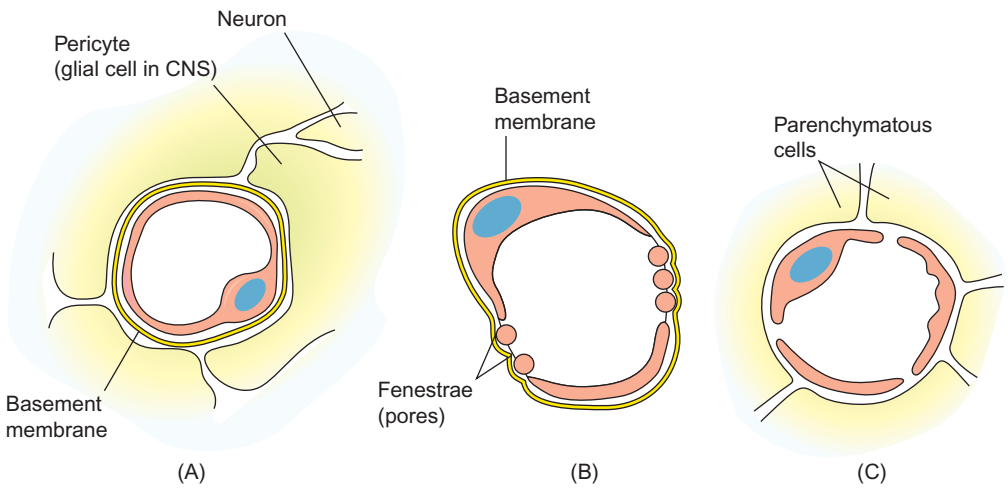

FIGURE 3.2 Diagram to show types of blood–tissue junction in capillary, venule, or sinusoid. (A) Continuous endothelium (transport of tissue nutrients and metabolites): central nervous system, connective tissue, skeletal and cardiac muscle, skin, lung. (B) Fenestrated endothelium (transport of secreted, excreted or digested materials): renal glomerulus, intestinal villi, choroid plexus, pancreas, endocrine glands. (C) Sinusoid (reticuloendothelial system): liver, spleen, bone marrow, adrenal, parathyroid.

into tissues. The affected part now shows the four cardinal signs of inflammation, being RED and WARM (vasodilation), SWOLLEN (vasodilation, cell and fluid exudate) and often PAINFUL (distension of tissues, presence of pain mediators).

Lymphatic capillaries also become dilated, taking up the inflammatory fluids and carrying them to local lymph nodes. There is a greatly increased turnover of plasma components in the inflamed tissue. Initially, the predominant cell is the neutrophil, a reflection of the situation in the blood, but neutrophils only live for a day or two in tissues, and as the acute inflammatory state subsides mononuclear cells become more prominent, especially macrophages, which phagocytose dead neutrophils and tissue debris.

The initial stages of the inflammatory response tend to be consistent, whatever the nature of the tissue insult, and this is partly because the changes are caused by the same mediators of acute inflammation. These include histamine (released from mast cells lying close to blood vessels), kinins (polypeptides derived from precursors in plasma; see Glossary) and products of complement activation by the alternative pathway (C3a and C5a). Some of the kinins are highly active and kallidin, for instance, a decapeptide formed from kallidinogen (an α_2 globulin) is about 15 times more active (on a molar basis) than histamine in causing inflammation. Most bacteria form inflammatory materials during their growth in tissues, but these are not very potent compared with the activation of C3 and other molecules by carbohydrates (e.g. polysaccharides) present on bacterial surfaces (see Figure 6.6). Macrophages, when they are stimulated, release a variety of inflammatory mediators and, in addition, immune-mediated inflammation results from interaction of microbial antigen with antibody (via C3a and C5a) or reaction of antigen with IgE antibody on mast cells. The final mediators include molecules such as TNF (tumour necrosis factor), ICAM-1 (intercellular adhesion molecule-1) and ELAM-1 (endothelial cell leucocyte adhesion molecule-1). Inflammatory responses, like other powerful tissue responses, must be controlled and terminated, and the mediators of inflammation not only have a variety of inhibitors but are also inactivated locally (e.g. kinins inactivated by kininases). At a later stage, prostaglandins (a family of 20-carbon fatty acid molecules) and leukotrienes (a group of biologically active lipids) come into play.[2] They are produced from leucocytes, endothelial cells and platelets, and they both mediate and control the response.

If inflammation is due to infection with one of the pyogenic bacteria and the infection continues, then the continued supply of inflammatory and chemotactic products from the multiplying bacteria maintains vasodilation and the flow of neutrophils to the affected area. There is an increase in the number of circulating neutrophils, because of an increase in the rate of release from the bone marrow. The bone marrow holds a vast reserve supply with 20 times as many neutrophils as are present in the blood. If the tissue demand continues, the rate of production in the bone marrow is increased, and circulating neutrophils may remain elevated in persistent bacterial infections such as subacute bacterial endocarditis. Neutrophil production in the bone marrow is regulated by certain colony-stimulating factors, and it is a serious matter if something goes wrong and the marrow supplies are exhausted. A fall in circulating neutrophils (neutropenia) during a bacterial infection is of ominous significance.

[2]The terminology becomes complicated. Eicosanoids are produced by metabolism of arachidonic acid and include leukotrienes, prostaglandins, thromboxanes and lipoxins.

Viruses produce inflammatory products in tissues in the form of necrotic host cell materials or antigen–antibody complexes, but these are less potent than bacterial products, and the acute inflammatory response is of shorter duration, neutrophils being replaced by mononuclear cells. Mononuclear infiltrates are also favoured in virus infections because the infected tissues themselves are often one of the sites for the immune response, with mononuclear infiltration and cell division.

After extravasation from blood vessels, leucocytes would not automatically move to the exact site of infection. Neutrophils show random movement in tissues and also a directional movement (chemotaxis) in response to chemical gradients produced by chemotactic substances. Monocytes show little or no random movement, but they too respond to similar chemotactic substances. Chemotactic substances such as leukotrienes, C3a and C5a are formed during the inflammatory response itself. Also, many bacteria, such as *Staphylococcus aureus* or *S. typhi*, form chemotactic substances, and thus automatically betray their presence and attract phagocytic cells. It would obviously be an advantage to an infectious agent if no inflammatory or chemotactic products were formed, but for most large microorganisms (bacteria, fungi, protozoa) these products seem an almost inevitable result of microbial growth and metabolism. However, some pathogens have evolved ways of interfering with the chemotactic process by producing substances that block chemotactic receptors. The early stages of the inflammatory response in particular are known to have an important protective effect against microorganisms. In experimental staphylococcal skin infections, for instance, if the early inflammatory response is inhibited by adrenalin, and the early delivery of plasma factors and leucocytes to the site of infection thus reduced, bacteria multiply more rapidly and produce a more severe lesion. Perhaps it is not surprising that many bacterial pathogens can suppress the early inflammatory response. For example the chemotactic inhibitory protein of *S. aureus* (Chips) bind to the C5a and fmp receptors on macrophages, blocking the recognition of C5a and formylated peptides.

If inflammation becomes more severe or widespread, it is generally modulated by increased output of corticosteroid hormones, but at the same time it is backed up by a general metabolic response in the body. This is called the *acute phase response.* The liver releases about 30 different proteins, including C-reactive protein and serum amyloid protein, which undergo 1000-fold increases in concentration, as well as mannose-binding protein, haptoglobulins (α_2-glycoproteins), protease inhibitors and fibrinogen. The exact function of these *acute phase proteins* is not clear, but they are protective; they fix complement, opsonize and inhibit bacterial proteases. Their presence is associated with an increased erythrocyte sedimentation rate. The patient may develop headache, muscle pains, fever and anaemia, with decreased iron and zinc and increased copper and ceruloplasmin in the serum. Proteins in muscle are broken down, partly to provide energy required during fever and fasting, and partly to provide amino acids needed by proliferating cells and for the synthesis of immunoglobulins and acute phase proteins.

Many of the features of the acute phase response appear to be due to the action of interleukin-1 (see Glossary), and also IL-6 and TNF released from macrophages and lymphocytes. It is a complex response, which on the whole would be expected to serve useful purposes, although some less obviously beneficial 'side effects' may be unavoidable.

Tissue Fluids

Tissue fluids normally contain variable amounts of plasma proteins, including IgG antibodies as discussed above. In the absence of specific antibodies and complement, tissue fluids make a good culture medium for most bacteria, but bacterial multiplication almost inevitably causes some inflammation. Powerful inflammatory events are set in motion when molecules on the bacterial surface (e.g. endotoxin) activate the alternative complement pathway. Larger amounts of IgG as well as activated complement components, will then be present in tissue fluids. At a later stage, secretory products from phagocytes (lysosomal enzymes, oxygen radicals, lactoferrin, etc.) will also be present, and finally tissue breakdown products and additional antimicrobial substances liberated from dead platelets, neutrophils and macrophages.

Lymphatics and Lymph Nodes

A complex network of lymphatics lies below the epithelium at body surfaces. After reaching subepithelial tissues, foreign particles of all kinds, including microorganisms, rapidly enter lymphatic capillaries after uptake by or passage between lymphatic endothelial cells. There is a particularly rich superficial plexus of lymphatics in the skin and in the intestinal wall. Microorganisms scratched or injected into the skin inevitably enter lymphatics almost immediately. The intestinal lymphatics not only take up microorganisms that have breached the epithelial surface but also have an important role in the uptake of fat in the form of chylomicrons.

Microorganisms in peripheral lymphatics are rapidly moved (within minutes) to the local lymph nodes strategically placed to deal with the flow of lymph before it returns to the blood. The rate of flow of lymph is greatly increased during inflammation, when there is increased exudation of fluid from local blood vessels and the lymphatics are dilated. Microorganisms carried to the node in the lymph are exposed to the macrophages lining the marginal sinus (Figure 3.3), and these cells take up particles of all types from the lymph and thus filter it. The efficiency of filtration depends on the nature of the particles, on the physiological state of the macrophages, and also on the particle concentration and flow rate, the efficiency falling off at high particle concentrations or high flow rates (see Chapter 5).

All infecting microorganisms are handled in the same way and delivered via lymphatics to the local lymph node. When there has already been microbial multiplication at the site of initial infection, very large numbers may be delivered to the node. The efficiency of the node as a defence post depends on its ability to contain and destroy microorganisms rather than allow them to replicate further in the node and spread to the rest of the body. The antimicrobial forces are the macrophages of the node, the neutrophils and serum factors accumulating during inflammation, and the immune response which is initiated in the node. Under normal circumstances, as the first trickle of microorganisms reaches the node, the most important event is the encounter with macrophages in the marginal sinus. Microorganisms escaping phagocytosis by these cells enter the intermediate sinuses where they run the gauntlet of a further set of macrophages before leaving the node. If there is an inflammatory reaction in the node, a substantial migration of neutrophils

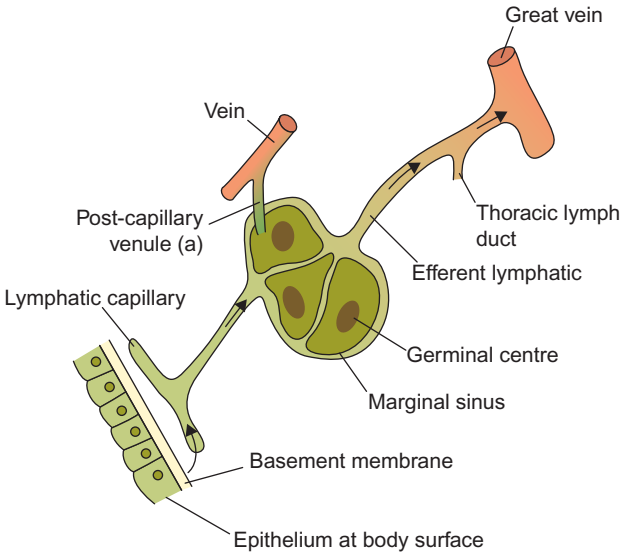

FIGURE 3.3 Diagram of the lymphatic system, showing pathways from body surface to venous system. (a) indicates a site of lymphocyte circulation from blood to lymph node and back to blood.

into the sinuses greatly increases the phagocytic forces and thus the filtering efficiency. There is usually a further node to be traversed before the lymph is discharged into the venous system.

As well as functioning as filters, the lymph nodes, of course, are sites where the immune response comes into play. Soon after infection, as inflammatory products of microbial growth arrive in the node, there is some swelling and inflammation. The microbial antigens, some of which are already associated with antigen-presenting cells encountered at the body surface, generate an immune response, and there is further swelling of the node as cells divide and additional lymphoid cells are recruited into the node from the blood. The ability of viruses and other intracellular microorganisms to bypass the defences of the node and spread to the bloodstream is discussed in Chapter 5.

Phagocytic Cells

Specialised phagocytic cells are divided into two main types: the macrophages, scattered through all the major compartments of the body (see Chapter 4) and the circulating neutrophils. The phagocytic cells to which microbes are exposed in the subepithelial tissues are the local macrophages (histiocytes) and also the cells arriving from the small blood vessels during inflammation. These comprise the blood monocytes which become macrophages after extravasation, and the neutrophils. From the time of the Russian zoologist, Elie Metchnikoff, who described phagocytosis in 1883, the importance of the phagocyte in defence against disease organisms has been accepted, and children have learnt of the white blood cells that act both as scavengers and policemen, removing debris, foreign particles and microorganisms. Because of the central importance of the phagocytic defence mechanisms, the subject will receive a chapter to itself.

NUTRITIONAL REQUIREMENTS OF INVADING MICROBES

In addition to being able to resist host defence mechanisms, a pathogenic organism – be it an obligate intracellular, facultative intracellular or extracellular pathogen – must also overcome the problem of obtaining essential nutrients if it is to be successful. Two examples illustrate this point. Iron is essential for bacterial growth but most iron is sequestered in the host and the concentration of free iron in body fluids is too low (*ca.* 10^{-18} M in serum) to support growth. In the host, iron is bound by both intracellular (ferritin, haemosiderin and haem) and extracellular (transferrin in serum and milk, and lactoferrin in milk), Fe-binding proteins with high association constants for iron. In order to overcome this problem, bacteria have evolved numerous ways of acquiring iron from the host in concentrations high enough for growth. For example, *Listeria monocytogenes* produces a soluble reductant which removes iron from transferrin. A common strategy by many bacterial pathogens is the synthesis of low molecular weight compounds called siderophores, which have an extraordinary affinity for iron. At the same time the bacteria express outer membrane proteins that act as receptors for the Fe-siderophore complexes so that Fe is taken up into the cell. *E. coli* has been extensively studied, and three classes of siderophore have been recognised; ferrichrome, the hydroxymates and aerobactin. *Salmonella* and *Shigella* spp. and *P. aeruginosa* produce more than one siderophore. *Salmonella* is also known to synthesise receptors for siderophores other than its own, which could be advantageous when present with other organisms, particularly in the competitive environment of the gut. The mycobacterial siderophores (Mycobactins) are lipid soluble and membrane associated, and exochelins are water soluble, extracellular and are the more important of the two.

Another common bacterial strategy for dealing with the Fe shortage involves the synthesis of new outer membrane proteins which interact directly with the host's own Fe-binding proteins. This is the method used by *Neisseria meningitidus* and *N. gonorrhoeae* which are able to scavenge iron directly from the host.

Perhaps the most dramatic example of Fe uptake and storage is exhibited by *Yersinia. Yersinia pestis* is the agent of bubonic plague and has been responsible for devastating epidemics throughout human history. This pathogen persists in wild rodent populations in many parts of the world except Australia, and is transmitted to humans by the bites of fleas. The blockage of the proventriculae of fleas by *Y. pestis* forces infected fleas to bite and subsequently regurgitate the infected blood meal into the bite wound of a new host. The ensuing bacteraemia in rodents completes the rodent–flea–rodent cycle essential for *Y. pestis* spread. However the ecology, pathogenicity and host range of *Yersinia pseudotuberculosis* (the predicted ancestor of *Y. pestis*) and *Yersinia enterocolitica* are quite different from *Y. pestis.* These are orally transmitted from contaminated food or water. As described in Chapter 2, they invade Peyer's patches and disseminate to mesenteric lymph nodes (where they multiply extracellularly) and occasionally beyond causing septicemic plague-like infections; normally these infections are self-limiting. Despite their disease-causing differences, the three *Yersinia* species do have some common pathogenic strategies, in particular the mechanism(s) for acquiring iron. *Yersinia* have two important sets of pathogenicity genes: the 70 kb virulence plasmid which has genes encoding proteins involved in

inhibition of phagocytosis to which we will return in Chapter 4, and chromosomal genes encoding virulence factors including an 'invasin' (involved in interaction with Peyer's patches) and the *pgm* (pigmentation) locus. Virulent *Y. pestis* strains accumulate huge quantities of exogenous haemin to form pigmented colonies on haemin agar (hence the alternative acronym for the locus, *hms).* Contiguous with the *hms* is *the ybt* gene cluster responsible for the synthesis of the siderophore yersiniabactin. In *Y. pseudotuberculosis, hms* and *ybt* are not contiguous, and in *Y. enterocolitica*, *hms* is absent. Inactivation of *hms* renders *Y. pestis* avirulent and unable to develop blockages in fleas, whereas inactivation of *ybt* in *Y. enterocolitica* hugely reduces virulence for laboratory animals. In fact, the *ybt* gene cluster has been designated the 'high pathogenicity island' (HPI) because biotype IB strains of *Y. pestis*, *Y. tuberculosis* and *Y. enterocolitica* (New World strains) all possess *ybt* biosynthetic genes and kill mice with very low infectious doses. In contrast, biotypes 2–5 (Old World strains) are much less virulent for mice and do not possess *ybt* genes. Accordingly, the capacity to acquire high concentrations of iron for metabolism is associated with a high virulence phenotype.

Other nutrients in short supply in the mammalian host are aromatic amino acids like tryptophan. Interestingly, the expression of the *trp* operon (which comprises the genes responsible for the synthesis of tryptophan) is controlled by Fe as well as by tryptophan levels. A functional aromatic biosynthetic pathway is absolutely vital for growth *in vivo* since aromatic amino acids are generated via chorismic acid. The latter is a branch point at which the biosynthesis of p-amino benzoic acid (PABA) also begins. PABA is required as a precursor of folic acid; it is as a competitor for PABA that the sulphonamide drugs work. By introducing lesions in one or more genes in this system (*aroA* and *aroD*), it has been possible to attenuate strains of pathogenic bacteria such as *S. typhimurium*, *E. coli*, and *Aeromonas salmonicida*, such that their initial invasive properties are unaltered but their ability to grow *in vivo* is severely restricted. In some cases, such crippled constructs can be used as vaccines to protect against subsequent infection or manipulated to carry genes encoding heterologous antigens which may offer immune protection.

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CHAPTER

4

Encounter of Microbes with Phagocytic Cells

OUTLINE

The phagocyte is the most powerful and most important part of the host's innate defences that can operate without delay. There are two major types of specialised phagocytic cells, the macrophage and the polymorphonuclear leucocyte which includes neutrophils, eosinophils, basophils and mast cells. In the subepithelial tissues, there are local resident macrophages, and as soon as an inflammatory response is induced, neutrophils arrive in large numbers after passing through the walls of small blood vessels.

Neutrophils arise in the bone marrow and are continuously discharged in vast numbers into the blood. The 3×10^{10} neutrophils that are present in normal human blood carry out their functions after leaving the circulation and entering sites of inflammation. These cells are nondividing, live only for a few days and each day about 10^{11} disappear from the blood, even in the absence of significant inflammation. Indeed, at any given time about half of them are adherent to or moving slowly along the walls of capillaries and post-capillary venules. This daily loss is balanced by entry into the blood from the bone

Mims' Pathogenesis of Infectious Disease.
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marrow and, to make some provision for sudden demands, the bone marrow contains an enormous reserve of about 3×10^{12} neutrophils.

Monocytes are circulating precursors of macrophages. They arise from stem cells in the bone marrow, and as soon as they leave the circulation and enter the tissue environment they differentiate into macrophages. Macrophages are a heterogeneous group of cells widely distributed throughout the body, where they serve a variety of functions in addition to phagocytosis (see Chapter 6). They are not as numerous as neutrophils, and there are no great reserves of macrophages in tissues (Figure 4.1). Fixed macrophages line the blood sinusoids of the liver (Kupffer cells), spleen, bone marrow and adrenals, and monitor the blood for effete cells, microorganisms or other foreign particles. Macrophages lining lymph sinuses in lymph nodes monitor the lymph, and the alveolar macrophages in the lung monitor the alveolar contents. The peritoneal and pleural cavities also contain

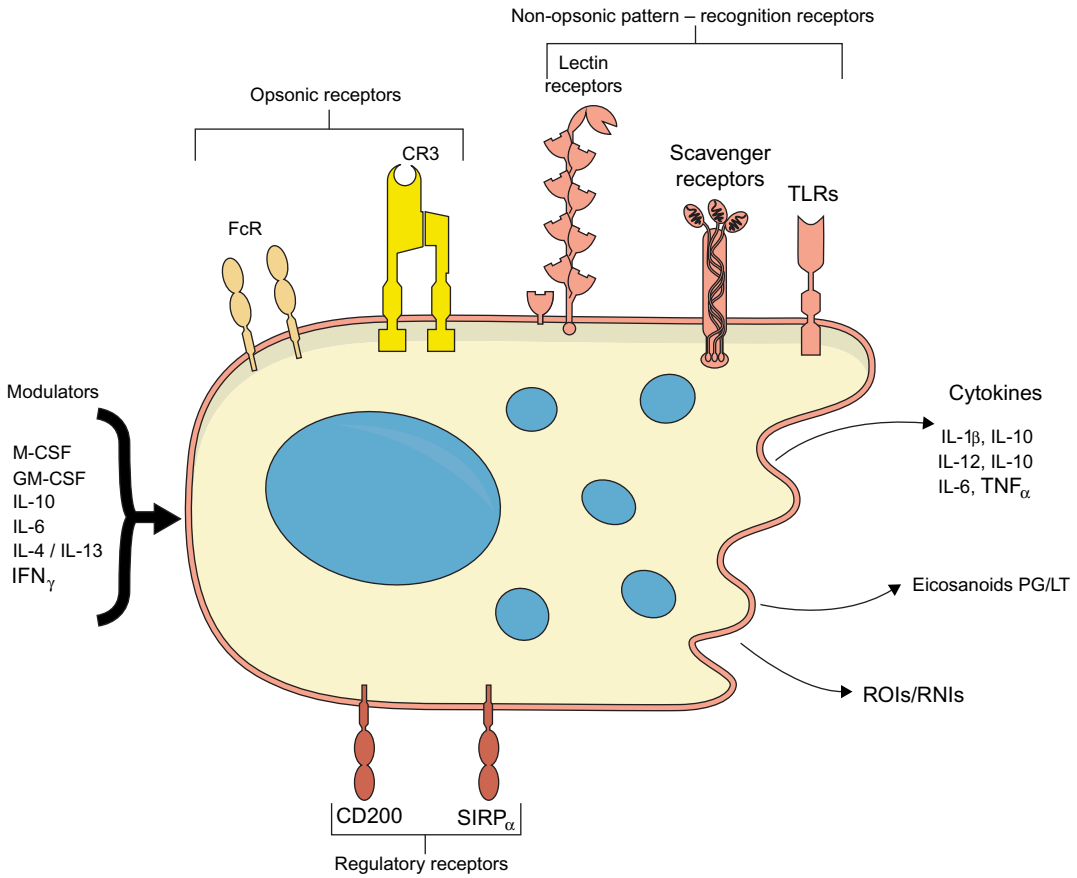

FIGURE 4.1 Macrophage receptors and functional molecules in phagocytosis. *Adapted from Male et al.* Immunology, *8th Edition, Elsevier, 2013, with permission.*

large numbers of macrophages. Macrophages, in fact, are strategically placed throughout the body to encounter invading microorganisms.

Phagocytosis is a basic type of cell function and is not restricted to macrophages and neutrophils. For instance, epidermal cells in the skin take up injected carbon particles, and intestinal epithelial cells and vascular endothelial cells also ingest certain marker particles, but this is on a very restricted scale compared with the professional phagocytes. Mere phagocytosis is not enough. If the ingestion of a microorganism is to be of service to the infected host, it must be followed by the killing and preferably intracellular digestion of the microorganism. Specialised phagocytic cells are therefore equipped with a powerful array of antimicrobial weapons and lysosomal enzymes.

CELL BIOLOGY OF PHAGOCYTOSIS

All cells sample their environment by the process of pinocytosis (the uptake of fluid and solutes). This process and that of receptor-mediated endocytosis use a clathrin-based mechanism to form endosomes. The newly formed endosomes mature by interaction with the other endocytic vesicles part of the early and late endocytic pathway and eventually interact with lysosomes where they undergo degradation. In contrast, phagocytosis is involved in the uptake of larger particles, is usually clathrin independent and occurs by an actin-dependent mechanism. Phagocytosis is a highly complex process whose outcome impacts on different areas of the immune and inflammatory responses, e.g. antigen presentation to T cells and effector cytokine responses.

Phagocytosis is an actin-mediated event that involves detection of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs) located on the plasma membrane of the phagocyte. PAMPs can be carbohydrates, lipopolysaccharide (LPS) or lipoprotein and found on bacteria and fungi, or dsRNA associated with viruses. PRRs are found in serum, on the plasma membrane and in the cytoplasm of phagocytes. Examples include carbohydrate receptors, scavenger receptors and Toll-like receptors (TLRs). There are several examples of the latter, including TLR4 that binds to LPS, TLR5 that binds to flagellin and TLR3 that interacts with dsRNA of viruses. Microorganisms can fix immunoglobulins and the C3 component of complement which triggers opsonic phagocytosis. Scavenger receptor and mannose receptor act as phagocytic PRRs, while others like Dectin-1 and FcgR serve dual roles transmitting inflammatory signals that activate NF-κB transcription, preceded by triggering actin polymerisation via Rac2, Cdc42 and RhoG. TLRs through their adaptor molecule MyD88 are also active in triggering NF-κB and controlling the maturation of the phagosome.

Other structures that appear in phagocytes during phagocytosis are inflammasomes. There are six inflammasomes so far described, each composed of a sensor receptor, an adaptor ASC, and pro-caspase-1 proteins. Upon activation, inflammosomes trigger caspase-1 to undergo autocatalytic cleavage which in turn cleaves the pro-forms of IL-1b and IL-18, leading to the genesis of pro-inflammatory cytokines. In addition, caspase-1 can also induce a form of inflammatory cell death termed pyroptosis (see Glossary).

PHAGOCYTOSIS IN POLYMORPHONUCLEAR LEUCOCYTES

Neutrophils generally carry out their functions after leaving the bloodstream, but they can under certain circumstances adhere to the endothelium of small blood vessels, especially in the lungs, and act as 'fixed' phagocytes. This happens, for instance, when Gram-negative bacteria or endotoxin enter the bloodstream, and probably depends on the action of complement.

As mentioned, there are several cell types which make up polymorphonuclear leucocytes (PMN): neutrophils, basophils, eosinophils and mast cells, each serving separate functions and distinguished by the staining reactions of their prominent cytoplasmic granules. The granules are lysosomes, consisting of membrane-lined sacs containing enzymes and other materials. The neutrophils are the most numerous, comprising 70% of the total leucocytes in blood. They have no mitochondria but plenty of glycogen as an energy source, which can be used under anaerobic conditions. They contain three types of granules: primary (Azurphil), secondary (specific) and tertiary (gelatinase), whose enzymes include peroxidase, alkaline phosphatase, acid phosphatase, ribonuclease, deoxyribonuclease, nucleotidases, glucuronidase, lysozyme and cathepsins. In addition, neutrophils (and macrophages) contain cationic peptides of 30–33 amino acids that are rich in cysteine and arginine and have a specific antibiotic-like activity by virtue of their pore-forming activity. They are called defensins, comprise *ca.* 30–50% of granular protein or 5% of total cellular protein, and are active against a range of pathogens as diverse as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E. coli*, *Cryptococcus neoformans*, Herpes simplex virus, Influenza virus and HIV. Defensin or defensin-like substances, e.g. LL-37, have pore-forming activity similar to the alpha defences. The granules also contain lactoferrin, a metal chelator protein that binds Fe over a wide pH range.

The eosinophils (1% of the leucocytes) are less effective than neutrophils in the phagocytosis and killing of microbes but they are especially active in the phagocytosis of immune complexes. For every circulating eosinophil, there are 300–500 in the extravascular tissues, and they are especially numerous in the submucosal tissues of the intestinal and respiratory tracts. Their granules contain, in addition to various enzymes, blockers of the inflammatory mediators (histamine, kinins and serotonin), five distinct cationic proteins and a major basic protein generated by immune complexes (see Chapter 6). A rise in the number of circulating eosinophils is a feature of certain parasitic and allergic diseases, and they are attracted into tissues by the eosinophil chemotactic factor released from mast cells. They bear C3b and Fc receptors and attach to and kill certain parasites (e.g. schistosomula) that are coated with a specific antibody, probably by discharge of the major basic protein.[1]

Basophils make up 0.5% of the total blood leucocytes, and their granules are especially rich in histamine and heparin. They closely resemble the mast cells seen in submucosal tissues and round blood vessels, and bear Fc receptors for IgE antibody. When antigen binds to IgE antibody on their surface the granules are discharged, and this leads to various 'allergic' inflammatory changes.

[1]The neutrophils also may attach in large numbers to the surface of the schistosomula, which is too big to be phagocytosed. Killing takes place if enough damage is inflicted in this combined assault. When polymorphs kill opsonised *Trichinella spiralis*, dozens of them may be seen attached to the surface of the parasite.

After extravasation from blood vessels, neutrophils would not automatically congregate at the exact site of microbial infection without any guidance. When seen in time-lapse cinephase movies, they display very active cell movement, travelling at up to 40 μm/min. One type of movement is random, in all planes, and to some extent this would bring the cells to the scene of infection. They also show chemotaxis, which is a directional cell movement in response to chemical gradients formed by the release of certain chemotactic materials in tissues. Many soluble bacterial products attract neutrophils in this way, as do the mediators generated by C3 and C5 components after antigen–antibody interactions (see Chapter 6), and various substances derived from host tissue.

Although phagocytosis is strikingly enhanced by these opsonins, and sometimes depends entirely on them, phagocytosis occurs during an infection, before antibodies have been formed, and this is a vital part of the 'early' defence system. A variety of objects including starch grains, yeasts, bacteria and polystyrene particles are adsorbed to the neutrophil surface and are phagocytosed without apparent need for antibodies. For example, mannose-binding lectin in serum reacts with carbohydrates on many bacteria, viruses, and fungi, and can opsonise them after attaching to a specific receptor on the phagocyte surface or activating the alternative pathway of complement. Familial deficiency of mannose-binding protein means susceptibility to meningococcal disease.

Phagocytosis is a familiar event in physical terms (Figure 4.2). The infolding of the plasma membrane to which particles are attached is due to the contraction of actin and myosin filaments (muscles) anchored to a skeleton of microtubules in the cytoplasm. As outlined above, the process is triggered by the attachment of particles to the receptors on the plasma membrane. Phagocytosis is associated with energy consumption involving oxidation of glucose via the hexosemonophosphate pathway – the respiratory burst. There is a 10- to 20-fold increase in the respiratory rate of the cell. There is also an increased turnover of membrane phospholipids. This is hardly surprising, because the multiple infoldings of the cell surface during active phagocytosis, in which up to 35% of the plasma membrane may be internalised, obviously requires synthesis of extra quantities of cell membrane.

As a result of phagocytosis, microorganisms are enclosed in membrane-lined vacuoles in the cytoplasm of the phagocytic cell, and subsequent events depend on the activity of the lysosomal granules (Figure 4.2). These move towards the phagocytic vacuole (phagosome), fuse with its membrane to form a phagolysosome, and discharge their contents into the vacuole, thus initiating the intracellular killing and digestion of the microorganism. The loss of lysosomal granules is referred to as degranulation. The process of ingestion, killing and digestion of a nonpathogenic bacterium by neutrophils can be followed biochemically by radioactive labelling of various bacterial components, and structurally by electron microscopy. When *E. coli* are added to rabbit neutrophils *in vitro*, phagocytosis begins within a few minutes. Nearly all neutrophils participate, each one ingesting 10–20 bacteria. Neutrophil granules then move towards the phagocytic vacuoles and fuse with them, delivering their contents into the vacuoles. The pH of the vacuoles becomes acid (pH 3.5–4.0), and this alone has some antimicrobial effect. Bacteria are killed (in the sense that they can no longer multiply when freed from the phagocytic cell) a minute or two later, before there is detectable biochemical breakdown of bacteria. Digestion then proceeds, first the bacterial cell wall components (detectable by the release from bacteria of radioactively labelled amino acids) and subsequently the contents of the bacterial cell.

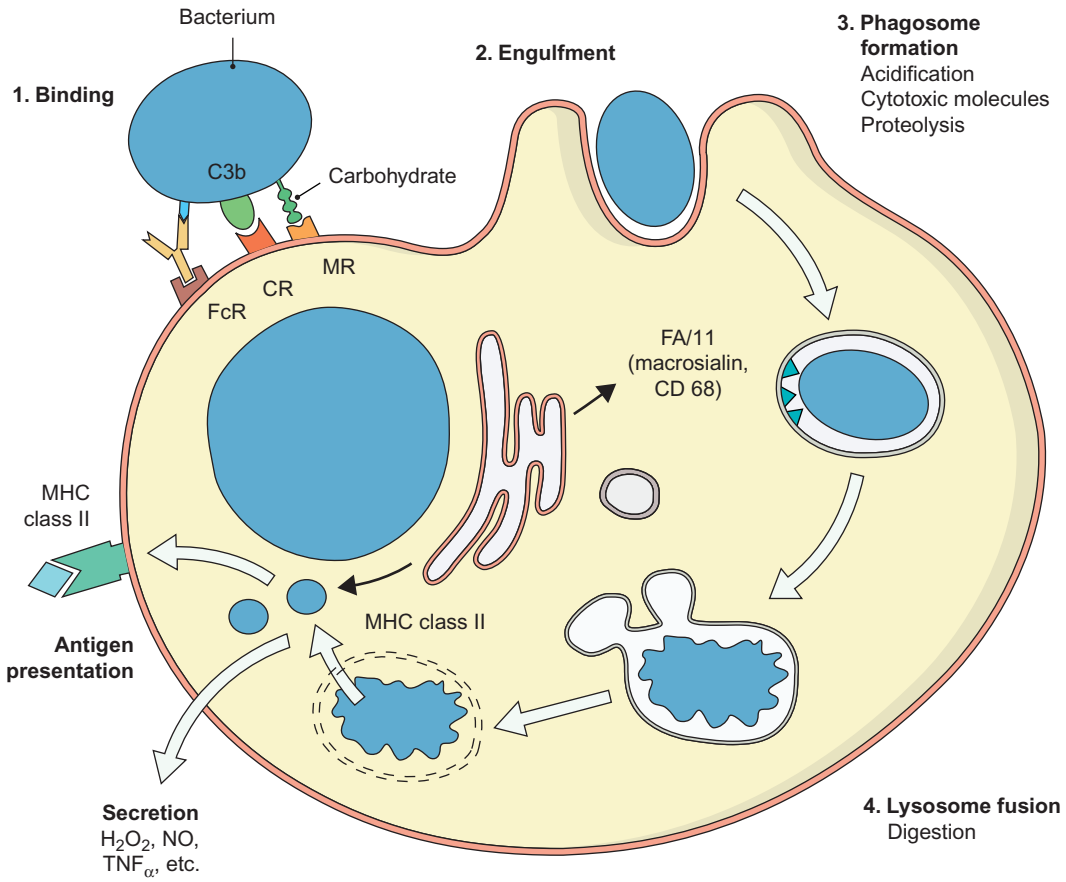

FIGURE 4.2 Stages in phagocytosis mediated by opsonic receptors. Note the diversity of functions carried out by macrophages.

By electron microscopy, the bacterial cell wall appears 'fuzzy' rather later, after about 15 min. The early killing is presumably associated with impaired functional integrity of the bacterial cell wall, the gross digestion of the corpse being detectable biochemically at a later stage, and changes in ultrastructural appearances later still.

The biochemical basis for the killing of bacteria and other microorganisms by neutrophils is complex, comprising various components. Although some of these components kill bacteria when added to them *in vitro*, their significance in the phagocyte is often not known.

1. Generation of reactive oxygen intermediates (ROIs), outlined in Figure 4.3. The brief burst of respiratory activity that accompanies phagocytosis is needed for killing rather than for phagocytosis itself, and membrane-associated NADPH oxidase is activated after phagocytosis has occurred. The following events taking place within the vacuole

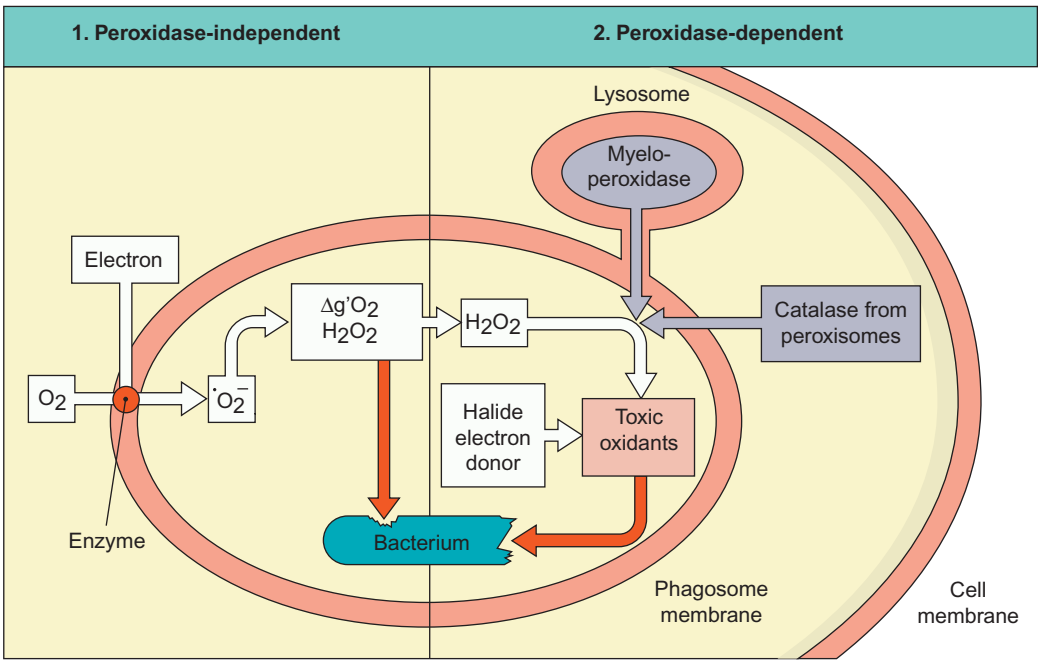

FIGURE 4.3 Oxygen-dependent microbicidal activity in the neutrophil.

are important. The oxygen produced gives rise to superoxide by the addition of one electron, and two superoxide molecules may interact (dismutate) and form hydrogen peroxide, either spontaneously or with the help of superoxide dismutase (SOD). The hydrogen peroxide in turn can be reduced to give the hydroxyl radical ($OH\cdot$). It can also undergo myeloperoxidase-mediated halogenation to generate hypochlorite (OCl^-) which not only disrupts bacterial cell walls by halogenation but also reacts with H_2O_2 to form singlet oxygen, which is possibly antimicrobial. Thus, free hydroxyl ($OH\cdot$) and superoxide (O_2^-) radicals, H_2O_2, $OC1^-$ and singlet oxygen ($\Delta g'O_2$) are all produced in neutrophils in the membrane of the phagosome, mostly by means of an electron transport chain, and involving cytochrome b. But it is not clear whether some or all of these products are responsible for killing or whether it also depends on other activities of the electron transport chain.

2. Oxygen-independent killing mechanisms. Oxygen-dependent killing is not the whole story. Neutrophils often need to operate at low oxygen tension, for instance where relatively anaerobic bacteria are multiplying, and such microorganisms are killed quite effectively in the absence of oxygen. There are a number of possible mechanisms. First, within minutes of phagocytosis the pH within the vacuole falls to about 3.5 and this would itself have an antimicrobial effect. Also, the granules delivered to the phagocytic vacuole contain certain antimicrobial substances. There are 'specific' granules and 'azurophil' granules, as well as the regular lysosomes. These contain, as mentioned above, not only myeloperoxidase but also lactoferrin, lysozyme, a vitamin B_{12}-binding protein, a variety of cationic proteins and acid hydrolases. Mice deficient in neutrophil

elastase and cathepsin G are highly susceptible to bacterial and fungal infections. Lactoferrin, which binds iron very effectively, even at a low pH would not kill but would deprive the phagocytosed microorganism of iron. The cationic proteins bind to bacteria and, under alkaline conditions, have a pronounced antibacterial action; they must act early, before the pH becomes acid. The most potent of them is bactericidal/permeability-increasing protein (BPI), which is active at picomolar concentrations. It binds to LPS on Gram-negative bacteria, damages their surface and inhibits their growth. Animals given BPI are protected against a wide range of Gram-negative bacteria. Exposure to BPI induces expression of a range of proteins in *Salmonella* and enteropathogenic *E. coli* (EPEC) including BipA. The latter is a remarkable protein belonging to the class of small GTPases involved in signal transduction. It is involved in resisting the cytotoxic effect of BPI, modelling of the EPEC-induced pedestal and flagella-mediated motility.

The acid hydrolases probably function by digesting the organisms after killing. The enzyme lysozyme hydrolyses the cross-links of the giant peptidoglycan molecules that form most of the cell wall of Gram-positive cocci (Figure 4.4). The cell wall is rapidly dissolved and the bacteria killed. Gram-negative bacteria have an additional LPS component incorporated into the outer surface of the cell wall, and this gives these bacteria relative resistance to the action of lysozyme.[2]

A novel protective mechanism mediated by neutrophils involves NETs structures that include DNA/histones and granular enzymes acting as traps for microorganisms in the

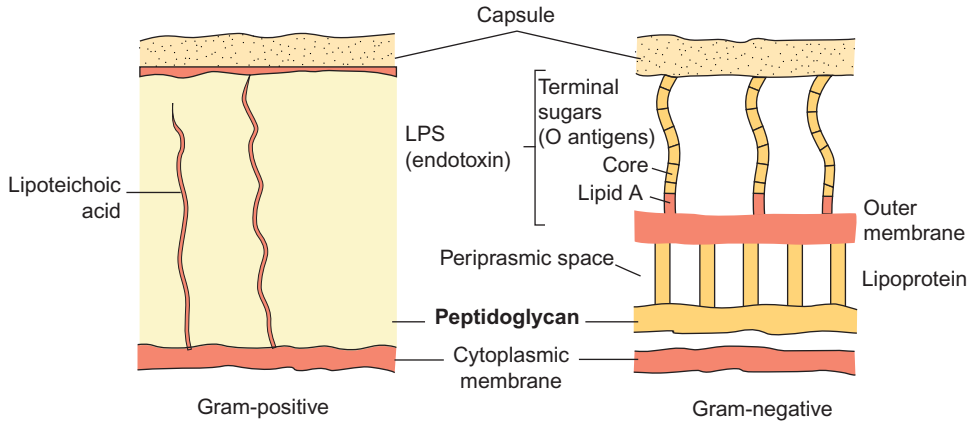

FIGURE 4.4 Comparison of Gram-positive and Gram-negative bacterial cell walls. Pili and flagella (the latter bearing H antigens in Gram-negative bacilli) are not shown. Peptidoglycan has lipoteichioic acid molecules extending through it, ±teichoic acid linked to peptidoglycan. The capsule may be protein or polysaccharide and is the site of the K antigen of Gram-negative bacilli.

[2]Granule proteins generally have to bind to the bacterial surface if killing is to occur, and a longer polysaccharide chain makes binding less effective.

cytoplasm. NETs are reactive against a wide range of pathogens which they kill by focusing high concentrations of antimicrobial substances.

Fusion of lysosomal granules with phagosomes is the prelude to intracellular digestion in phagocytes and is closely comparable with the process by which a free-living protozoan such as *Amoeba* digests its prey. In both cases, the phagocytic vacuole becomes the cellular stomach. Under certain circumstances, neutrophil granules fuse with the cell surface rather than with the phagocytic vacuole, and the contents of the granule are then discharged to the exterior, producing local concentrations of lysosomal enzymes in tissues and often giving rise to severe histological lesions. Antigen–antibody complexes induce this type of response in neutrophils, and the resultant tissue damage is exemplified in the blood vessel wall lesions in a classical Arthus response. On other occasions, lysosomes fuse with the phagocytic vacuole before phagocytosis is completed and the vacuole internalised. Lysosomal enzymes then pass to the exterior of the cell to give what is referred to as 'regurgitation after feeding'. This occurs after exposure to certain inert particles or to antigen–antibody complexes. Since neutrophils live for no more than a day or two, their death and autolysis inevitably leads to the liberation of lysosomal enzymes into tissues. When this occurs on a small scale, macrophages ingest the cells and little damage is done, but on a larger scale the accumulation of necrotic neutrophils and other host cells, together with dead and living bacteria, and autolytic and inflammatory products, forms a localised fluid product called pus. This product, resulting from the age-old battle between microorganism and phagocyte, can be thin and watery (streptococci), thick (staphylococci), cheesy (*Mycobacterium tuberculosis*), green (*P. aeruginosa* pigments) or foul smelling (anaerobic bacteria). Before the advent of modern antimicrobial agents, a staphylococcal abscess could contain more than half a litre of pus.

PHAGOCYTOSIS IN MACROPHAGES

The processes of adsorption, ingestion and digestion of microorganisms in macrophages are in general similar to those in neutrophils, but there are important differences.

Macrophages exhibit great changes in surface shape and outline, but do not have the neutrophil's striking ability to move through tissues. They show chemotaxis, but the chemotactic mediators are different from those attracting neutrophils. This contributes to the observed local differences in macrophage and neutrophil distribution in tissues. Macrophages also have a different content of lysosomal enzymes, which varies with the species of origin, the site of origin in the body and the state of activation (see Chapter 6). They do not contain the cationic proteins found in neutrophil granules, but they do contain defensin peptides and an equivalent, but not the same, oxygen-dependent antimicrobial system. This gives rise to differences in their ability to handle ingested microorganisms. Thus, although the fungus *C. neoformans* is phagocytosed by human neutrophils and then killed by chymotrypsin-like cationic proteins and the oxygen-dependent system, the same fungus survives and grows readily after phagocytosis by human macrophages. The antimicrobial armoury of human neutrophils also gives them a major role in the killing of the fungus *Candida albicans*, whereas macrophages are much less effective. Indeed, for many bacteria, neutrophils

show a bactericidal activity that is superior to that of monocytes and macrophages. This is because opsonised phagocytosis is often more rapid in neutrophils, and there is a greater generation of the antibacterial species of oxygen. On the other hand, macrophages live for long periods (months, in man) compared with neutrophils (days, in man). Neutrophils are very much 'end cells', delivered to tissues with a brief life span and limited adaptability, whereas macrophages are capable of profound changes in behaviour and biochemical make-up in response to stimuli (see Chapter 6). When neutrophils have discharged their lysosomal granules into phagosomes, the cells, rather than the granules, are renewed. Macrophages, on the other hand, retain considerable synthetic ability, so that they can be stimulated to form large amounts of lysosomal and other enzymes. Also, because of their longer life in tissues, it is common to see macrophages loaded with phagocytic vacuoles whose contents are in all stages of digestion and degradation. Certain materials, particularly the cell walls of some bacteria, are only degraded very slowly or incompletely by macrophages.

Macrophages, like neutrophils, express receptors for the Fc portion of IgG and IgM immunoglobulins, and complement, so that immune complexes or particles coated with immunoglobulins and complement are readily adsorbed. Macrophages also have the ability to recognise and adsorb to their surface various altered and denatured particles such as effete or aldehyde-treated erythrocytes. However, mere adsorption of microorganisms to the cell surface does not necessarily lead to phagocytosis. Certain mycoplasma, for instance, attach to macrophages and grow to form a 'lawn' covering most of the cell surface, but are not phagocytosed unless antibody is present. Macrophages are also secretory cells and liberate about 60 different products ranging from lysozyme to collagenase. These may be important in antimicrobial defence as well as in immunopathology (see Chapter 6).

Another important difference is the ability of many macrophages, especially when activated, to generate reactive nitrogen intermediates (RNIs), the nitric oxide (NO) pathway (Figure 4.5). Among its many activities (on the vascular system, on neurons, on platelets, etc.), NO is microbicidal, being effective against a range of organisms including mycobacteria and *Leishmania* spp. Bacteria produce an enzyme (NO dioxygenase) that detoxifies NO, and if this capacity is removed they become exquisitely sensitive to NO. Paradoxically, it is doubtful if tetrahydrobiopterin is made by human macrophages and the role of the NO pathway in the antimicrobial function in human macrophages *in vivo* is not clear. However, other nonimmunological cells (fibroblasts, endothelial cells, hepatocytes and cerebellar neurons) are known to generate RNI, although less markedly than macrophages, and RNI may represent an important basic mechanism of local resistance against intracellular pathogens.

After the microorganism has been killed, the subsequent disposal of the corpse is only of concern to the host. Most microorganisms are readily digested and degraded by lysosomal enzymes. But the microbial properties that give resistance to killing sometimes also give resistance to digestion and degradation, because the cell walls or capsules of certain pathogenic bacteria are digested with difficulty. Group A streptococci, for instance, are rapidly killed once they have been phagocytosed, but the peptidoglycan–polysaccharide complex in the cell wall resists digestion, and streptococcal cell walls are sometimes still

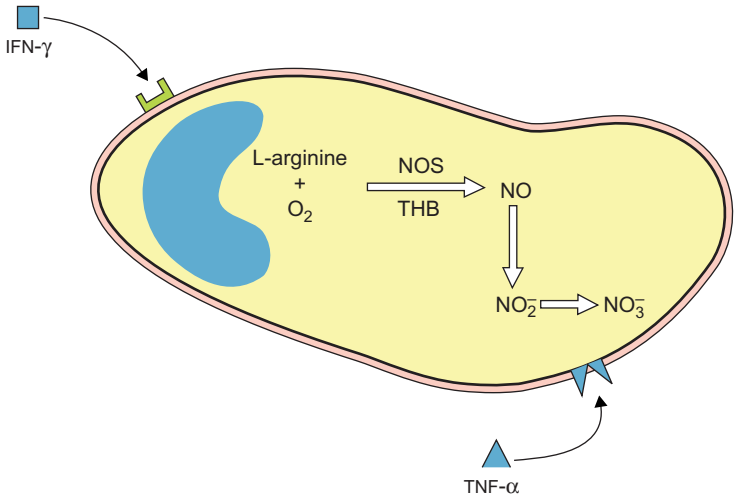

FIGURE 4.5 Schematic representation of the NO pathway in murine macrophages. Nitric oxide synthetase (NOS) mediates the addition of O_2 to the guanidino N of L-arginine to form NO. This is rapidly converted to NO_2^- and NO_3^-. Precisely which RNI is involved and by what mechanism killing takes place is not clear. Tetrahydrobiopterin (THB) is an essential cofactor for NOS but this is not present in human macrophages. The pathway is blocked by the arginine analogue N^G-monomethyl-L-arginine. The process is subject to modulation by several cytokines but two seem to be very important. The synthesis of NOS is activated by interferon-γ (IFN-γ) and the subsequent steps optimised by tumour necrosis factor-α (TNF-α). The latter may arise from the macrophage stimulated by IFN-γ in the first place – an autocrine effect.

visible in phagocytes a month or so after the infection has terminated.[3] The waxes on the outer surface of certain mycobacteria are not readily digested by lysosomal enzymes and it is possible that this is why such bacteria (e.g. the causative agent of feline leprosy, *Mycobacterium lepraemurium*) are difficult to kill. Although saprophytic mycobacteria have a similar type of covering, it may have particular properties in *M. lepraemurium*.

MICROBIAL STRATEGY IN RELATION TO PHAGOCYTES

As has been discussed earlier, microorganisms invading host tissues are first and foremost exposed to phagocytes, and the encounter between microbe and phagocyte has played a vital role in the evolution of multicellular animals, all of which, from the time of their origin in the distant past, have been exposed to invasive microorganisms. The central importance of this ancient and perpetual warfare between the microbe and the phagocytic cell was clearly recognised by Metchnikoff over a 100 years ago.

[3]Because the capsules or cell walls of streptococci, pneumococci, mycobacteria, *Listeria* and other bacteria pose problems for lysosomal enzymes and are not readily digested in phagocytes, bacterial fragments are sometimes retained in the host for long periods. This can lead to interesting pathological or immunological results (see Chapter 8).

Microorganisms that readily attract phagocytes, and are then ingested and killed by them, are by definition unsuccessful. They fail to cause a successful infection. Phagocytes, when functioning in this way, have an overwhelming advantage over such microorganisms. Most successful microorganisms, in contrast, have to some extent at least succeeded in interfering with the antimicrobial activities of phagocytes, or in some other way avoiding their attention. The contest between the two has been proceeding for so many hundreds of millions of years that it can be assumed that, if there is a possible way to interfere with or otherwise prevent the activities of phagocytes, then some microorganisms will almost certainly have discovered how to do this. Therefore, the types of interaction between microorganisms and phagocytes will be considered from this point of view.

Many microbial factors can inhibit the operation of host defence mechanisms without doing any damage to the host in the form of pathology, i.e. there is no toxic activity. For example, we now know that bacterial pathogens such as *S. aureus* produce an array of different molecules which target specific components of the host immune response, particularly the stages involved in phagocytosis.

Microbes that are noninfectious for man are dealt with and destroyed by the phagocytic defence system just as in the case of the nonpathogenic bacteria in neutrophils as described above. Nearly all microorganisms, indeed, are noninfectious and it is only a very small number that can infect the vertebrate host, and an even smaller number that are significant causes of infection in man. The ways in which microorganisms meet the challenge of the phagocyte will be classified for simplicity (Figure 4.6, Table 4.1).

Inhibition of Chemotaxis or the Mobilisation of Phagocytic Cells

Various substances released from bacteria attract phagocytes, but their activity is generally weak. Other bacterial substances react with complement to generate powerful chemotactic factors such as C5a. Formylated peptides, a by-product of bacterial translation, are also chemotactic. Microorganisms can avoid the attentions of phagocytic cells by inhibiting chemotaxis, and as a result of this the host is less able to focus neutrophils and macrophages into the exact site of infection. Some bacterial toxins inhibit the locomotion of neutrophils and macrophages. The streptococcal streptolysins which kill phagocytes can suppress neutrophil chemotaxis in even lower concentrations, apparently without adverse effects on the neutrophil. *Clostridium perfringens* θ toxin has a similar action on neutrophils. There are good methods available for the quantification of chemotaxis, and it is possible that other pathogenic bacteria will be shown to produce inhibitors. The chemotactic inhibitory protein of *S. aureus* (Chips) is a secreted protein which binds to the C5a and formylated peptide receptors on neutrophils. This blocks the detection of a chemotactic concentration gradient by the neutrophil and accordingly inhibits its capacity to sense and target invading *S. aureus* cells.

Both neutrophils and macrophages arise from stem cells in the bone marrow, and their rate of formation is greatly increased during infection, so that blood leucocyte counts reach two to four times normal levels. This is associated with increased blood levels of certain factors that stimulate colony formation by leucocyte precursors. Four of these colony-stimulating factors, which are glycoproteins, active at very low concentrations (10^{-11}–10^{-13} M), have

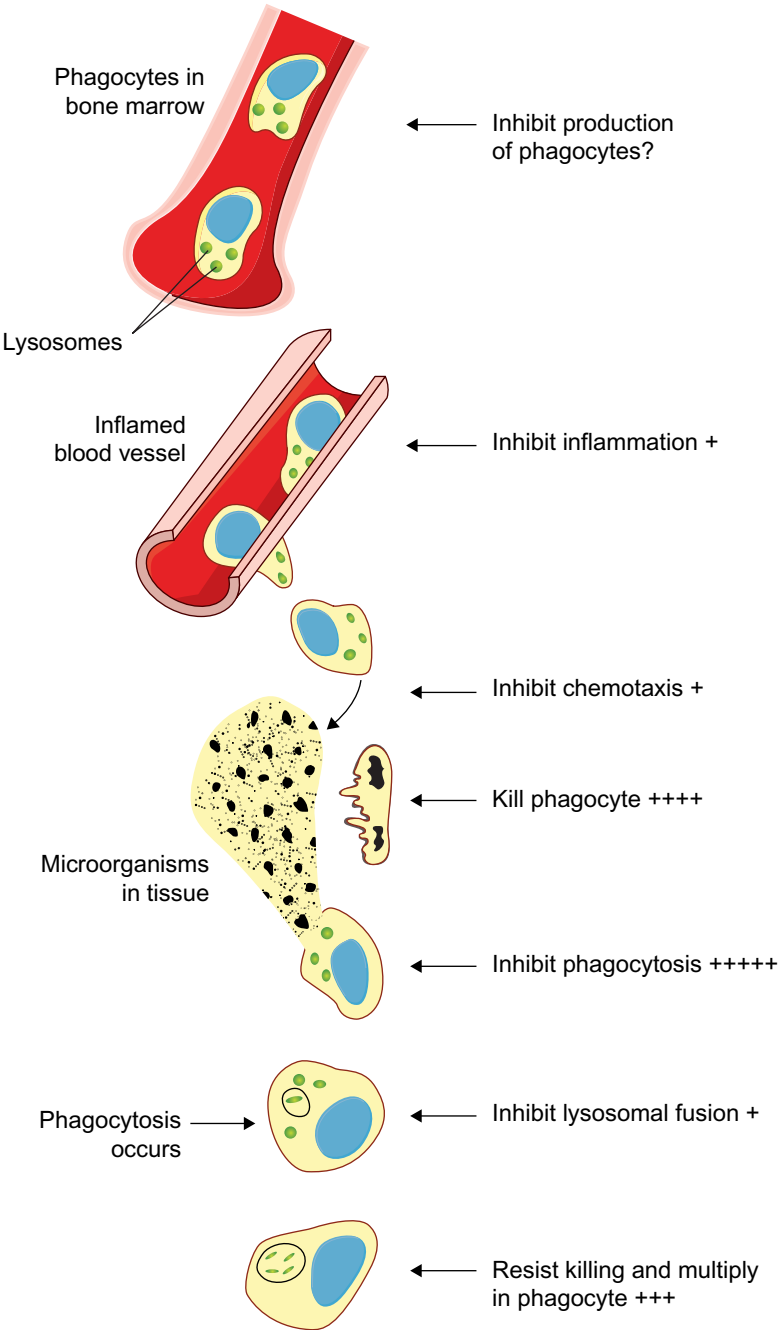

FIGURE 4.6 Antiphagocytic strategies available to microorganisms. The extent to which strategies are actually used by microorganisms are indicated by pluses.

TABLE 4.1 Examples of Types of Bacterial Interference with Phagocytic Activities

Microorganism	Type of Interference	Mechanism or Responsible Factor
Streptococcus pyogenes	Kill phagocyte	Streptolysin induces lysosomal discharge into cell cytoplasm
	Inhibit neutrophil chemotaxis	
	Resist phagocytosis	Streptolysin
	Resist digestion	M-like protein
		Hyaluronic acid capsule
Staphylococci	Kill phagocyte	Leucocidin (e.g. PVL)
	Inhibit opsonised phagocytosis	SpA binds Fc portion of Ab;
	Resist killing	Chips
	Block chemotaxis	Polysaccharide capsule in some strains
Bacillus anthracis	Kill phagocyte	Lethal factor of tripartite toxin
	Resist killing	Capsular polyglutamic acid
Haemophilus influenzae	Resist phagocytosis (unless Ab present)	Polysaccharide capsule
Streptococcus pneumoniae	Resist digestion	
Klebsiella pneumonia		
Pseudomonas aeruginosa	Kill phagocyte	Exotoxin A kills macrophages; also cell-bound leucocidin Exopolysaccharide)
	Resist phagocytosis (unless Ab present)	
	Resist digestion	
Escherichia coli	Resist phagocytosis (unless Ab present)	O antigen (smooth strains)
	Resist killing	K antigen (acid polysaccharide)
	Kill macrophages	K antigen
Salmonella spp.	Resist phagocytosis (unless Ab present)	Vi antigen
	Resist killing; survival in macrophages	Secreted products of SPI-2
	Kill phagocyte	Secreted products of SPI-1
Clostridium perfringens	Inhibit chemotaxis	θ-toxin
	Resist phagocytosis	Capsule
Cryptococcus neoformans	Resist phagocytosis	Capsular polyuronic acid
Treponema pallidum	Resist phagocytosis	Capsular polysaccharide
Yersinia pestis	Kill phagocyte	Yop virulon proteins
Mycobacteria	Resist killing and digestion	Cell wall component
	Inhibit lysosomal fusion	
Brucella abortus	Resist killing	Cell wall substance
Toxoplasma gondii	Inhibit attachment to neutrophil	
	Inhibit lysosomal fusion	
Plasmodium berghei	Resist phagocytosis	Capsular material

been identified. They are produced in many tissues, and their concerted action is needed for the production and final differentiation of neutrophils (also eosinophils) and macrophages. They also help control the activity of differentiated cells. Clearly, if it were possible for microorganisms to release substances that inhibited the formation or action of colony-stimulating factors, and thus seriously impair the phagocytic response to infection, some of them might be expected to do so. There is a decrease rather than an increase in blood neutrophils during certain infections such as typhoid and brucellosis, but there is so far no evidence that this is due to effects on colony-stimulating factors.

Inhibition of Adsorption of Microorganism to Surface of Phagocytic Cell

Many microorganisms tend to avoid phagocytosis without being obviously toxic for phagocytes. As a rule, it is not possible to distinguish between a failure to adsorb and a failure to ingest the microorganism. Since our understanding of adsorption is so slight, our understanding of failure to adsorb is equally inadequate. Yet the distinction can sometimes be made, as when pilated (virulent) gonococci attach to neutrophils but are not ingested or killed. *Mycoplasma hominis* remains extracellular when added to human neutrophils *in vitro*, and it appears that there is no firm adsorption of the mycoplasmas to the neutrophil surface, although in the presence of antibody to the mycoplasmas there is adsorption, ingestion and digestion. The reason for the failure in adsorption is not clear, but it may be because the mycoplasmas damage the neutrophil, which shows increased oxidation of glucose and defective killing of phagocytosed *E. coli.* If neutrophils are added to the protozoan parasite *Toxoplasma gondii in vitro*, the mobile neutrophils are seen to turn aside from the toxoplasmas, indicating perhaps a failure of attachment. Antibody-coated or dead toxoplasmas, on the other hand, are successfully phagocytosed and digested by neutrophils. Macrophages, it may be noted, ingest the live parasite and support its growth.

Many viruses will not attach to, and therefore cannot infect, a cell unless a specific receptor is present on the cell surface (see Chapter 2). When the virus cannot grow in the phagocyte it would be an advantage to avoid being taken up and destroyed, but so far it has not been possible to associate avoidance of phagocytosis with virus pathogenicity. When, however, a virus infects and grows in the phagocytic cell this may be an important part of the infectious process (see Chapter 5), especially if the phagocyte is so little affected that it carries the infecting virus from one part of the body to another.

Inhibition of Phagocytosis — Opsonins

Microbial products that kill phagocytes may at lower concentrations interfere with their locomotion or their phagocytic activity, for instance by inhibiting protein synthesis. A more direct challenge to the phagocyte is provided by the various microorganisms whose surface properties prevent their phagocytosis. As mentioned above, it is not usually possible to distinguish between inhibition of adsorption to the phagocytic cell and inhibition of phagocytosis which follows adsorption.

Many important pathogenic bacteria bear substances on their surface that inhibit phagocytosis (see Table 4.1). Clearly it is the bacterial surface that matters. The phagocyte physically encounters the surface of the microorganism, just as the person knocked down by a car encounters the hard metal exterior of the vehicle, and the phagocyte has no more immediate interest in the internal features or antigens of the microorganism than the person knocked down has of the upholstery or luggage inside the car. Resistance to phagocytosis is sometimes due to a component of the bacterial cell wall, and sometimes it is due to a capsule enclosing the bacterial wall, secreted by the bacterium. Classical examples of antiphagocytic substances on the bacterial surface include the M-like proteins of streptococci and the polysaccharide capsules of pneumococci. These bacteria owe their success to their ability to survive and grow extracellularly, avoiding uptake by phagocytic cells.

M-like proteins on the surface of streptococci are associated with resistance to phagocytosis mediated through different mechanisms including binding of factor H which disrupts the complement pathway, and binding of IgG through its non-specific Fc region which results in immobilised antibody which is the incorrect orientation for recognition by Fc receptors on circulating phagocytes. In a similar fashion, staphylococcal protein A binds the Fc region of IgG through 4 or 5 IgG-binding domains and may be a significant factor in the lack of success to date in inducing a protective immune response in humans against *S. aureus* infection.

The polysaccharide capsule of the pneumococcus is likewise associated with resistance to phagocytosis and with virulence. It takes less than 10 encapsulated virulent bacteria to kill a mouse after injection into the peritoneal cavity, but 10,000 bacteria are needed if the capsule is removed by hyaluronidase. As with pathogenic streptococci, phagocytosis takes place more readily via Fc and C3b receptors, when the bacterial surface has been coated with specific antibody and C3b deposited. Unencapsulated strains of bacteria are coated with C3b without the need for antibody, after activation of the alternative pathway, but this is inhibited by neuraminic acid components of the capsule. If a mouse is rendered incapable of forming antibodies to the capsule, infection with a single bacterium is then enough to cause death. It is not clear why the capsule confers resistance to phagocytosis; perhaps its slimy polysaccharide nature makes the phagocytic act difficult for purely mechanical reasons. Although antibody is needed for phagocytosis in a fluid medium, it is known that phagocytosis takes place without antibody on the solid surface lining of an alveolus or lymphatic vessel (or on a piece of filter paper!), where the physical act of phagocytosis is favoured, and the phagocyte can 'corner' and get round the bacterium. Pathogenic bacteria with similar polysaccharide capsules include *Haemophilus influenzae* and *Klebsiella pneumoniae*. Patients with agammaglobulinaemia have repeated infections with streptococci and these encapsulated bacteria. Their neutrophils fail to take up and destroy bacteria because opsonising antibodies cannot be produced. Polysaccharide capsules are not necessarily associated with virulence, since they occur in free-living nonparasitic bacteria. Presumably they have functions other than the antiphagocytic one, perhaps giving protection against phages and antimicrobial agents such as bacteriocins.

Anthrax and plague bacteria also have capsules that are associated with virulence. Bacteria of the *Bacteroides* group are normally commensal, but can form abscesses, often together with other microorganisms, and they have polysaccharide capsules. Pathogenic strains of *E. coli* and *Salmonella typhi* have thin capsules consisting of acidic polysaccharide (K antigen), which in some way make phagocytosis difficult. Perhaps this is because (in the absence of antibody) the encapsulated strains do not activate complement via the alternative pathway, and are therefore poorly opsonised. Gram-negative bacteria also have cell walls containing an LPS complex (endotoxin), and the somatic (O) antigens occur in the polysaccharide side chains (Figure 4.4). Bacteria with certain types of O antigen have a colonial form designated as smooth, and they show an associated virulence, with resistance to phagocytosis except in the presence of antibody. Rough colonial forms lack these particular antigens, which are determined by immunodominant sugars in the polysaccharide side chains, and are not virulent, showing no resistance to phagocytosis.

The parasitic trypanosomes causing African sleeping sickness circulate in the blood, from which they are transmitted to fresh hosts by biting tsetse flies. The bloodstream

forms have a pronounced surface coat with an outer carbohydrate layer, which perhaps inhibits phagocytosis of the parasites by reticuloendothelial cells (see Chapter 5) and enables the parasitaemia to continue.

Some microorganisms pose purely mechanical problems for the phagocytic cell without specifically preventing phagocytosis. There are difficulties with motile microorganisms, whether motility is due to flagella (Gram-negative bacteria, *Trichomonas vaginalis*) or to amoeboid movement (*Entamoeba histolytica*). Immobilising antibodies may be necessary. The sheer size of a microorganism can be a problem. A single macrophage will be unable to phagocytose a large microorganism, and macrophages attempting to phagocytose the advancing tip of fungal hyphae are just carried along by the hyphal growth. In such situations, several macrophages must cooperate and if necessary form syncytial giant cells, as in the response to fibres and other large foreign objects.

As mentioned above, both neutrophils and macrophages have specific surface receptors for the Fc fragment of IgG and IgM antibodies and also for the C3b product of complement activation (see Chapter 6). This ensures that microorganisms coated with antibody or complement are opsonised. Cells other than neutrophils and macrophages lack these receptors, and here attachment and phagocytosis of particles coated with antibody is not promoted but even inhibited. Opsonised microbes are not only taken up but also killed more rapidly in the phagocyte. For instance, in the early stages of typhus, the rickettsiae multiply in macrophages after phagocytosis, but later, when antibodies have formed, the antibody-coated rickettsiae are rapidly phagocytosed and killed, and eventually digested.

Opsonisation without specific antibody takes place following deposition of C3b on the bacterial surface after activation of the alternative complement pathway and attachment to the C3b receptor on the phagocyte. It is an important host defence early in infection, before antibodies are formed, and the following can be considered as microbial 'strategies' to prevent this type of opsonisation. Encapsulated strains of *S. aureus* appear to activate and bind complement without the need for antibody, but are not opsonised and phagocytosed. For example, surface protein ClfA binds to complement factor I, and SdrE binds to factor H resulting in decreased opsonophagocytosis. In the case of Group A streptococci, the outer covering of M protein prevents complement activation by the alternative pathway. Strains of *E. coli* with K1 capsular polysaccharide are pathogenic for newborn infants and show an associated resistance to opsonisation by the alternative complement pathway. Secreted proteins may also be produced to interfere with complement. For example, staphylokinase activity converts host plasminogen into plasmin which has protease activity for both IgG and C3b deposited on the bacterial cell surface. In addition, other enzymes such as C5a peptidase made by Streptococci, and IgA protease made by numerous bacteria including *Neisseria* spp. have specific activity for degradation of opsonins and thus promote avoidance of opsonophagocytosis.

There are a number of ingenious ways in which bacteria and other microorganisms avoid inactivation by host antibodies, or even avoid eliciting antibodies (see Chapter 7). One example will be given here, since it involves phagocytosis (Figure 4.6). As discussed previously, staphylococcal protein A binds to IgG via the Fc portion, and there are about 80,000 binding sites on each bacterium resulting in a major inhibition of opsonophagocytosis. Similar IgG-binding molecules such as M-like protein, protein G and ZAG are

present on many streptococci. Viruses of the herpes group code for Fc receptors, induced on the surface of infected cells, and this is a further indication that antibody-binding molecules are useful to infecting microorganisms. The antibody molecules are not only bound in a useless 'upside-down' position to the microbe or the infected cell, but also, by their presence at this site, they interfere with the access of specific antimicrobial antibodies or cells.

Inhibition of Fusion of Lysosome with Phagocytic Vacuole

Clearly, if the phagocytosed microorganism is not exposed to intracellular killing and digestive processes, it has the opportunity to survive and multiply within the host cell. Mycobacteria can enter macrophages via C3b receptors without inducing the respiratory burst and *Mycobacterium leprae* has phenolic glycolipid-1 on its surface, which scavenges ROI, thereby protecting the pathogen. Pathogens such as *M. tuberculosis*, *Chlamydia* and *Legionella* spp. produce cell wall components which are released from the phagosome and modify lysosomal membranes to inhibit fusion (*M. tuberculosis* produces sulfatides). Virulent *Salmonella typhimurium* also inhibits fusion and divides within unfused vacuoles. This is in contrast to the events after uptake of nonvirulent *M. tuberculosis*, when lysosomal fusion is general, phagocytic vacuoles receive lysosomal contents, and bacilli are killed.

The intracellular protozoan parasite *T. gondii* is phagocytosed by macrophages, inducing its own engulfment by actively inserting a specialised 35 nm diameter cylinder into the macrophage.[4] But in a large proportion of the vacuoles there is no lysosomal fusion, and the toxoplasmas multiply, eventually killing the cell. Mitochondria and lengths of endoplasmic reticulum surround these vacuoles, presumably in response to chemical stimuli arising from the toxoplasmas, and perhaps playing a part in nourishment of the parasite. The adenylate cyclase toxin of *B. pertussis* which increases intracellular cAMP inhibits phagosome–lysosome fusion and leads to an increase in growth in macrophages; toxin-defective bacteria show a 100-fold fall in growth, compared to the parent strain. Inhibition of fusion is an active process and does not generally occur when microorganisms are killed or coated with antibody beforehand.

Escape from the Phagosome

After capture in a phagosome, a microorganism can still evade antimicrobial forces by escaping at an early stage from the phagosome and entering the cytoplasm. There are now good examples of this phenomenon. We have already met with *Shigella* which can escape from vacuoles and spread to adjacent cells. When *Listeria monocytogenes* infects mouse macrophages only a proportion of incoming bacteria escape into the cytoplasm. The bacteria are taken into phagosomes which are acidified, conditions necessary for the activity of listeriolysin, a vital virulence determinant mediating escape from the vacuole. Within the

[4]*Toxoplasma gondii* can also invade a large variety of nonphagocytic cells. Little is known about attachment mechanisms or receptors, but the parasite secretes substances that help penetration, and the process is an active one, help being given by the host cell.

first hour following phagocytosis, most bacteria are killed in the phagosomal compartment due to the transfer of lysosomal enzymes to about two-thirds of these vacuoles. By electron microscopy, only 14% of the total number of organisms are found in the cytoplasm which includes those that had just escaped and those that had already started to multiply. Multiplication is rapid thereafter with a doubling time of 40 min, with clear evidence of actin-mediated spread to adjacent cells. Two phospholipases C (PLC-A and PLC-B) are also involved in this process. Phospholipase A seems to render the phagolysosomal membrane susceptible to the damaging effects of listeriolysin O, thereby allowing the organisms to escape. PLC-B is required to accomplish escape from the double membrane in order to infect adjacent cells, in a manner similar to that described for *Shigella* (see Chapter 2). For viruses, escape involves fusion of the virus envelope with the phagosome membrane so that the nucleocapsid core is set free in the cytoplasm. There is evidence that the phenomenon also occurs with *M. leprae*, *Rickettsia mooseri*, and the trypomastigote form of *Trypanosoma cruzi*. These can be seen free, often multiplying, in the cytoplasm of macrophages. Escape is generally prevented when the microorganism is coated with antibody.

Resistance to Killing and Digestion in the Phagolysosome

Many successfully infectious microorganisms resist killing and digestion in the phagocytic vacuole. For those whose multiplication is for the most part extracellular, this ability to survive rather than suffer death and dissolution in the phagocyte may possibly add to their success in the infected host. Other microorganisms, however, are specialists in intracellular growth and some of them grow in phagocytes. Certain viruses depend for their success on infecting the phagocyte after avoiding killing and digestion in the phagolysosome; macrophages rather than neutrophils are important. In the case of reoviruses, exposure to lysosomal enzymes actually initiates the 'uncoating' of the virus particle in the cell and thus helps virus multiplication. The cells susceptible to reoviruses, however, are not necessarily specialised phagocytes. Many other viruses have specialised mechanisms for entering susceptible nonphagocytic cells; their fate in phagocytic cells is not necessarily important. Polio- and rhinoviruses, for instance, are taken up, killed and digested in phagocytic cells, but they nevertheless successfully infect target cells in the upper respiratory tract and alimentary canal and are shed profusely from these sites.

Bacteria, as a result of phagocytosis, enter phagocytic cells more commonly than any other type of host cell, and intracellular bacteria cannot establish a successful infection unless they resist killing and then grow in the phagocyte. Thus, macrophages are important sites of bacterial growth in infections with *Mycobacteria*, *Brucella*, *Listeria*, *Trypanosoma*, *Nocardia* and *Yersinia pestis*.[5] In some instances the microorganism escapes from the

[5]*Yersinia pestis* is the causative agent of the plague (from the Latin *plaga*, a blow), an often lethal infection transmitted to man by fleas from infected rats or other rodents, which can also spread from man to man by the respiratory route. In the fourteenth-century epidemics of the Black Death, it is estimated to have killed a third of the people of Europe. The bacteria are able to grow in the phagolysosome of macrophages when Ca^{2+} concentrations reach low levels ($\leq 100\ \mu M$) and produce several potent toxins.

phagosome or inhibits lysosomal fusion, but *M. lepraemurium*, *Listeria monocytogenes*, *Y. pestis* and virulent strains of *S. typhimurium* can grow in the phagosome in spite of lysosomal fusion. Neutrophils are less important sites of microbial growth, partly because of their short life span, but their powerful lysosomal enzymes take a heavy toll of ingested bacteria that show no particular resistance to killing and digestion.

Once a microorganism has been phagocytosed, the most important thing is whether or not it is killed in the phagocyte. When nonvirulent *E. coli* is phagocytosed by neutrophils it is soon killed, but bacterial macromolecular machinery proceeds for a while after death of the bacterium. Most microorganisms are killed after phagocytosis, but the bacteria or protozoa that infect phagocytes must allow themselves to be taken up by these cells, and their success hinges in the first place on their resistance to killing (Table 4.1).

Many pathogenic bacteria show a degree of resistance to killing and sometimes also to digestion in the phagolysosome, as indicated in Table 4.1. Catalase, by destroying H_2O_2 might protect bacteria from killing, and catalase-rich strains of staphylococci and *L. monocytogenes* show better survival inside neutrophils. SOD produced by Streptococci, on the other hand, generates H_2O_2, which can have inhibitory effects on staphylococci which are competing for the same niche, e.g. nasopharynx.

In the case of *Salmonella*, resistance to intracellular killing is partly mediated by the production of factors such as SOD, which inhibits reactive oxygen and nitrogen species. In addition, metal ion transporters appear to be important for survival. Salmonella has the ability to sense the hostile environment of the phagosome (probably low pH) by the two-component regulator system phoP/phoQ which is activated within acidified phagosomes and which controls the expression of a wide array of different virulence factors. Factors encoded by salmonella pathogenicity islands play an important role in survival. In particular, SPI-2-encoded type 3 secretion system (T3SS) inhibits phagosome maturation leading to formation of a salmonella-containing vacuole (SCV) and interferes with the endocytic pathway which in turn prevents transport of hydrolytic enzymes to the lysosome. Furthermore, a T3SS-dependent actin coating of intracellular Salmonella may prevent lysosomal fusion.

GROWTH IN THE PHAGOCYTIC CELL

The ways in which microorganisms avoid being phagocytosed and killed have been discussed above. An equally satisfactory victory over the phagocyte is achieved when the microorganism uses it as a site of growth. The microorganism now allows itself to be phagocytosed, but resists killing and digestion, and then multiplies, deriving nourishment from the phagocytic cell. As was pointed out in the preceding section, neutrophils have such a brief life span that they are rarely important sites for microbial growth. Virulent bacteria tend to remain viable if they are phagocytosed by neutrophils, but intracellular growth is generally slight compared with the growth of bacteria in extracellular fluids. Macrophages, by comparison, live for long periods. Many microorganisms have, as it were, come to accept eventual phagocytosis by macrophages as inevitable, and are able to multiply inside the cell (Table 4.2). They have learnt how to induce the macrophage to protect and feed them, rather than destroy and digest them. Sometimes mitochondria and

TABLE 4.2 Examples of Microorganisms that Regularly Multiply in Macrophages

Viruses	Herpes-type virus
	Hepatitis viruses of mice
	HIV
	Measles, distemper
	Poxviruses
	LCM
	Lactate dehydrogenase-elevating virus of mice
	Aleutian disease of mink (see Glossary)
Bacteria	*Rickettsia rickettsi*
	Rickettsia prowazeki
	Mycobacterium tuberculosis
	Mycobacterium leprae
	Listeria monocytogenes
	Brucella spp.
	Legionella pneumophila
Fungi	*Cryptococcus neoformans*
Protozoa	Leishmanias
	Trypanosomes
	Toxoplasmas

ribosomes are recruited to the edge of the phagosome, where they perhaps play a part in bacterial nutrition and growth. This ability to grow in macrophages is often a key property of successful invasive microorganisms (see Chapter 5).

Bacteria, fungi and protozoa often multiply inside phagocytic vacuoles. Nourishment of the parasite takes place across the membrane of the vacuole, and host materials must be made available to the parasite. Certain coccidias, for instance, induce the host cell to extrude material into the vacuole and then take it up by endocytosis (see Glossary). Macrophages parasitised by *T. gondii* appear to be giving biochemical support to the invader in a most hospitable fashion. Microvilli from the host cell extend into the vacuole which is surrounded by strips of endoplasmic reticulum and mitochondria.

Certain viruses grow in macrophages and in a few instances, such as the highly successful lactate dehydrogenase-elevating virus of mice, the macrophage is the only cell in the body that is infected. Viruses do not generally infect by phagocytosis as this leads to their destruction by lysosomal enzymes, but by endocytosis (non-enveloped and most enveloped viruses), or by fusion with the plasma membrane (some enveloped viruses) as with other types of cell (see below).

KILLING THE PHAGOCYTE

The most straightforward antiphagocytic approach is to kill the phagocyte, and many successful infectious bacteria do this. Some, as they multiply in tissues, release soluble materials that are lethal for phagocytes. Part of the success of pathogenic streptococci and staphylococci is attributable to their ability to kill the phagocytes that pour into foci of infection. Pathogenic streptococci release haemolysins (streptolysins) which lyse red blood cells and are much more active weight for weight than haemolysins such as bile salts or saponin, but which also have a more important toxic action on neutrophils and macrophages. Within 1–2 min of its addition to neutrophils, streptolysin O causes the neutrophil granules to explode and their contents are discharged into the cell cytoplasm. The lysosomal enzymes, when confined to a phagocytic vacuole, help the cell by performing valuable digestive functions, but when enough are released into the cell cytoplasm in this way, they act on cell components and within a minute or two the cytoplasm liquefies and the cell dies. The streptolysin, by damaging the lysosomes, makes them function as 'suicide bags'. Streptolysin S has an even more potent action on membranes. Various haemolysins (α, β) are released also by pathogenic staphylococci, which can impact on phagocyte function, in addition to a family of bi-component leukocidins which have been well studied in recent years. For example, the Panton-Valentine leukocidin made by some *S. aureus* strains, including the USA300 epidemic clone, binds to the CsaR and C5L2 complement receptors on neutrophils and has cytotoxic activity which leads to the release of inflammatory mediators which likely contribute to the severe symptoms associated with necrotising pneumonia. *L. monocytogenes* secretes listeriolysin which acts like streptolysin. The lethal factor (LF) of the tripartite anthrax toxin is a zinc metalloprotease which is potently cytotoxic for macrophages.

Y. enterocolitica has long been regarded as a paradigm for studying intracellular pathogens. However, it is now abundantly clear that it is essentially an extracellular pathogen with an ability to survive in lymphoid tissue resisting phagocytosis by killing phagocytes. The high pathogenicity island of *Yersinia* encodes the Yop virulon, a 70 kb plasmid encoding more than 50 genes, comprising an amazingly sophisticated system for resisting the immune system of the host. *Yersinia* spp. is one of many Gram-negative pathogens that can synthesise a Type III secretion apparatus spanning both inner and outer membranes which have a pool of effector molecules ready for secretion through the specialised apparatus. Upon contact with eukaryotic cell membranes, a sensor interacts with a receptor on the bacterial cell, causing removal of the stop valve and addition of further components to the secretion apparatus which allows pore-forming fusion with the target cell membrane, thus creating an 'injectosome'. Through this injectosome, preformed effector molecules are introduced into the cell which inhibit phagocytosis and cytokine release and kill the cell.

In general, neutrophils are more readily killed by toxins than are macrophages, possibly because their lysosomes are more easily discharged. Invaders with good lysosomal weaponry of their own, such as virulent strains of the protozoan parasite *E. histolytica*, can kill neutrophils by mere contact (see Chapter 8). Others exert their toxic action on the phagocyte after phagocytosis has taken place, releasing cytotoxic substances which pass directly through the vacuole membrane and into the cell. The phagocyte can be said to have died of food poisoning. For instance, virulent *Shigellae* kill mouse macrophages after phagocytosis, whereas avirulent *Shigellae* fail to do so, and are themselves killed and digested.

Certain *Chlamydia* multiply in macrophages after phagocytosis and destroy the cell by inducing the discharge of lysosomal contents into the cytoplasm. Virulent intracellular bacteria of the *Mycobacterium*, *Brucella* and *Listeria* groups owe much of their virulence to their ability to multiply in macrophages, although the macrophage is often destroyed in the end by mechanisms, which with one or two exceptions are not known. Finally, Salmonella have the capacity for another major pathogenicity island-encoded pathogenic mechanism, SPI-1 induced apoptosis (programmed cell death) of macrophages which results in the release of bacteria after proliferation and organ dissemination.

We conclude this section by describing the interaction of *Legionella pneumophila* with phagocytes as it brings together many of the aspects dealt with separately in the preceding sections. *L. pneumophila* is essentially a bacterial parasite with an ability to cause a respiratory infection (Legionnaire's disease) in humans. *L. pneumophila* infects type I and II alveolar epithelial cells and macrophages. It cannot grow in cell-free lung lavage from normal or infected guinea pigs, is killed by neutrophils, but successfully infects and grows in macrophages.

In the environment, *Legionella* spp. are ubiquitous and parasites of protozoa. Bacterial transmission to humans occurs through droplets generated from environmental sources such as cooling towers and shower heads, but at present we do not know what constitutes the 'infectious particle'. Initial attachment to both protozoa and mammalian cells is mediated by pili and (for mammalian cells) adsorbed C3b. Once internalised, the bacteria form a Legionella-containing vacuole (LCV) which assists in avoiding recognition by the cellular immune system. Of note, Legionella can maintain a neutral pH in the phagosome and avoid acidification. It is well established that the dot/Icm type 4 secretion system (T4SS) is essential for Legionella survival within LCV in macrophages. This specialised transfer system enables the effective translocation of over 270 protein effectors which may involve considerable functional redundancy. Many of the effectors manipulate the host vesicular transport system. A number of them target the endocytic pathway and can interfere with lysosomal protein trafficking, and inhibit lysosomal fusion. In addition, some effectors can subvert host-innate immune defence pathways, including NF-κB production and apoptotic signalling.

ENTRY INTO THE HOST CELL OTHER THAN BY PHAGOCYTOSIS

Although the usual way in which a particle enters a cell is by phagocytosis, so that the particle is enclosed in a phagocytic vacuole, there are other methods of entry. Electron microscope studies indicate that some bacteria, for instance, adsorb to the cell surface and enter the cytoplasm directly after inducing a local breakdown in the plasma membrane. The plasma membrane is reformed immediately. Shigellas and pathogenic salmonellas appear to enter intestinal epithelial cells in this way, and other bacteria show the same behaviour in tissue culture cells. It may be a less frequent occurrence in specialised phagocytic cells. Protozoa have a complex structure and can utilise their own lysosomal enzymes to penetrate host cells. Trypanosomes, *Eimeria*,[6] *T. gondii* and *E. histolytica* enter susceptible cells by active

[6]Various species of *Eimeria* (a protozoan parasite) cause contagious enteritis, or coccidiosis, in all domestic animals. Ingested oocysts invade intestinal epithelial cells and the entire life cycle with schizonts, merozoites and gametocytes takes place in these cells.

penetration, and the active end of the parasite has vesicles containing lysosomal enzymes that aid the penetration process. When a malaria parasite penetrates a red blood cell, a specialised projection (conoid end) on the malarial merozoite makes contact with the red cell surface. The parasite then injects a lipid-rich material from special glands (rhopteries), and it seems that this material is inserted into the red cell membrane, whose area is thus increased. As the merozoite actively enters the red cell, the membrane stays intact, but there is now enough of it to form an invagination and accommodate the advancing parasite.

If viruses enter cells by phagocytosis, they are destroyed by hydrolytic enzymes. Most non-enveloped and enveloped viruses enter cells by endocytosis, but a few types of enveloped virus enter instead by fusion at the plasma membrane at the cell surface. It commences with the virus attachment proteins binding to a critical number of cell receptor molecules. This triggers endocytosis – the invagination of the plasma membrane into small virus-sized depressions coated on the cytoplasmic side with a cellular protein (clathrin), giving them the name of 'coated pits'. These then detach from the plasma membrane and become vesicles free in the cytoplasm. At this stage, viruses still have to release their genome and deliver it across the vesicle-membrane into the cytoplasm. Non-enveloped viruses achieve this after destabilisation and permeabilisation of the virus particle, which results from interaction with cell receptors alone, or in conjunction with the reduction of the internal pH of the vesicle (to about 5.5–6) by the importation of protons by a cellular pump. The genomes of endocytosed enveloped viruses are released into the cytoplasm after fusion of the virion and vesicle membranes. It involves the destabilisation of the lipids of both membranes so that they can fuse together to form a single continuous membrane. All viral envelope proteins have a buried hydrophobic region which at this time enters and disrupts the structure of the membrane as a prelude to fusion. Exposure of this 'fusion peptide' is triggered by the low pH conditions referred to above. Fusion at the plasma membrane by viruses such as HIV and paramyxoviruses (like measles and Sendai viruses) takes place in exactly the same way except that it occurs at neutral pH.

Most of the discussion has dealt with the ways in which microorganisms can avoid intracellular digestion. There are one or two instances where exposure to lysosomal enzymes is actually necessary for the multiplication of microorganisms. Spores of *Clostridium botulinum* are said to germinate in cells only after the stimulus of exposure to lysosomal contents.

CONSEQUENCES OF DEFECTS IN THE PHAGOCYTIC CELL

The importance of the phagocytic cell in defence against microorganisms is illustrated from observations on diseases where there are shortages or defects of phagocytic cells. A serious shortage of neutrophils, with $<1000\,\mu m^{-3}$ in the blood (normal 2000–5000 mm^{-3}), is seen in acute leukaemia or after X-irradiation, and predisposes to infection with Gram-negative and pyogenic Gram-positive bacteria. There are also one or two inherited shortages. Blood neutrophil counts are about one-tenth of normal in a significant proportion of Yemenite Jews (benign neutropenia), although surprisingly they seem little the worse for it except for a susceptibility to periodontal disease. But certain naturally occurring defects in the function of phagocytes have more serious consequences, and studies of

these defects have thrown much light on normal phagocyte function. Unfortunately, the defects are often multiple so that interpretation is not easy.

Children with chronic granulomatous disease, usually an X-linked recessive trait, have neutrophils that look normal and show normal chemotaxis and phagocytosis, but there is defective intracellular killing of bacteria. The gene that is abnormal has been cloned, and it appears to code for an essential component in the phagocyte's NADPH oxidase system. There is no respiratory burst, and the superoxide radical and H_2O_2 are therefore not generated (Figure 4.3), and associated with this there is increased susceptibility, especially to staphylococcal and Gram-negative bacterial infections. In spite of undiminished immune responses to infection, patients suffer recurrent suppurative infections with bacteria of low-grade virulence such as *E. coli*, *Klebsiella* spp., staphylococci and micrococci, and usually die during childhood. Neutrophils are present in foci of infection but cannot kill the microorganisms and are eventually taken up by macrophages, leading to the formation of a chronic inflammatory lesion called a granuloma (see Chapter 8). Interestingly, the patients have normal resistance to streptococcal infections because streptococci are catalase negative and can themselves generate H_2O_2 without destroying it. H_2O_2, with the help of the cell's myeloperoxidase, then generates hypochlorite and singlet oxygen. As might be expected, patients with severe glucose 6-phosphate dehydrogenase (G6-PD) deficiency also suffer from infection with catalase-positive organisms, because they too fail to generate superoxide and H_2O_2. Patients with myeloperoxidase deficiency show delayed killing of bacteria in neutrophils but normal resistance to bacterial infections, and surprisingly an increased susceptibility to *C. albicans*.

Another example of a neutrophil defect is seen in Chediak-Higashi disease, which occurs in mice, mink, cattle, killer whales and man, and here too there is increased susceptibility to certain infections. Neutrophils contain anomalous giant granules (lysosomes) and the basic defect is probably in microfilaments. Phagocytosis and even lysosomal enzyme content appear normal, but there is defective chemotaxis, defective lysosomal fusion and delayed bacterial killing.

As well as shortages or defects in the quality of phagocytic cells, there may also be defects in the delivery system by which phagocytes are focused and assembled in the infectious foci where they are needed. Neutrophils show defective chemotaxis (as well as phagocytosis) in a variety of rare conditions such as the lazy leucocyte syndrome, due to a disorder of the cell membrane, and the actin dysfunction syndrome where actin is not polymerised as normal to form microfilaments, so that the 'muscle' system of the cell is defective. Impaired digestion and disposal of microbial antigens is likely to be associated with immunopathology, but little is known of this aspect of phagocyte function.

Abnormalities in phagocyte function are not uncommon in certain acute infections such as Gram-negative bacteraemia and also in otherwise normal patients with recurrent staphylococcal infections. Presumably the abnormality could cause the infections, but at times it reflects the antimicrobial activities of the infectious agent.

Some of the clinical conditions are complex and of varied origin, often with multiple defects. In chronic mucocutaneous candidiasis, for instance, some patients have impaired cell-mediated immunity and others have defective macrophage function. They suffer from persistent and at times severe infection of mucous membranes, nails and skin with the normally harmless yeast-like fungus *C. albicans*.

SUMMARY

In summary, the encounter between the microorganism and the phagocytic cell is a central feature of infection and pathogenicity. Phagocytes are designed to ingest, kill and digest invaders, and the course of the infection depends on the success with which this is carried out.

Virulent microorganisms have developed a great variety of devices for countering or avoiding the antimicrobial action of phagocytes. Although substances produced by or present on microbes may at first sight appear to have a useful function, not all will prove to be of practical importance in the infected host. Microbial killing and digestion in phagocytes is still only partly understood, but it is important to conceive logically of the ways in which microorganisms can avoid being ingested, killed and digested. Most viruses do not infect phagocytes, and the exceptions do so by endocytosis or fusion, not phagocytosis.

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CHAPTER

6

The Immune Response to Infection

OUTLINE

Vertebrates have two major defences against pathogens: the innate and the adaptive immune responses. Innate immunity is composed of a number of humoral factors circulating in blood such as cytokines, chemokines and complement, and a variety of leucocytes including macrophages, neutrophils and natural killer (NK) cells. The advantage of the innate defence against pathogens is the speed and diversity of the response. The components of innate immunity are already present in the circulation poised to respond rapidly to pathogens. In contrast, the adaptive immune response takes several days before it is fully operational. The adaptive response is mediated by lymphocytes and can be distinguished from the innate response by specificity for antigen and memory of an antigen encounter. Communication between different molecules and cells is of paramount importance for the detection and control of the relevant pathogen. In this context, the macrophage and neutrophil are at the vanguard of the innate response. They respond to tissue damage directed by the inflammatory response and follow an orderly exit from the bloodstream to enter the site of infection, guided in part by chemokines. The neutrophil is the first leucocyte on the scene; it then recruits macrophages, and serum proteins also enter due to the leakiness of the blood vessel wall. The leucocytes set about marshalling the defenses and controlling the infection.

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DOI: http://dx.doi.org/10.1016/B978-0-12-397188-3.00006-8

Detecting the presence of a pathogen involves interactions between structures on the pathogen, referred to as pathogen-associated molecular patterns (PAMPs), and their receptors, namely pattern recognition receptors (PRRs). PAMPs can be carbohydrates, lipopolysaccharide or lipoprotein found on bacteria and fungi. Virus PAMPs can be dsRNA, non-methylated CpG DNA or in the case of something like paramyxoviruses, the envelope glycoprotein. PRRs are found in serum, on the plasma membrane and in the cytoplasm (or cytoplasmic vesicles) of phagocytes. Examples include carbohydrate receptors, scavenger receptors and Toll-like receptors (TLRs). There are several examples of the latter, including TLR4 that binds to LPS, TLR5 that binds to flagellin and TLR3 that interacts with dsRNA of viruses. Recognition of a PAMP results in signal transduction through an adaptor molecule (e.g. MyD88) and the initiation of an immune response commencing with the recruitment and activation of polymorphs and macrophages that participate in phagocytosis (see Chapter 4) and NK cells that cope with virus-infected cells. The blood vessels associated with the lesion become leaky, enabling serum proteins such as complement, acute phase proteins and defencins to enter the tissue and contribute to controlling the infection and supporting tissue repair. TLRs therefore occupy a key position in the link between the innate and adaptive responses.

The two arms of the adaptive immune response are mediated by different types of immunologically reactive lymphocytes, which were originally named according to their origin into B (bursa in birds or bone marrow and foetal liver in mammals) and T (thymus) dependent cells. These two types of cells can be distinguished through the expression of specific cell surface molecules. B cells are concerned with the antibody response and T cells with initiating the cell-mediated immune (CMI) response. Antigen recognition is by B-cell and T-cell receptors. These are structurally similar yet recognise different forms of antigen. Both receptors are composed of heavy and light chains (Figure 6.1) and each chain is further divided into variable (V) and constant (C) regions. It is the variable region that binds to antigen. Each lymphocyte contains a unique antigen receptor clonally derived following somatic recombination and somatic mutation and pairing of VH and VL chains. In mammals, this diversity is enormous and estimated to be of the order of 10^9 lymphocytes which corresponds to 10^9 antigenic determinants, and more than matches the diversity of antigens presented by microorganisms.

The receptor for antigen on B cells is the B-cell receptor composed of immunoglobulin M (IgM) and co-receptors Igα and Igβ. IgM can exist in two forms: a monomeric membrane bound receptor and a secreted pentameric form. When antigen enters the host for the first time there will be a few B cells that react with it specifically. Following this encounter these B cells become activated and clonally expand in germinal centres. Some B cells differentiate to form plasma cells (the main antibody synthesising cells) and others form memory B cells. Memory is one of the main features of the adaptive immune response and the basis of vaccination (see Chapter 12). During B-cell activation, the IgM producing B cells can undergo heavy chain class switching to produce IgA, IgG or IgE. The antigen specificity of the original IgM molecule may undergo further change resulting in selection of high-affinity antibody molecules, a process termed affinity maturation.

There are two types of T-cell receptor α/β and γ/δ. The dominant type is α/β which is associated with the two main T-cell populations: $CD4^+$ helper T cells and $CD8^+$ cytotoxic

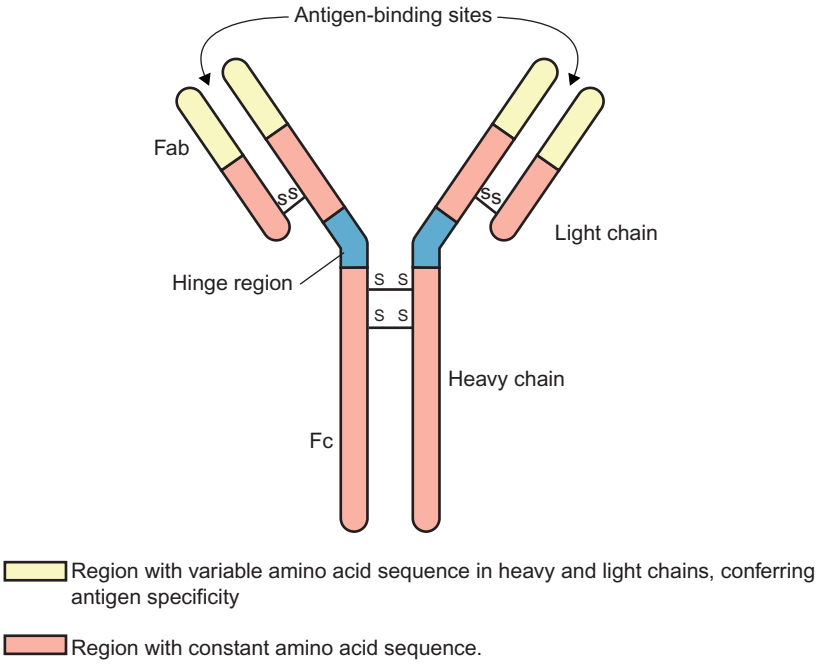

FIGURE 6.1 Basic Y-shaped (four-chain) structure of immunoglobulin G molecule.

T cells. Whereas the B-cell receptor binds to continuous and discontinuous epitopes as part of the three-dimensional structure of antigen, the T-cell receptor can only recognise short linear peptides in association with molecules from the major histocompatability complex (MHC). This remarkable adaptation is the vertebrate answer to identifying intracellular pathogens.

There are two forms of MHC relevant to antigen recognition. MHC class I is associated with endogenously derived peptide present in cytoplasm following the digestion of newly synthesised proteins (e.g. viral) by proteolytic enzyme complexes called proteasomes. The peptides (around 9 amino acids in length) are transported to the endoplasmic reticulum (ER) via a peptide transporter called TAP. In the ER, the peptide binds to a grove in the MHC class I heavy chain. This structure is then stabilised by β2 microglobulin (light chain) to create the tri-molecular complex. The complex is transported to the plasma membrane where it is recognised by the TCR on a $CD8^+$cytotoxic T lymphocyte (CTL). An important feature of this mechanism is that antigens can be produced early in the infectious cycle before a virus has had the chance to assemble new virions. The other important feature of MHC class I is that it is found on virtually all nucleated cells, making this a powerful system for dealing with intracellular pathogens.

A second family of polymorphic MHC molecules is MHC class II. These molecules are found only on professional antigen presenting cells (APC), namely dendritic cells, B cells

and macrophages. These APCs take up antigen exogenously and process it in a different way to the endogenous MHC class I pathway. As MHC class II molecules are formed in the ER or recycled from the plasma membrane, they acquire a protein called the invariant chain which blocks the premature loading of peptide. In view of the significance of the invariant chain to adaptive immunity, it is somewhat surprising that viruses have so far failed to disrupt expression. Peptide selection takes place in multivesicular bodies where the invariant chain is lost and replaced by peptide. This structure then traffics to the plasma membrane and is presented to $CD4^+$ T cells.

A critical link between the innate and adaptive immune responses are dendritic cells (DC). DC are the 'lead' APC responsible for the 'priming' of naive $CD4^+$ T cells which is essential for the induction of the adaptive immune response. Although B cells and macrophages present antigens to recognise antigen, this is only effective once the T-cell response has been initiated by DC. There are numerous populations of DC strategically placed in lymphoid tissue to encounter microbes or their antigens, and at the same time they are in close proximity to lymphoid cells.

Following activation by TLRs, DCs settle down in lymph nodes and the spleen, putting out dendritic processes and thereby increasing the surface area available for contacting a T cell. When a TCR engages a peptide presented by MHC class II, a number of additional receptor ligand interactions take place. This includes the co-stimulatory molecules CD28-, CD80, CD4, and LFA-1 and ICAM-1. These molecules form an immunological synapse, an activating structure that initiates T-cell proliferation. The net effect is the activation and proliferation of T cells. When infection occurs for the first time there will be a few T cells (estimated to be 1 in 100,000 CTLs) that recognise viral antigen and respond. Within the next four days, this number rises to 1 in 50 CTLs reactive to viral antigen. This equates to a doubling time of $\sim$2 h, making the lymphocyte the most rapidly dividing vertebrate cell. Equally dramatic is that many CTL responses are dominated by only one or two clones. For example, MCMV infection of C57Bl mice results in 95% of the CTL response and is dominated by a single CTL clone, reactive against nine amino acids of a viral transcription factor. This is a characteristic feature of MHC class I restricted T cells. The situation is different for MHC class II restricted T-helper cells where a number of antigen-specific T-cell clones can be detected with varying frequencies. Once initiated, the subsequent rounds of cell division are driven by IL-2, a T-cell growth factor. By the second week, the effector cell frequency starts to decline as the result of apoptosis and the establishment of memory T-cells. The induction, expansion and function of T cells are summarised in Figure 6.2.

$CD4^+$ T cells are a heterogeneous group of lymphocytes performing many functions in the immune response. They are distinguished by the cytokines they produce and the expression of particular transcription factors, e.g. FoxP3, a transcription factor strongly associated with T-reg cells, and GATA3, which is produced by IL-4 and IL-9 secreting T cells, primarily associated with immunity to helminth parasites (Figure 6.3).

T-helper cells can be further subdivided into subpopulations distinguished from each other by the type of cytokines produced and particular transcription factors. The two main subpopulations associated with infection are Th1 and Th2. Th1 cells are characterised by the expression of interleukin-2 (IL-2) and interferon-γ (IFN-γ). Their main function is to stimulate proliferation of all T-cell populations (both $CD4^+$ and $CD8^+$ T cells) via IL-2, as

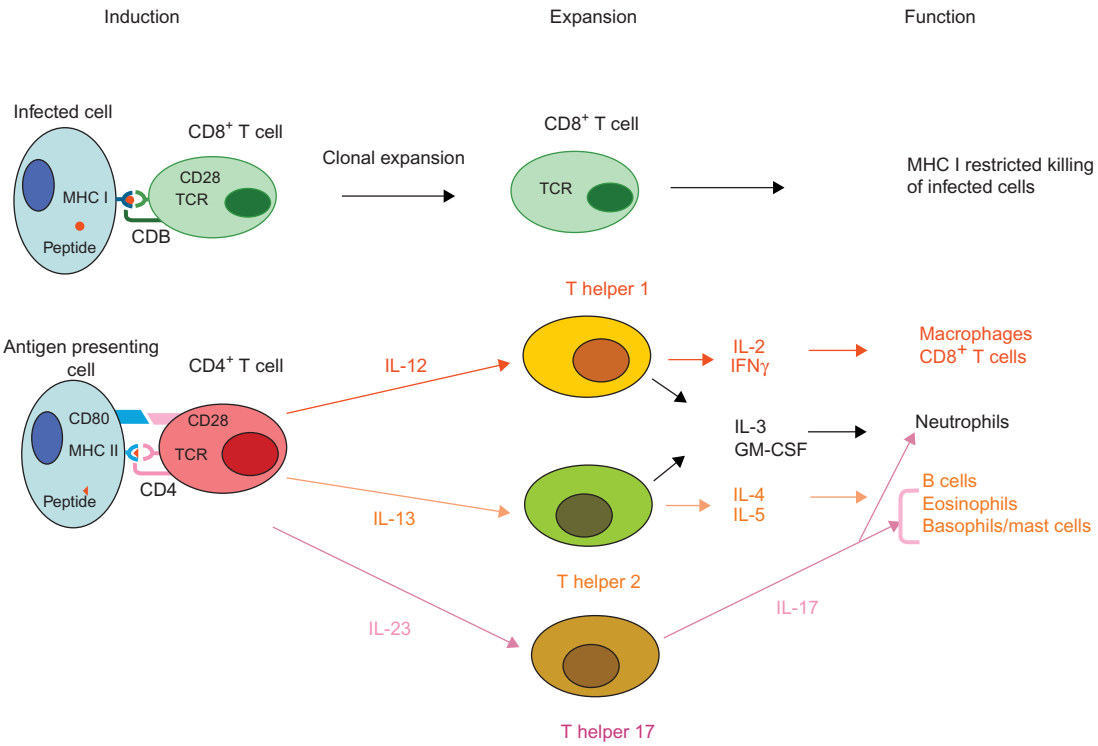

FIGURE 6.2 Induction and expression of the immune response. Virus-infected cells present endogenous antigen via MHC class I, resulting in the activation of $CD8^+$ T cells. Antigen presenting cells present exogenous antigen via MHC class II, resulting in the activation of $CD4^+$ T cells. Key accessory molecules involved in T-helper cell activation include CD80 and CD28. Once activated, $CD8^+$ T cells undergo proliferation and clonal expansion. Depending on the cytokines present, Th cells differentiate into Th 1, Th 2 or Th 17 cells, each of which drives a different immune response.

well as to activate tissue macrophages via IFN-γ. IFN-γ is the principal effector mechanism in the defence against intracellular bacteria (e.g. Mycobacteria, Brucella, Rickettsia) and intracellular parasites (e.g. Leishmania, Eimeria, Babesia). It does this by activating macrophages and stimulating them to produce the enzymes that trigger the major intracellular killing mechanisms. In contrast, Th2 cells produce IL-4, IL-5 and IL-10. Th2 cells influence B-cell activation, proliferation and immunoglobulin production. IL-4 stimulates B-cell growth and heavy chain switch from IgM to IgG, IgE and IgA and stimulates high affinity antibody synthesis. These cytokines also induce proliferation of basophils/mast cells by IL-4 and the proliferation and differentiation of eosinophils by IL-5. These T-cell subpopulations can inhibit as well as stimulate; Th1-produced IFN-γ can act to suppress Th2 cells and Th2-produced IL-4 can inhibit some Th1 responses.

Both Th1 and Th2 cells produce IL-3 and granulocytes-macrophage colony stimulating factor (GM-CSF). The main effect of these is to activate and induce proliferation of

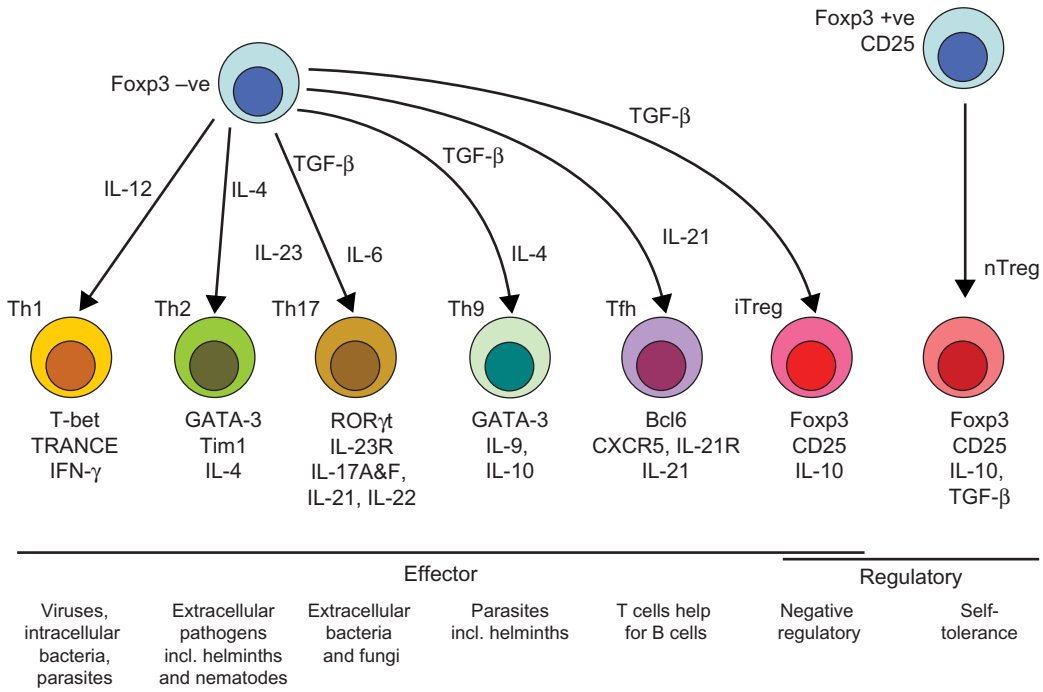

FIGURE 6.3 Heterogeneity of CD4 T cell and T regulatory cells in mice and humans. The figure highlights the role of cytokines and transcription factors in defining the subsets. *Courtesy of Prof. Peter Kaiser, the Roslin Institute, University of Edinburgh.*

neutrophils and macrophages. Neutrophils are the major phagocytic cells in the blood and are the principal cells associated with acute inflammation. They function chiefly in the defence against extracellular bacteria (see Chapter 4). Depending on the nature of the antigen and the route of infection or immunisation, one particular Th subset will predominate. For example, microbial infection of skin will favour Thl cells, where delayed-type hypersensitivity (DTH) responses are important, whereas infections involving parasitic worms will favour Th2 cells, where IgE antibody is an important effector mechanism. T-cell cytokines are critical molecules in a number of immunological reactions. A summary of the cytokines and their actions is given in Table 6.1.

$CD4^+$ cells are important in driving proliferation and effector function of $CD8^+$ cytotoxic T cells (CTL) in the primary immune response. In particular, they are very important in establishing CTL memory. Macrophages and Th1 cells also participate in DTH responses which can be visualised in the skin as a swelling at the site of infection.

When an immune response is initiated, powerful forces are set in motion, which can be advantageous, but at times disastrous for the individual (see Chapter 8). So that each response can unfold in a more or less orderly fashion, it is controlled by a combination of stimulatory and inhibitory influences. The latter includes antigen control and the activity of regulatory T cells (characterised by the expression of FoxP3, a transcription factor)

producing immunosuppressive cytokines (IL-10 and TGFβ). Antigen itself acts as an important regulatory agent. Following its combination with antibody and uptake by phagocytic cells, it is catabolised and begins to disappear from the body. Since it is the driving force for an immune response, this response dies away as antigen disappears.

TABLE 6.1 Cytokines Involved in Responses to Microbial Infection

Cytokine	Source	Action
IL-1	Macrophage, monocytes, B cells	Co-stimulator of T cells. Activates macrophages.
IL-2	Th1 cells	Induces proliferation of T cells and activates NK cells. Antibody production
IL-3	Activated Th cells, Mast cells, NK cells, eosinophils, endothelium	Growth and differentiation of precursor cells in bone marrow
IL-4	Th2 cells. Mast cells; macrophage	B-cell proliferation and differentiation
IL-5	Th2 cells. Mast cells; eosinophils	Induces differentiation of B cells and activates eosinophils
IL-6	Macrophage, Th2 cells, B cells, astrocytes, endothelium	B- and T-cell growth and differentiation
IL-7	Bone marrow and thymus stromal cells	Differentiation and proliferation of lymphoid progenitor cells, B, T, and NK cell survival, development, and homoeostasis
IL-8	Macrophage, lymphocytes, epithelial cells, endothelial cells	Chemotaxis of neutrophils
IL-9	Th2 cells	Stimulates mast cells
IL-10	Monocytes; Th2 cells; $CD8^+$ T cells, mast cells, macrophages, B cells	Activates B cells; inhibits Th1 cytokine production
IL-12	B cells; T cells; macrophages; dendritic cells	Activates NK cells and directs $CD4^+$ T cells to Th1 responses
IL-13	Activated Th2 cells; mast cells; NK cells	Induces proliferation of B cells, inhibits Th1 cells and the production of macrophage inflammatory cytokines
IL-17	Th17 cells	Increases inflammatory cytokines
IL-18	Macrophage	Increase NK cell activity
IL-21	Th17 cells (other Th cells produce lower levels); NK cells	Activation and proliferation of $CD8^+$ T cells, NK cytotoxicity, B-cell proliferation (CD40 driven) isotype switching, differentiation of Th17 cells
IL-23	Macrophage, dendritic cells	Th17 cell development; Activates NK cells, Induces inflammation
IFN-γ	Lymphocytes	Activates most lymphoid cells
TNF-α	Macrophage, NK cells, $CD4^+$ T cells	Activation of macrophages. Induces inflammation and fever
TNF-β	Lymphocytes	Activation of macrophages. Induces inflammation and fever
TGF-β	Macrophage	Downregulates proinflammatory cytokines
GM-CSF	T cells: macrophage	Proliferation of granulocytes and macrophages

Immune responses can therefore be regulated by controlling the concentration and location of antigen. A small amount of specific antigen or cross-reactive antigens from other sources is thought to be important for the maintenance of certain types of immunological memory. As already discussed above, cytokines are powerful regulators of the immune response (Table 6.1). Whereas some of these factors activate the immune system, others can exert inhibitory effects. For example, transforming growth factor-β (TGF-β) is a potent inhibitor of T- and B-cell proliferation. Other cytokines such as IFN-γ inhibit IL-4 activation of B cells, whereas IL-4 and IL-10 inhibit IFN-γ activation of macrophages and hence DTH reactions. T cells producing these cytokines can therefore be thought of as regulator or suppressor cells. Excessive production of any one of these cytokines may lead to an inappropriate balance between antibody and CMI responses, or to a more generalised immunosuppression affecting the immune response to other microorganisms (see Chapter 7).

In a naturally occurring infection, the infecting dose generally consists of only a small number of microorganisms, whose content of antigen is extremely small compared with that used by immunologists, and quite insufficient on its own to provoke a detectable immune response. But the microorganism then multiplies, and this leads to a progressive and extensive increase in antigenic mass. Antibodies of various types and reactivities are produced in all microbial infections and are directed not only against antigens present in the microorganism itself but also against the soluble products of microbial growth, and in the case of viruses against the virus-coded enzymes and other proteins formed in the infected cell during replication. Of the antigens present in the microorganism itself, the most important ones in the encounter between microorganism and host are those on the surface, directly exposed to the immune responses of the host. Antibody responses to internal antigenic components are generally less important, although they are often of great help in detecting past infection, and may play a part in immune complex disease (see Chapter 8). Internal, or non-structural virus proteins, produced in an infected cell, are presented by MHC I and so can act as targets for cytotoxic T cells.

There are three other important adjuncts to the immune response. These are complement, phagocytic cells (macrophages and neutrophils) and NK cells, which are described under separate headings later. Each is involved in various types of immune reactions.

ANTIBODY RESPONSE

Types of Immunoglobulin

By the time they reach adult life, all animals, including man, have been exposed to a wide variety of infectious agents and have produced antibodies (immunoglobulins) to most of them. Serum immunoglobulin levels reflect this extensive and universal natural process of immunisation. The major circulating type of antibody is immunoglobulin G (IgG). It has the basic four-chain immunoglobulin structure in the shape of a Y, as illustrated in Figure 6.3, and a molecular weight of around 150,000. The molecule is composed of two heavy and two light polypeptide chains held together by disulphide bonds. For a given IgG molecule the two light chains are either kappa (κ) or lambda (λ), and both

heavy chains are gamma (γ). The antigen-binding ends of the light and heavy chains have a unique amino acid sequence for a given antibody molecule and are responsible for its specificity, while the remaining sequence of the chains is identical throughout a given class of antibody. The molecule can be split into three parts by papain digestion. Two of these (Fab) represent the arms of the Y and contain the antigen-binding sites; the third part (Fc) has no antigen-binding sites, but carries domains that activate complement and combine with receptors on the surface of neutrophils and macrophages. This last activity of the Fc fragment mediates attachment of antibody-coated microorganisms to the phagocyte, giving the antibody opsonic activity. The Fc fragment also contains the domains responsible for the transport of IgG across the placenta of some mammals. IgG can pass the placenta in primates, including man, but not in rodents, cows, sheep or pigs. Most IgG is in the blood, but it is also present in smaller concentrations in extravascular tissues including lymph, peritoneal, synovial and cerebrospinal fluids. Its concentration in tissue fluids is increased as soon as there is inflammation, or when it is being synthesised locally. There are four subclasses of IgG in man, which differ in heavy chains and in biological properties such as placental passage, complement fixation and binding to phagocytes. The amounts present in serum are also different, but almost nothing is known of their relative importance in infectious diseases.

Serum IgM is a polymer of five subunits, each with the basic four-chain structure but with a different heavy chain (μ), and has a molecular weight of around 900,000. Because it is such a large molecule, it is confined to the vascular system. Its biological importance is first that, molecule for molecule, it has five times the number of antigen-reactive sites as IgG. It therefore has high avidity and is particularly good at agglutinating microorganisms and their antigens. It also has five times the number of Fc sites and therefore at least five times the complement-activating capacity. A mere 30 molecules of IgM attached to *E. coli* can ensure its destruction by complement, whereas 20 times as many IgG molecules are required. Also, IgM is formed early in the immune response of the individual. An infectious disease can be regarded as a race between the replication and spread of the microorganisms on the one hand, and the generation of an antimicrobial immune response on the other. A particularly powerful type of antibody that is produced a day or two earlier than other antibodies may often have a determining effect on the course of the infection, favouring earlier recovery and less severe pathological changes. As each immune response unfolds, the initially formed IgM antibodies are replaced by IgG antibodies (via the process called class switching), and specific IgM is thus only detectable during infection and for a short while after recovery. The presence of IgM antibodies to a microbial antigen therefore indicates either recent infection or persistent infection. A pregnant woman with a recent rubella-like illness would have rubella IgM antibodies if that illness was indeed rubella. Measles virus occasionally persists in the brain of children instead of being eliminated from the body after infection, and the progressive growth of virus in the brain causes a fatal disease called subacute sclerosing panencephalitis. The onset of disease may be 5–10 years after the original measles infection, but IgM antibodies to measles are still present because of the continued infection.

IgM antibodies are not only the first to be formed in a given immune response, but were also the first to be formed in evolution. They are the only antibodies found in a primitive vertebrate such as the lamprey. IgM antibodies are also the first to be found during

the development of the individual. After the fifth to sixth month of development, the human foetus responds to infection by forming almost entirely IgM antibodies, and the presence of raised IgM antibodies in cord blood suggests intrauterine infection. The only maternal antibodies that can pass the placenta to reach the foetus are IgG (IgM is too large to cross the placenta), and thus the presence of IgM antibodies to rubella virus in a newborn baby's blood shows that the foetus was infected.

Secretory IgA is the principal immunoglobulin on mucosal surfaces and in milk (especially colostrum). It is a dimer, consisting of two subunits of the basic four-chain structure with heavy chains, and as the molecule passes across the mucosal epithelium, it acquires an additional 'secretory piece'. Secretory IgA has a molecular weight of around 385,000. It does not activate complement; although monomeric IgA–antigen complexes do activate the alternative complement pathway. It has to function in the alimentary canal, and the secretory piece gives it a greater resistance to proteolytic enzymes than other types of antibody. In the submucosal tissues, the IgA molecule lacks a secretory piece and enters the blood via lymphatics to give increased serum IgA levels in mucosal infections.

In the intestine, that seething cauldron of microbial activity, immune responses are of immense importance but poorly understood. On the one hand, commensal inhabitants are to be tolerated, but on the other hand, protection against pathogens is vital. Powerful immunological forces are present. The submucosa contains nearly 10^{11} antibody-producing cells, equivalent to half of the entire lymphoid system, and in man there are 20–30 IgA producing cells per IgG producing cell. Immune responses are probably generated against most intestinal antigens and the sheer number of these antigens is formidable. It is a daunting prospect to unravel immune events and understand control mechanisms in this dark, mysterious part of the body. It has become clear that in some species most of the intestinal secretory IgA comes from bile. Although some of the IgA produced by submucosal plasma cells attaches to the secretory piece present on local epithelial cells and is then extruded into the gut lumen, most of it reaches the blood. In the liver, IgA attaches to the secretory piece which is present on the surface of hepatic cells, and is transported across these cells to appear in bile. This is important in the rat, but perhaps less so in man. One consequence of the IgA circulation is that, when intestinal antigens reach subepithelial tissues, they can combine with specific IgA antibody, enter the blood as immune complexes and then be filtered out and excreted in bile as a result of IgA attachment to liver cells.

There is a separate circulatory system that involves the IgA producing cells themselves. After responding to intestinal antigens, some B cells enter lymphatics and the bloodstream, from whence they localise in salivary glands, lung, mammary glands and elsewhere in the intestine. Localisation at these sites is achieved by recognition of particular receptors on vascular endothelial cells called addressins. In this way, specific immune responses are seeded out to other mucosal areas, where IgA antibody is produced and further responses to antigen can be made.

IgA antibodies are important in resistance to infections of the mucosal surfaces of the body, particularly the respiratory, intestinal and urogenital tracts. Infections of these surfaces are likely to be prevented by vaccines that induce secretory IgA antibodies rather than IgG or IgM antibodies. However, most patients with selective IgA deficiencies do not show undue susceptibility to infections of mucosal surfaces, probably because there are compensatory increases in the concentration of IgG and IgM antibodies

on these surfaces. Those that are more susceptible generally have associated deficiencies in certain IgG subclasses.

IgE is a minor immunoglobulin only accounting for 0.002% of the total serum immunoglobulins, and it is produced especially by plasma cells below the respiratory and intestinal epithelia. It has a marked ability to attach to mast cells and includes the reagenic antibodies that are involved in anaphylactic reactions. When an antigen reacts with IgE antibody attached to a mast cell, mediators of inflammation (serotonin, histamine, etc.) are released. Thus, if a microorganism, in spite of secretory IgA antibodies, infects an epithelial surface, plasma components and leucocytes will be focused on to the area as soon as microbial antigens interact with specific IgE on mast cells. IgE is considered to be important in immunity to helminths. Larval forms coated with IgE antibodies are recognised by eosinophils and destroyed.

In humans, intestinal antibody is measured in duodenal or jejeunal aspirates, or in faeces (coproantibody). Antibody from the entire gut can be sampled by 'intestinal lavage', when an isotonic salt solution is drunk until there is a watery diarrhoea, which is collected, heat inactivated, filtered and concentrated.

IgD antibodies are present on the surface of B lymphocytes; however, secreted IgD is also seen. IgD-secreting B cells in the human upper respiratory are involved in responses to bacteria which infect the respiratory tract. Secreted IgD 'arms' basophils and other cells of the innate immune system, stimulating release of proinflammatory mediators.

The antibody response takes place mostly in lymphoid tissues (spleen, lymph nodes, etc.) and also in the submucosa of the respiratory and intestinal tracts. Submucosal lymphoid tissues receive microorganisms and their antigens directly from overlying epithelial cells, and lymphoid tissues in spleen and lymph nodes receive them via blood or lymphatics. Initial uptake and handling is by macrophages and dendritic cells, following which antigens are delivered to $CD4^+$ T cells.

On first introduction of an antigen into the body, the antibody response takes several days to develop. Pre-existing antigen-sensitive B lymphocytes encounter antigen via the immunoglobulin receptor (IgM). The antigen is internalised and processed via the exogenous pathway and presented in association with MHC class II molecules to activated T-helper cells. T-cell help is provided via CD40 activation and/or cytokine receptors on B cells, e.g. IL-4 receptor (Figure 6.1). The B cells then:

1. divide repeatedly, forming a clone of cells with similar reactivity (clonal expansion), some of which remain after the response is over, as memory cells;
2. differentiate, developing an ER studded with ribosomes, in preparation for protein synthesis and export;
3. synthesise specific antibody. The fully differentiated antibody-producing cell is a mature plasma cell. Each clone of cells forms immunoglobulin molecules of the same class and the same antigenic specificity.

Although the majority of antibody production occurs following T-cell help, B cells can also become activated directly by polymeric antigens (antigens with repeating epitopes) which cause cross-linking of specific immunoglobulin receptors. This is commonly seen with bacteria, but is also observed with viruses such as polyoma virus, rotavirus and vesicular stomatitis virus. T-cell-independent antibody responses are largely confined to the IgM

isotype and have low affinity and short-lived memory, and are the only type of antibody responses to non-protein antigens, such as carbohydrates. However, these responses can be protective and in the race to stem the dissemination of pathogens in the host such antibody responses may provide a key defence. Every infection is a race between the ability of the invading microbe to multiply and cause disease, and the ability of the host to mobilise specific and nonspecific defences – a delay of a day or so on the part of the host can be critical.

In a natural infection the initial microbial inoculum is small, and the immune stimulus increases in magnitude following microbial replication. Small amounts of specific antibody are formed locally within a few days, but free antibody is not usually detectable in the serum until about a week after infection. As the response continues and especially when only small amounts of antigen are available, B cells producing high-affinity antibodies are more likely to be triggered, so that the average binding affinity of the antibody increases as much as 100-fold. The role of antibody in recovery from infection is discussed in Chapter 9, the relative importance of antibody and cell-mediated immunity depending on the microorganism. On re-exposure to microbial antigens later in life, there is an accelerated response in which larger amounts of mainly IgG antibodies are formed after only one or two days. The capacity to respond in this accelerated manner often persists for life and depends on the presence of 'memory cells'.

Antibodies to a given microbial antigen remain in the serum, often for many years. Since the half-life of IgG antibody in man is about 25 days, antibody-forming cells are continually active. In some instances, microorganisms remain in the body after the original infection (a persistent infection) and can continuously stimulate the immune system. In other instances, it seems clear that antibody levels are kept elevated partly by repeated re-exposure to the microbe, which gives subclinical re-infections and boosts the immune response. This is known to occur with whooping cough, measles and other infections. Sometimes, however, antibodies remain present in the serum for very long periods in the absence of persistent infection or re-exposure. For instance, five of six individuals who suffered an attack of yellow fever in an epidemic in Virginia, USA, in 1855 were found to have circulating antibodies to yellow fever 75 years later. There had been no yellow fever since the time of the original epidemic. Similarly, evidence from isolated Eskimo communities in Alaska shows that antibody to poliomyelitis virus persists for 40 years in the absence of possible re-exposure. How can one explain such long-lived antibody responses? Antigen can persist on the surface of follicular dendritic cells (another member of the dendritic cell family involved specifically with presenting antigen to B cells) in lymphoid follicles for up to eight weeks, falling short of that required for long-lived serological responses in humans. Plasma cells have also been recorded to survive in the bone marrow for long periods, far in excess of what had previously been predicted for the half-life of these cells in lymph nodes and spleen. It is possible that plasma cell survival in the bone marrow involves some sort of bystander help, for example IL-15, to maintain cell division and antibody production.

As a general rule, the secretory IgA antibody response is short-lived compared with the serum IgG response. Accordingly, resistance to respiratory infection tends to be short-lived. Repeated infection with common cold or influenza viruses often means infection with an antigenically distinct strain of virus, but re-infections with respiratory syncytial virus or with the same strain of parainfluenza virus, for instance, are common.

Re-infection of the respiratory tract or other mucosal surfaces is more likely to lead to signs of disease, because of the short incubation period of this type of infection. After re-infection with a respiratory virus there can be clinical disease within a day or two, before the immune response has been boosted and can control the infection. This is in contrast to re-infection with something like measles or typhoid, which are generalised infections, and the long incubation period gives ample opportunity for the immune response to be boosted and control the infection long before the stage of clinical disease (Figure 6.4).

The newborn infant has acquired the IgG antibodies of the mother via the placenta and so has some protection against most of the infections that she has experienced. There is also transfer of secretory IgA antibodies initially via the milk which is human colostrum containing 2–40 mg/m1 IgA. This maternal 'umbrella' of antibodies lasts for about six months in man, and the infant encounters many infectious agents while still partially protected. Under these circumstances the infectious agent multiplies, but only to a limited extent, stimulating an immune response without causing significant disease. The infant thus acquires active immunity while partially protected by maternal immunity. Very occasionally, a mother has not encountered a common microorganism and therefore has no immunity to transfer to her offspring. Certain virus infections, such as herpes simplex and rubella, are especially severe in the totally unprotected small infant, causing systemic illness and often death. There are other major differences between the response to infectious agents of immature and adult individuals. They are due to age-related differences in the immune response, in the inflammatory response, in tissue susceptibility, etc. and are considered in Chapter 11. As the child encounters the great variety of natural infections, total serum antibody levels rise, reaching adult levels by about five years of age. Immunological reactivity reaches peak levels in the adolescent or young adult, but falls off detectably in old individuals. This makes old people less resistant to primary infections, and less capable of keeping certain latent infections under control (see Chapter 10).

Protective Action of Antibodies

Antibodies are formed against a great variety of microbial components and products. The larger microorganisms have more components and products because they have more genes. The presence of antibody indicates present or past infection, but only some of the antibodies have a significant protective function. Protective antibodies generally combine with antigenic components on the surface of microorganisms and prevent them attaching to cells or body surfaces, prevent them from multiplying, and sometimes kill them. The antimicrobial actions of antibodies can be categorised as follows:

1. Antibodies promote phagocytosis and subsequent digestion of microorganisms by acting as cytophilic antibodies or opsonins (see below).
2. Antibodies combining with the surface of microorganisms may prevent their attachment to susceptible cells or susceptible mucosal surfaces (streptococci, gonococci, rhinovirus).
3. Antibodies to microbial toxins or impedins neutralise the effects of these molecules.

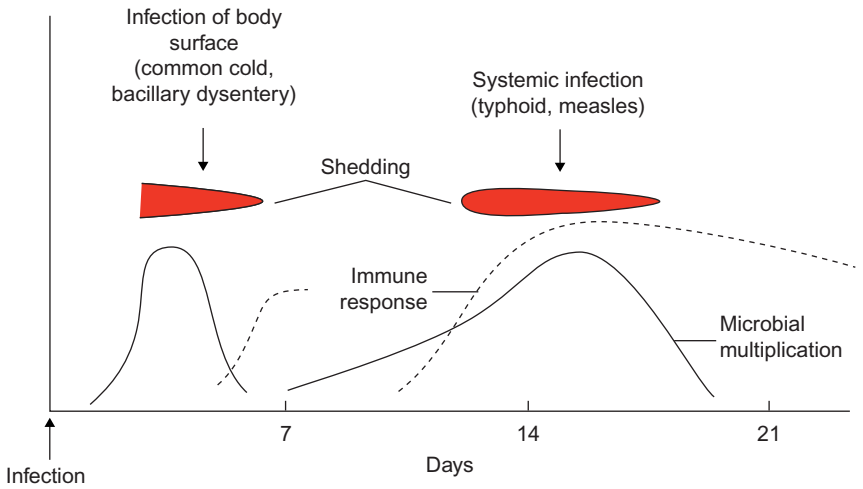

FIGURE 6.4 Distinction between infections of body surfaces and systemic infections.

4. By combining with microbes or antigens and activating the complement system, antibodies induce inflammatory responses and bring fresh phagocytes and more serum antibodies to the site of infection. This can have pathological as well as antimicrobial results (see Chapter 8).
5. Antibodies combining with the surface of bacteria, enveloped viruses, etc., may activate the complement sequence and cause lysis of the microorganism (e.g. *Vibrio cholerae*, *E. coli*, parainfluenza virus, *Mycoplasma pneumoniae*). Host cells bearing new antigens on their surface as a result of virus infection are lysed in the same way, often before virus replication is completed (see Chapter 9).
6. Antibodies enable certain leucocytes to kill infected host cells bearing viral or other foreign antigens on their surface. These include monocytes, neutrophils and NK cells, which act by recognising IgG antibody specifically attached to the target cell surface. Bacteria such as *Shigella* and meningococci can also be killed in this way. NK cells are present in blood and lymphoid tissues and bear receptors for the Fc region of IgG (FcγRIII or CD-16). Antibody-dependent cell cytotoxicity (ADCC) of this type is more efficient per antibody molecule than complement-dependent cell killing and is therefore more likely to be relevant *in vivo*.
7. Antibodies combining with the surface of microorganisms agglutinate them, reducing the number of separate infectious units and also, at least with the smaller microorganisms, making them more readily phagocytosed because the clump of particles is larger in size. Agglutinated virions are compromised in the ability to bind to the virus receptor and so are unable to infect cells.
8. Antibodies attaching to the surface of motile microorganisms may render them nonmotile, perhaps improving the opportunities for phagocytosis.
9. Antibodies combining with extracellular microorganisms may inhibit their metabolism or growth (malaria, mycoplasmas).

T-CELL-MEDIATED IMMUNE RESPONSE

When T cells leave the thymus they enter into the peripheral circulation, touring the lymphoid system on the look out for foreign antigens. They are found in discrete areas in lymphoid organs, notably around the splenic arterioles and paracortical areas of lymph nodes; also in the blood and lymph. About 90% of the recirculation takes place from blood to lymph nodes via the post-capillary venules and then via lymphatics back to the blood (Figure 6.5). This trafficking of lymphocytes is dependent upon the recognition of selective ligands on endothelial cells (called addressins or homing receptors) that act as 'postal codes' enabling lymphocytes to identify the correct location. Key addressins associated with entry into lymph nodes are GlyCAM-1 (glycosylation-dependent cell adhesion molecule 1) and CD34. Recognition of these addressins by T cells involves L-selectin, which binds to GlyCAM-1, and LFA-1 (lymphocyte functional antigen 1), a member of the integrin family. The remaining 10% of cells leave capillaries in various parts of the body, moving through the tissues, entering lymphatics and passing through local lymph nodes. This last route is particularly important in the small intestine, where a different set of addressins operate by MAdCAM-1 (mucosal addressin cell adhesion molecule 1) which interacts with lymphocytes expressing the integrin $\alpha 4/\beta 7$. A given T cell circulates about once in 24 h in a man, and once in 2 h in a mouse.

T cells become arrested in lymph nodes when they encounter antigen of the appropriate specificity presented on the surface of a dendritic cell. As mentioned above, dendritic cells acquire antigens at sites of infection where they become activated and migrate via the lymphatics to the local lymph node. In this environment dendritic cells differentiate, put out dendritic processes and express key accessory molecules important for interacting with and stimulating bound T cells. For example, a $CD4^+$ T cell will engage MHC class II complexed with foreign peptide with the α/β TCR and CD4 (which helps to stabilise the interaction) and also binds the accessory molecules CD40 and B7 on the dendritic cell; these react with the CD40 ligand and CD28, respectively, present on the T cell. A $CD8^+$ T cell would initially recognise MHC class I, but the accessory molecules would be the same as for $CD4^+$ T cells. These interactions are critical events in the evolution of the adaptive immune response as they lead to the activation and clonal expansion of both $CD8^+$ and $CD4^+$ T-cell populations (Figure 6.2).

Dendritic cells continue to transport antigens from the site of infection, thereby servicing new waves of T cells that become attracted to the lymph node. After around 4–5 days[1] clonally selected T cells begin to leave the node via the efferent lymphatics and join the bloodstream. They now target the tissue where the infection is raging. Identification of, and access to, the site of infection from the bloodstream involves the T cells detecting a gradient of chemokines, such as IL-8 and RANTES. These chemokines are released from damaged tissues and aid the exit of T cells via endothelial cells which must display the appropriate addressins.

[1]The time taken for some responses to manifest depends upon the infecting organism and the immune competence of the host. In the majority of virus infections, cytotoxic T cells are first detected in lymph nodes or spleen 4–5 days after infection. A delayed hypersensitivity response to vaccinia virus is positive within one week, whereas the same response to infections, such as tuberculosis, brucellosis and leishmaniasis, is not seen for several weeks.

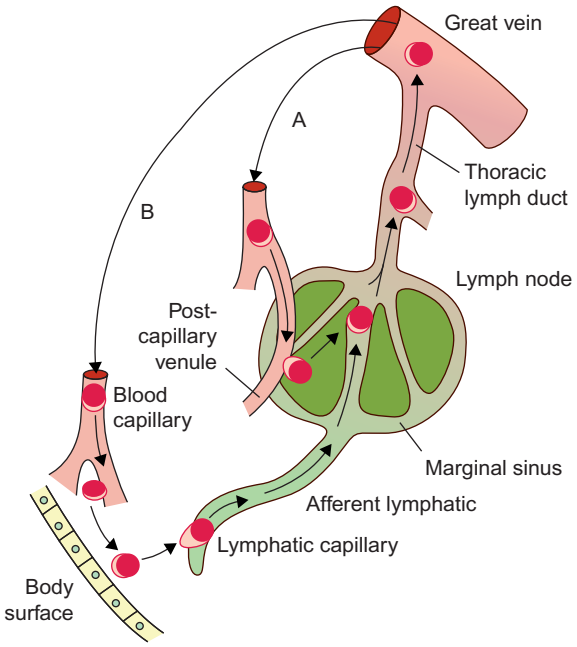

FIGURE 6.5 Lymphocyte recirculation. Recirculating lymphocytes in man are mostly T lymphocytes; approximately 90% of recirculation is by Route A and 10% by Route B.

Once at the site of infection, T cells will encounter macrophages and neutrophils, part of the advanced guard of the innate immune defences. Macrophages provide an additional stimulus to $CD4^+$ T cells following interaction with MHC class II, resulting in the release of a variety of cytokines with antimicrobial activity. A list of some of the common T-cell-derived cytokines and their properties is given in Table 6.1. The infected tissue now undergoes further change with the recruitment of more monocytes from the blood. As they enter the tissue, they become activated by IFN-γ released from activated T cells, predominantly $CD4^+$ T cells. This in turn causes dramatic changes in macrophages resulting in antimicrobial activity. The tissue becomes swollen and is characterised by mononuclear cell infiltration, the hallmark of a DTH reaction. These reactions are particularly important for controlling intracellular bacterial infections such as *Listeria monocytogenes* and *M. tuberculosis*.

Cytotoxic T cells ($CD8^+$ T cells) entering a site of infection will sample the MHC class I molecules on infected cells through their TCR. Specific recognition will lead to T-cell activation and the release of IFN-γ, but in addition they become cytolytic, killing infected cells by inducing apoptosis (see Glossary). Cytolysis involves intimate contact between the T cell and target cell, resulting in the T cell delivering a 'lethal hit' in the form of perforin, a molecule similar to C9 of the complement system which forms a 'plug' in cell membranes causing cell lysis and granzymes (proteases). Perforin inserts into the membrane of the target cell, enabling passage of the granzymes, resulting in cell death. The cytotoxic cell then disengages and homes on to another target. This is an efficient and rapid killing mechanism, capable of destroying virus-infected cells in minutes, well before new virions are assembled and released. Perforin is important in this process, since mice lacking the perforin gene are unable to eliminate infection caused by LCMV (lymphocytic choriomeningitis virus). However, in some systems perforin is not the

main mechanism of killing; rather Fas/Fas ligand assumes this role. Fas is a member of the TNF receptor family found on several cell types. These receptors have 'death' domains which, when activated by Fas ligand on $CD8^+$ T cells, lead to target cell death by apoptosis. CD8 T-cells are not the only T-cells able to mediate cytolytic reactions; CD4 T-cells can also perform this role, although this is restricted to cells expressing MHC class II.

As the effector phase of the T-cell response unfolds, there follows a period of silencing with effector cell numbers declining and conversion to a memory population. Memory T cells can be distinguished from naive T cells (yet to encounter antigen) by the presence of particular membrane markers. The commonest marker involves different isoforms of CD45. The high molecular weight isoform of CD45, CD45RA, is found on naïve T cells and the low molecular weight isoform, CD45RO, is found on memory T cells. It is still unclear whether memory cells arise directly from naïve cells or whether they arise from effector cells. The purpose of immunological memory is to provide a group of cells capable of a rapid response to pathogens on successive encounters. This is clearly the case in DTH responses where mononuclear cell infiltration can be seen within 24–48 h of antigen challenge. A positive response is seen by skin swelling at the site of injection (forearm in man, ear or footpad in mouse). The word 'delayed' is used to contrast it with antibody-mediated responses which appear within an hour. This type of test can be used in the clinic to determine prior exposure to an infectious agent. For example, the tuberculin test is used in determining previous exposure to tuberculosis. The test involves delivering mycobacterial antigens into the skin. Those with a positive response have at some time been infected or are at present infected with tuberculosis, with related mycobacteria, or with the attenuated mycobacteria in the BCG vaccine. Those with a negative response have never been infected or have been infected but have recovered and eliminated bacteria from the body. The response may also be negative early after infection before T-cell immunity has had time to develop, or in acute disseminated infection where the T-cell response is feeble.

A question of major importance in immunology is how is memory established and maintained? The concensus is that antigen is not needed for the maintenance of CD8 and CD4 memory. Experimental evidence showed that antigen primed T cells when transferred to mice lacking antigen and MHC class I and II maintained the antigen primed T cells, whereas naïve T cells required MHC for their survival. An important driver of CD8 T-cell memory is IL-15 and to a lesser extent IL-7. The mechanism for triggering low-level activation is not known.

We know less about the population of T cells that express the γ/δ TCR. They are distributed throughout the body and, in some species, notably cattle and sheep, can account for up to 60% of all T cells. These cells do not appear to recognise peptides presented by MHC molecules, but associate directly with various structures, such as stress proteins, non-classical MHC molecules and glycolipids. A subpopulation of γ/δ T cells exists at epithelial surfaces, intraepithelial lymphocytes, which are thought to play an early defensive role in pathogen-induced damage of the epithelium.

NK CELLS

NK cells are part of a family of innate lymphoid cells (ILC) that are distinguished by the absence of rearranged antigen receptors, the hallmark of T and B cells in the adaptive

immune response. Other ILCs include Rorψ + ILCs and type 2 ILCs, which play an important role in the immune response to bacteria and extracellular parasites. Along with NK cells they represent the first line of defence against a wide range of pathogens.

These large granular lymphocytes constitute a separate lineage of lymphocytes characterised by the membrane markers CD-56 and CD-16 and the absense of RAG recombinase-dependent clonal antigen receptors. NK cells function *in vivo* by recognising target cells that lack or have reduced MHC class I expression, a feature observed in many virus infections. Indeed it seems that NK cells evolved to counter this very threat. Although NK cells were first described back in the 1970s, it is not until recently that there has been progress on the identification of NK cell receptors and their ligands. These turn out to be a diverse collection of receptors and ligands and include: killer immunoglobulin-like receptors (KIRs), c-type lectin-like, LILR, NCRs, Ly49 (mouse) and CD-16, with a variety of ligands such as HLA-C, HLA-E, influenza haemagglutinin and many more. A key feature of NK cell receptors is the activating and inhibitory effects following ligand binding. Whether inhibitory or activating responses prevail depends on the type of cytoplasmic tail of the receptor, i.e. ITIM (immunoreceptor tyrosine inhibitory motifs) or ITAM (immunoreceptor tyrosine activating motifs).

NK cells are generally considered as belonging to the innate immune response. However, recent evidence shows that NK cells can establish memory to certain antigens, a feature associated primarily with the adaptive immune response. An example of this phenomenon is murine CMV infection of C57Bl6 mice. These mice express the Ly49H activating receptor on NK cells which interacts with the viral protein m157. Upon infection Ly49H positive NK cells expand in the presence of IL-15. After around five days the NK cell numbers begin to contract but sufficient numbers remain in the liver as memory cells.

NK cells like neutrophils and macrophages are rapidly mobilised to sites of infection where they play an important effector role limiting the spread of infection by either killing virus-infected cells (this is by a perforin-dependent mechanism) or through the production of cytokines such as IFN-γ and TNF-α, both potent activators of phagocytic cells, critical for the defence against intracellular bacteria such as *L. monocytogenes*. NK cells also express the low-affinity receptor for IgG (Fcγ RIII or CD-16). This enables IgG-coated target cells to be recognised and rapidly killed in a process called ADCC. NK cells are the major exponents of this process.

MACROPHAGES, NEUTROPHILS AND MAST CELLS

Macrophages, because of their phagocytic prowess and their location in many tissues, are inevitably important in the uptake of invading microorganisms, and they have important functions as phagocytes whether or not an immune response has been generated. They are involved in the initiation of immune responses to infection, as described above, and are also important in the expression of the immune response seen at a later stage in the infection. In this they operate in close association with both antibodies and T cells.

Neutrophils are also of extreme importance, operating in association with antibody and complement. They are mainly present in the blood and do not continuously monitor the

tissues and fluids[2] of the body. They are, however, rapidly delivered to tissues as soon as inflammatory responses are initiated (see Chapter 3). They are short-lived; during an infection macrophages are always having to deal with dead neutrophils containing microorganisms in various stages of destruction and digestion. Both neutrophils and macrophages bear Fc and C3b receptors on their surfaces which promote the phagocytosis of immune complexes or microorganisms coated with antibody. By preparing microorganisms for phagocytosis in this way, specific antibodies and complement act as opsonins. Complement also often increases the virus-neutralising action of antibody, presumably by adding to the number of molecules coating the virus particle and further preventing its attachment to susceptible cells. When a microorganism is coated with antibody it undergoes a different fate after phagocytosis. *Toxoplasma gondii*, for instance, normally manages to enter macrophages without triggering an oxidative metabolic burst but this antimicrobial response does occur when the parasite is coated with antibody, and is presumably triggered by Fc-mediated phagocytosis. In the case of viruses, antibody can promote uptake and degradation by macrophages. With viruses like dengue that can infect macrophages, small amounts of cross reactive but non-neutralising antibody, often generated following infection by a different strain of dengue, actually enhance infection of these cells, presumably by enhancing the uptake or altering the intracellular fate of the virus. The Fc receptor becomes a Trojan horse allowing entry to the cell.

When C3 is associated with antibody on the surface of a microorganism, it often increases the degree of opsonisation. IgM antibodies attached to *Pseudomonas* or other Gram-negative bacilli may even require complement before there is opsonisation. Sometimes, however, C3 is activated on the microbial surface by the alternative pathway and acts as an opsonin independently of antibody. This may be important early in pneumococcal infection, for instance, when there is not much antibody available. Opsonised phagocytosis is the principal method of control of infections with microorganisms such as the streptococcus, staphylococcus or encapsulated pneumococcus, the antibody response and complement acting in conjunction with phagocytic cells.

Macrophages also help give expression to the T-cell response, and this seems particularly important in the case of microorganisms such as mycobacteria, *Leishmania*, herpesviruses, brucellas and lymphogranuloma inguinale (*Chlamydia trachomatis*), that survive and multiply within phagocytes and other cells. When sensitised T cells encounter specific antigen, they release a number of cytokines, with a profound effect on macrophages. Some cytokines induce inflammation and are chemotactic, bringing circulating macrophage precursors (monocytes) to the site of the reaction, and others inhibit their movement away from the site. Merely accumulating macrophages at a focus of infection is sometimes enough to control the infection but, especially for microorganisms that are not easily killed in macrophages, something more than this is often needed. Thus there are other cytokines, especially IFN-γ, that activate macrophages, causing them to develop increased phagocytic and digestive powers.

The increased phagocytosis can be demonstrated directly by the uptake of particles or microorganisms, and is also evident by increased attachment and spreading on a glass

[2]Sometimes, however, circulating neutrophils are arrested in capillaries, especially in the lung, and can then phagocytose microorganisms present in the blood.

surface, in what can be regarded as a heroic attempt to phagocytose the entire vessel in which the macrophages are contained. The increased digestive powers are associated with increased lysosomes and lysosomal enzyme content, and there is also an increased ability to generate oxygen radicals. As a result of these changes, macrophages show increased ability to destroy ingested microorganisms. For instance, in mice that have recently developed a T-cell response to tuberculosis, macrophages are activated and have an increased ability to ingest and destroy tubercle bacilli. Indeed, resistance to tuberculosis in man is largely attributable to the antibacterial activity of activated macrophages. Mouse macrophages activated in this way by tuberculosis also show increased ability to ingest and destroy certain unrelated intracellular bacteria such as *L. monocytogenes*, and protozoa such as *Leishmania*. In other words, the macrophage is activated by cytokines following an immunologically specific interaction between lymphocyte and microbial antigen, but expresses this reactivity nonspecifically against a wider range of microorganisms. Some of the cytokines necessarily have a restricted local area of action, but activated macrophages are not confined to the immediate vicinity of the lymphocyte encounter with antigen. Macrophages elsewhere in the body are often affected, suggesting that the mediators (or the activated macrophages) spread throughout the body. Activation lasts only for a short time and is no longer detectable a week after termination of the infection. In persistent infections such as tuberculosis, macrophages can remain activated for longer periods because of the continued expression of the T-cell response.

Macrophages are also activated during the course of certain virus infections and can express this reactivity against unrelated microorganisms. For instance, when mice are infected with ectromelia (mousepox) virus and six days later injected intravenously with *Listeria*, the reticuloendothelial macrophages in the spleen show an increased ability to ingest and destroy these bacteria. Macrophages activated in infections by viruses that grow in macrophages may show increased resistance to the infecting virus, and sometimes they are also resistant to infection with unrelated viruses. Macrophage activation is important in protozoal infections, and specific antibody responses may add to the macrophage's antimicrobial capacity. In a resistant host, *Leishmania* parasites are destroyed after phagocytosis by activated macrophages, and unrelated microorganisms such as *Listeria* are also killed. Nonactivated macrophages, in contrast, generally support the growth of both *Leishmania* and *Listeria.* Normal macrophages support the growth of *T. gondii*, but after activation during the infection they increase H_2O_2 production 25-fold and kill the parasite.

Like macrophages, mast cells are also strategically located in tissues throughout the body, acting as an early warning system for intrusive pathogens. Mast cells are important initiators of inflammation, where they are able to respond to a variety of mediators associated with bacteria, viruses, parasites, as well as complement components (C3a, C5a), cytokines (TNF-α, IL-12, stem cell factor) and IgE, a product of Th2-mediated immune responses. Mast cells respond to these stimuli by the rapid release from cytoplasmic granules of proinflammatory mediators (e.g. histamine, proteases) and cytokines (TNF-α, IL-6). These substances have powerful effects on tissues, causing, for example, bronchoconstriction, increased gastrointestinal motility and increased vascular permeability leading to the accumulation of phagocytes at sites of infection. Mast cells are an important component in host defence against parasitic worms. They achieve this in various ways, for example, by

promoting the expulsion of worms through inflammatory mediators causing rapid convulsive movements of the gut, i.e. 'throwing out' the parasite, or by recruiting eosinophils to mediate ADCC on IgE antibody-coated parasites (see Chapter 9). The importance of mast cells in protective immunity has been studied using mast cell-deficient mice, which show a reduced ability to eliminate endo- and ecto-parasites (e.g. biting insects), and to control certain forms of bacterial infection, e.g. bacterial peritonitis. Whereas mast cells do have phagocytic potential and can engulf bacteria, it is thought that protection against bacterial peritonitis occurs via production of TNF-α.

Although mast cells also mediate allergic disorders, some of which can be fatal, one must reconcile these pathological mechanisms with the potential benefits to animals and presumably early man in terms of the evolutionary pressures to acquire and retain defence mechanisms to counter the diversity of macroparasite infections.

COMPLEMENT AND RELATED DEFENCE MOLECULES

Complement is a complex series of interrelated proteins present in normal serum. It functions by mediating and amplifying immune reactions. The first component (C1) is a complex of three proteins, C1q, C1r and C1s. It is activated in the classical complement pathway, after Clq combines with immunoglobulin (IgG or IgM) in immune complexes (antibody bound to antigen).[3] The immune complex may be free in the tissues or located on a cell surface following the reaction of specific antibody with a cell surface antigen. The activated first component is an enzyme system and acts on the next component to form a larger number of molecules of the second component's enzyme. This in turn activates larger amounts of the next component, and so on, producing a cascade reaction (Figure 6.7). A single molecule of activated C1 generates thousands of molecules of the later components and the final response is thus greatly amplified. The later complement components have various biological activities, including inflammation and cell destruction, so that an immunologically specific reaction at the molecular level can lead to a relatively gross response in the tissues.

After activation of the C1 components, C4 and then C2 are activated to form a C3-convertase, and this in turn acts upon C3 to generate C3a, which has chemotactic and histamine-releasing activity. The residual C3b becomes bound to the antigen–antibody complex, and the whole complex can now attach to C3b receptors present on macrophages and neutrophils. The complex also attaches to C3b receptors on non-phagocytic cells (platelets and red cells) in some species and this is called immune adherence. In the blood it can lead to aggregation and lysis of platelets with release of vasoactive amines. C5 is the

[3]Fc sites on the immunoglobulin are slightly altered as a result of the combination with antigen, and the altered Fc sites bind to the C1q fraction of C1. Each C1q must bind to at least two Fc sites and this means that there must be several IgG molecules close together on the immune complex. With IgM, several Fc sites are present on a single molecule, and IgM therefore activates complement much more efficiently. Although in Figure 6.6, antibody is shown attached to the complex throughout the sequence, the amplification phenomenon leads to the formation of thousands of additional and separate molecules of the later components.

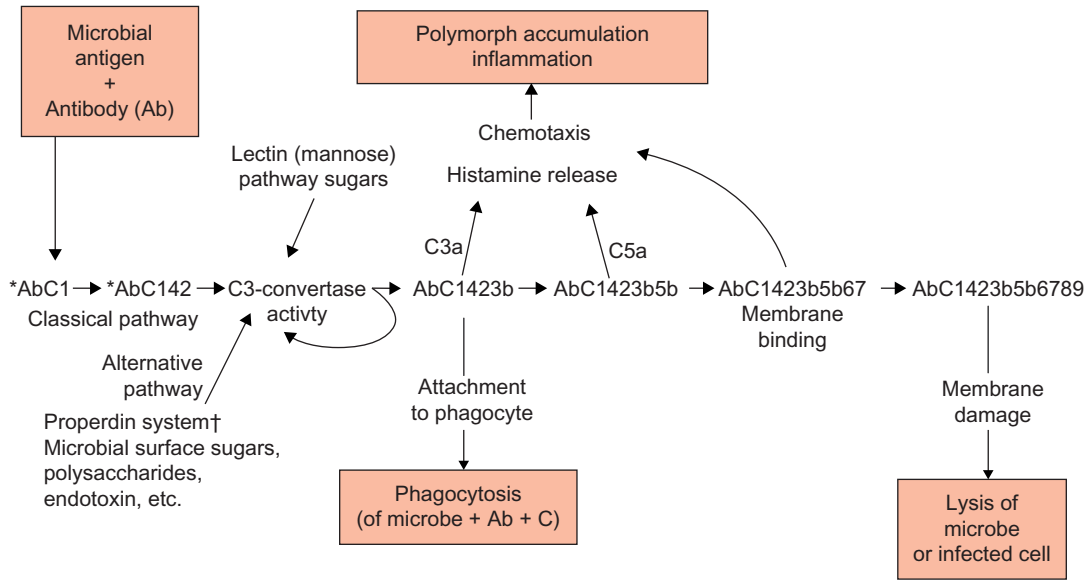

FIGURE 6.6 The complement activation sequence and antimicrobial actions.

next component to be activated, forming C5a with additional chemotactic and histamine-releasing activity. C5b remains with the complex and binds with C6 and C7, and finally with C8 and C9. The membrane attack complex is formed when the last component (C9) is polymerised to form a pore, and is inserted so as to traverse the cell membrane. This allows a net influx of Na^+ and water, resulting in death of the cell.

Each activation must be terminated somehow, rather than snowball into generalised activation. The complement sequence is therefore controlled by a number of built-in safety devices in the form of inhibitors (regulatory proteins) and unstable links in the complement chain. The activated components have a short (ms) half-life and therefore cannot diffuse through the body and affect distant tissues.

Substances such as microbial polysaccharides and endotoxin can activate the complement system independently of antigen–antibody reactions and C1. These are directly involved in the activation of C3 and this process is referred to as the alternative complement pathway. Activation of the alternative pathway involves the formation of a convertase between C3 and factor B (C3bBb – shares homology with C2bC4b convertase). This binds to bacterial surfaces where it activates more C3, resulting in more C3b deposited. This amplification system very quickly leads to the entire microbial surface being covered in C3b. The whole process is strongly regulated by two inhibitory proteins, factors H and I, which degrade C3b. The fact that the complement cascade can be activated without the need for an antigen–antibody reaction may be important in certain infectious diseases. The peptidoglycan of the cell wall of staphylococci, or the polysaccharides on the surface of the pneumococcus, for instance, could activate the alternative pathway very early in infection before specific antibodies have been formed, leading to antibacterial effects as

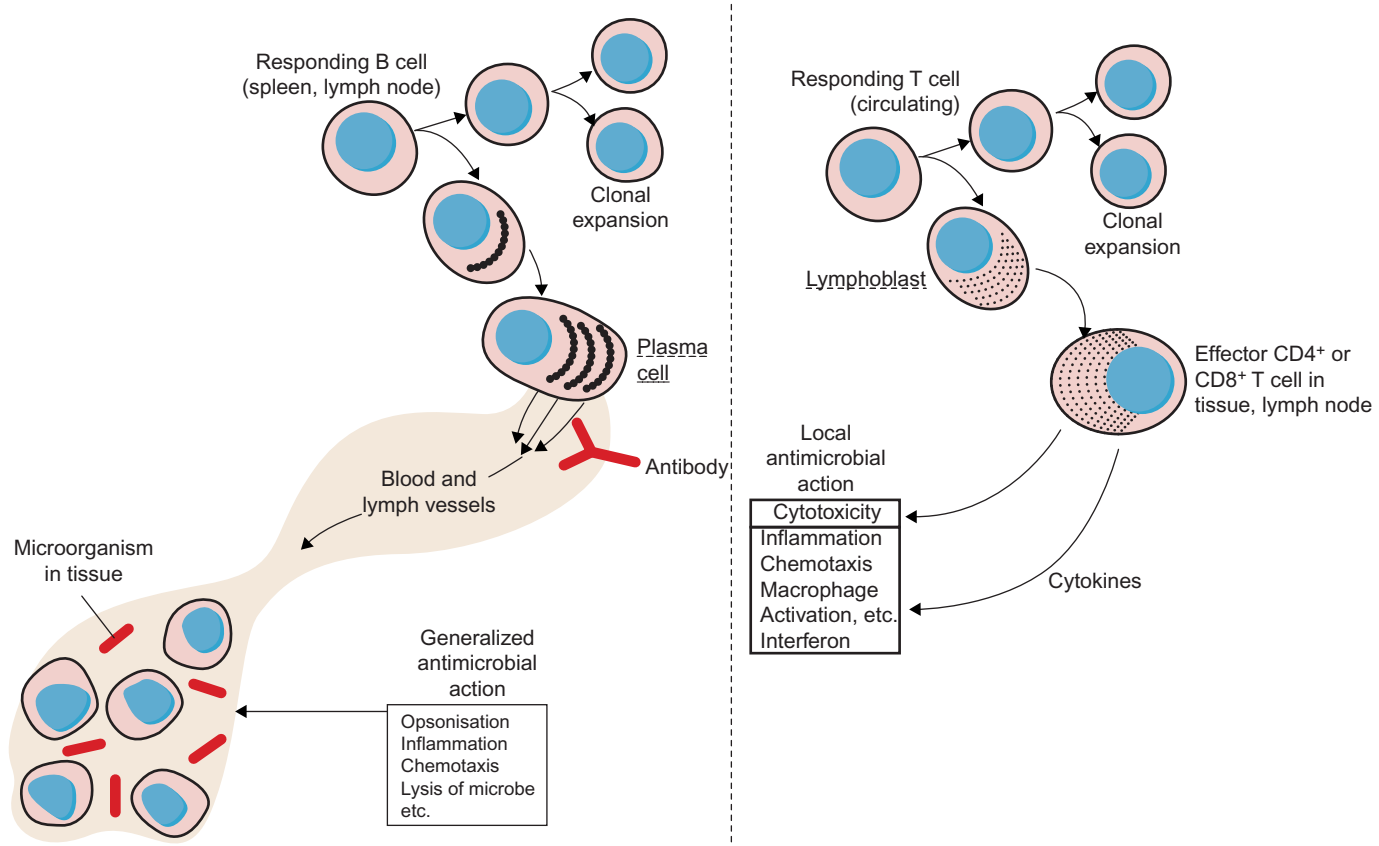

FIGURE 6.7 Comparison of antimicrobial action of B and T lymphocytes. Antibody tends to act at a distance from the plasma cell, whereas effector T-cell responses generally require the local presence of the effector cell.

described later.[4] The alternative pathway (so called because scientists discovered it after the 'classical' pathway) is probably an ancient defence system and appeared in evolution before the classical pathway.

Complement is capable of causing considerable inflammation and tissue damage, especially because of the amplification phenomenon. Once the sequence is activated, there are four principal antimicrobial functions, each of which is enhanced when both classical and alternative pathways are involved (Figure 6.6).

1. The inflammation induced at the site of reaction of antibodies with microbes or microbial antigens focuses leucocytes and plasma factors on to this site.
2. The chemotactic factors attract neutrophils to the site.
3. The C3b component bound to complexes attaches to C3b receptors on phagocytes and thus acts as an opsonin, promoting phagocytosis of microbes and microbial antigens.
4. Where antibody has reacted with the surface of certain microorganisms (Gram-negative bacilli, enveloped viruses, etc.) or with virus-infected cells, the later complement components are activated to form the membrane attack complex. Small pores, 9–10 nm in diameter, appear in the wall of Gram-negative bacilli, for instance, and lysozyme (present in serum) completes the destructive effect. Cells infected with budding viruses and bearing viral antigens on their surface (see Figure 9.2) can be destroyed by complement after reaction with specific antibody, even at an early stage in the infectious process (see also Chapter 9).

The binding or fixing of complement to immune complexes forms the basis of the complement fixation test. In the test for antibody, a known antigen is used in the reaction and vice versa. Complement is added to the reaction mixture and, if there has been a specific antigen–antibody interaction, this complement is fixed and is no longer detectable. The test for complement is by adding sheep red blood cells coated with specific antibody: if complement is present the cells are lysed, but if it has been used up (fixed) the cells are not lysed.

Another series of molecules acting as a first line of defence against microorganisms are the collectins, found in serum and various tissues. The name is derived from their structure, i.e. a collagen 'stalk', a neck region and a globular carboxy-terminal C-type (calcium-dependent) lectin-binding domain. Included in this family are the mannose-binding protein (MBP), lung surfactant proteins A and D and serum bovine conglutinin. The structure of MBP is similar to C1q in that it resembles a 'bunch of tulips'. Furthermore, MBP is able to substitute for Clq in binding Clr and Cls in activating the classical pathway. These proteins recognise patterns of carbohydrates on the surface of bacteria, viruses and parasites (e.g. MBP binds mannose, fucose, *N*-acetyl glucosamine), where they mediate both complement-dependent and complement-independent protective responses. The lung surfactant proteins have been shown to function in defence against *Pneumocystis carinii* and *Cryptococcus neoformans*, two important respiratory pathogens in immunocompromised hosts, probably by aiding their phagocytosis by alveolar macrophages.

[4]On the other hand, Babesia activate the alternative pathway and depend on this for entry into susceptible erythrocytes, which bear C3b receptors.

CONCLUSIONS CONCERNING THE IMMUNE RESPONSE TO MICROORGANISMS

Each T or B cell is committed to respond to a particular epitope. The initial encounter with this epitope, whether in lymphoid tissues or elsewhere in the body is a small-scale event. The purpose of the response, especially when the antigen is from an infecting microorganism, is to turn this microscopical event into a larger event as soon as possible, so that both antibody and T cells can be brought into action on a significant scale. Both types of immune reactive cell are small, and each must differentiate, generating the cytoplasmic machinery needed for synthesis of antibodies (B cell) or cytokines involved in the induction and expression of immunity (T cell). The stimulated cell also gives rise to a dividing population of cells with the same specific immune reactivity, and the response is thus magnified.

Plasma cells can persist for many years as an effector-memory population for the most part in lymphoid tissues, and antigens are brought to them via blood or lymph. The antibodies formed circulate through the body, acting at a distance from the plasma cells that are situated in lymphoid tissues. Antibodies needed on mucosal surfaces must pass through an epithelial cell layer onto these surfaces, and they are produced by plasma cells situated just below these surfaces. Antibodies bathe tissues and mucosal surfaces where they can react with microbes and microbial antigens in a mostly useful antimicrobial fashion. At the site of the antigen–antibody interaction in tissues, complement is activated and inflammatory responses are generated so that antibodies, phagocytes and more immune reactive cells are delivered to the scene of action.

In contrast, the ability to recognise and destroy infected host cells depends on the local action of individual sensitised T cells. The body's population of sensitised T cells must therefore be circulated throughout the tissues of the body like antibodies, and especially through the lymph nodes to which microbes and their antigens are brought from the tissues. In this way, microbial antigens can be recognised wherever they are and the T-cell response initiated. The response, involving an accumulation of mostly lymphocytes and macrophages, can be generated locally in the tissue and also more centrally in lymph nodes or spleen. Microbial antigens are most commonly presented to the immune system at the periphery of the body. Langerhans cells in the skin, dendritic cells in submucosal lymphoid tissues and local lymph nodes are involved, and there is a tendency for T-cell responses to predominate. At a later stage in the response, central immune tissues in the spleen are also active. Sometimes, however, there is a reversal of this normal sequence, and antigens are presented directly to central immune tissues. There is then a tendency for the antibody response to be dominant. This is a generalisation, but it may have some bearing on problems of tolerance and suppression (see Chapter 7) and on the subject of T cells versus antibody in recovery from infection (see Chapter 9).

Immune reactions are specifically triggered by T and B cells by virtue of their ability to recognise antigen in a highly specific manner. Macrophages and the dendritic cell family, by processing and presenting antigens, exercise a controlling influence at this stage, and are in close physical association with T and B cells. Macrophages, neutrophils, NK cells and complement play an important part as effectors and amplifiers of the reaction in tissues.

The part played by antibodies, T cells, NK cells, neutrophils, macrophages and complement in recovery from microbial infections is discussed at greater length in Chapter 9.

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CHAPTER

7

Microbial Strategies in Relation to the Immune Response

OUTLINE

The very existence of successful infectious agents indicates that host defences do not constitute an impenetrable barrier for microorganisms. Infections are common. There are more than 400 distinct microorganisms that infect man alone, and all of us, by the time of death, have experienced at least 150 different infections. Many of these infections are asymptomatic, but a disease sometimes appears before the microorganism has been controlled and eliminated. In many instances, as discussed in Chapter 10, the infection is not eliminated but persists in the body. Persistence represents a failure of the host's antimicrobial forces — forces that can be regarded as having been designed to eliminate invading microorganisms from tissues. The infecting microorganism can then continue to cause pathological changes or continue to be shed from the body.

Once the epithelial surfaces have been penetrated, the major host defences are Natural Killer (NK) cells, complement, phagocytic cells, interferon and later antibody and T cells. These constitute a mighty hexad whose action is described in Chapters 6 and 9. Generally

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speaking, if there is a way in which host defences can be successfully bypassed or overcome, then at least some microorganisms can be expected to have 'discovered' this. Microorganisms evolve very rapidly in relation to their host, so that most of the feasible antihost strategies are likely to have been tried out and exploited. The microbial devices for overcoming the phagocytic cell system and thus contributing to invasiveness, virulence or persistence have been discussed in Chapter 4. This chapter is largely devoted to an account of the microbial strategies that have been developed to overcome or bypass the immune response. As new strategies are revealed, one has the impression that microbes know more about the immune response than the immunologists! Strategies for interference with immune defences are summarised in Table 7.3.

INDUCTION OF IMMUNOLOGICAL TOLERANCE

Tolerance is an immunologically specific reduction in the immune response to a given antigen. As discussed here, it is due to a primary lack of responsiveness, rather than to an active suppression of the immune response. If there is a feeble host immune response to the relevant antigens of a microorganism, the process of infection is facilitated and the possibility of persistence increased. This does not involve a general failure of the host immune response of the type discussed in Chapter 9, but a particular weakness in relation to an antigen or antigens of a given microorganism.[1] Sometimes it is said that a particular microbial component is a 'poor antigen', an observation that suggests tolerance to this antigen. All microorganisms except the smallest viruses have numerous antigens on their surfaces and, if infection with a given microorganism is to be favoured, then the immunological weakness must be in relation to the microbial antigens that are important for infectivity, invasiveness or persistence. Also, because in a given infection either antibody or T cells may be the most important antimicrobial force (see Chapter 9), there must be a weakness in that arm of the immune response to which the microorganism is most susceptible. Tolerance can involve either antibodies or T cells to some extent independently. Tolerance to infectious organisms is rarely absolute, with no trace of an immune response to an antigen, but even slight specific weakness (or slowness) in a host may favour a microorganism (see Chapter 11). There are a variety of ways in which tolerance, defined in this way, can arise.

Prenatal Infection

There is commonly a degree of tolerance to a microorganism when infection occurs during foetal or early post-natal life. At one time it was thought that any antigen present in the foetus during development of the immune system was regarded as 'self', and that as a result there was no immune response to it. It is now clear that immune responses do occur under these

[1]Most tolerance is exerted at the level of the T cell, whether by failure to respond or following the generation of suppressor/regulatory T cells. B cells, however, are susceptible to 'tolerisation' by antigen during their development.

circumstances, but they are often weak and fail to control an infection.[2] For instance, rubella virus infects the human foetus, causing congenital malformations, and although the foetus receives rubella antibodies (IgG) from the mother and makes its own IgM antibody response to the infection, the T-cell response is particularly poor, enabling the virus to persist during foetal life and for long periods after birth. In the mouse, lymphocytic choriomeningitis (LCM) virus is transmitted vertically (see Glossary) via the egg, so that the foetus is infected from the earliest stages of development. The congenitally infected mouse nevertheless makes a feeble antibody response (and no antiviral T-cell response) to the virus, but this fails to control the infection, and virus persists in most parts of the body for the entire life of the animal. In contrast to this, when adult mice are infected for the first time with LCM virus, they develop both antibody and a vigorous CD8 T-cell response, and the T-cell response becomes a pathogenic force that can lead to tissue damage and death (see Chapter 8). A similar situation exists in bovine virus diarrhoea virus (BVDV) infection of cattle. BVDV also induces tolerance when infection occurs *in utero* between 100 and 150 days gestation. In this instance, no antibody or T-cell response is made to the virus, which persists in the adult in a non-pathogenic form. However, infection of adult cattle with a pathogenic virus of the same strain leads to virus growth and disease in the absence of an immune response. If a different BVDV strain or another microorganism infects the tolerised cattle, then an immune response is generated to this.

The above are examples of antigen-specific interference with the function of the immune response. However, some antigens can act in a more 'non-specific' way, yet have a dramatic effect on the development of T cells and subsequent virus infection. These are called 'superantigens' and they are produced by certain bacteria (staphylococci, streptococci; see Chapter 8) and viruses (e.g. retroviruses). They function by binding to major histocompatability complex (MHC) class II molecules and interact with the T-cell receptor via the Vβ chain (all T cells belonging to a particular Vβ family, whatever their antigen specificity, will interact). In certain bacterial infections, the superantigen induces T-cell proliferation resulting in the release of large quantities of cytokines. However, if T cells encounter these antigens early in their development (i.e. in the thymus), they become deleted and hence no T cell with that Vβ chain is detected in the spleen or lymph node. This is seen in mice carrying the retrovirus MMTV (mouse mammary tumour virus). This virus is integrated into the germ line and produces a superantigen that results in the clonal deletion of particular T-cell subsets. The superantigen also causes a clonal expansion of B cells, required by the virus for its growth and also as a vehicle to spread to the main target organ, the mammary gland. Interestingly, mice deprived of those T cells are not compromised in their ability to recognise and respond to other microorganisms, but they are resistant to re-infection by MMTV.

Desensitisation of Immune Cells by Circulating Antigens

Tolerance to a given microorganism can arise when large amounts of microbial antigen or antigen–antibody complexes are circulating in the body. For instance, patients suffering

[2]Tolerance to the vast majority of 'self-antigens' is complete since these antigens persist in the body and maintain the tolerogenic signal. Therefore, for a microorganism to utilise this method of persistence, it must survive in the host following a prenatal infection without producing overt disease, i.e. exist in a non-pathogenic form.

from disseminated coccidiomycosis or cryptococcosis, both fungal infections, show antibodies but little or no T-cell response to the microorganisms. This is referred to as a state of anergy. It seems to be due to excessive amounts of circulating fungal antigen and the T-cell response to unrelated microorganisms is not affected. Those suffering from kala-azar (visceral leishmaniasis) or diffuse cutaneous leishmaniasis have a defective T-cell response to the protozoal antigens, again associated with the presence of circulating leishmania antigens and resulting in systemic spread and chronicity of the infection. Antibodies are formed, at least in kala-azar, a severe generalised form of leishmaniasis, but this is not enough by itself; if there is to be recovery and healing, a good T-cell response is also necessary, enabling sensitised lymphocytes to destroy host cells infected with *Leishmania* microorganisms. A possible mechanism for tolerisation (desensitisation) of specifically reactive circulating T cells by antigen is as follows. When they are circulating through the body, T cells fail to make their usual intimate association with dendritic cells, B cells, etc. Instead, the T-cell response becomes diluted among various tissues leading to a reduction in the critical cell mass required for activation. In this environment, T cells may encounter antigen on non-professional antigen-presenting cells that lack the necessary co-stimulator molecules for activating T cells and instead may deliver a tolerogenic signal. Under these circumstances, CD4 and CD8 T cells lose their ability to respond to the specific antigen and become anergised. In a similar way, developing B cells can be rendered immunologically impotent by direct exposure to circulating antigen.

In chronic infection with viruses such as HIV, HepC, HepB and LCMV, CD8 T cells can suffer from 'exhaustion' arising from continued stimulation during virus infection. It differs from other forms of T-cell anergy in that it generally takes longer to develop, i.e. several months as opposed to days for other forms of anergy. There follows a stepwise reduction in cytokine expression with interleukin-2 (IL-2) disappearing first and then IFN-γ. Some T cells recover their antiviral activity, whereas others undergo apoptosis.

After infection with *Treponema pallidum*, there is an initial T-cell response, as detected by lymphocyte transformation *in vitro* in the presence of treponemes. This initial response disappears as the bacteria multiply and spread throughout the body, and lymphocytes from patients with early secondary syphilis fail to respond *in vitro* to *T. pallidum*. Later in the secondary stage, weeks or months after infection, lymphocyte reactivity reappears, delayed skin reactions are demonstrable, granulomata appear in lymph nodes and the infectious process is finally brought under control. It is not known why lymphocytes from patients with early secondary syphilis fail to respond to the infecting bacteria. Antigen-specific suppression is a possibility, or T cells become anergised due to high levels of circulating antigen, or alternatively, T cells become sequestered in particular tissues and do not appear in the peripheral blood where sampling for reactive T cells would normally occur.

Molecular Mimicry

If a microbial antigen is very similar to normal host antigens, the immune response to this antigen may be weak or absent, giving a degree of tolerance. An example of this is the scrapie agent which shares a similar amino acid sequence to a host protein (PrP), thus rendering the scrapie agent invisible to the immune system. The mimicking of host

antigens by microbial antigens is referred to as molecular mimicry. The hyaluronic acid capsule of streptococci, for instance, appears to be identical to a major component of mammalian connective tissue. The commonest resident bacteria of the normal mouse intestine are *Bacteroides*, and these share antigens with mouse intestine. Cross-reactions are seen, even with foetal mouse intestine, which absorbs antibacterial antibodies from serum. Mice are known to be generally rather unresponsive to *Bacteroides* antigens, and it is tempting to suggest that this facilitates establishment of these bacteria as lifelong intestinal commensals (see Chapter 2). Generally, however, there is little evidence that molecular mimicry is a cause of poor immune responses. On the contrary, there is good evidence that antibodies formed against microorganisms sometimes cross-react with host tissues and therefore cause disease. A good example of this is coxsackie B virus-induced myocarditis. Patients with inflammatory cardiomyopathy have antibodies that cross-react with peptides from coxsackie B3 proteins and with peptides from cellular adenine nucleotide translocator. Other examples include antigens in *T. pallidum* that cross-react with components in normal tissues, and antigens in Epstein–Barr virus that cross-react with human foetal thymus, but this does not prevent antibodies being formed and providing the basis for the Wasserman and the Paul Bunnell (heterophile antibody) tests, respectively. There are one or two infections in which antibodies react with normal uninfected host cells. For instance, in atypical pneumonia caused by *Mycoplasma pneumoniae*, antibodies to heart, lung, brain and red blood cells may be formed. The antibodies to red blood cells (called cold agglutinins) very occasionally cause haemolytic anaemia.

Molecular mimicry, in summary, sounds like a good idea from the point of view of the infecting microorganism, and there are observations suggesting that it occurs, but so far there is not very convincing evidence. Indeed, it seems likely that antigenic determinants of microbes may resemble those of the host purely by accident rather than by sinister microbial design, and sometimes the common sequences reflect basic biological functions, common to many living creatures. It was found that about one-third of 800 different monoclonal antibodies to defined virus antigens cross-reacted with normal host tissue components. Computer searches for shared amino acid sequences between viral polypeptides and host components such as myelin basic protein showed that shared stretches of 8–10 amino acids, which could give cross-reactive immune responses, were quite common. Only a few viral polypeptides and one or two host components have been tested, and cross-reactive responses would turn out to be very common indeed, if other host components and the polypeptides of other viruses, protozoa, bacteria, etc. were examined. Although these phenomena are examples of molecular mimicry, it would be unreasonable to suggest that they have any meaning in terms of microbial strategies. Rather the host, in responding to such an immense variety of different microbial antigens, is always in danger of responding accidentally, as it were, to its own tissues. The resulting autoimmune response, however, only rarely leads to harmful, immunopathological results (see Chapter 8).

Conclusion About Inducing Tolerance

Usually, when there is a weak immune response to a microbial antigen, it is not known which of the above mechanisms is responsible. There is, for instance, a very weak antibody response to the microorganisms present in the normal intestinal tract of

mice. These microorganisms have been present during the evolution of the host animal. They are symbiotic in the sense that they may supply nutrients to the host and tend to prevent infection with other more pathogenic microorganisms (see Chapter 2). Perhaps the immune response is poor because the bacteria share antigens with the mouse intestine, as mentioned above. Perhaps mice have a genetically determined immunological weakness as regards these microorganisms. Perhaps infection shortly after birth has induced a large degree of tolerance. Perhaps large amounts of antigen are constantly absorbed from the intestine, desensitising lymphocytes and inducing tolerance. At present we do not have enough evidence to decide between these possibilities. In man, the urinary tract is commonly infected with *Escherichia coli*, and the frequency of different bacterial serotypes is in proportion to their frequency in the faecal flora. Strains rich in the polysaccharide K antigens, however, are more likely to invade the kidneys. Children with pyelonephritis due to *E. coli* show a correspondingly poor antibody response to these antigens, but the cause of the poor response is not known. Sometimes one suspects tolerance to a microorganism, but there is no evidence. For instance, when species of dermatophyte fungi of animal origin infect the skin of man, there is inflammation followed by healing and relative resistance to re-infection. But with species of fungi adapted to man, there tends to be less inflammation, a more chronic infection and less resistance to re-infection. This sounds as if it could be due to a weak immune response to antigens of the human type of fungus.

Studies of autoimmunity have revealed that in normal people there are lymphocytes that respond to autoantigens. Autoimmune disease is avoided by suppressing these responses. This leads to the possibility that specific suppression is commoner than primary unresponsiveness (clonal deletion or clonal anergy). Indeed, the autoimmune phenomena seen in certain infectious diseases (see Chapter 8) could be attributed to a breakdown in specific immune control mechanisms. Also, there are indications that, in certain persistent infections, a weak response to microbial antigens is due to antigen-specific suppression rather than to a shortage of responding cells. The following section deals with immunosuppression in infectious diseases.

IMMUNOSUPPRESSION

General Immunosuppression

A large variety of microorganisms cause immunosuppression in the infected host. This means that the host shows a depressed immune response to antigens, unrelated to those of the infecting microorganism. Infectious agents that multiply in macrophages or lymphoid tissue (viruses, certain bacteria and protozoa) are especially likely to do this. For instance, during the acute stage of measles infection, patients with positive tuberculin skin tests become temporarily tuberculin negative. The exact mechanism is not clear and may be complex. The virus grows in monocytes and could for instance induce production of immunosuppressive cytokines (e.g. IL-10) that inhibit the induction of Th1 responses (e.g. delayed-type hypersensitivity (DTH) responses), or affect the expression of Major Histocompatibility Complex (MHC) or accessory molecules on cells. Even after vaccination with live attenuated measles virus,

immune responses are depressed, and there is a reduction in cutaneous sensitivity to poison ivy, lasting several weeks. Depressed T-cell or antibody responses to unrelated antigens have also been described in people with mumps, influenza, Epstein–Barr virus and cytomegalovirus infections. Immunosuppression is a feature in mice infected with cytomegalovirus, LCM virus, murine leukaemia virus or *Toxoplasma gondii*, and in cattle infected with rinderpest virus. Patients with certain types of malaria, trypanosomiasis, leishmaniasis and lepromatous leprosy show reduced responses to various unrelated antigens and vaccines.[3]

At present we do not know how such a variety of microorganisms inhibit immune responses. Interference with the immune functions of dendritic cells, macrophages and lymphocytes is important.[4] The reduced responses may partly be due to 'antigenic competition' rather than actual suppression of responses by the infecting microorganisms. Antigenic competition might be expected when an urgent, generalised response to an invading microorganism commandeers a large proportion of the available space, uncommitted cells, etc., in lymphoid tissues. When the spleen, for instance, is enlarged, a seething mass of cells responding with maximum immunological effort against the invader would leave less space and fewer cells for responses to unrelated antigens.

Interest in virus-induced immunosuppression received a great stimulus with the appearance of the acquired immunodeficiency syndrome (AIDS) in 1979. In this disease the infecting virus (HIV, human immunodeficiency virus) infects both CD4 T cells and macrophages. This results in serious loss of immune reactivity and particularly of T-helper function.

In severely affected patients, the immune deficit allows a variety of persistent yet normally harmless infections (*Pneumocystis carinii*, cytomegalovirus, tuberculosis, toxoplasmosis, candidiasis, etc.) to become active, and these, together with various other infections, eventually prove fatal. HIV is responsible for the immunosuppression that gives the other microorganisms the opportunity to cause the lethal disease. (HIV also causes a late-onset, independently evolving disease of the brain.) The virus persists in the body, and patients remain infectious for life. Feline leukaemia virus (also a retrovirus) causes a similar condition in cats, and infected cats are more likely to die of secondary infection than of the leukaemia itself. People who are HIV positive but asymptomatic have virus-specific antibody, T-cell responses and low levels of infectious virus. This state continues for years, but symptoms of AIDS eventually appear, coinciding with a decline in immune responses and a concomitant increase in virus. This could be regarded as a useful result from the point of view of the virus, favouring persistence and transmission to fresh hosts during the asymptomatic period, the more general and disastrous immunosuppression being an 'unfortunate' side effect.

[3]There is a specific unresponsiveness to *Mycobacterium leprae*. Suppressor T cells obtained from skin lesions have been shown to inhibit the response of other T cells specifically to *M. leprae* antigens, perhaps by lysing cells that are presenting these antigens. The suppressor T cells are possibly induced by the terminal sugars on a leprosy-specific phenolic glycolipid.

[4]Interference with the recirculation and homing of T cells (see Chapter 6) would be a theoretically attractive strategy for a microbe. Pertussis toxin (pertussigen) has this effect, but its relevance *in vivo* is not known.

Antigen-Specific Suppression and Regulatory T Cells

A general immunosuppression induced in the host is of no particular significance for an infectious agent if it merely promotes infection by unrelated microorganisms. If the immunosuppression is to be of value, it must involve the response to the infecting microorganism itself, facilitating its spread, multiplication and persistence. A number of infectious agents, especially viruses, induce this type of immune suppression. In other words, their strategy is to suppress host immune responses specifically to their own antigens. The phenomenon occurs with other infectious agents, such as leprosy and in tuberculosis, where there are reduced DTH and IL-2 responses to tubercular antigen (PPD) but normal responses to streptococcal antigens.

Antigen-specific suppression might enable a virus to persist indefinitely in the body (see Chapter 10). Also, many human viruses (measles, mumps) have an incubation period of 10 days or more because they take time to spread in the body, multiply and be shed to infect a new host. Immune responses might normally be expected to curtail the infection before the full sequence of events had unfolded, and it would therefore be advantageous for such viruses to suppress responses to their own antigens. The argument applies even more to hepatitis B and rabies viruses, with incubation periods of several months.

The most audacious strategy would be to infect the very tissues in which the immune response is generated, and interfere with it, in other words, to evade immune defences and invade immune tissues. It turns out that infection of lymphoreticular tissues is very common in systemic and in persistent virus infections (Table 7.1). There are several possible mechanisms for antigen-specific suppression. During a normal response, there is careful control over the distribution and concentration of antigen, especially in lymphoid tissue. Antigens are normally delivered to lymphocytes in minute quantities and in an appropriate setting after processing by dendritic cells and macrophages. If larger amounts of antigen are liberated locally in these tissues by an invading virus, a disordered response is to be expected, perhaps by tolerising T or B cells. Another mechanism would be for the invading virus to infect preferentially the T or B cells that responded specifically to its own surface antigens, and either inactivate or destroy these cells. This would eliminate the clones of cells that might otherwise generate a specific antiviral response. Finally, the invading virus might generate antigen-specific suppressor/regulatory cells (see Chapter 6) or other suppressor factors, an example of which are regulatory T cells (Treg cells).

Treg cells are induced in a variety of immunological reactions where they dampen potentially pathological responses, e.g. autoimmune diseases by maintaining a state of tolerance. There are broadly two forms of Treg cells: one develops in the thymus referred to as natural Tregs (nTregs) and the other is induced in the periphery (iTregs). There are many examples of chronic infection where the induction of Treg cells creates a suppressive environment favouring the persistence of viruses such as HepC and HepB. Treg cells mediate their effector activity in a variety of ways including release of immunosuppressive cytokines such as IL-10, TGF-β and IL-35, through cytolysis and by interfering with the function of dendritic cells. Some of the above possibilities involve antigen-specific unresponsiveness in addition to active immunosuppression.

TABLE 7.1 Infection of Lymphoreticular Tissues by Viruses and Other Infectious Agents Exhibiting Systemic Infection or Persistence

Infectious Agents	Host
VIRUSES	
Adenoviruses (L)	Man
Epstein–Barr virus (L)	Man
Kaposi's sarcoma herpesvirus (L)	Man
Cytomegalovirus (M)	Man, mouse.
Leukaemia virus (L, M)	Mouse, etc.
Visna virus (M)	Sheep
LCM virus (L, M, DC)	Mouse
Murine gammaherpesvirus (L, M)	Mouse
Thymic necrosis (L)	Mouse
Measles (L)	Man
Rubella (L, M)	Man
Lactic dehydrogenase virus (M)	Mouse
Infectious bursal disease virus (L)	Chicken
Aleutian disease virus (M, DC)	Mink
Equine infectious anaemia (M)	Horse
African swine fever virus (M)	Pig
HIV (L, M, DC)	Man
OTHER INFECTIOUS AGENTS	
(see also Table 4.2)	
Scrapie (DC)	Mouse, sheep
Theileria parva (L)[a]	Cattle

L, lymphocytes known to be infected; M, macrophages known to be infected; DC, dendritic cells known to be infected.
[a]A tick-borne protozoal parasite responsible for East Coast fever, an important cattle disease in East Africa. The parasite invades lymphocytes, becomes associated with the mitotic apparatus and stimulates cell division. But the infected lymphocytes are later killed by other immune cells. Presumably the parasite betrays its presence by allowing its antigens to be displayed on the infected cell.

Another 'clever' example of unresponsiveness is when the microorganism exploits 'holes' in the immunological repertoire of the host. Immune responses to given antigens, as noted earlier, are controlled by immune response genes. Successful microorganisms therefore would tend to develop surface antigens that are poorly seen and poorly responded to by the host.

ABSENCE OF A SUITABLE TARGET FOR THE IMMUNE RESPONSE

There are various ways in which intracellular microorganisms can avoid exposing themselves to immune forces. They evade host immune responses as long as they stay inside infected cells and allow at the most a low density of microbial antigen to form on the cell surface. This is what happens in dorsal root ganglion cells persistently infected with varicella zoster virus (see Chapter 10), in circulating lymphocytes infected with Epstein–Barr virus, and in most of the cells of a mouse infected with persistent murine leukaemia virus. In Epstein–Barr virus-infected B cells, EBNA-1 (Epstein–Barr nuclear antigen) is responsible for maintaining the latent viral genome and, because it persists during virus latency, it is a potential target for cytotoxic CD8 T cells. However, this protein is able to resist proteolysis by the proteosome thus avoiding processing and presentation via MHC class I molecules. Malaria parasites are present in liver cells during the exoerythrocytic stages of infection and, during this silent latent period, the parasite avoids stimulating or presenting a target for the immune response. Even when the malaria parasites are growing in red blood cells and causing the disease, their very presence inside the red blood cells protects them from circulating antibodies. The merozoites that emerge from infected red cells are only briefly exposed to antibodies before entering fresh uninfected cells. Some of the parasite components, however, may be present on the surface of the infected red cell, which then becomes a less protected site.[5]

Some of the intracellular microorganisms that expose their antigens on the infected cell surface benefit from a host-mediated mechanism for disposal of those antigens. This involves antibodies and depends on the phenomenon called capping. Substances can move in the fluid matrix of the cell membrane and, when microbial antigens on the cell surface react with a specific antibody, the antigen–antibody complex moves to one pole of the cell (capping). Here the complex is either shed or taken into the cell by endocytosis. The antibodies that should have prepared the infected cell for immune destruction are diverted from this purpose and used to rid the cell of microbial antigens, making it less susceptible to immune lysis. Capping is frequently observed *in vitro* on cells infected with various enveloped viruses, notably measles and herpes simplex viruses. However, although a desirable strategy for hiding membrane associated antigens, there is little evidence to suggest it works *in vivo*. In certain protozoa, capping by antibody can lead to the loss of the microbe's own surface antigens. It occurs with *T. gondii* and with *Leishmania*, but its importance in resistance to host immune defences is not known.

Intracellular microorganisms also escape the action of antibodies if they spread directly from cell to cell without entering the extracellular fluids. This is seen when herpes simplex virus spreads progressively from cell to cell through cytoplasmic 'tunnels' in the presence of potent neutralising antibody. This virus also avoids detection by the immune system when travelling within axons of sensory nerves. Some enveloped viruses (corona and flaviviruses) avoid displaying their antigens on the cell surface by budding into cytoplasmic

[5]Red blood cells infected with *Plasmodium falciparum* express on their surface a receptor for the cell surface ligand – ICAM-1, present on endothelial cells. Its significance for the parasite is not clear, but it would promote binding of the infected red cell to post-capillary venules and could be a mechanism for the sludging of red cells in cerebral blood vessels in cerebral malaria.

vesicles. Virions are then released directly into the external medium by fusion of the vesicle with the plasma membrane. Cells formed by division of an infected cell are also infected without virus entering extracellular fluid. For example, cells derived from the ovum of a mouse infected with leukaemia virus are all infected, whether or not virus is released from the cell, and in the newborn infant with rubella virus, a cell that was initially infected in the foetus has given rise to a group of infected progeny cells in spite of the presence of neutralising antibodies. The ability to stay inside cells certainly contributes to the success of persistent intracellular microorganisms, without in most cases being the sole factor.

An entirely different approach for a virus is the concealment of an antigenic site on the virion, behind a carbohydrate moiety. A single point mutation creates the glycosylation site and renders the site invisible to the immune system so that antibody is no longer made against it. The reverse mutation can restore the site. This situation occurs naturally in influenza A and rabies viruses, and presumably confers some selective advantage.

MICROBIAL PRESENCE IN BODILY SITES INACCESSIBLE TO THE IMMUNE RESPONSE

Many viruses persist in the infected host and are shed to the exterior via the saliva (herpes simplex virus, cytomegalovirus, rabies virus in vampire bats), milk (cytomegalovirus in man, MMTV) or urine (polyomavirus in mice). The surface of the infected cell can be said to face the external world as represented by the lumen of the salivary gland, mammary gland or kidney tubule (Figure 7.1). As long as virus particles and viral antigens are only formed on the lumenal surface of the cell and there is little or no cell destruction, it is difficult for T cells or antibodies to reach the site and eliminate the infection. This is the case for murine cytomegalovirus infection, where T cells efficiently control the productive infection in all tissues, except the salivary gland where the clearance of virus is markedly delayed. Secretory IgA antibodies could react with viral antigens on the infected cell surface, but complement is unlikely to be activated and the cell would not be destroyed. IgA antibodies could also react with extracellular virus particles but would at the most render them non-infectious, again without acting on the source of the infection. The same considerations apply to epidermal infection with human wart viruses (Papilloma virus), or to infection of the epidermis lining the chicken's feather follicle with Marek's disease virus. In the case of a wart, restricted viral gene expression occurs at early stages in the differentiation of the epidermis with structural gene expression, viral DNA replication and virus particles shed only within the keratinised epithelium. This clever strategy bypasses the host defences, resulting in a persistent infection. The anatomy of an established wart indicates a 'silent' lesion with no signs of an immune response. However, staying on the 'right' side of the basement membrane is essential for the survival of the virus since breakdown results in the appearance of the immune response in particular cytotoxic CD8 T cells that recognise early viral antigens destroying infected cells. Similar mechanisms exist for fungi that grow in the dead keratinised cells of skin and hair.

Bacteria that are present and multiply in the lumen of glands, tubes and tubules also enjoy some freedom from immune forces. In rats persistently infected with *Leptospira*, for instance, the bacteria multiply in the lumen of kidney tubules and are shed in urine. If the urine enters water in a river or puddle, it remains infectious and can cause leptospirosis in

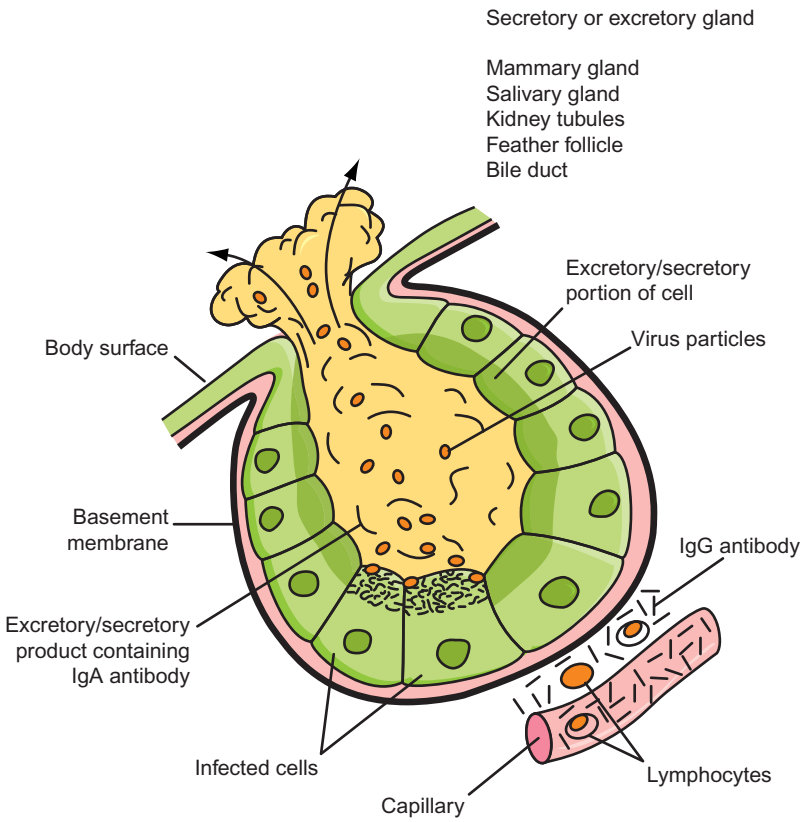

FIGURE 7.1 Viral infections of cell surfaces facing the exterior.

man. Commensal intestinal bacteria, unless they are very closely associated with the intestinal epithelium, enjoy similar freedom, and it is therefore impossible to eliminate these bacteria by artificially inducing immune responses against them. Other bacteria, such as *Brucella abortus* in the cow, persistently infect mammary glands and are shed in the milk. In typhoid carriers, the bacteria colonise scarred avascular sections of the biliary or urinary tract, and are thence shed, often in large quantities, into the faeces or urine. Bacteria may also lurk in biliary or renal stones, and in a similar way the staphylococci in the devascularised bone of patients with chronic osteomyelitis are protected from host defences.

INDUCTION OF INAPPROPRIATE ANTIBODY AND T-CELL RESPONSES

Many types of antibody molecule are formed against a given antigen, reacting with different antigenic determinants (epitopes) on the molecule. For instance, studies with monoclonal antibodies (see Glossary) have shown that there are many different epitopes on a protein such as the haemagglutinin expressed on the surface of influenza virus, but only those

located within five particular antigenic sites are capable of neutralising infectivity. Also, antibodies tend to have a range of avidities (see Glossary). If the antibodies formed against a given microorganism are of low avidity, or if they are mostly directed against unimportant antigenic determinants on the microorganism, then they will only have a weak antimicrobial action and there are likely to be difficulties in controlling infection with that particular microorganism. For instance, there are several persistent (lifelong) virus infections of animals in which antibodies are formed and react specifically with the surface of the infecting virus but fail to render it non-infectious. The virus–antibody complexes are therefore infectious and they circulate in the blood. These viruses include LCM and leukaemia virus infections in mice, and also Aleutian disease virus in mink. In the latter disease, there is a stupendous immune response on the part of the infected animal, with a fivefold increase in total IgG levels and viral antibody titres of 1/100,000. The antibody is not only of no antiviral value but causes life-threatening immunopathological damage. Since there is also no effective T-cell response to these infections, and since these viruses grow in host cells without harming them, the infections persist for life.

Non-neutralising antibodies of this sort are particularly important if they combine with microbial antigen and block or sterically hinder the binding of any good-quality neutralising antibodies that may also be present. Antibodies to LCM virus formed in infected mice are known to have this property.

It is not known how commonly ineffective antibodies are induced in other microbial infections, but if they are induced, the antimicrobial task of the host is certainly made more difficult. The antibodies formed in patients with syphilis (a persistent infection) are only very feebly antimicrobial. Although they combine with the surface of the treponemes and perhaps aid phagocytosis (act as opsonins), they cause little neutralisation and, after 36 h treatment with antibody plus complement, most of the treponemes remain infectious.

From a microorganism's point of view, it would also be an advantage to be able to induce the host to make the wrong type of immune response. There is a tendency for the antibody and the T-cell response to given antigens to vary inversely, and if for a given infection the host's major antimicrobial force was the T-cell response, the microorganism could with advantage induce the formation of a strong antibody response. This could be done by inducing the formation of Th2 rather than Th1 cell responses. Parasitic worms induce strong Th2 immune responses, benefiting the host by producing IgE antibodies important for the expulsion of the parasite. However, co-infection of parasitic worms with other microorganisms can inadvertently deviate normally protective Th1 responses against the microorganism to a poorly protective Th2 response. This can be considered a form of opportunism, which on the one hand can benefit a microbe but at the same time may have adverse effects on the host, i.e. a more severe infection may ensue. Deviation of immune responses from strong Th1-mediated CMI responses can also occur when microorganisms infect the host through different routes. For example, herpes simplex virus infection of the epidermis promotes strong Th1 activity, measured as DTH, but a weaker antibody response. In contrast, infection via the bloodstream induces strong antibody responses but negligible CD4 (Th1) cell responses. Once these pathways are set in motion, it is difficult to reverse them, i.e. Th1-mediated CMI can no longer be induced in the host against the virus once it has been delivered in the bloodstream. Infections with Gram-negative bacteria such as *Salmonella typhi* are controlled by macrophages and T cells, and it has been suggested that endotoxin (see

Chapter 8), which acts as a general B-cell (antibody) stimulator, directs the host response in favour of antibody rather than T-cell activation of macrophages, to the benefit of the infecting bacteria. Patients with lepromatous leprosy or pulmonary tuberculosis show poor CMI responses to the invader, suggesting that the bacteria have induced an inappropriate immune response in a susceptible type of host.

Persistent protozoal infections such as malaria and African trypanosomiasis are characterised by the formation of very large amounts of antibody. But most of this appears to have little or no protective value in the host, although it sometimes shows some *in vitro* inhibition of parasite motility, viability, multiplication or metabolism. Although some of these antibodies are directed against microbial antigens, most are truly non-specific in the sense that they do not react at all with any microbial antigens.[6] Some react with host tissues, such as the heterophile antibodies (see Glossary) and the antibodies to DNA, Schwann cells and cardiac myofibrils, that are seen in the various types of trypanosomiasis, and this raises the question of autoimmune damage (see Chapter 8). Similar antihost antibodies occur in certain virus infections such as those caused by Epstein–Barr virus and cytomegalovirus. The basis for these irrelevant or excessive antibody responses is B-cell proliferation induced by the infection, often referred to as polyclonal clonal activation. This is seen in malaria, lepromatous leprosy and also in infection with *M. pneumoniae*, *Trypanosoma* species, Epstein–Barr virus and many other microorganisms. It would make sense if it reflected microbial interference with host immune responses (see Chapter 10), but its significance in these important infectious diseases is still shrouded in mystery. For viruses such as MMTV and Epstein–Barr virus that grow in B cells, proliferation of these cells can be interpreted as a useful strategy.

ANTIBODIES MOPPED UP BY SOLUBLE MICROBIAL ANTIGENS

The antimicrobial action of antibodies is to a large extent due to their attachment to the surface of microorganisms. Antibody on the microbial surface prevents entry into susceptible cells, promotes uptake by phagocytes, activates complement lysis of the microorganism, etc., as discussed in Chapter 6. One strategy that microorganisms could use to defend themselves against the antibody weapon would be to liberate their surface components in soluble form into tissue fluids. These surface components would combine with and 'neutralise' antibody before it reached the microorganism.

Soluble antigens are liberated into tissue fluids in most microbial infections, but it is not often that these are known to be surface antigens. Most normal tissue cells bud off tiny membrane-bound blebs of cytoplasm into surrounding fluids. Cells infected with budding viruses produce virus particles, but they probably also liberate tiny blebs of cytoplasm whose limiting membrane contains viral antigens. The 20 nm particles present in the serum of patients and carriers with hepatitis B virus infection are produced in this way. There are up to 10^{13} particles per millilitre of serum. Polysaccharide antigens from *Candida* contain mannan,

[6]Adults in West Africa chronically infected with malaria show seven times the normal (European) rate of IgG production, on a body weight basis. Much of this is associated with malaria infection because it is reduced by 30% after several years of prophylactic antimalarial therapy. Only 5% of the circulating IgG, however, is found to react with malarial antigens.

and this material, which is present in the serum of patients suffering from candidiasis, inhibits lymphocyte proliferation in response to *Candida* antigens. Perhaps *Candida* polysaccharides are handled abnormally in these patients, allowing free mannan to stay in the circulation and interfere with macrophage responses. In certain bacterial infections, surface polysaccharides are liberated. Bacterial polysaccharides are detectable in the serum in pneumococcal pneumonia, and in the serum and cerebrospinal fluid in fulminating meningococcal meningitis. The surface polysaccharide in *Pseudomonas aeruginosa* is also released from multiplying bacteria *in vitro* and presumably *in vivo*. Even endotoxin is released in small amounts into the surrounding fluid by Gram-negative bacteria. Antigens from *Trypanosoma cruzi*, *Candida albicans*, *T. gondii*, *Plasmodium* spp. and *Babesia* spp. are present in serum during systemic infections. The phenomenon may prove to be a common one. But in spite of the theoretical advantages for the microorganism, it is not known whether the released surface components mop up enough antibody or inactivate enough T or B cells to be of significance in the infection.

LOCAL INTERFERENCE WITH IMMUNE FORCES

There are several ways in which microorganisms, without preventing the generation of immunity, interfere with the local antimicrobial action of immune forces. For instance, a few microorganisms induce the formation around themselves of a capsule or cyst. Cysts are formed in certain protozoal infections, but they occur inside cells, especially macrophages, and protect the microorganism from destruction by the host cell rather than from host immune forces inside macrophages in the central nervous system, muscle and lung.[7] Shielded by the tough wall of the cyst, the microorganism multiplies without provoking a host reaction, and thousands of parasites may be present in a single cyst in the chronic stage of the disease. Cysts that restrict the access of antibody and phagocytic or immune cells are best seen with helminth parasites. The dog tapeworm, for instance, lives in the alimentary canal of dogs, and enormous numbers of eggs are present in faeces. When humans ingest these eggs, the parasites develop and travel from the intestine to reach the lung or liver, where hydatid cysts are formed. A cyst consists of larval worms inside a firm capsule made up of parasite and host components. The cyst gradually grows larger, in spite of the antibody and cell-mediated immune response of the host, often reaching the size of a coconut, and may cause serious disease. The natural hosts for the cysts are grazing animals such as sheep, which ingest the eggs; after the sheep has been killed and eaten by a predatory carnivore such as the dog, the larval worms in the cyst grow to form adult worms in the intestine.

Virulent staphylococci produce a coagulase and this acts on prothrombin at the site of infection, which leads to the deposition of a layer of host fibrin around the bacteria, resulting in an abscess. It is possible that this restricts the local access of host cells, and that the bacteria are also disguised immunologically, so that they are less readily identified as targets for immune responses. There are other examples from bacterial infections. The K antigens on the surface of *E. coli* are closely associated with the pathogenicity of these bacteria. They can

[7] *T. gondii* is primarily a parasite of members of the cat family, in whom it infects intestinal epithelium and is shed in faeces. Human infection is worldwide and is common in the United Kingdom, but nearly always symptomless.

mediate attachment of *E. coli* to intestinal epithelial cells and certain K antigens can increase the ability of *E. coli* to grow in the kidney or other sites. First, the K antigen is a polysaccharide and makes phagocytosis a more difficult task for host cells (see Chapter 4). Also, some of them are poor immunogens, possibly resembling host polysaccharides. Finally, the K antigen interferes with the alternative pathway of complement activation, so that the bacteria escape this early antibacterial defence mechanism.

One theoretically simple strategy for bacteria would be to produce an enzyme that destroys antibodies, and it has been shown that pathogenic strains of the gonococcus, a human pathogen, liberate a protease that specifically cleaves human IgA1 subtype antibodies. The protease acts at the site of a Pro-Thr peptide bond in the hinge region of the heavy chain (see Figure 6.1). The significance of this enzyme *in vivo* is not yet clear because antigen-combining sites on the cleaved fragments would remain intact, but such enzymes are obviously likely to be important and might help account for the apparent indifference of the gonococcus to the host's antibody response. A similar enzyme is produced by the meningococcus, a frequent resident in the nasopharynx, by many strains of *Haemophilus influenzae* and *Streptococcus pneumoniae* and also by *Streptococcus sanguis*, one of the common commensal bacteria of the mouth. In each case the bacteria producing the protease are normally exposed to secretory IgA antibody. The staphylococci provide a more plausible example of the local interference with the action of antibody by bacteria. Virulent staphylococci have a factor called staphylococcal protein A in the cell wall that is excreted extracellularly, and this inhibits phagocytosis by attaching to the Fc portion of the antibody molecule and preventing the effective recognition of bacteria via Fc receptors on circulating phagocytes. *P. aeruginosa* produces an elastase that inactivates the C3b and C5a components of complement and thus tends to inhibit opsonisation and the generation of chemotactic and other inflammatory responses. Perhaps this contributes to invasiveness; *Pseudomonas* infections tend to show minimal inflammatory responses. Factors that inhibit the action of complement or opsonising antibodies are perhaps also produced by other bacteria, but even when such factors are discovered and defined, it can be difficult to decide how important they are in the actual process of infection. *Entamoeba histolytica*, for example, produces proteases that degrade C3a and C5a and the amoebic adhesin prevents assembly of C8, C9 into the membrane attack complex.

Gram-negative bacteria have a very complex cell wall, consisting primarily of a membrane-like arrangement of phospholipids, lipopolysaccharide (LPS or endotoxin; Figure 4.4) and protein. In virulent strains of bacteria, the polysaccharide chains project from the general bacterial surface, carrying on their tips the important O antigens of the cell wall. The O antigens are the key targets for the action of host antibody and complement, but when this reaction takes place on the end of the polysaccharide chains, a significant distance external to the general bacterial cell surface, complement fails to have its normal lytic effect. Such bacterial strains are virulent because of this resistance to host immune forces. Their colonies happen to have a smooth appearance on agar surfaces and they are therefore called 'smooth' strains. If the projecting polysaccharide chains are shortened or removed, antibodies react with O antigens on the general bacterial surface or very close to it, and complement can then lyse the bacteria. Strains without the projecting polysaccharides are therefore non-virulent and they have a 'rough' colonial morphology. Gram-negative bacteria therefore can be thought of as protecting themselves from the

damaging consequences of antibody and complement reactions by having antigens that project a short distance out from the bacterial cell surface.

Recent studies have highlighted the importance of LPS in determining the *in vivo* phenotype of gonococci. Gonococci in urethral exudates resist complement-mediated killing by human serum. In most cases, this resistance is lost after one subculture in laboratory media. A terminal Galβ1−4G1cNAc site on a conserved 4.5 kDa LPS surface component is sialylated by host-derived cytidine 5′-monophosphate-*N*-acetyl neuraminic acid (CMP-NANA). The transfer is catalysed by a gonococcal sialyltransferase. Not only does this prevent lysis by complement but confers on the organism the ability to avoid contact with and ingestion by phagocytes and masks LPS from reacting with specific antibodies. It also, paradoxically, prevents the uptake of the organisms by endothelial cells. This latter property is probably of importance in the extracellular phase of transmission. Presumably, in order for re-infection to occur, the organisms must undergo desialylation, presumably by host enzymes. Meningococci − Groups A, B and C − show similar properties to the gonococcus except that for Groups B and C where the sialylation requires endogenous CMP-NANA. In one Group B meningococcal epidemic, almost all cases had sialylated LPS and were virulent in a mouse model of infection; in contrast, most carrier isolates were not sialylated and were not virulent in mice.

Certain bacteria activate complement via the alternative pathway (without antibody) and this promotes opsonisation and thus increases host resistance early in infection. Group A streptococci would activate the alternate pathway were it not for their special outer covering. These bacteria are poorly opsonised by complement alone, but when the M protein on the pili is gently removed with trypsin to expose peptidoglycan, the alternative pathway is activated, the bacteria bind complement and are then efficiently opsonised and phagocytosed. Encapsulated strains of *Staphylococcus aureus* activate the alternative pathway and bind complement, but C3 is somehow hidden by capsular material and the bacteria are not opsonised.

The DNA viruses have evolved a number of strategies to combat the local actions of the immune response. These include viral genes that mimic: (a) cytokines and chemokines, (b) receptors for cytokines and chemokines, (c) MHC molecules, (d) antiapoptotic factors and cell cycle proteins and (e) complement regulatory proteins. These genes have been acquired by viruses during their evolution by acts of molecular piracy from the cell. The viral genes show close homology with cellular genes and perform similar functions. Thus many act as decoy proteins subverting the action of the normal cellular counterpart. Vaccinia virus disrupts cytokine and chemokine responses through production of soluble receptors against TNF, IFN-γ, IFN-α/β, IL-1β and through the production of chemokine binding proteins (CBPs, a new set of regulatory molecules so far only found in vaccinia and herpes viruses). A similar armory is found in the gammaherpes viruses which possess cellular homologues of IL-10 (Epstein−Barr virus), IL-6, MIP-1α/β (KSH virus), IL-8 receptor and CBP (MHV-68, KSH virus). These viral cytokines and chemokines (virokines) act on the relevant target cell in a way analogous to their cellular counterpart. This could involve recruiting target cells to sites of infection in order to infect them. Lymphocytes are the principal site of latency in gammaherpes virus infection (see Chapter 10). To ensure target cells survive to support a latent infection, members of the gammaherpes viruses also carry the antiapoptotic genes, vBcl-2 and vFLIP. Viral Bcl-2 has been shown to protect cells from

death induced by TNF and presumably serves a similar function *in vivo* by preventing premature B-cell death, thus insuring virus survival in the B-cell compartment.

Surviving the attentions of NK cells and cytotoxic T cells is an important goal for all viruses. The herpes viruses and adenoviruses have solved this problem by interfering with the expression of MHC molecules on the cell surface. To inhibit cytotoxic T-cell recognition of MHC class I molecules, cytomegalovirus and adenovirus produce proteins that either anchor MHC class I in the endoplasmic reticulum or block their progression through the Golgi complex. This prevents the MHC molecules reaching the cell surface and being recognised by T cells. Herpes simplex virus and cytomegalovirus also inhibit peptide transport into the endoplasmic reticulum by producing a protein that binds to the TAP molecules responsible for this transport. Reducing MHC class I expression is a key strategy for outwitting cytotoxic T cells; however, this strategy only serves to alert NK cells to these target cells (see Chapter 6). To overcome this and outwit NK cells, human and murine cytomegalovirus encode an MHC class I–like protein on the surface of infected cells which can interact with NK cells, instructing them to ignore the infected cell.

The fact that pox viruses and herpes viruses encode proteins designed to disrupt the complement system suggests that viruses see this system as a threat. Pox viruses are able to inactivate C4b through production of C4B-BP-like molecules and herpes simplex virus gC on the surface of the virus, or the infected cell binds C3b and thereby inhibits the complement cascade. The gammaherpes virus MHV-68 expresses a homologue of a complement regulatory protein. Deleting this gene from the virus genome attenuates the virus when used to infect mice. This indicates that subduing the effects of complement is important for the pathogenesis of these viruses. Herpes simplex virus and human cytomegalovirus induce virus-coded Fc receptors on virions and on the surface of infected cells. This could be useful for the virus by binding IgG non-specifically to the cell surface and thus protecting it from immune lysis. Fc receptors are present on staphylococci (protein A), on certain types of streptococci (ZAG protein and protein G) and on trypanosomes, and could protect these microorganisms in a similar fashion. The Fc and C3 receptors present on the various forms of *Schistosoma mansoni* conceivably aid the survival of this large parasite in the host.

REDUCED INTERFERON INDUCTION OR RESPONSIVENESS

The interferons are cytokines and play a major role in the innate defence against virus infection by augmenting NK cell, dendritic cell and macrophage activity, and promoting an antiviral state in cells, a form of intracellular immunity (see Chapter 9). This gives the IFNs an important role in early defence, before the adaptive immune response has been generated. Their production is stimulated by foreign macromolecules (Chapter 9); IFN-α and IFN-β are induced in all cell types in response to double-stranded RNA, and IFN-γ is produced following the activation of T cells and NK cells (see Chapter 6 and 9).

Viruses are generally sensitive to interferon and they can evade this host defence mechanism if they fail to induce interferon in the host or if they are resistant to the action of interferon. There are a number of approaches whereby viruses can disrupt the IFN response. These include: (i) interfering with host gene expression and/or protein synthesis, e.g. the NSs protein of Bunyamwera virus inhibits cellular mRNA transcription by interfering with RNA

polymerase; (ii) inhibiting IFN signalling, e.g. soluble IFN receptors that mimic αβIFN receptors (poxviruses), and inhibition of signalling through STAT 1 and 2 rapid degradation of STATs (HSV, NDV (Newcastle Disease Virus), etc.); (iii) blocking the action of IFN-induced antiviral enzymes, e.g. prevents the IFN-induced phosphorylation of eIF2α, so inhibiting the initiation of translation and blocks the activation of a dsRNA-dependent protein kinase (PKR – an interferon-induced enzyme) by producing a protein that competes for dsRNA (pox viruses).

Influenza encodes a 230 aa protein called NS1 which has a multifunctional role in subverting IFN responses. NS1 is a major virulence determinant associated with the highly pathogenic avian influenza viruses (see Chapter 11). It disrupts the intracellular antiviral activity of PKR and 2′5′-oligoadenylate synthetase/RNAse; it suppresses apoptosis, interferes with viral morphogenesis and also impacts on viral mRNA translation.

There are a few persistent virus infections in which interferon is not induced. Mice persistently infected with LCM or leukaemia virus do not produce detectable interferon in spite of the continued multiplication of virus. This is also true of mouse cells infected *in vitro* with these viruses, although virus multiplication is readily inhibited when interferon is added. The infected mice form interferon normally when infected with other viruses, so the defect is specifically in relation to these particular viruses. Mink infected with Aleutian disease virus fail to respond with interferon production, although they give normal responses to other interferon inducers. Here there is an additional feature, because the infecting virus appears to be insensitive to the action of interferon. Presumably the cells of mice have difficulty in recognising LCM or leukaemia virus nucleic acid as foreign, but nothing is known about this. Some persistent viruses such as human adenoviruses tend to be insensitive to interferon, and others (e.g. hepatitis B) are poor inducers of interferon. The fact that many viruses have evolved mechanisms for evading interferons (inducing less, or becoming insensitive to its action) suggests in itself that interferons are an important part of host defences. Mice with experimentally disrupted IFN-α/β receptor genes are highly susceptible to many viruses (e.g. vesicular stomatitis virus (VSV), Semliki Forest virus, herpesviruses).

ANTIGENIC VARIATION

One way in which microorganisms can avoid the antimicrobial consequences of the immune response is by periodically changing their antigens. They present a moving target to the immune response.

Antigenic Variation Within the Infected Individual

This happens in a few bacterial and protozoal infections where it is an important factor promoting their persistence in the body. The spirochaetal microorganism *Borrelia recurrentis* is transmitted from person to person by the body louse and causes relapsing fever. After infection, the bacteria multiply and cause a febrile illness until the onset of the immune response a week or so later. Bacteria then disappear from the blood because of antibody-mediated lysis or agglutination, and the fever falls. But antigenically distinct

mutant bacteria then arise in the infected individual so that 4–10 days later bacteria reappear in blood and there is another febrile episode, until this in turn is terminated by the appearance of a new set of specific antibodies. Ensuing attacks become progressively less severe, but there may be up to ten of them before final recovery. The disease is called relapsing fever because of the repeated febrile episodes, each caused by a newly emerging antigenic variant of the infecting bacterium.

Some protozoa have a similar antigenic versatility. Sleeping sickness is a disease of man in Africa caused by parasitic protozoa of the *Trypanosoma brucei* group and is spread by biting (tsetse) flies. The infection spreads systemically to the lymph nodes and blood and is characterised by recurrent fever and headache. In the later stages, the central nervous system is involved to give chronic meningoencephalitis, occasionally with the condition of lethargy from which the disease gets its name. The surface coat of the trypanosome is 12–15 μm thick and is composed of carbohydrate and a glycoprotein of molecular weight 65,000. During the infection, an antigenically new coat is produced spontaneously in about 1 in 10,000 trypanosomes, to give a series of antigenic variants. These arise by changes in gene expression rather than by mutation, and a single clone of trypanosomes can express hundreds of variants. The systemic stage of the infection consists of a series of parasitaemic waves, each wave being antigenically different from preceding and successive waves. During this time the immune system is constantly trying to catch up, as it were, with the trypanosomes. A large part (about 10%) of the genome of the trypanosome is taken up with the different surface coat genes, but this is a worthwhile investment for the parasite, allowing it to stay for long periods in the blood and also offering a dismal outlook for a vaccine.

Parasitic worms have even greater opportunities for this type of hide-and-seek with the immune response. Schistosomiasis is a common disease of man in Egypt and elsewhere in Africa, caused by trematodes (flukes) of the genus *Schistosoma*. There is a larval stage of the parasite in the blood, and the adult worm lives in the veins around the bladder and rectum, causing frequent, painful and bloody urination. The adult worm liberates antigens into the blood, and although the antibodies formed are effective against new larval invaders, they have no effect on the adult because it is safely covered with a layer of host antigens. This ensures that the adult worms remain few in number and prevents overcrowding in the host.

Antigen variation in *Neisseria gonorrhoeae* contributes to the pathogenicity of this resourceful parasite. During the initial stages of infection, adherence to epithelial cells of the cervix or urethra is mediated by pili, but equally efficient attachment to phagocytes would be undesirable. Hence rapid switching on and off of the genes controlling pili is necessary at different stages of the infection. Changes are also seen in the expression of the outer-membrane proteins of the bacteria. Finely tuned control of expression of the genes for pili and outer-membrane proteins, giving changes in adherence to different host cells, in resistance to cervical proteolytic enzymes, in cytotoxicity, etc., are presumably necessary for successful infection, spread through the body, growth and shedding of these bacteria.

The different strains of gonococci circulating in the community also show great antigenic variation in pili and in outer-membrane proteins, which helps account for the multiple attacks of gonorrhoea that can occur in an individual. *Pilin*, the protein subunit of the pili, consists of constant, variable and hypervariable regions (analogous with immunoglobulin molecules) and genetic rearrangements and recombinations occurring in the repertoire of pilin genes forms the basis for the antigenic variation.

The above microorganisms are complex enough to be capable of undergoing a series of antigenic variations during the course of a single infection. The lentiviruses (visna in sheep, equine infectious anaemia in horses and HIV in man) cause persistent infections of long duration and show antigenic variation within a given infected individual. In HIV, replication errors, like those of other RNA viruses, are not checked because there are no proofreading mechanisms. Consequently, mutations can arise in key antigenic molecules such as gp120 leading to evasion of antibody defences. The HIV gp120 mutations are more extensive than say those in the H and N of influenza virus, probably because there is more opportunity for these to occur; HIV replicates more widely in the body than influenza virus and for a longer period. Mutations can also appear in viral proteins recognised by CD8 T cells, resulting in (a) failure of key antigenic peptides to bind to MHC class I molecules or (b) loss of a critical amino acid involved in binding peptide to the T-cell receptor. This could influence the existing repertoire of memory T cells leading to a failure in immune surveillance and favouring the emergence of new antigenic variants within the host. For many viruses, antigenic variation occurs within one individual, but the variants are rarely seen in a second individual.

Antigenic Variation at the Population Level

When antigenic variants are transmitted, however, they can accumulate as the virus spreads in the host community, so that eventually a strain is formed that differs antigenically from the original to such an extent that it can come back and re-infect the population. This occurs especially with infections limited to mucosal surfaces, where resistance is often of limited duration (see Chapter 6) and there is a strong selective advantage for virus strains with altered antigenicity. The time between initial infection and shedding is only a few days, and an antigenically altered virus variant can infect, replicate and be shed from the body before a significant local secondary immune response is generated. In contrast to this, re-infection with viruses such as rubella, measles or mumps, which cause systemic infection, is less likely. The incubation period is two to three times as long as in a respiratory virus infection, and the secondary immune response has time to come into action and prevent the spread of infection through the body (Figure 6.4). The growth of virus in the skin and respiratory tract that occurs late in the incubation period is therefore prevented, and there is no shedding of virus to the exterior. Partly for these reasons, systemic viruses such as rubella, measles, poliomyelitis or mumps tend to be of uniform character (monotypic) antigenically, and all known isolates worldwide are neutralised by antiserum produced against any other isolate.

The significance of antigenic variation is well illustrated by influenza viruses. Both influenza A and influenza B viruses evolve continuously, undergoing small antigenic changes due to point mutations, deletions and insertions, as the virus spreads through the community. This operates on a worldwide scale and is called antigenic drift. As new variants appear, they replace the previous variant, so that within about four years a given individual can be re-infected with an antigenic variant that has been gradually generated by infection of other individuals. This results in local epidemics. Foot and mouth disease virus also evolves by antigenic drift. Influenza A virus, however, shows in addition

'antigenic shift', which results in the creation of new pandemic strains. These probably occur by genetic recombination between human and animal virus strains in a doubly infected host. It is thought that the animal virus 'reservoir' consists of populations of susceptible birds (mainly sea birds and ducks), which are known to harbour their own strains of influenza A virus. Recombinants between different type A influenza viruses are readily formed because the influenza virus genome consists of eight segments which can re-assort independently of each other. To be of pandemic potential, antigenic shift must involve the RNAs encoding one or both of the surface components of the virus, either haemagglutinin (H) or neuraminidase (N), but any of the RNAs can be shifted. Very occasionally the recombinant virus shows major antigenic differences from previous human influenza A virus strains, having H or N antigens of bird origin, and is at the same time capable of infecting and being efficiently transmitted in man. Initial infection of man with the new strain perhaps takes place in parts of the world where people live in close association with domestic birds. The entire world's population, with no previous immunological experience of such a virus, is completely susceptible. In modern times, pandemic strains of influenza A virus have arisen to give major global outbreaks in 1918, 1957 (Asian flu) and 1968 (Hong Kong flu). All have originated in Asia. Influenza occurs only in the winter and the new shift variants spread from the Southern Hemisphere winter in June northwards to peak around December in the Northern Hemisphere. Inevitably some infected individuals on one side of the world will carry the infection to the opposite hemisphere, but efficient person-to-person spread and an epidemic will have to wait until the winter. Pandemic strains are designated according to the H or N antigens (Table 7.2). Although antigenic shift causes a dramatic increase in the amount of influenza, it is a comparatively rare event, and antigenic drift is in fact responsible for a far greater number of influenza epidemics. Monitoring of the antigenic evolution of influenza A and B viruses is carried out by local, national and international laboratories coordinated by the World Health Organization (WHO). It is the responsibility of the WHO to advise the vaccine manufacturers when a significantly different strain of influenza has emerged.

As a viral adaptation for the overcoming of host immunity, antigenic variation is more likely to be important in longer-lived species such as the horse or man, where there is a need

TABLE 7.2 Pandemic Strains of Influenza Virus in Modern Times

Time of Pandemic	Strain Designation
1918–1919[a]	H1N1
1957–1958 ('Asian flu')	H2N2
1968–1969 ('Hong Kong flu')	H3N2
2009–2010 (swine flu 2009)	H1N1

H, haemagglutinin; N, neuraminidase.
[a]RNA sequences were identified from the HA of the 1918–1919 virus. This was done from an Alaskan victim of the epidemic, using paraffin-embedded lung sections and the actual lung of a corpse frozen in the permafrost. The sequence has been useful for reconstructing the virus and testing in mice. It has a similar pathology to that caused by H5N1. However, why this virus causes increased mortality in 15–45 year olds remains a mystery.

for multiple re-infection during an individual's lifetime if the virus is to remain in circulation, and if the virus does not have the ability to become latent (Chapter 10). In shorter-lived animals such as chickens, mice or rabbits, on the other hand, populations renew themselves rapidly, and fresh sets of uninfected individuals appear fast enough to maintain the infectious cycle. Human respiratory viruses are among the most successful animal viruses in the world. Some show regular antigenic variation and in others local immune responses are weak. Because of assured increases in human numbers and density, these viruses are perhaps entering their golden age, with an almost unlimited supply of susceptible hosts in the foreseeable future, and poor chances of control by vaccination (see Chapter 12). In the past 10 years, we have witnessed the emergence of some particularly aggressive strains of avian influenza A viruses: of these the N5N1 and H7N9 viruses both appear to have been transmitted directly from poultry to humans, although they have not been shown to transmit from human to human. Interestingly, the H7N9 virus does not cause disease in chickens making it doubly difficult to track the spread of the virus to humans.

The bacteria responsible for superficial infections also tend to show something similar to immunological drift, with the appearance of new variants or subtypes that can re-infect the individual. Staphylococci and streptococci, for instance, exist in a great variety of antigenic types and can perhaps be regarded as antigenic drift. Some of the 150 surface proteins of *M. pneumoniae* show great antigenic variation. Among the intestinal bacteria, *E. coli* shows a similar antigenic variety, but it is not clear that this has the immunological significance suggested. Just as with virus infections, the bacteria that cause systemic infections are relatively conservative, antigenically speaking and tend to be monotypic in type, as for instance with plague, tuberculosis, syphilis, typhoid, etc.

A summary of microbial interference with or avoidance of immune defences is set out in Table 7.3.

TABLE 7.3 Microbial Interference with or Avoidance of Immune Defences

Type of Interference/Avoidance		Mechanism	Example	Status
Induction of	Ineffective antibody	Antibody of poor specificity or affinity fails to neutralise or opsonise	LCM virus *T. pallidum*	+ +
	Blocking antibody	Ineffective antibody bound to microbe blocks action of 'good' antibody or immune cells	Disseminated gonorrhoea?	±
	Enhancing antibody	Antibody bound to microbe enhances infection of phagocyte by attaching to Fc receptor	Dengue virus	++
	No antibody	No neutralising antibody	African swine fever virus	++
Destruction of antibody		Liberation of IgA protease	Gonococcus	+
			H. influenzae	+
			Streptococci	+

(*Continued*)

TABLE 7.3 (Continued)

Type of Interference/Avoidance	Mechanism	Example	Status
Switch-on of T cells or of B cells non-specifically, non-productively	Polyclonal activation of T cells (by superantigen) of B cells	Staphylococcal toxins (see Chapter 8)	+
		Epstein–Barr virus	±
		M. pneumonia	±
Antigenic variation	Microbial antigens vary within individual host	Trypanosomiasis	++
		Relapsing fever	++
		HIV	++
	Microbial antigens vary within host population	Influenza virus	++
		Streptococci	+
Infection in bodily site inaccessible to antibody and immune cells	Persistent infection of glands, etc. inaccessible to circulating antibody and immune cells (see Figure 7.1)	Cytomegalovirus	++
		Rabies virus	++
		Marek's disease virus	++
'Silent' infection of host cell without making it vulnerable to immune lysis	Failure to display microbial antigen on infected cell surface	Herpes simplex virus	++
		Epstein–Barr virus	++
	Loss of microbial antigen by capping	Measles virus	+
Fc receptors present on microbe or induced on infected host cell	IgG antibodies non-specifically bind to microbe or infected cell in 'upside down' position and block immune lysis/ opsonisation, etc.	Staphylococci (protein A)	+
		Certain streptococci	+
		Herpes simplex virus	?
		Cytomegalovirus	?
Induction of immunological tolerance and antigen-mediated suppression of T and B cells	Involves clonal deletion or clonal anergy of T or B cells, and induction of Treg cells leading to suppression of T-cell responses	LCM virus	+
		BVDV	++
		Lepromatous leprosy	?
		Hepatitis B and C viruses	?
Antibodies mopped up by microbial antigens	Microbial surface antigens in extracellular fluids combine with and 'divert' antibodies	Hepatitis B virus	?
		Pneumococcal infection	?
Molecular mimicry	Microbial antigens mimic host antigens, leading to poor antibody response	*M. pneumonia*	?
Lack of recognition by T cells	Interference with the expression of MHC molecules	Adenovirus	+
		Cytomegalovirus	+
		Vaccinia	+

(*Continued*)

TABLE 7.3 (Continued)

Type of Interference/Avoidance	Mechanism	Example	Status
		Herpes simplex virus	+
		Vaccinia virus	+
Inhibition of cytokines, chemokines and complement proteins	Synthesis of 'imitation' cytokines or of soluble receptor (for IL-1, IL-8, TNF, IFN-γ, C3b) by viruses	Kaposi's sarcoma herpes virus Herpes simplex (C3b rec)	+ + +
Prevention of interferon action	Synthesis of intracellular protein inhibitors	Vaccinia virus Adenovirus	+ +
	Induce little or no interferon	LCM virus	±
Concealment of antigenic site	Mutation to provide a nearby glycosylation site	Influenza A virus Rabies virus	++ ?

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CHAPTER

8

Mechanisms of Cell and Tissue Damage

OUTLINE

The damage inflicted by pathogens on their hosts is the result of direct and indirect collateral effects resulting from the activity of virulence factors performing specific functions involved in pathogenesis. It is the different types of damage caused which result in the symptoms of disease which allow diagnosis and implementation of appropriate treatment and control measures. The impact on the host of microbial damage depends very much on the tissue involved. Damage to muscle in the shoulder or stomach wall, for instance, may not be serious, but in the heart the very existence of the host depends on a strong muscle contraction continuing to occur every second or so, and here the effect of minor functional changes may be catastrophic. The central nervous system (CNS) is particularly vulnerable even to slight damage. The passage of nerve impulses requires normal function in the neuronal cell membrane, and viruses especially have important effects on cell membranes. Also a degree of cellular or tissue oedema that is tolerable in most tissues may have serious consequences if it occurs in the brain, enclosed in that more or less rigid box, the skull. Therefore, encephalitis and meningitis tend to cause more severe illness than might be expected from the histological changes themselves. Oedema is a serious matter also in the lung. Oedema fluid or inflammatory cell exudates appear first in the space between the

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alveolar capillary and the alveolar wall, decreasing the efficiency of gaseous exchanges. Respiratory function is more drastically impaired when fluid or cells accumulate in the alveolar air space. The effect of tissue damage is much less in the case of organs, such as the liver, pancreas or kidney, which have considerable functional reserves. More than two-thirds of the liver must be removed before there are signs of liver dysfunction.

Cell damage has profound effects if the endothelial cells of small blood vessels are involved. The resulting circulatory changes may lead to anoxia or necrosis in the tissues supplied by these vessels. Here too, the site of vascular lesions may be critical, effects on organs such as the brain or heart having a greater impact on the host, as discussed above. Rickettsiae characteristically grow in vascular endothelium and this is an important mechanism of disease production. By a combination of direct and immunopathological factors, there is endothelial swelling, thrombosis, infarcts, haemorrhage and tissue anoxia. This is especially notable in the skin and forms the basis for the striking rashes in typhus and the spotted fevers. These skin rashes, although important for the physician, are less important for the patient than similar lesions in the CNS or heart. It is damage to cerebral vessels that accounts for the cerebral disturbances in typhus; involvement of pulmonary vessels causes pneumonitis, and involvement of myocardial vessels causes myocardial oedema. For example, in Q fever, rickettsiae sometimes localise in the endocardium, and this causes serious complications.

Sometimes an infectious agent damages an organ, and loss of function in this organ leads to a series of secondary disease features. The signs of liver dysfunction are an accepted result of infections of the liver, just as paralysis or coma is an accepted result of infection of the CNS.

There are many diseases of unknown aetiology for which an infectious origin has been suggested. Sometimes it is fairly well established that an infectious agent can at least be one of the causes of the disease, but in most instances it is no more than a hypothesis, with little or no good evidence. For conditions as common and as serious as multiple sclerosis, cancer and rheumatoid arthritis, it would be of immense importance if a microorganism were incriminated, since this would give the opportunity to prevent the disease by vaccination or treat it with anti-microbials. For example, Borna disease (BD) has classically been described as a chronic, progressive meningoencephalomyelitis, causing both neurological and behavioural symptoms in horses and sheep. Experimental infection of tree shrews (*Tupaia glis*) with BD virus however results in very little overt disease, but afterwards the male is no longer able to enact the ritual courtship behaviour, which (as students well know) is an essential preliminary to mating in all primates. Thus it can be said that infection with BD virus renders the male psychologically sterile. Presumably the virus in some way alters the functioning of neurons concerned in this particular pathway. All other behavioural and physiological aspects appear normal. BD virus is not known to occur in man, but speculation about an analogous human situation is fuelled by the finding of BD virus-specific antibodies in patients with psychiatric/behavioural disorders. Since the aetiology of such diseases raises interesting problems in pathogenesis, the present state of affairs is summarised in Table 8.1, which includes some of the human diseases whose infectious origin is probable, possible, conceivable or inconceivable.

Causal connections between infection and disease states are particularly difficult to establish when the disease appears a long time after infection. It was not too difficult to

TABLE 8.1 Microorganisms Postulated as Causes of Human Diseases of Uncertain Aetiology

Disease	Features	Microorganism	Pathogenic Mechanism	Comments
Juvenile diabetes	Onset early in life; sensitive to insulin	Coxsackie B viruses	Infection and damage of islets of Langerhans; secondary immune phenomena	Accounts for some cases
		Mumps Rotavirus Rubella		No direct evidence Late result congenital rubella
Crohn's disease	Granulomatous inflammation of intestine	Mycobacteria *E. coli* Viruses	Not clear; secondary immune phenomena	No good evidence
Ulcerative colitis	Inflammation of colon	Viruses	Not clear; secondary immune phenomena	No good evidence
Multiple sclerosis	Demyelinating disease of CNS. Waxes and wanes	Epstein–Barr, HHV6	Autoimmunity triggered by presentation of brain autoantigens in the envelope of a succession of different viruses	Epidemiological link with Epstein–Barr virus
Rheumatoid arthritis	Chronic inflammation and damage to joints	Mycoplasmas		Cause arthritis in animals but no evidence for man
		Viruses (Epstein–Barr, rubella, parvovirus B 19)		No good evidence
Paget's disease of bone	Localised deformation of bone	Measles virus	Persistent infection of osteoclasts P62, IL-6 induction	Good evidence
Ankylosing spondylitis	Chronic arthritis of spine	*Klebsiella* spp.	Immune response to bacterial antigen cross-reacts with joint antigen, giving autoimmune damage	Strong association with HLA B27 genotype
Chronic fatigue syndrome	Tiredness, muscle weakness, lasting months or years	Epstein–Barr virus? HHV6, etc.	Unknown Upset of hypothalamic–adrenal axis	Some cases
Alzheimer's disease	Presenile (<55 years) dementia	'Slow virus'?	Infectious agent replicates slowly in brain, destroying cells	Some cases?
Senile dementia	Loss of neurons; very common at 65 + years			No evidence

(*Continued*)

TABLE 8.1 (Continued)

Disease	Features	Microorganism	Pathogenic Mechanism	Comments
CANCER				
Carcinomas	Nasopharyngeal carcinoma	Epstein–Barr virus	Transformation of epithelial cell	Susceptibility gene in Chinese people
	Cervical/penile carcinoma	Papillomaviruses	Transformation of epithelial cell	Associated with sexual promiscuity
	Carcinoma of liver	Hepatitis B virus	Transformation of hepatic cell	Liver cancer especially common in those with persistent Hepatitis B infection
	Skin cancer (basal cell carcinoma)	Papillomaviruses	Ultraviolet light as co-carcinogen	Evidence in animals but so far not in humans
	Stomach cancer	*H. pylori*	Chronic inflammation	Association (in small proportion of cases) is with chronic gastritis and ulcer (role of host genes, diet, cofactors?)
Lymphomas	Burkitt's lymphoma	Epstein–Barr virus Malaria	Transformation of B lymphocyte plus cofactor	Strong evidence
	Hodgkin's disease	Epstein–Barr virus	Transformation of B lymphocyte	Possible early role in HL-induction in some patients
Leukaemias		Retroviruses	Transformation of white cell precursor	Cause leukaemia in animals, and certain T-cell leukaemias in humans (HTLV 1 and 2)

prove and accept that the encephalitis that occasionally occurs during or immediately after measles was due to measles virus. But it was hard to accept that a very rare type of encephalitis (subacute sclerosing panencephalitis or SSPE), occurring up to 10 years after apparently complete recovery from measles, was also due to measles virus and this was only established after the eventual isolation of a mutant form of measles virus from brain cells. 'Slow' infections, in which the first signs of disease appear a long time after infection, are now an accepted part of our outlook. The disease Kuru occurred in New Guinea and was transmitted from person to person by ritual cannibalism. The incubation period in man appears to be 12–15 years, and the disease was caused by an infectious proteinaceous agent known as a prion that grew in the brain. This was established when the same disease appeared in monkeys several years after the injection of material from the brain of Kuru patients. A similar agent termed the scrapie agent infects sheep, cattle, mice and other animals and also has an incubation period representing a large portion of the

lifespan of the host. In both Kuru and SSPE, the agent was eventually shown to be present in the brains of patients. If in a slow infection, the microorganism that initiated the pathological process is no longer present by the time the disease becomes manifest, then the problem of establishing a causal relationship will be much greater. This may possibly turn out to be true for diseases like multiple sclerosis and rheumatoid arthritis. Liver cancer in humans and certain leukaemias in mice, cats, humans and cattle can be caused by slow-type virus infections. Cancer or leukaemia appears as a late and occasional sequel to infection. The virus, its antigens or fragments of its nucleic acid are often detectable in malignant cells.

One important factor that often controls the speed of an infectious process and the type of host response is the rate of multiplication of a microorganism. Often the rate of multiplication in the infected host, in the presence of anti-microbial and other limiting factors, and when many bacteria are obliged to multiply inside phagocytic cells, is much less than the optimal rate in artificial culture. A microorganism with a doubling time of a day or two will tend to cause a more slowly evolving infection and disease than one that doubles in an hour or less (Table 8.2).

It is uncommon for an infectious agent to cause exactly the same disease in all those infected. Its nature and severity will depend on infecting dose and route, and on the host's age, sex, nutritional status, genetic background and so on (see Chapter 11). Many infections are asymptomatic in more than 90% of individuals, clinically characterised disease occurring in only an occasional unfortunate host, as 'the tip of the iceberg'. Asymptomatically infected individuals who may continue to shed pathogen are important because they are not identified, move normally in the community, and play an important part in transmission.

TABLE 8.2 Growth Rates of Microorganisms Expressed as Doubling Times

Microorganisms	Situation	Mean Doubling Time
E. coli, staphylococci, streptococci, etc.	*In vitro*	20–30 min
S. typhimurium	Mouse spleen	5–12 h
	In vitro	30 min
Tubercle bacillus	*In vitro*	24 h
	In vivo	Many days
FUNGI		
C. albicans	*In vitro* (37°C)	30 min
Dermatophytes	*In vitro* (28°C)	1–24 h
T. pallidum	*In vivo* (rabbit)[a]	30 h
Leprosy bacillus	*In vivo*[a]	2 weeks
P. falciparum	*In vivo* or *in vitro*[b]	8 h

[a]Cannot be cultivated *in vitro*.
[b]Erythrocyte or hepatic cell.

This chapter deals with demonstrable cell and tissue damage or dysfunction in infectious diseases. But one of the earliest indications of illness is malaise, or 'not feeling very well'. This is distinct from fever or a specific complaint such as a sore throat and, although it is difficult to define and impossible to measure, we all know the feeling. It can precede the onset of more specific signs and symptoms, or accompany them. Sometimes it is the only indication that an infection is taking place but almost nothing is known of the basis for this feeling. 'Toxins', of course, have been invoked and the early response to pyrogens before body temperature has actually risen and may play a part. Interferons may have something to do with it because pure preparations of human α- or β-interferons cause malaise and often headaches, and muscle aches after injection into normal individuals. Soluble mediators of immune and inflammatory responses, such as interleukin-1 (IL-1; see Glossary) or other cytokines doubtless also play a part. Several cytokines induce release of prostaglandin E2 which, in addition to its effect on fever, reduces the pain threshold in neurons, and this could account for aches and pains.

INFECTION WITH NO CELL OR TISSUE DAMAGE

Before giving an account of the mechanisms by which pathogens induce damage in the host, it is important to remember that many infectious agents cause little or no damage. Indeed, it is of some advantage to the microorganism to cause minimal host damage, as discussed in Chapter 1. Many virus infections fall into this category. Thus, although infection with rabies or measles viruses nearly always causes disease, there are many enterovirus, reovirus and myxovirus infections that are typically asymptomatic. Even viruses that are named for their common association with disease (poliomyelitis, influenza, Japanese encephalitis) may also be associated with infections in which an antibody response is the only sign of the presence of the pathogen, and tissue damage is too slight to cause detectable illness. There is a tendency for persistent viruses to cause no more than minor or delayed cellular damage during their persistence in the body, even if the same virus has a more cytopathic effect during an acute infection, e.g. adenoviruses and herpes simplex (see Chapter 10). A few viruses are remarkable because they cause no pathological changes at all in the cell, even during a productive infection in which infectious virus particles are produced. For instance, mouse cells infected with lymphocytic choriomeningitis (LCM) (see Glossary) or murine leukaemia virus show no pathological changes. The recently identified Torque Tenoviruses (TTVs) are ubiquitous in the human population and appear to establish persistent infections; however, no concrete association with any disease has been demonstrated. Throughout the life of the animal, virus and viral antigens are produced in the cerebellum, liver, retina, etc. without discernible effect on cell function. But sometimes there are important functional changes in infected cells which lead to a pathological result. For example, the virus infects growth-hormone-producing cells in the anterior pituitary. Although the cells appear perfectly healthy, the output of growth hormone is reduced, and as a result of this, suckling mice fail to gain weight normally and are runted.

As discussed previously, there are many millions of commensal bacteria which make up the microbiota, which serve important functions for their host. Of course, these bacteria

are not typically involved in causing damage. Bacteria such as meningococci and pneumococci, whose names imply pathogenicity, spend most of their time as harmless inhabitants of the normal human nasopharynx: only occasionally do they have the opportunity to invade tissues and give rise to meningitis or pneumonia. However, when bacteria invade tissues, they almost inevitably cause some damage, and this is also true for fungi and protozoa. Some of the damage may not be severe in nature. For example, *Treponema pallidum* produces no toxins, does not cause fever and attaches to cells *in vitro* without harmful effects. Leprosy and tubercle bacilli eventually damage and kill the macrophages in which they replicate, but pathological changes are to a large extent caused by indirect mechanisms (see below). In patients with untreated lepromatous leprosy, the bacteria in the skin invade blood vessels, and large numbers of bacteria, many of them free, may be found in the blood. In spite of the continued presence of up to 10^5 bacteria/ml of blood, there are no signs or symptoms of septicaemia or toxaemia. *Mycobacterium leprae* can be regarded as a very successful parasite that induces very little host response in these patients, even when the bloodstream is invaded.

DIRECT DAMAGE BY MICROORGANISMS

Cell and tissue damages are sometimes due to the direct local action of the microorganism. However, in many cases it is not clear how the death of cells results from virus infection. Virus infections result in a shutdown of RNA synthesis (transcription), protein synthesis (translation) and DNA synthesis in the host cell, but often these are too slow to account for the death of the cell. After all, cells like neurons never synthesise DNA, and the half-life of most proteins and even RNAs is at least several hours. A possible alternative mechanism is the alteration of the differential permeability of the plasma membrane. This is important as the cell has a high internal K^+ concentration and low Na^+ concentration, while the reverse is true of body fluids. Viruses do alter membrane permeability, but the unresolved question is whether or not this is responsible for the death of the cell or whether it is merely an after effect.

In many virus infections (including human immunodeficiency virus (HIV), adenoviruses, herpesviruses, influenza virus and picornaviruses), the cells commit suicide by a mechanism called 'programmed cell death' or 'apoptosis'. This is the natural process by which the body controls cell numbers and rids itself of superfluous or redundant cells during development. Cells do not disintegrate but round up and are then removed by phagocytes. Apoptosis in virus infections can be regarded as a host strategy for destroying infected cells. The chromatin condenses round the edge of the nucleus and a cellular endonuclease cleaves the DNA into 180–200 base pair fragments. The cell membrane forms blebs but stays intact while the cell as a whole breaks up into smaller bodies. The suicide process is more controlled, almost more dignified, than mere disintegration and necrosis. In the latter there is early loss of membrane integrity, spillage of cell contents and random break-up of DNA.

Some viruses encode proteins whose function is to inhibit apoptosis, so allowing the virus to replicate and new virions to be produced before the cell dies. Conversely some viruses appear to induce apoptosis, perhaps as a means of evading the immune response; apoptotic cells are not efficiently recognised by the immune system.

There are two more characteristic types of morphological change produced by certain viruses, and these were recognised by histologists more than 50 years ago. The first are inclusion bodies, parts of the cell with altered staining behaviour which develop during infection. They often represent either cell organelles or virus factories in which viral proteins and/or nucleic acids are being synthesised and assembled. Herpes group viruses form intranuclear inclusions, rabies and poxviruses intracytoplasmic inclusions, and measles virus both intranuclear and intracytoplasmic inclusions. The second characteristic morphological change caused by viruses is the formation of multinucleate giant cells. This occurs, for instance, when HIV 'fusion' proteins (gp120−gp41) present on the surface of an infected cell attach to CD4 receptors in the plasma membranes of neighbouring cells; membranes then fuse and multinucleate cells are formed. This fusion mimics the fusion event that occurs when an enveloped virus binds to the surface of an uninfected cell and the virus membrane and cellular membranes fuse, so allowing entry of the virus genome and proteins to the cell. This cell−cell fusion can also be observed following infection with paramyxovirus (measles, respiratory syncytial virus (RSV)) and certain herpes viruses.

Before leaving the subject of direct damage by viruses, one supreme example will be given. Here the direct damage is of such a magnitude that the susceptible host dies a mere 6 h after infection. If Rift Valley Fever virus, an arthropod-borne virus infecting cattle, sheep and man in Africa, is injected in very large doses intravenously into mice, the injected virus rapidly infects nearly all hepatic cells. Hepatic cells show nuclear inclusions within an hour and necrosis by four hours. As the single cycle of growth in hepatic cells is completed, massive liver necrosis takes place, and mice die only 6 h after initial infection. The host defences in the form of local lymph nodes, local tissue phagocytes, etc. are completely overcome by the intravenous route of injection, and by the inability of Kupffer cells to prevent infection of hepatic cells. Direct damage by the replicating virus destroys hepatic cells long before immune or interferon responses have an opportunity to control the infection. The experimental situation is artificial, but it illustrates direct and lethal damage to host tissues after all host defence mechanisms have been overwhelmed.

Most rickettsiae and *Chlamydia* damage the cells in which they replicate, and it is possible that some of this damage is due to the action of toxic microbial products. This action, however, is confined to the infected cell, and toxic microbial products are not liberated to damage other cells. Mycoplasma (see Table A.3) can grow in special cell-free media, but in the infected individual they generally multiply while attached to the surface of host cells. As studied in culture and on the respiratory epithelium, they 'burrow' down between cells, inhibit the beat of cilia and cause cell necrosis and detachment. The mechanism is not clear. If a complete lawn of mycoplasma covers the surface of the host cell, some effect on the health of the cell is to be expected, but it is possible that toxic materials are produced or are present on the surface of the mycoplasma.

Intracellular bacterial pathogens generally damage the cells in which they replicate (see Chapter 4). *Listeria*, *Brucella* and *Mycobacteria* are specialists at intracellular growth, and the infected phagocyte is slowly destroyed as increasing numbers of bacteria are produced in it. Bacteria such as staphylococci and streptococci grow primarily in extracellular fluids but can also invade and proliferate in epithelial and endothelial cells. They are also ingested by phagocytic cells, and virulent strains of bacteria in particular have the ability to destroy the phagocyte in which they find themselves or can avoid killing and proliferate

within the phagocytes, as described in Chapter 4. Many bacteria cause extensive tissue damage by the liberation of toxins into extracellular fluids. Various toxins have been identified and characterised. Most act locally, but a few cause pathological changes after spreading systemically through the body.

Dental caries provides an interesting example of direct pathological action. Colonisation of the tooth surface by *Streptococcus mutans* leads to plaque formation, and the bacteria held in the plaque utilise dietary sugar and produce acid. Locally produced acid decalcifies the tooth to give caries. Caries, arguably the commonest infectious disease of Western man, might logically be controlled by removing plaque, withholding dietary sugar, or vaccinating against *S. mutans.* However, fluoride in the water supply or in toothpaste has been the method of choice and has been very successful. It acts by making teeth more resistant to acid.

MICROBIAL TOXINS

This is a huge and growing part of our subject and we need to define the term toxin, a task which is more difficult than one might think. An attempt was made by Bonventre who in 1970 defined toxins as a 'special class' of poisons which differ from, for example, cyanide or mercury by virtue of their microbial origin, protein structure, high molecular weight and antigenicity. This view is too embracing, because it includes proteins of doubtful significance in disease and also too restrictive, because it excludes non-protein toxic complexes such as endotoxin. Another suggestion is that toxin must include all naturally occurring substances (of plant, animal, bacterial or whatever origin) which, when introduced into a foreign host, are adverse to the well-being or life of the victim. This, too, is unsatisfactory because some substances – potent toxins within the scope of this definition – are being used in some contexts as therapeutic agents! Perhaps it is pointless to strive for an all-embracing definition, although the obvious differences between bacterial and fungal toxins warrant the continued use of the appropriate prefix. For example, bacterial toxins are usually of high molecular weight and hence antigenic, whereas fungal toxins tend to be low molecular weight and not antigenic.

The problem of definition is compounded because there are substances (aggressins) which help to establish an infective focus as well as those whose action is uniquely or largely responsible for the disease syndrome. Also there are substances known to be produced by bacteria *in vitro*, whose properties on *a priori* grounds make them potential determinants of disease, but which have not been shown to play a role *in vivo.*

For many toxins, there is considerable understanding of the genetic basis of toxin expression, secretion, assembly and activity, the resolution of the three-dimensional structure of toxins, and their biochemical modes of action. We now know a great deal about the spread of some virulence determinants in bacterial populations via bacteriophages and other transmissible genetic elements, the conditions under which toxins are expressed both *in vitro* and *in vivo*, how to disassemble complex protein toxins and form chimeric derivatives of known and potential use as therapeutic agents, and how to use some of the deadliest poisons known to man in treating certain physiological disorders. Elucidation of biochemical modes of action has resulted in toxins being used increasingly as important

tools for the dissection of cell biological processes. Also, some new insights as to the role(s) of toxins in disease causation have been developed. The latter is the result of using isogenic toxin-deficient mutants *in vivo*, using more relevant biological test systems and concentrating more on the effects of sublethal doses of toxin and less on the effects of injecting a toxin bolus into some animal. It is beyond the scope of this book to attempt to cover all these subjects, so only an outline treatment will be given with some examples.

Protein Toxins

These are either secreted by or released upon lysis from both Gram-positive and Gram-negative bacteria, and historically referred to as exotoxins. They are proteins, some of which are enzymes. When liberated locally they can cause local cell and tissue damage. Those that damage phagocytic cells and are therefore particularly useful to the microorganism have been described in Chapter 4. Those that promote the spread of bacteria in tissues have been referred to in Chapter 5. A selection of some protein toxins follows.

Toxins Which Act Extracellularly

Helicobacter pylori is a specific human pathogen affecting billions of people worldwide. It is transmitted via the orofaecal route and colonises the seemingly inhospitable niche of the stomach. Some 20% of infected patients can develop ulcers or stomach cancer. An essential virulence factor of *H. pylori* is a potent urease which is synthesised in vast quantity by the organism, and (at least in culture) released by autolysis and efficiently absorbed on to the surface of viable organisms. As noted in Chapter 2, it is important in local neutralisation of stomach acidity thereby allowing *H. pylori* to penetrate the protective mucus layer overlying the lining of the stomach where the organism attaches to gastric epithelial cells. However, urease is now considered by some as a toxin which acts outside cells, since NH_3, the product of urease activity, is toxic to cells.

Proteases and *hyaluronidases*, which help the spread of bacteria through tissues, have already been mentioned in Chapter 5. Here we consider toxins which act on extracellular substances and are responsible for many of the main characteristics of the diseases caused by the infecting organism. *Pseudomonas aeruginosa* elastase, and one of at least six proteases of *Legionella pneumophila*, both induce fibrinopurulent exudation in the rat lung (a model for *P. aeruginosa*-induced pneumonia in human cystic fibrosis) and the guinea-pig lung (a model for legionnaires' disease), respectively. These characteristics almost certainly arise from the release of oligopeptides from extracellular matrix components of the host which are chemotactic for leucocytes and fibroblasts. The *L. pneumophila* protease is the same major secretory protein (the zinc metalloprotease) already considered in Chapter 4 in relation to survival within macrophages.

Staphylococcal exfoliative toxins (epidermolytic toxins) are important in staphylococcal bullous impetigo and 'scalded skin syndrome', a disease of newborn babies. The disease is characterised by a region of erythema which usually begins around the mouth and, in 1–2 days, extends over the whole body. The most striking feature of the disease is that the epidermis, although apparently healthy, can be displaced and wrinkled like the skin of a ripe peach by the slightest pressure. Soon large areas of epidermis become lifted by a layer

of serous fluid and peel at the slightest touch. Large areas of the body rapidly become denuded in this way and the symptoms resemble those of massive scalding. The toxin causes cleavage of desmoglein 1, a desmosomal adhesion molecule (desmosomes are specialised cell membrane thickenings through which cells are attached to each other) in the stratum granulosum.

Toxins Which Damage Membranes

Some toxins destroy membranes by virtue of their proteolytic activities, and some by their ability to degrade lipid components, while others are pore-forming or detergent-like in their mode of action.

PROTEASES

In addition to their action on protein components of lung connective tissue referred to above, *P. aeruginosa* elastase and the zinc metalloprotease of *L. pneumophila* are believed to destroy cell membranes by their proteolytic activity. This is the probable reason for the haemorrhage associated with lung infections caused by these pathogens, i.e. effects on type I alveolar epithelial and endothelial cells.

PHOSPHOLIPASES

CLOSTRIDIUM PERFRINGENS α-TOXIN A large number of bacterial enzymes are phospholipases, some of which, but by no means all, are important toxins. A good example is the α-toxin of *C. perfringens*, the organism most commonly associated with gas gangrene. It is strictly anaerobic and occurs as a normal inhabitant in the large intestines of man and animals; its spores are ubiquitous in soil, dust and air. *C. perfringens* does not multiply in healthy tissues but grows rapidly when it reaches devitalised and therefore anaerobic tissues. This could be after contamination of a natural wound with soil or dust, particularly on battlefields or in automobile accidents, or after contamination of a surgical operation site with clostridia from the patient's own bowels or skin. After abortions, particularly in the old days before antibiotics, intestinal clostridia often gained access to necrotic or devitalised tissues in the uterus and set up life-threatening infections. Invasion of the blood was common and soon resulted in death, the clostridia localising and growing in internal organs such as the liver after death. *C. perfringens* has various enzymes that enable it to break down connective tissue materials, including collagen and hyaluronidase, thereby facilitating spread of the infection along tissue planes. Most of these enzymes are toxic to host cells and tissues, but α-toxin is easily the most important one. It is dermonecrotic, haemolytic (a feature seen mainly in tissues close to the focus of infection but sometimes responsible for large-scale intravascular haemolysis in infected patients), causes turbidity in lipoprotein-rich solutions and is lethal. While it is still true that these activities are all due to one molecular species, they are not (as was once thought) different expressions of the one enzymic activity.

Historically, *C. perfringens* α-toxin was the first bacterial toxin to be characterised as an enzyme: it is a zincmetallophospholipase C (PLC) which removes the head group, phosphoryl choline, from phosphatidyl choline and from sphingomyelin. It is of undoubted importance in gas gangrene. Toxoid prepared by formalin-treated toxin will protect sheep against infection caused by *C. perfringens*. The main basis of toxicity is the consequence of the ability of the α-toxin, in sublytic doses, to cause profound metabolic changes arising from release

of phospholipid derivatives. The activation of the arachidonic acid cascade results in the production of leukotrienes (increasing vascular permeability), prostaglandins and thromboxanes (causing inflammation, muscle contraction and platelet aggregation). This toxin also upregulates expression of endothelial leucocyte adhesion molecule-1 (ELAM-1), intercellular adhesion molecule-1 (ICAM-1) and neutrophil chemoattractant-activator IL-8, thereby impairing delivery of phagocytes to the site of infection. Clostridial illness can be mild or very severe according to the extent of bacterial spread, and the quantity of toxins that are formed and absorbed. Since the bacteria grow and produce their toxins only in devitalised tissues, the most important form of treatment is to remove such tissues. Clostridia are strictly anaerobic and exposure of the patient to hyperbaric oxygen (pure oxygen at 2–3 atmospheres in a pressure chamber) has been found useful in addition to chemotherapy.

Staphylococcal β-toxin (haemolysin) is a good example of a sphingomyelinase with an important role in virulence. It has activity on a number of different cell types including erythrocytes and may contribute to iron acquisition by release of haemoglobin from red blood cells. Recently, it has been reported to play a role on biofilm production by *Staphylococcus aureus*. It is noteworthy that in contrast to bovine mastitis strains of *S. aureus*, many human strains contain a bacteriophage encoding immune evasion factors inserted in the β-toxin gene resulting in loss of β-toxin expression. These data imply that beta toxin may be more important in some animal infections in comparison to humans.

PORE-FORMING TOXINS

A variety of bacterial pathogens produce specialised pore-forming toxins with an array of receptors, cell specificities and activities. Here we will discuss some selected examples.

CHOLESTEROL-BINDING CYTOLYSINS This is a large family of pore-forming toxins, also known as 'SH-activated cytolysins', made by many different species of Gram-positive bacteria, not all of which are pathogens. They are lethal, cardiotoxic, antigenically related, and their lytic and lethal activities are blocked by cholesterol. Interaction with cholesterol is thought to be the key primary event in their interaction with susceptible membranes, which leads to the impairment of the latter; cholesterol plays no further part in the subsequent damage process. However, the role of cholesterol has been interpreted in terms of mediating the oligomerisation process (illustrated in Figure 8.1) which leads to membrane damage. Examples of cholesterol-binding cytolysins (CBCs) from pathogenic species include streptolysin O and S made by streptococci, perfringolysin O made by *C. perfringens*, listeriolysin made by *Listeria monocytogenes*, and pneumolysin (PLY) made by *Streptococcus pneumonia.* Despite the similarities which warrant their inclusion in the same toxin group, they play entirely different roles in disease causation by the organisms expressing these toxins. A good example to go into more detail is PLY.

PNEUMOLYSIN

This protein is produced by the pathogen *S. pneumoniae* (pneumococcus) which causes bacteraemia, pneumonia, meningitis and otitis media in humans. PLY is different from all other members of this group in that it is not actively secreted by the pathogen but remains in the cytoplasm until released by lysis of the pneumococcus. This toxin is a four-domain molecule which oligomerises and forms a pore after cholesterol binding. It possesses a

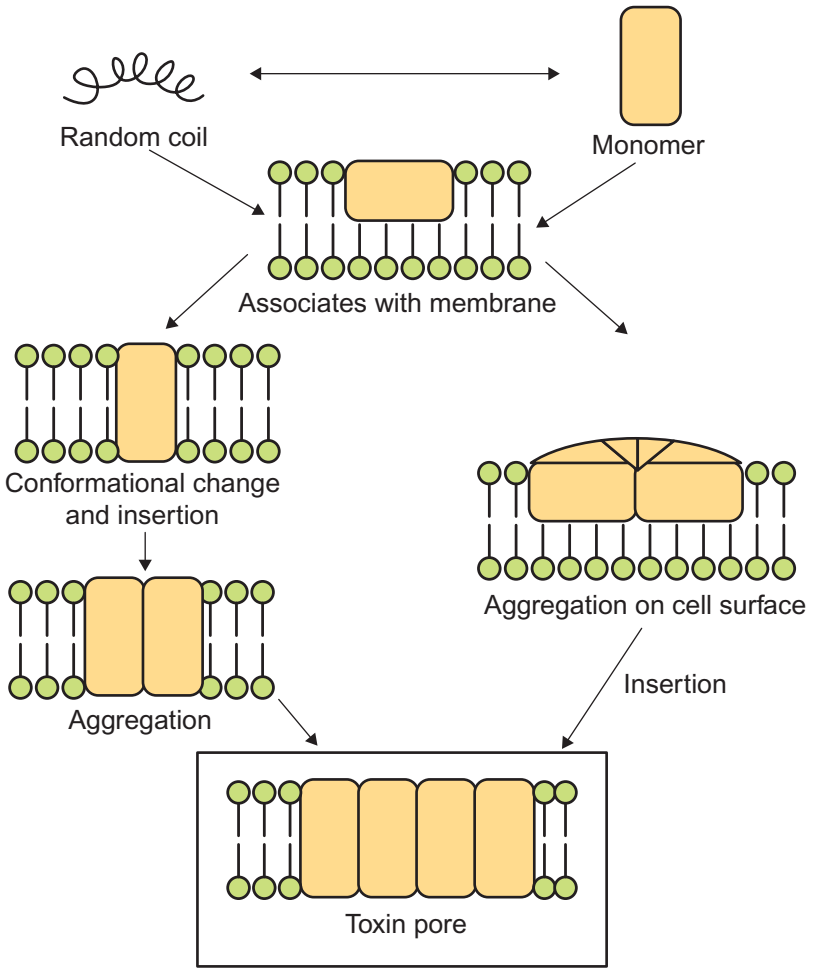


FIGURE 8.1 Pore formation by pore-forming toxins. Newly synthesised proteins are soluble. On interaction with cell membranes they undergo conformational changes which allow reorganisation on and insertion into target cell membranes. Cholesterol is involved as primary receptor or mediator of aggregation for the CBC group. Others have specific receptors, but staphylococcal δ-toxin does not. *Reproduced with permission from Mitchell et al., 1992, Figure 1.*

number of different functions in pathogenesis and has haemolytic activity and induces inflammation of the lung conferring the ability to replicate in the lung and invade the bloodstream, and altering alveolar permeability, also inhibiting cilial beat in respiratory mucosa. An important function is the activation of the classical pathway of complement which presumably assists evasion of complement activity directed towards bacterial cells. PLY can influence the expression of a number of host genes and multiple host-associated signal transduction pathways and is considered to be a neurotoxin. PLY has also been implicated in causing sensorineural deafness associated with meningitis caused by the pneumococcus (Figure 8.2).

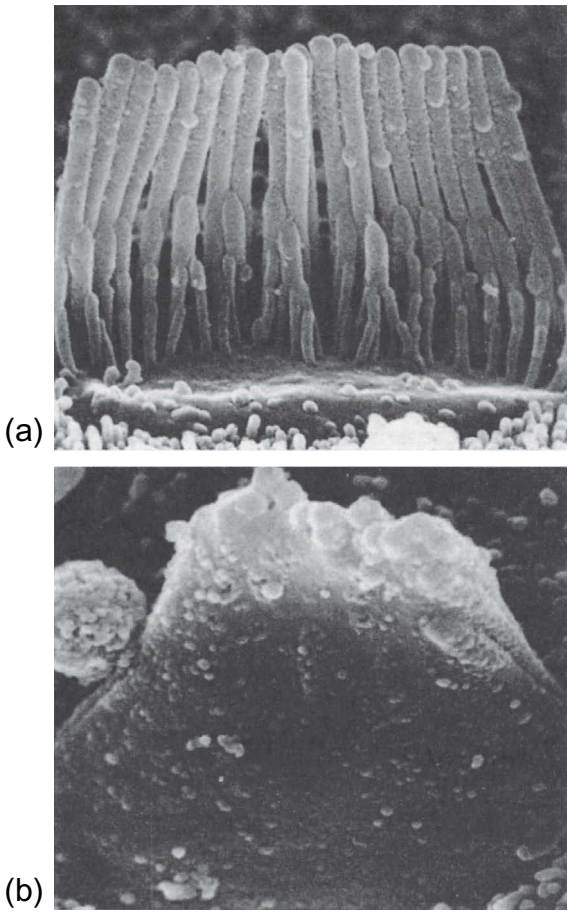


FIGURE 8.2 The effect of PLY on the hair cells of the inner ear of a guinea pig. (a) A scanning electron micrograph of normal hair cells, (b) hair cells after exposure to PLY; note disappearance of hairs. Hearing depends on the transmission to the hair cells of pressure waves generated in the fluid-filled chamber (scala tympani) of the cochlea. This causes lateral displacement of the hairs. Inelastic links between hairs in different rows results in membrane deformation, opening of ion channels and influx of ions. This generates an action potential in the underlying auditory nerves. *Kindly provided by Drs M. P. Osborne and S. D. Comis, Department of Physiology, The Medical School, University of Birmingham, UK.*

Attempts to develop protective anti-pneumococcal vaccines have hitherto been based on the type-specific capsular polysaccharides. Unfortunately, there are at least 90 known types and current vaccine preparations comprise a blend of polysaccharides from some 23 types. Currently, efforts to develop a broadly effective vaccine based on genetically engineered PLYs fused to other *S. pneumoniae* antigens are demonstrating some promise.

RTX TOXINS This group of toxins has been designated RTX (repeats in toxin) toxins by virtue of a common structural feature – the presence of an array of a nine amino acid repeat (*ca.* 10–40) to which Ca^{2+} binds thereby activating the toxins which form membrane pores of varying sizes. They constitute the largest group of bacterial pore-forming toxins and are widespread among Gram-negative pathogens. In general, the role of RTXs in disease is not clear but three examples are given where RTXs are important. *Escherichia coli* α-haemolysin, regarded as the prototype of this group, is important in extraintestinal infections caused by this organism; the toxin is active against a broad range of mammalian cells. Leukotoxin from *Pasteurella haemolytica* exhibits narrow target cell and host specificities; it specifically kills ruminant leucocytes and

is important in bovine pneumonic pasteurellosis. The third example is the 'invasive' adenylate cyclase toxin of *Bordetella pertussis*. This toxin is unique among this group in that it is a large bifunctional toxin: it has both haemolytic (Hly) and adenylate cyclase (AC) activities, hence the designations AC-Hly, AC toxin, CyaA, and is known to be important in the early stages of respiratory tract colonisation. Strictly it is the haemolysin part of the molecule which belongs to the RTX family and its main function appears to be in translocation of the AC moiety into the cell where cAMP levels are elevated with ensuing pathophysiological sequelae.

STAPHYLOCOCCAL α-TOXIN Staphylococci produce a range of toxins, some of which we have already met. The α-toxin is easily the most studied from a biophysical point of view and is considered the main cytolysin produced by *S. aureus*. Like streptolysin O and staphylococcal δ-toxin, it is secreted as a water-soluble protein and undergoes self-induced oligomerisation on cell membranes to form heptameric pores. Recently, the receptor for α-toxin was identified as a disintegrin and metalloprotease 10 (ADAM-10) molecule, which upon binding relocate to calveolin-enriched lipid rafts which lead to clustering of signal molecules. Ultimately the interaction leads to upregulation of ADAM-10 metalloprotease activity leading to the cleavage of E-cadherin and disruption of epithelial barrier function. This in turn leads to acute lung injury. The toxoid (inactive) version of α-toxin is currently being examined as a potential vaccine.

DETERGENT-LIKE TOXINS Phenol soluble modulins (PSMs) represent a new family of staphylococcal toxins which include the classical staphylococcal δ-toxin involved in various aspects of pathogenesis. PSMs are amphipathic alpha helical peptides with their own unique secretory transport system, and they have potent activity on neutrophils after phagocytosis, and contribute to biofilm formation, dissemination, colonisation and interspecies competition. Of note, δ-toxin has recently been shown to exacerbate the symptoms of atopic dermatitis by binding and activating mast cells.

BINARY TOXINS These comprise two proteins, only one of which is toxic but the other is necessary at some stage for manifestation of toxicity. A good example is the staphylococcal leukocidins which belong to a very large family of binary leukocidins. Each leukocidin consists of two proteins – S (so called because it elutes slowly) and F (it elutes fast) from an ion-exchange column. S binds first to cell receptors, several of which have been recently identified (important in defining target cell specificity), followed by F which acts synergistically with S to create functional pores in the target membrane. There are at least six class S proteins and five class F proteins which can give rise to *ca*. 30 biologically active combinations, a fact which could be highly significant in that some strains produce more than one binary leukocidin. Although various S–F combinations exhibit different target cell specificities, most are active against PMNs. For example, Panton–Valentine leukocidin is highly active against human PMNs binding to the human complement receptor C5aR and causes release of leukotriene B4, IL-8, histamine and tissue degradative enzymes, which likely accounts for some of the respiratory tissue damage and severe symptoms associated with necrotising pneumonia. In addition leukocidin ED binds to the CCR5 receptor on neutrophils and T-lymphocytes, leading to disruption of phagocytosis which promotes survival in mouse models of infection.

Toxins with Intracellular Targets

Many toxins have intracellular targets. There is intense interest in seeking to understand the mechanism(s) of uptake of the active moieties of toxins whose targets are intracellular. This is driven by the desire to understand fundamental mechanisms in cell biology and to develop selective 'cytotoxic therapies' in clinical medicine as well as to unravel the molecular mechanisms of disease causation. To reach an intracellular target, a protein must first be translocated across the cytoplasmic membrane. There are at least three ways in which this can be achieved: self-translocation across cytoplasmic membrane, direct injection and receptor-mediated endocytosis.

SELF-TRANSLOCATION

The best example of self-translocation across cytoplasmic membrane known to date: the invasive adenylate cyclase of *B. pertussis* described above.

DIRECT INJECTION

A good example is the *P. aeruginosa* exoenzyme S (PES). PES is a single polypeptide with no receptor-binding component or translocation domain (see below): it is 'injected' directly across cell membranes by a mechanism functionally similar to that already described for the translocation of Tir by *E. coli* (see Chapter 2). PES is activated by a cytoplasmic protein FAS, and ADP-ribosylates by the small G-protein Ras resulting in the collapse of the cytoskeleton (see Chapter 4).

RECEPTOR-MEDIATED ENDOCYTOSIS

There are several variations on the receptor-mediated endocytosis theme reflecting the structure of the toxins; in some cases the process involves the subversion of normal processes used by the host cell to regulate movement and organisation of cellular membranes and substituent components. Toxins first bind to their respective receptors and become internalised via coated pits, vesicles or caveolae, into endosomes from which they still must escape into the cytoplasm.

Three types of toxin with intracellular targets have been recognised, reflecting their genetic origin. Some toxins consist of a single peptide, the product of a single gene, which undergoes post-translational modification into A and B fragments which are covalently linked (Figure 8.3). The A fragment is the 'active' toxiphore and the B fragment bears the receptor-binding domain and also mediates translocation of A into the cytoplasm. Examples include diphtheria toxin (DT), *P. aeruginosa* exotoxin A (PEA) (Figure 8.4), and the clostridial neurotoxins (BoNT and TeTx) (Figure 8.5).

A second group of toxins are the products of separate genes, giving rise to A and B subunits which non-covalently associate into stable complexes. They are also designated A–B type toxins, in which the number and nature of B subunits vary, but the connotations of A and B are as for DT. Examples include classical cholera toxin (CT), *E. coli* heat labile enterotoxins (LTs), pertussis toxin (PT), Shiga toxin (ShT) and Shiga-like toxin (ShLT) (Figure 8.4).

A third group of toxins are the products of separate genes giving rise to different proteins which are functionally equivalent to A and B subunits. These proteins do not associate into stable complexes but must act in concert to express toxicity and are known as

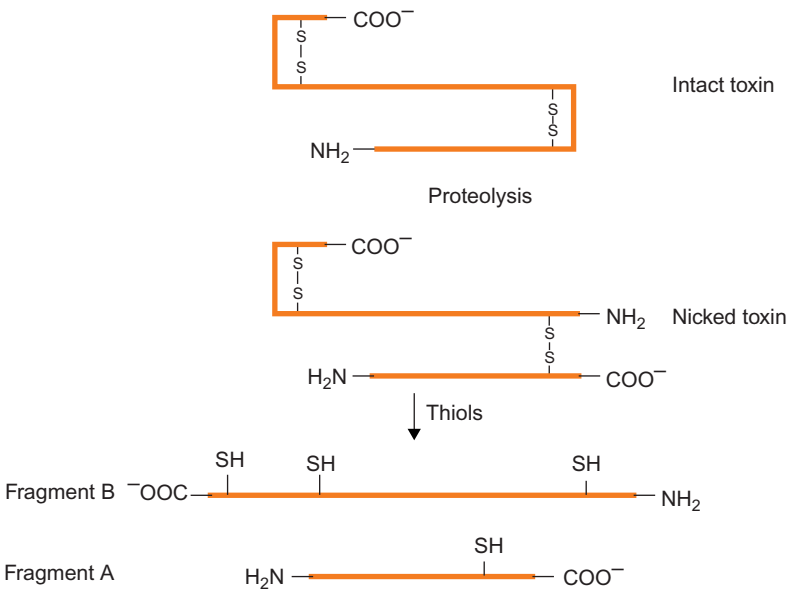


FIGURE 8.3 DT: post-translational modification of the single peptide into A and B fragments. The toxin is synthesised as a single polypeptide but is cleaved (nicked) by proteases into two fragments designated A and B, held together by an –S–S– bond. The latter is reduced during translocation of the A fragment into the cytosol. The B fragment consists of a receptor domain (R) which recognises the DT receptor – an EGF-like precursor which happens to be widely distributed throughout all organ systems – and a T domain which facilitates the translocation of A into the cytoplasm (see Figure 8.4).

binary (or bi-component) toxins. Examples include anthrax toxins and *Clostridium botulinum* C2 toxin (Figure 8.4).

TRANSLOCATION OF TOXIPHORE INTO THE CYTOPLASM

DIRECT ESCAPE FROM ENDOSOME DT B fragment binds to its receptor (a precursor of heparin-binding epidermal growth factor (EGF)-like growth factor), undergoes conformational change in the acidified endosome and inserts into the endosomal membrane, pulling the C terminus of the A fragment across the membrane. The –S–S– bridge is exposed to the cytosol, reduced, thereby freeing A to enter the cytosol (Figure 8.6). A similar mechanism operates with anthrax lethal factor (LF) and oedema factor (EF) toxins but in this case a third protein, protective antigen (PA), acts as the functional equivalent of DT B (Figure 8.7).

ROUTE TO ENDOPLASMIC RETICULUM For most if not all other toxins the route to an intracellular target is much more complex. Newly formed toxin-containing endosomes enter those vesicular trafficking pathways which lead to the trans-Golgi network (TGN), through the Golgi and further into the endoplasmic reticulum (ER). Using the example of CTA, in the Golgi apparatus, the A1 protein is recognised by the ER chaperone, protein disulfide isomerase, unfolded and delivered to the membrane, where the ER-oxidase-Ero1

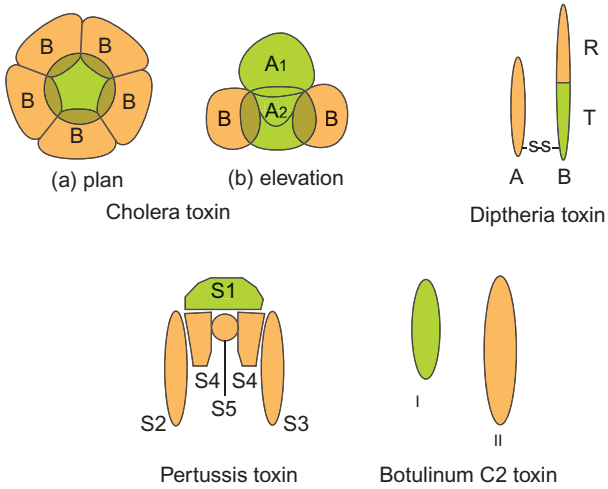


FIGURE 8.4 Schematic structure of three types of A–B-type toxins. The hatched regions are the binding/translocation-facilitating parts ('B subunits'). DT is synthesised as a single peptide (see Figure 8.3). CT is represented in plan and elevation views; *E. coli* LTs are structurally and functionally very similar to CT. CTA comprises CTA1–A2. A1 is the toxiphore which is held in association with B via A2. A2 has no known enzymic activity but plays some as yet undefined part in toxicity. Differences in CT A2 and LT A2 have been implicated as part of the reason for the lesser severity of disease caused by ETEC. PT B subunits are heterogeneous. Botulinum C2 toxin is a two-component binary toxin, in which two proteins do not form stable complexes prior to cell attachment. Not to scale. *Modified with permission from Madshus and Stenmark, 1992, Figure 1.*

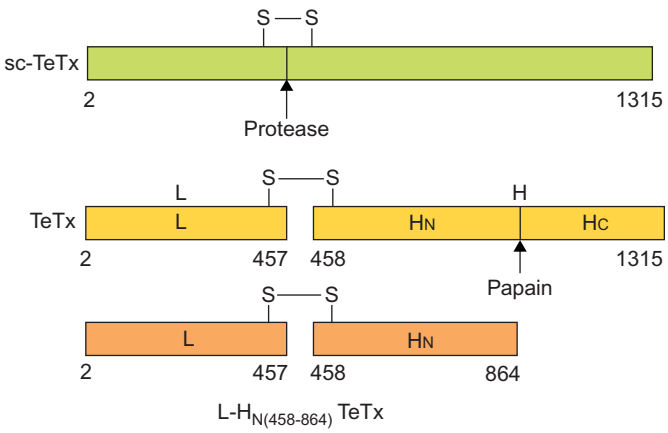


FIGURE 8.5 Structure/nomenclature of tetanus and botulinum neurotoxins. sc-TeTx: single chain non-cleaved tetanus toxin. TeTx: tetanus toxin after proteolytic activation. L and H: intact light and heavy chains, respectively. L-$H_{N(458-864)}$ TeTx: intact L chain linked to a fragment of H chain (from residue 458 at the N-terminal end to residue 864). H_N and H_C: N-terminal and C-terminal parts, respectively, of the H chain. The corresponding nomenclature for botulinum toxin type A is BoNT/A, and for type B, BoNT/B, etc. *Reproduced with permission from Witholt et al., 1992.*

triggers the release of the A1 protein. As the A1 protein moves from the ER into the cytoplasm by the Sec61 channel, it refolds and avoids deactivation as a result of ubiquitination. The C-terminal sequence *lysine–aspartate–glutamate–leucine* (the KDEL motif) in the A subunit of CT and related sequences in LT and PEA, is normally found in proteins which, having been processed in the Golgi, are returned to and are trapped by the ER which recognises the KDEL motif, thereby preventing such proteins being lost to the cell via exocytotic trafficking. Then, either in the TGN or the ER, CTA is reduced, freeing CTA_1, the

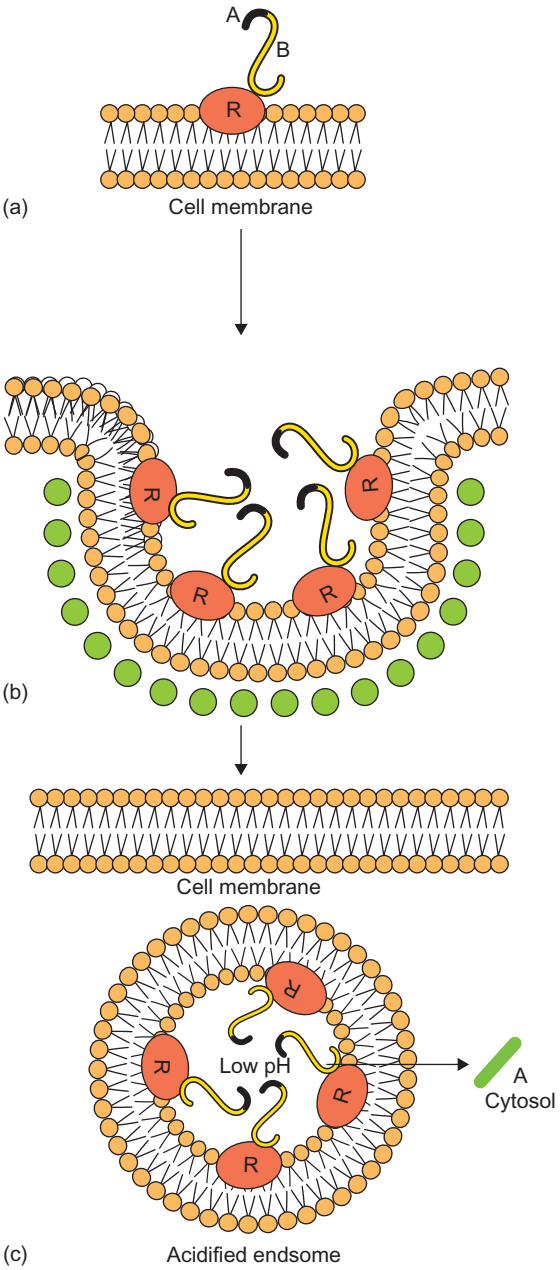


FIGURE 8.6 Direct escape of DT A fragment and anthrax LF and EF toxins from acidified endosome, (a) DT binds via B fragment to its receptor (R) in the cell membrane. (b) These complexes migrate to clathrin-coated pits. (c) This gives rise to acidified endosomes which induces conformational changes in B, insertion of B into the membrane and escape of A fragments into the cytosol. The only other known examples of direct escape of toxin into the cytosol are anthrax EF and LF (see Figure 8.7).

CT toxiphore (Figure 8.4). CT is activated by ADP-ribosylating factors (ARFs) which are also known to be involved in vesicular trafficking. Thus, by anterograde transport (the term given to secretory pathways from ER to the plasma membrane), CTA_1 may well be ferried in vesicles to the basolateral membrane with which they fuse, thereby depositing CTA_1 near to the target, adenylate cyclase (Figure 8.8).

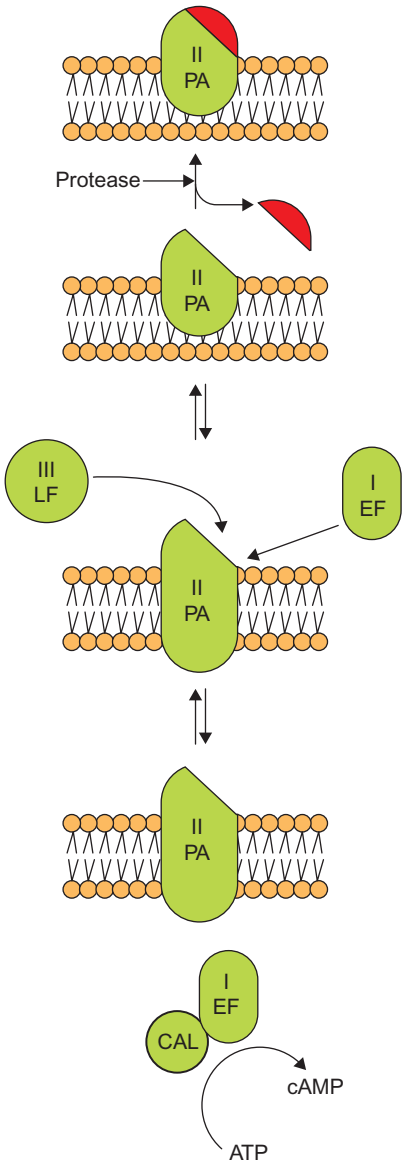


FIGURE 8.7 Mode of entry and action of anthrax toxins. PA interacts with the cell membrane and forms heptameric oligomers. After proteolytic cleavage, PA sites are exposed which bind EF, thereby facilitating translocation of EF into the cytosol directly from acidified endosomes as described for DTA (Figure 8.6). EF must be rapidly inactivated since washing toxin-treated cells results in a rapid loss of adenylate cyclase activity. EF interacts with calmodulin (CAL) to become an active adenylate cyclase enzyme in nearly all cells. Interaction of PA with EF and subsequent internalisation of EF is blocked by prior binding of LF. In contrast to EF which is active in many cells, LF protease is only active in macrophages. This model explains the characteristic hypovolaemic shock syndrome (cAMP is a potent secretagogue), cytotoxicity to macrophages, and the immunogenicity of PA.

INTRACELLULAR TARGETS

The targets for some of the intracellular toxins are listed in Table 8.3, and illustrated in Figures 8.9–8.13.

Superantigens: Toxins with Multiple Biological Activities

The superantigen group of toxins represent a large family of strain-dependent toxins made mostly by *S. aureus* and Group A streptococci. To date at least 23 different members of the family have been identified among *S. aureus* isolates alone. While most *S. aureus*

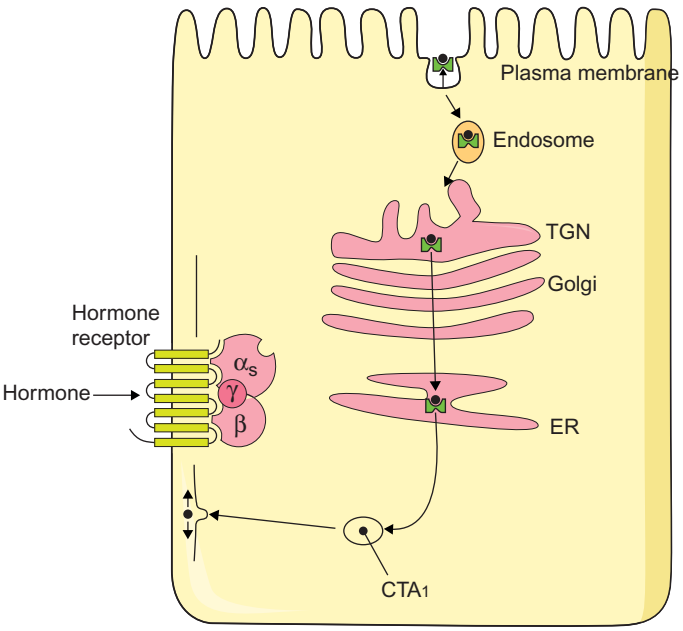


FIGURE 8.8 Mode of entry of CT. This represents a much more complex route to an intracellular target. As described in the text it involves interaction of CTB subunits (shaded part of molecule) with ganglioside receptor GM1 (vertical arrow), receptor-mediated endocytosis, retrograde transport of the endosome to the TGN, through the Golgi to the ER, and anterograde transport of liberated CTA1 from ER in vesicles directed to the basolateral membrane, the intracellular location of adenyl cyclase. This mechanism is operative at least for several other toxins.

superantigens are encoded on mobile genetic elements and are therefore found only in a subgroup of strains, one recently identified superantigen known as SEIX is encoded in a stable region of the genome and is therefore made by the great majority of strains. The toxins are superantigens by virtue of their ability to bind to major histocompatibility (MHC) class II molecules, outside the antigen-binding grove. They are presented as unprocessed proteins to certain T lymphocytes expressing specific T-cell receptor (TCR) motifs located in the variable domain of the β-chain (Vβ) of the TCR (see Chapters 6 and 7). Nanogram to picogram quantities of superantigen will stimulate up to 20% of all T cells, compared with only 0.001–0.00001% T cells stimulated by conventional presentation of antigen to TCR. As a consequence of this huge proliferation of T cells and expression/release of aberrantly high levels of cytokines and other mediators, many biological systems are affected causing lethality/shock. This represents an important interference with a coordinated immune response, and the widespread polyclonal activation and cytokine release can be regarded as a microbial strategy, a 'diversion' of host immune defences. Ironically, the superantigen not only expands the circulating T-cell population but also reacts with developing T cells in the thymus, causing the same subpopulation to decline (see Chapter 7). It seems probable that these effects on immune cells represent a more important biological function of these toxins than the one responsible for the characteristics of disease; the latter may be no more than an 'accidental' phenomenon. For example, it may be that superantigens' main role is to subvert the T-cell response in localised infections leading to immune avoidance and persistence. It turns out that similar molecules are formed by Yersinia, mycoplasma and by certain retroviruses (e.g. the Mls antigen of mouse mammary tumour virus).

It has been shown experimentally (or proposed) that immune stimulation, cytokine release, induction of capillary leak, shock and lethality are related to the superantigenicity

TABLE 8.3 Target Proteins for Intracellular Toxins

Toxin Group Organism	Toxin	Preferred Targets		
		GTP-Binding Proteins	ATP-Binding Proteins	Other Targets
RIBOSYLTRANSFERASES (ADPRASES)				
C. diphtheriae	DT	Elongation factor 2 (EF2); see Figures 8.9 and 8.10		Vimentin[a]
P. aeruginosa	Exotoxin A (PEA)	Elongation factor 2 (EF2); see Figures 8.9 and 8.10		
V. cholerae	CT	α_s subunit of G_s ($\alpha_s\beta\gamma$) regulator of adenylyl cyclase; [b] see Figure 8.11		
E. coli	Heat-labile toxins LTI and LTII cytotoxic necrotising factor (CNF1)	α_s subunit of G_s ($\alpha_s\beta\gamma$) regulator of adenylyl cyclase; see Figure 8.11 Rho G-protein		
B. pertussis	Pertussigen	α_i subunit of G_i ($\alpha_i\beta\gamma$) regulator of adenylyl cyclase; [c] see Figure 8.11		
C. botulinum	C2 toxin		Non-muscle actin, γ smooth muscle actin; see Figure 8.13	
Iota group[d]				
C. perfringens *Clostridium spiroforme*	Iota toxin *C. spiroforme* toxin		All mammalian actin isoforms	
C. difficile	ADPRase J			
C. botulinum	C3 ADPRase	Rho G-protein		
Clostridium limosum	ADPRase (similar to *C. botulinum* C3)			
GLYCOSYLTRANSFERASES (LARGE CLOSTRIDIAL TOXINS)				
C. difficile	TcdA and TcdB	Rho, Rac G-proteins		
Clostridium sordelli	TcsL	Rac (and other) G-proteins		
Clostridium novyi	Tena	Rho, Rac G-proteins		
SHT, SHLT				
S. dysenteriae	ShT			Ribosomes; see Figure 8.10
E. coli	ShLT			Ribosomes; see Figure 8.10

(*Continued*)

TABLE 8.3 (Continued)

Toxin Group Organism	Toxin	Preferred Targets		
		GTP-Binding Proteins	ATP-Binding Proteins	Other Targets
'INVASIVE' ADENYLATE CYCLISES				
B. anthracis	EF			Activated by calmodulin; ATP
B. pertussis	AC-Hly			Activated by calmodulin; ATP
PROTEASES				
C. botulinum *C. tetani*	Neurotoxin (BoNT) Neurotoxin (TeTx)			Proteins involved in release of neurotransmitters; see Figure 8.12
B. anthracis	Lethal toxin (LF)			Protein kinase kinases 1 and 2

[a]Vimentin: intermediate filament protein.
[b]CT A1–A2 will catalyse a range of reactions involving transfer of ADP-ribose to other substrates.
[c]Pertussigen will catalyse the ADP-ribosylation of G proteins involved in several transmembrane signalling events.
[d]*C. perfringens and C. spiroforme* toxins and *C. difficile* ADP-ribosyltransferase form an iota subgroup, in that antibodies will cross-react within this group but not with *C. botulinum* C2 toxin. Only within the iota group are the binding components interchangeable. The iota group will also modify all mammalian actin isoforms.

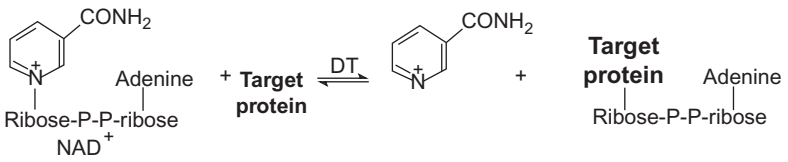


FIGURE 8.9 ADP-ribosylation reaction. This enzymic reaction is common to a wide range of toxins with different target proteins (see Table 8.3).

of these proteins. However, there are other biological activities of these toxins which are not mediated by their superantigenicity. Some of these activities are common to all, and others specific to certain members of this group. The red skin rash elicited by the streptococcal toxins (which gave rise to the original nomenclature 'erythrogenic' toxins) is regarded as a secondary hypersensitive effect, but pyrogenicity is the result of direct action on the hypothalamus as well as release of IL-1 and TNF-α from macrophages.

Staphylococci cause food poisoning on a worldwide scale but particularly in countries such as France which consume large quantities of unpasteurised cheese. Infection rates are under-reported, probably because it is normally a self-limiting gastrointestinal disease. Onset of disease is rapid after consumption of food contaminated with a subgroup of the superantigen family (enterotoxins) which have emetic activity (capacity to induce vomiting). The main features of the disease are diarrhoea and severe vomiting, the latter being due to enterotoxin stimulation of the vagus nerve.

In addition, some strains of *S. aureus* cause toxic shock syndrome (TSS), a multisystem disease. Originally, TSS was seen characteristically in menstruating women whose tampons

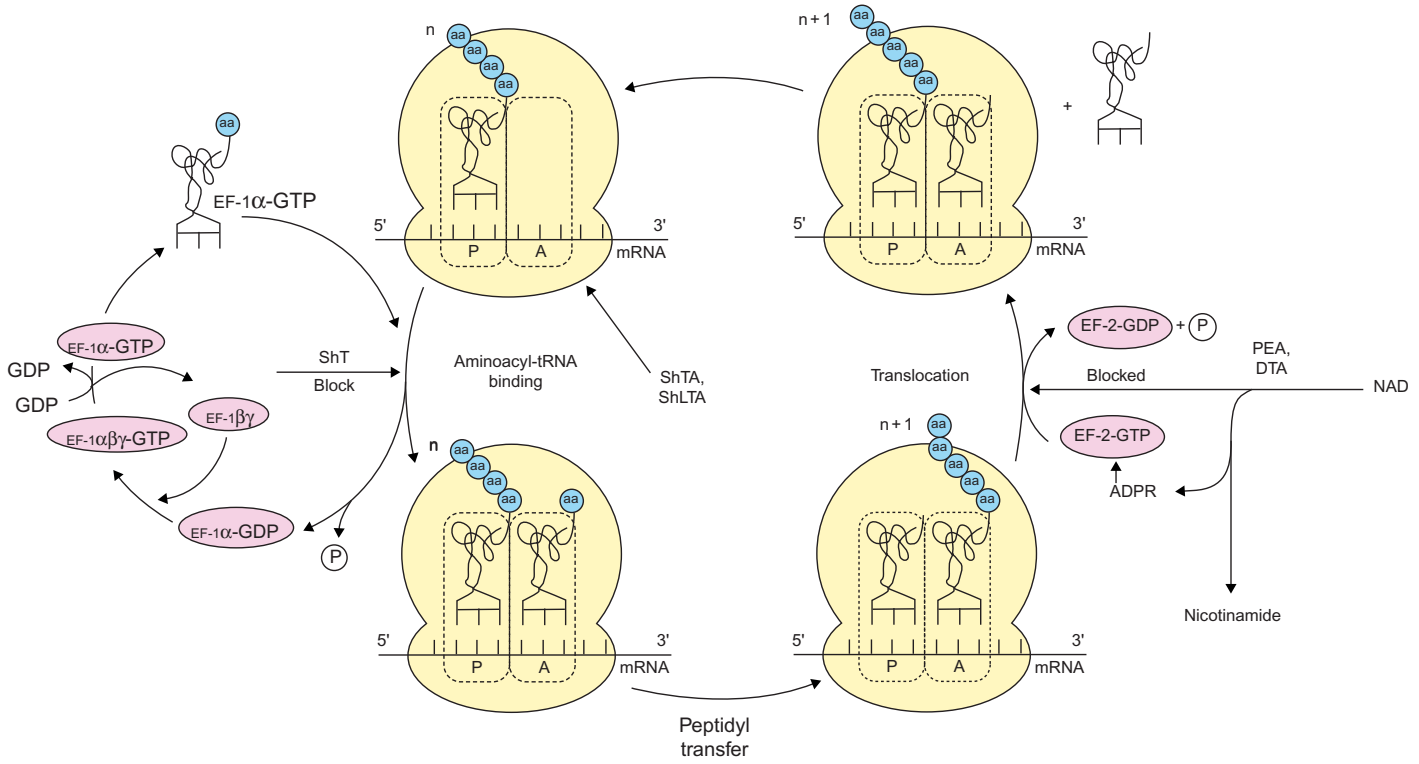


FIGURE 8.10 Inhibition of protein synthesis by DT, *P. aeruginosa* toxin A (PEA), ShT, ShLTs and poliovirus. The schema shows a round of peptide elongation and illustrates the key role played by two enzymes, EF-1 and EF-2. EF-1-GTP interacts with aminoacyl-tRNA; this complex is docked into site A, EF-l-GTP becomes EF-1-GDP and is recycled as shown. After peptidyl transfer, EF-2-GTP catalyses transfer of the extended peptide to site P, and is itself autocatalytically converted to EF-2-GDP. DTA and PEA each ADP-ribosylates diphthamide (a modified histidine) in EF-2-GTP, which can no longer translocate the newly elongated peptide from the A site to the P site. The ShTA fragment is a specific *N*-glycosidase which cleaves an adenine residue from near the 3′ end of the 28S ribosomal RNA. This depurination results in failure of EF-1-dependent binding of aminoacyl-tRNA to site A and hence inhibits protein synthesis. Poliovirus achieves selective inhibition of host protein synthesis at an earlier stage than is depicted here. Host mRNA is first modified (capped), then bound to the small ribosomal subunit; poliovirus mRNA is not capped. The function of a cap-binding protein, which recognises and binds host mRNA to the ribosome, is inhibited by a poliovirus virion protein thereby allowing differential translation of virus messenger RNA. EF-1α, nucleotide-binding protein; EF-1αβγ, nucleotide exchange protein. *Modified with permission from Riis et al., 1990, Figure 1.*

harboured multiplying staphylococci. It is due to a toxin called toxic shock syndrome toxin 1 (TSST-1). TSS is characterised by sudden onset of fever, vomiting, diarrhoea, an erythematous rash followed by peeling of the skin, hypotensive shock, impairment of renal and hepatic functions and occasionally death. We now know that TSS is not confined to menstruating women. Non-menstrual TSS presents with essentially the same signs as menstrual TSS and is caused by other staphylococcal enterotoxins (SEs). TSST-1 is isolated only from menstrual cases of TSS: this toxin has the ability to cross the vaginal mucosal barrier whereas the other SEs do not. Streptococcal TSS (STSS), a life-threatening disease caused by streptococci, is also a well-recognised clinical entity, probably corresponding to the severe cases of scarlet fever described in the older literature.

Significance of Toxins in Disease

It is important to point out that, while the outstanding advances made in our knowledge of toxin structure and mode of action at the cellular level can be exploited in a remarkable way (see below), it is important to remember that such knowledge by itself does not tell the whole story of the pathogenesis of infectious disease. To illustrate this some examples are given below.

CHOLESTEROL-BINDING CYTOLYSINS

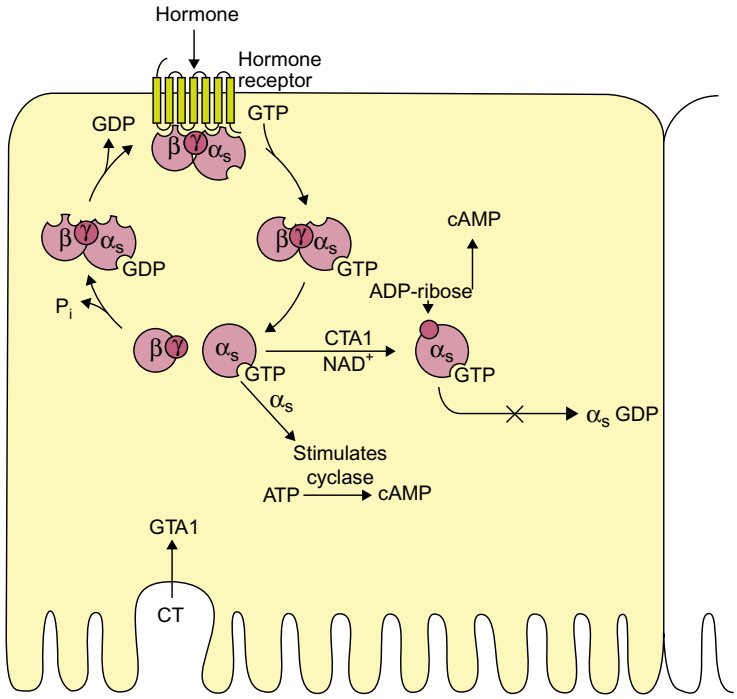
It is obvious from our consideration of these toxins that the elucidation of their lytic activities towards red cells in terms of a fundamentally similar mechanism, by itself tells us nothing about their respective roles in disease. Moreover, as already outlined above, PLY is now known to be a multifunctional molecule whose relevance in disease varies with the infection setting!

CORYNEBACTERIUM DIPHTHERIAE

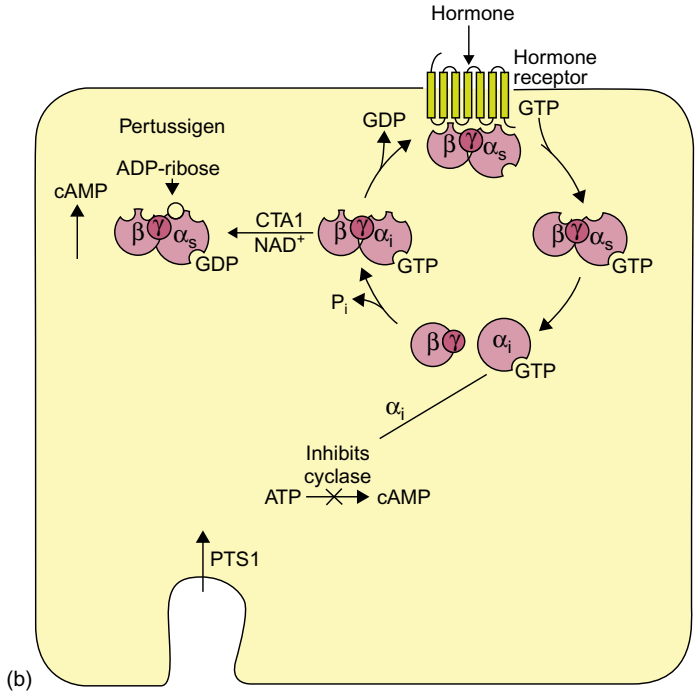
C. diphtheriae produces DT which is of unquestionable importance in causing diphtheria. Sustained active immunisation with DT toxoid has made diphtheria a clinical rarity in advanced countries. Failure to continue this policy resulted in a huge diphtheria epidemic in the early 1990s in the states comprising the former USSR. *C. diphtheriae* organisms multiply on the epithelial surfaces of the body (nose, throat, skin) but do not penetrate deeply into underlying tissues. The infection on the body surface causes necrosis of mucosal cells with an inflammatory exudate and the formation of a thick 'membrane' (hence the name *C. diphtheriae:* Gr., *diphthera* = membrane) and if the infection spreads into the larynx there may be respiratory obstruction. The toxin probably assists colonisation of the throat or skin by killing epithelial cells and polymorphs. DT can also be disseminated from the infection site and has important actions, especially on the heart and nervous system. The toxin is encoded by a lysogenic corynephage β whose transcription is controlled by an iron-dependent repressor, emphasising the importance of *C. diphtheriae* Fe metabolism *in vivo.*

SHT AND SHLT

Shigella dysenteriae 1 is the cause of bacillary dysentery. For a long time it was thought that ShT was the principal cause of this disease. However, in Chapter 2, the importance of gut invasiveness in *Shigella* infections was emphasised. While it is not at all clear how ShT can be involved in the watery diarrhoea phase of dysentery, it is perceived as exacerbating


Hormone
Hormone receptor
GDP
GTP
cAMP
ADP-ribose
P_i
CTA1
NAD^+
α_s GDP
Stimulates cyclase
ATP
cAMP
GTA1
CT

(a)


Hormone
Hormone receptor
GTP
GDP
Pertussigen
ADP-ribose
cAMP
CTA1
NAD^+
P_i
α_i
Inhibits cyclase
ATP
cAMP
PTS1

(b)

FIGURE 8.11 Mode of action of CT, *E. coli* LT toxins and pertussigen (PT). There are five main features in this diagram.

1. The production of cAMP by adenyl cyclase. Cyclic AMP is an important second messenger involved in the intracellular amplification of many cellular responses to external signals including hormones. The nature of the physiological response reflects the differentiation of the cell responding to the stimulus. For, example, in gut cells the response would be altered ion transport and hence fluid secretion; in muscle cells it would be glycogen breakdown in response to the call for more energy. The production of cAMP is controlled both positively (a) and negatively (b) at two levels. Interaction of hormone and receptor releases the heterotrimeric ($\alpha\beta\gamma$) G-protein regulator complex which, upon binding GTP, dissociates into α-GTP and $\beta\gamma$. The α-subunit may be stimulatory (α_s) and activate adenyl cyclase (as in (a)) or inhibitory (α_i) and inhibit adenyl cyclase (as in (b)); adenyl cyclase is not shown structurally in the diagram. In gut cells the receptor would be on the non-luminal basolateral side enabling enterocytes to respond to stimuli from the circulation.
2. The second level of control involves endogenous GTPase properties of both α_s and α_i subunits of the G-protein regulator: α_s-GDP and α_i-GDP are inactive.
3. The level of cAMP may be affected by physiological stimuli or by perturbation of the normal regulatory cycle as illustrated, by CT and LTs in enterocytes (a) or PT in a pancreatic B cell (b).
4. CTA1 ADP-ribosylates α_s-GTP which promotes continued dissociation of the heterotrimer and also inactivates the endogenous GTPase activity. Hence stimulation of the cyclase continues. LTs act in a similar manner.
5. PTS1 ADP-ribosylates the α_i-GDP$\beta\gamma$-heterotrimer which can no longer associate with the receptor or lose GDP to undergo another cycle of GTP activation; active cyclase can no longer be turned off. In pancreatic cells this results in loss of inhibition of insulin secretion.

Note: the α-GTP subunits are functionally analogous to the monomeric GPTases described in Figure 4.1. *Adapted with kind permission from Gierschik, 1992, Figure 4.*

the bloody diarrhoea phase rather than initiating it. In contrast there is now evidence that ShLTs are important in haemorrhagic colitis (HC) and haemolytic urea syndrome (HUS) caused by ShLT-producing strains of enterohaemorrhagic (EHEC) *E. coli:* EHEC has the capacity to progress disease beyond a watery diarrhoea stage to HC and HUS; the latter is characterised by renal failure, thrombocytopenia and microangiopathic haemolytic anaemia. This virulence attribute is due to possession of one or more of a family of ShLTs. As in dysentery, the role of ShLT in the causation of watery diarrhoea is controversial and indeed it may not be absolutely necessary. Importantly, the normal reservoir for EHEC is not humans but the terminal rectum of the cow, where it colonises without causing clinical symptoms of disease. This suggests that the potent effects of ShLT made by EHEC causing infections of humans are unlikely to be the main purpose of their expression and that there is likely to be another less pathological role in its main niche. This may involve promotion of colonisation through subtle effects on the local bovine gut epithelium.

VIBRIO CHOLERAE AND CT

The classic paradigm for bacterial watery diarrhoea is cholera caused by *V. cholerae* in the small intestine. *V. cholerae* colonises the upper small intestine by adhering to epithelial cells. Water and electrolytes are lost through the intact epithelial cells into the small intestine. As the multiplying bacteria increase in numbers and more and more epithelial cells are affected, the absorptive capacity of the colon is overwhelmed and there is profuse watery diarrhoea, as much as 1 l/h in severe cases. The massive loss of isotonic fluid with excess of sodium bicarbonate and potassium leads to hypovolaemic shock, acidosis and haemoconcentration. Anuria develops, and the collapsed, lethargic patient may die in

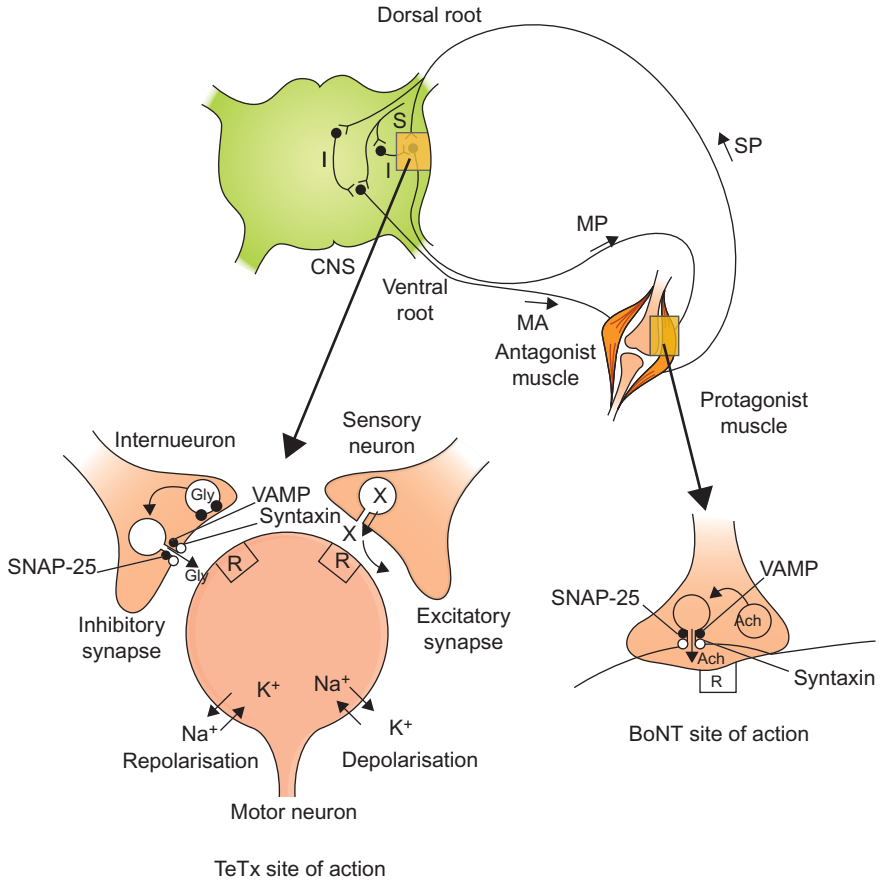


FIGURE 8.12 Sites and mode of action of clostridial neurotoxins BoNT and TeTx. This figure has three main features.

1. Reflex arc (top). Mechanism for inhibiting the antagonists to a muscle contracting in response to stretch. Muscles are reciprocally innervated with sensory and motor neurons, although for clarity this is shown only for the protagonist muscle. On stretch, the stretch receptors generate an impulse which is transmitted along the afferent sensory (S) neuron of the protagonist (P) muscle. This SP neuron enters the spinal cord by the dorsal root and synapses with the motor neuron supplying the protagonist muscle (MP) and with an interneuron (I) which in turn synapses with the motor neuron supplying the antagonist muscle (MA); the efferent motor neurons leave the spinal cord by the ventral root. At the SP/MP synapse an excitatory transmitter is released which induces an impulse in MP which leads to contraction of protagonist muscle. However, excitation of I causes release of an inhibitory transmitter at the I/MA synapse which leads to relaxation of the antagonist muscle. Note that the basic reflex arc has been shown for simplicity but TeTx acts mainly on voluntary muscles.
2. A simplified version of the biochemical events occurring in synapses (lower left). Excitatory and inhibitory synapses, neurotransmitter release and action. Gly, glycine; R, receptors of neurotransmitters; X, hitherto uncharacterised (candidates include glutamate, dopamine, ATP, substance P, and somatostatin).
3. Sites of neurotoxin action (lower left and right). The predominant site of action of TeTx is the intermotor neuron synapse; the exocytotic machine is interfered with by the endopeptidase action of TeTx on VAMP. BoNT acts at the neuromuscular junction, inhibiting the release of acetyl choline (Ach) by its proteolytic action on VAMP (types B, D and F), or SNAP (types A and E), or syntaxin (type C).

Amplified from Stephen and Pietrowski, 1986, Figures 18 and 19.

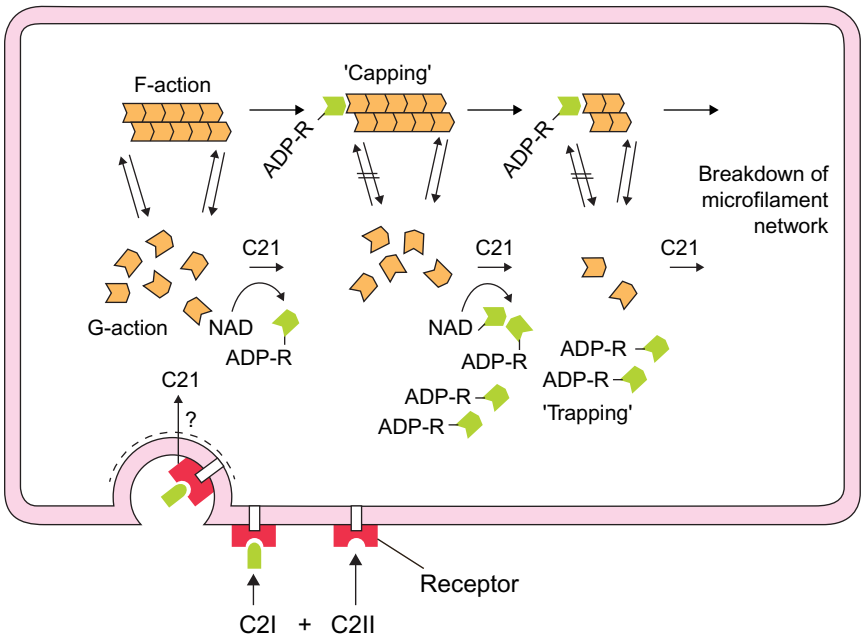


FIGURE 8.13 Mode of action of actin-ADP-ribosylating toxins. *C. botulinum* C2 toxin component 2II binds to the cell membrane followed by C2I. The latter is internalised and upsets the equilibrium between polymerisation and depolymerisation of actin. ADP-ribosylation of actin inhibits its polymerisation and turns G-actin into a capping protein which binds to the fast-growing (concave) ends of actin filaments. Capping of the concave ends increases the critical concentration for actin polymerisation. Since the slow-growing (pointed) ends of actin filaments are free, depolymerisation of actin occurs at these ends. Released actin is substrate for the toxin and will be withdrawn from the tread-milling pool of actin by ADP-ribosylation, i.e. trapped. Both reactions will finally induce the breakdown of the microfilament network. *Reproduced with permission from Aktories et al., 1992, Figure 2.*

12–24 h. Lives are saved by replacing the lost water and salts, but the patient recovers as affected cells are shed and replaced in the normal fashion. The infection is particularly severe in children who easily develop low levels of plasma potassium. However, on a global scale this greatly feared disease, cholera, is only responsible for less than 1% of the total deaths due to diarrhoea.

The genome of *V. cholerae* contains genetic elements which are important in *V. cholerae* virulence: CTXϕ (the genome of a filamentous bacteriophage) which encodes the CT, and a large pathogenicity island VPI (for *V. cholerae* pathogenicity island). VPI is the integrated genome of another large filamentous bacteriophage (VPIϕ) and encodes the toxin co-regulated type IV gilus (Tcp). Of the numerous colonisation factors known to be produced by *V. cholerae*, only Tcp has been proven to be important in human disease. Tcp is a remarkable entity; its subunit TcpA is a coat protein of VPIϕ, but it also acts as a receptor for CTXϕ and mediates interbacterial adherence. Thus, as a result of sequential infection by two 'pathophages', *V. cholerae* acquires the ability to colonise the human gut and secrete classical CT, which is a potent enterotoxin. The integration into the chromosome of these phage genomes brings their expression under the control of regulatory genes in the

ancestral chromosome, whilst the replication of phages enables their interbacterial spread. CT is an 'AB'-type toxin in which the pentameric B subunit recognises and binds to its cell receptor (GM1 ganglioside) thereby initiating the internalisation of the active A subunit (CTA_1) and elevation of cAMP.

Studies on human jejunal biopsies show that cholera is not a purely pathophysiological disease but a pathological one, involving changes in the microvasculature and enteric nerve fibres, degranulation of argentaffin cells, mucosal mast cells and eosinophils; the extent of these changes correlated with clinical severity of disease. Despite the undoubted importance of CT in the causation of the disease, and the potent antigenicity of CT, it is now recognised that a large number of other virulence determinants are involved, and that protective immunity is very largely antibacterial. It is stopping effective colonisation which is important rather than neutralisation of the toxin. This has been partially achieved by using killed whole cell vaccines. Several attempts have been made in the laboratory to manipulate virulent strains genetically (in practice this means deleting or inactivating the known toxin genes) such that the attenuated strain will colonise the gut and stimulate local immune responses, and thereby prevent colonisation of the gut by virulent strains. To date, attenuated strains have been developed which fulfil these criteria, but these induce a mild transient diarrhoea, which has prevented their adoption into vaccination programmes. The most widely used current vaccine is an oral one which includes a combination killed bacteria (bacterin) and CT B subunit.

BORDETELLA PERTUSSIS TOXIN (PERTUSSIGEN)

Whooping cough (pertussis) is a severe respiratory tract infection characterised by prolonged paroxysmal coughing, attacks of which continue long after infection has cleared. The disease is capable of striking all ages but is particularly prevalent and severe in young children, where hospitalisation is required in about 10% of cases. The causative agent, *B. pertussis*, is transmitted aerially from the respiratory tract of an infected individual to that of a susceptible host. The organism attaches via several adhesins – filamentous haemagglutinin, fimbriae and the 69 kDa outer-membrane protein, pertactin – to the mucosal surface between cilia, and multiplies there during the incubation period of the disease, which is commonly around seven days. The infection then manifests as a slight fever and catarrh which is often indistinguishable from a common cold. However, 1–2 weeks later bouts of uncontrollable coughing begin. It is this paroxysmal coughing, along with the notorious 'whoop' as the child attempts to draw breath, which characterises the disease. The paroxysmal coughing stage often lasts for several weeks and no treatment is fully effective in controlling the symptoms. The only proven means of controlling whooping cough is vaccination but, in the United Kingdom at least, sporadic reports of vaccine-induced brain damage in infants has diminished public acceptance of the vaccine. However, it should be noted that permanent encephalopathy (brain damage) is a recognised though rare consequence of whooping cough infection.

Without doubt, pertussigen (PTx), whose biochemical mode of action is described above, is an exceedingly important virulence determinant of *B. pertussis:* PTx toxic activities including histamine sensitisation, hyper-insulinaemia followed by hypoglycaemia, induction of leukocytosis and IgE induction are all observed after infection and administration of PTx; these toxic properties of PTx are abolished when the ADPR activity of PTx

is inactivated. PTx non-toxic activities – mitogenicity, haemagglutination, platelet activation, mucosal adjuvanticity – are triggered by PTx B subunits. Much current work is being devoted to producing immunogenic, completely non-toxic preparations of PT by genetic manipulation of the gene encoding the S1 subunit (Figure 8.4); in clinical trials in Italy, such engineered vaccines have been shown to be both safe and effective.

However, *B. pertussis* also produces other potentially important toxins including AC-Hly involved in colonisation (see above), dermonecrotic toxin (DNT; formerly known as heat-labile toxin), and two non-protein toxins – trachéal cytotoxin (TCT) and endotoxin. DNT is lethal for mice and causes skin lesions in rabbits and guinea pigs. It is of doubtful significance in humans but important in atrophic rhinitis in pigs caused by *Bordetella bronchiseptica*[1]. TCT is a small glycopeptide which destroys ciliated epithelial cells and is almost certainly responsible for some of the observed histopathological damage in *B. pertussis* infections.

CLOSTRIDIAL NEUROTOXINS

Tetanus occurs in man and animals when *Clostridium tetani* spores germinate in an infected wound and produce their toxin; all strains of *C. tetani* produce the same toxin. Spores are ubiquitous in faeces and soil and require the reduced oxygen tension for germination provided locally in the wound by foreign bodies (splinters, fragments of earth or clothing) or by tissue necrosis as seen in most wounds, the uterus after septic abortion, or the umbilical stump of the newborn. The site of infection may be a contaminated splinter just as well as an automobile or battle injury. It also reaches the CNS by travelling up other peripheral nerves following blood-borne dissemination of the toxin through the body. The B part of the toxin binds to dissialogangliosides (GD2 and GD1b) on the neuronal membrane and movement of the protein across that membrane into the neuron is promoted by the presence of a translocation domain. The motor nerves in the brainstem are short and therefore the cranial nerves are among the first to be affected, causing spasms of eye muscles and jaw (lockjaw). There is also an increase in tonus of muscles round the site of infection, followed by tonic spasms. In generalised tetanus there is interference with respiratory movements, and without skilled treatment the mortality rate is about 50%.

Botulism[2] is caused by *C. botulinum*, a widespread saprophyte present in soil and vegetable materials. *C. botulinum* contaminates food, particularly inadequately preserved meat or vegetables, and produces a powerful neurotoxin. The botulinum toxin is destroyed at 80°C after 30 min – of great importance to the canning industry – and there are at least seven antigenically distinct serotypes (A-G) produced by different strains of bacteria but which have a pharmacologically similar mode of action. It is absorbed from the intestine and acts on the peripheral nervous system by binding to the synaptic vesicle protein SV2 on neurons, interfering with the release of acetylcholine at cholinergic synapses of neuromuscular junctions. Somewhere between 12 and 36 h after ingestion there are clinical signs suggesting an acute neurological disorder, with vertigo, cranial nerve palsies and finally death

[1]DNT is similar to the dermonecrotic toxin of *Pasteurella multicoda* also involved in porcine atrophic rhinitis. They inactivate the GTPase activities of Rho proteins, resulting in cytoskeletal changes affecting osteoblasts.

[2]*Botulus* (Latin) = sausage. In 1793 a large sausage was eaten by 13 people in Wildbad in Germany; all became ill and six died. The disease was subsequently referred to as botulism.

a few days later with respiratory failure. A less typical form of botulism occurs in small infants. The spores, present in honey applied to rubber teats, appear to colonise the gut, so that the toxin is produced *in vivo* after ingestion. The high potency of botulinum toxins and the specificity and reversibility has found numerous applications in the treatment of various clinical syndromes and cosmetically for the reduction of facial wrinkling (Botox).

ANTHRAX TOXIN

Anthrax is a disease of animals, particularly sheep and cattle, and to a lesser extent man, caused by infection with *Bacillus anthracis.* Infection takes place following the ingestion of spores, the inhalation of spores or in most cases by the entry of spores through abraded skin. The spores germinate inside macrophages and then the bacteria form a toxin which kills macrophages, increases vascular permeability and gives rise to local oedema and haemorrhage. Infection of the skin in man leads to the formation of a lesion (malignant pustule; a *black* eschar, hence *B. anthracis;* Gr. *anthrakos* = coal) consisting of a necrotic centre surrounded by vesicles, blood-stained fluid and a zone of oedema and induration. In severe infections (nearly all cases of anthrax inhalation are fatal) there is septicaemia with toxic signs, loss of fluid into tissues, with widespread oedema and eventually death. Anthrax in man occurs mainly in those whose work brings them into contact with infected animals. It is not a common disease in the United Kingdom, and the usual source of infection is imported bones, hides, skins, bristles, wool and hair, or imported fertilisers made from the blood and bones of infected animals.

The anthrax toxin complex consists of three components, factor I (EF), factor II (PA) and factor III (LF), none of which are toxic by themselves, but in binary combinations exhibit two types of activity. PA in the form PA83 binds to the receptors tumour endothelium marker-8 (TEM8) and capillary morphogenesis protein 2 (CMG2) before being proteolytically cleaved into a peptide which can form ring-shaped oligomers. Once bound to LF and EF, the complexes are endocytosed into an acidic vacuole in the cell before moving into the cytosol. PA and LF form a binary proteolytic cytotoxin which kills macrophages (see Chapter 4) but not any other cell type, whereas PA and EF form a binary toxin which will elevate cAMP levels (Figure 8.7) in nearly all types of cell.

CLOSTRIDIUM DIFFICILE

C. difficile represents a classic example of the difficulty in interpreting disease mechanisms in terms of characterised enzyme activities ascribed to toxins relevant in disease. *C. difficile* is now established as the most common nosocomial enteric pathogen causing pseudomembranous colitis, antibiotic-associated colitis and antibiotic-associated diarrhoea. The most important defence against this opportunistic pathogen is the normal colonic microflora, although the microbial species responsible for and the mechanisms whereby they suppress the growth of *C. difficile* are still not understood. Disruption of the normal ecosystem by antibiotics can result in colonisation by *C. difficile* which, if of the right pathotype, will cause diarrhoea or, more seriously, pseudomembranous colitis. Production of proteolytic and hydrolytic enzymes and capsule, expression of fimbriae and flagella, chemotaxis and adhesion to gut receptors may all play a part in the pathogenesis of *C. difficile*-induced disease by facilitating colonisation or by directly contributing to tissue

damage. However, toxins A and B (TcdA, TcdB) are thought to be the primary virulence determinants of this pathogen in the context of antibiotic-associated gastrointestinal disease. The toxins have identical enzyme specificities – they glucosylate the same serine residue in target proteins, the Ras family of GTPases, leading to the disruption of vital signalling pathways within the cell. However, B is *ca.* 1000-fold more cytotoxic to cultured cells than A, but does not cause fluid secretion in the gut on its own, whereas A does. Over the years there has been considerable controversy in assigning relative importance to the toxins in the virulence of *C. difficile*. Recently it was shown using a hamster model that both toxins are capable of cytotoxicity and both toxins are important in pathogenesis. Overall, the toxins were responsible for many of the symptoms associated with *C. difficile* infections.

Fungal Exotoxins

Many fungi contain substances that are harmful when taken by mouth, and there are two diseases that result from the ingestion of food containing preformed fungal toxins. As with *C. botulinum*, the disease is caused without the need for infection. *Aspergillus flavus* infects groundnuts (monkey nuts) and produces a very powerful toxin (aflatoxin). Contaminated (badly stored) groundnuts used to prepare animal feeds caused the death of thousands of turkeys and pigs in the United Kingdom in 1960 and the survivors of intoxication nearly all developed liver cancer. As such, it is now clear that these toxins can have carcinogenic properties. Human disease has not yet been associated with this toxin. *Claviceps purpurae* is a rust fungus affecting rye, and it produces toxins (ergotamine especially) that give rise to ergot poisoning when contaminated grain is eaten. Mushrooms and toadstools have long been recognised as sources of poisons and hallucinogens.

Cell-Associated Toxins

Unlike the toxins already discussed in this chapter, there is a group of toxins which are distinct structural components and are not released into the surrounding medium in any quantity except upon death and lysis of the bacteria. The toxins typically comprise well-recognised structural entities which on *a priori* grounds must have key functions in the organism: they are found in the outer membranes of Gram-negative organisms. There are two chemically distinct types of toxin considered: lipopolysaccharide (endotoxin; LPS) and protein. The bulk of this section is taken up with endotoxin.

Many pathogenic organisms, however, are pathogenic partly by virtue of possessing various types of surface structure important in conferring virulence. These include, for example, adhesins which are important in colonising body surfaces or a variety of surface molecules (which may or may not be inside capsules) that render them resistant to phagocytosis. But the majority of adhesins and antiphagocytic determinants are themselves non-toxic.

The Gram-negative bacterial cell wall is subject to considerable variations in both the composition of LPS and in the number and nature of the proteins found in the outer-cell membrane.

Endotoxins

Endotoxins are part of the outer membrane of Gram-negative bacteria. It has been known for many years that the cells (alive or dead) or cell extracts of a wide variety of Gram-negative bacteria are toxic to man and animals. The literature on this subject is vast, sometimes confusing and often controversial; here we can give no more than a brief outline. Some of the diseases in which endotoxin may play an important role include typhoid fever, tularaemia, plague and brucellosis, and a variety of hospital-acquired infections caused by opportunistic Gram-negative pathogens, which include *E. coli*, *Proteus, P. aeruginosa*, *Enterobacter*, *Serratia* and *Klebsiella*. In addition, endotoxin has been intensively studied as a possible causative agent of shock arising from post-operative sepsis or other forms of traumatic injury in which the normal flora of the gut is often the source of endotoxin.

The toxins we have considered so far have been protein (or at least part protein) in nature but, in contrast, endotoxin is a complex LPS. It is also much more heat stable than protein toxins and much less easily toxoided. In addition to lethality, endotoxin displays a bewildering array of biological effects.

Location in Cell Envelope

The complex nature of the multilayered Gram-negative bacterial envelope is shown in Figure 8.14 (see also Figure 4.3). The outer membrane is composed of a bimolecular leaflet arrangement as are other membranes but has a different composition from the cytoplasmic membrane. The LPS is unique in nature, only found in Gram-negative bacteria, and is, or contains within it, what we designate endotoxin. Immunoelectron microscopy indicates that LPS exists in the outer leaflet of the membrane and extends outwards up to 300 nm; it is on, rather than in, the cell. Thus it is evident that the term endotoxin is a misnomer which derives from the era when toxins were considered to be either exotoxins, which were synthesised and secreted by the viable organism, or endotoxins, which were intracellular and released only upon lysis.

Structure

LPS consists of three regions: polysaccharide side chains, core polysaccharide, and lipid A which consists of a diglucosamine backbone to which long-chain fatty acids are linked (Figure 8.14). The relationship of this type of molecule to the outer membrane is also shown in Figure 8.14. The long-chain fatty acids interdigitate between the phospholipids in the outer leaflet and may also be linked (or interact) with lipoproteins, which in turn may or may not be covalently anchored to the rigid peptidoglycan. The polysaccharide side chains project outwards.

This structure is not invariant. For example, many organisms when first isolated give rise to colonies with a smooth appearance on agar but on subculture produce colonies with a rough appearance. In general, 'smooth' strains of pathogenic species are more virulent than rough strains. This S→R conversion is accompanied by a loss of region I side chains, which contain the deoxy and dideoxy sugars found in these LPS complexes. In addition to these somewhat drastic changes involving loss of side chains, it is possible to induce major compositional changes by manipulating the growth rate of these organisms in a chemostat. Thus the LPS of *Salmonella enteriditis* when grown with a mean

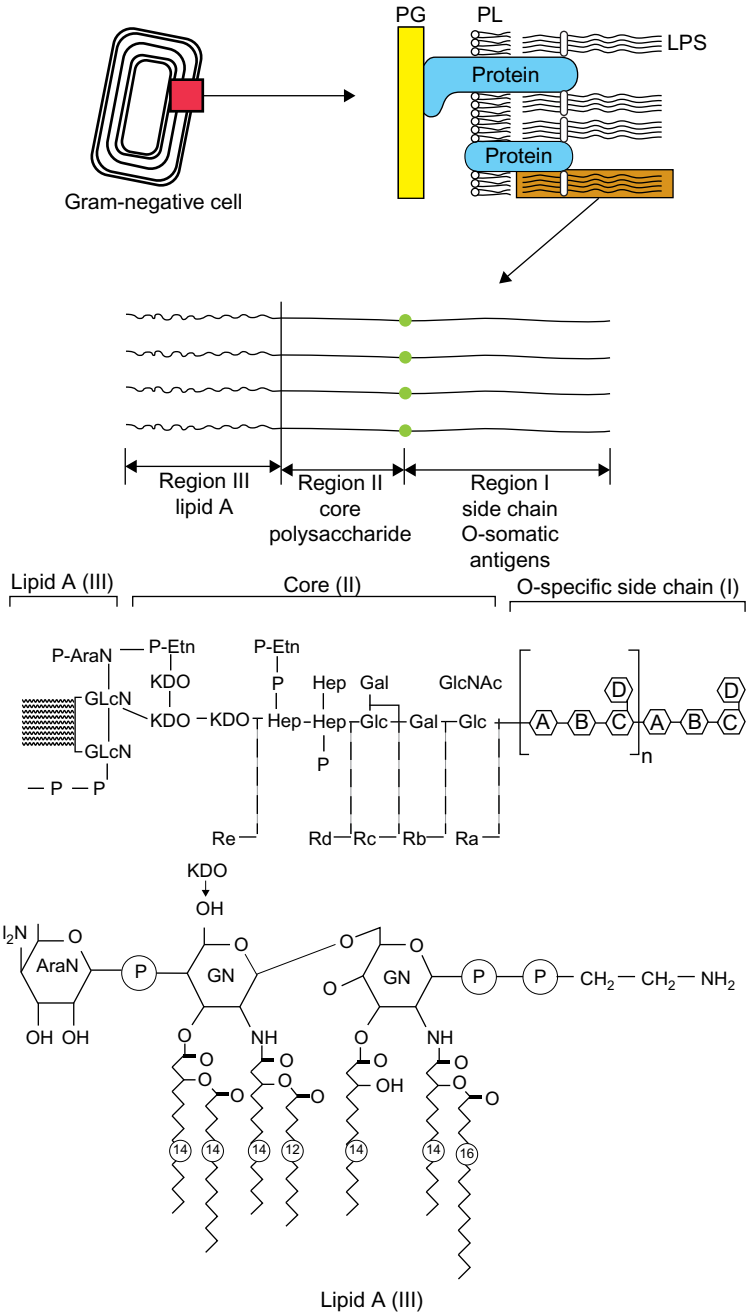


FIGURE 8.14 General structure of *Salmonella* LPS. See text for fuller explanation. Abbreviations: PG, peptidoglycan; PL, phospholipid; A-D, sugar residues; Glc, D-glucose; Gal, D-galactose; GlcN, D-glucosamine; GlcNAc, *N*-acetyl-D-glucosamine; Hep, L-glycerol-D-*manno*-heptose; KDO, 2-keto-3-deoxy-D-*manno*-octonate; AraN, 4-amino-L-arabinose; P, phosphate; EtN, ethanolamine; ~ ~ ~, hydroxy and non-hydroxy fatty acids; Ra–e, incomplete forms of LPSs. The structures indicated are typical of the Enterobacteriaceae and the Pseudomonadaceae. *H. influenzae*, *Neisseria meningitidis*, *B.*, *Acinetobacter calcoaceticus* and *Bacteroides fragilis* have less complicated LPS structures in that they do not possess the equivalent of the O-somatic side chains. *Chlamydiae* possess only lipid A and the inner core region comprising lipid A and KDO.

generation time of 20 min is nearly totally deficient in tyvelose (a dideoxy sugar), possesses 85% of the galactose and 150% of the glucose contents of LPS obtained when the generation time is 50 min. These genotypic S organisms exhibit an R-phenotype in terms of their vastly reduced O-agglutinability (see below); such observations are potentially very important in the context of the *in vivo* phenotype and pathogenicity, since it is well known that the growth rate of *Salmonella typhimurium* in mice is 10–20 times lower than *in vitro*.

Immunochemistry and Seroclassification

The extent to which lipid A is common between different genera is uncertain, but it is not likely to vary tremendously. The core polysaccharide structure is the same or very similar within groups of the Enterobacteriaciae. Thus polysaccharides from salmonellae are similar to each other, but differ from those of *E. coli* strains. However, within a group such as the salmonellae, there is a wide variation in the composition and detailed structures of the side chains, a fact which is exploited in the Kauffman–White scheme for classifying salmonellae, giving rise to several thousand serotypes.

The side chains carry the O-somatic antigen specificities of which there are far more than can readily be accounted for on the basis of the known number of sugars involved in the basic repeating units. In the side chains are found a range of deoxy and dideoxy sugars. The general principles governing the relationship between the various chemotypes and serotypes are now well understood; the multiplicity of antibody specificities evoked may be explained in terms of antibodies which can recognise different aspects of one three-dimensional structure.

Biological Properties

Lipid A is the primary toxiphore, but the polysaccharide plays an important part in conferring solubility upon, and optimising the size of micellar aggregates of LPS, hence affecting biological activity. However, the immune status of the test animal may affect toxicity: as normal animals produce antibodies to the antigenic determinants on the surface of normal gut organisms (including O-somatic antigens), some of the biological effects of endotoxin may be mediated by hypersensitivity mechanisms.

The range of biological properties of endotoxin is quite bewildering and the mode(s) of action very complicated. Included among those effects which might play a role in Gram-negative bacterial infections are abortion, pyrogenicity, tolerance (not immune tolerance), the Schwartzmann phenomenon, hypotension and shock, and lethality, but the precise part played by LPS in these phenomena in Gram-negative infections is far from clear. LPS causes the release of vasoactive substances, activates the alternative pathway of the complement cascade and also activates factor XII (Hageman factor), the first step of the coagulation cascade, which sometimes results in disseminated intravascular coagulation. Many, perhaps nearly all, of the actions of LPS are due to the stimulation of cytokine release from macrophages and other cells. There is an effect on the circulation, leading ultimately to vascular collapse. The vascular regions most affected differ from species to species; in man and sheep the main changes are found in the lungs. LPS has powerful immunological actions, which is surely no accident; as well as activating the complement system, it induces IL-1 production and is a potent B-cell

mitogen. Man is one of the most sensitive of all species to the pyrogenic action of endotoxin. A dose of 2 ng/kg of body weight injected intravenously into man causes the release of the endogenous pyrogen IL-1 and TNF from macrophages, which act on the hypothalamus to give an elevation of body temperature within an hour. It is possible that the pyrogenic action of LPS helps to generate fever in Gram-negative bacterial infections, but LPS is not the only bacterial factor capable of inducing a febrile response. For example, recall the Gram-positive bacterial superantigens discussed earlier in this chapter.

In spite of all these toxic actions, there have been suggestions that some of the responses to LPS (by macrophages, polymorphs) could be advantageous to the host, possibly assisting in the recognition and destruction of bacteria. Could it be that host responses to LPS are, like the complement or the clotting systems, useful in moderation but harmful in excess? There are reports that, when animals with less vigorous responses to LPS are infected, they suffer fewer symptoms but permit greater growth of bacteria.

Very large numbers of Gram-negative bacteria are normally present in the intestines (see Chapter 2), their continued death and exit in the faeces being balanced by multiplication in the lumen. There is a continuous, inevitable low-grade absorption of endotoxin from the intestine.[3] Absorbed (endogenous) endotoxin enters the portal circulation and is taken up and degraded by reticuloendothelial cells, mainly Kupffer cells in the liver. Continuous exposure to endotoxin probably has profound effects on the immune system and on the histology of the intestinal mucosa, stimulating development of the immune system in the immature individual, but there are no obvious pathogenic consequences. Normal people have low levels of antibody to endotoxin as a result of this continuous exposure. The sick individual may be much more susceptible to endogenous endotoxin, perhaps because of defects in removal by Kupffer cells.

After trauma or after genito-urinary instrumentation, endotoxin is detectable in peripheral blood but this leads to no particular signs or symptoms. When large amounts of endotoxin enter the blood, there are profound effects on blood vessels with peripheral vascular pooling, a drastic fall in blood pressure, collapse and sometimes death. Thus, if enough endotoxin enters the blood during massive Gram-negative bacterial sepsis, the vasomotor action of endotoxin becomes important and shock intervenes.[4] In experimental animals endotoxin also causes vasodilation and haemorrhage into the intestinal mucosa, and sometimes haemorrhage into the placenta with abortion, but these actions do not appear to be important in all Gram-negative bacterial infections.

[3]In addition, various antigens are absorbed in small quantities from the intestine, and in normal individuals antibodies are formed against various food proteins and to some extent against resident intestinal bacteria (see Chapter 2). Kupffer cells remove any antigen–antibody complexes formed locally in the intestine and prevent them from entering the systemic circulation.

[4]It must be remembered that endotoxin is only one of the pathways to shock in infectious diseases. Shock is also seen for instance in leptospiral and rickettsial infections, in gas gangrene, and in sepsis due to Gram-positive bacteria (see above).

To summarise, endotoxin, although studied so carefully and for so long, has not yet been shown to play a definitive role as a toxin in the pathogenesis of any infectious disease. But, in spite of its effects on various host defence systems including polymorphs, lymphocytes, macrophages, complement, and on endothelial cells and platelets, its overall role in infection is still not clear. It can, however, cause shock when Gram-negative bacteria invade the blood. It is for this reason that considerable effort in recent years has gone into the development of antilipid A antibodies for use as therapeutic agents to combat shock in such situations; the success rate is only partial and the expense enormous. It may be feasible to develop synthetic derivatives which would neutralise the biological activity of lipid A. However, the characteristics of the O-antigen polysaccharide are sometimes important in determining virulence: certain chemotypes are important in resisting phagocytosis.

General Observations on Toxins

Considerable space has been given to toxins because they are being intensively investigated as possible virulence determinants and have a major impact on the pathology of infectious diseases. The account illustrates the complexity of host–microbe interactions when analysed at the molecular level. Most toxins are liberated from the microbial cell and can be studied with greater facility than many of the more elusive determinants of pathogenicity. But remember that microbes that replicate inside host cells are less likely to form powerful toxins because they cannot afford to damage at too early a stage the cell in which they are multiplying. Thus, toxins are not prominent products in intracellular infections due to Mycobacteria, *Brucella*, *Rickettsiae*, *Mycoplasma* or *Chlamydia*, and viruses do not form toxins.

Although a single molecule of a toxin-like DT is enough to kill a cell, other toxins may do no more than impair cell function when present in sublethal concentrations. This can lead, for instance, to defective function in immune or phagocytic cells. Low concentrations of the streptococcal streptolysins will inhibit leucocyte chemotaxis. At even lower concentrations the toxins can be potent inducers of cytokines. The PLY of *S. pneumoniae* and anthrax lethal toxin make monocytes release IL-1 and TNF-α at 10^{-15} and 10^{-18} molar concentrations, respectively.

The ability to form toxins, whether encoded by the chromosome or by plasmids, is subject to strong selective pressures. If toxin production puts a microorganism at a serious disadvantage, it will tend to disappear. If it is advantageous it will be maintained, and will spread through the microbial population, just as the genetic changes that confer resistance to anti-microbial drugs are selected for when these drugs are widely used. It is therefore not unreasonable to ask how many of the well-known toxins are actually useful to the microbe as well as being important in causing disease in the host (Table 8.4). Although for some toxins, the specific advantage they confer to the bacteria is unclear, it is highly likely there is an important function provided which contributes to bacterial survival or transmission. The potent damaging effects of some toxins may not be representative of the true benefit which the toxin provides to the bacteria.

TABLE 8.4 Examples of Possible Usefulness of Toxins to Microorganisms

Microorganism	Toxin	Disease Production by Toxin	Value of Toxin to Microorganism
C. diphtheriae	DT	Epithelial necrosis Heart damage Nerve paralysis	Epithelial cell and polymorph destruction assists colonisation
C. tetani *C. botulinum*	Tetanus toxin Neurotoxin	Muscle spasm, lockjaw Paralysis	Could killing the host be worthwhile? A dead, putrefying corpse is a fine growth medium for these anaerobic, basically saprophytic bacteria
Shigella spp.	ShT	Exacerbates diarrhoea, dysentery Neurological effects	Diarrhoea aids transmission
V. cholerae	CT	Diarrhoea	Diarrhoea aids transmission
B. anthracis	Anthrax toxin(s)	Oedema, haemorrhage Circulatory collapse	Kills phagocytes. Also a dead host, teeming with spores, can be a good reservoir for transmission
L. pneumophila	Proteases, etc.	Contribute to lung pathology	Possible role in resisting phagocytic destruction by free-living amoebae
Staphylococcus pyogenes	Superantigens, enterotoxins	Toxic shock diarrhoea, vomiting	All are powerful T-cell mitogens (superantigens, see Chapters 7 and 8). Possible role in diverting T cells from anti-bacterial activity
S. pyogenes	'Erythrogenic toxin' (SPEA)	Scarlet fever	
P. aeruginosa	Exotoxin A proteases, elastase, etc.	Various clinical diseases	Possible role in free-living existence
B. pertussis	Pertussigen	Whooping cough	Cough aids transmission; interferes with T-cell migration
S. pneumoniae	PLY	Promotes bacteraemia Sensorineural deafness	Weakens host defences (polymorphs, complement)
Yersinia pestis	Endotoxin Other toxins	Severe systemic disease	Kills phagocytes
Various Gram-negative bacteria	Endotoxin	Contributes to disease, septic shock	LPS acts as B-cell mitogen. Possible role in diverting B cells

INDIRECT DAMAGE VIA INFLAMMATION

In infectious diseases there is nearly always a certain amount of direct microbial damage to host tissues, as discussed above. Host cells are destroyed or blood vessels injured as a direct result of the action of microbes or their toxins. Blood vessel injuries account for much of the disease picture in rickettsial infections (see above). Inflammatory materials are liberated from necrotic cells, whatever the cause of the necrosis. Also many bacteria

themselves liberate inflammatory products and certain viruses cause living infected cells to release inflammatory mediators. Therefore it is not always clear how much of the inflammation is directly microbial rather than host in origin. For instance, peptidoglycan of *Haemophilus influenzae* type b causes acute inflammation when introduced into the cerebrospinal fluid of adult rats. Probably much of this is caused by inflammatory mediators from the host; TNF-α is detectable in cerebrospinal fluid of most cases of purulent bacterial meningitis in humans and there are raised concentrations in the serum of patients with *Plasmodium falciparum* malaria. But inevitably the host (see Chapter 3) generates inflammatory and other tissue responses, and these responses sometimes account for the greater part of the tissue changes. Pathological changes can then be regarded as occurring indirectly as a result of these responses to the infection. Inflammation causes redness, swelling, pain and sometimes loss of function of the affected part (see Chapter 6) and is generally a major cause of the signs and symptoms of disease. Indirect damage attributable to the host immune response is discussed separately below. In most diseases direct and indirect types of damage both make a contribution to pathological changes, but in a given disease one or the other may predominate.

In a staphylococcal abscess the bacteria produce inflammatory materials, but they also kill infiltrating polymorphs whose lysosomal enzymes are thereby liberated and induce further inflammation. This type of indirect non-immunological damage is sometimes important in streptococcal infections. Virulent streptococci produce various toxins that damage phagocytes and also bear on their surfaces substances that impede phagocytosis (see Chapter 4). Nevertheless, with the help of antibody, all streptococci are eventually phagocytosed and killed and the infection terminated. Unlike the staphylococci, however, killed group A streptococci pose a digestive problem for phagocytic cells. The peptidoglycan component of the streptococcal cell wall is very resistant to digestion by lysosomal enzymes. Hence macrophages laden with indigestible streptococcal cell walls tend to accumulate in sites of infection. Lysosomal enzymes, including collagenase, leak from these macrophages, causing local destruction of collagen fibres and the connective tissue matrix. Macrophages secrete many other substances, some of which may contribute to cell and tissue damage. Many macrophages eventually die or form giant cells, sometimes giving rise to granulomatous lesions. In this way, persistent streptococcal materials sometimes cause chronic inflammatory lesions in the infected host. An additional immunopathological contribution to the lesions is to be expected if the host is sensitised to peptidoglycan components. Other pathogenic microorganisms that are digested with difficulty by phagocytes include *Listeria*, *Shigella*, *Candida albicans* and, of course, *Mycobacteria*, but the importance of this in the pathogenesis of disease is not generally clear.

INDIRECT DAMAGE VIA THE IMMUNE RESPONSE (IMMUNOPATHOLOGY)

The expression of the immune response necessarily involves a certain amount of inflammation, cell infiltration, lymph node swelling, even tissue destruction, as described in

Chapter 6. Such changes caused by the immune response are classed as immunopathological. Sometimes they are very severe, leading to serious disease or death, but at other times they play a minimal part in the pathogenesis of disease. With the possible exception of certain vertically transmitted virus infections and the transmissible spongiform encephalopathies (prion diseases), there are signs of an immune response in all infections. Therefore it is to be expected that there will nearly always be some contribution of the immune response to pathological changes. Often the immunological contribution is small, but sometimes it forms a major part of the disease. For instance, in tuberculosis (TB) the pathological picture is dominated by the operation of a strong and persistent cell-mediated immunity (CMI) response to the invading bacillus. In the classical tubercle a central zone of bacilli with large mononuclear and giant cells, often with some necrosis, is surrounded by fibroblasts and lymphocytes. Mononuclear infiltrations, giant cells and granulomatous lesions are characteristic pathological features of TB. When macrophages are killed by intracellular mycobacteria, the lysosomal enzymes and other materials released from the degenerating cell contribute to chronic inflammation as in the case of the streptococcal lesions referred to above.

The mere enlargement of lymphoid organs during infectious diseases is a morphological change that can often be regarded as pathological. The lymph node swelling seen in glandular fever, for instance, is an immunopathological feature of the disease, and the same can be said of the striking enlargement of the spleen caused by chronic malaria and other infections in the condition known as tropical splenomegaly.

As often as not the relative importance of direct microbial damage as opposed to immune and non-immune inflammatory reactions had not yet been determined, but the picture is clearer in most of the examples given below.

In one important human disease, pathological changes are certainly immunopathological in nature, but not enough is known about it to classify the type of reaction (Table 8.5). This disease is rheumatic fever, which follows group A streptococcal infections of the throat. It is the commonest form of heart disease in many developing countries, where it currently affects 30 million children. Antibodies formed against a streptococcal cell wall or membrane component also react with the patient's heart muscle or valves, and myocarditis develops a few weeks later. Many strains of streptococci have antigens that cross-react with the heart, and repeated infections with different streptococci cause recurrent attacks of rheumatic fever. There is genetic predisposition to the disease, based either on a particular antigen present in the heart of the patient or on a particular type of antibody response.

A number of microorganisms have antigens similar to host tissue components so that in the course of responding immunologically to such infections the host is vulnerable to autoimmune damage (e.g. ankylosing spondylitis). The antibodies to host components such as DNA, IgG, myofibrils and erythrocytes that are seen in trypanosomiasis, *Mycoplasma pneumoniae*, and Epstein–Barr virus infections appear to result from polyclonal activation of B cells. It is not clear how important these autoimmune responses are in pathogenesis, but they reflect fundamental disturbances in immunoregulation.

Four types of immunopathology can be distinguished according to the classification of allergic reactions by Coombs and Gell, and microbial immunopathology will be described under these headings (see Table 8.5).

TABLE 8.5 Immunopathological Reactions and Infectious Diseases

Reaction	Mechanism	Result	Example from Infectious Disease
Type 1 Anaphylactic	Antigen + IgE antibody attached to mast cells → histamine, etc. release	Anaphylactic shock Bronchospasm Local inflammation	Contribution to certain rashes? Helminth infections
Type 2 Cytotoxic	Antibody + antigen on cell surface → complement activation or ADCC	Lysis of cell bearing microbial antigens	Liver cell necrosis in hepatitis B?
Type 3 Immune complex	Antibody + extracellular antigen → complex	*Extravascular complex*	
		Inflammation ± tissue damage	Allergic alveolitis
		Intravascular complex	
		Complex deposition in glomeruli, joints, small skin vessels, choroid plexus → glomerulonephritis, vasculitis, etc.	Glomerulonephritis in LCM virus infection (mice) or malaria, HIV, Hepatitis B (man) Prodromal rashes Fever
Type 4 Cell-mediated (delayed)	Sensitised T lymphocyte reacts with antigen; lymphokines liberated; cytotoxicity triggered	*Extracellular antigen*	
		Inflammation, mononuclear accumulation, macrophage activation	Acute LCM virus disease in mice Certain virus rashes TB, leprosy (granulomas)
		Tissue damage	
		Antigen on tissue cell	
		T lymphocyte lyses cell	An *in vitro* classic, but difficult to demonstrate *in vivo*

Type 1: Anaphylactic Reactions

These depend on the reactions of antigens with reaginic (IgE) antibodies attached to mast cells via the latter's Fc receptors. The reaction takes place mostly at the body surfaces, resulting in the release of histamine, eosinophil and neutrophil chemotactic factors, leukotrienes and heparin from mast cells, and the activation of serotonin and plasma kinins. If the antigen–antibody interaction takes place on a large enough scale in the tissue, the histamine that is released can give rise to anaphylactic shock, the exact features depending on the sensitivity and particular reaction of the species of animal to histamine. Guinea pigs suffer from bronchospasm and asphyxia, and in man there are similar symptoms, sometimes with a fall in blood pressure and shock. This type of immunopathology, although accounting for anaphylactic reactions to horse serum or to penicillin, is not important in infectious diseases. When the antigen–IgE antibody interaction takes place at the body surface, there are local inflammatory events, giving rise to urticaria in the skin, and hay fever or asthma in the respiratory tract. This local type of anaphylaxis may play a

part in the pathogenesis of virus infections of the upper respiratory tract (e.g. common cold, RSV infections of infants), or in skin rashes in infectious diseases.

Type 1 reactions are common in helminth infections perhaps because IgE antibodies have an important role in protection against these parasites. The IgE antigen reaction, by causing inflammation, summons up from the blood anti-microbial forces such as polymorphs, antibodies and complement components. A dramatic Type 1 reaction can follow rupture of a hydatid cyst of *Echinococcus granulosus* (the dog tapeworm). Slow leakage of worm antigens means that mast cells are sensitised with specific IgE antibody, and the sudden release of antigen can cause life-threatening anaphylaxis. When the larvae of *Ascaris lumbricoides* pass through the lung on their journey from blood to intestine, they can give rise to IgE-mediated respiratory symptoms, with infiltration of eosinophils.

Type 2: Cytolytic or Cytotoxic Reactions

Reactions of this type occur when antibody combines with antigen on the surface of a tissue cell, and either activates the complement sequence whose membrane attack complex kills the cell, or triggers cytotoxicity by K cells (NK cells or phagocytes with Fc receptors). K (killer) cell cytolysis is referred to as antibody-dependent cellular cytotoxicity (ADCC). The antibody-coated cell is destroyed. As discussed in Chapter 6, the same reaction on the surface of a microorganism (e.g. enveloped virus) constitutes an important part of anti-microbial defences, often leading to the destruction of the microorganism. Cells infected with viruses and bearing viral antigens on their surface are destroyed in a similar way.

Clearly the antibody-mediated destruction of infected cells means tissue damage, and it perhaps accounts for some of the liver necrosis in hepatitis B, for instance, and probably in yellow fever. Infected cells can also be destroyed by sensitised lymphocytes or NK cells independently of antibody (see below).

In certain infections antibodies are formed against host erythrocytes and these cells are particularly sensitive to lysis. The haemolysis in malaria is caused by antibodies to parasite-derived antigens that have attached to red cells, rather than by autoantibodies to red cells themselves. In pneumonia due to *M. pneumoniae* (atypical pneumonia), antibodies (cold agglutinins) are formed against normal human group O erythrocytes. Haemolytic anaemia is occasionally seen, and there is reticulocytosis (see Glossary) in 64% of patients. The lesions in the lungs are perhaps based on cell-mediated immunopathological reactions.

Type 3: Immune Complex Reactions

The combination of antibody with antigen is an important event, initiating inflammatory phenomena that are inevitably involved in the expression of the immune response. In the infected host, these inflammatory phenomena are most of the time of great anti-microbial value (see Chapter 6). But there are nevertheless immunopathological features of the infection, and immune complex reactions sometimes do a great deal of damage in the infected individual. The mechanisms by which antigen–antibody reactions cause inflammation and tissue damage are outlined in Figure 8.15. IgA immune complexes are

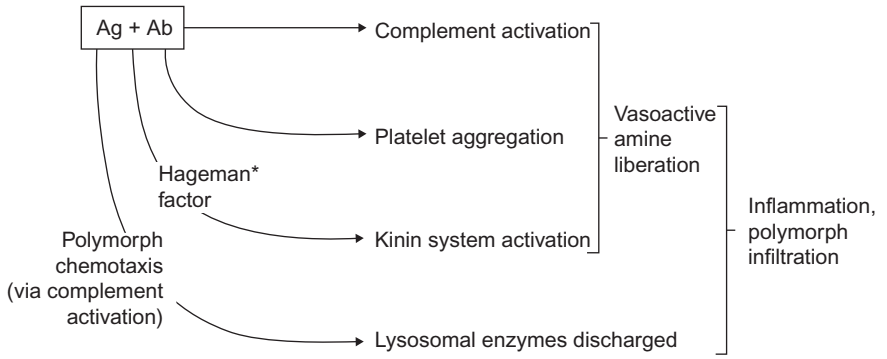


FIGURE 8.15 Mechanisms of inflammation and tissue damage induced by antigen–antibody reactions. *Activation of Hageman factor can lead to blood coagulation.

generally less harmful. Antigens absorbed from the intestine can combine locally with IgA antibody and the complex then enters the blood, to be filtered out in the liver and excreted harmlessly in bile.

When the antigen–antibody reaction takes place in extravascular tissues, there is inflammation and oedema with infiltration of neutrophils. If soluble antigen is injected intradermally into an individual with large amounts of circulating IgG antibody, the antigen–antibody reaction takes place in the walls of skin blood vessels and causes an inflammatory response. The extravasating neutrophils degenerate and their lysosomal enzymes cause extensive vascular damage. This is the classical Arthus response. Antigen–antibody reactions in tissues are not usually as serious as this, and milder inflammatory sequelae are more common as in the case of allergic alveolitis (see below).

Glomerulonephritis and Vasculitis

When the antigen–antibody reaction takes place in the blood to give circulating immune complexes, the sequelae depend to a large extent on size and on the relative proportions of antigen and antibody. If there is a large excess of antibody, each antigen molecule is covered with antibody and is removed rapidly by reticuloendothelial cells, which have receptors for the Fc portion of the antibody molecule (see Chapter 4). When equal amounts of antigen and antibody combine, lattice structures are produced, and these form large aggregates whose size ensures that they are also rapidly removed by reticuloendothelial cells. If, however, complexes are formed in antigen excess, the poorly coated antigen molecules are not removed by reticuloendothelial cells. They continue to circulate in the blood and have the opportunity to localise in small blood vessels elsewhere in the body. Complexes are deposited in the glomeruli of the kidneys, the choroid plexuses, joints and ciliary body of the eye. Factors may include local high blood pressure and turbulent flow (glomeruli), or the filtering function of the vessels involved (choroid plexus, ciliary body). In the glomeruli the complexes pass through the endothelial windows (Figure 8.16) and come to lie beneath the basement membrane. The smallest-sized complexes pass through the basement membrane and seem to enter the urine. This is probably the normal mechanism of disposal of such complexes from the body.

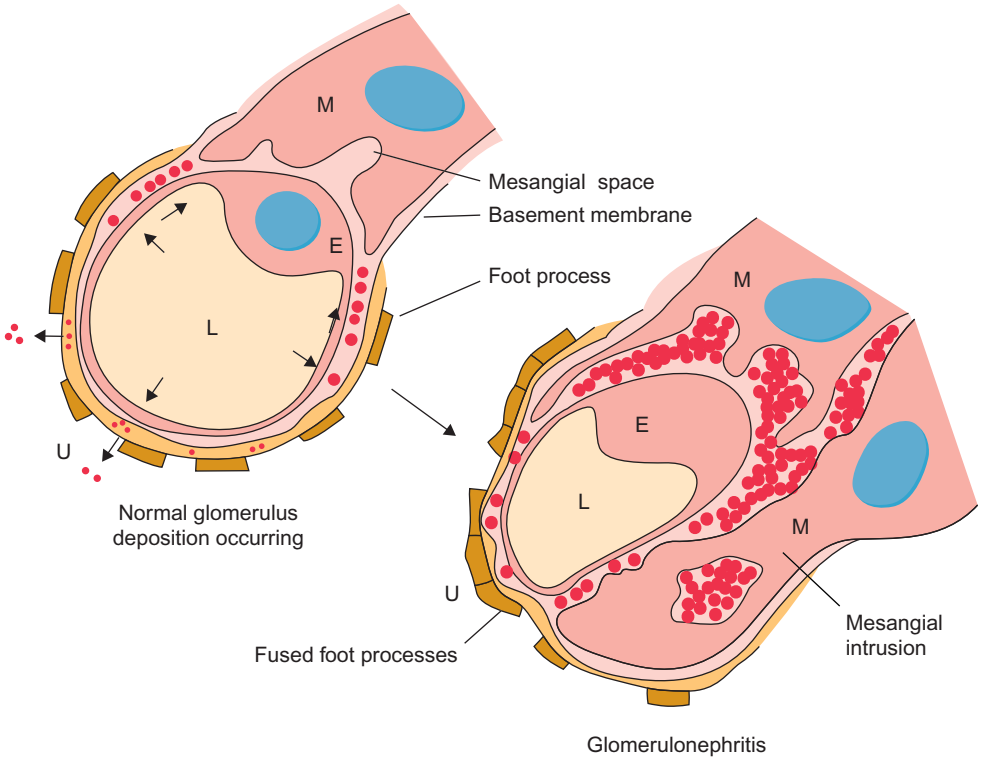


FIGURE 8.16 Immune complex glomerulonephritis. Arrows indicate the movement of immune complex deposits, some moving through to the urine and others (larger deposits) being retained. M, mesangial cell; U, urinary space; L, lumen of glomerular capillary; E, endothelial cell (contains 100 nm pores or windows).

Immune complexes are formed in many, perhaps most, acute infectious diseases. Microbial antigens commonly circulate in the blood in viral, bacterial, fungal, protozoal, rickettsial, etc. infections. When the immune response has been generated and the first trickle of specific antibody enters the blood, immune complexes are formed in antigen excess. This is generally a transitional stage soon giving rise to antibody excess, as more and more antibody enters the blood and the infection is terminated. Sometimes the localisation of immune complexes and complement in kidney glomeruli[5] is associated with a local inflammatory response after complement activation. There is an infiltration of neutrophils, swelling of the glomerular basement membrane, loss of albumin, even red blood cells, in the urine and the patient has acute glomerulonephritis. This is seen following streptococcal infections, mainly in children (see below). As complexes cease to be formed the changes are reversed, and complete recovery is the rule. Repeated attacks or persistent

[5]Cells in kidney glomeruli, in joint synovium and in choroid plexuses bear Fc or C3b receptors. This would favour localisation in these tissues.

deposition of complexes leads to irreversible damage, often with proliferation of epithelial cells following the seepage of fibrin into the urinary space.

Under certain circumstances, complexes continue to be formed in the blood and deposited subendothelially for long periods. This happens in certain persistent microbial infections in which microbial antigens are continuously released into the blood but antibody responses are only minimal or of poor quality (see below). Complexes are deposited in glomeruli over the course of weeks, months or even years. The normal mechanisms for removal are inadequate. The deposits, particularly larger complexes containing high molecular weight antigens or antibodies (IgM), are held up at the basement membrane and accumulate in the subendothelial space together with the complement components. As deposition continues, they gradually move through to the mesangial space (Figure 8.16) where they form larger aggregates. Mesangial cells (macrophages of the kidneys) one of whose functions is to deal with such materials, enlarge, multiply and extend into the subepithelial space. If these changes are gradual there are no inflammatory changes, but the structure of the basement membrane alters, allowing proteins to leak through into the urine. Later the filtering function of the glomerulus becomes progressively impaired. The pathological processes continue, some glomeruli ceasing to produce urine and the individual has chronic glomerulonephritis.

Circulating immune complex deposition in joints leads to joint swelling and inflammation, but in choroid plexuses there are no apparent pathological sequelae. Circulating immune complexes are also deposited in the walls of small blood vessels in the skin and elsewhere, where they may induce inflammatory changes. The prodromal rashes seen in exanthematous virus infections and in hepatitis B are probably caused in this way. If the vascular changes are more marked, they give rise to the condition called erythema nodosum, in which there are tender red nodules in the skin, with deposits of antigen, antibody and complement in vessel walls. Erythema nodosum is seen following streptococcal infections and during the treatment of patients with leprosy. When small arteries are severely affected, for instance in some patients with hepatitis B, this gives rise to periarteritis nodosa.

Immune complex glomerulonephritis occurs as an indirect immunopathological sequel to a variety of infections. First there are certain virus infections of animals. The antibodies formed in virus infections can act to neutralise any free virus particles, thus terminating the infection (see Chapter 6), but the infection must persist if antigen is to continue to be released into the blood and immune complexes formed over long periods. Non-neutralising antibodies help promote virus persistence because they combine specifically with virus particles, fail to render them non-infectious, and at the same time block the action of any 'good' neutralising antibodies that may be present. Immune complexes in antigen excess are formed in the blood when the persistent virus or its antigens circulates in the plasma and reacts with antibody which is present in relatively small amounts. Virus infections with these characteristics are included in Table 8.6. In each instance complexes are deposited in kidney glomeruli and sometimes in other blood vessels as described above. In some there are few if any pathological changes (LDV and leukaemia viruses in mice), probably because there is a slow rate of immune complex deposition, whereas in others glomerulonephritis (LCM virus in mice, hepatitis B and C and HIV in man) or vasculitis (ADV in mink) is seen.

TABLE 8.6 The Deposition of Circulating Immune Complexes in Infectious Diseases

Microbe	Host	Kidney Deposits	Glomerulo-nephritis	Vascular Deposits
Leukaemia virus	Mouse, cat	+	±	–
Lactate dehydrogenase virus (LDV)	Mouse	+	±	–
LCM virus	Mouse	++	+	±
Aleutian disease virus (ADV)	Mink	+	+	++
Equine infectious anaemia virus	Horse	+	+	+
Hepatitis B virus	Man	+	–	+
S. pyogenes	Man	+	+	–
Malaria (nephritic syndrome)	Man	+	+	–
T. pallidum (nephritic syndrome in secondary syphilis)	Man	+	+	?
Infectious causes of chronic glomerulonephritis[a]	Man	++	++	–

[a]Nephrologists and pathologists distinguish 10 different types of glomerulonephritis, some of them infectious in origin, the immune complexes being deposited directly from blood or formed locally in glomeruli.

A persistent virus infection that induces a feeble immune response forms an ideal background for the development of immune complex glomerulonephritis and is often seen in HIV-infected patients. There are one or two other microorganisms that occasionally cause this type of glomerulonephritis, and it is seen, for instance, in chronic quartan malaria and sometimes in infective endocarditis. In both these examples microbial antigens circulate in the blood for long periods. However, immune complex deposition does not necessarily lead to the development of glomerulonephritis, and immune complexes are detectable in the glomeruli of most normal mice and monkeys. Even in persistent virus infections the rate of deposition may be too slow to cause pathological changes as with LDV and leukaemia virus infections of mice (see Table 8.5).

Immune complex glomerulonephritis occurs in man as an important complication of streptococcal infection, but this is usually acute in nature with complement activation and inflammation of glomeruli, as referred to above. Antibodies formed against an unknown component of the streptococcus react with circulating streptococcal antigen, perhaps also with a circulating host antigen, and immune complexes are deposited in glomeruli. Streptococcal antibodies cross-reacting with the glomerular basement membrane or with streptococcal antigen trapped in the basement membrane may contribute to the picture. Deposition of complexes continues after the infection is terminated, and glomerulonephritis develops a week or two later. The streptococcal infection may be of the throat or skin, and *Streptococcus pyogenes* types 12 and 49 are frequently involved. Glomerulonephritis is also an important consequence of Lyme disease in dogs, caused by the tick-borne pathogen *Borrelia burgdorferi*.

Allergic Alveolitis

When certain antigens are inhaled by sensitised individuals and the antigen reaches the terminal divisions of the lung, there is a local antigen–antibody reaction with formation of immune complexes. The resulting inflammation and cell infiltration causes wheezing and respiratory distress, and the condition is called allergic alveolitis. Persistent inhalation of the specific antigen leads to chronic pathological changes with fibrosis and respiratory disease. Exposure to the antigen must be by inhalation; when the same antigen is injected intradermally, there is an Arthus-type reaction, and IgG rather than IgE antibodies are involved.

There are a number of microorganisms that cause allergic alveolitis. Most of these are fungi. A disease called farmer's lung occurs in farm workers repeatedly exposed to mouldy hay containing the actinomycete *Micromonospora faeni*. Cows suffer from the same condition. A fungus contaminating the bark of the maple tree causes a similar disease (maple bark stripper's disease) in workers in the United States employed in the extraction of maple syrup. The mild respiratory symptoms occasionally reported after respiratory exposure of sensitised individuals to TB doubtless have the same immunopathological basis.

Other Immune Complex Effects

In addition to their local effects, antigen–antibody complexes generate systemic reactions. For instance, the fever that occurs at the end of the incubation period of many virus infections is probably attributable to a large-scale interaction of antibodies with viral antigen, although extensive CMI reactions can also cause fever.

Systemic immune complex reactions taking place during infectious diseases can give rise to a serious condition known as disseminated intravascular coagulation. This is seen sometimes in severe generalised infections such as Gram-negative septicaemia, meningococcal septicaemia, plague, yellow fever and fevers due to hantaviruses. Immune complex reactions activate the enzymes of the coagulation cascade (Figure 8.15), leading to histamine release and increased vascular permeability. Fibrin is formed and is deposited in blood vessels in the kidneys, lungs, adrenals and pituitary. This causes multiple thromboses with infarcts, and there are also scattered haemorrhages because of the depletion of platelets, prothrombin, fibrinogen, etc.

Immune complex immunopathology is probable in various other infectious diseases. For instance, the occurrence of fever, polyarthritis, skin rashes and kidney damage (proteinuria) in meningococcal meningitis and gonococcal septicaemia indicates immune complex deposition. Circulating immune complexes are present in these conditions. Immune complexes perhaps play a part in the oedema and vasculitis of trypanosomiasis and in the rashes of secondary syphilis.

Type 4: Cell-Mediated Reactions

Although antibodies often protect without causing damage, the mere expression of a CMI response involves inflammation, lymphocyte infiltration, macrophage accumulation and macrophage activation as described in Chapter 6. The CMI response by itself causes pathological changes, and cytokines such as TNF and INF play an important part.

This can be demonstrated as a delayed hypersensitivity reaction by injecting tuberculin into the skin of a sensitised individual. The CMI response to infection dominates the pathological picture in TB, with mononuclear infiltration, degeneration of parasitised macrophages and the formation of giant cells as central features. These features of the tissue response result in the formation of granulomas (see Glossary) which reflect chronic infection and accompanying inflammation. There is a ding-dong battle as the host attempts to contain and control infection with a microorganism that is hard to eliminate. The granulomas represent chronic CMI responses to antigens released locally. Various other chronic microbial and parasitic diseases have granulomas as characteristic pathological features. These include chlamydial (lymphogranuloma inguinale), bacterial (syphilis, leprosy, actinomycosis) and fungal infections (coccidiomycosis). Antigens that are disposed of with difficulty in the body are more likely to be important inducers of granulomas. Thus, although mannan is the dominant antigen of *C. albicans*, glucan is more resistant to breakdown in macrophages and is responsible for chronic inflammatory responses.

Fibrosis is a feature of chronic infection with some viruses such as hepatitis B, hepatitis C and Epstein–Barr viruses. The hepatitis viruses replicate in parencymal cells (hepatocytes) of the liver and become targeted by cytotoxic T cells. These cells fail to resolve the infection and promote chronic inflammation leading to extensive fibrosis and scar formation. Eventually this lead to liver failure and/or hepatocellular carcinoma. A similar mechanism is responsible for the condition of chronic fibrosing alveolitis.

The lymphocytes and macrophages that accumulate in CMI responses also cause pathological changes by destroying host cells. Cells infected with viruses display vitrus peptides in 10 context of MHC I on their surface and so are targets for CMI responses as described in Chapters 6 and 9. Infected cells, even if they are perfectly healthy, are destroyed by the direct action of sensitised T lymphocytes, which are demonstrable in many viral infections. In glandular fever, cytotoxic T cells react against Epstein–Barr virus-infected B cells to unleash an immunological civil war that is especially severe in adolescents and young adults. Antigens from *Trypanosoma cruzi* are known to be adsorbed to uninfected host cells, raising the possibility of autoimmune damage in Chagas' disease, caused by this parasite.[6] It is also becoming clear that cells infected with certain protozoa (e.g. *Theileria parva* in bovine lymphocytes) have parasite antigens on their surface and are susceptible to this type of destruction. Little is known about intracellular bacteria.

The most clearly worked out example CMI mediated immunopathology is seen in LCM virus infection of adult mice. When virus is injected intracerebrally into adult mice, it grows in the meninges, ependyma and choroid plexus epithelium, but the infected cells do not show the slightest sign of damage or dysfunction. After 7–10 days, however, the mouse develops severe meningitis with submeningeal and subependymal oedema, and

[6]Chagas' disease, common in Brazil, affects 12 million people and is transmitted by blood-sucking insects. After spreading through the body during the acute infection, the parasitaemia falls to a low level and there is no clinical disease. Years later a poorly understood chronic disease appears, involving heart and intestinal tract, which contain only small numbers of the parasite but show a loss of autonomic ganglion cells. An autoimmune mechanism is possible, because a monoclonal antibody to *T. cruzi* has been obtained that cross-reacts with mammalian neurons.

dies. The illness can be completely prevented by adequate immunosuppression, and the lesions are attributable to the mouse's own vigorous $CD8^+$ T-cell response to infected cells. These cells present processed LCM viral peptides on their surface in conjunction with MHC class I proteins, and sensitised $CD8^+$ T cells, after entering the cerebrospinal fluid and encountering the infected cells, generate the inflammatory response and interference with normal neural function that cause the disease. The same cells destroy infected tissue cells *in vitro*, but tissue destruction is not a feature of the neurological disease. In this disease the $CD8^+$ T cells probably act by liberating inflammatory cytokines. It may be noted that the brain is uniquely vulnerable to inflammation and oedema, as pointed out earlier in this chapter. The infected mouse shows the same type of lesions in scattered foci of infection in the liver and elsewhere, but they are not a cause of sickness or death. LCM infection of mice is a classical example of immunopathology in which death itself is entirely due to the cell-mediated immune response of the infected individual. This response, although apparently irrelevant and harmful, is nevertheless an 'attempt' to do the right thing. It has been shown that immune T cells effectively inhibit LCM viral growth in infected organs. However, a response that in most extraneural sites would be useful and appropriate turns out to be self-destructive when it takes place in the CNS.

Another type of T-cell-mediated immune pathology is illustrated by influenza virus infection of the mouse. When inoculated intranasally, the virus infects the lungs and causes a fatal pneumonia in which the airspaces fill up with fluid and cells. The reaction is massive and the lungs almost double in weight. Effectively the animal drowns. The cause is an influx of virus-specific $CD8^+$ T cells. Normally when an appropriate number of T cells had entered the lungs, the T cells would issue a feedback response to prevent such overaccumulation, but it is thought that influenza virus infects the T cells and inhibits this control process, so that the lungs are eventually overwhelmed. The virus does not multiply in or kill the infected T cells, and it is presumed that it undergoes limited gene expression.

One human virus infection in which a strong CMI contribution to pathology seems probable is measles. Children with thymic aplasia show a general failure to develop T lymphocytes and CMI, but have normal antibody responses to most antigens. They suffer a fatal disease if they are infected with measles virus. Instead of the limited extent of virus growth and disease seen in the respiratory tract in normal children, there is inexorable multiplication of virus in the lung, in spite of antibody formation, giving rise to giant cell pneumonia. This indicates that the CMI response is essential for the control of virus growth. In addition there is a total absence of the typical measles rash, and this further indicates that the CMI response is also essential for the production of the skin lesions. Cell-mediated immune responses also make a contribution to the rashes in poxvirus infections.

OTHER INDIRECT MECHANISMS OF DAMAGE

Stress, Haemorrhage, Placental Infection and Tumours

Sometimes in infectious diseases, there are prominent pathological changes which are not attributable to the direct action of microbes or their toxins, nor to inflammation or immunopathology. The stress changes mediated by adrenal cortical hormones come into this

category. Stress is a general term used to describe various noxious influences and includes cold, heat, starvation, injury, psychological stress and infection. An infectious disease is an important stress, and corticosteroids are secreted in large amounts in severe infections. They generally tend to inhibit the development of pathological changes, but also have pronounced effects on lymphoid tissues, causing thymic involution and lymphocyte destruction. These can be regarded as pathological changes caused by stress. It was the very small size of the thymus gland as seen in children dying with various diseases, especially infectious diseases, that for many years contributed to the neglect of this important organ, and delayed appreciation of its vital role in the development of the immune system.

Appreciation of the effects of stress on infectious diseases and the immune response in particular has led to the establishment of the science of neuroimmunology. Properly controlled experiments are difficult to mount but it is clear that the nervous system affects the functioning of the immune system. The pathways of this communication are still poorly understood, but there is a shared language for immune and neural cells. For example, neural cells as well as immune cells have receptors for interleukins, and lymphocytes and macrophages secrete pituitary growth hormone. Work on *Mycobacterium bovis* grew out of observations from the turn of the century that stress appears to increase the death rate in children with TB. In one type of experiment, mice were stressed by being kept in a restraining device where movement was virtually impossible. This resulted in the reduction of expression of MHC class II antigens on macrophages, which correlated with increased susceptibility to infection. Similarly stressing mice infected with influenza virus caused several immunosuppressive events including reduction of inflammatory cells in the lung and decreased production of IL-2. Suppression of antibody responses is found in people suffering a type of stress familiar to students—examinations! The best responses to hepatitis B vaccine in students immunised on the third day of their examinations were found in those who reported the least stress. Finally, in a double-blind trial at the Common Cold Research Unit in England with five different respiratory viruses, it was ascertained in human volunteers that stress gave a small but statistically significant increased likelihood of an individual developing clinical disease.

Pathological changes are sometimes caused in an even more indirect way as in the following example. Yellow fever is a virus infection transmitted by mosquitoes and in its severest form is characterised by devastating liver lesions. There is massive mid-zonal liver necrosis following the extensive growth of virus in liver cells, resulting in the jaundice that gives the disease its name. Destruction of the liver also leads to a decrease in the rate of formation of the blood coagulation factor, prothrombin, and infected human beings or monkeys show prolonged coagulation and bleeding times. Haemorrhagic phenomena are therefore characteristic of severe yellow fever, including haemorrhage into the stomach and intestine. In the stomach the appearance of blood is altered by acid, and the vomiting of altered blood gave yellow fever another of its names, 'black vomit disease'. Haemorrhagic phenomena in infectious diseases can be due to direct microbial damage to blood vessels, as in certain rickettsial infections or in the virus infection responsible for haemorrhagic disease of deer. They may also be due to immunological damage to vessels as in the Arthus response or immune complex vasculitis, to any type of severe inflammation, and to the indirect mechanism illustrated above. Finally there are a few infectious diseases in which platelets are depleted, sometimes as a result of their combination with immune complexes

plus complement, giving thrombocytopenia and a haemorrhagic tendency (see also disseminated intravascular coagulation, p. 287). Thrombocytopenic purpura is occasionally seen in congenital rubella and in certain other severe generalised infections.

Infection during pregnancy can lead to foetal damage or death not just because the foetus is infected, but also because of infection and damage to the placenta. This is another type of indirect pathological action. Placental damage may contribute to foetal death during rubella and cytomegalovirus infections in pregnant women.

Certain viruses and even some bacteria undoubtedly cause cancer (leukaemia viruses, human papillomaviruses, several herpes viruses in animals, *H. pylori*—see Table 8.1) and this is to be regarded as a late pathological consequence of infection. Sometimes the host cell is transformed by the virus and converted into a tumour cell, the virus either introducing a transforming gene into the cell, activating expression of a pre-existing cellular gene or inactivating the cell's own fail-safe tumour suppressor gene. DNA viruses require the cell to provide the nucleotides etc. required for new virus genomes and so have evolved proteins, which are necessary for virus replication, to drive the cell into S phase, where such 'building blocks' are produced. In the normal life cycle of these viruses, the cell will die as the virus completes its life cycle; however, in abortive infections where cell lysis occurs, these cells can continue to proliferate and transformation is often therefore an 'accidental' consequence of infection. The transforming genes of retroviruses are known as viral *onc* (*vONC*) genes. These v*ONC* genes themselves originate from cellular oncogenes (cONC) which were taken up (transduced) into the genome of infecting viruses during infection. cONC are essential genes expressed within the host cell, where they play a role in normal growth and differentiation, often coding for recognised growth factors (e.g. human platelet-derived growth factor). The expression of these cellular genes is normally very tightly controlled; however, following transduction by a retrovirus they come under the control of the highly active virus promoter (LTR) and are expressed in infected cells. Additionally these transduced genes can be mutated within the virus-forming chimeric proteins with virus proteins, the net effect of this is to remove post-translational control of the activity of these proteins resulting in not only overexpression but constitutive activation.

Transformation has been extensively studied *in vitro*, and the features of the transformed cell have been described (changed surface and social activity, freedom from the usual growth restraints).

Co-infections

Increasingly, it is becoming appreciated that simultaneous infection with two different microorganisms is a relatively common occurrence. Although some infections generate antimicrobial responses such as interferon production and macrophage activation which would make a second infection less likely, co-infections are most common when local defences have been damaged by the first invader. The pathological results may be much more severe because there is a second infectious agent present. This can be considered as another mechanism of pathogenicity. Classical instances involve the respiratory tract. The destruction of ciliated epithelium in the lung by viruses such as influenza or measles allows normally non-pathogenic resident bacteria of the nose and throat, such as the pneumococcus or *H. influenzae*, to invade the lung and cause secondary pneumonia. If these bacteria enter the

lung under normal circumstances, they are destroyed by alveolar macrophages or removed by the mucociliary escalator. In at least one instance the initial virus infection appears to act by interfering with the function of alveolar macrophages. Mice infected with parainfluenza 1 (Sendai) virus show greatly increased susceptibility to infection with *H. influenzae*, and this is largely due to the fact that alveolar macrophages infected with virus show a poor ability to phagocytose and kill the bacteria. Specialised respiratory pathogens such as influenza, measles, parainfluenza or rhinoviruses damage the nasopharyngeal mucosa and can lead in the same way to secondary bacterial infection, with nasal catarrh, sinusitis, otitis media or mastoiditis. The normal microbial flora of the mouth, nasopharynx or intestine is always ready to cause trouble if host resistance is lowered, but under normal circumstances they hinder rather than help other infecting microorganisms (see Chapter 2).

One interesting example of exacerbation of infection occurs in mice dually infected with influenza virus and microorganisms such as *S. aureus* or *H. influenzae*. Under these conditions, animals with an existing influenza infection are more susceptible to secondary infections with *S. aureus*. It is considered that a major cause of mortality in the great 1918 flu pandemic was severe pneumonia due to secondary infection with *S. aureus*. For an influenza virion to be infectious, the viral haemagglutinin protein needs to be proteolytically cleaved by a host protease. If the appropriate protease is in short supply or lacking completely, virions are formed but they are not infectious. Under these circumstances, the haemagglutinin can be cleaved extracellularly by microbial proteases with resulting increased amounts of infectious virus and disease. It has been shown that *S. aureus* can produce proteases which may activate the influenza haemagglutinin and exacerbate infection.

As a final example of dual infections, microorganisms that cause immunosuppression can activate certain pre-existing chronic infections. In measles, for instance, there is a temporary general depression of CMI; tuberculin-positive individuals become tuberculin negative, and in patients with TB the disease is exacerbated. In the acquired immunodeficiency syndrome (AIDS) immunosuppression by HIV activates a variety of pre-existing persistent infections.

DIARRHOEA

In the context of the damage inflicted on the host during infection, diarrhoea deserves a separate section, since it is one of the commonest types of illness in developing countries and a major cause of death in childhood. Particularly in infants, who have a very high turnover of water relative to their size, the loss of fluid and salt soon leads to life-threatening illness. In 1998, diarrhoea was responsible for 2.2 million deaths worldwide in children under five years old. In villages in West Africa and Guatemala, the average 2–3-year-old child has diarrhoea for about two months in each year.[7] Diarrhoea also

[7]Diarrhoea on a massive scale is not always confined to developing countries. There was a major outbreak of *Cryptosporidium* infection in Milwaukee, United States, in 1993 with more than 400,000 cases; 285 of these were diagnosed in the laboratory and they suffered watery diarrhoea (a mean of 12 stools a day) for a mean of nine days. The small (4–5 μm) oocysts, probably from cattle, had entered Lake Michigan and then reached the community water supply because of inadequate filtration and coagulation treatment.

interacts with malnutrition and can cause stunted growth, defective immune responses and susceptibility to other infections. Fluid and electrolyte replacement is a simple, highly effective, life-saving treatment that can be used without determining the cause of the diarrhoea. Oral rehydration therapy (ORF) means giving a suitable amount of salt and sugar in clean water, and this is something that can be done by the mother. Diarrhoea is also a common affliction of travellers from developed countries, and business deals, athletic successes and holiday pleasures can be forfeited on the toilet seats of foreign lands. The most reliable prophylaxis is to 'cook it, peel it, or forget it'. Most attacks of diarrhoea are self-limiting. Diarrhoea means the passage of liquid faeces,[8] or faeces that take the shape of the receptacle rather than have their own shape. This could arise because of increased rate of propulsion by intestinal muscles, giving less time for reabsorption of water in the large bowel, or because there was an increase in the amount of fluid held or produced in the intestine. In many types of infectious diarrhoea, the exact mechanism is not known. Diarrhoea, on the one hand, can be regarded as a microbial device for promoting the shedding and spreading of the infection in the community, or, on the other hand, as a host device to hasten expulsion of the infectious agent. Diarrhoea is a superb mechanism for the dissemination of infected faeces and there is no doubt that strains of microbes are selected for their diarrhoea-producing powers. The advantages to the host of prompt expulsion of the infectious agent were illustrated when volunteers infected with *Shigella flexneri* were given Lomotil, a drug that inhibits peristalsis. They were more likely to develop fever and had more difficulty in eliminating the pathogen.

Before attempting to explain the pathophysiology of diarrhoeal disease, the normal structure and function of gut will be considered. The main function of the gut is the active inward transport of ions and nutrient solutes which is followed by the passive movement of water. The driving force is the Na^+/K^+ ATPase situated in the basolateral membrane of enterocytes on the villus (), which maintains a low intracellular (Na^+), thus creating the electrochemical gradient favourable for Na^+ entry and a high regional (Na^+) in the intercellular spaces; $C1^-$ follows Na^+. A similar situation exists in crypt cells: Na^+/K^+ ATPase drives secretion. The key difference is the location of the carrier systems responsible for the facilitated entry of the actively transported species. In villus cells the carriers are present in the brush border, whereas in crypt cells they are located in the basal membrane: this is responsible for the vectorial aspects of ion/fluid traffic in villus/crypt assemblies. However, it is clear that several factors in addition to enterocytes are involved in regulating fluid transport in the gut; these include the enteric nervous system and the anatomy of the microcirculation. The latter plays a profoundly important role in the uptake of fluid. This is illustrated in Figure 8.17, which shows the existence of zones of graded osmotic potential. At the tips of villi in adult human gut, osmolalities range from 700 to 800 mOsm/kg H_2O, which would generate huge osmotic forces. Thus, current perceptions are that enterocytes are responsible for generating this gradient and the blood supply acts as a countercurrent multiplier which amplifies the gradient in a manner analogous to the loops of Henle in the kidney. The hypertonic zone has been demonstrated directly in whole villi of infant mice in terms of the changing morphology of erythrocytes: in the lower regions of villi they show characteristic discoid morphology, whereas in the upper

[8]Liquid faeces are not abnormal in all species. The domestic cow experiences life-long diarrhoea, but presumably does not suffer from it.

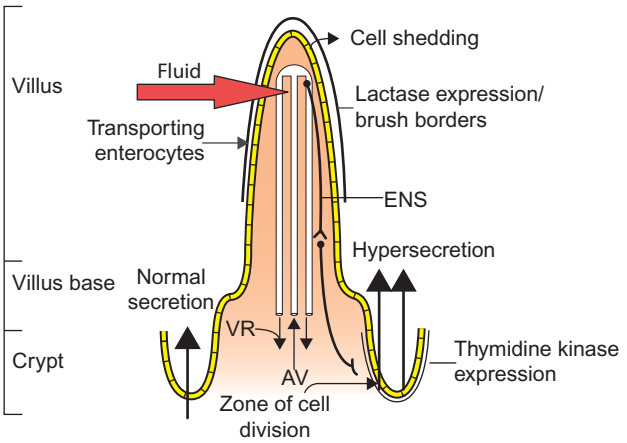


FIGURE 8.17 Small intestinal villus: simplified schema of integrated structure and function. Note the central arterial vessel (AV) which arborises at the tip into a capillary bed drained by a subepithelial venous return (VR). Movement of sodium into VR creates a concentration gradient between VR and AV, causing absorption of water from AV and surrounding tissue. This results in a progressive increase in the osmolarity of incoming blood moving into the tip region through to VR. Tip osmolarity is about three times higher than normal. Hyperosmolarity has been demonstrated in man and can be inferred in mice from the morphology of erythrocytes which changes during ascent of the same vessel from base to tip regions of villi. The intensity of shading indicates a vertical increase in osmolarity. The left crypt represents normal physiological secretion and the right crypt hypersecretion. ENS, the enteric nervous system, is depicted schematically and not anatomically.

region they are crenated, indicating a hyperosmotic environment. The hypertonicity is dissipated if the blood flow is too slow and washed out if too fast. It is the villus unit rather than enterocytes by themselves that is responsible for fluid uptake. Another consequence of the microcirculatory anatomy is that villus tip regions are relatively hypoxic. In addition, neonatal brush borders contain disaccharidases (principally lactase) which break down non-absorbable disaccharides (e.g. lactose) into constituent absorbable monosaccharides.

Villus tips and crypts are regarded as the anatomical sites of physiological absorption and secretion respectively. Fluid transport is a bidirectional process in the healthy animal with net absorption in health and net secretion in disease. The balance between absorption and secretion is poised at different points throughout the intestinal tract, reflecting differences in both structure and function. Proximal small intestine is relatively leaky; in contrast the colon is a powerfully absorptive organ.

Finally, crypts are the principal sites of cell regeneration, replacing cells which migrate up the epithelial escalator. The epithelium is renewed in approximately 3–5 days. At villus tips senescent cells are shed.

Diarrhoeal disease can result from interference with almost anyone, or a combination of these systems. The range of intestinal pathogens and the types of disease they cause is illustrated in Tables 8.7 and 8.8. Non-invasive pathogens like *V. cholerae* and enterotoxigenic *E. coli* (ETEC) secrete toxins which perturb the ion transport systems. Invasive non-histotoxic pathogens, such as some *Salmonella* strains (see Chapter 2) and rotavirus, invade villus tip cells which are then shed into the intestinal lumen. Invasive histotoxic pathogens, such as some strains of *Salmonella* (see Chapter 2), cause rapid toxin-mediated

TABLE 8.7 Production of Diarrhoea by Microorganisms Shed in Faeces

Infectious Agent	Diarrhoea	Site of Replication
Rotaviruses	+	Intestinal epithelium
Parvoviruses (dogs)	+	Intestinal epithelium (crypt cells)
Intestinal adenoviruses (types 40, 41)	+	Intestinal epithelium
Intestinal coronaviruses[a]	+	Intestinal epithelium
Norwalk virus group (caliciviruses)	+	Intestinal epithelium
V. cholerae	+	Intestinal lumen
C. difficile	+	Intestinal lumen
C. jejuni	+	Intestinal epithelium
E. coli	+	Varies[b]
Shigella	+	Intestinal epithelium
Salmonella sp.	±	Intestinal epithelium (varies)
Salmonella typhi	+	Intestinal lymphoid tissue, liver, biliary tract
Cryptosporidium	+	Intestinal epithelium
Giardia lamblia	+	Attached to intestinal epithelium
E. histolytica	+	Invasion of intestinal epithelium

[a]Described for pigs, foals, calves, sheep, dogs, mice, man and turkeys; maximum susceptibility in the first few weeks of life.
[b]Strain ETEC remains in the lumen; EIEC is similar to *Shigella*, EHEC reaches subepithelial tissues.

detachment of epithelial cells. Experimental rotavirus infections have been studied in great detail allowing us to delineate intermediate stages between initial infection, through clinical diarrhoea to recovery from infection.

Campylobacter jejuni does not figure in our treatment so far despite the fact that *C. jejuni* and related species are the most common bacterial cause of diarrhoea in many industrialised countries. It is known that motility and adherence are critical stages of infection which then lead to cytoskeletal rearrangement, host cell death, and then tight junction disruption and cytokine induction which results in loss of epithelial cell function. This then leads to a disrupted barrier, poor absorptive functions and ultimately the symptoms of disease. The clinical picture of the pathogenesis of *C. jejuni* infection may be summarised as follows. In developing countries the most common clinical presentation is mild watery diarrhoea, whereas in developed countries disease often manifests as a severe inflammatory diarrhoea. No evidence has yet been found to suggest that the watery type and severe bloody type of diarrhoeas can be explained in terms of a *C. jejuni* equivalent of the ETEC and EHEC mechanisms described above. Current thinking proposes that the different disease patterns reflect the immunological status of the host. Those with full immunity experience no clinical disease, whereas those with no pre-immunity experience the full-blown bloody diarrhoea and those with partial immunity, watery diarrhoea. The incubation period can range from 1–7 days and acute diarrhoea can last for 1–2 days with abdominal

TABLE 8.8 Types of Intestinal Infection

Types of Infection	Microorganism	Disease
Microorganism attaches to epithelium of small intestine, rarely penetrates and causes disease (diarrhoea), often by forming a toxin(s) which induces fluid loss from epithelial cells	*V. cholerae*	Cholera
	E. coli (certain strains)	Infantile gastroenteritis (certain types) or mild cholera-like disease in adults (travellers' diarrhoea)
	Giardia lamblia	Calf diarrhoea Giardiasis
Microorganism attaches to and penetrates epithelium of large intestine (*Shigella*) or ileum (*Salmonella*), causing disease by shedding/killing epithelial cells (exotoxin?) and inducing diarrhoea	*Shigella* spp.	Bacillary dysentery
	Salmonella (certain species)[a]	Salmonellosis Coliform enteritis or dysentery
	E. coli (certain strains)	Piglet diarrhoea
	C. jejuni	Diarrhoea, enteritis in man[b]
	Human diarrhoea viruses	Gastroenteritis
Subepithelial penetration uncommon	*Eimeria* spp.	Coccidiosis in domestic animals (may cause diarrhoea and blood loss)
	E. histolytica	Amoebic dysentery
Microorganism attaches to and penetrates intestinal wall. Also invades subepithelial tissues, sometimes (typhoid, hepatitis A) spreading systemically	*Salmonella typhi* and *Salmonella paratyphi*	Enteric fever (typhoid) Salmonellosis (severe form)
	Salmonella (certain species)	Calf enteritis
	E. coli (certain strains)	Varied
	Hepatitis A virus, reoviruses, enteroviruses	Hepatitis

[a]There are more than 1,000 serotypes of *Salmonella*, distinct from *Salmonella typhi* and *Salmonella paratyphi*. They are primarily parasites of animals, ranging from pythons to elephants, and their importance for man is their great tendency to colonise domestic animals. Pigs and poultry are commonly affected, and human disease follows the consumption of contaminated meat or eggs.
[b]Other campylobacters cause sepsis, abortion and enteritis in animals.

pain which may persist after diarrhoea has stopped. Diarrhoeal stools often contain fresh blood, mucus and an inflammatory exudate with leucocytes; bacteremia may also occur though it is rarely reported. Infected mucosae may be oedematous and hyperaemic with petechial haemorrhages. The disease, even its severe form, tends to be self-limiting, despite the fact that organisms may be isolated for several weeks after resolution of the symptoms. We do, however, know that there is a strong correlation between infection with *C. jejuni* and Guillain–Barré syndrome which is the most notable complication of *C. jejuni* infection. Guillain–Barré syndrome is a peripheral neuropathy, and one possible cause may be an autoimmune phenomenon arising from molecular mimicry between the polysaccharide side chains of *C. jejuni* and neural gangliosides.[9]

Rotaviruses are known to invade intestinal epithelial cells and cause diarrhoea in man, foals, dogs, pigs, mice, etc. Extensive multiplication takes place and very large amounts of virus (10^{11} particles/g) are shed in faeces. The conventional wisdom is that tips of villi especially are affected, leading to reduced absorption of fluid from the lumen. In addition, destruction of enterocytes leads to a loss in lactase resulting in an accumulation of lactose in the gut causing an osmotic flux of fluid into the intestine. A major study of rotavirus-induced

[9]Guillain–Barré syndrome is also associated with certain virus infections.

diarrhoea in neonatal mice provides a different model of this important disease of children. The main features of this model are summarised in Figure 8.18. Oral infection of the gut induces ischaemia in villi, followed by hypoxia, enterocyte damage, and shortening of villi. The perception is that it is the induction of ischaemia and not viral replication *per se* that results in these changes. It is during rapid resynthesis of the atrophied villi that maximum

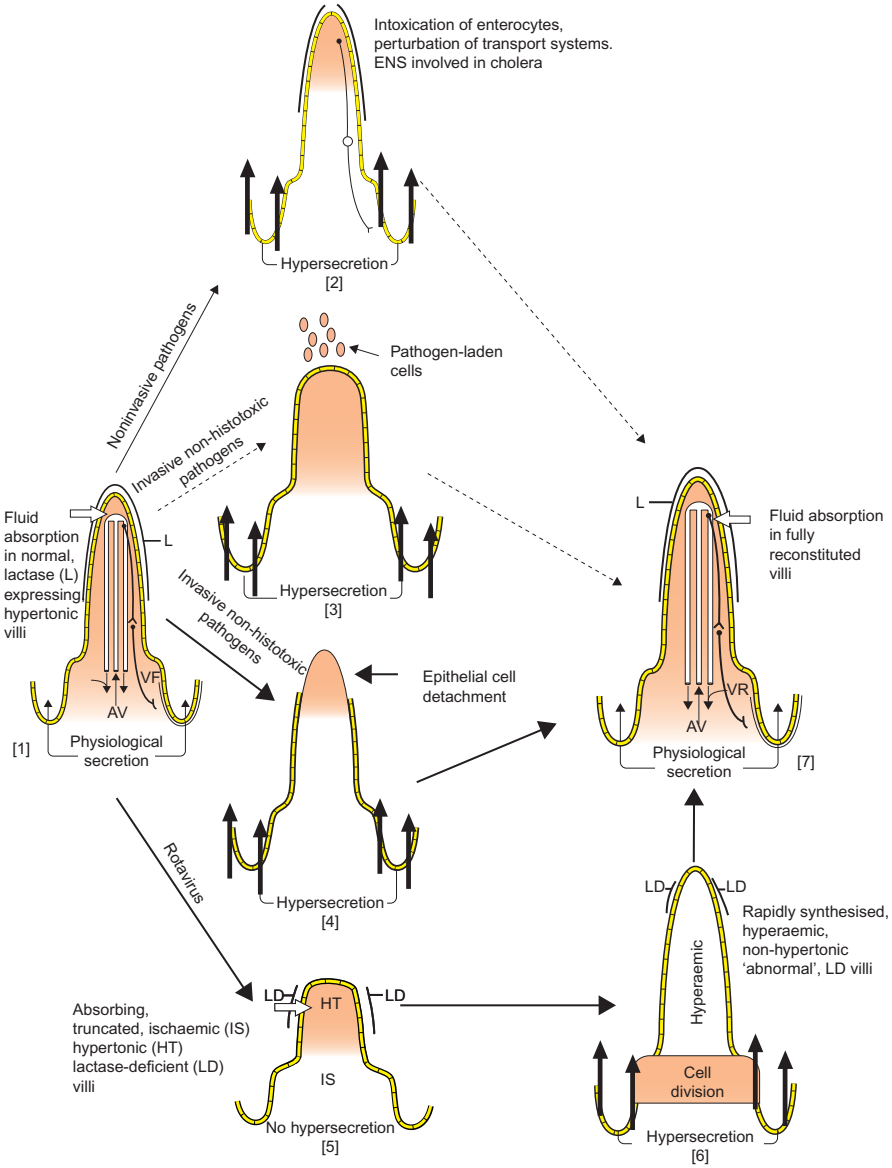


FIGURE 8.18 Diarrhoeal mechanisms: initial stages and (for rotavirus) some intermediate stages in disease progression. This represents a schematic summary of the text on diarrhoeal disease.

diarrhoea occurs due to the transient accumulation of excess NaCl in dividing cells. Unusually for viruses, an enterotoxin has been isolated which is the product of one of the viral genes (NSP4). Administration of NSP4 alone has been shown to cause a dose-dependent diarrhoea. NSP4 has been shown to block the apical sodium glucose symporter in rabbits, contributing to the Na imbalance. Prolongation of diarrhoea is seen to be due to the hyperaemic state of the newly reconstructed villi which reduces the hypertonicity of villi. Resolution of the diarrhoea occurs when microcirculation is restored to normal with concomitant restoration of hypertonic tip zones in villi.

The preceding description of the self-limiting diarrhoea induced by rotavirus in neonatal mice is that of a basic response probably applicable to many diarrhoeas, since the features of the post-peak phase have often been reported or can be inferred in other infections. However, the observed pathology will be different according to age, host species, or the inducing pathogen. For example, in rotavirus-infected lambs, villus atrophy and crypt hypertrophy occur (the latter indicative of crypt cell division) but as in mice, infected lambs are not lactose intolerant. In rotavirus-infected swine piglets, crypt hypertrophy occurs but villus atrophy is severe, the animals are lactose intolerant and mortality is high; a similar situation exists for the coronavirus, transmissible gastroenteritis (TGE) virus of swine. The latter has often been used as the model for infantile diarrhoea but the question is whether human infants are more like piglets or lambs. Clinical studies have shown that recovery from *mild*, acute gastroenteritis of rotavirus origin occurs within two weeks irrespective of the carbohydrate ingested. Clearly, the severity of disease and the clinical outcome will depend on the extent of 'vertical' villus/crypt involvement and the regions of intestine infected. When villus erosion is severe, then lactose may cause an 'osmotic' purge or be fermented by intestinal bacteria to short-chain fatty acids which stimulate secretion in the colon. Astroviruses, Norwalk virus, caliciviruses and certain adenoviruses all cause gastroenteritic disease by infecting enterocytes. However, parvoviruses cause severe intestinal disease in dogs by virtue of their predilection for the mitotically active crypt cells which is the cause of the near-complete erosion of villi similar to that seen after exposure to sublethal doses of irradiation.

Entamoeba histolytica causes lysis of target cells apparently by direct contact with the cell membrane. This pathogen produces under *in vitro* conditions an array of virulence determinants including the GAL/GALNac lectin involved in initial adherence, Kerp1 and 2 involved in liver abscess formation, glycosylphosphatidylinositol (GPI)-anchored lipophosphoglycans (LPGs) and cysteine proteases that round up cells, pore-forming proteins, collagenases and oligosaccharidases and neurotransmitter-like compounds; the latter can induce intestinal fluid secretion.

Although much research has been focused on toxins, their mode of action, and their role in disease, it is useful to compare different types of intestinal infection and to refer to the concept of *food poisoning*. Types of intestinal infection are set out in Table 8.8. Food poisoning is a loosely used term, and usually refers to illnesses caused by preformed toxins in food, or sometimes to illnesses that come on within a day or so after eating contaminated food. Food may be contaminated with plant poisons, fungal poisons (e.g. poisoning due to *Amanita phalloides*), fish poisons,[10] heavy metals, as well as with bacterial toxins or bacteria.

[10]Ingestion of scombroid fish (mackerel, etc.) containing large amounts of histamine or similar substances leads to headache, flushing, nausea and vomiting within an hour.

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CHAPTER

9

Recovery from Infection

OUTLINE

If there is to be recovery from an infection, it is first necessary that the multiplication of the infectious agent is brought under control. The microbe must decrease in numbers and cease to spread through the body or cause progressive damage. This is accomplished by immunological and other factors whose action is now to be described. The average multiplication rate of various microorganisms in the infected host as shown by doubling times is nearly always longer than in artificial culture under optimal conditions. This in itself reflects the operation of anti-microbial forces. In the process of recovery from an infectious disease, damaged tissues must of course be repaired and reconstituted. Sometimes the microorganism is completely destroyed and tissues sterilised, but often this fails to take place and the microorganism persists in the body, in some instances continuing to cause minor pathological changes. The individual is nevertheless said to have recovered from the acute infection and is usually resistant to re-infection with the same microorganism. Persistent infections are dealt with in Chapter 10.

IMMUNOLOGICAL FACTORS IN RECOVERY

The mechanisms of recovery from a primary infection are not necessarily the same as those responsible for resistance to re-infection (see below). For instance, antibody to

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measles is of prime importance in resistance to re-infection and susceptible children can be passively protected by the antibody present in pooled normal human serum. But, compared with T-cell-mediated immunity (CMI), antibody plays only a small part in the recovery from initial infection with measles virus. Antibody, T cells, NK cells, complement, phagocytes and cytokines such as interferon are involved in the response to nearly all infections and, without any doubt, are together responsible for recovery. They constitute a formidable anti-microbial force, and the relative importance of the individual components in recovery is now becoming understood. The application of the 'omic' technologies, i.e. high throughput proteomics, and transcriptome analysis using microarray analysis, or more recently RNAseq, coupled with the use of gene 'knockout' mice, has been tremendously important in evaluating, for example, the role of cytokines and chemokines in the recovery process. This has led to understanding that the immune response is a very plastic system where the loss of one gene can invariably be compensated for by other gene products.

If there is one cell that can be labelled with a multifunctional role in recovery from infection, then that cell is the macrophage (see Chapters 3, 4, 6 and 8). They play a pivotal role in both the induction and the expression of CMI responses; they are at the centre of the inflammatory responses and are key cells in the repair of tissue damage.

One major difficulty in assessing the importance of immune responses is that nearly all microorganisms are very complex, with large numbers of antigens. Various tests for antibody and T cells are carried out, but it is not always possible to test the response to a defined antigen, or to know precisely which antigens are important for infection and pathogenicity.

Antibody

The different types of antibody and the ways in which they have an anti-microbial action are listed in Chapter 6. Antibody actions against microorganisms are further discussed at the end of this chapter under the section 'Resistance to Re-infection'.

In some infections, antibody plays a major part in the process of recovery. For instance, viruses producing systemic disease, with a plasma viraemia, are controlled primarily by circulating antibody. This seems to be so in yellow fever and poliomyelitis virus infections. Children with severe agammaglobulinaemia are unable to form antibodies to poliovirus, and are about 10,000 times more likely than normal individuals to develop paralytic disease (which is generally of a chronic type) after live virus vaccination.[1] They have normal CMI and interferon responses, normal phagocytic cells and complement, but lack the specific antibody which must be produced if virus multiplication and spread to the central nervous system are to be inhibited.

[1]Agammaglobulinaemics are also susceptible to pneumococcal infections. Theoretically, opsonisation of these bacteria should occur after activation of the alternative complement pathway (Chapter 6), but antibody appears to be needed for optimal uptake and killing by phagocytes. Antibody may also be needed for lysis of virus-infected host cells after complement pathway activation.

Antibody on its own can neutralise virus infectivity and inhibit the activity of bacterial toxins. Virus neutralising antibodies can be of the IgG, IgA or IgM immunoglobulin isotypes. The amount of antibody required to induce neutralisation is unclear. Some early studies on the kinetics of neutralisation by high affinity antibodies suggested a single hit mechanism, meaning that one virus particle is neutralised by one molecule of antibody. The single hit hypothesis has recently been challenged in favour of multiple hit kinetics with estimates of 10 antibody molecules required to neutralise HIV-1. Multiple hit kinetics are more likely, and in fact are essential, if aggregation of virions by bivalent antibodies is to occur.

Neutralising antibodies can act by blocking the interaction between a cell receptor and the virus attachment proteins present on the virion surface. For non-enveloped viruses, such as the picornaviruses, attachment takes place via interaction with a limited number of structures present on the capsid. For rhinoviruses this is a narrow surface depression (or 'canyon') that surrounds each of the 12 capsid vertices. In contrast there are hundreds of virus glycoproteins present on the surface of an enveloped virus and it is likely that at any one time (i) not all of these are recognised by antibody and (ii) that only one antibody is bound per virus protein, i.e. for the flu HA spike, which has five sites recognised by neutralising antibody, only one of these sites may be occupied on a single HA molecule. The size of an antibody molecule and the distribution of these sites on HA would support this view. In addition to sterically hindering the interaction between virus and receptor, antibodies can interfere with uncoating, by either triggering a stage of uncoating prematurely or preventing uncoating by cross-linking surface structures on the virion. Some antibodies inhibit fusion (the first stage of uncoating) probably by preventing the conformational change in the virus proteins required to allow fusion to occur. Rhinovirus capsids are known to undergo a pH-mediated conformational change within the endosome, allowing the virus genome to escape the endosome. It is possible that antibodies can prevent this change, so blocking genome release.

Antibody also promotes the uptake and digestion of virus by phagocytic cells, so that the virus–antibody complex is finally taken up and disposed of. As antibodies are bivalent (IgG) they can bind to two virions simultaneously, cross-linking the virions and so creating large aggregates or clumps of virus. This decreases the ability of the virus to interact with cells and targets the virus for phagocytosis. In all the mechanisms outlined above, complement can act to increase the effectiveness of antibody. Non-neutralising antibodies are also produced during the immune response and are likely to contribute to recovery by activating complement or by interacting with Fc receptors on NK cells/macrophages.

As discussed above, antibodies neutralise free virus particles liberated from cells, but, despite the help of complement and of phagocytes with Fc receptors, are less able to influence events in infected cells. Action on the infected cell seems necessary for recovery from the above virus infections. The destruction of cells infected with viruses takes place in various ways but depends on the mechanism of virus maturation in the cell. The non-enveloped viruses, such as poliovirus or papilloma viruses, replicate and produce fully infectious particles inside the cytoplasm. These particles consist of the nucleic acid with its protein coat (capsid) and are exposed to antibody when liberated from the cell (Figure 9.1). Enveloped viruses are liberated by a process of budding from cell

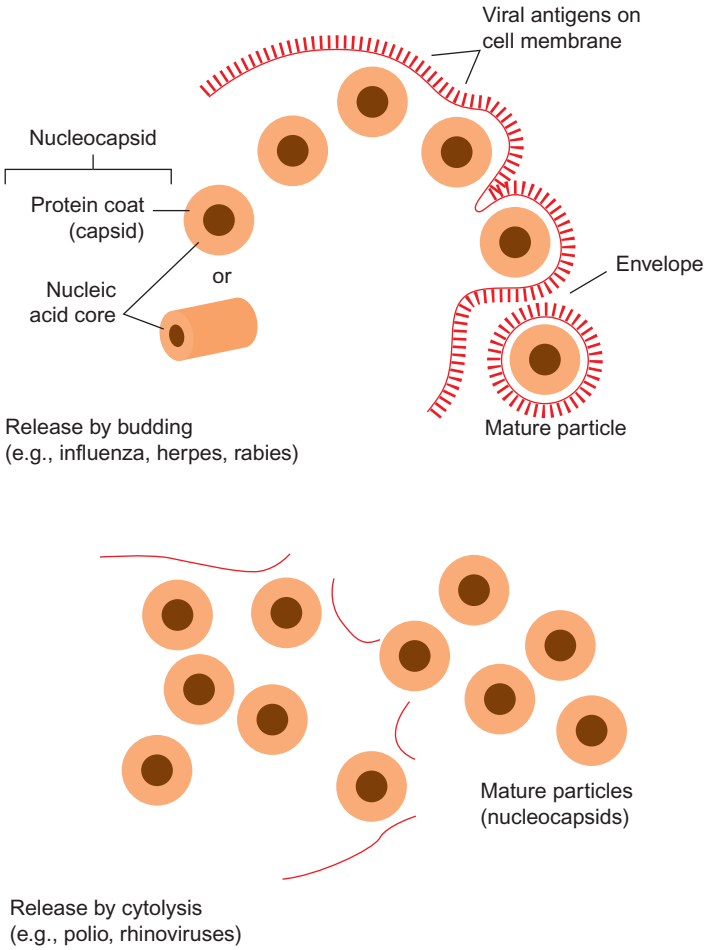

FIGURE 9.1 Diagram to illustrate mechanisms of virus release from infected cell. Nucleocapsids may be spherical (herpes viruses) or tubular (influenza). Budding may also take place from nuclear membrane (herpes viruses) or from the membrane lining cytoplasmic vacuoles (coronaviruses, flaviviruses).

membranes. The viral genome and nucleoproteins or capsids become closely associated with the cell membrane, either the cytoplasmic membrane or in some cases (e.g. herpes, pox) with an internal membrane and acts as an *initiation* point for the viral envelope proteins (Figure 9.1). The virus particle matures by budding through the altered membrane, acquiring an envelope as it does so. There are two important consequences of this mechanism of virus maturation. First, virus can be released even though the cell remains alive and intact. Second, the foreign viral glycoproteins appearing on the cell surface are recognised by host antibody and an immune response is generated with the infected cell as the target. The significance of this is that the infected cell can be destroyed before virus has been liberated as viral glycoproteins often appear on the cell surface early in the replication process, before progeny virus particles have been formed.

Various bacteria have been shown to make specific attachments to epithelial surfaces and here secretory IgA antibodies are significant. IgA antibodies are formed in most

infections of mucosal surfaces whether bacterial, viral or due to other microorganisms. They tend to prevent re-infection, but if formed early enough in the primary infection they could block the attachment of the microorganism to susceptible cells or cell surfaces and thus interfere with the spread of infection. Their actual function in recovery, however, is doubtful. As was pointed out earlier, virus infections that are limited to epithelial surfaces and do not have a time-consuming spread of infection through the body have incubation periods of no more than a few days. There is little opportunity for the slowly evolving immune response to play an important role in recovery, and virus replication is often inhibited before there has been a detectable IgA response. On the other hand, it must be remembered that antibodies (IgG or IgA) can be produced locally within two days after experimental respiratory tract infections, for instance, and they would not be detected routinely when bound to viral antigens at this stage. But interferon is produced by the first infected cell and is likely to have an important local antiviral action. If the process of infection takes longer, then secretory IgA antibodies have more opportunity to aid recovery. When the intestinal protozoan *Giardia lamblia* causes symptoms, these are not seen until 6–15 days after infection. A role for secretory IgA antibodies is indicated because patients with a shortage of these antibodies show troublesome and persistent giardial infection.

The antibody response to streptococci, staphylococci and various encapsulated bacteria such as the pneumococcus is of particular importance. These are the common pyogenic (pus-forming) infections. For its anti-bacterial function, antibody needs to operate together with phagocytic cells and complement and, if either of these are missing, resistance to pyogenic infections is impaired. Children with agammaglobulinaemia suffer repeated infections with pyogenic bacteria. The spleen is an important site of antibody formation, and when the spleen has been removed surgically, or rendered incompetent in children with sickle cell disease, there is increased susceptibility to such infections. On the other hand, many bacterial infections (tuberculosis, syphilis, typhoid, gonorrhoea) can persist or can re-infect in spite of the presence of large amounts of antibody. This is discussed more fully in Chapter 7, and it is a reminder of the frequent inability of antibodies to ensure recovery.

Antibodies are vital in recovery from diseases caused by toxins, such as diphtheria and tetanus. As soon as antibodies have been formed to neutralise the powerful toxins and prevent further tissue damage, recovery is possible; without antibodies the other anti-bacterial forces may operate in vain. In diphtheria the patient often recovers and is immune to the toxin without having controlled the infection itself, and remains a carrier.

In the case of protozoa, such as malaria, antibody must be directed against the relevant stage of the microorganism (especially the merozoite) and also against the relevant antigen on the microorganism. Merozoites are the forms that specifically enter red blood cells and parasitise them, and protective antibodies coat the merozoite surface and inhibit this absorption, at the same time promoting phagocytosis by the reticuloendothelial system (RES).

Host defences against fungi are less clearly defined but there are indications that CMI is more important than antibody. Disseminated infection with certain fungi (*Coccidioides*, *Histoplasma*) occurs even in the presence of high antibody titres, and in such cases there is usually no CMI demonstrable by skin tests, suggesting that T-cell responses matter most. Local infections with fungi elicit good CMI responses but poor antibody responses, and

the patient recovers. Severe mucocutaneous candidiasis is seen in those with defective CMI, in spite of normal antibody production. Although CMI is central for recovery from the majority of fungal infections, antibody with anti-fungal properties are produced during infection as determined by monoclonal antibodies against membrane antigens of *Cryptococcus neoformans* and *Candida albicans.* Such antibodies when injected into infected mice protect against infection. Therapeutic antibodies are being used to control a number of infectious diseases.

Small microorganisms such as viruses may have no more than one (HIV, human immunodeficiency virus) or two different proteins on their surface. The surface of influenza virus, for instance, consists of 500 or more haemagglutinin trimers, interlaced with about 100 neuraminidase tetramers. Antibodies to either antigen protect against infection, although the haemagglutinin contains the major neutralisation sites. Antibody to the neuraminidase inhibits its enzymatic activity. It does not prevent infection of the cell but prevents the dissemination of newly formed virus and thus hinders the spread of infection. This occurs because neuraminidase is required to detach haemagglutinin from sialic acid at the point on the cell membrane where the virus emerges. One can begin to work out the mechanisms of antibody protection in a relatively simple microorganism of this sort. Larger microorganisms, however, generally have many different proteins and carbohydrates on their surface. Some of these will be concerned with vital steps in the process of infection, and antibodies to specific neutralisation sites on these will be protective. Antibodies to other antigenic sites on these structures and even to some complete structures will not be protective, and when they are attached to the microbial surface may even physically interfere with (block) the action of protective antibodies. In addition, a large assortment of irrelevant antibodies are produced to internal components of the microorganism. Antibodies themselves differ in the firmness of the combination they make with antigens and may be of high or low avidity (see Glossary). Thus the quality of the antibody also matters. Protection by antibody is therefore a complicated matter, and if there is no protection in spite of the presence of large amounts of antibody, one has to ask first what components of the microbe these antibodies are combining with, and whether these antibodies have the relevant specificity for the job. Second, one needs to ask whether the antibody itself is of sufficient quality and of the appropriate isotype.

Cell-Mediated Immunity

As presented in Chapter 6, T cells are a heterogeneous population of cells as reflected by cytokine profiles and effector function. In particular, CD4 T cells assume a central role in coordinating cells from the innate response in recovery from infection. This is reflected in tissue responses in the host that bear the hallmarks of T-cell involvement, with the infiltrating cells consisting primarily of lymphocytes and macrophages. The nature of the particular T-cell subset will depend on the pathogen. For example, Th17 cells are important in recovery from extracellular bacteria and fungal infections including *Staphylococcus aureus*, whereas intra-cellular bacteria such as tuberculosis, brucellosis, listeriosis, tularaemia, syphilis and tuberculoid leprosy are controlled by Th1 cells. In *Leishmania* infection,

recovery is associated with the development of a Th1 response. This is orchestrated by the production of interleukin-12 (IL-12) from infected macrophages which acts on either natural killer (NK) cells or $CD4^+$ T cells to produce interferon-γ (IFN-γ) and tumour necrosis factor (TNF), which in turn feeds back on macrophages to induce nitric oxide, an important molecule in controlling this parasite. The blockade of IL-12 activity *in vivo*, either by neutralising antibodies or the use of IL-12-deficient mice, leads to the development of a Th2 response which fails to protect the host from a generalised parasite infection, through a lack of nitric oxide production. Similar mechanisms operate in recovery from *Listeria* infection, illustrating the central role of IL-12 and IFN-γ in the evolution of Thl-protective immune responses. In some situations persistence of antigen, as with *Mycobacterium tuberculosis*, can lead to protracted Th1 responses resulting in chronic inflammation. These responses are characteristic of delayed-type hypersensitivity which can be demonstrated in a specific manner by skin testing.

As pointed out earlier, CMI develops in many other infections but is not very clearly associated with recovery. On infection with *Streptococcus pyogenes*, for instance, delayed hypersensitivity develops to the streptococcal products streptokinase and streptodornase, but it is less important than antibody in recovery from infection.

The clearest picture about CMI in recovery comes from certain virus infections, particularly herpes viruses, poxviruses, influenza virus. All viruses utilise the cellular translation machinery to produce virus proteins and so a proportion of these proteins enter the MHC I processing pathway in a manner similar to all other cellular proteins. It can be argued that MHC I/$CD8^+$ T-cell responses evolved primarily to combat virus infections, as destruction of infected host cells has long been considered a feature of viral rather than other infections. There is now, however, evidence for this occurring with other infections. Host cells infected *in vitro* with protozoa (*Plasmodia* and *Theileria*), rickettsia (*Coxiella burnetii*) and with certain bacteria (*Listeria*) can present pathogen derived peptides in the context of MHC I. This is achieved as a natural consequence of degradation of intracellular antigens by the proteolytic machinery of the infected cell and the presentation of antigenic peptides by MHC class I. Consequently, whether a cell is infected by an enveloped or non-enveloped virus, a bacterium or a parasite, antigens can be recognised by cytotoxic T cells (CTLs).

The immune mechanisms for the destruction of cells bearing foreign antigens on their surface can be summarised as 'burns', 'pores' or 'poisons' and are as follows:

1. As mentioned above (Chapter 6), T-cell receptors only recognise peptides in association with MHC class I proteins (CD8 T cells) or class II proteins (CD4 T cells) on the target cell surface. Any viral protein can be processed in this way and usually internal virion proteins or non-virion (e.g. nuclear-transcription factors) proteins provide the major target for T cells. In order to destroy a target cell, T cells must become activated. Once this is achieved, the activated CTL makes intimate contact with the target cell membrane and delivers a lethal hit. The T cell then disengages and moves to another target. Killing of target cells occurs by one of two mechanisms. One mechanism involves releasing the contents of cytotoxic granules containing perforin, which deposits 'pores' in the membrane of the target cell, and granzyme B, which enters the

cell through the pores to act as a 'poison' in triggering apoptosis. Perforin is homologous to C9, the pore-forming component of complement. Both proteins polymerise on contact with the target cell breaching the membrane and producing pores through which electrolytes and other molecules flow causing cell damage. In contrast to apoptosis, cellular necrosis may occur (possibly as a result of large amounts of perforin being deposited) in which there is a leakage of cell components and K^+ ions, an influx of water and Na^+ ions, and the target cell swells up and dies. NK cells also use the perforin lytic mechanism to kill target cells. A second method that triggers cell death is the interaction of Fas (a TNF-like receptor on target cells) with the Fas ligand (on T cells), said to be the 'kiss of death', due to the activation of the 'death' domain in the cytoplasmic tail of Fas. This results in the initiation of a cascade of cellular proteases leading to apoptosis. An additional cytolytic mechanism involves the cytokine IL-7. This cytokine mediates killing of persistent virus-infected cells involving interaction with the intracellular protein arih2. IL-7 is presently undergoing clinical trials against a variety of persistent viral and bacterial infections.

2. Macrophages, polymorphs (neutrophils, basophils and eosinophils) and NK cells have the ability to destroy target cells with the assistance of specific antibody (antibody-dependent cell-mediated cytotoxicity (ADCC)). Antibody combines with antigen on the infected cell surface, and the killer cell attaches to the antibody-coated cells via the Fc receptor. The process is enhanced when complement is activated, the C3b molecules deposited on the cell surface being recognised by mononuclear and phagocytic cells that bear C3b receptors. The final killing mechanism is not clear, but the killer cell releases oxygen radicals and hydrogen peroxide (see p. 92) which 'burn' the target cell. The Fc receptors for IgG and IgE on eosinophils enable them to kill multicellular parasites such as schistosomes after adhering in large numbers to the antibody-coated surface of these parasites. This involves releasing toxic proteins (e.g. major basic protein and eosinophil cationic protein) directly onto the parasite surface to 'burn' holes in the tegument, enabling eosinophils to enter the parasite to deliver the *coup de grace*. These proteins are so toxic they can also damage mammalian cells, so the eosinophil carefully seals the area on the parasite where the proteins are delivered. Why the eosinophil is not destroyed is a mystery.

Destruction of infected cells is not the only mechanism available to T cells in controlling a virus infection. In hepatitis B virus (HBV) infection of the liver and in herpes simplex virus infection of neurons, $CD8^+$ T cells act to 'cure' the infection rather than kill the cells. This has been demonstrated in a transgenic mouse model of HBV infection in which every hepatocyte expresses viral genomes. By delivering immune T cells to these mice, the infection is readily controlled, but widespread destruction of hepatocytes is not observed. The key protective mechanism is IFN-γ, released by the activated $CD8^+$ T cells, which blocks virus replication and rids the cells of the viral genome. A similar mechanism operates in HBV infected chimpanzees, the other natural host for this virus. In neurons infected with herpes simplex virus, it is likely that similar $CD8^+$ T-cell control mechanisms occur, since neurons expressing late virus proteins (an indicator of the late stages of virus replication) can be prevented from cell death and 'cured' of this productive infection. However, the virus may persist in a latent form. This strategy benefits the virus in terms of its survival

as a latent infection and also the host in retaining the function of these irreplaceable cells. A decision on whether HBV or herpes virus-infected cells are killed or cured could be related to the amount of MHC class I expression on the infected cell. When MHC expression is high, these cells can be targeted by CTLs and killed. When MHC expression is low, CTLs may have difficulty directly engaging the target cell but can still influence virus replication through the local release of IFN-γ. This further illustrates the diversity of antiviral mechanisms at the disposal of the host.

The sequence of events with herpes virus, poxvirus and measles virus infections appears to be as follows: at sites of virus multiplication, T lymphocytes in the course of their normal movements through the body encounter viral peptides that are complexed with MHC proteins on the surface of a dendritic cell or other antigen-presenting cell. When a T cell encounters the antigen to which it is specifically sensitised, it becomes activated and divides to give fresh supplies of specifically sensitised T cells. These can react with any cell presenting the relevant peptide in association with a MHC molecule. Cytokines are liberated to attract macrophages and other leucocytes and focus them onto the site of infection. Infected cells are destroyed or cured by cytotoxic T lymphocytes and other cells, and virus material and cell debris is phagocytosed and disposed of by activated macrophages. Similar events occur in lymph nodes to which virus or virus antigens have been brought by lymphatic drainage.

The best way of discovering the function of a bodily mechanism or organ is to see what happens when it is removed. In experimental infections, CMI can be inactivated without affecting antibody or interferon responses, and changes in the disease are then studied.

A defined depletion of T cells can be achieved by treatment with monoclonal antibodies specific for CD4 or CD8 proteins. This approach enables T cells or other cells to be depleted at any stage in the immune response to infective agent. An alternative method is to use transgenic 'knockout' mice in which the gene encoding the protein of interest is inactivated at the DNA level. This powerful technology enables selected defects in host defence to occur, resulting in deficiencies of, for example, IFN-γ, TNF, IL-2, IL-4, IL-10 or their receptors; CD8 and CD4 T-cell function (disrupt CD8 or CD4 genes) and B-cell function (disrupt expression of IgM). Most of these gene knockout mice develop normally and remain well but show increased susceptibility to intra-cellular infections caused by various viruses, bacteria or protozoa. The picture is however complex. Deleting one cytokine or cell function upsets a delicate network of anti-microbial forces. Often a different defence mechanism takes over the function of the one that has been deleted. In other words, there is a redundancy in host defence mechanisms, as might be expected as an evolutionary response to infectious agents that often evade or interfere with these mechanisms.

At the clinical level, albeit without the precise focusing achieved in the knockout mice, evidence for the importance of CMI in the control of infections comes from studies on patients with defective CMI. Very rarely, infants are born with an absent or poorly developed thymus gland (thymic aplasia or hypoplasia). Their T lymphocytes fail to differentiate and develop, giving rise to severe CMI deficiency. Although their T-cell-dependent antibody response is also defective, they make a normal T-cell-independent antibody response (mainly IgM) (see Chapter 6 and Glossary). Thymic aplasia gives some insight into the importance of CMI in infectious diseases. Affected infants show a normal ability to control most bacterial infections, but a greatly increased susceptibility to infections with various viruses and certain other

intra-cellular microorganisms. After measles infection, for instance, there is no rash in these children, but an uncontrolled and progressive growth of virus in the respiratory tract, leading to fatal giant cell pneumonia. Evidently the CMI response controls the infectious process and at the same time plays a vital role in the development of skin lesions. In the days when affected children were vaccinated against smallpox with vaccinia virus, the virus grew as usual in epidermal cells at the inoculation site to give an increasing zone of skin destruction. In normal infants there was an inflammatory response at the edges of the lesion after 6–8 days and this led to inhibition of virus growth, then scabbing and healing of the lesion. The infant with thymic aplasia, however, did not show this response and the destructive skin lesion continued to enlarge, occupying an ever-increasing area of the arm and shoulder. The infection could be controlled by local injection of immune lymphocytes from a closely related donor, but not by antibody. Infants with this type of immune deficiency also tend to suffer severe generalised infections with herpes simplex virus. In addition, they show increased susceptibility to other intra-cellular microorganisms. When they are vaccinated against tuberculosis with live BCG vaccine, the attenuated bacteria (instead of undergoing limited growth with induction of a good CMI response) multiply in an uncontrolled fashion and may eventually kill the patient. The CMI response is therefore necessary for the control of infection with intracellular bacteria of this type.

The CMI response may have additional anti-microbial effects in chronic infections with certain intra-cellular organisms. When the microorganism persists as a source of antigenic stimulation and the CMI-induced influx of mononuclear cells continues, a granuloma may be formed (see below). The focus of infection tends to be walled off, and this is often associated with the inhibition of microbial growth. Granulomas are a feature of respiratory tuberculosis, contributing to pulmonary fibrosis. Granulomas, however, can result from chronic accumulation of immune complexes as well as from chronic local CMI reactions (see Chapter 8).

INFLAMMATION

Inflammation, whether induced by immunological reactions, tissue damage or microbial products, plays a vital role in recovery from infection (see also Chapters 3 and 6). Inflammation is necessary for the proper functioning of the immune defences because it focuses all circulating anti-microbial factors onto the site of infection. The circulating anti-microbial forces that arrive in tissues include polymorphs, macrophages, lymphocytes, antibodies, activated complement components and materials like fibrin that play a part in certain infections. The increased blood supply and temperature in inflamed tissues favour maximal metabolic activity on the part of leucocytes, and the slight lowering of pH tends to inhibit the multiplication of many extracellular microorganisms. The prompt increase in circulating polymorphs during pyogenic infections is caused in the first place by the release of cells held in reserve in the bone marrow, but there is also an increase in the rate of production. Monocyte release and production is controlled independently. At least four colony-stimulating factors, all glycoproteins, control the mitosis of polymorph and macrophage precursors, and their final differentiation and activity. They are present in increased amounts in serum during infection, and in animals the serum levels are dramatically raised by the injection of endotoxin.

Circulating polymorphs show increased functional activity during pyogenic infections and readily take up and reduce a certain yellow dye (nitroblue-tetrazolium), forming dark blue deposits in the cytoplasm. An increase in the proportion of polymorphs showing this reaction reflects their increased activity, but the test is of no value in the diagnosis of pyogenic infections because of false-positive and false-negative results. In any case, increased reduction of the dye is not necessarily associated with increased bactericidal activity.

When inflammation becomes severe or widespread, there is a general body response with the appearance of acute-phase proteins in the blood. As a result, two classical changes can be detected in the blood. The first is an increase in the erythrocyte sedimentation rate (ESR), and this is a clinically useful indication that inflammation or tissue destruction is occurring somewhere in the body. The exact mechanism of the increase is not understood. The second change is the appearance in the blood of increased quantities of a β-globulin synthesised in the liver and detected by its precipitation after the addition of the C carbohydrate of the pneumococcus. It is therefore called C-reactive protein and other acute-phase proteins are formed as a result of the action of mediators such as IL-1, IL-6 and TNF. Very small amounts are present in the blood of normal individuals, but there is a 1,000-fold increase within 24 h of the onset of inflammation. After binding to substances derived from microorganisms and from damaged host cells, it activates the complement system, acts as an opsonin, and possibly serves a useful function. Both the ESR and C-reactive protein changes are non-specific sequelae to inflammation of any sort, whether infectious or non-infectious.

When the infection is persistent, inflammation may become chronic, lasting weeks or months. Infections do not generally last for long periods if they induce acute polymorphonuclear inflammation; the battle between host and microbe is decided at an early stage. Chronic inflammation depends on a constant leakage of microbial products and antigens from the site of infection. The type of infection that persists and causes chronic inflammation is generally an intra-cellular bacterial or fungal or chlamydial infection. In these infections there is a chronic CMI response, with proliferation of lymphocytes and fibroblasts in infected areas, a steady influx of macrophages, and the formation of giant and epithelioid cells. Episodes of tissue necrosis alternate with repair and the formation of granulation tissue, then fibrous tissue. It is a ding-dong battle between microorganisms and host antimicrobial forces. The resulting granuloma (see also above) can be regarded as an attempt to wall off the infected area. Chronic infections with chronic inflammation and granuloma formation include tuberculosis, syphilis, actinomycosis, leprosy, lymphogranuloma inguinale and coccidiodomycosis. Chronic viral infections are not associated with chronic inflammatory responses, probably because virus growth is often defective and no more than minute amounts of antigen are liberated.

COMPLEMENT

Complement has been discussed and invoked on many occasions in Chapters 6–8 and in this chapter. It should be remembered that some of the complement components are quite large molecules and do not readily leave the circulation except where there is

local inflammation. Complement can carry out anti-microbial activities in the following ways:

1. *Complement lysis.* Complement reacts with antibody (IgG and IgM) that has attached to the surface of infected cells or to the surface of certain microorganisms, and destroys the cell or microorganism after making holes in the surface membrane. Gram-negative bacteria are killed in this way and also enveloped viruses such as rubella and parainfluenza (although as mentioned above, lack of complement does not exacerbate these virus infections). Because of the amplification occurring in the complement system (see Chapter 6), especially when the alternative pathway is also activated, antibody attached to the surface of a microorganism is more likely to induce complement lysis than it is to neutralise it. Complement lysis is therefore perhaps more important when antibody molecules are in short supply, early in the immune response. Bacteria with surface polysaccharide components can activate complement without the need for antibody (see 5, below), as can host cells infected with viruses such as measles. In the latter case, alternative pathway activation by itself does not do enough damage to kill the cell. Presumably, less severe membrane lesions can be repaired; antibody as well as complement must be present for lysis.
2. *Complement opsonisation.* Complement reacts with antibody attached to the surface of microorganisms, providing additional receptor sites for phagocytosis by cells bearing the appropriate complement receptors, like polymorphs or macrophages. Phagocytosis is also promoted by antibody attached to the microorganism because of the Fc receptors on phagocytes, but when complement is activated there are many more molecules of C3b present as a result of the amplification phenomenon. Therefore complement often has a more pronounced opsonising effect than antibody alone and for some bacteria, such as the pneumococcus, opsonisation actually depends on complement. Complement opsonisation is important when the antibody is IgM, because human phagocytes do not have receptors for the Fc region of IgM. Complement can also act as an opsonin though not always so effectively, in the absence of antibody (see 5, below).
3. *Complement-mediated inflammation.* Specific antibodies react with microbial antigens that are either free or on the surface of microorganisms. Following this antigen–antibody reaction, complement is activated, with generation of inflammatory and chemotactic factors (C3a and C5a). These substances focus anti-microbial serum factors and leucocytes onto the site of infection.
4. *Complement-assisted neutralisation of viruses.* In the case of viruses coated with antibody, complement adds to the mass of molecules on the virus surface and may hinder attachment of virus to susceptible cells. In some situations complement can mediate neutralisation of virus coated with a non-neutralising antibody. This will depend on the antibody isotype and presumably the density of antibody on the surface of the virus. Some viruses (murine leukaemia virus, Sindbis virus) can directly activate the complement system by interaction of virion envelope proteins with Clq or C3, resulting in the neutralisation of infectivity.
5. *Complement-assisted cell lysis.* C3b deposition on infected host cells not only opsonises (see above) but also augments ADCC and antibody-dependent complement-mediated lysis of cells.

6. *Complement opsonisation via alternative pathway*. Complement reacting with endotoxin on the surface of Gram-negative bacilli, with capsular polysaccharide of pneumococci, etc., or with *Candida*, is activated via the alternative pathway and C3b-mediated opsonisation takes place. It seems likely that this is important in natural resistance to infection.

Unfortunately, there is little direct evidence that the above anti-microbial activities of complement are in fact important in the body. The rare patients with C3 deficiency develop repeated pyogenic infections, and C3-deficient mice show increased susceptibility to plague and to staphylococcal infections. Mice with C5 deficiency (controlled by a single gene) are more susceptible to *Candida* infection, probably because of inadequate opsonisation. Patients with C5–C8 deficiencies, however, are often particularly susceptible to disseminated or recurrent neisserial infection. In this case the bactericidal rather than the opsonising action of complement seems important. But observations on complement deficiencies are probably too limited to draw firm conclusions and there have been few clearly defined deficiencies. The system is a highly complex one, with alternative pathways, positive feedback amplification and multiple inhibitors. A similar complement system occurs in a wide range of vertebrates and it must be assumed that such a complex, powerful system confers some biological advantage, presumably by giving resistance to microbial infections.

INTERFERONS

The interferons are cytokines, members of a family of cell-regulatory proteins produced by all vertebrates. There are several types of interferon, but only four relevant to studies on infectious diseases: alpha (α), beta (β), gamma (γ) and lambda(λ). Alpha and beta interferons are very similar and are made by nearly all cells in the body, including epithelial cells, neurons and muscle cells in response to viral and other infections (bacteria, mycoplasma, protozoa). There are 12 human alpha interferon genes and one beta interferon gene encoded on the short arm of chromosome 9. Gamma interferon is produced by NK cells and by T lymphocytes following antigen-specific stimulation. Only one gene exists for gamma interferon, encoded on chromosome 12. There are three lambda interferons (λ1 or IL29, λ2 or IL28a, λ3 or IL28b). These interferons are very similar to IFN α/β in that they are induced upon virus infection, but differ in their tissue distribution. They are particularly active in the respiratory system as potent inhibitors of influenza virus. All are cytokines with immunoregulatory functions as well as the anti-microbial action described below.

Viruses are the most important inducers of IFN-α and -β, the stimulus to the cell being the double-stranded RNA formed during virus replication (Figure 9.2). Interferons act on uninfected cells, binding to a cell surface receptor and activating a number of genes involved in immunity to viruses. Some of these gene products (e.g. 2′5′A synthetase/RNAseL) target viral messenger RNA and others (e.g. PKR) inactivate polypeptide chain elongation, blocking viral protein synthesis. Interferons are exceedingly potent *in vitro*, being active at about 10^{-15} M. They have no direct action on virus itself and do not interfere with viral entry into the susceptible cell. The interferons produced by different species of animals are to a large extent species specific in their action. Interferon liberated from

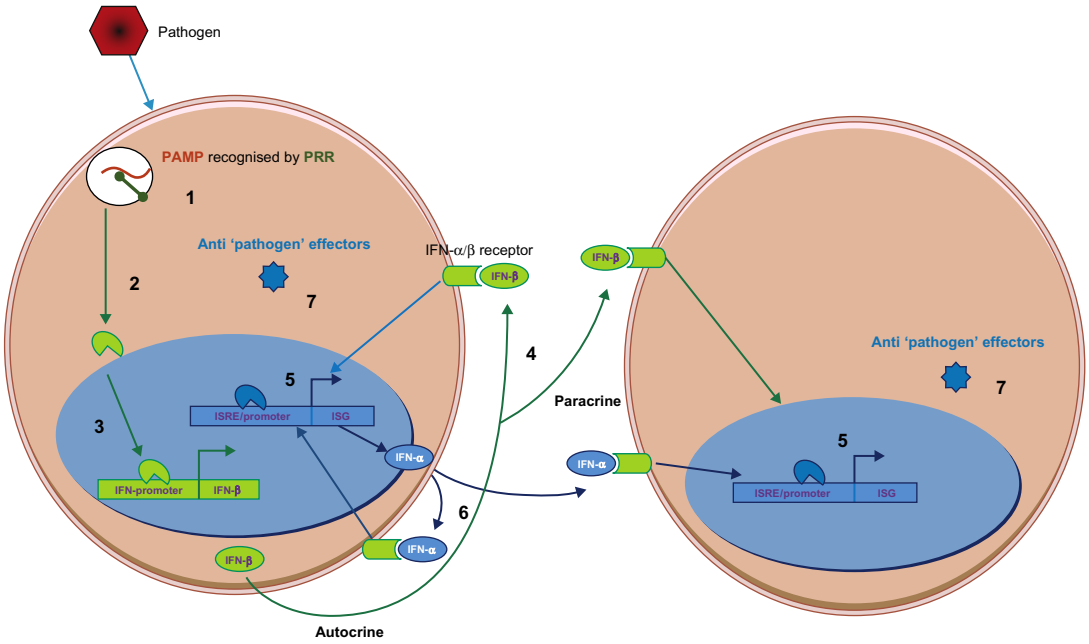

FIGURE 9.2 Mechanism of induction and expression of α and β interferon. Following infection with a pathogen, PAMPs (e.g. LPS) are recognised by cellular PRRs (e.g TLR4) (step 1). This triggers a signal transduction cascade (e.g. involving MyD88 and NF-κB) (step 2) which results in the stimulation of IFN-β gene expression (step 3). IFN-β is released from the cell and binds to the IFN-α/β receptor on both the infected cells (autocrine) and adjacent uninfected cells (paracrine) (step 4). This receptor binding stimulates a further signal transduction event (mediated in part by the JAK/STAT pathway) which in turn activates expression of interferon-stimulated genes (ISGs) via recognition of interferon-stimulated response elements (ISRE) (step 5). This also induces IFN-α expression which acts on the infected and uninfected cells to potentiate the response (step 6). The ISGs (e.g. PKR, RNAseL and Mx) act to inhibit pathogen replication (step 7).

infected cells can reach other cells in the vicinity by diffusion and establishes an antiviral state which protects them from infection. A cell is thus protected from infection with all viruses for a period of up to 24 h. It would seem inevitable that interferon is important in recovery from virus infections, whether on epithelial surfaces or in solid tissues. Interferons are also induced by non-viral agents such as rickettsiae and other bacteria, and will protect cells from various non-viral intra-cellular microorganisms and have other effects on host resistance. They activate NK cells and control T-cell activity by upregulating the expression of MHC proteins and thus the concentration of available peptide antigen.

It is now clear that interferon plays a central role in limiting virus infection *in vivo*. Evidence for this comes from various experimental approaches. The most dramatic are mice lacking the receptor for IFN-α/β (deleted by transgenic 'knockout' technology) which means they are unable to respond to interferon produced during a virus infection. Such animals are highly susceptible to many virus infections, including herpes simplex, MHV-68 and Semliki forest virus. Alpha/beta interferon can also be selectively inhibited in mice

by treatment with antibody to interferon. When this is done, enhanced susceptibility to certain virus infections is observed. Interferon has also been given passively to experimental animals and can be effectively induced by the administration of a synthetic ds-RNA preparation (poly I: poly C). Antiviral results are demonstrable in experimental infections, and are most clearly seen in infections of epithelial surfaces such as the conjunctiva or respiratory tract, and when treatment is begun before rather than after infection.

In humans, naturally occurring deficiencies in IFN are rare, partly because, for IFN-α at least, there are so many different genes involved. A study of 30 children who suffered from recurrent respiratory tract infections identified four with impaired interferon production. When these particular children were infected with common cold viruses, IFN-α could not be detected in nasal washings. Their peripheral blood leucocytes also failed to produce IFN-α on repeated testing *in vitro*, although INF-γ production was normal.

Interferon would seem to be the ideal antiviral chemotherapeutic agent for use in man, being produced naturally by human cells, non-immunogenic and active against a broad spectrum of viruses. However, it does cause influenza-like symptoms. So far, results in human patients have not been dramatic. For instance, volunteers infected intranasally with rhinoviruses and other respiratory viruses have been given either poly(I:C) or repeated very large doses of purified human interferon by the same route, but with only slight protection. However, it has proved useful in clearing up some cases of chronic hepatitis B infection and is being used to treat hepatitis caused by the flavivirus, hepatitis C. HBV downregulates the expression of MHC class I proteins on infected hepatocytes, thus preventing $CD8^+$ T cells from destroying infected cells. By treating with IFN-γ or IFN-α, expression of MHC class I proteins is upregulated and CTLs can act. Initially the patient may become ill from the effects of interferon, but eventually a virus-free liver regenerates. Here interferon is exercising its regulatory function as well as its antiviral effect. A strain of mice can be created in which the IFN-γ gene or its receptor has been inactivated or 'knocked-out' transgenically. In the absence of pathogens, mice developed normally, but they were more susceptible to the intra-cellular bacteria *Mycobacterium bovis* and *Listeria monocytogenes*, and to vaccinia virus (but not to influenza virus). The multiplicity of effects of this interferon was demonstrated by impairment in these mice of the functions of macrophages and NK cells, reduction of macrophage MHC class II proteins, uncontrolled proliferation of splenocytes and a reduction in the amount of antigen-specific IgG2a. Further support for the importance of interferons in anti-microbial defences comes from the discovery that certain viruses have gene products that interfere with the antiviral action of interferon (see Chapter 7).

MULTIMECHANISTIC RECOVERY: AN EXAMPLE

Although the host factors responsible for recovery have been described separately, they generally act together. Recovery is multimechanistic. *Salmonella typhimurium* infection of mice is the most widely used model for typhoid-like disease caused by *Salmonella typhi* in man. The system involves intravenous injection of mice with organisms and, over a period of several days, estimation of the bacterial populations present in liver and spleen (whose mononuclear cells represent the main battleground in this infection). Sublethal infection

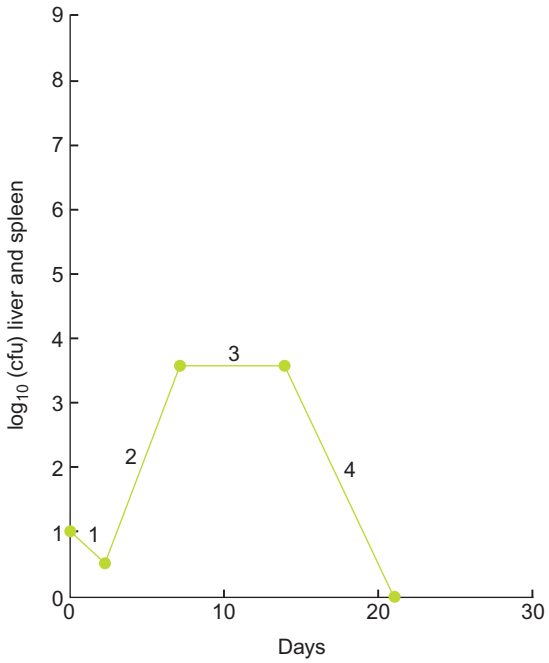

FIGURE 9.3 The four phases of a sublethal *Salmonella* infection in mice. Phase 1: initial inactivation of a large fraction of the challenge inoculum. Phase 2: exponential growth in the RES over the first week. Phase 3: plateau phase in which growth is suppressed. Phase 4: clearance of the organisms from the RES. cfu, colony-forming units.

proceeds in at least four distinct phases, schematically depicted in Figure 9.3, and with some *Salmonella* this confers solid immunity to re-challenge.

Phase 1: Initial inactivation of the inoculum. This is a constant finding representing the transition from the *in vitro* to the *in vivo* phenotype. The decline is due to immunologically non-specific uptake and destruction in macrophages of the RES. It is enhanced when animals are pre-treated with opsonising antibody, as would be expected.

Phase 2: Exponential growth in the RES. This occurs during the first week with an estimated doubling time for *Salmonella* of *ca.* 2–5 h; killing rates are also slow. Three factors can affect phase 2: (i) Inoculum dose. By increasing the dose the pattern of phases 1 and 2 remains the same but raised to a higher level. When the inoculum reaches LD_{50} or higher (see Glossary) no phase 3 is observed; phase 2 continues till lethal numbers (10^8–10^9) are reached. With very high doses, the slope of phase steepens and the time to death shortens. (ii) Virulence of the bacteria. Increase in the slope of phase 2 is also a function of the virulence of the strain. (iii) Innate resistance of the host. A gene *ity* (immunity to typhimurium) expressed through macrophages controls phase 2. A similar situation exists for *Leishmania donovani*, *M. tuberculosis* BCG, and *Mycobacterium lepraemurium.*

Phase 3 is essential for the host to survive. It is not mediated by T cells but requires continued production of TNF-α, which stimulates the production of IFN-γ. Studies with the Listeria model indicate that TNF-α is produced by macrophages which stimulate NK cells to release IFN-γ, which in turn activates newly recruited macrophages. It is of interest that, during the plateau phase, mice show a manifest macrophage-mediated immunosuppression towards other antigens.

Phase 4 is the clearance phase that does require the presence of T cells, causing macrophage activation. CD8 as well as CD4 T cells are involved. Again host genes play an important role in this phase.

This example illustrates the importance of a coordinated multimechanistic approach by the innate and adaptive immune responses in resolving a complex host–pathogen interaction.

TEMPERATURE

In man, the mean daily body temperature is 36.8°C with a daily variation of only 1.3°C, the maximum being at about 18.00 h, the minimum at about 03.00 h. This almost constant body temperature, like the almost constant level of blood sugar, illustrates Claude Bernard's dictum that *'La fixité du milieu intérieur est la condition de la vie libre'*. If the individual is to function steadily in spite of changes in the external environment, the internal environment must remain constant. The brain is one of the most sensitive parts of the body to departures from normality. At body temperatures below 27.7–30°C people become unconscious, at 40.5°C or above they become disoriented and may be maniacal; above 43.3°C they are comatose. A rise in body temperature is one of the most frequent and familiar responses to infection, whether the infection is largely restricted to body surfaces (common cold, influenza) or is obviously generalised (measles, typhoid, malaria). During fever the appetite is often lost and headache may result from dilation of meningeal blood vessels. The temperature rise is largely due to an increase in heat production, and the raised metabolic rate, together with reduced food intake, results in a high excretion of nitrogen in the urine. There is rapid wasting of body fat and muscles if the fever is prolonged.

A common mediator of the febrile response is IL-1 and, in addition, IL-6 causes fever by acting on the hypothalamus, whereas TNF (in the LPS fever model in rats) tends to reduce an already elevated temperature.

IL-1 is present in inflammatory exudates and in the plasma during fever and acts on the temperature-regulating centre in the anterior hypothalamus, resetting the body thermostat. IL-1 is produced by macrophages and certain other cells, and as little as 30–50 ng causes fever in rabbits. Fever is a common accompaniment of generalised antigen–antibody reactions. For instance, rabbits immunised with bovine serum albumin develop fever when injected with this antigen. Systemic virus infections such as the exanthems (see Glossary) are characterised by an asymptomatic incubation period during which virus replicates and spreads through the body, followed by a sudden onset of illness with fever. The febrile reaction is mainly due to the immune response to the virus; hence its relatively sudden onset a week or two after infection. The CMI response as well as the antibody response is involved. Antigen–antibody reactions, in addition to causing fever, can also give rashes, joint swelling and pain, even glomerulonephritis (see Chapter 8). The first signs of illness in hepatitis B, before jaundice, are often 'allergic' in nature and mediated by antigen–antibody interactions, with fever, joint pains and fleeting rashes.

The generalised CMI response in the infected host is also a cause of fever, e.g. in tuberculosis, brucellosis and perhaps staphylococcal and cryptococcal infections. Tuberculin added to alveolar macrophages from an immunised animal induces IL-1, and patients with chronic brucellosis develop fever when injected with 10 g of purified brucella.

Certain bacterial products are pyrogenic. The peptidoglycan in the cell wall of staphylococci causes monocytes to liberate IL-1. More importantly, endotoxins from Gram-negative bacteria also have this effect, as little as 2 ng of *Salmonella* endotoxin kg^{-1} causing fever in man. Endotoxin is present in the circulation during systemic infection with Gram-negative bacteria, but tolerance to endotoxin-induced fever develops quite rapidly, and endotoxin itself probably makes no more than a partial contribution to the febrile response, even in infections such as typhoid and dysentery. There is no good evidence that other microbial products or toxins cause fever other than by immunological mechanisms. In the old days before penicillin, pneumococcal pneumonia used to give one of the highest fevers known in man with dramatic and severe onset, the temperature often rising to 40°C within 12 h. These bacteria, however, have no endotoxin or other pyrogens and the mechanisms were presumably immunological.

When human volunteers were infected with influenza virus, those with the most IL-6 and IFN-γ in nasal fluids had higher temperatures, as well as more virus, more mucus production and more symptoms. In the case of influenza virus infection of ferrets, which is the best animal model for transmission of human influenza, there is a direct correlation between virulence and viral pyrogenicity. IL-1 is released locally in the respiratory tract as a result of virus–phagocyte interaction. Since fever is such a constant sequel of infection, it is natural to suppose that it has some anti-microbial benefit. Thomas Sydenham in the seventeenth century wrote that 'Fever is a mighty engine which nature brings into the world for the conquest of her enemies'. Bodily functions are profoundly disturbed by fever. Metabolic activity is increased in phagocytic cells, and studies *in vitro* show that there are large increases in T-cell proliferation and in antibody production at febrile temperatures. The evidence, however, is disappointing. Temperature-sensitive mutants of certain viruses are often less virulent, and experimental virus infections can sometimes be made more severe by preventing fever with antipyretic drugs. When fever is induced in infected animals by raising the environmental temperature, there are also other complex physiological changes, making it difficult to interpret such experiments. In two bacterial infections, gonorrhoea and syphilis, the microbes themselves are actually killed by febrile temperatures, but in the natural disease these temperatures are rarely reached. Before the introduction of antibiotics, patients with these two diseases were infected with malaria in order to induce body temperatures high enough to eradicate the infection (following which the malaria was treated with quinine).

If fever is of value to the host, one might expect microbes to attempt to prevent it. Vaccinia virus, which normally fails to cause fever in mice, produces a soluble receptor for IL-lβ (the fever mediator) and virus strains lacking the gene for this receptor do cause fever. We may ask whether T pallidum actively inhibits the fever response.

Fever is costly in energy and is an ancient bodily response, having evolved with the vertebrates over hundreds of millions of years. Perhaps one day some more convincing evidence will emerge to give substance to Sydenham's eloquent convictions.

TISSUE REPAIR

Once the multiplication of the infecting microorganism has been controlled, and the microorganism itself perhaps eliminated from the body, the next step in the process of recovery is to tidy up the debris and repair the damaged tissues. In other words

pathogenesis is followed by 'pathoexodus'. Four examples will be given, in the skin, respiratory tract, liver and the foetus. At the molecular level a profusion of mediators are involved. Cytokines, because of their effects on cell growth and differentiation, play a part at all stages in the repair process.

In the skin

During recovery from a boil, for instance, the sequence of events is as follows. Superficial tissue debris, including necrotic epidermis, inflammatory cells and plasma exudate, dries off as a scab. This gives mechanical protection, acts as a barrier to further infection, and can be shed to the exterior after repair is completed. Below the scab, phagocytic cells clear up the debris and fibroblasts move in, multiply and lay down a mucopolysaccharide matrix over the underlying intact tissues. New blood vessels are formed, and later on lymphatics, by the sprouting of the endothelial cells of neighbouring vessels into the fibroblast matrix. The newly formed capillaries advance into the damaged zone at 0.1–0.6 μm a day. They are fragile and leaky, and there is a continuous extravasation of polymorphs, macrophages and fibroblasts into the matrix. As seen from the surface, each collection of capillary loops in the fibroblast matrix looks like a small red granule and this soft vascular material is therefore called granulation tissue. It bleeds easily and with its rich blood supply and abundant phagocytic cells is well protected against infection. Meanwhile, epidermal cells at the edges of the gap have been multiplying. The newly formed layer of cells creeps over the granulation tissue, and the epidermis is thus reconstituted. Fibroblasts in the granulation tissue lay down reticulum fibres and later collagen. If the area of epidermal cell destruction is large, and when underlying sebaceous glands, hair follicles, etc. are destroyed, a great deal of collagenous fibrous tissue is formed to repair the gap. The newly formed collagen in fibrous tissue contracts and tends to bring the skin edges together. Contracting collagen can strangle an organ like the liver, but in the skin it merely forms a scar. A scar is a characteristic sequel to vaccination with BCG, or to a bacterial infection involving sebaceous glands, as seen in severe acne.

In the Respiratory Tract

After infection with a rhinovirus or influenza virus, there are large areas where the epithelial cells are destroyed, mucociliary transport is defective and the underlying cells are vulnerable to secondary bacterial infection. Phagocytic cells must now ingest and dispose of tissue debris, and the epithelial surface must be reconstituted by a burst of mitotic activity in adjacent epithelial cells. To some extent, pre-existing cells can slide across the gap but repair depends on mitosis in cells at the edges. The process of repair takes several days, and the mechanism is the same whether the damage is caused by viruses, bacteria or chemicals. Epithelial regeneration is particularly rapid in respiratory epithelium, and also in conjunctiva, oropharynx and mucocutaneous junctions, but it is delayed if the infection continues. After chronic bacterial or chemical damage, there is an increase in mucus-producing goblet cells in the respiratory epithelium, and sometimes impairment of

mucociliary mechanisms, resulting in the condition called chronic bronchitis. As a rule, however, recovery is complete.

In the Liver

During recovery from focal hepatitis, polymorphs and macrophages are active in areas of tissue damage, phagocytosing dead and damaged hepatic cells, Kupffer cells, biliary epithelial cells, inflammatory cells and microorganisms. As this proceeds, neighbouring hepatic cells and bile duct epithelial cells divide to replace missing cells. This, together with cell movement and rearrangement, leads to remodelling of the lobules and the restoration of normal appearances. If supporting tissues have been significantly damaged, and particularly if there are repeated episodes of necrosis, healing involves scar formation. When this is widespread it is referred to as cirrhosis, the bands of fibrous tissue dividing up the organ into irregular islands. The regenerating islands enlarge to form nodules, the fibrous tissue thickens and contracts and there is obvious distortion of structure, with circulatory impairment, biliary obstruction and liver dysfunction.

In most tissues, repair with restoration of structural integrity can be achieved by fibrous tissue formation. Recovery of function depends more on the ability of differentiated cells in damaged tissues to increase their numbers again and thus restore functional integrity. Liver cells or epithelial cells have a great capacity for mitosis, and the intestinal epithelium, respiratory epithelium or liver can be restored to normal without great difficulty. In the case of cardiac muscle, striated muscle or brain, the differentiated cells show little if any mitotic capacity and destruction in these tissues results in a permanent deficit in the number of cells. This may be of no consequence in a muscle as long as firm scar tissue repairs the damage, but it may be important in the central nervous system. Anterior horn cells destroyed by poliovirus cannot be replaced, and if enough are destroyed there will be a permanent paralysis, although some restoration of function takes place by learning to use muscles more effectively and by the recovery of damaged anterior horn cells.

In the Foetus

Tissue repair in the foetus is in some ways easier and in others more difficult. In general there is a very great capacity for repair and reconstitution of damaged tissues. Primitive mitotic cells abound, organs are in a state of plasticity, and in the developmental process itself tissue destruction and repair accompanies mitosis and construction. On the other hand, at critical times in foetal life, there is a programmed cell division and differentiation in the course of constructing certain major organs. If one of these organs is damaged at this critical time, the developmental process is upset and the organ is malformed. This is what happens when rubella virus infects the human foetus during the first three months of pregnancy. Depending on the exact organ system being formed at the time of foetal infection, there may be damage to the heart, eyes, ears or brain, resulting in congenital heart disease, cataract, deafness or mental retardation in the infant. Other infections (see Table 5.3) affect particularly the central nervous system of the foetus (toxoplasmosis, cytomegalovirus, syphilis) and sometimes bones and teeth (syphilis).

If the foetal infection is severe, as is the rule with vaccinia virus or with most bacteria, foetal death and abortion is the inevitable consequence. There are only a small number of microorganisms that infect the foetus and interfere with development without proving fatal. This type of nicely balanced pathogenicity is needed if the infected foetus is to survive and be born with a malformation. Even the infections that cause malformations (teratogenic infections) are sometimes severe enough to kill the foetus. In most congenital infections the microorganism remains present and is detectable in the newborn infant (cytomegalovirus, rubella, syphilis, etc.), often persisting for many years. It is a striking feature of most teratogenic foetal infections (rubella, cytomegalovirus, toxoplasmosis) that the mother suffers a very mild or completely inapparent infection.

Certain microorganisms infect the foetus and damage developing organs, but are then eliminated from the body. The damaged organs are formed as best as possible, and at birth there are no signs that the malformation was caused by a microorganism. Tissues are sterile, and no inflammatory responses are visible histologically. Thus when a pregnant hamster is infected with K virus (a polyomavirus), there is infection of the dividing cells that are to form the molecular layers constituting the bulk of the cerebellum. These cells are destroyed, the cerebellum therefore fails to develop normally, and the newborn hamster shows severe signs of cerebellar dysfunction, although it is perfectly well in every other way. The affected cerebellum is small and greatly depleted of cells, but there is no evidence of past microbial infection.

RESISTANCE TO RE-INFECTION

Resistance to re-infection depends on the immune response generated during primary infection. Passive immunisation with antibody is known to protect humans against measles, hepatitis A, hepatitis B, rabies, etc., and the passively acquired (maternal) immunity of the newborn child or calf to a great variety of infections is another example of the resistance conferred by specific antibody. Most resistance to re-infection is antibody-mediated. IgG antibodies generally continue to be formed in the body many years after the initial infection; IgA antibodies are less persistent than IgG antibodies. Even if antibody levels are undetectable, memory B cells from the initial infection are often present in large enough numbers to give an accelerated (anamnestic) response within a few days of re-infection. This is especially important in infectious diseases with incubation periods measured in weeks because there is time enough for the anamnestic response to operate and terminate the infection during the incubation period, before production of clinical disease. Sometimes resistance to re-infection is maintained by repeated subclinical infections, each of which boosts the immune response. For instance, children catching rubella at school can re-infect their immune parents sub-clinically and this is detected by a rise in antibody levels. Resistance to rubella, diphtheria and perhaps other infectious diseases is maintained in this way.

Antibodies protect against infection in a number of ways. For instance, they attach to the microbial surface and promote its uptake by phagocytic cells, acting as opsonins. Other antibodies protect against re-infection by combining with the microbial surface and blocking attachment to susceptible cells or body surfaces. Microorganisms that need

to make specific attachments are listed in Table 2.1. However, circulating IgG or IgM antibodies coat polioviruses, coxsackie viruses or adenoviruses, and act by interfering with viral uncoating rather than by blocking attachment to susceptible cells. Secretory IgA antibodies are particularly important because they can act on the microorganism before its attachment to a body surface. They do not act as opsonins, and they do not lyse microorganisms because there is no complement on body surfaces, and in any case they fix complement poorly. But by preventing the attachment of microorganisms such as *Vibrio cholerae* to intestinal epithelium, the gonococcus to urethral epithelium, or *Chlamydia* to the conjunctiva, IgA antibodies can ensure that these microorganisms are carried away in fluid secretions rather than initiate infection. Acquired resistance to infection of the surface of the body is often of short duration. For instance, resistance to gonorrhoea or parainfluenza viruses following natural infection seems to last only for a month or so, and in childhood repeated infections with respiratory syncytial virus and *Mycoplasma pneumoniae* are common. Presumably the IgA antibodies that mediate resistance are short-lived and IgA memory cells do not generate a good enough or rapid enough secondary response.

Resistance to re-infection, since it is immunological in nature, refers especially to the antigenic nature of the original infecting microorganism. Resistance to measles or mumps means resistance to measles or mumps wherever or whenever they occur, because these viruses are essentially of only one type (monotypic) immunologically. Resistance to the disease influenza or poliomyelitis, however, depends on the separate acquisition of resistance to a number of distinct antigenic types of influenza or polioviruses. Resistance to streptococci depends on the acquisition of antibodies to the M protein in the bacterial cell wall, and since there are at least 10 types of M protein that circulate quite commonly in communities (40–50 types of M protein altogether), repeated infections with *S. pyogenes* occur as antibodies are gradually developed against the various types. Often, however, different serological types of a given microorganism show some overlap so that antibodies to one type can confer partial resistance to another.

When resistance to a disease appears not to develop, the possibility of multiple antigenic types must be considered. There are multiple antigenically distinct types of gonococcus, for instance, a fact that helps account for successive attacks of gonorrhoea. Numerous attacks of non-specific urethritis are to be expected because of the variety of microorganisms that cause this condition. In one study, 40% of attacks were due to *Chlamydia*, but there are 12 known antigenic types.

Resistance to re-infection can also be mediated by CMI. The CMI response generated on primary infection lasts for weeks or perhaps months rather than years, and there is an accelerated CMI response on re-infection, although less vigorous than in the case of antibodies. Nearly always, a persistent infection is needed to give continued CMI resistance and infections showing this are usually intra-cellular in nature. For instance, resistance to re-infection with tuberculosis, syphilis and possibly malaria depends on the active presence of the microorganism in the body, with continuous stimulation of the antibody and CMI responses. In most of these instances, resistance to re-infection is CMI-mediated. There are a few examples, however, such as measles, in which recovery from primary infection is largely due to CMI, but resistance to re-infection is attributable to antibody.

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CHAPTER

10

Failure to Eliminate Microbe

OUTLINE

There are many infections in which the microorganism is not eliminated from the body, but persists in the host for months, years or a lifetime. Examples of persistent infections are given in Table 10.1. One way of looking at persistent infections is to regard them as failures of the host defence mechanisms which are designed to eliminate invading microorganisms from tissues. There are various ways in which the host defence mechanisms can fail and various methods by which the microbes can overcome them. Microbial adaptations to the encounter with the phagocytic cell are described in Chapter 4 and the ways in which the immune responses are by-passed are described in Chapter 7. Persistent infections usually follow on from an initial acute infection. Primary infection with herpes simplex virus causes an acute stomatitis with herpetic (literally meaning creeping, or snake-like, which gives the virus its name) lesions on the oral mucosae, and this is followed by a persistent infection. Persistent infections are not usually significant causes of acute illness, but they are particularly important for five reasons:

1. They enable the infectious agent to persist in the community
2. They can be activated in immunosuppressed patients, and sometimes (e.g. herpes simplex) in normal people (latency)
3. Some are associated with immunopathological disease
4. Some are associated with neoplasms
5. Some are immunosuppressive (human immunodeficiency virus; HIV) and permit disease caused by other normally harmless persistent microorganisms.

Mims' Pathogenesis of Infectious Disease.
DOI: http://dx.doi.org/10.1016/B978-0-12-397188-3.00010-X

TABLE 10.1 Examples of Persistent Infections (Mainly Human)

Microorganism	Site of Persistence	Infectiousness of Persistent Microorganism	Consequence	Shedding of Microorganism to Exterior
VIRUSES				
Herpes simplex	Dorsal root ganglia	–	Activation, cold sore	+
Varicella zoster	Dorsal root ganglia	–	Activation, zoster	+
Cytomegalovirus	Lymphoid tissue	–	Activation ± disease	+
	Salivary glands	+	None known	+
HHV6	Lymphoid system	±	None known	±
Epstein–Barr virus	Lymphoid tissue	–	Lymphoid tumour	–
	Epithelium	++	Nasopharyngeal carcinoma	–
	Salivary glands	+	None known	+
Hepatitis B	Liver (virus shed into blood)	+	Chronic hepatitis; liver cancer	+
Adenoviruses	Lymphoid tissue	–	None known	+
Polyomavirus (mice)	Kidney tubules	+	None known	+
Polyomaviruses	Kidney	–	Activation (pregnancy, immunosuppression)	+
Leukaemia viruses	Lymphoid and other tissues	±	Late leukaemia	–
Measles	Brain	±	Subacute sclerosing panencephalitis	–
HIV	Lymphocytes, macrophages	+	Chronic disease	+
CHLAMYDIA				
Trachoma	Conjunctiva	+	Chronic disease and blindness	?
Psittacosis	Lung (rarely in man)	?	None known	–
	Spleen (of bird)	±	Activation	+
RICKETTSIA				
Rickettsia prowazeki	Lymph node	?	Activation	+
Rickettsia burneti (sheep)	Spleen?	–	Activation; source of human Q fever	

(*Continued*)

TABLE 10.1 (Continued)

Microorganism	Site of Persistence	Infectiousness of Persistent Microorganism	Consequence	Shedding of Microorganism to Exterior
BACTERIA				
Salmonella typhi	Gall bladder	+	Intermittent shedding in urine, faeces	+
	Urinary tract	+		+
Mycobacterium tuberculosis	Lung or lymph node (macrophages?)	?	Activation, tuberculosis in middle age	+
Treponema pallidum	Disseminated	±	Chronic disease	–
PROTOZOA				
Plasmodium vivax	Liver	?	Activation, clinical malaria	+
Toxoplasma gondii	Lymphoid tissue, muscle, brain	±	Activation, neurological disease	_
Trypanosoma cruzi	Blood, macrophages	±	Chronic disease	–

Persistent infections cannot by definition be acutely lethal; in fact they tend to cause only mild tissue damage or disease in the host. The comparatively mild diseases caused by persistent infections with adenoviruses, herpesviruses, typhoid or malaria can be contrasted with the serious diseases caused by the nonpersistent microorganisms of plague, cholera, yellow fever or paralytic poliomyelitis.

In certain acute infections the patient appears to recover, but there is later a relapse. Following typhoid, for instance, 8–10% of patients suffer relapses, although usually mild. In such instances the infection is not strictly persistent, but it seems as if the host's immune forces need repeated stimulation before there is complete elimination of the infectious agent.

LATENCY

Any form of persistent infection endows a microorganism with a greatly enhanced ability to remain in the host population as well as in the infected individual, and latency (which means that the latent object is present but not apparent) represents an extreme manifestation of persistence. By definition, the microorganism is not detectable in infectious form during the latent period of infection.

The significance of persistence in latent form becomes clear when measles is compared with chickenpox. Measles is not normally a persistent infection, and after an individual has been infected and suffered the characteristic illness and rash, the immune response controls the infection and eliminates the virus from the body. Immunity to reinfection is

lifelong, and a continued supply of fresh susceptible hosts must be found if the virus is to persist in the community. The virus does not survive for long outside the body, and therefore cannot persist in a community unless there is, at all times, someone actually infected with measles. Before the general use of measles vaccine, measles used to come to towns and cities every few years, infecting the susceptible children who had appeared since the last epidemic, and then disappearing again. The virus had to be re-introduced into the community at intervals because there were not enough susceptible children appearing to keep the infection going all the time. From studies of island communities, it has been shown that the minimum population to maintain measles without introduction from the outside is about 500,000. The herpesvirus, varicella zoster virus (VZV), also causes an acute infection which manifests as chickenpox. However, in contrast to measles, this acute infection is always the prelude to the establishment of a lifelong persistent infection. During childhood infection with VZV, the virus ascends to the dorsal root ganglia of sensory nerves supplying the affected skin areas and stays there in an essentially noninfectious state after recovery and elimination of virus from the rest of the body (Figure 10.1). The disease, chickenpox, disappears temporarily from the community. Virus in the dorsal root ganglia is kept under control by cell-mediated immunity (CMI), but the strength of this CMI response weakens as individuals age, and there is an increasing likelihood in older people that latency will be broken and one of the ganglia will produce enough infectious virus to spread down the peripheral nerve to the skin. This disease is called zoster (shingles) and is characterised by a crop of vesicles which is restricted to the distribution of that particular nerve, i.e. to a single dermatome. The vesicles are rich in virus which is capable of causing chickenpox in any susceptible children who have appeared in the community. Studies of island communities have shown that chickenpox can maintain itself indefinitely in a community of $<$1000 individuals. The viruses that maintain themselves in small, completely isolated Indian communities in the Amazon basin are therefore persistent viruses (Table 10.1) rather than nonpersistent viruses such as polio, influenza or measles. This was shown from antibody surveys carried out shortly after first contact of these communities with the outside world. Other infections which can be maintained in small populations are those in which there is persistent shedding of the microorganism (typhoid, tuberculosis, see below) or in which there is a reservoir of infection in some other host species (yellow fever, plague).

Chickenpox is described as a persistent infection characterised by latency; in fact, the sequence of events is an acute infection, apparent recovery from the original infection (the latent phase) and later in life a second acute infection with disease as the virus reappears and is once again shed to the exterior.

Herpes simplex virus gives rise to an exactly comparable latent infection. Infection normally occurs during infancy or early childhood, and causes a mild acute illness with stomatitis and slight fever. The virus travels up the axons of sensory nerves to the trigeminal ganglion supplying the mouth and related areas, and after apparent recovery from the initial infection, virus remains latent in neurons in the ganglion (Figure 10.2). At intervals later in life virus can be activated in the ganglion, travel down the nerve and cause a vesicular eruption, usually round the lips or nostrils. This is called a cold sore, and virus from the cold sore can infect a susceptible individual. Certain individuals (about 10% of the population in the United Kingdom) are particularly prone to cold sores. Factors that

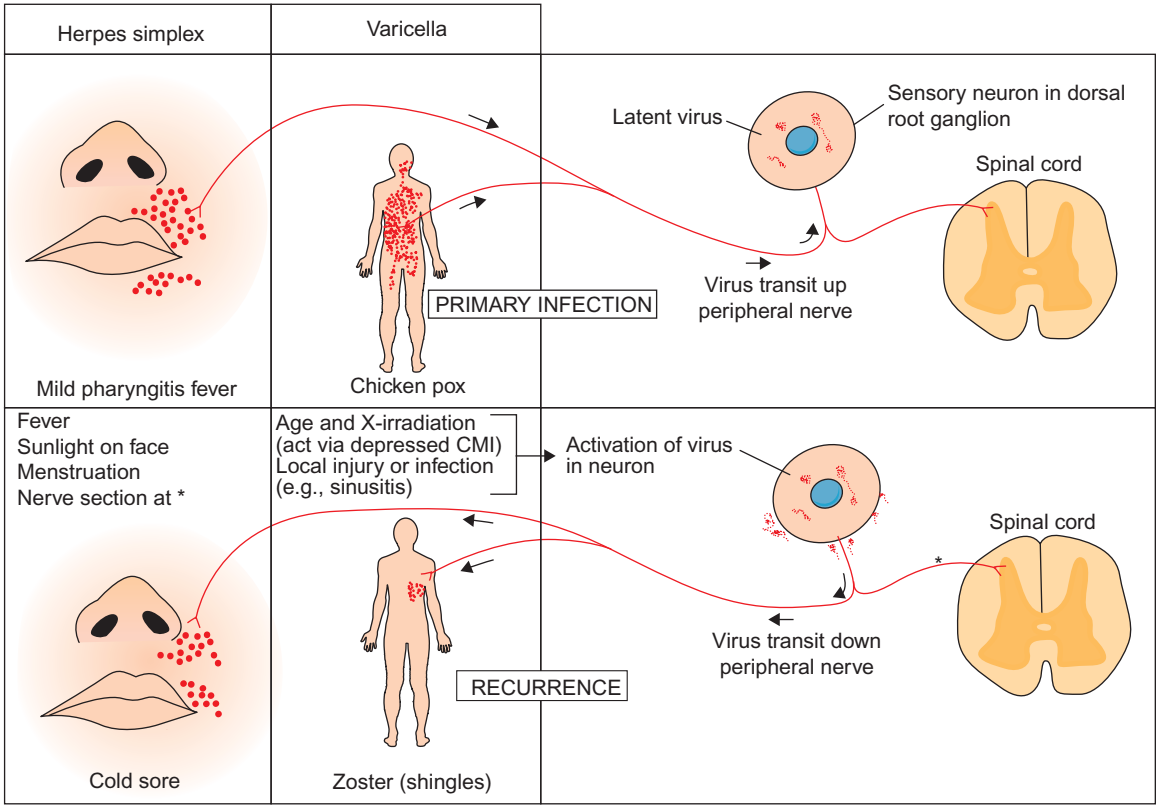

FIGURE 10.1 Mechanism of latent herpes simplex and VZV infection in man.

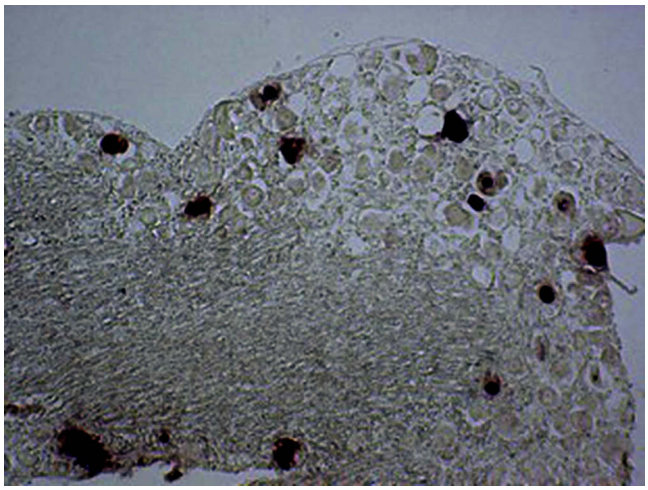

FIGURE 10.2 Detection of LATs of herpes simplex virus by *in situ* hybridisation. The picture shows a section of a dorsal root ganglion from a mouse infected with virus 30 days earlier. At this time there is no infectious virus present and no viral proteins are expressed in the neuron. During this period of virus latency, the only viral activity is the expression of LATS, which are non-protein coding RNAs that give rise to stable introns, and small RNAs including a set of miRNAs. *By kind permission of Professor S. Efstathiou, Department of Pathology, University of Cambridge, Cambridge, U.K.*

reactivate virus in the ganglion include colds and other fevers, menstruation and psychological factors, often referred to as 'stress'. The mechanism by which these factors induce reactivation is not understood. Sunlight is also well known to activate cold sores. It probably acts on the skin around lips or nostrils, causing cellular damage and inflammation and stimulating sensory nerves and thus the latent virus in the ganglion, or by causing a subclinical, spontaneously reactivating lesion to become an overt lesion. The eruption is restricted to the area supplied by the particular sensory ganglion that was involved during the original childhood infection. Although the mucosal membranes of the mouth and genitalia are the most common sites of infection and reactivation, herpes simplex may infect other areas of the body. A small child falls and hurts its knee, the knee is kissed better by an aunt with a cold sore, and the knee is now the primary site of infection. Recurrent 'cold sores' in this individual involve the knee. During the latent stage, herpes simplex virus DNA is present in neurons in dorsal root ganglia. The latent state seems to be delicately balanced; the virus can be reactivated merely by exposing without touching the trigeminal ganglion at operation. The mechanism of latency and reactivation of herpes simplex virus has been the subject of much study; however to date no definitive answer to the question of what controls this process has been forthcoming. In an acute infection, activation of transcription of the virus genome depends on the interaction of a virion protein, VP16, with a cellular protein(s). One theory is that latency arises either when the activating cellular proteins are absent, or repressor proteins bind to VP16 and/or bind to the activating cellular proteins, with the result that normal transcription of virus genes is inhibited. During HSV latency there is very limited transcription of a specific class of viral RNAs known as latency-associated transcripts (LATs) (Figure 10.2). Virus mutants lacking LAT sequences can still establish a latent infection and undergo reactivation of virus and the function of LATs is still the focus of much research. Neurons harbour multiple copies of the herpesvirus genome, with a range of copy numbers in individual cells. The viral genome is not integrated with host DNA and it is not in its usual linear form. Instead it circularises and exists in the cell in a free episomal form. The amount of viral DNA in

sensory ganglia does appear to correlate with the frequency of reactivation. Furthermore, reactivation only occurs in a small fraction of the neurons that contain viral DNA.

It has been suggested from experimental work with mice that reactivation is quite common in ganglion cells, but immune responses generally suppress virus replication before the full pathogenic sequence can be enacted. There is evidence to suggest that there is a limited amount of CMI surveillance in the nervous system and that interferon produced by these T cells plays a role in restricting virus reactivation. Fluctuations in the level of this surveillance may play a role in reactivation. Following reactivation and initiation of virus gene expression, the virus must first travel down the axon using the normal cytoskeletal transport system (at about 9 μm/h), then infect dermal cells and finally epidermal cells, before a lesion is produced. Looked at this way, each clinical lesion represents a failure to control the growth and spread of reactivating virus. In other words, reactivation is a two-stage process. In the case of herpes simplex virus and varicella zoster, the first stage takes place in the ganglion and occurs spontaneously, while the second requires the spread of virus from nerve endings to dermal and epidermal cells, where it is exposed to the immune system; hence infection can be controlled and the lesions resolved. After virus has reactivated in sensory neurons, sensations such as itching are generated in the areas supplied by affected neurons, perhaps before virus actually reaches the skin. When pseudorabies virus, a similar virus, multiplies in sensory neurons in pigs, these phenomena are prominent enough to give the condition the name 'mad itch'. Herpes simplex reactivations can occur without visible skin or mucosal lesions. At times, therefore, immune forces seem to control the infection before skin lesions can be produced. Whatever the stage of reactivation that is under immune control, it is clear that CMI is involved. The CMI response to varicella zoster wanes as we age, whereas responses to other persistent infections such as herpes simplex and cytomegalovirus are unaffected, possibly due to differences in the underlying reactivation rate and hence 'reboosting' of the immune system by HSV and CMV. Thus the incidence of VZV reactivation to cause shingles (zoster) increases with age.

Brill–Zinsser disease is a rickettsial example of latency. Following complete clinical recovery from typhus (caused by *Rickettsia prowazekii*), the rickettsia can become latent in lymph nodes or the reticuloendothelial system. Months or years later, unknown influences cause the latent infection to be activated and the individual suffers a disease similar to the original typhus with the rickettsia once again present in the blood. If the human body louse is present, it can acquire the infection following a blood feed and transmits it as typhus to susceptible individuals. Sheep can become latently infected with *Coxiella burneti*. The infection may reactivate in late pregnancy, and very large numbers of organisms are then shed in urine, faeces, amniotic fluid and placenta. A stable infectious aerosol is formed which can cause Q fever in susceptible farmers or veterinary surgeons.

Malaria provides a classic example of protozoal latency. After clinical recovery, particularly from *vivax* malaria, the parasite disappears from the blood circulation and enters a state of latency in the liver. The specialised liver form is called a hypnozoite. Subsequently, often after many years, the parasite in the liver re-infects red blood cells to give a fresh clinical attack of malaria. Malarial latency is particularly striking in those from temperate climates who become infected in the tropics, return home, and suffer an attack of malaria many years later. In endemic areas, latently infected patients act as a reservoir for the parasite and this represents an obstacle to attempts to eliminate malaria.

Tuberculosis sometimes gives a type of latent infection. The bacteria remain dormant in the body after the initial infection and recovery, and can later be reactivated to give clinical disease. In the days before BCG vaccination, many town and city dwellers were infected in early life, but in most cases the infection in the lung or lymph node was controlled and remained subclinical, giving rise to a healed primary focus. After the age of about 40, perhaps with the general age-related decrease in the strength of CMI, there is an increasing likelihood that the bacteria in a primary focus will become active again and cause clinical disease. Respiratory tuberculosis in the middle-aged patient generally arises in this way.

Viable But Noncultivable Forms

Study of bacterial persistence and latency is complicated by the occurrence of viable but noncultivable forms. This is an interesting phenomenon exhibited by certain Gram-negative non-spore-forming and non-cyst-forming bacteria (including *Campylobacter jejuni*, *Vibrio cholerae*, *Vibrio fulnificus* (an estuarine bacterium) and *Salmonella enteritidis*). Such organisms are triggered by a variety of different environmental conditions to assume peculiar morphologies and are then extremely difficult (or to date, impossible) to cultivate *in vitro.* Failure to recover them reflects our ignorance of the necessary culture conditions, rather than indicating that they are dead! In fact, success in reverting some 'noncultivable forms' to normal forms has been reported. Long-term persistence of *Chlamydia* species is reported not only *in vitro* but also in birds, animals and humans. The mechanism is not clear, but it may be important in conditions such as trachoma, Reiter's disease and chronic pelvic disease with infertility in women. Noncultivable *Chlamydia* species are sometimes detectable *in vivo* by specific antibody and DNA probes. Chlamydial reticulate bodies (RBs) replicate but are not in themselves infectious. Their replication is complex and is particularly susceptible to the availability of nutrients, especially amino acids. In addition, restriction of the supply of amino acids also leads to the production of abnormal forms similar to those induced by penicillin. It could be that failure to cultivate *Chlamydia* from sites *in vivo*, where their presence can be demonstrated, is due to the very low infectivity for cell cultures of abnormal forms induced by the unfavourable nutritional status of certain environments.

PERSISTENT INFECTION WITH SHEDDING

Persistence is the state where microorganisms can be found continuously in the individual, and in this particular category they are shed more or less continuously, often for many years, without causing further significant disease. After recovery from typhoid, for instance, bacteria sometimes persist for long periods in the gall bladder. Scarred, avascular areas of the gall bladder are colonised, where the bacteria enjoy a certain freedom from the blood-borne antimicrobial forces of the host. Typhoid bacilli are discharged intermittently into the bile and thus the faeces. Two to five per cent of typhoid cases become faecal excretors and nearly all of them are women, because gall bladder damage and scarring is more common in women. Such carriers of typhoid are important sources of infection as

they are apparently healthy. 'Typhoid Mary' was a carrier who was employed as a cook in the United States, and moved from one place to another, cooking for eight different families and causing more than 200 cases of typhoid before she was finally caught and pensioned off. Typhoid can also persist in the urinary tract, giving urinary spread of disease from the carrier. One serious outbreak (310 cases, with 43 deaths) of typhoid in Croydon, London, in 1937 was traced to a carrier who had been employed during work on water pipes supplying the affected area, and had urinated on nearby ground and contaminated the water supply.

A carrier state is also seen in certain bacterial infections of the body surfaces. After recovery from diphtheria, scarlet fever or whooping cough, the bacteria often persist in the nasopharynx for many months, serving as a source of infection for susceptible individuals. The mechanisms of persistence are not understood, but it must be remembered that the normal resident commensal bacteria of the nasopharynx are by definition also persistent, and often include potentially pathogenic bacteria such as the meningococcus, the pneumococcus and pathogenic strains of group A streptococcus or *Staphylococcus aureus*. It is uncommon for these bacteria to give trouble; for every person suffering from meningococcal disease there are about 1000 unaffected carriers. The fact that bacteria capable of causing diphtheria, scarlet fever or whooping cough can also persist in this site is perhaps not surprising.

Entamoeba histolytica often causes a persistent infection, and cysts can be shed in the faeces for many years after recovery from amoebic dysentery or after subclinical infection. The cysts are highly resistant and infectious. *Entamoeba* persistence is not unexpected because several other *Entamoeba* species are regular human commensals. Persistent asymptomatic carriage also occurs with other intestinal protozoal pathogens, including *Cryptosporidium*, *Giardia* and *Blastocystis*.

Persistent virus infections include Epstein–Barr virus and herpes simplex virus infections, and these are shed in saliva, often for long periods after the initial infection. Epstein–Barr virus is detectable in throat washings for at least several years. Herpes simplex virus reappears in the mouth later in life if there are cold sores, and repeated tests on given individuals have shown that the virus is also intermittently present in oral secretions at other times, resulting from reactivation of latent virus without the development of a lesion. Hepatitis B virus persists in the blood for long periods, and perhaps for life in up to 10% of adults and 90% of neonates. About 0.2–0.5% of individuals in Western Europe, Australia and North America are carriers, many of them apparently normal and healthy, however the incidence is higher in Eastern Europe, SW Asia, Central and Southern America (2–7%) and in Tropical Africa, SE Asia and parts of China where the incidence is 8–20% (WHO; Hepatitis B). The blood of a carrier is infectious and can be transmitted to susceptible individuals via blood transfusions, the contaminated syringes of drug addicts, and the tattooist's, acupuncturist's or ear-piercer's needle. In the case of hepatitis C, the commonest cause of post-transfusion hepatitis, up to 90% of infected adults become carriers in spite of apparently vigorous antibody and CMI responses.

Other viruses are shed in urine. Hantaviruses for instance cause a natural infection of mice and other rodents and establish infection in the kidney, whence it is discharged into the urine. The infection is persistent and urine is the major vehicle for the spread of infection between individuals although infection of the lungs has been seen with some viruses

and host species. When the host animal is in contact with humans or invades their dwellings, human infection can occur resulting in haemorrhagic fever with renal syndrome (primarily in Europe and Asia), or hantavirus cardiopulmonary syndrome (primarily in North and South America). Other important human diseases acquired from animal urine in this way include leptospirosis, Lassa fever or Hantaviruses. Cytomegalovirus is present in the urine of about 10% of children under the age of five in London, but it is unlikely that this is important in the spread of infection. Certain viruses, such as cytomegalovirus in man, are shed persistently in milk, and among the bacteria both *Brucella* and tubercle bacilli are present in the milk of persistently infected cows. Pasteurisation of milk was introduced worldwide in an attempt to prevent the spread of brucellosis and tuberculosis caused by drinking raw milk.

There are several human infections in which the microorganism often persists in tissues for long periods and at the same time causes chronic disease. These include tuberculosis, leprosy, syphilis, brucellosis and trachoma. In these infections, the disease is the result of a long drawn out battle between the microorganism and the immune and tissue defences of the host, sometimes one and sometimes the other gaining the upper hand. In the case of leprosy and tuberculosis, bacteria continue to be shed to the exterior and infect others. Each of these infections is a tribute to the ability of the microorganism to survive and multiply in the face of host defence responses, and the progressive tissue damage is partly a direct result of bacterial activity, but largely attributable to the host responses.

An unusual group of infectious agents persists in the body after infection and gives rise to progressive and fatal neurological disease after prolonged incubation periods. This includes scrapie, bovine spongiform encephalopathy (BSE), chronic wasting disease of deer, kuru and Creutzfeld–Jacob disease. Scrapie is a naturally occurring disease of sheep that has been present in Europe and the United Kingdom for about 300 years. The brain is involved, and the disease is so called because affected sheep itch, and scrape themselves against posts and fences to relieve this symptom. In the laboratory, scrapie is transmissible to mice and other animals, and the feeding of infected sheep's heads to mink on mink farms in the United States has given rise to the disease called transmissible mink encephalopathy. BSE appeared mainly in dairy cattle in the United Kingdom in the 1980s and, like the mink disease, resulted from the adaptation of scrapie to a new host species. The agent responsible for BSE has been passed to mice and indeed resembles scrapie, but importantly it is not identical and has presumably undergone adaptation to grow in cattle. Unfortunately BSE, unlike scrapie, can be transmitted by the oral route to humans, in whom it causes a fatal CJD-like disease (new variant CJD) which was first recorded in the United Kingdom in 1995; as of January 2014 there had been 177 confirmed cases of nvCJD.

Kuru was a fatal neurological disease of humans in Papua New Guinea, spread by ritual cannibalism. It was restricted to the Fore tribes, and a total of 3700 cases occurred in a population of 35,000. The condition was not communicated directly from person to person; however, following death, the bodies of the deceased were eaten by relatives as a mark of respect. Cannibalism in New Guinea has now died out and so has the disease. The last person with kuru died in 1998. A suggested origin of kuru was from a single Fore native who may have spontaneously developed Creutzfeld–Jacob disease in the early 1900s.

Creutzfeld–Jacob disease is a rare neurological disease of humans, occurring sporadically all over the world, caused by infectious agents similar to those of scrapie and kuru,

but with an unknown mode of transmission. About 10% of cases occur in certain families.

In all these diseases, the incubation period represents a large fraction of the life span of the host – months to years with mouse models of scrapie (varies with mouse strain and scrapie strain), two years with sheep scrapie, 4–6 years with BSE in cattle, and 4–20 years with kuru in humans. During this time the microbial agent steadily increases in amount, first in lymphoid tissues and then in the brain. Cells infected are neurons, follicular dendritic cells, and probably astrocytes and B cells. The process of infection and production of pathological changes is slow but proceeds inexorably and no antibody is formed. One prominent pathological feature is a fine vacuolation in the brain and this group of diseases was therefore called the 'spongiform encephalopathies'.

These causative agent(s) of these diseases have not been unequivocally identified. Scrapie can be transmitted using the brain tissue of an affected sheep, the agent is resistant to heat, autoclaving, formalin and organic solvents and has not been shown to contain either DNA or RNA. The pathological changes in the brain are associated with conversion of a host-coded prion protein (PrP^{c}) present in neurons in the normal brain, into a modified form, the scrapie prion protein (PrP^{sc}). This is resistant to normal proteolytic processing, accumulates in synaptic structures and results in death of neurons, possibly by apoptosis. The presence of PrP^{sc} in the brain is a diagnostic feature of this group of diseases. This has led to the hypothesis that PrP^{sc} is the actual infectious agent. However, it has not been explained how the different incubation periods which are so characteristic of the 15–20 different strains of scrapie agent in mice can be attributed to, and coded for, by a normal host protein. Either there is an as-yet unrecognised nucleic acid component or the PrP protein exists in multiple conformations. Transgenic 'knockout' mice which lack the PrP gene seem normal in all respects except that they cannot be infected with scrapie. Transgenic mice which contain different numbers of copies of the PrP^{c} gene show a correlation between copy number and susceptibility, once a certain level of expression of PrP^{c} is reached. Scrapie occurs spontaneously in these mice. In sheep there is an association between the susceptibility to scrapie infection and particular alleles of the PrP^{c} gene. The United Kingdom is currently attempting to remove scrapie from the national sheep flock by a process of selective breeding for the resistant alleles.

EPIDEMIOLOGICAL SIGNIFICANCE OF PERSISTENT INFECTION WITH SHEDDING

There are obvious advantages to a microorganism if it persists in the host and is shed from the body for long periods after the initial infection. Maintenance of the infection in a host community is made easier, and herpes simplex virus, VZV, tuberculosis, typhoid and other infections have already been discussed from this point of view.

The epidemiological advantages of prolonged shedding of microorganisms to the exterior are well illustrated in the cases of myxomatosis and cholera. They are not truly persistent infections, but show the results of an increased period of shedding during the acute disease and convalescence. Each provides an excellent example of the natural evolution of an infectious disease. Myxomatosis is a virus disease of rabbits, spread mechanically by

biting arthropods. When introduced into Australia in 1950 in an attempt to control the non-native rabbit population, it caused nearly 100% mortality in the population. But rabbits were never eliminated from Australia because within the next five years or so a new and more stable host–microbe balance evolved. There was a change in both the virus and the host species. First, rabbits with a genetically based susceptibility to myxomatosis were weeded out, leaving a rabbit population that was by nature more resistant, only 25% of them dying after infection with virulent virus. Second, the virus changed. Infected rabbits develop virus-rich swellings on the ears and face, and these skin lesions serve as sources of infection for the mosquitoes that carry virus to other rabbits. In the early stages of the Australian epidemic, when the infection was very severe, rabbits died a few days after developing these swellings. Later, however, a strain of virus emerged which was much less lethal and allowed the rabbit with the virus-rich swellings to live for a week or two, even to survive, and this gave greatly increased opportunities for virus spread by mosquitoes. The less lethal strain of virus therefore replaced the original virulent strain in the rabbit population.

Cholera is an intestinal infection caused by *V. cholerae* and is transmitted by faecal contamination of water supplies, and it is spread with great rapidity and efficiency in crowded human communities in the absence of satisfactory sanitary arrangements. This was so in the nineteenth century in London, and nowadays in India or parts of the Middle East, and is all too familiar in refugee camps throughout the world. Classically there is a very short illness, characterised by vomiting, diarrhoea, dehydration and shock, which is often lethal within 24 h. The bacteria persist in water for a week or so and convalescent patients may continue to excrete bacteria in faeces for a few weeks. Two sero groups of *V. cholerae*, 01 and 0139, cause outbreaks of cholera. *V. cholerae* 01 has two biotypes, classical and El Tor. El Tor induces a milder or indeed asymptomatic infection than the classical strains; however, infected people excrete bacteria for longer periods than in classical cholera. El Tor is also less readily inactivated and can thus spread from person to person by contact, feeding utensils, etc., as well as by faecal contamination of drinking water. The El Tor strain has largely replaced classical strains of cholera, mainly because it is shed from the patient for a longer period and does not depend for its transmission on a contaminated water supply.

PERSISTENT INFECTION WITHOUT SHEDDING

A large proportion of the microorganisms that persist in the body are rarely if ever shed to the exterior. Their importance is for the individual rather than for the community. Most of them give rise to no ill effects, but one or two may cause trouble if the immune responses are weakened, and one or two can ultimately cause cancer. Most of them are viruses, and viruses have a unique ability to persist and multiply in cells, often in a defective (noninfectious) form. Many adenoviruses, for instance, persist in lymphoid tissues after initial infection, causing no disease, but are still recoverable from normal adenoids or tonsils. There is little or no infectious virus in these tissues because of effective control by immune or other mechanisms, but when the tissue is removed and placed in a culture where the controls are no longer present, the infectious virus appears. Adenoviruses are

recoverable from one-third of all adenoids and tonsils removed during the first decade of life, and they must be regarded as part of the normal microbial flora of man. Certain herpesviruses, including Epstein–Barr virus, cytomegalovirus, human herpes virus type 6 (HHV6) and Marek's disease virus in chickens, also show persistent infection of lymphoid tissue. In the case of Epstein–Barr virus, circulating B cells contain viral DNA but no infectious virus, whereas myeloid cells are the target in HHV6. Cytomegalovirus is present in monocytes of about 5% of normal people (e.g. healthy blood donors), so that infection can occur as a result of blood transfusions.

In the early days of tissue culture, when normal monkey and human kidney cells were used for the propagation of polio and other viruses, a number of viruses were isolated from the kidneys of normal individuals. These included reoviruses, measles virus, cytomegaloviruses, and a papovavirus (SV40) in monkey kidneys. There is a final group of persistent viral infections that are nearly always completely harmless to the host, but sometimes, often a very long period after initial infection, they cause malignant change. These are the retroviruses that cause mammary tumours in mice, and leukaemias (sometimes other types of tumour) in mice, humans, other mammals and in chickens. Retroviruses are RNA viruses that contain a reverse transcriptase enzyme, which transcribes viral RNA into cDNA as a necessary part of the replication cycle. This DNA is integrated into the genome of the infected cell and becomes part of it. Thus if a germ cell is infected, the viral genome is transferred from one generation to the next, via the offspring. This is an example of vertical transmission and these viruses are endogenous retrovirus. They start off as infectious, but soon accumulate mutations and deletions, and eventually lose infectivity, remaining as DNA in the host genome, no more than 'retroviral fossils' — protected, replicated and handed down the generations as if they were the host's own genes. This surely represents the ultimate, the final logical step in parasitism! It becomes difficult to determine which is host and which is parasite, and the word infection loses much of its meaning.

Why are the retrovirus sequences so common, and why are there often multiple copies of them? For example, the human genome contains 50–100 copies of human endogenous retrovirus-W (HERV-W); it could be that they are such successful parasites it is just too difficult to keep them out of or to get rid of them from host DNA. The possible role of endogenous retroviruses in diseases is not well understood. They have been implicated in several conditions including cancer and multiple sclerosis (and other autoimmune diseases) and pose a problem in attempts to develop xenotransplantation approaches; a human adapted pig organ for example may express an endogenous PERV which could infect the intended human recipient. It has long been hypothesised that endogenous retroviruses may play a role in foetal development. In a recent study, inhibition of expression of a sheep endogenous retrovirus (enJSRV) using siRNA technology showed that an endogenous retrovirus-derived protein is essential to allow normal placentation and hence pregnancy.

For an infection transmitted exclusively vertically, via the egg or sperm, there is of course no need for the virus ever to mature into an infectious particle. Its continued presence in the descendent host generations is ensured. Other retroviruses more regularly undergo a full cycle of replication in cells throughout the body, and are shed in saliva, milk, blood, etc., to infect other individuals. These can be transmitted horizontally and are called exogenous retroviruses. Cats infected with feline leukaemia virus, for instance, excrete in their saliva up to 10^6 infectious doses ml.

The lentiviruses ('slow' viruses) are another group of retroviruses, which include visna of sheep and goats, equine infectious anaemia of horses, and HIV. All are persistent infections, causing chronic disease and showing antigenic variation in the infected host. As is so often the case in persistent virus infections, macrophages and lymphocytes are infected.

The phenomenon of integration of viral genome into host cell genome is not unfamiliar to microbiologists. It is a feature of the so-called temperate (nonlysogenic) infection of bacteria with bacteriophages (viruses of bacteria). The infection is harmless, and the bacteriophage genome expresses only those few proteins which are necessary for the maintenance of the lysogenic state. The diphtheria toxin of *Corynebacterium diphtheriae* is a protein encoded by a lysogenic phage. Following treatment with certain inducing agents (or occasionally spontaneously) control processes are upset and the infection reverts to a lytic one in which infectious phage is produced and the host bacterium is destroyed.

SIGNIFICANCE FOR THE INDIVIDUAL OF PERSISTENT INFECTIONS

Persistent infections that are normally held in check by immune defences can be activated when immune defences are weakened. This is a major feature in AIDS. It also occurs when patients undergoing transplant surgery are given immunosuppressive drugs, and the persistent but normally harmless cytomegalovirus for instance is activated in most patients within a month or two, often giving rise to fever, pneumonitis or hepatitis. Even warts are activated and appear sometimes in large numbers. The CMI response is depressed in patients with certain tumours of lymphoid tissues, such as Hodgkin's disease, and these patients may suffer from activation of persistent tuberculosis, varicella zoster or cytomegalovirus infections. Not all persistent infections are activated, there is no evidence for an increased incidence of herpes simplex cold sores because CMI impairment is specific for varicella zoster rather than herpes simplex.

Persistent infections induce persistent immune responses, and these, although failing to eliminate the microorganism, sometimes cause pathological changes. The continued immune response to infections, such as tuberculosis and syphilis, leads to chronic disease, as mentioned above. In many cases the granuloma is the characteristic lesion formed around persistent foci of infection, making a major contribution to the disease itself. Persistent infections are often associated with persistence of microorganisms or microbial antigens in the blood. Circulating immune complexes are formed under these circumstances and can give rise to a number of pathological changes, including glomerulonephritis. If the lesions at the sites of microbial persistence are trivial, immune complex formation is sometimes the major disease process. This seems to be the case in some types of chronic glomerulonephritis in man.

One important consequence of persistent infection and of major significance for the individual is that persistent microorganisms may eventually induce tumour formation. The viral leukaemias, sarcomas, mammary carcinomas and leukoses of mice, cats, chickens and other animals are caused by persistent retroviruses, when present in individuals of suitable age and genetic constitution. In man, certain types of leukaemia are caused by human T-cell leukaemia viruses 1 and 2 (HTLV1 and 2). The human wart is a benign

tumour caused by a persistent virus, and cancer of the cervix is now very closely associated with a few of the sexually transmitted papilloma (wart) viruses. Burkitt's lymphoma and nasopharyngeal carcinoma are caused by Epstein–Barr virus, and liver cancer by hepatitis B virus. Very few people infected with these viruses develop a malignant tumour, and various cofactors are probably necessary, but have not yet been identified.

CONCLUSIONS

Microbial persistence, in summary, is a common sequel to viral, chlamydial and intracellular bacterial infections. Many of the severe infections causing illness and death in communities (poliomyelitis, plague, yellow fever, cholera) are not persistent and the microorganisms are eliminated from the body after recovery. Persistent infections are often important from the microbe's point of view, enabling it to be maintained in small or isolated host communities. Persistent infections also generally present problems in the development of vaccines (see Chapter 12). They are becoming relatively more important, both for the individual and for the community, as the nonpersistent infections are eliminated by public health measures and by vaccination. Not only may they reactivate and cause troublesome infections in immunocompromised or immunosuppressed patients, but some of them can cause malignant tumours.

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CHAPTER

11

Host and Microbial Factors Influencing Susceptibility

OUTLINE

A host may be susceptible to infection by a given microorganism but rarely suffers harmful effects. In the old days, everyone was susceptible to infection with polioviruses or tubercle bacilli, but relatively few became paralysed or developed pulmonary tuberculosis. Not only this, but host susceptibility to infection often varies independently of susceptibility to disease. From the microorganism's point of view, infectiousness or transmissibility is not the same as pathogenicity. Transmissibility in fact depends on the extent of shedding of microorganisms from the infected individual, on the stability of microorganisms outside the host, and on the ease with which infection is established in new hosts. Each of these factors shows great variation. Variations in the ease with which infection is established are illustrated in Table 11.1. It can be seen that the dose required to produce infection, disease or death depends on the microorganism, the route of infection, the host, and on other factors.

The word virulence is sometimes used to refer to the infectiousness or transmissibility of a microorganism, but the word as used here will refer instead to its pathogenicity, or ability to cause damage and disease in the host. An infection can be totally harmless and asymptomatic or lead to a lethal disease, depending on the results of the encounter between microorganism and host. The characteristics of both the microbe (seed) and the host (soil) contribute to the outcome of an infection, and either can exercise a determining

Mims' Pathogenesis of Infectious Disease.
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TABLE 11.1 Examples of Variations in the Dose of Microorganisms Required to Produce Infection, Disease or Death in the Host

Microorganism	Host	Routes of Infection	Minimally Infectious (ID_{50}), Disease Producing (DD_{50}) or Lethal Dose (LD_{50})[a]
Rhinovirus	Man	Nasal cavity	1 $TCID_{50}$[b] (DD_{50})
		Conjunctiva	16 $TCID_{50}$ (DD_{50})
		Posterior pharyngeal wall	200 $TCID_{50}$ (DD_{50})
Salmonella typhi	Man	Oral	$\leq 10^5$ bacteria (DD_{50})
Shigella dysenteriae	Man	Oral	10 bacteria (DD_{50})
Vibrio cholerae	Man	Oral	10^8 bacteria (DD_{50})
		Oral (together with bicarbonate)	10^4 bacteria (DD_{50})
Giardia lamblia	Man	Oral	10 cysts (ID_{50})
Mycobacteriumtuberculosis	Man	Inhalation	1–10 bacteria (ID_{50})
Ectromelia (mousepox) virus: virulent strain	Mouse (C57BL or WEHI strain)	Footpad	1–2 virus particles[c] (ID_{50})
	Mouse (WEHI strain)	Footpad	25 virus particles (LD_{50})
	Mouse (C57BL strain)	Footpad	10^7 virus particles (LD_{50})

Examples from man, together with one example from an experimental animal (mouse) to show host genetic effects (mouse strain differences).
[a]'50' means that this dose produces the effect in 50% of those inoculated.
[b]Tissue culture infectious doses.
[c]By electron microscopy.

influence. To put it as a platitude, it takes two (microbe and host) to make an infection or a disease. Some of the host and microbial factors influencing susceptibility to disease are discussed in this chapter.

GENETIC FACTORS IN THE MICROORGANISM

The microorganism's ability to infect a given host is genetically determined and many microorganisms infect only one particular host species. For instance, measles, trachoma (*Chlamydia trachomatis*), typhoid (*Salmonella typhi*) and our warts (papilloma virus) are exclusively human infections. Others are less specific, rabies and anthrax seemingly capable of infecting all mammals. Pathogenicity or virulence is also a function of the microbial genome. Virulence is recognised to depend on coordinated expression of numerous genes, whose products mediate adherence, antiphagocytic activity, immune evasion, production

of toxins, etc. Environmental stimuli inform a bacterium about its changing surroundings, and for a pathogen these may vary enormously according to the stage of the infection process. Such changes may be sensed in physical terms (pH, temperature, osmolarity), nutrient availability, or in terms of hostile host defences. Some examples have already been given earlier: *Shigella* invasion (see Chapter 2) and *Salmonella* intraphagocytic survival (see Chapter 4). The latter is an example of a member of the family of 'histidine protein kinase/response (2-component) regulators' a number of which are commonly encoded by bacterial pathogens. The first component is a sensor protein – a protein kinase – which is sensitive to fluctuations in one or more environmental parameters. In response to the environmental signal, it autophosphorylates at a conserved histidine residue. The second component is a regulatory protein which is phosphorylated at a conserved aspartic acid residue by the activated kinase, and this alters some cellular function, usually at the level of transcription. When the genes which respond to the stimulus are co-regulated by a common regulator, they are described as a regulon. A group of regulons which responds independently to the same stimulus is called a stimulon. Some examples are given in Table 11.2. Recently another family of regulators based on small RNA molecules have been identified to play an important role in the regulation of virulence for many bacterial pathogens.

TABLE 11.2 Examples of Two Component Systems for Regulating the Expression of Bacterial Virulence Components

Genes *Acronym*; Function Controlled	**Initial Signal**	**Pathogens**
ompR/envZ Outer membrane proteins	Osmotic stress	*Salmonella typhimurium* *Shigella flexneri*
phoPlphoQ Phosphatase; intraphagocytic survival	Multifactorial	*S. typhimurium*
bug *Bordetella* virulence gene; adhesins and toxins	Temperature	*Bordetella* spp.
algR Alginate synthesis in the cystic fibrosis lung	Osmotic stress	*Pseudomonas aeruginosa*
pilA/pilB Adhesion pili	?	*Neisseria gonorrhoeae*
agr Accessory gene regulator; expression of range of toxins	?	*Staphylococcus aureus*
toxR Single protein system with both sensing and kinase activity, expression of cholera toxin and toxin co-regulated pili	Osmotic stress, temperature, pH, amino acid availability	*Vibrio cholerae*
mry Product activates gene-encoding M protein	CO_2	*Streptococcus pyogenes*

Changes in virulence may arise from apparently trivial changes in the genome. Minor changes in the M protein that coats group A streptococci can lead to major changes in bacterial virulence, and a single amino acid change in the haemagglutinin of influenza virus can convert a relatively avirulent strain into a more virulent strain. The differences between variola minor and variola major (smallpox) used to be a matter of life and death when human beings were infected, but are only detectable with difficulty in the laboratory. A fresh pandemic strain of influenza A virus is able to spread readily in the community because one of the surface proteins, haemagglutinin, of the virus shows a major difference from pre-existing strains and therefore no one has immunity to infection.

The pathogenicity for a given host is often dramatically altered following the repeated growth of a microorganism under unfamiliar circumstances outside the body. The laboratory passage of pathogenic viruses in cultured cells often leads to great reductions in pathogenicity (attenuation) in the original host, and this has been a standard procedure for the production of live virus vaccines. The attenuated strain is a genetic variant. A similar phenomenon is seen with bacteria. For instance, BCG (Bacille Calmette–Guérin) vaccine consists of a strain of bovine tubercle bacillus, highly attenuated after 350 subcultures over the course of 15 years in glycerine–bile–potato culture medium. Also, gonococci cultivated in artificial media after isolation from the human urethra show rapid change to an almost nonpathogenic form.

When the last edition of this book was published, little was known of the genetics of animal viruses because, as the majority of viruses have RNA genomes, it was not easy to find good genetic markers and use classical approaches such as recombination. Then, the genomic sequence of only a few viruses was known, now there are thousands of whole genome sequences available for both DNA and RNA viruses. This increased knowledge along with the development of reverse genetic systems which allow the generation of replicating viruses from cloned DNA (cDNA in the case of RNA viruses) has resulted in an explosion of information on the role of individual virus proteins, and indeed viral nucleic acid structure, in determining the pathogenic outcome of infection. All viruses are mutable, and the selection of mutant viruses is the mechanism for the changes that take place during attenuation. However, RNA viruses accumulate more mutations than DNA viruses as they have no 'proof reading and error correction' mechanism to detect and put right any mistakes. A classic example of the evolution of virus virulence is the attenuation of myxomavirus following attempts to use this virus to control the feral rabbit population in Australia in the 1950s (see Chapter 10). Sequencing of the genomes of the original 1950s strains and the attenuated strains that emerged, showed that attenuation was due to mutations in multiple genes and also that different isolates with similar virulence profiles had different mutations, i.e. virulence is a complex and multigenic trait.

For bacteria, mutation is also one of the important types of change in genetic constitution. The progeny of a single bacterial cell are not genetically homogeneous; a small proportion of them are mutants. The mutation rate for a given genetic change varies between 1 in 10 and 1 in 10^{10}, and the spontaneous mutants only replace the original type if they are favoured (selected) by the environment. Similarly, mutations which have a lethal or harmful effect will be lost from the population immediately or over time. The largest virus genomes belong to the poxviruses with sizes ranging up to 300,000 bp. Bacterial genomes are an order of magnitude larger and the first bacterial genome sequence (of *Haemophilus*

influenzae) was completed in 1995 approximately 10 years after the first large DNA virus (EBV) was sequenced. There are now many thousands of bacterial genome sequences publicly available. Bacterial genomes typically contain about 500 to 5,000 genes, and mutations may result in changes in structure, biochemical activity, antigenic properties, ability to produce toxins, etc., any of which could lead to changes in pathogenicity. Much of bacteriology consists of a study of mutants, especially those that acquire new antigens or toxins. Smooth–rough variation is an important type of mutation affecting pathogenicity. Certain bacteria owe their pathogenicity to a surface component or capsule that interferes with phagocytosis by polymorphs and macrophages (see Chapter 4). This surface material often gives the bacterial colonies formed on artificial media a 'smooth' appearance. The surface material is lost during long periods of growth of bacteria in the laboratory, and in the host this leads to more efficient phagocytosis and decreased pathogenicity. The colonies now have a 'rough' appearance. Smooth–rough variations are common in salmonellas, shigellas and pneumococci, and represent bacterial mutations. Genetic changes in bacteria may involve extrachromosomal genetic elements called plasmids (see Glossary). Plasmids also often carry determinants for toxin production (e.g. *E. coli* enterotoxin, *S. aureus* exfoliative toxin), or for colonisation (pili of enteropathogenic *E. coli)* or invasiveness (*Shigella flexneri*). Plasmids are transferred between bacteria and are important in the transfer of antibiotic resistance between intestinal bacteria.

An individual with trypanosomiasis is persistently infected because the parasite undergoes periodic changes in its surface coat proteins, termed variant surface glycoproteins (VSGs). The *T. brucei* genome contains at least 1,250 different VSG genes at up to 20 expression sites; however, only one VSG gene is expressed at a given time. When an antibody response is mounted to this VSG, the majority of the trypanosomes are killed by complement mediated lysis. A small proportion however switches to express a new VSG, resulting in a parasite which is not recognised by pre-existing antibody.

MicroRNAs (miRNAs) are short (21–23nt) RNAs that act as post-transcriptional inhibitors of gene expression. Cellular miRNAs expressed in the nucleus are derived from primary transcripts (pri-miRNAs) that are processed via the enzyme Drosha to form a shorter preliminary miRNA (pre-miRNA). These pre-miRNAs are exported from the nucleus and once in the cytoplasm are further cleaved by the enzyme dicer to produce a transient double-stranded precursor, where one strand is designated the miRNA or guide strand and the complementary strand is designated the miRNA* or passenger strand. The miRNA is stably incorporated into the RNA-induced silencing complex (RISC) and via the interaction of a seed region (nucleotides 2–7 or 2–8 at the 5′ end of the miRNA) guides the RISC to a target mRNA transcript. This results in repression of protein synthesis by a number of mechanisms including mRNA degradation and inhibition of translation.

To date, over 250 virus-encoded miRNAs have been identified, the majority from herpesviruses. Herpesvirus-encoded miRNAs have been shown to regulate both cellular and viral gene expression and to influence cell processes including proliferation. In the herpesvirus Marek's disease virus (MDV), which causes a lymphoproliferative disease in chickens, deletion of a single virus-encoded miRNA abrogates virus-induced cellular transformation. In another herpesvirus, Epstein–Barr virus (EBV), the causative agent of glandular fever and B-cell tumours, a cluster of miRNAs has been implicated in

controlling virus-induced B-cell proliferation and transformation. The MDV-encoded miRNA is an orthologue of a cellular miRNA, miR155, which is essential for normal B-cell differentiation and antibody production, is involved in inflammation by regulating members of the TNF-receptor family and their ligands, and is involved in regulating interferon (IFN) and $CD8^+$ T-cell responses to pathogens. Thus it appears that a virus has either evolved to mimic a key cellular miRNA, important in the biology of its target cells, or has pirated a copy of the cellular miRNA. This type of molecular piracy, whereby cellular genes appear to have been acquired by viruses from the benefit of the virus is well documented from protein-coding genes, especially in poxviruses and herpesviruses.

The innate immune system maintains an essential role in host defence due to the ability of PRRs to recognise PAMPs (see Glossary). PRRs are evolutionarily conserved in most vertebrate, and invertebrate, species, highlighting their key role in innate immune response to pathogens. Despite the diversity and efficacy of PRRs, pathogens have evolved various strategies to subvert immune recognition and successfully replicate in host cells. A common strategy involves the manipulation of recognition signalling cascades, such as the NF-κB, PKR, and RIG-1 pathways. Several Gram-negative bacteria, e.g. *E. coli*, *Shigella* sp. or *Salmomella* sp. use a type of 'molecular syringe' called a type II secretion system to inject virulence factors directly into the host cell. Some of these virulence factors directly target NF-κB whilst others target IKK, which normally activates NF-κB by phosphorylating IκB, so releasing active NF-κB. Viruses also act to inhibit NF-κB signalling; adenoviruses express a virally encoded RNA which competes with dsDNA for binding to PKR, poxviruses express proteins which inhibit PKR signalling directly. Adenoviruses also inhibit IKK whilst African swine fever virus produces a functional homologue of IκB. Hepatitis C virus inhibits the degradation of IκB. Since NF-κB activation leads to expression of type 1 IFNs (e.g. IFNα and β) — the main antiviral effectors — inhibition of IFN transcription has a profound effect on the ability of the host to restrict early stages of virus infection. The NS1 protein of influenza A viruses is a non-essential virulence factor (viruses lacking a functional NS1 can still replicate) that plays a major role in restricting the innate responses during infection. NS1 limits IFNα/β mediated antiviral responses both by inhibiting IFN expression and the downstream effects of IFN-induced effectors such as PKR.

Many bacteria are able to prevent phagocytosis by forming a phagosome-like membrane-enclosed vacuole. Following internalisation, *S. enterica* uses various proteins to rearrange the phagocytes actin cytoskeleton allowing the formation of a modified phagosome, where the bacteria can replicate. The bacterium also drives the translocation of host endosome markers to the vacuole membrane, which prevents the maturation of the phagosome and digestion of the phagosome contents, i.e. the bacterium.

Concealment from the innate immune system within an endocytic vacuole is also seen with other pathogens: bacteria (*Ehrlichia risticii*, *Mycobacterium avium complex*, and *M. bovis*), fungi (*Histoplasma capsulatum*) and protozoan parasites (*Leishmania donovani*). Pathogens can also evade phagocytosis by escaping from the phagosome before it fuses with the lysosome and induces degradation. *Listeria monocytogenes*, *S. flexneri* and *Leishmania* spp. secrete pore-forming proteins (PFPs) which insert into the phagosomal membrane and form a pore, which enables the secretion of other membrane-degrading enzymes and the escape of the pathogen from the phagosome.

GENETIC FACTORS IN THE HOST

Susceptibility to infectious disease is always influenced and is sometimes determined by the genetic constitution of the host. An impressive example of individual differences in susceptibility that are assumed to be to a large extent genetic in origin was provided in Lubeck in 1926. Living virulent tubercle bacilli instead of vaccine was inadvertently given to 249 babies. There were 76 deaths, but the rest developed minor lesions, survived, and were alive and well 12 years later. The infecting material and the dose were in each case identical. More recent studies on infection rates in children have shown that children whose biological parents died of infectious disease are more likely to die of infection than children whose adoptive parents died of infectious disease, a clear case of genetics playing a greater role than environmental factors.

Using rapid, high-throughput sequencing technologies, an entire human genome can now be analysed and individual nucleotide variations identified in less than a month. It is possible to design microarrays to detect single nucleotide polymorphisms (SNPs), and using these 'SNP chips', large cohort studies can be carried out relatively quickly, linking particular SNPs to disease susceptibility. The application of these techniques in genome-wide association studies (GWAS) has revolutionised our understanding of genetic susceptibility, and advances in technology will accelerate this understanding. A detailed discussion of the results of these rapidly evolving studies is outside the scope of this book; however, what is clear is that susceptibility is most often polygenic, and in many cases involves genes in either the innate or adaptive immune responses, or both.

There are, however, examples where the genetic or molecular basis of susceptibility or resistance is reasonable, well studied and understood. People with the sickle cell trait show a markedly decreased susceptibility to malaria. Malarial merozoites parasitise red blood cells and metabolise haemoglobin, freeing haem and utilising globin as a source of amino acids. A single gene present in these individuals causes a substitution of the amino acid valine for glutamic acid at one point in the β-polypeptide chain of the haemoglobin molecule. The new haemoglobin (haemoglobin S) becomes insoluble when reduced and precipitates inside the red cell envelope, distorting the cell into the shape of a sickle. In the homozygote there are two of these genes and the individual suffers from the disease sickle cell anaemia, but in heterozygous form (sickle cell trait) the gene is less harmful, and provides a resistance to severe forms of falciparum malaria that ensures its selection in endemic malarial regions. In normal RBCs, the parasite interacts with host actin microfilaments to transport a parasite protein to the cell surface, and this essential (for the parasite) interaction is disrupted in cells in which haemoglobin S aggregates are present. This possibly accounts for the resistance to malaria of those with the sickle cell trait. A similar protection against malaria is conferred on those with another type of abnormal haemoglobin called haemoglobin C, produced by a single amino acid change in the polypeptide chain, and which also interferes with the interaction with actin. CD234, a glycoprotein which is present on the surface of red blood cells, is a chemokine receptor and is also called the Duffy antigen after the patient in whom it was first identified. Red blood cells from most West Africans, in contrast to those from Europeans, lack the Duffy antigen which acts as

receptor for *Plasmodium vivax*, a human malarial parasite. This type of malaria is almost unknown in West Africa.

People with the sickle cell trait (heterozygotes) do not suffer clinically, but the homozygotes often die during childhood. They not only develop anaemia (their red blood cells are more fragile because they sickle and unsickle under normal circumstances while circulating), but they also show increased susceptibility to infection. The basis of differential human susceptibility to tuberculosis is beginning to be understood. A combination of studies of twins, linkage studies and more recently GWAS has begun to dissect the genetic basis of the susceptibility. As might be expected, genes involved in the immune response to this pathogen are strongly linked to resistance/susceptibility; MHC/HLA, Toll-like receptors, cytokines (e.g. IL-1, IL-6, 1L-18) and chemokines (RANTES and MCP-1) have all been implicated in playing a role in susceptibility. Since its emergence in the early 1980s, HIV infection has resulted in approximately 36 million deaths and as of 2014 a similar number of people were living with the infection. The reduction in mortality rate can to a large extent be attributed to the introduction of aggressive and effective antiviral intervention strategies; however, it is becoming clear that within the human population there is a degree of genetic resistance both to HIV infection and to the progression to AIDs. HIV enters the cell via binding to its cellular receptor, the CD4 molecule. A second interaction is also required with a co-receptor, most often a chemokine receptor, either CCR5 or CXCR4. A 32 bp deletion in the CCR5 gene has been linked to resistance to HIV infection, homozygotes for this mutation (approximately 1% of the European population) are highly resistant to HIV infection, and heterozygotes progress more slowly to AIDs. A number of studies have confirmed that individuals with HLA types B*57:01, B*27:05, and B*14 are resistant to HIV infection whereas HLA C*57 is associated with progression to AIDS.

Genetic influences in man are often difficult to dissociate from nutrition and other socioeconomic factors. Measles, for example, is largely a mild disease in developed countries but can have a mortality rate of up to 50% in severely malnourished children in the Third World. The genetic effect is clearly shown to be distinct from environmental effects in studies of the occurrence of diseases such as tuberculosis in identical twins who have lived apart. In one classical study with tuberculosis, it was shown that 87% of identical twins also had the disease, whereas only 26% of nonidentical twins were affected. The identical twins, moreover, showed a similar type of clinical disease, which has also been shown for leprosy. The picture is much clearer in certain experimental infections in animals. For instance, the susceptibility of mice to certain viruses and to enteric bacteria such as *Salmonella* is under genetic control, and susceptible and resistant strains have been developed by breeding. Susceptibility of mice to *Salmonella* infections is under the control of many genes. In one instance, that of susceptibility to the lethal effect of intracerebrally injected yellow fever virus, resistance is inherited as a single dominant genetic factor. The basis of resistance in the brain is not understood, but it presumably involves the susceptibility of neurons to infection with yellow fever virus. Resistance to mouse hepatitis virus is also under simple genetic control and this seems to operate by restricting virus growth in liver macrophages, and thus preventing infection of liver cells. Genetic factors presumably control the behaviour and characteristics of macrophages, and macrophages play a central role as determinants of pathogenicity in many viral, bacterial and other infections.

Species' differences in susceptibility to infection, of course, are also genetically determined, and in some instances the mechanisms have been identified. Guinea pigs are resistant to South American strains of *Yersinia pestis*, and this is because asparagine, a bacterial growth requirement, is missing from guinea pig serum, which contains the enzyme asparaginase. Bacterial growth is accordingly slower, giving time for the immune response to control the infection. Susceptibility of the bovine placenta to *Brucella abortus* is associated with the presence of a bacterial growth stimulant, erythritol. This is not present in the human placenta which is therefore resistant to infection. Accordingly, cows but not people abort when infected with *B. abortus*.

Isolated human populations generally show a high mortality after the first encounter with traders, invaders or explorers from Europe or Asia. Although some of this is due to combat, social disruption, etc., most of it appears to be due to the new infectious diseases encountered such as measles and tuberculosis. One suggestion is that this is based on MHC genetics. It is an advantage for a host population to have a good deal of diversity (polymorphism) in MHC genes controlling immune responses. This makes it more likely that there will be a suitable MHC molecule to accommodate peptides from any novel infectious agent, so that an immune response can be initiated. We know that many of the populations from historically isolated islands and continents show less MHC polymorphism than those from areas where contact between different peoples was more common,[1] and perhaps this accounts for their great susceptibility to new infections.

There is a good example in mice of the independent control of immune responses to different microbial antigens. Adult mice of most strains infected with LCM (lymphocytic choriomeningitis) virus generally show severe pathological changes as a result of the cell-mediated immune (CMI) response to the virus. Virus multiplies in exactly the same way in mice of the C57BL strain, but they do not develop disease because they have a weak T-cell response to LCM virus antigens. However, mice of the C57BL strain generate a vigorous immune response to ectromelia (mousepox) virus. This virus grows in the liver and is often lethal in many strains of mice, but the vigorous immune response of C57BL mice ensures the early inhibition of virus multiplication in the liver and allows them to survive. In both these examples, C57BL mice show greater resistance to disease, resistance to LCM virus disease because of a weaker immune response and resistance to the disease mousepox because of a stronger immune response.

Age of Host

There are hardly any infectious agents that cause exactly the same disease in infancy, adult life and old age. Susceptibility is generally greater in the very young and the very old, as for example in Q fever, typhoid, bacillary dysentery or bacterial pneumonia. The greater susceptibility is for a number of different reasons. In the first place, immune responses are weaker in immature and in ageing individuals. Men over the age of 40 years show a gradual decline in the magnitude of antibody and CMI responses to standard antigens. In the elderly, T cell and also phagocyte function tend to be decreased.

[1]Europeans, sub-Saharan Africans and East Asians have 30–40 different MHC (A, B) alleles, whereas native North Americans and natives of Polynesia or Papua New Guinea have only 10–20.

Similar changes are seen in mice. All types of infections therefore tend to be controlled less successfully at these ages, but at the same time there is less immunopathology. However, the evidence for an overall reduction in resistance to infectious disease in elderly people is not impressive.

Infections in infants sometimes spread rapidly and prove fatal without the evolution of the characteristic clinical and pathological changes seen in adults. The infant's greater freedom from immunopathology is illustrated in LCM virus infection of mice. Adult mice die when virus is injected into the brain, as a result of the CMI response to infection, but infant mice remain well because their response is much weaker. Certain latent infections such as varicella zoster virus (VZV) are kept under control by CMI, and in older people with failing CMI these infections are more likely to undergo activation. Thus, older people show an increased incidence of reactivation of VZV in dorsal root ganglia resulting in herpes zoster.

This is distinct from age-related differences in the *incidence* of infection. It is not surprising that most infections are commonest in children, especially after first contact with other children at school. Exposure can be an important factor. Immunological immaturity makes newborn animals highly susceptible to viral, bacterial and other infections. Some of the human infections that are also more severe in early infancy become progressively less severe as the child grows up. The commonest infantile infections are those that cause respiratory illness, and those that give diarrhoea and vomiting. Infections that are prevalent in the community, however, will at some time have infected the mother, and the transfer of maternal antibodies (IgG) to the infant via placenta or milk confers protection during this vulnerable period of life. In the rare human infant who is born without maternal IgG, viruses such as herpes simplex or varicella zoster or cytomegalovirus can cause lethal diseases. Newborn mice also are notoriously highly susceptible to a great variety of experimental virus infections, their mothers not having provided them with antibody. In man, IgM antibodies are not transferred from mother to infant and, in so far as these antibodies are important in resistance to Gram-negative bacterial sepsis, infants are particularly susceptible to coliform sepsis. Maternal antibody, whether to herpes simplex, malaria or streptococci is transferred mainly via the placenta in man, but in other animals transfer through the milk is more important. A newborn foal, for instance, takes a feed of antibody-rich (especially in IgA) milk (colostrum) within minutes of birth and thereby acquires a protective umbrella against a great number of infectious agents.

Age-related differences in susceptibility are at times attributable to physical or physiological differences. The increased susceptibility of old people to respiratory infection is partly due to factors such as the loss of elastic tissue round alveoli, weaker respiratory muscles and a poorer cough reflex. Both old people and infants sometimes fail to show the usual signs of infection, such as fever. The lungs of infants are particularly susceptible to whooping cough and other bacterial pneumonias, partly because the airways are narrow and more readily blocked by secretion and exudate. Infants are also the first to suffer the effects of fluid and electrolyte loss, so that infections characterised by fever, vomiting or diarrhoea tend to be more serious at this time of life. Often the reasons for increased susceptibility in infancy are not clear. Respiratory syncytial virus, for instance, often causes serious illness in infancy with croup, bonchiolitis or bronchopneumonia. In adults, the virus causes a minor upper respiratory infection, but in early life there is invasion and

growth of virus in the lower respiratory tract. It is not known whether this is because respiratory epithelium and alveolar macrophages are more vulnerable to infection than in older individuals, or because host defences are less effective. As an example of age-related susceptibility based on a local physiological difference, the skin of children becomes less susceptible to fungus infections (ringworm) at puberty, and this is connected with the marked increase in sebaceous secretions at puberty. However, the same increase in sebaceous secretion at puberty leads to greater susceptibility to the skin disease acne, induced by bacteria originating from the sebaceous glands. It is thought that lipases from *Proprionibacterium acnes* hydrolyse triglycerides in sebum to form fatty acids that are responsible for the inflammation in the lesions (and scars on the skin as well as psychological).

Certain virus infections are usually milder in childhood, and more likely to be severe when primary infection occurs in adult life. These include varicella zoster, mumps, poliomyelitis and EBV infections. VZV often causes pneumonia in adults, and mumps involves the testicle after puberty, giving a troublesome orchitis. Infections with polioviruses are nearly always asymptomatic in early childhood. When polioviruses first came and caused a 'virgin soil' epidemic in certain isolated Eskimo communities in the 1940s and in the island of St. Helena in 1947, there was a strikingly high incidence of paralysis in adults, but mostly inapparent infections in childhood and old age. Where infection during childhood is the rule, paralytic disease might be expected to be less common. In developed countries, on the other hand (North America, northern Europe, etc.), where there has been a certain amount of interruption of the faecal–oral spread of infection, poliovirus infection was often delayed until adolescence or adult life, and as a result paralytic disease had been quite common (until the development of vaccines). EBV is excreted in saliva, and in developing countries most individuals are infected as young children, undergoing an inapparent infection. In developed countries, where childhood infection is less common, first infections often occur in adolescence or early adult life, following the extensive salivary exchanges that take place during kissing. In this age group, and in these countries, therefore, EBV causes the more serious disease, glandular fever. It is not known why these infections are more severe in adults, but a more powerful immunological contribution to pathology and disease might be suspected.

Sex of Host

In most species studied, males are more susceptible to infection by all classes of pathogen and in a number of studies this susceptibility has been linked to steroid hormone levels. This is in part due to differences in the way in which male and female sex hormones regulate immune responses; however, other hormone-regulated genes also play a role.

Females generally have higher IgG and IgM levels, develop stronger T-cell-mediated responses, and are less susceptible to many infections. In mice, males have a lower antibody response than females following infection with the fungus *Candida albicans*, and castrated males have a similar response to those seen in females. This suggests a role for testosterone in suppressing the immune response. A similar situation is seen in humans where men and women who have low estradiol levels (in the luteal phase of their

menstrual cycle) have lower antibody levels than women in the luteal phase where estradiol levels are higher. The role of sex hormones is however not that straightforward; treatment of female mice with oestrogen leads to an increase in IFNγ levels leading to an increased resistance to leishmania infection. Similarly treated mice are however more susceptible to Listeria, Salmonella and Toxoplasma infections possibly due to a decrease in NK cell and macrophage activity and reductions in IL-2 and TNF-α levels. Hormonal changes during pregnancy are complex and it is well recognised that pregnancy is linked to a significant depression in cell-mediated immunity. Various new hormones appear, along with changes in levels of oestrogens, progesterone and corticosteroids. In humans, pregnancy is associated with an increased reactivation of latent herpesvirus infections, e.g. HSV, EBV and CMV and this can lead to serious neonatal infections. Pregnancy is also associated with an increased susceptibility to other infections, e.g. influenza A, hepatitis A and B, tuberculosis and the fungal infection *Coccidioides immitis*.

The increased susceptibility to infectious disease of pregnant women should be distinguished from the susceptibility of the foetus. The pregnant woman can be regarded as the site of development of a novel set of tissues, including the foetus, placenta, lactating mammary gland, etc., each providing a new and possibly susceptible target for infectious agents. The foetus is exquisitely susceptible to nearly all microorganisms, but access is normally restricted by the placenta. Microorganisms that can infect the placenta, such as syphilis, toxoplasmosis, cytomegalovirus, rubella and smallpox, are then at liberty to infect the foetus (see Table 5.3).

Malnutrition of the Host

Malnutrition can interfere with any of the mechanisms that act as barriers to the multiplication or progress of microorganisms through the body. It has been repeatedly demonstrated that severe nutritional deficiencies will interfere with the generation of antibody and CMI responses, with the activity of phagocytes, and with the integrity of skin and mucus membranes. Often, however, nutritional deficiencies are complex, and the identification of the important food factor is difficult. This is reflected in the use of inclusive terms such as 'protein-calorie malnutrition'. Also, at times, it is impossible to disentangle the nutritional effects from socioeconomic factors such as poor housing, crowding, inadequate hygiene and microbial contamination of the environment. Poverty is a close and ancient companion of infection. Worldwide, the most lethal infections in terms of the total numbers killed each year are respiratory infections (all types), acquired immunodeficiency syndrome (AIDS) and diarrhoeal infections (all forms). The deaths are mostly in developing countries, where communicable diseases still stand as the main public health problem. The period just after weaning is often the most vulnerable, when the nutritional state is poor, and there are many common microorganisms still to be encountered. A study of children in Guatemalan villages provides a good illustration of the synergism between malnutrition and infection. A group of children were studied individually from birth, and their colonisation by various microorganisms and parasites was recorded. There was a loss in body weight after the sixth month, at the time of weaning from breast milk to a deficient diet. Further interruptions in weight gain, and sometimes temporary weight loss, were

correlated with measles, various respiratory infections, and infection with *Shigella* and *Entamoeba histolytica.* Body weights at two years of age were sometimes little more than half of those of American children, and there was often almost no gain in weight during the second and third year of life.

Nutrition is affected when there is a bacterial overgrowth in the upper small intestine. This is common in developing countries and is associated with heavy bacterial contamination of water supplies. The increased numbers of bacteria degrade bile salts to cause malabsorption of fat (steatorrhoea); they impair absorption of carbohydrate, they bind vitamin B_{12}, whose shortage leads to anaemia, and they further interfere with absorption of nutrients when they produce enterotoxins. Children with malnutrition had more than 10^5 bacteria ml^{-1} in the upper jejunum (and as many as 10^7–10^8 ml^{-1}), whereas normal children had $<10^4$ bacteria ml^{-1}.

It seems clear that protein deficiency tends to depress in particular the CMI response, which, together with reduced C3 levels, lowered production of secretory IgA and reduced killing of bacteria by polymorphs, causes an increased susceptibility to many infectious diseases. Children with protein deficiency, the extreme form being represented by the clinical condition called kwashiorkor, are very susceptible to measles. This is a result of their weaker CMI response to the infection, the lowered resistance of mucosal surfaces of the body, and perhaps to the higher contamination of the environment with the microorganisms that cause secondary infections. All the epithelial manifestations of measles are more severe. Life-threatening secondary bacterial infection of the lower respiratory tract is common, as well as otitis media, sinusitis, etc. Conjunctivitis occurs, especially if there is associated vitamin A deficiency, and at times progresses to severe eye damage and blindness. The tiny ulcers in the mouth that constitute Koplik's spots in normally nourished children can enlarge to form massive ulcers or necrosis of the mouth (cancrum oris). Instead of an occasional small focus of infection in the intestine, there is extensive intestinal involvement with severe diarrhoea, which exacerbates the nutritional deficiency. Increased susceptibility to herpes simplex and *Pneumocystis carinii* Pneumonia (also increased in HIV patients) infection, and to Gram-negative septicaemia is also seen in protein deficiency. Because of the effect on CMI, there is greater susceptibility to tuberculosis. Tuberculosis has often been noted to increase in frequency in times of famine, and this has also been observed in the inmates of concentration camps.

On the other hand, it looks as if certain infections are less severe in malnourished individuals. Typhus, for instance, is said to cause a higher mortality in well-fed than in malnourished individuals, and clinical malaria was suppressed in Somali nomads during the 1970s' famines, only to be reactivated five days after refeeding. It is unclear why malnourished individuals are sometimes less susceptible to infection. A decrease in the host inflammatory and hypersensitivity responses would be expected, and perhaps those diseases which appear to be less severe are those with an immunopathological component to the symptoms.

Vitamin A, B and C deficiencies are known to lead to impaired integrity of mucosal surfaces, which in turn causes increased susceptibility to infection, and adds to the complexity of the picture. In developing countries, the severity of measles is greatly reduced when children are given vitamin A supplements. There may be a pre-existing vitamin A deficiency, but measles itself causes reduced vitamin A levels. Mortality is lowered and ocular

damage, in particular, is less severe. The effect is not only on the integrity of epithelial surfaces. Children given these supplements show less depletion of T lymphocytes and increased production of measles-specific IgG antibody, compared with untreated children with measles. The commonest mineral deficiency is iron and, by affecting certain enzyme systems, this can increase susceptibility to infection. For example, it causes reduced myeloperoxidase activity in phagocytes, with less hydroxyl radical formation, and this means defective killing of bacteria (see Chapter 4). Zinc, selenium, and vitamin E deficiency, especially in the elderly, can reduce immune and phagocytic function. Chronic diarrhoea leads to zinc deficiency, and by giving zinc supplements to children in New Delhi, Brazil and China, the incidence of cough, pneumonia and diarrhoea was reduced.

STRESS AND HORMONAL FACTORS

Hormones have an important role in maintaining homoeostasis and in regulating many physiological functions in the body and we have already discussed the effect of sex hormones on disease outcome.

'Stress' as a scientific term is difficult to define; it can be, and is, used to describe any process which perturbs normally physiological processes and by this definition infection is a stress. Stress is most commonly, however, taken to mean some type of psychological or mental stress and for many years it was unclear as to the mechanistic link between stress and immune modulation. It is now clear that the immune, endocrine and nervous systems are tightly linked and that neuronal signals can modulate the immune response via the so-called 'neuroimmune axis'. The complex interplay between the nervous and immune systems can be summarised as follows: (i) immune responses can be regulated and modulated; (ii) stimulation of specific regions of the brain can modulate immune responses; (iii) activation of the immune system correlates with changes in the neurotransmitter levels and brain activity; (iv) cytokines and neurotransmitters are produced by both the immune and nervous system facilitating this crosstalk between the systems.

During stress glucocorticoids, catecholamines and neuroendocrine factors are released into the circulation of the host. Corticosteroids have a complex and wide range of actions; the most important for infectious diseases are the glucocorticosteroids, which inhibit inflammation and depress immune responses. Corticosteroids also stabilise cell membranes and lysosomes, giving cells some protection against damage or destruction. Inflammation makes an important contribution to tissue damage and pathology in infectious disease and endogenous corticosteroids play an important role in limiting this inflammation and so limiting immunopathology. The innate immune and inflammatory responses are however also central to limiting the infection. This dual role is reflected in the results of giving corticosteroids in infectious disease. Herpes simplex keratoconjunctivitis or encephalitis, for instance, is temporarily improved by corticosteroids because of the reduction in local inflammation, but this reduction in inflammation means that the infection can spread more readily. The net effect is to make the disease worse. For the same reasons, a large number of different experimental infections in animals are made more severe by corticosteroid administration.

Although corticosteroids are used therapeutically, the key question is what effect the individual's own corticosteroids have on the course of an infectious disease. It is first necessary to say something about the function of the corticosteroid response to stress. Small areas of tissue injury give rise to quite severe but nevertheless locally useful inflammation, mediated by various inflammatory factors. If exactly the same response took place in multiple sites of infection in the body or in response to more extensive tissue injury, the immediate overall result in terms of vasodilation and loss of fluid into tissues would be harmful. An individual who is infected or wounded may need to retain bodily functions for running or fighting, and the effect of multiple unmodified local inflammatory responses might well be incapacitating. When inflammation occurs on a large scale, therefore, it is an advantage to make an overall reduction in its severity, so that the general impact on the host is lessened. This is a teleological way of looking at the function of corticosteroid hormones, which also makes sense of their metabolic function in mobilising energy sources. The response to stress of the autonomic nervous system, involving adrenalin-mediated changes in preparation for bodily action (fight or flight) is more obviously interpreted in these terms. During an infection there is an increase in the rate of corticosteroid secretion, just as in response to other bodily stresses such as hunger, injury or exposure to cold. Rises in urinary 17-ketosteroids are seen, for instance, in Q fever and sandfly fever infections in man. There is also an increased rate of utilisation of corticosteroids by tissues. Inflammatory and immune responses thus take place against the dampening and modifying background of increased corticosteroid levels, which ensure that continued bodily function and balance (homoeostasis) is maintained. When the corticosteroid response is depressed, as in individuals with Addison's disease (where the adrenal glands do not produce sufficient steroid hormones), the consequences of infection or tissue injury are very severe, and affected patients therefore have to be given increased doses of corticosteroids during infections. Conversely in Cushing's syndrome where there is excessive production of corticosteroids from the adrenal cortex there is also a greatly increased susceptibility to infection.

It is clear that increased levels of corticosteroid hormones are necessary for a successful host response to infectious disease. Administering additional amounts of corticosteroids is not necessarily of value unless the host's own corticosteroid response is known to be subnormal, or if it is for the moment more important to reduce inflammation than to control infection. Otherwise, additional corticosteroids tend to promote the infection by decreasing the effectiveness of antimicrobial forces, as discussed above.

When corticosteroids are given, they can not only make any infection that happens to occur at the time more severe, but also reactivate persistent infections that are normally held in check by immune forces. Tuberculosis in man is often activated or made worse by corticosteroid administration. Stress tends to act in the same way, probably because of increased secretion of corticosteroids. One classical example in animals is psittacosis, a chlamydial infection of parrots and budgerigars. These birds normally carry the microorganism as a persistent and harmless infection, localised in the spleen. Following the stress of transport in cages, exposure to strange surroundings or inadequate diet, the infection is activated in the bird, and the microorganism begins to be excreted in the faeces. Human infection can then take place by inhalation of particles of dried droppings from the cage, causing the troublesome disease psittacosis, with pneumonia as a common feature.

In humans, stress in the form of anxiety calls into action the same physiological changes which were designed to deal with physical stresses (Figure 11.1). For instance, in a university boat race the crew had increases in corticosteroid production that enabled them to sustain the physical stress of the race, but the coxswain was found to have an increase of equal magnitude, despite exerting significantly less physical effort. Psychological stress is one of the main factors which many sufferers link to the recurrence (reactivation) of oral and genital herpes. The latent infection in neurons is maintained, at least in part, by a constant immune surveillance and IFN production which appears to inhibit virus replication. Stress-induced immune modulation as described above could reduce this block to replication leading to reactivation and the development of lesions.

The adrenal cortex itself is not often involved in infectious diseases but, if it is, the infection in the cortex tends to be extensive. Examples include tuberculosis and histoplasmosis in man and various viral, bacterial, fungal and protozoal infections in experimental animals. Infectious agents localising in the adrenal cortex encounter a high concentration of corticosteroid hormones originating from cortical cells. Antimicrobial forces are therefore weakened locally, and the infection is exacerbated. Active adrenal foci of infection are often seen at a time when foci elsewhere in the body are healing.

There is usually a change in susceptibility to infection during pregnancy, as discussed earlier in this chapter, and this is due to hormonal changes. The male sex hormones

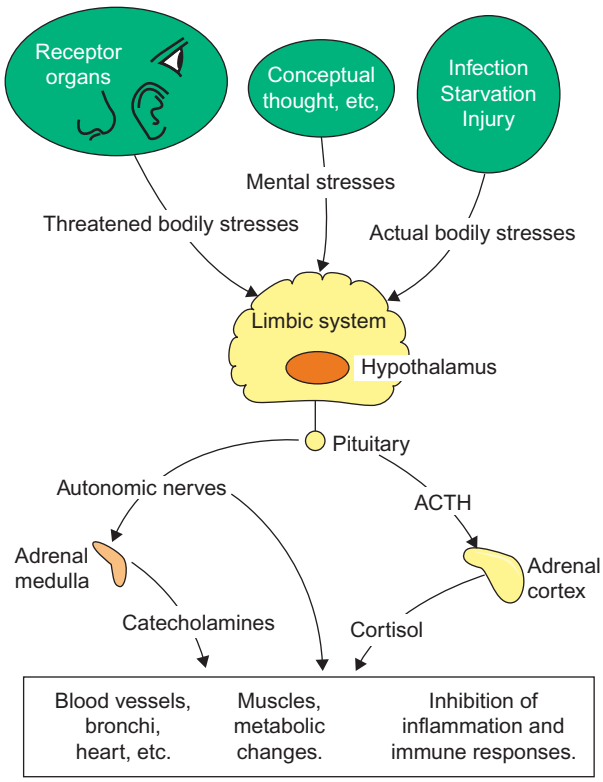

FIGURE 11.1 Diagrammatic representation of stress mechanism in man. Various cytokines (IL-1, IL-6 and tumour necrosis factor) act on the hypothalamus, and IL-1 and IL-2 on the pituitary gland.

responsible for the changes in the testicle at puberty can be regarded as causing this organ's susceptibility to mumps virus infection. Clearly, there are hormones that control the health and well-being of cells and tissues in all parts of the body and, in this sense, serious hormonal disturbances could always affect the course of infectious diseases.

OTHER FACTORS

A host of miscellaneous factors influence the course of infectious diseases, and some of them merit particular mention. Certain lung conditions resulting from the inhalation of particles have an important effect on respiratory infection. Silicosis is a disease due to the continued inhalation of fine particles of free silica. It occurs in coal miners and in various industries where sandstone and similar materials are used. There is a great increase in susceptibility to tuberculosis, which is more likely to cause serious or fatal disease. This is because lung macrophages, which play a central role in resistance to respiratory tuberculosis, become damaged or destroyed following the phagocytosis of the free silica particle. When intact macrophages containing nonlethal amounts of silica phagocytose tubercle bacilli, the bacteria grow faster, the cell dies, and the progeny bacteria are released sooner.

Nowadays, most people spend 90% of their lives indoors, and air exchange with the outside world is much less than it used to be, but some exposure to atmospheric pollutants is inevitable. The air is polluted in many towns and cities, especially with substances derived from the combustion of commercial, domestic and automobile fuels. These include SO_2, nitrogen oxides, CO, ozone, benzene, acid aerosols, and also particles. Although these particles form a small proportion of the total mass of particles suspended in air, they are important because they include small ($<2\ \mu m$ diameter) particles, which are stable, penetrate deep into the lungs and may bear acidic gases or contain toxic elements such as lead. In many countries, pollutants have been reduced by clean air laws, catalytic converters, and the use of lead-free fuels. The commonly measured pollutants are SO_2 and particulates (smoke). For both, the upper limit (24 h mean) recommended by the World Health Organisation (WHO) is $20-50\ \mu g/m^3$, but these values are commonly exceeded. Can atmospheric pollution increase the severity of respiratory infections? It has been reported that people with chronic bronchitis produce larger volumes of morning sputum and note a worsening of symptoms when SO_2 values in air reach $250\ \mu g/m^3$, and there is an increase in respiratory mortality when levels exceed $750\ \mu g/m^3$. In the great London smog of 1952, before the Clean Air Bill greatly improved the quality of London air, SO_2 levels reached $8000\ \mu g/m^3$, and there were 4000 excess respiratory deaths. The morbidity and mortality, however, is seen in those with chronic respiratory disease (chronic bronchitis, etc.) in the very old and in other susceptible individuals.

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CHAPTER

12

Vaccines and How They Work

OUTLINE

INTRODUCTION

The greatest achievement in medicine in the twentieth century has probably been the great reduction in the incidence of infectious disease. Smallpox has been eliminated, and most of the old scourges such as cholera, diphtheria and typhoid have been brought under control, at least in the developed countries of northern America, northern Europe, Australia, etc. giving us the opportunity to die of other things later in life. This revolution in infectious diseases was in the first place the result of dramatic improvements in sanitation and public health, which provided clean water supplies, adequate disposal of sewage and better housing. The downward trends in many infectious diseases were in progress early in the twentieth century, well before antibiotics had been invented.

Improvements in water supplies and sewage disposal obviously have a great impact on enteric diseases such as cholera and typhoid. Better housing and nutrition have had an important influence on other diseases. Tuberculosis, referred to as the Great White Plague in the cities of nineteenth-century Europe, and notoriously promoted by crowding and poverty, has been steadily declining, at least in the developed world as a cause of death, as standards of housing and nutrition have improved. Infectious diseases like typhus and plague have receded as people and their dwellings have become free from the lice, fleas and rats that were necessary for the spread of these diseases. But all these infections are still present in the world and the people of developed countries are protected from them only so long as they continue to be protected from lice, fleas, rats, poverty, crowding and

Mims' Pathogenesis of Infectious Disease.
DOI: http://dx.doi.org/10.1016/B978-0-12-397188-3.00012-3

contaminated food and water. Once an infectious agent has been totally eradicated on a global scale, it cannot of course return. This is difficult to achieve with infections such as malaria, plague and yellow fever because they have vectors and animal reservoirs, but infections restricted to man and involving no other host can be totally eradicated if all human infection is prevented. Smallpox came into this last category, and it was totally eradicated from the world by a relentless vaccination programme carried out by the World Health Organisation (WHO). Smallpox eradication was also made easier because the virus does not persist in the body and therefore cannot reactivate. Poliovirus is another example, where it is hoped to bring about complete eradication of disease within the next few years. Although many infectious diseases were already declining following general improvements in public health, the decline was greatly accelerated by the development of vaccines to prevent diseases and antibiotics to treat infections. Vaccines, used on a large scale, have been a major antimicrobial force in the community. Many infections, especially virus infections such as measles and poliomyelitis, have receded wherever effective vaccines have been used. Some vaccines are better than others. Yellow fever has proved to be one of the best vaccines, while vaccines against typhoid and cholera have so far remained comparatively unsatisfactory. There has been a failure to develop effective vaccines for many important human diseases such as trachoma, human immunodeficiency virus (HIV), malaria, syphilis and gonorrhoea. However, recent improvements in vaccine design strategies such as reverse vaccinology are providing success stories and hope for the future.

What Is a Vaccine?

A vaccine is a material originating from a microorganism or other parasite that when delivered to an uninfected host induces an immunologically mediated resistance to disease.

What Do We Ask of an Ideal Vaccine?

1. That it promotes effective resistance to the disease, but not necessarily to the infection
2. That resistance lasts as long as possible
3. That vaccination is safe, with minimal and acceptable side effects. The smallpox vaccine, which remained more or less unchanged for more than a 100 years, would never have been licensed if introduced now. Rather lower safety standards are acceptable for most veterinary vaccines. A vaccine, even if not completely safe, should be safer than exposure to the disease, assuming that the risk of exposure is significant. Attitudes to a given vaccine's safety depend on whether the safety of the individual or the protection of the community is under consideration. When a vaccine gives protection to the community, the community owes a debt to any individual damaged by the vaccine
4. That the vaccine is stable and will remain potent during storage and shipping. The fact that yellow fever virus can be freeze-dried and transported unrefrigerated in the tropics has been a great asset favouring the success of this vaccine. Poliovirus cannot be successfully freeze-dried, but vials containing the live (oral, Sabin) vaccine show a colour change when overheated
5. That the vaccine is reasonably cheap, if it is for large-scale use, or for use in developing countries

6. In many veterinary vaccines, due in part to trade constraints on infected animals, or meat from infected animals, it is desirable to be able to differentiate vaccinated from naturally infected animals.

GENERAL PRINCIPLES

Effective resistance to infection or disease depends on the vaccine having certain properties, and there are often different requirements for different types of infection. Some important general principles are discussed below.

The Vaccine Should Induce the Right Type of Immune Response

The relative importance of antibody and T cells in resistance to disease has been discussed in Chapters 6 and 9. Vaccines should induce the type of immunity that is relevant for the particular microorganism. Resistance to tuberculosis or typhoid seems to require effective T-cell-mediated immunity, whereas resistance to influenza or poliomyelitis requires a good antibody response.

The Vaccine Should Induce an Immune Response in the Right Place

For resistance to infections of epithelial surfaces, it is more appropriate to induce secretory IgA antibodies than circulating IgG or IgM antibodies. Thus, secretory IgA antibodies might give valuable protection against influenza or cholera, but not against rabies or yellow fever which by-pass epithelial surfaces and enter the body through bite wounds. For this type of infection an IgG response is most beneficial.

The Vaccine Should Induce an Immune Response to the Right Antigens

A given microorganism contains many different antigens, many hundreds or thousands of antigens in the case of protozoa, fungi and bacteria, and in virus infections from as little as three (polyomavirus) to more than 100 (herpes and poxviruses) are produced. Immune responses to many of these antigens develop during infection. Antibody-mediated resistance to infection, however, depends principally on immune responses to the smaller number of antigens on the surface of the microorganism.

Resistance to Some Infectious Diseases Does Not Depend on Immunity to the Infectious Agent

In certain infections such as tetanus and diphtheria, disease is entirely due to the actions of toxins as discussed in Chapter 8. Immunity to the disease requires only an effective antibody to the toxin. For the production of a vaccine, therefore, a toxin is modified by chemical or physical treatment (alcohol, phenol, ultraviolet irradiation) so that it is no

longer toxic, but maintains its antigenic character. The resulting toxoid can function as a very effective vaccine when combined with an adjuvant.

There are Important Differences in Principle Between Killed and Live Vaccines

The primary response to an antigen is classically distinguished from the secondary response. After the first injection of an antigen, the immune response begins and at the same time the antigen itself is generally degraded and disposed of in the body. The second injection of antigen now induces a greatly enhanced response, and subsequent injections give further boosts (Figure 12.1). Each killed vaccine must therefore be given in repeated doses if an adequate immune response and resistance is to be induced. The microorganisms in live vaccines, on the other hand, multiply in the host after administration. The antigenic mass contained in the vaccine itself is small but it is increased many thousand times following growth of the microorganism in the body. The effective dose is greatly amplified in this way, and the primary response merges into the secondary immune response, giving a high level of immunity (Figure 12.1). Only one dose of vaccine is therefore needed to produce satisfactory immunity. Nearly all the successful viral vaccines, both medical and veterinary, consist of living attenuated virus. Examples of different types of vaccine are given in Table 12.1, and differences between live and killed vaccines are summarised in Table 12.2.

Factors Determining the Duration of Resistance

Clearly, the longer protection lasts the better; no vaccine would prove popular if an injection was required every six months throughout life. The duration of resistance to disease depends to some extent on the type of infection. In the case of systemic infections with an incubation period of a week or two, a low residual level of immunity gives resistance to disease, because even if re-infection does occur, the immune response is boosted during the incubation period and the infection is terminated before the onset of disease. Repeated subclinical booster infections may be important in maintaining immunity to diseases such as measles and rubella.

We have little understanding of the factors responsible for the long-lasting immune responses to microorganisms that are seen in the absence of persistent infection or re-infection. Immunity conferred by the live yellow fever virus vaccine, for instance, is probably lifelong, although the infection is not a persistent one, and viable virus is apparently completely eliminated from the body. Live vaccines give longer lasting protection than killed ones, if the infectious agent persists in the body and produces antigens, to give continuous stimulation of immune responses (BCG, Marek's disease vaccine for poultry).

The Concept of Attenuation

It would seem ridiculous to use the naturally occurring disease agent as the vaccine, because it would tend to cause the disease that one wishes to prevent. In the early days

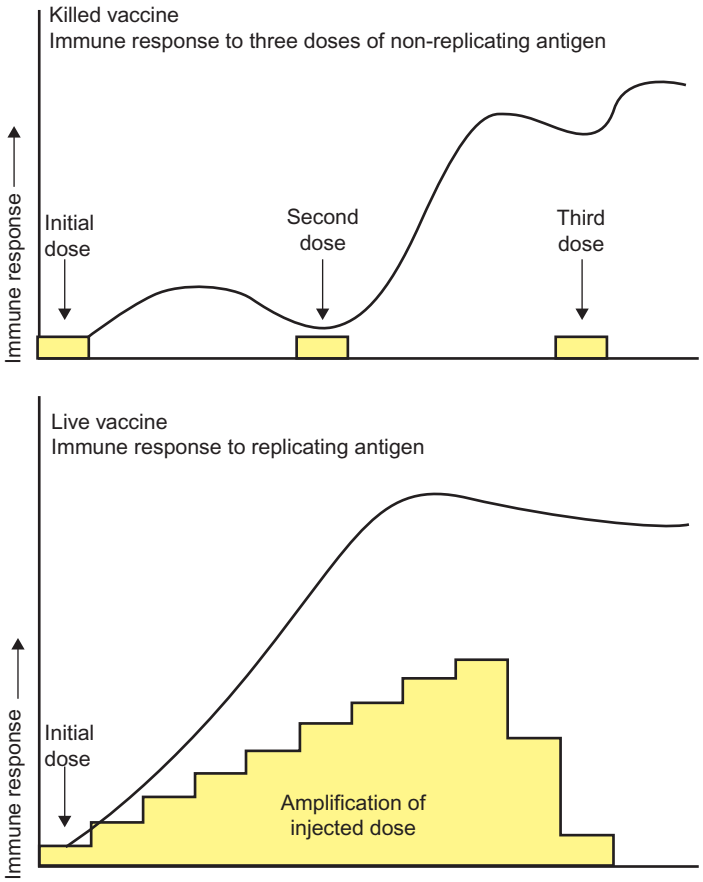

FIGURE 12.1 Comparison of immune responses to live and to killed vaccines.

of smallpox vaccination, however, living virus from the scabs of smallpox patients was used as a vaccine. Lady Mary Wortley Montagu, wife of the British Ambassador to Turkey, brought this type of vaccination (variolation) to England over 250 years ago. It was effective, but could be fatal, and was made illegal in 1840 when Jenner developed his calf lymph vaccine. The most common way to reduce the pathogenicity of a microorganism is by repeated growth (passage) in cell culture (measles, Sabin polio, varicella zoster virus) or on bacterial growth media (BCG). This is an empirical procedure, depending on the fact that prolonged passage of a microorganism in an artificial system tends to select mutants better suited to growth in that system than in the original host. For instance, influenza and respiratory syncytial virus mutants have been produced that grow poorly at 37°C, the temperature of the lower respiratory tract, but well at the temperature of the nose, 33°C. These temperature-sensitive (ts) mutants multiply after instillation into the nose and induce immunity, but are unable to spread to the lower respiratory tract.

TABLE 12.1 Types of Vaccines

Vaccine	Live Vaccines	Killed Vaccines
Viral	Smallpox	Poliomyelitis (Salk)
	Rubella	Influenza
	Measles	Hepatitis A
	Poliomyelitis (Sabin)	Hepatitis B[a]
	Yellow fever	
	Mumps	
	Varicella zoster	
Bacterial	BCG[b]	Cholera
		Typhoid
		Whooping cough
Bacterial polysaccharide vaccines		Pneumococcus[c] – 23 antigenically distinct polysaccharides
		Meningococcus – serogroups A and C[d]
		Haemophilus influenzae b[c]
Rickettsial		Typhus
Bacterial toxoid vaccines	Diphtheria, tetanus	

[a]The first vaccine produced by recombinant DNA technology after the cloning of the surface antigen (HBSag).
[b]BCG is derived from a strain of *M. bovis* isolated in 1908 from a cow with mastitis; after 230 subcultures over the course of about 15 years, the bacteria had lost their virulence. Highly effective in children, but less so in prevention of pulmonary tuberculosis in adults. We await a new twenty-first century vaccine for tuberculosis.
[c]There are 84 pneumococcal serotypes but most serious illnesses are due to the 23 more common types. Children <2 years old generally give poor antibody responses to polysaccharide vaccines. Capsular polysaccharides are being used to produce vaccines to other bacteria, such as *Haemophilus influenzae*. Polysaccharides are T-independent antigens and their immunogenicity in infants can be enhanced when they are converted to T-dependent antigens by conjugation with protein 'carriers'.
[d]Unfortunately, most cases of meningitis in the United Kingdom and United States are due to serogroup b, but this particular polysaccharide is poorly immunogenic in man.

TABLE 12.2 Comparison of Live and Killed Vaccines

Live	Killed
Must be attenuated by passage in cell culture or bacteriological media	Can be produced from fully virulent microorganisms, e.g. poliovirus (Salk), typhoid vaccines
Smaller number of microorganisms needed	Large number of microorganisms needed
Tend to be less stable	Tend to be more stable
Adjuvant not required	Adjuvant often required
Can often be given by a natural route	Generally given by injection
Induces antibody and T-cell responses	Induces antibody but poor T-cell responses
Possibility of spread of infection to unvaccinated individuals	Spread is not possible

Unless the microorganism can be conveniently cultivated artificially such attenuation is impossible. Attenuation has usually been a 'blind' procedure, and the microorganism has to be tested for virulence during its continued cultivation in the laboratory. Nowadays attenuation can be carried out in using a more directed and rational approach. For example, reverse genetic techniques are now being used to generate influenza vaccines. Influenza A virus is an RNA virus with a genome consisting of eight segments, each of which can be considered to express one protein (some segments express more; however, for the purpose of this explanation we will consider it as one segment: one antigen). It is now possible to create recombinant viruses which contain, for example, seven segments from an attenuated strain with one segment, for example, expressing the haemagglutinin protein, derived from the strain which we wish to vaccinate against.

The process of attenuation must be taken far enough so that the vaccine does not cause disease. An early live measles vaccine (Edmonston strain) caused fever and a rash, and human gammaglobulin was administered at the same time to decrease the severity of the vaccine disease. Attenuation, however, must not be taken too far, because the microorganism may then fail to replicate fully enough to induce a good immune response.

The Concept of Monotypic Microbes

Certain microorganisms are antigenically much the same, i.e. they do not vary much between different strains, so that resistance to disease, once established, is secure. This is so for polio, measles, yellow fever or tuberculosis. Sometimes a given disease is caused by a number of microorganisms which differ antigenically, and resistance to only one of them will not provide resistance to the disease. There are dozens of antigenically distinct types of streptococci, for instance, and resistance to streptococcal infection is not complete until there have been immune responses to them all. The same is true for the common cold, which can be caused by more than 100 antigenically distinct viruses belonging to at least five different groups. Some microorganisms are undergoing repeated antigenic changes during the course of their circulation in the community. The most relevant example of this is influenza virus in man, which continually and subtly alters its antigenic makeup, requiring the continual development of new vaccines against the currently circulating strains.

Adjuvants

Adjuvants are materials that increase the immune response to a given antigen without being antigenically related to it. Compounds such as aluminium salts have long been used as adjuvants, and in diphtheria and tetanus vaccines the toxoids are combined with aluminium hydroxide or phosphate. The aluminium salt converts the soluble toxoid into a particulate precipitate and thus increases immunogenicity. Various oils are also effective as adjuvants with the vaccine material generally administered as a water-in-oil emulsion. Influenza vaccine in some countries is administered using an oil in water adjuvant. A somewhat similar approach is the use of liposomes produced from natural and synthetic

lipids or so-called ISCOMs (immunostimulatory complexes), produced using saponin, a component of tree bark. In both cases, the uptake of and presentation by antigen-presenting cells is improved significantly. For many years, mycobacterial products were used as adjuvants, and Freund's original complete adjuvant consists of killed, dried mycobacteria (usually *Mycobacterium tuberculosis*) suspended in mineral oil. Mycobacterial adjuvants can cause granulomas and are not now acceptable for human or animal use. Muramyl dipeptide, a water-soluble component of mycobacterial cell walls, has been used in conjunction with other components such as liposomes to increase adjuvant potency. In recent years, vaccines comprising cytokines as adjuvants have been explored with variable success. Most of these feature as veterinary vaccines, where cost is an overriding consideration.

Recent advances in our understanding of the innate immune system and mechanisms of antigen presentation have allowed the rational development of adjuvant components. Toll-like receptors (TLRs) are pattern recognition receptors that play a central role in activation of the innate immune response. TLR agonists such as double-stranded RNA (TLR3), bacterial lipopolysaccharide derivatives (TLR4); bacterial flagellin (TLR5), single-stranded RNA (TLR7, 8) and CpG oligonucleotides (TLR9) have all been utilised in adjuvant formulations.

The Age at Which Vaccines Should Be Given

Human infants are born with a supply of maternal IgG antibody derived from the placental route, and they thus acquire resistance to all infections to which the mother had antibody-mediated immunity. They also receive secretory IgA antibodies in colostrum and milk, and these provide some protection against intestinal infections. Live polio vaccine (Sabin) is less likely to immunise in the first few months of life because secretory IgA antibodies from maternal milk inhibit the growth of the vaccine virus.

The first encounter with many microorganisms thus takes place under an umbrella of maternal immunity, and when infection takes place it is likely to be mild yet at the same time significant enough to generate some immunity in the infant. Maternal antibody persists for up to six months after birth and vaccines, particularly live vaccines, are likely to be less effective if given before this time. Diphtheria, tetanus and whooping cough vaccines are not given until the infant is 3–6 months old. Certain infections, however, such as measles and whooping cough are particularly severe in infants and very young children (two-thirds of deaths from whooping cough occur during the first year of life), and there is a need to give protection as soon as possible after maternal immunity has faded. Pertussis vaccination, therefore, is commenced at three months to give protection during the first year of life. The complications of diphtheria and pertussis vaccination are commoner in older individuals, and this is another reason for giving these vaccines early in life. In the poultry industry, the use of *in ovo* technology (vaccination of eggs) has been a major breakthrough in the large-scale vaccination of chickens against troublesome pathogens, such as Mareks disease virus, infectious bursal disease virus and Newcastle disease virus. Combination vaccines against several viruses are possible without compromising survival at hatch.

COMPLICATIONS AND SIDE EFFECTS OF VACCINES

Many vaccines contain a number of compounds which are either an unavoidable consequence of the production and purification of the antigen, or are added to either stabilise the vaccine during transport or to act as an adjuvant. In some cases, these components can lead to adverse consequences in the vaccinated individual. For example, most influenza vaccines contain very low amounts of egg proteins (the virus is propagated in eggs), and some individuals have experienced an allergic reaction to this; in some countries cell culture based vaccines are licensed for use in these individuals. Many vaccines contain low levels of antibiotics as a preservative and some also contain the organomercurial thiomersal as a preservative. The inclusion of these compounds, albeit at levels which are designated as safe by regulatory authorities, is a cause for concern by a number of groups and individuals.

In addition to preventing infection in the individual, many vaccines also work to induce herd immunity, and by doing so reduce the number of susceptible/infectious individuals able to transmit the disease. If a significant number of individuals decline to be vaccinated then this reduces herd immunity and previously controlled disease may re-emerge. An example of this is the measles, mumps and rubella triple vaccine (MMR) which was linked by one (subsequently discredited) study to an increased incidence of autism in vaccinated children. Due to parental concerns, this resulted in a dramatic fall in the number of children receiving the vaccine and an increase in the number of reported cases of measles in several areas.

The Development of New Vaccines

Many vaccines contain large numbers of irrelevant antigens, derived either from the microorganism itself or from the culture system used to produce it. It would be better to replace these crude soups with cocktails of defined polypeptides. Sometimes it does not matter if the relevant protective antigen or antigens are not known, as long as relatively clean preparations of virus are available, as in the case of the inactivated rabies vaccine produced from human diploid cells. However, for many bacterial and protozoal infections we still need to know more about the role of microbial surface components in pathogenesis.

There are a few bacterial diseases where the relevant antigen has been identified. Capsular polysaccharides can be used to induce protection against pneumococcal, meningococcal or *Haemophilus influenzae* infections (Table 12.1). Capsular materials are readily obtained by growing bacteria in the laboratory, but it may be noted that many of the genes that code for the enzymes that synthesise the capsule have been cloned. The K88 and K99 adhesins that are responsible for the attachment of *E. coli* to the gut wall of piglets and calves can be used to immunise the pregnant mother whose antibodies will then protect the newborn animal against *E. coli* diarrhoea. Also, the diseases due to the action of a toxin can be approached by developing a toxoid vaccine, as is used very successfully for Tetanus, and recent studies have suggested that vaccination of rabbits with cytolytic or superantigenic toxins may offer protection against challenge with MRSA. Capsular polysaccharides that promote T-cell-independent immune responses are notoriously poor immunogens in infants. However, when such polysaccharide antigens are coupled to a carrier protein, strong T-cell-dependent immune responses are induced with IgG antibodies and long-lived memory

responses. This approach revolutionised vaccination against *H. influenzae* b, a cause of pneumonia and central nervous system disorders in infants, and has now opened new avenues for producing meningococcal and pneumococcal vaccines in the future.

The problem of growing the microorganisms can be solved by genetic engineering. If the DNA that codes for the relevant antigen (e.g. hepatitis B surface antigen) can be obtained, it is incorporated into a plasmid which is introduced into a bacterium (*E. coli*) or a yeast (*Saccharomyces cerevisiae*). The antigen can then be bulk-produced from cultures. All hepatitis B vaccine is now produced in this way.

An excellent example of a vaccine derived by recombinant techniques involves the human papilloma virus (HPV) and protection against cervical cancer. There are two vaccines currently available against HPV 16 and 18 (the tumour-forming strains) and both involve expression of the viral surface protein L1. Aggregates of L1 form into virus-like particles (VLP) that are free of nucleic acid and therefore the potential of the vaccine to replicate, making it very safe. This vaccine has now been adopted as part of the UK immunisation programme for girls aged 12–13.

It is also possible to develop live avirulent vaccines by removing or inactivating the genes that confer virulence. For instance, the gene for the cholera toxin has been cloned in *E. coli*, altered by mutation and then re-introduced into virulent *Vibrio cholerae*. This gives a strain of bacteria that multiplies without producing the toxin when given orally, and induces immunity to cholera.

Transgenic plants are potentially an exciting prospect for mass production of vaccines deliverable in an edible form. To produce vaccine-transgenic plants, the gene for a protective antigen is introduced under the control of plant-specific DNA regulatory sequences and integrated into the genome of the plant. This vaccine antigen can then be transferred from generation to generation in the normal way. Surprisingly, this has been slow on the uptake despite early successes including the introduction into potato tubers of the capsid protein of Norwalk virus (a virus associated with acute gastroenteritis), the LT fusion protein of *E. coli* and CT-B subunit of cholera toxin. A clinical trial involving eating raw potatoes containing the LT fusion protein demonstrated specific IgG and IgA antibodies sufficient to overcome a high dose of virulent *E. coli*. If these antigens can still retain immunogenicity after they are cooked, then this will make a tremendous impact on delivering vaccines on a large scale to developing countries, where enteric diseases are a major problem.

A potentially exciting development in vaccination has been the use of nucleic acid vaccines. In this situation, nucleic acid encoding a foreign antigen (vaccine) is directly introduced into tissue, resulting in the transfection of host cells and the expression of the foreign protein. DNA vaccines are composed of a bacterial plasmid, with a strong viral promoter, the gene of interest and polyadenylation termination signals. The plasmid can also act as an adjuvant by containing CpG motifs. Delivery of DNA vaccines is by intramuscular injection or by gene gun, a process involving the plasmid DNA being coated on gold particles that are then shot into skin by a high-pressure gas jet. This process has been shown to promote antigen presentation by both the MHC class I and class II pathways. An early example of this technique was the introduction of the gene encoding influenza haemagglutinin into the muscle of mice, resulting in the induction of both T-cell and antibody immunity which protected the animals against an influenza A virus challenge. DNA

vaccines have been used widely to protect animals against a variety of pathogens, including those against dangerous pathogens and against pathogens where the antigens have proved difficult to isolate in any quantity, particularly those of complex parasites.

Attenuated Viruses and Bacteria as Carriers

Finally, it is possible to introduce the gene for any given viral protein into the genome of an avirulent virus that can then be administered as a live vaccine. The foreign viral protein is produced in infected cells and induces an immune response. This has been done mostly with vaccinia virus, into which genes from viruses such as hepatitis B, influenza, rabies, herpes simplex, HIV and foot-and-mouth disease have been introduced. This has the supreme advantage that genes from up to 10 or more different infectious agents could be introduced into the same strain of vaccinia virus, a single inoculation of which would simultaneously immunise against a wide range of infections. Unfortunately, smallpox-vaccinated people cannot be immunised in this way, and vaccinia virus is not considered safe enough for people by modern standards. Such concerns have resulted in the genetic engineering of highly attenuated vaccinia virus and the use of canary poxvirus as vaccine vehicles. The latter is unable to grow fully in mammalian cells, but is capable of initiating protective immune responses to antigens by the virus. Other virus vectors are being investigated, including adenovirus and cytomegalovirus (CMV). CMV has achieved spectacular results protecting monkeys against Simian Immunodeficiency virus (SIV). By removing SIV genes responsible for disrupting host $CD8^+$ T-cell responses, a huge $CD8^+$ T-cell response to SIV antigens is induced not only in the primary response but also in the secondary and tertiary responses. This represents a real possibility of developing a vaccine against HIV infection.

Bacteria have also been used to deliver antigens to the immune system. Here foreign genes introduced into the chromosome or into plasmids of attenuated *Salmonella typhimurium* and mycobacteria BCG have been used successfully to initiate immune responses at mucosal surfaces. In the case of intestinal pathogens, the avirulent bacteria, given orally, colonise and multiply, the polypeptides from the pathogens are produced, and gut immunity develops against the intestinal pathogen.

REVERSE VACCINOLOGY

The post-genomics era which benefitted from the existence of whole genome sequence data for many microbial pathogens has revolutionised the approach to bacterial vaccine design. The availability of information on all of the coding sequences for bacterial pathogens facilitates an objective assessment of the bacterial proteins likely to represent antigens and candidate vaccine components. Typically an *in silico* genome sequence analysis will identify genes encoding proteins likely to be surface exposed and involved in host–pathogen interactions. Comparison of multiple genome sequences from diverse clinical strains will indicate which of the predicted antigens are highly conserved between strains, an important characteristic for universal efficacy. Candidate proteins can then be further screened by *in vitro* functional assays including antibody neutralization and

opono-phagocytosis assays. Pools of multiple proteins may then be tested in animal models to determine their protective efficacy against challenge with the pathogen, before trials can be carried out on the natural host. Such an approach has been successful in developing a novel vaccine against *Neisseria meningitidis* serogroup B, a major cause of meningitis in infants. The vaccine is made of surface proteins of the bacterium and after detailed functional investigation *in vitro*, was demonstrated to be effective in phase 3 trials in infants. It is hoped that a similar approach may be active against other major pathogens of humans and animals.

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Appendix

TABLE A.1 Global Disability-Adjusted Life Years (DALYs) for Major Infectious Diseases in 2010

Disease	DALYs[a] (Thousands)	Percentage Change 1990–2010
Tuberculosis/AIDS	130,944	65
Diarrhoeal diseases	282,982	−48
Lower respiratory infections	115,227	−44
Tropical diseases	108,739	4.7
INDIVIDUAL PATHOGENS		
Malaria	82,685	19
HIV/AIDS	81,547	350
Tuberculosis	49,396	−19
Pneumococcal pneumonia	26,906	−38
Haemophilus influenza B pneumonia	21,315	−51
Respiratory Syncytial virus pneumonia	20,472	−54
Influenza	19,244	−40
Rotavirus enteritis	18,650	−55
Typhoid and paratyphoid fevers	12,239	32
Measles	10,420	−80

[a]DALYs is a measure of disease burden that takes into account not only premature death but also the prevalence, severity and duration of illness.
Data taken from Murray et al., 2012. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010 (vol. 380, p. 2197, 2012). *Lancet* **381**, 628–628 (2013).

TABLE A.2 Bacteria of Human Importance

Organism	Diseases	Other Features
GRAM-POSITIVE COCCI		
Staphylococcus aureus	Boils, septicaemia, food poisoning, osteomyelitis, endocarditis, necrotising pneumonia	Common commensal. Associated with antibiotic resistance (particularly methicillin; MRSA)
Streptococcus pyogenes	Tonsillitis, scarlet fever, erysipelas, septicaemia Necrotising fasciitis	Also causes glomerulonephritis, and rheumatic fever, with immunopathological basis
Streptococcus viridans group (*Streptococcus sanguis*, etc.)	Infective endocarditis	Oral commensals settle on abnormal heart valves during bacteraemia
Streptococcus mutans	Dental caries	Regular inhabitant of mouth; initiates plaque on tooth surface
Streptococcus pneumoniae	Pneumonia, otitis, meningitis	Normal upper respiratory tract commensal can spread to infected or damaged lungs
GRAM-NEGATIVE COCCI		
Neisseria gonorrhoeae	Gonorrhoea	Obligate human parasite
Neisseria meningitidis	Meningitis	Obligate human parasite; increased upper respiratory carriage in epidemics
GRAM-POSITIVE BACILLI		
Corynebacterium diphtheriae	Diphtheria	Natural host man. Non-invasive disease due to toxin
Bacillus anthracis	Anthrax	Pathogen of herbivorous animals who ingest spores. Occasional human infection
Clostridium spp.	Tetanus, gas gangrene, botulism	Widely distributed in soil and intestines
GRAM-NEGATIVE BACILLI		
E. coli	Urinary tract infections, gastroenteritis, HUS	Normally inhabit the intestine (man and animals). Many antigenic types
Salmonella spp.	Enteric fever; food poisoning	*Salmonella typhi* – natural host man; invasive. Other *Salmonella* – 1000 species, mainly animal pathogens
Shigella spp.	Bacillary dysentery	Obligate parasite of man. Local invasion only
Proteus spp.	Urinary tract and wound infection	Common in soil, faeces. Occasionally pathogenic
Klebsiella spp.	Urinary tract and wound infection, otitis, meningitis, pneumonia	Present in vegetation, soil, sometimes faeces. Pathogenic when host resistance lowered
Pseudomonas aeruginosa	Urinary tract and wound infection, respiratory infections (CF patients)	Common human intestinal bacteria. Resists many antibiotics

(*Continued*)

TABLE A.2 (Continued)

Organism	Diseases	Other Features
Haemophilus influenzae	Pneumonia, meningitis	Human commensal. Invades damaged lung
Bordetella pertussis	Whooping cough	Specialised human respiratory parasite
Yersinia pestis	Plague	Flea-borne pathogen of rodents. Transfer to man as greatest infection in human history
Brucella spp.	Undulant fever	Pathogens of goats, cattle and pigs with secondary human infection
Legionella pneumophila	Legionnaire's disease	Respiratory pathogen of man, often acquired from contaminated air-conditioning units
Vibrio cholerae	Cholera	Obligate parasite of man. Non-invasive intestinal infection
Helicobacter pylori	Peptic ulcer	Infection widespread. Pathogenesis unclear, but cytotoxin is important and urease helps with residence in acid environment
ACID-FAST BACILLI		
Mycobacterium tuberculosis	Tuberculosis	Chronic respiratory infection in man; killed 1.5 million in 1998. Enteric infection with bovine type via milk
Mycobacterium leprae	Leprosy	Obligate parasite of man. Attacks skin, nasal mucosa and nerves. About 1 million lepers in the world
MISCELLANEOUS		
Treponema pallidum	Syphilis	Obligate human parasite. Sexual transmission. Related nonvenereal human bacteria
Actinomyces israeli	Actinomycosis	Normal inhabitant of human mouth
Leptospira spp.	Leptospirosis (Weil's disease, etc.)	Mostly pathogens of animals. Human infection from urine of rats, etc.
Mycoplasma spp.	Pneumonia, urethritis	Airborne transmission of *M. pneumoniae*. Lack true cell wall − hence no staining with Gram stain and no sensitivity to beta-lactams
Rickettsia spp.	Typhus, spotted fevers, Q fever (*C. burnetii*)	Obligate intracellular parasites. Gram negative. Acquired from animal reservoir via biting arthropods or aerosol (*C. burnetii*)
Chlamydia spp.	Trachoma, urethritis, pneumonia	Obligate intracellular parasites. Acquired by direct human contact, or from infected bird (psittacosis)

TABLE A.3 Fungi and Protozoa of Human Importance

Organism	Diseases	Other Features
FUNGI		
Candida albicans	Thrush, dermatitis, etc.	Normally present on body surfaces; occasionally pathogenic
Dermatophytes (*Trichophyton* spp., *Epidermophyton* spp., *Microsporum* spp.)	Ringworm	Infection of skin, hair, nails (e.g. *Tinea pedis* – athlete's foot) Some species acquired from animals
Cryptococcus neoformans	Meningitis	Occurs in bird droppings, soil; causes skin, lung, CNS disease in immunocompromised
Blastomyces spp.	Blastomycosis	Soil fungi in the Americas; systemic infection in man
Histoplasma capsulatum	Histoplasmosis	Soil fungus in the Americas; can give lung lesions and systemic illness in man
Pneumocystis carinii	Pneumonia	Worldwide infection, causes disease in immunocompromised
PROTOZOA		
Plasmodia (four species)	Malaria	Mosquito transmitted; persistent infection in man
Toxoplasma gondii	Toxoplasmosis	Widely distributed in animals and birds; transplacental infection in man; genito-urinary infection in both sexes, often asymptomatic
Trichomonas vaginalis (flagellate)	Trichomoniasis (urethritis, vulvovaginitis)	
Giardia lamblia (flagellate)	Low-grade intestinal disease	Water-borne outbreaks occur
Trypanosoma spp. (flagellate)	Trypanosomiasis	Transmission by biting insects (animal reservoir); three species pathogenic for man
Leishmania spp. (flagellate)	Leishmaniasis (kala-azar, oriental sore, etc.)	Transmission from animal host to man via sandflies
Entamoeba histolytica	Amoebic dysentery	Invasion of intestinal mucosa; may spread to liver
Balantidium coli (ciliate)	Dysentery	Infection of man from pigs

TABLE A.4 Viruses of Human Importance

Genome	Virus Family	Example	Comments
ssDNA	Parvovirus	Parvovirus B19	In infants causes fifth disease or slapped cheek disease, so called because of the characteristic red rash on the cheeks
dsDNA	Poxvirus	Smallpox	Smallpox has been eradicated and samples are now only held by Centers for Disease Control and Prevention (CDC) in the United States and the State Research Center of Virology and Biotechnology VECTOR in Koltsovo, Russia
	Herpesvirus (*herpes* = creeping)	Herpes simplex virus (HSV or HHV1, HHV2) Varicella zoster (VZV or HHV3) Epstein–Barr virus (HHV4), Cytomegalovirus (HHV5), HHV6, HHV7, Kaposis sarcoma herpesvirus (HHV8)	Establish latency and persist for life of host. Reactivation can lead to recurrence of disease, e.g. VZV causes chicken pox following primary infection. Latent VZV can reactivate to cause shingles
	Adenovirus (*adeno* gland)	Types 1–57	Typically mild respiratory disease
	Papillomavirus	Over 100 types	Causes warts and can cause cervical carcinoma. A vaccine is available to protect against development of cervical carcinoma
	Hepadnavirus	Hepatitis B	Chronic infection can lead to hepatocellular carcinoma
dsRNA	Reovirus	Rotavirus	Major cause of infant diarrhoea
ssRNA (+ ve sense)	Picornavirus	Poliovirus	Polio is almost eradicated
		Rhinovirus	Major cause of the common cold
		Echovirus	Acute febrile illness in newborns, aseptic meningitis

(*Continued*)

TABLE A.4 (Continued)

Genome	Virus Family	Example	Comments
	Togavirus (*toga* = cloak)	Alphaviruses: Chikungunya, Ross river virus Rubivirus: Rubella	Arboviruses: replicated in and transmitted by arthropods Rubella (German Measles) in children
	Flavivirus (*flavi* = yellow)	Flaviviruses: Yellow fever, West Nile virus, Dengue Hepaciviruses: Hepatitis C	Arthropod-borne viruses: *Arboviruses*: Cause febrile illness and haemorrhagic fevers Hepatitis
	Coronavirus (*corona* = crown)	SARS (Severe Acute Respiratory Syndrome coronavirus) MERS-CoV (Middle East Respiratory Syndrome coronavirus) 229E, OC43	SARS: Major global outbreak in 2004. High mortality rate MERS-CoV: Severe outbreaks beginning 2012, mainly in the Middle East. High mortality rate Common cold
	Calicivirus (*calyx* = cup)	Norovirus: Norwalk virus	Winter vomiting and diarrhoea (cruise ship virus)
ssRNA (-ve sense)	Orthomyxovirus (*myxo* = mucin)	Influenza A, B, C	Influenza
	Paramyxovirus	Measles Mumps Parainfluenza 1−4 Respiratory syncytial virus	Respiratory and/or generalised infection. PI-3 major cause of respiratory disease in infants
	Bunyavirus	Rift Valley Fever Crimea-Congo haemorrhagic fever Hantavirus Sin nombre	Arboviruses: Replicate in arthropod host. Fever, pulmonary failure
	Rhabdovirus (*rhabdo* = bullet)	Rabies Bat Lyssavirus	Rabies
	Arenavirus (*Arena* = sand)	Lassa fever, Pichinde, Tacaribe	Infects rodents and passed to humans via contact with urine and faeces. Haemorrhagic fevers
	Filovirus	Ebola and Marburg	Haemorrhagic fevers. Very high mortality rate (>60%)
RNA genome replicating via DNA intermediate	Retrovirus	HIV, HTLV-1	AIDs, Adult T-cell leukaemia

Where a different genus of the same family is listed under "Example" the genus is underlined.

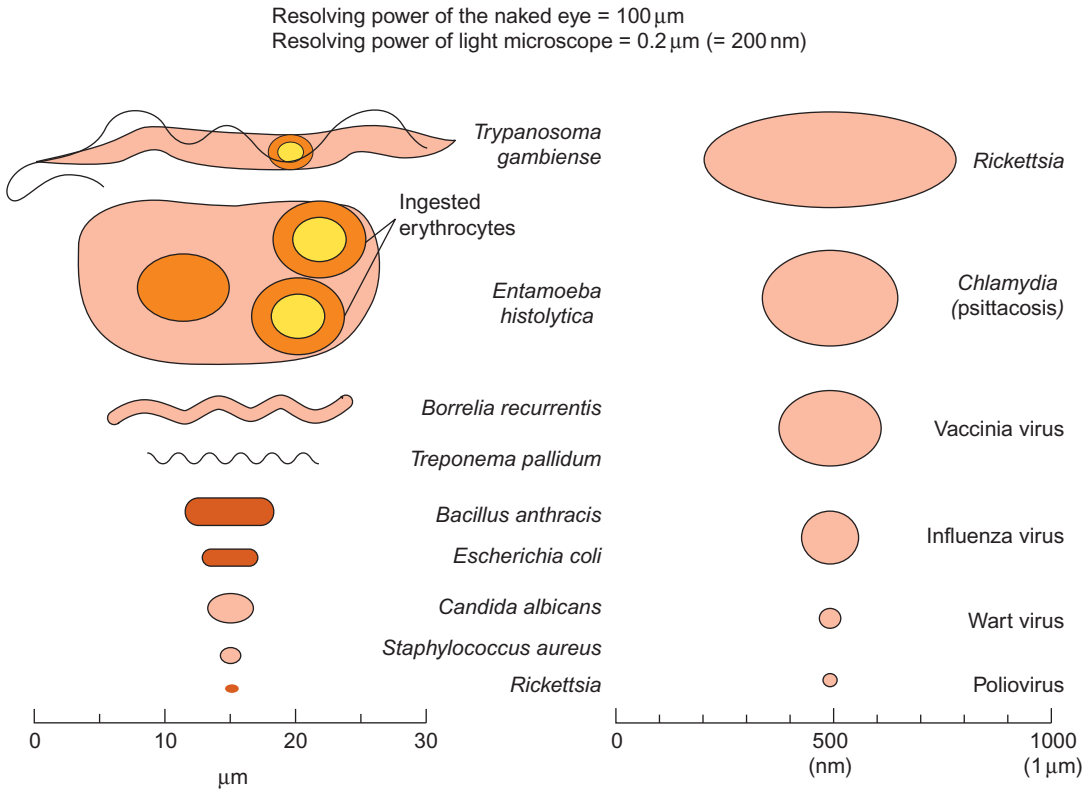

FIGURE A.1 Relative size of microorganisms.

Conclusions

One of the most important conclusions from this survey of microbial infection and pathogenicity is that various microorganisms have developed many strategies that enable them to overcome or by-pass host defences. Microorganisms evolve rapidly compared with their vertebrate host species and can generally be expected to be one step ahead. They are also quick to take advantage of changes in the host's way of life, and the comparatively recent increases in human population density, and global mobility, have been exploited, especially by the respiratory viruses. For similar reasons, the venereal route of infection arose from changes in human behaviour. Modern syphilis appears to have originated from an ancestral non-venereal spirochaete similar to yaws, which infected the skin in warm countries and was spread by contact. The venereal form arose in the towns and cities of temperate countries where skin-to-skin contact was reduced because people wore clothes, and mucosal spread offered greater possibilities.

Unless an infectious agent is transmitted effectively it will not survive. Transmission is an essential requirement. It is a striking feature of human infections acquired from arthropods and from animal reservoirs that few of them are transmitted sequentially from person to person. If human to human transmission occurs at all, it often fails to be maintained for more than a few transfers (Lassa fever, monkeypox). Infections of this type depend on human proximity to infected arthropods, birds, or mammals. They are often restricted geographically and they are eliminated when the source of infection is eliminated (e.g. rabies, malaria, yellow fever). In contrast, nearly all the infections that spread directly from person to person maintain themselves independently of arthropods and animals.

Transfer of microorganisms via urine, faeces and food has greatly decreased as a result of public health reforms, at least in developed countries, but aerosol and mucosal (kissing, venereal) transfer occurs with ever-increasing efficiency. Additionally, the increase in intravenous drug use has led to an increase in diseases spread via contaminated shared needles. The mucosal route of infection is more difficult to control. Mucosal contacts are part of loving and caring, which is at the core of man's humanity, and as long as people are people, microorganisms will have the opportunity to spread in this way.

Control of the spread of respiratory infections poses even greater problems. For the foreseeable future, they will predominate in our crowded communities. One person soon infects scores of others and new infections, such as pandemic strains of influenza, now spread throughout the world with formidable speed. SARS spread rapidly across the world from the initial outbreaks in China, due almost entirely to air travel. Likewise the 2009 swine flu outbreak spread across the world in only a few weeks. This is the type of infection that could present a major threat to the human species. Accordingly, it is

important to study more thoroughly the aspects of pathogenesis that are concerned with respiratory transmission and to identify the genetic determinants that would confer on a sexually or faecally transmitted microorganism the capacity to be transferred by aerosol. In fact, the pathogen factors required for effective transmission are poorly understood in general because of the paucity of good models of transmission. Also, because most of our new infections come from arthropods or animals, we need to know more about the pathogenic determinants that enable them to infect us and be transmitted between us. Since it first appeared in Africa some time before the 1960s, human immunodeficiency virus (HIV) has already changed and become more readily transmissible by the sexual route. Perhaps there is an unsurmountable pathogenic block to respiratory transmission, but in acquired immunodeficiency syndrome (AIDS) patients the virus is known to be present in alveolar macrophages and bronchoepithelial cells. A further short step into respiratory epithelium could convert HIV into a true major threat to our species.

The incidence of hepatitis C (previously called non-A, non-B hepatitis) has also risen dramatically in recent years. It is thought that the original, low efficiency route of infection was by sexual transmission, or via blood contamination during ritual procedures such as circumcision or scarification. Changes in modern lifestyles meant that recently the virus was spread more efficiently via blood transfusion (although screening has now stopped this route). The major route now appears to be via contaminated needles.

Because microorganisms can evolve so rapidly, there is a real possibility that a particularly unpleasant one could emerge at any time. For instance, there have been no influenza A pandemics of very great significance since the 1918 outbreak. If a new strain appeared that spread with great facility and was at the same time highly lethal, say by invading cardiac muscle to produce myocarditis, the human population could be decimated before vaccines controlled it or a more stable type of virus-host balance emerged. The appearance in the late 1990s of the highly pathogenic H5N1 influenza A virus was of great concern for two reasons: (1) it affected mainly younger individuals and (2) it appeared to spread directly from chickens to humans, a route not previously commonly seen. Thankfully human to human transmission of this virus is exceedingly inefficient. However, the potential for new, highly pathogenic and transmissible strains is worrisome. Developments in genomics have allowed examination of the emergence of new pathogen clones of bacterial species such as *S. aureus* and *E. coli* and we can now trace the origins of new strains and their subsequent spread both locally and on a global scale. Such studies may in the future assist us to estimate the emergence of new pathogens with capacity for harming human or animal health.

It is important to contrast the incidence of infectious disease in different parts of the world, and to remember the differences between the 'haves' and the 'have nots'. In developing countries, there are millions of unnecessary deaths from infection each year (see Table A.1), and these are due, directly or indirectly, to poverty. In developing countries, many of the old infections have been eliminated, but various latent, persistent, and opportunistic infections remain, especially in those kept alive by modern medicine. Globally, resistance to antibiotics is reaching crisis proportions, and it is widely accepted that alternatives are urgently needed.

Advances in technology made since publication of the last edition of this book have enabled a vastly improved understanding of pathogen biology and evolution, of how the

host responds to infection and how the pathogen manipulates that response. It is likely that the remarkable pace of discovery will continue in the coming years which promise further exciting developments in our understanding of infectious diseases. In turn it is hoped that the vast numbers of people who continue to suffer from infectious diseases will benefit from this knowledge through improved measures for control of infections.

Glossary

Active immunity Immunity acquired actively, following infection or immunisation by vaccines.

ADCC Antibody-dependent cell-mediated cytotoxicity.

Addison's disease Disease resulting from destruction of adrenal glands, characterised by weakness, debility and very great susceptibility to the stress of infection, trauma, etc. Other features include spontaneous hypoglycaemia and pigmentation.

Adjuvant A material that enhances the immune response to an antigen.

Agglutination Clumping together of proteins (in antigen–antibody reactions) or microorganisms, or red blood cells (haemagglutination).

Agonist Something that stimulates a biological response by occupying cell receptors.

Anamnestic response Secondary immune response (see Primed).

Anterior horn cells The main motor neurons in the anterior horn (as seen in cross-section) of the spinal cord, supplying striated muscle.

Antigen presentation Display on the cell surface of processed peptides in combination with MHC proteins.

Antigen processing Proteolytic digestion of a protein to form peptides, which combine with MHC proteins to be presented on the cell surface to specifically reactive T cells.

Antigenic determinant (=epitope) The small site on the antigen to which antibody attaches. Large antigens such as proteins carry several different antigenic determinants on the molecule, against which several different antibodies are formed.

Antigenic site A cluster of epitopes/antigenic determinants (q.v.).

Apoptosis Death of a cell in which it rounds up and is phagocytosed. It is not lysed. Involved in the natural regulation of cell numbers in tissues and occurs as the result of signals transmitted by adjoining cells. Is triggered by some viruses and inhibited by others.

Arthus response Inflammatory reaction formed at the site where antigen is given to an animal possessing precipitating antibody to that antigen. Characteristically, oedema, haemorrhage and necrosis appear after a few hours (immediate hypersensitivity), and complement, polymorphs and platelets are involved in the reaction.

Attenuated Reduced in virulence for a given host, often as a result of continued growth of a microorganism in an artificial host or culture system.

Autoimmunity Immunity (humoral or cell-mediated) to antigens of the body's own tissues. Can cause tissue damage and disease, but also occurs as a harmless consequence of tissue damage.

Avidity Refers to the strength of binding of antibodies to multivalent antigens. (Affinity is a more precisely used term referring to the strength of binding of one antibody combining site to a monovalent antigen.)

Bacterial cell wall Constitutes up to 20% dry weight of cell. Basically peptidoglycan (= mucopeptide = polymer of aminosugars cross-linked by peptide chains) containing components unique to microorganisms (e.g. muramic acid). Peptidoglycan may constitute nearly all of the cell wall (certain Gram-positive bacteria), sometimes with additional polysaccharides and teichoic acids. Gram-negative bacterial cell walls are mostly lipopolysaccharides and lipoproteins, with little mucopeptide.

Bacteriocin Complex bacteriocidal substance released by certain bacteria, active against related bacteria, e.g. colicins produced by *E. coli*; pyocins produced by *Pseudomonas aeruginosa*.

Basement membrane A sheet of material up to 0.2 μm thick lying immediately below epithelial (and endothelial) cells and supporting them. Contains glycoproteins and collagen and to some extent acts as a diffusion barrier for microorganisms. Thickness and structure varies in different parts of the body.

B cells Population of lymphoid cells derived from bone marrow, developing without the need for the thymus. Differentiate to form antibody-producing cells. Compose 10–20% circulating lymphocytes in man.

Capsid Protein coat enclosing the nucleic acid core of a virus.

CD (cluster differentiation) antigens Antigens on cell surfaces, serving various functions and used to identify different cell types (e.g. CD4 on helper T cells). More than 300 different CD antigens have been identified.

Cell-mediated immunity (CMI) Specific immunity mediated by and transferrable to other individuals by cells (T cells), not by serum.

Challenge Administration of antigen or pathogen to provoke an immune reaction, usually in a primed individual.

Chemokines Small molecular weight molecules acting as chemoattractants and activators of lymphocytes and macrophages. There are four families defined by the position of the first two cysteines in their sequence: CC (27 members), CXC (15 members), CX3C (1 member), and C (2 members). They bind specifically to a seven-transmembrane G-protein-coupled receptor, for which there exists an equally diverse family of chemokine receptors.

Coccus Spherical or ovoid bacterium.

Colicins See Bacteriocin.

Commensal (table companion) Associated with a host, often deriving nourishment from host, but neither beneficial nor harmful.

Complement An enzymic system of serum proteins, made up of nine components (C1–C9) that are sequentially activated in many antigen–antibody reactions. It is a unique cascade system in which a small triggering event is amplified into a large response. Complement can also be activated directly, without an initial antigen–antibody reaction; this is the alternate complement pathway. Complement is involved in immune lysis of bacteria, and of some viruses and other microorganisms. It plays a part in phagocytosis, opsonisation, chemotaxis and the inflammatory response.

Connective tissue Forms an all-pervading matrix, connecting and supporting muscles, nerves, blood vessels, etc. Consists of a muco-polysaccharide 'ground substance' containing cells (fibroblasts, histiocytes, etc.), collagen and elastic fibres.

C-reactive protein A protein with subunits of M_r 24,300 that happens to react with the C carbohydrate of the pneumococcus. It is synthesised in the liver and is detectable in the serum when inflammation or tissue necrosis has taken place. It binds to substances from microorganisms and damaged tissues, activating the complement system.

Cushing's syndrome A disease resulting from excessive secretion of hormones from the adrenal cortex. Patients show wasting of muscle and bone, fat deposits on face, neck and back, and small blood vessels are easily ruptured.

Cytokines A group of at least 20 proteins, including interferons and interleukins. They are the hormones of the immune system, mediating interactions between immune cells, and having pathological as well as protective actions on infectious diseases.

Defective virus replication Incomplete virus replication, with production only of viral nucleic acid, proteins or non-infectious virus particles.

Defensins Peptides present in tears and in phagocytes that act against and destroy many microbes (bacteria, viruses, fungi) by punching holes in outer membranes.

Delayed-type hypersensitivity (DTH) Hypersensitivity reaction visible 1–2 days after introduction (usually intradermally) of antigen into a sensitised individual. An expression of cell-mediated immunity (cf. Arthus reaction).

Dendritic cell A large, specialized antigen-presenting cell with long tree-like (dendritic) processes, present in lymphoid tissues; not phagocytic and does not bear Fc receptors. Similar to Langerhan's cell in skin.

Dorsal root ganglia A series of ganglia lying dorsal to the spinal cord (as seen in cross-section). Contain cell bodies of principal sensory neurons, each receiving impulses along fibres from skin, etc., and sending impulses along shorter fibres to spinal cord.

ELAM-1 Endothelial cell leucocyte adhesion molecule-1. (CD62E). Inflammatory mediator, binds to polymorphs, enabling them to stick to endothelial cells lining capillaries and venules.

Enanthem Lesions of mucosae (e.g. mouth, intestines) in virus infections (cf. Exanthem).

Endocytosis The uptake of material by the cell into membrane-lined vesicles in the cytoplasm. The term includes pinocytosis (uptake of fluids) and phagocytosis (uptake of particles).

Endotoxin Toxic component associated with cell wall or microorganism. Generally refers to lipopolysaccharide of Gram-negative bacilli, the toxic activity being due to lipid A.

Enterotoxin Toxin acting on intestinal tract.

Envelope Membrane of virus derived from infected host-cell membrane.

Exanthem Skin rash in virus infections (cf. Enanthem).

Exotoxin Toxin actively secreted (e.g. cholera toxin) or released by autolysis from microorganism (e.g. tetanus toxin).

Fimbriae (pili) Thread-like processes (not flagella) attached to cell walls of certain bacteria, often mediating attachment to host epithelial cell.

Fomites Comprehensive word for patients' bedding, clothes, towels, and other personal possessions that may transmit infections.

Genome The complete genetic material of an organism, e.g. chromosome and plasmids, etc.

Germinal centre A rounded aggregation of lymphocytes, lymphoblasts, dendritic cells and macrophages. Germinal centres develop in primary nodules (follicles) of lymphoid tissue in response to antigenic stimuli.

Gram-negative Losing the primary violet or blue during decolorisation in Gram's staining method. The method, developed by Hans Gram, a Danish physician, in 1884, gives a simple and convenient distinction between groups of bacteria. The staining reaction reflects differences in cell wall composition (see Figure 4.4), but the mechanism is not clear.

Gram-positive Retaining the primary violet or blue stain in Gram's method.

Granuloma A local accumulation of densely packed macrophages, often fusing to form giant cells, together with lymphocytes and plasma cells. Seen in chronic infections such as tuberculosis and syphilis.

Haemolysis Destruction of red blood cells. Caused by bacterial toxins or by the action of complement on red cells coated with specific antibody.

Hapten A small molecule which is antigenic (combines with antibody) but is not immunogenic, i.e. does not induce an immune response *in vivo* unless attached to a larger (carrier) molecule.

Heat shock proteins (hsp) A family of proteins that control the correct folding of other proteins, acting as 'molecular chaperones'. They are induced in both microbe and phagocyte during the stress of infection (e.g. raised body temperature); and also have immunological roles (e.g. in antigen-processing).

HLA see MHC.

Horizontal transmission The transmission of infection from individual to individual in a population rather than from parent to offspring.

Humoral immunity Specific immunity mediated by antibodies.

ICAM-1 Intercellular adhesion molecule-1.

Immune complex A complex of antigen with its specific antibody. Immune complexes may be soluble or insoluble, and may be formed in antibody excess, antigen excess, or with equivalent proportions of antibody and antigen. They may contain complement components.

Immune tolerance An immunologically specific reduction in immune responsiveness to a given antigen.

Immunopathology Pathological changes partly or completely caused by the immune response.

Infarction Obstruction of blood supply to a tissue or organ.

Inflammasomes Large intracellular multiprotein complexes that play a central role in innate immunity.

Integrin A large family of cell adhesion receptors (e.g. fibrinogen receptor, laminin receptor) expressed on many cell types, mediating adhesion of cells to each other or to extracellular components. After interaction of integrin with ligand, vital signals affecting differentiation, proliferation, etc. are transmitted to the cell interior.

Interferons Proteins produced in response to pathogens which mediate communication between host cells to stimulate immune protective responses for pathogen clearance.

Interleukins Cytokines; proteins (at least 35 in humans) that carry vital signals between different immune cells.

Interleukin-1 Produced by macrophages, promotes activation and mitosis of T and B cells. Causes fever as well as a variety of effects on muscle cells, fibroblasts and osteoblasts.

Interleukin-2 Produced by T (especially Th) cells; essential for the continued proliferation (clonal expansion) of activated T cells.

Interleukin-3 Multicolony stimulating factor; stimulates precursor cells (e.g. in bone marrow) to divide and form colonies of polymorphs, monocytes, etc.

In vitro 'In glass', that is to say not in a living animal or person.

In vivo In a living animal or person.

Kinins Low molecular weight peptides generated from precursors in plasma or tissues and functioning as important mediators of inflammatory responses. C2 kinin is derived from complement, and other kinins from α_2-globulins.

Latency Stage of persistent infection in which a microorganism causes no disease, but remains capable of activation and disease production.

LD_{50} (lethal dose 50) Dose that kills 50% of test animals/cells. A direct measure of virulence.

Legionellosis Infection with *Legionella pneumophila*. The bacteria colonise cooling towers, creeks, showerheads, air conditioning units, etc., and are inhaled after becoming airborne. Some patients develop pneumonia.

Leucocytes Circulating white blood cells. There are about 9,000 mm^{-3} in human blood, divided into granulocytes (polymorphs 68–70%, eosinophils 3%, basophils 0.5%) and mononuclear cells (monocytes 4%, lymphocytes 23–25%).

Lymphokine A cytokine released by primed lymphocyte on contact with specific antigen. Involved in signalling between immune cells. Important in CMI; cf. monokine (e.g. IL-1) produced by monocytes and macrophages.

Lysosome Cytoplasmic sac present in many cells, bounded by a lipoprotein membrane and containing various enzymes. Plays an important part in intracellular digestion.

Lysozyme An enzyme present in the granules of polymorphe, in macrophages, in tears, mucus, saliva and semen. It lyses certain bacteria, especially Gram-positive cocci, splitting the muramic acid-β-(1→4)-*N*-acetylglucosamine linkage in the bacterial cell wall. It potentiates the action of complement on these bacteria. Presumably lysozyme is not exclusively an antibacterial substance because large amounts are present in cartilage. It is present in glandular cells in the small intestine, especially in the Brazilian ant bear, where its chitinase-like activity may help with the digestion of insect skeletons.

Memory cells Sensitised cells generated during an immune response, and surviving in large enough numbers to give an accelerated immune response on challenge.

MHC (major histocompatibility complex) A region of the genome coding for immunologically important molecules.

Class I MHC molecules are HLA (human leucocyte antigen A, B, C) in man and H2 (K, D, L) in mice. They are associated with β_2 microglobulin and expressed on the surface of nearly all cells. They confer uniqueness on the cells of each individual and ideally the class I characteristics of donor and recipient should be matched for successful organ transplantation.

Class II MHC molecules (HLA-DP, DQ, DR in man; H-2 IA, IE in mice) are present on antigen-presenting cells (some macrophages, dendritic cells, Langerhans cells).

Micro RNA (miRNA) Small (21–24 nucleotides in length) non-coding RNAs, which are able to regulate gene expression at the post-transcriptional level.

Microarray A technique where thousands of short oligonucleotides of known sequence are immobilised on a solid substrate. This is often called a 'microarray chip'. The oligonucleotides (or clusters of these) are designed to be complementary to the mRNA of an individual gene and allows the measurement of expression levels of large numbers of genes simultaneously.

Monoclonal antibody A given B cell makes antibody of a certain class, avidity and specificity. Serum antibody consists of the separate contributions from tens of thousands of B cells. Dr Caesar Milstein discovered how to induce an individual B cell to divide and form a large enough population (clone) of cells to give bulk quantities of the unique antibody. This is a monoclonal antibody.

Natural antibodies Antibodies present in normal serum, reacting with a wide range of organisms. To a large extent they reflect specific responses to previous subclinical infections; for example, normal sera lyse many Gram-negative bacteria because of antibodies induced by the normal intestinal flora.

NF-κβ Nuclear factor κβ. Protein complex involved in cellular responses to a wide range of stimuli. Acts to stimulate specific gene expression. Plays a key role in regulating the immune response to infection.

Nosocomial infection An infection acquired in hospital.

Nucleocapsid Viral nucleic acid enclosed in a capsid consisting of repeating protein subunits.

Opsonin (Greek *opson*, a seasoning or sauce) Serum component that combines with antigen or the surface of a microorganism and promotes its phagocytosis by polymorphs or macrophages.

Otitis media Infection and inflammation of the middle ear.

PAMPs see Pathogen associated molecular patterns.

Passive immunity Transfer of preformed antibodies to non-immune individual by means of blood, serum components, etc., e.g. maternal antibodies transferred to foetus via placenta or milk, or immunoglobulins injected to prevent or modify infections.

Pattern recognition receptor (PRR) Proteins expressed by cells which recognise PAMPs of pathogens and act to stimulate the innate immune response.

Pathogen associated molecular patterns (PAMPs) Structural elements of microbes essential for their survival and recognised by PRRs.

Pathogenic Producing disease or pathological changes.

Persistent infection An infection in which the microorganism persists in the body, not necessarily in a fully infectious form, but often for long periods or throughout life.

Phage typing Traditional typing method whereby strains of *Salmonella typhi*, *Staphylococcus aureus*, or *Mycobacterium tuberculosis* can be distinguished on the basis of their different susceptibility to a battery of bacteriophages.

Pili see Fimbriae.

PKR Protein Kinase R. Plays a key role in the cellular response pathogens and other stressors. Activated by double-stranded RNA (dsRNA).

Plaque forming units (p.f.u.) A measure of the relative amount of infectious virus in a sample.

Plasma cell B cell which has differentiated to form rough surfaced (ribosome studded) endoplasmic reticulum with basophilic cytoplasm. It is the major antibody-producing cell.

Plasmid A small extrachromosomal piece of genetic material in bacterium, replicating autonomously in the cytoplasm. It may carry 50–100 genes. Plasmids are common in Gram-negative bacilli and also occur in staphylococci. The workhorse of molecular cloning.

Pleural and peritoneal cavities Potential cavities surrounding organs of thorax and abdomen. Lined by 'mesothelial' membrane and containing macrophages and other cells.

Polyclonal activator Something that activates many clones of lymphocytes. Infections that activate B cells in this way cause the formation of large amounts of circulating antibody directed against unknown antigens as well as against the infectious agent, and often against host tissue antigens.

Primary infection The first infection with a given microorganism.

Primed Exposed to antigen for the first time to give a primary immune response. Further contact with the same antigen leads to a secondary immune response.

Prion Infectious particle containing neither DNA nor RNA, consisting of host protein (Pr^{c}) that has been converted into a self-replicating form (Pr^{sc}). Thought to be the infectious particle causing scrapie, kuru, Creutzfeld-Jakob disease (CJD), bovine spongiform encephalopathy (BSE), etc.

Properdin system Consists of Factor A (a serum protein), Factor B (a β-glycoprotein) and properdin. Not completely defined and role not understood, but may have antibacterial and antiviral action. It is an alternative pathway for the activation of complement, in which C1, C2 and C4 are short-circuited.

Proteome The entire set of proteins expressed within a cell.

Proteomics This term covers a number of techniques used to investigate the proteome.

Pyogenic Causing production of pus.

Pyrogen A substance causing fever.

Pyroptosis A form of apoptosis associated with antimicrobial responses during inflammation.

Reservoir Animal (bird, mammal, mosquito, etc.) or animals in which a microorganism maintains itself independently of human infection.

Reticulocytosis Presence in blood of increased numbers of an early form of red cell (reticulocyte), due to increased rate of production in bone marrow.

Reticuloendothelial system A system of cells that take up particles and certain dyes injected into the body. Comprises Kupffer cells of liver, tissue histiocytes, monocytes, and the lymph node, splenic, alveolar, peritoneal and pleural macrophages.

Reverse vaccinology Approach for vaccine design based on predicting antigens from genome sequences.

RIG-I A key pattern recognition receptor which is involved in activation of innate immune responses.

RNASeq A set of techniques which allows the sequencing of all RNA molecules within a cell.

Schistosomiasis (=bilharzia) A disease with urinary symptoms common in many parts of Africa. Caused by the fluke (trematode) *Schistosoma haematobium*; larvae from infected snails enter water and penetrate human skin.

Shedding The liberation of microorganisms from the infected host.

SSPE (subacute sclerosing panencephalitis) A rare complication of infection with measles virus, occurring in about 1 per 100,000 cases. The incubation period of about 10 years classifies it as a 'slow' virus infection. Non-infectious mutant virus slowly spreads through the brain causing deterioration of brain function and death.

Stress Physical or mental disturbance severe enough to initiate a coordinated response originating in the cortex and hypothalamus, and involving either the autonomic nervous system or pituitary–adrenal axis. Catecholamines and corticosteroids are released in an attempt to counter the harmful systemic effects of the disturbance (or often the threatened disturbance in the case of mental stress).

Superantigen Exotoxin produced by some bacteria, esp. staphylococci and streptococci, which stimulate activation of large numbers of T-cells resulting in immune dysregulation and loss of T-cell function.

Symbiotic Living in a mutually beneficial association with the host.

Systemic infection Infection that spreads throughout the body.

T cells (T lymphocytes) Population of lymphoid cells whose development depends on the presence of the thymus. Responsible for cell-mediated immunity. Compose 75% circulating lymphocytes in man. Distinguished by having on their surface CD4 proteins or CD8 proteins which define their reactivity with cells bearing MHC I or II proteins, respectively. All helper T cells are CD4.

Teleology Doctrine that biological phenomena generally have a purpose, serving some function.

T-independent antigen Antigen that directly stimulates a B cell to form antibody without the need for a helper T cell. These antigens (e.g. polysaccharides) have repeated determinants that cross-link Ig receptors on B cells. The antibodies formed are mostly IgM.

Titre (1) A measure of units of antibody per unit volume of serum, usually quoted as a reciprocal of the last serum dilution, giving antibody-mediated reaction, e.g. 120. (2) Measure of units of virus per unit volume of fluid or tissue. Usually given in $\log_{10}$ units per ml or g, e.g. $10^{5.5}$ p.f.u. ml^{-1}.

TNF (tumour necrosis factor) A cytokine, first recognised as a product of activated macrophages. Plays a role in disease production as well as in host defence.

Toxoid Toxin rendered harmless but still capable of acting as antigen.

Transcriptome The entire set of RNAs expressed within a cell (see RNAseq, microarray).

Transformation A change in the behaviour of a cell, for instance after infection with an oncogenic virus, so that it acquires the properties of a cancer cell. Transformed cells undergo continued mitosis so that the cells in a monolayer are not inhibited from growth by contact with neighbouring cells, and continue to multiply and form a heap of cells. The word also refers to changes in a lymphocyte associated with onset of division.

Tuberculin test A skin test for delayed hypersensitivity to antigens from *Mycobacterium tuberculosis.* In man the antigen is introduced into the skin by intradermal injections (Mantoux test) or by multiple puncture (Heaf test and tine test).

Vector As used in this book, the word refers to an arthropod that carries and transfers an infectious agent. Quite separately, a vector means a replicating genetic unit such as a virus or a plasmid, which will carry and replicate a segment of foreign DNA that has been introduced into it.

Vertical transmission The transmission of infection directly from parent to offspring. This can take place *in utero* via egg, sperm, placenta, during birth (contact with infected birth canal), or postnatally via milk, blood, contact.

Viraemia Presence of virus in the bloodstream. Virus may be associated with leucocytes (leucocyte viraemia) or free in the plasma (plasma viraemia).

Virion The complete virus particle.

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