OKLAHOMA NOTES

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Microbiology & Immunology

Richard M. Hyde

Oklahoma Notes

Basic-Sciences Review for Medical Licensure
Developed at
The University of Oklahoma at Oklahoma City, College of Medicine

Suitable Reviews for:
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Preface to the Oklahoma Notes

In 1973, the University of Oklahoma College of Medicine instituted a requirement for passage of the Part I National Boards for promotion to the third year. To assist students in preparation for this examination, a two-week review of the basic sciences was added to the curriculum in 1975. Ten review texts were written by the faculty: four in anatomical sciences and one each in the other six basic sciences. Self-instructional quizzes were also developed by each discipline and administered during the review period.

The first year the course was instituted the Total Score performance on National Boards Part I increased 60 points, with the relative standing of the school changing from 56th to 9th in the nation. The performance of the class has remained near the national candidate mean (500) since then, with a mean over the 12 years of 502 and a range of 467 to 537. This improvement in our own students' performance has been documented (Hyde et al: Performance on NBME Part I examination in relation to policies regarding use of test. J. Med. Educ. 60:439–443, 1985).

A questionnaire was administered to one of the classes after they had completed the boards; 82% rated the review books as the most beneficial part of the course. These texts have been recently updated and rewritten and are now available for use by all students of medicine who are preparing for comprehensive examinations in the Basic Medical Sciences.

RICHARD M. HYDE, Ph.D.
Executive Editor

PREFACE

The material in this text was compiled to serve as a study guide for a review of microbiology and immunology suitable for preparing for Part I of the National Board of Medical Examiners (NBME) exam. I have assumed that you, the reader, have had a comprehensive course covering this discipline. In-depth presentation of material will not be found in this review: You are urged to consult other study aids (lecture notes, textbooks, etc.) for detailed explanations of material that you find troublesome.

In general, the text of the book is on the left side of each page; questions, illustrations, summary sentences or phrases, and other study aids are on the right. This format has the intent of getting you involved in the review process. Use a highlighter, put boxes around key statements, answer the questions, and fill in the blanks as you work through the book. Your reward will be proportional to your effort (i.e., no pain, no gain).

There are five proficiency examinations in this book, one for each major area of coverage. In addition, a comprehensive examination will be found at the end of the book. Performance data for each is given to help you evaluate your own preparedness. Some questions may cover material not detailed in the book: Be sure that you know the answer to these questions as well, since this is just another form of review.

Contributions by the following individuals are gratefully acknowledged: Drs. G. S. Bulmer, J. J. Ferretti, D. C. Graves, M. H. Ivey, M. P. Lerner, L. V. Scott, J. R. Sokatch, and C. Weeks, and Ms. Dawn H. Struthers (artwork).

R.M.H.

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REVIEW OF MICROBIAL PHYSIOLOGY

Definitions

Autotrophs - organisms which are able to use CO_2 as the sole carbon source.

Axial filament - proteinaceous organelles of spirochetal forms.

Bacterial cell wall - barrier against osmotic lysis; composed of peptidoglycan inner layer and various surface layers which differ in the different bacteria.

Capsule - usually carbohydrate layer outside the cell wall of many bacteria - anti-phagocytic function.

Cell membrane - bilayered structure inside bacterial cell wall. Contains enzymes responsible for synthesis of cell wall, etc., as well as transport of solutes and oxidative phosphorylation.

Enterochelins - iron chelating compounds found in enteric bacteria (e.g., E. coli, Salmonella) which are important in survival in vivo as they permit the organism to accumulate ferrous ions essential for growth.

Flagella - proteinaceous organelles of motility in rod-shaped bacteria.

Heterotrophs - organisms that require the major portion of their carbon from organic sources.

Metachromatic granules - polymetaphosphate inclusions found in many bacteria. Their presence is useful in identification of the diphtheria bacillus.

Mesosomes - irregular convoluted invaginations of the cell membrane-functions include a role in DNA replication, and secretion.

Pili - proteinaceous appendages on gram negative bacteria; function in conjugation and/or adherence to host cell membranes.

Cell Wall

The cell wall of bacteria protects the cell against osmotic lysis. Cell walls of Gram positive and Gram negative bacteria both have peptidoglycan (mucopeptide) as the innermost layer of the cell wall. They differ in the nature of the surface layers.

The cell wall of Gram positive bacteria contains from 40-90% peptidoglycan, while cell walls of Gram negative bacteria contains only 5-10% peptidoglycan. In Gram positives, the next layer is carbohydrate, composed of ribitol teichoic acid, and the outermost layer of the cell wall is composed of two or three kinds of protein. The lipoteichoic acids of group A streptococcal cell walls are involved in the organism's adherence to epithelial cells.

Cell walls of Gram negative enteric bacteria are composed of an outer layer of lipoprotein-lipopolysaccharide (endotoxin), a middle layer of protein, and an innermost layer of peptidoglycan. The cell membrane, which is not a part of the cell wall, appears as a double layered structure immediately below the cell wall.

The basic unit of peptidoglycan is a disaccharide-tetrapeptide containing N-acetylmuramic acid, N-acetylglucosamine, D-alanine, L-alanine, D-glutamic acid or its derivative, D-isoglutamine and a basic amino acid, usually diaminopimelic acid. The basic units of mucopeptide are cross-linked to each other to form a tight meshwork which surrounds and protects the entire cell.

Lipopolysaccharides are composed of lipid A, core and O-antigen. Core is subdivided into inner core, or backbone, and outer core.

Complete the table below:

Gram Negative Cell Wall Zone	Chemical Component
Outer Membrane	_____
	_____
	_____
Middle Layer	_____
Peptidoglycan	_____
Cytoplasmic membrane	_____

Porins are "holes" in the outer membrane portion of the cell wall of Gram negatives which allow free diffusion of molecules less than 600 daltons through the membrane. They also serve as attachment sites for phage.

The mucopeptidase, lysozyme, hydrolyzes the linkage between N-acetylmuramic acid and N-acetyl glucosamine causing

1. the peptidoglycan layer to disappear.
2. the cell to become an osmotically-fragile.
3. Both
4. Neither

(Answer on next page)

Cell Membranes

The ultrastructural appearance of membranes is bilayered, i.e., structures with the lipid oriented so that the non-polar (fat soluble) fatty acid side chains face the interior and the polar (water soluble) glycerol esters face the exterior of the membrane. Proteins are sandwiched between phospholipid molecules.

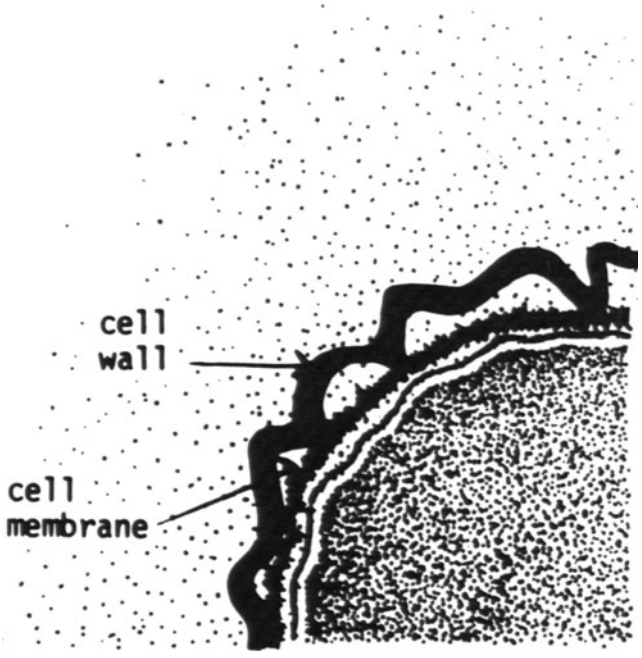
Isolated cell membranes of both Gram positive and Gram negative bacteria are approximately one-third lipid, mostly phospholipid, and two-thirds protein; occasionally polysaccharide is also attached to the membrane. There are at least three kinds of proteins associated with membranes of bacteria;
1) biosynthetic enzymes which are responsible for the synthesis of the external layers of the cell, in particular the membrane, cell wall and capsule,
2) transport enzymes responsible for the transport of water-soluble materials from the medium into the cell, and
3) the cytochrome enzymes and other enzymes of the electron transport system.

The cell membrane is a living, biologically active structure; it acts as a permeability barrier. The lipid bilayer acts as a barrier to the passage of water-soluble chemicals. A second function of the cell membrane is to serve as a site for synthesis of peptidoglycan, lipopolysaccharide and capsule. The third important function of the cell membrane is to serve as the site of electron transport and oxidative phosphorylation in aerobic and facultative bacteria.

Mesosomes

Mesosomes are invaginations of the membrane which appear to be enclosed in a sac. They are similar in chemical composition to the membrane but are low in enzymes associated with the electron transport system. Septal mesosomes appear to be involved in cell division (they are associated with DNA). Lateral mesosomes function in secretion.

The outer membrane of the cell wall of Gram negatives is somewhat selective, and is not as permeable to antibiotics as is the cell wall of Gram positives. Hence, the former organisms have become more important in human medicine during the antibiotic era.

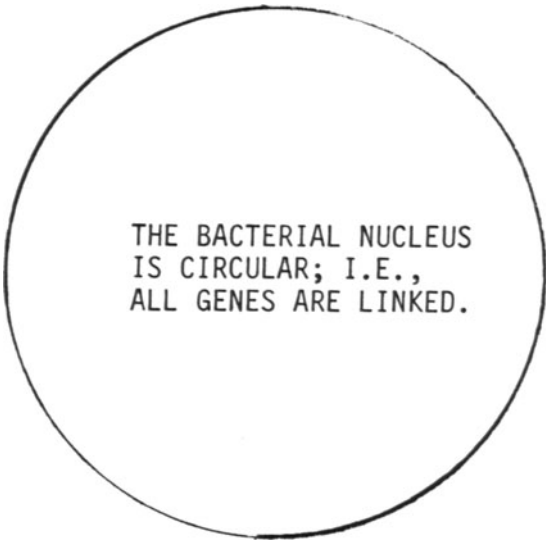


Answer to question about lysozyme's effect on bacterial cell wall = 3.

INTRACYTOPLASMIC STRUCTURES

Bacterial Nucleus (Nucleoid)

The double-stranded DNA nucleus of bacteria is prokaryotic. There is no evidence of any kind of organization such as that which occurs in eukaryotic nuclei. The bacterial nucleus is usually attached to the membrane or to the mesosome. It is composed of a single, circular chromosome.



Ribosomes

Ribosomes are the only structural organelle in the bacterial cytoplasm. They are numerous, and mostly grouped in chains (polysomes).

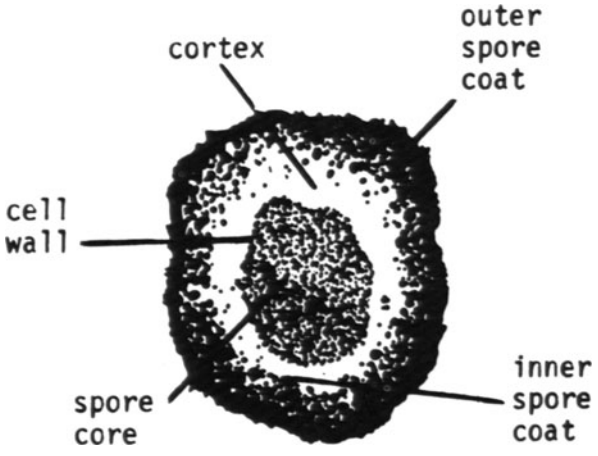
Cytoplasmic Inclusions

Several species of bacteria form lipid inclusions which appear to be a source of reserve energy for the organism. Metachromatic granules are thought to be composed of polymetaphosphate. The function of metachromatic granules is not clear but may be related in some fashion to energy metabolism. Starch or glycogen granules have also been detected in bacteria.

Prokaryotes do not have basic proteins (e.g. histones) associated with the DNA. They also lack a nuclear membrane.

Spores

Bacterial spores are dormant forms of the species whose function is survival under adverse conditions. Most spore-forming bacteria are Gram positive rods in the family Bacillaceae. They are composed of a bacterial nucleoid surrounded by a cell membrane and several layers known as the outer and inner coats which appear to be composed of highly stable proteins such as keratin, possibly with some phospholipoprotein containing Ca^{++} dipicolinate. The mature spore is a dormant organism characterized by a very low metabolic rate. Spores are highly resistant to heat, light, desiccation, and other deleterious agents.



EXTERIOR STRUCTURES

Flagella

Flagella, the organelles responsible for motility of bacteria, are located either at the ends of the cell (polar flagellation) or over the entire surface (peritrichous flagellation). There are three parts of the flagellum; filament, hook and basal body. The flagella filament is composed of an elastic protein named flagellin. These are the H antigens of motile bacteria.

Axial Filaments

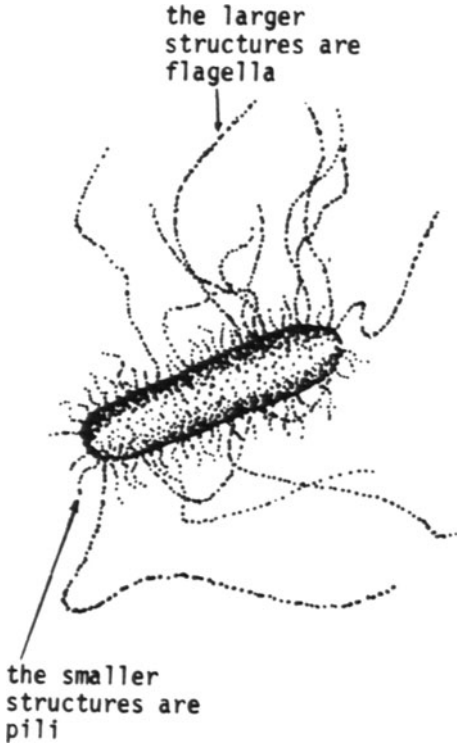
The organelles responsible for motility of spirochetes are called axial filaments. They are composed of protein and have a hook at the proximal end which is attached to the cell.

Pili (Fimbriae)

Pili are short, hair-like, protein structures which occur on a large number of Gram negative species. Host cell selectivity may be directed by pili, e.g., the pili of the gonococcus has an affinity for the columnar epithelium of the urethra.

The sex pilus is found only on male strains of bacteria which are capable of donating their DNA by conjugation. These pili are usually named after the fertility agent carried by the strains such as F-pilus and Hfr pilus. DNA of the donor is transported to the recipient (the female cell) via the tubule of the sex pilus.

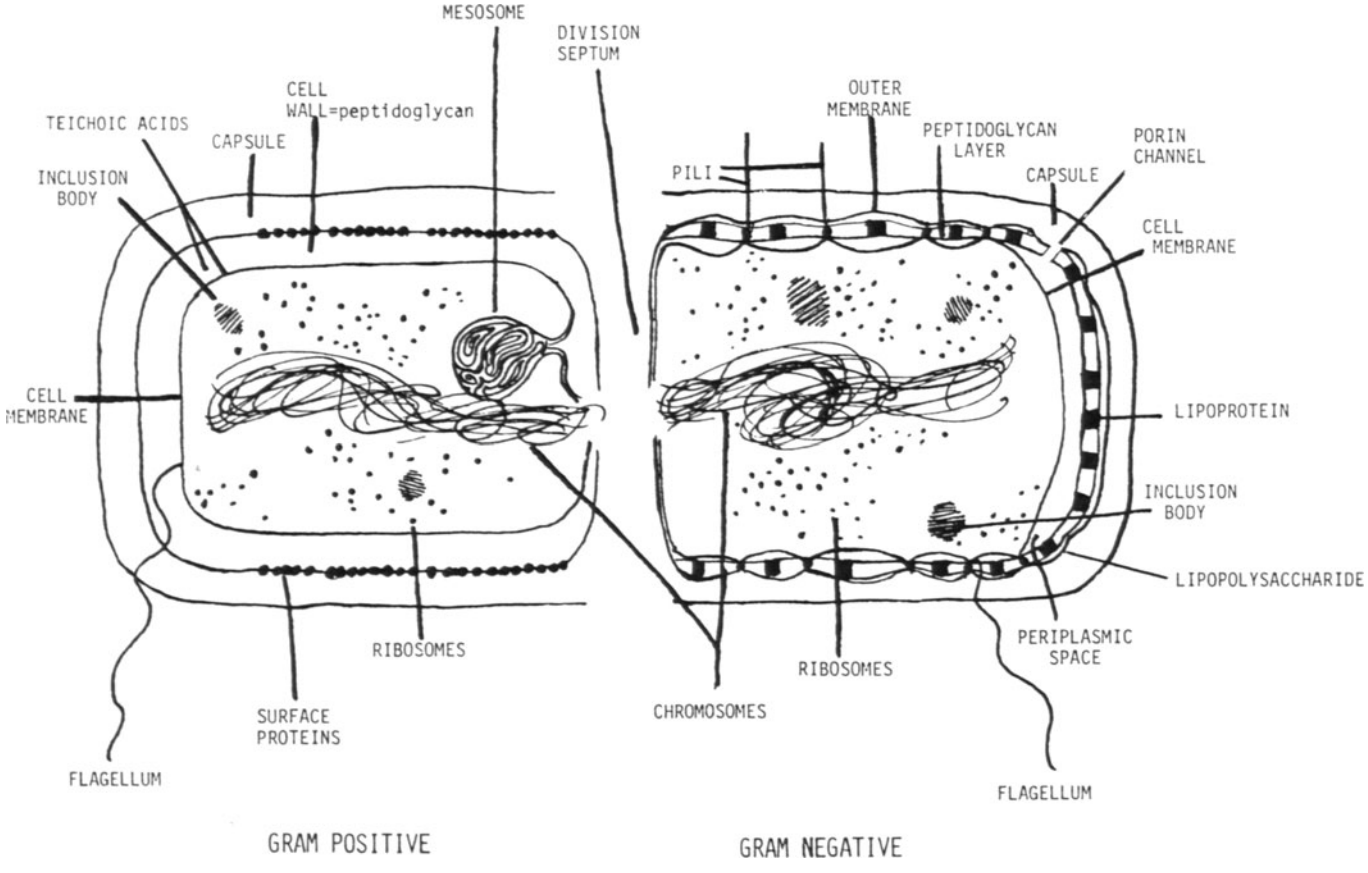
Bacterial chemotaxis occurs when toxins, (e.g. sugars) react with chemoreceptors in the membrane or periplasmic space. Methyl-accepting proteins relay signals from the receptors to the flagellar apparatus. S-adenosyl methionine serves as the methyl donor. The signal influences flagellar rotation such that the organism moves toward, or away from, the taxin depending upon the direction of rotation of the flagella.



Capsules

Capsules of most species of bacteria are polysaccharide, but in the genus Bacillus, there are capsules of poly-D-glutamic acid. Polysaccharide capsules vary from relatively simple structures such as hyaluronic acid which is a linear polysaccharide composed of N-acetylglucosamine and glucuronic acid, to the highly branched polysaccharides formed by Streptococcus pneumoniae. Although there are usually no more than three or four sugars in capsules of Streptococcus pneumoniae, they are branched polysaccharides and are unique enough that specific antisera to the capsules can be formed. Some 80 strains of Streptococcus pneumoniae have been identified on the basis of the antigenic properties of their capsules.

Capsules of certain species of bacteria inhibit phagocytosis and thereby enhance the ability of the organism to establish an infection, i.e., they are antiphagocytic virulence factors.



GROWTH OF BACTERIA

Source of Carbon

Autotrophs are organisms which are able to use carbon dioxide as the sole carbon source. Heterotrophs require the major portion of their carbon in the form of organic carbon although almost all heterotrophs require some carbon dioxide.

Microorganisms which are pathogenic for man are heterotrophs/autotrophs.

Physical Requirements for Growth

The temperature for optimum growth of most bacteria is between 20-40 C; these are mesophiles. Bacteria which grow in association with warm blooded animals have optimum growth temperatures in the vicinity of 35-40 C.

Microorganisms which are pathogenic for man are mesophiles/psychrophiles.

The growth rate is affected by the osmotic pressure of the medium. Most bacteria are able to tolerate 1-2% salt, but the growth drops off rapidly as the salt concentration increases above this level. Haloduric organisms (e.g., S. aureus) can grow in the presence of high salt concentrations.

Certain pathogenic microorganisms are haloduric; this fact is used in the design of selective media for their isolation from clinical specimens. Name a medium used for Staphylococcus aureus:

_____

The effect of pH on the growth of bacteria is as might be predicted - bacteria grow best at pH values near neutrality.

Bacteria which require oxygen for growth are aerobes; strict aerobes will grow only in the presence of oxygen. Bacteria which grow in the absence of oxygen are anaerobes. Strict anaerobes are unable to grow in the presence of oxygen and are apparently poisoned by it; they lack a functional electron transport system and are unable to produce energy by oxidative phosphorylation. They also lack catalase and superoxide dismutase.
Most pathogenic bacteria are facultative anaerobes; they can grow with or without oxygen.

What is the genus name of a strict anaerobe which is a sporeformer?

_____

What is the genus name of a strict anaerobe which predominates in the gut?

_____

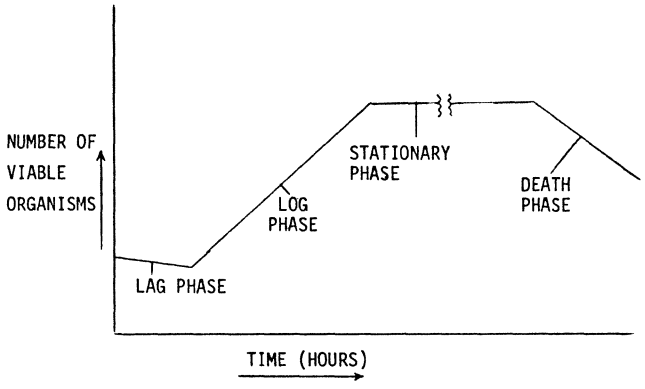
(See the Pathogenic Bacteriology section for answers to the above)

Measurement of Bacterial Populations

Bacteria reproduce by binary fission, an asexual process which results in two genetically identical daughter cells. Each division is a generation and it follows that each generation leads to a doubling in cell count. The growth rate is the number of generations per unit time and is obtained by dividing the number of generations by the time interval required to increase the cell count to that level. The generation time is the time required for one generation.

Phases of Growth

There are four distinct phases of growth. The first phase of the growth is the lag phase. The second phase is the exponential or logarithmic phase of growth. During this phase, total and viable counts are very nearly equal. In the third phase of growth, the stationary phase, growth has stopped. Since growth is an exponential increase of protoplasm, the last generation will use as much as one half of the nutrients in the medium which means that the medium suddenly becomes unable to support growth. Usually, it is the energy source which becomes limiting, but it can also be a vitamin or amino acid which is necessary for growth. Growth may also stop because of an increase in toxic products such as acid.



A fourth phase of the growth curve, the death phase, can occur. There is a decline in the population of bacteria. The medium is no longer able to support growth and the cell cannot maintain life indefinitely.

BACTERIAL METABOLISM

Exoenzymes

Bacteria frequently excrete enzymes which digest large, insoluble molecules into small, soluble molecules which can pass through the cell membrane.

Transport

Kinetics - pinocytosis does not occur in bacteria and nutrients must be transported into the cell in a soluble form. Velocity of transport follow kinetics described by the Michaelis-Menten equation for enzyme reactions.

Characteristics - Bacteria use facilitated diffusion, active transport, and group translocation to transport substrates. The stereoisomer which is biologically active is selectively transported. Transport systems responsible for transport of substrates which are catabolized are usually inducible (except glucose which is constituitive.) Active transport and group translocation require energy in order to concentrate substrate inside the cell and, in the case of group translocation, to phosphorylate the substrate. Transport proteins are located in the cell membrane. Facilitated diffusion and active transport are accomplished by binding proteins which reversibly, but selectively, absorb substrate from solution.

Transport of Amino Acids occurs by active transport without chemical modification. One system may be responsible for the transport of more than one amino acid.

Transport of sugars occurs mainly by active transport and group translocation and occasionally by facilitated diffusion. Group translocation occurs only with sugars and is affected by the phosphotransferase system.

Gases (such as oxygen) water, and some ions (such as sodium) are transported into the cell by passive diffusion.

Porin channels formed by protein trimers in the outer membrane of Gram negative cell walls allow passive diffusion of molecules < 600 daltons (trisaccharides or tetrapeptides).

The four types of transport seen in bacteria are

1. _____
2. _____
3. _____
4. _____

Which of the above

- require energy?
- require a specific binding protein?
- is involved in amino acid transport?
- is involved in sodium transport?

ENERGY METABOLISM

Fermentation

Fermentation is the anaerobic metabolism of a substrate. The Embden-Meyerhof pathway is the most common pathway used for sugar fermentation. Energy is mobilized during fermentation in phosphoryl groups of molecules such as adenosine triphosphate and phosphenol-pyruvate. Some anaerobic and facultative bacteria also ferment amino acids for energy.

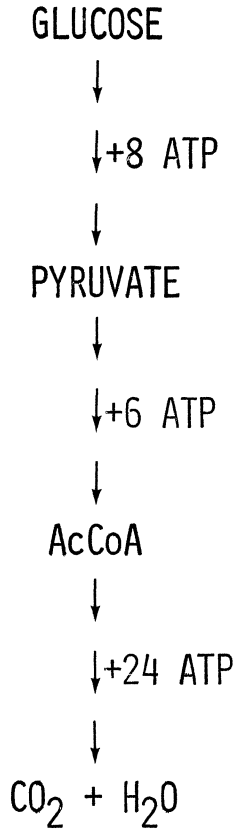
Aerobic Energy Metabolism

The reduction of oxygen to water by NADH is a rich source of energy which takes place in aerobic and facultative bacteria through the action of the electron transport system. Some of the energy is trapped by oxidative phosphorylation and the rest is lost as heat.

The electron transport system of bacteria is located in the cell membrane and is composed of cytochrome enzymes, lipid cofactors such as vitamin K and coenzyme Q, and coupling factors, the latter being involved in oxidative phosphorylation.

Aerobic Carbohydrate Metabolism

The most common mechanism for aerobic metabolism of carbohydrates in bacteria is a combination of the Embden-Meyerhof pathway and the tricarboxylic acid cycle. The latter is used for the aerobic oxidation of products of the metabolism of carbohydrates, amino acids and lipids. Hexoses are oxidized to pyruvate, which is oxidized to acetyl-CoA which then enters the tricarboxylic acid cycle by condensation with oxaloacetic acid to citrate. Fatty acids formed by the hydrolysis of triglycerides are also oxidized to acetyl-CoA. Amino acids may be oxidized to pyruvate, acetyl-CoA, oxaloacetate, fumarate or succinate.



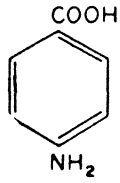
Which type of metabolism produces more energy: aerobic or anaerobic?

How much more?

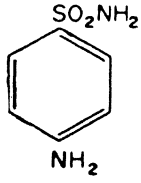
Sulfonamides

Action

Sulfonamides are bacteristatic, that is, they inhibit growth but do not kill; these drugs depend on the immune system of the host to remove and kill the infecting bacteria. Para-aminobenzoic acid and many other natural products such as thymine, purines, serine and methionine may overcome the action of sulfa drugs, thus sulfonamides are often found to be ineffective in sites of extensive tissue destruction.
Sulfonamides inhibit the condensation of 2-amino-4-hydroxy-6-dihydropteridinyl-pyrophosphate with para-aminobenzoic acid. Sulfonamide condenses with pteridine pyrophosphate forming an analogue of dihydropteroic acid. They act as allosteric inhibitors of dihydropteroate synthase. The drug is most effective against those bacteria which are able to synthesize their own folic acid.

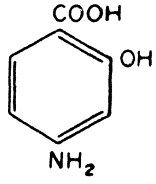


Para-aminobenzoic acid



Sulfanilamide

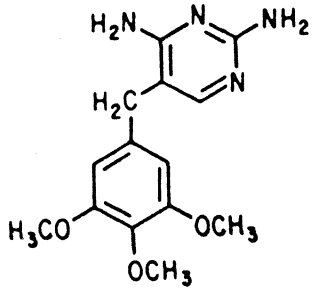
The low toxicity of sulfonamides for man is understandable since man is unable to synthesize folic acid. The concentration of folic acid in tissue is either too low to reverse the action of sulfonamides or sulfa sensitive bacteria are impermeable to folic acid.



Para-aminosalicylic acid

Resistance

The most frequently observed naturally occurring resistance to sulfonamides is associated with the presence of an R factor. Resistance appears to be due to the production of an altered dihydropteroate synthase.



Trimethoprim

Clinical Use

Sulfonamides are used for urinary tract infections, some upper respiratory tract infections, for shigellosis, and for trachoma and inclusion conjunctivitis. They are also used in combination with a dihydrofolate reductase inhibitor, trimethoprim, which gives enhancement of antibacterial action.

Other Antimetabolites

Para-aminosalicylic acid is also an analogue of para-aminobenzoic acid with many actions similar to sulfonamide. Para-aminosalicylic acid is bacteristatic and inhibits the condensation of 2-amino-4-hydroxy-6-dihydropteridinyl-pyrophosphate and para-aminobenzoic acid. The action of para-aminosalicylic is also reversed by para-aminobenzoic acid. The most important use of para-aminosalicylic acid is for the treatment of tuberculosis.

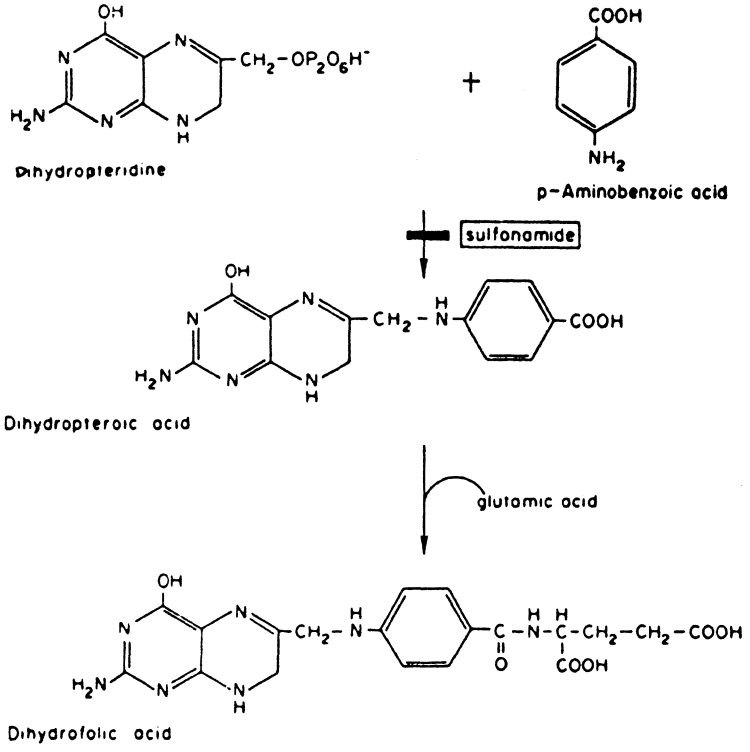
Isoniazid is a bactericidal agent which is also used for treatment of tuberculosis, frequently in combination with para-aminosalicylic acid. The mode of action is isoniazid is to inhibit synthesis of mycolic acids, an important component of the mycobacterial cell wall.

Sulfone derivatives such as diaminodiphenylsulfone (Dapsone) have been the drugs of choice for treatment of leprosy, however, recently rifamycin has been used with promising results.

Which amtimetabolite(s)
- inhibit dihydrofolate reductase?

- dihydropteroic acid synthase?

- inhibit mycolic acid synthesis?

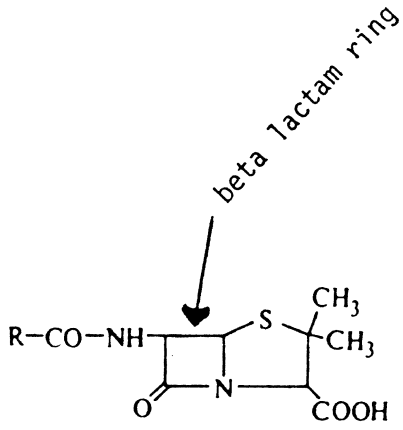


CHEMOTHERAPEUTIC AGENTS - ANTIBIOTICS

INHIBITORS OF CELL WALL SYNTHESIS

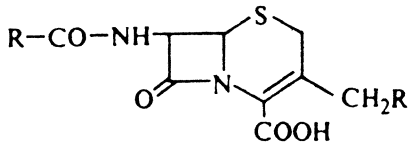
Penicillins and Cephalosporins

Structure
These antibiotics have a similar chemical structure, the common element being the β-lactam ring. They also have a similar mode of action. Benzylpenicillin, or penicillin G, has the disadvantages that it is hydrolyzed by acid, which limits its oral use, and is inactivated by penicillinase. Semisynthetic penicillins have substituted acyl groups which make them stable to acid, resistant to penicillinase, or both.



Penicillins

Mode of action.
These are bactericidal agents which inhibit peptidoglycan synthesis. Their molecular configuration is similar to that of the D-alanyl-D-alanine terminus of the pentapeptide side chain and they react with the transpeptidase forming an inactive complex.



Cephalosporins

Resistance
Bacterial resistance to penicillins is usually the result of a β-lactamase whose production is governed by a plasmid. β-Lactamases hydrolyze the β-lactam ring. Penicillinases are active against penicillins but relatively inactive against cephalosporins.

Clinical Use
Benzylpenicillin is used for infections caused by bacteria which are not resistant to penicillins, in particular, infections caused by streptococci, diplococci, Neisseria and Treponema. Methicillin, and oxacillin are used for infections caused by bacteria which form penicillinase. Oxacillin has the advantage that it can be used orally. Ampicillin and Carbenicillin are more effective against Gram negative bacteria than are the other penicillins. The cepholosporins are used mostly against penicillin-resistant staphylococci since penicillinase from these organisms does not inactivate cepholosporins. Cephalosporins can also be used in patients allergic to penicillins.

Resistance to penicillin is usually the result of the production of an enzyme,

_____,
the production of which is governed by an independently replicating genetic unit known as a

_____.

Other Inhibitors of Wall Formation

D-Cycloserine owes its antibacterial action to the fact that it is an analogue of D-alanine and competes with it for transport by the D-alanine-glycine transport system. D-Cycloserine is a competitive inhibitor of the alanine racemase and D-alanyl-D-alanine synthetase, both of which are important enzymes in the synthesis of peptidoglycan. The most important use of cycloserine is as a second line drug in the treatment of tuberculosis.

D-cycloserine is a competitive inhibitor of two enzymes, _____, and _____, both of which are important in _____ _____ synthesis.

Vancomycin is an antibiotic which inhibits the transfer of the disaccharide-pentapeptide from the phospholipid carrier to the cell wall acceptor. It binds to D-alanyl-D-alanine. Vancomycin is more active against Gram positive bacteria than Gram negative bacteria.

Vancomycin inhibits the transfer of disaccharide-pentapeptide from the _____ carrier to the _____ acceptor.

Bacitracin is a polypeptide antibiotic produced by Bacillus species which is active against Gram positive bacteria and Neisseria. Bacitracin is used only topically. Bacitracin inhibits the hydrolysis of lipid pyrophosphate to lipid phosphate thereby preventing its reuse in mucopeptide synthesis. It inhibits production of monophosphate carrier protein.

ANTIBIOTICS WHICH INHIBIT PEPTIDOGLYCAN SYNTHESIS (BACTERICIDAL ACTION)

CYTOPLASM	MEMBRANE	CELL WALL
D cycloserine a competitive inhibitor of alanine racemase and D-alanyl-D-alanine synthetase.	Bacitracin inhibits the lysis of lipid pyrophosphate thereby limiting available substrate for mucopeptide synthesis.	Penicillins and Cephalosporins react with transpeptidase forming an inactive complex Vancomycin inhibits transfer of disaccharid pentapeptide to the cell wall.

CELL MEMBRANE INHIBITORS

These antibiotics interact with the membrane of the cell and alter its osmotic properties. The membrane becomes "leaky" and allows the escape of potassium ions and vital metabolites. They are also able to react with mammalian cell membranes, and hence are quite toxic.

Microbial and Mammalian membranes are quite similar, hence drugs which affect their antibacterial action via membrane action are likely to be toxic. They cause _____ _____.

Polymyxins are a family of decapeptides. They are active against gram negative bacteria only. Because of the extreme toxicity, they are used primarily in the treatment of serious Pseudomonas infections.

Polymyxin B is used in the treatment of infections caused by _____.

Polyenes are macrolide antibiotics. The two most important are nystatin and amphotericin B. These agents selectively inhibit organisms that have sterols in their membranes, hence they are active against the FUNGI but have no toxicity for prokaryotic forms such as bacteria due to the absence of sterols in the bacterial cell membrane. They disrupt the integrity of the sterol containing cell membrane.

Amphotericin B reacts with fungal membranes due to their content of _____.

Nystatin is highly toxic and is only used for topical fungal infections (e.g., candidiasis). Amphotericin B is used parenterally; nephrotoxicity is a major complication of its use.

Imidazoles are synthetic agents which exhibit anti-fungal activity. Miconazole and ketoconazole are clinically the most useful. The former is used topically or intravenously; ketoconazole is effective when administered orally. Both of these compounds interfere with ergosterol synthesis.

Ketoconazole interferes with the synthesis of _____.

REVIEW

Penicillin and other inhibitors of cell wall formation are bactericidal/bacteristatic?

Antifungal antibiotics include _____, _____, and _____.

Antifungal imidazole compounds include _____, and _____, both of which interfere with the synthesis of _____.

INHIBITORS OF PROTEIN SYNTHESIS

Streptomycin

Structure
Streptomycin is an aminoglycoside antibiotic.

Action
Streptomycin is a bactericidal drug with several effects on growing bacteria. The lethal effect seems to be a result of its inhibition of protein synthesis by preventing initiation. Streptomycin binds to the 30S ribosome; the binding site has been identified as a ribosomal protein designated S12.

Resistance
Resistance to streptomycin is associated with the presence of an R factor (plasmid). There are two enzymes which cause a chemical modification of streptomycin making it ineffective as an antibiotic. Streptomycin-spectinomycin adenyl transferase catalyzes the transfer of the adenyl portion of adenosine triphosphate to the 3' hydroxyl of N-methyl-L-glucosamine. The other streptomycin-modifying enzyme is streptomycin phosphotransferase which also selects the 3' hydroxyl of N-methyl-L-glucosamine as its site of action.

Resistance to streptomycin is due to enzymes produces by the organism, namely _____, or _____.

Clinical use
Streptomycin is bactericidal for most Gram negative bacilli but not for most Gram positive bacteria. It is a first line drug for the treatment of tuberculosis, usually with para-aminosalicylic acid and isoniazid. Streptomycin is also used frequently in the treatment of genitourinary tract infections caused by Gram negative bacilli. Streptomycin is the drug of choice for tularemia and plague and is used for brucellosis in combination with one of the tetracyclines. Streptomycin, when used for long periods of time, will cause damage to the eighth cranial nerve, resulting in loss of hearing.

Aminoglycoside antibiotics are bactericidal/bacteristatic.

OTHER AMINOGLYCOSIDES

Kanamycin, Amikacin, and Gentamicin

Activity
These are also bactericidal antibiotics with an action similar to streptomycin. They also cause misreading in protein synthesis.

Resistance
Resistance to these antibiotics is associated with the presence of an R factor.

Clinical use
These antibiotics have pharmacological properties similar to streptomycin. They are bactericidal for Gram negative bacilli, Mycobacterium tuberculosis and Staphylococcus.

Amikacin is used in treatment of group B and listerial meningitides.

Kanamycin is used for genitourinary tract infections especially those caused by Proteus. It is also a drug of choice for Listeria.

Gentamicin is an excellent drug for treatment of septicemias caused by Gram negative enteric bacilli, Serratia and Pseudomonas. When used for systemic infections caused by Pseudomonas aeruginosa, it is usually used with carbenicillin although the two drugs cannot be mixed in the same solution since carbenicillin inactivates gentamicin.

Aminoglycoside antibiotics have toxicity for the _____ cranial nerve, causing _____.

TETRACYCLINES

Structure
The tetracyclines are a family of antibiotics with a four ring structure.

Activity
Tetracyclines are active against a wide variety of microorganisms. Sensitive organisms include not only Gram positive and Gram negative bacteria, but also rickettsia, mycoplasma and chlamydia. Tetracyclines are bacteristatic drugs which inhibit protein synthesis. They bind to the 30S ribosome and inhibit binding of aminoacyl-tRNA to the acceptor site of this ribosome.

Resistance
Resistance is associated with the presence of an R factor in Gram negative bacilli which confers resistance against all tetracyclines. Unlike other R factor-mediated resistance, however, resistance to tetracyclines appears to be due to an impaired ability to transport the drug.

Tetracycline antibiotics bind to the 30S ribosome and inhibit binding of _____.

Clinical use
Tetracyclines are absorbed from the gastrointestinal tract, and therefore can be used orally. They are first line drugs for treatment of infections caused by rickettsia, mycoplasma and chlamydia. Tetracyclines are also used for the treatment of cholera and brucellosis and for treatment of infections caused by bacteria which have become resistant to the penicillins and other antibiotics.

Tetracyclines are bactericidal/static.

One of the serious side effects of therapy with the tetracyclines and to a lesser extent with penicillin and the aminoglycosides is superinfection by resistant organisms. Superinfection occurs in the gastrointestinal tract, oral cavity and vagina, usually after oral administration of antibiotics. Resistance organisms which predominate in these cases are Staphylococcus aureus, Candida albicans, Pseudomonas and Proteus. Tetracyclines are also deposited in teeth during calcification and may produce a yellow stain when used in large doses in children.

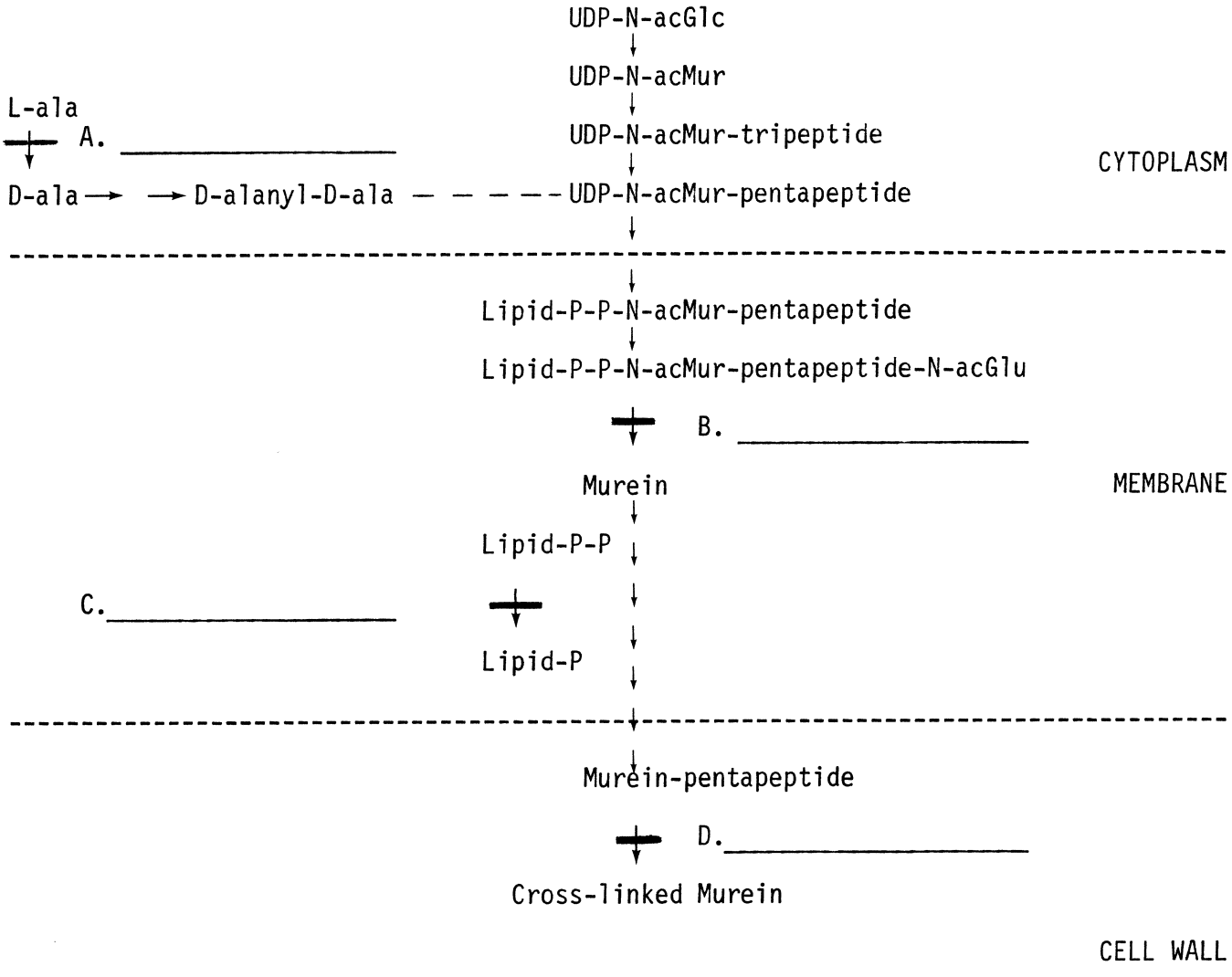
Tetracycline drugs are active against many bacteria, including _____, _____, _____, _____, and _____.

REVIEW OF ANTIBIOTICS WHICH AFFECT CELL WALL SYNTHESIS

Fill in the blanks with the appropriate antibiotics. (Answers on next page)

A. _____ B. _____

C. _____ D. _____



CHLORAMPHENICOL

Activity
Chloramphenicol is a broad spectrum antibiotic which is bacteristatic for both Gram positive and Gram negative bacteria, rickettsia and chlamydia. Chloramphenicol is one of several antibiotics which bind to the 50S ribosome, others being the macrolide antibiotics such as erythromycin and the lincomycins. Chloramphenicol's effect on protein synthesis appears to be the result of the interference with peptide bond formation.

Resistance
Resistance to chloramphenicol in Gram negative bacilli is also associated with the presence of an R factor which is responsible for the formation of an enzyme, chloramphenicol acetyltransferase, which catalyzes the formation of the mono- and diacetyl derivatives of chloramphenicol with acetyl coenzyme A resulting in inactivation of the drug.

Clinical use. It should be used primarily for typhoid fever, H. influenzae meningitis, anaerobic infections and for those bacterial infections resistant to other drugs. Aplastic anemia associated with the use of chloramphenicol has restricted the use of this drug.

Clindamycin, although chemically unlike chloramphenicol, has a similar mechanism of action, (i.e., it blocks peptide bond formation). The major use for clindamycin is in therapy of anaerobic infections.

REVIEW OF ANTIBIOTICS THAT INTERFERE WITH PROTEIN SYNTHESIS

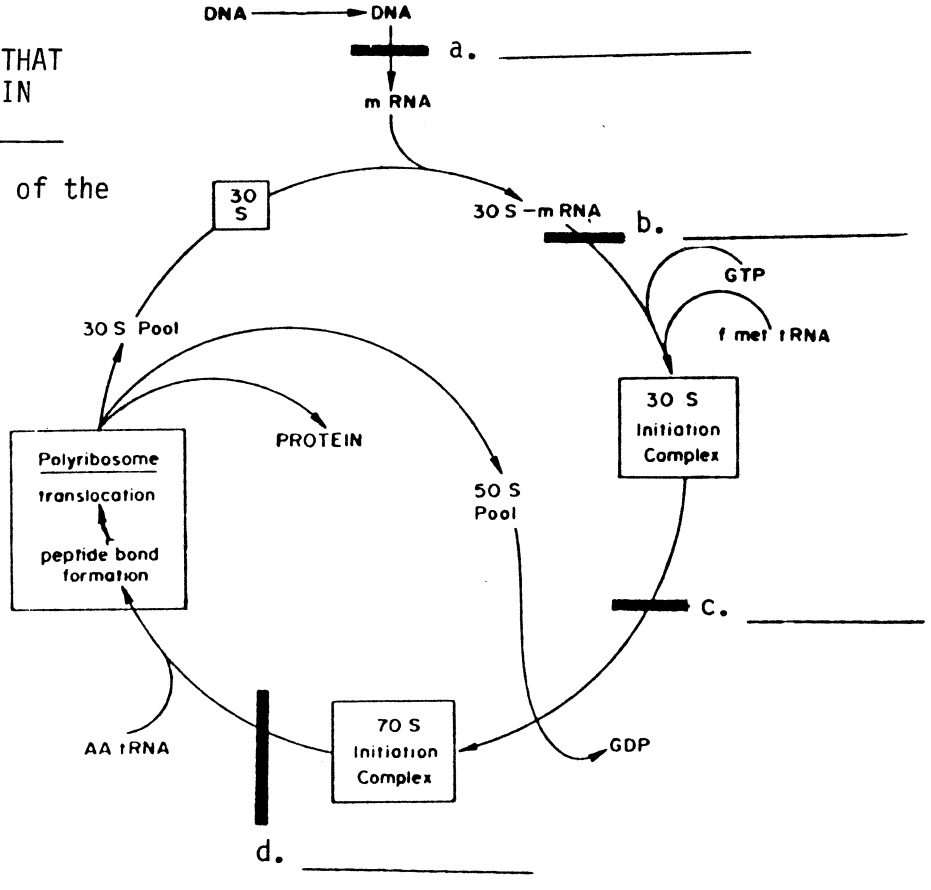
In the blank write the name of the appropriate antibiotic.
(answers on next page)

A. _____

B. _____

C. _____

D. _____



ANSWERS
A=cycloserine
B=vancomycin
C=bacitracin
D=penicillin or cephalosporin

ERYTHROMYCIN

Structure
This is the most important of the macrolide antibiotics. These agents contain a macrocyclic lactone ring to which 1 or more sugars are attached.

Activity
Erythromycin reacts with the 50S ribosomal subunit and seems to block the translocation step in protein synthesis by inhibiting the release of charged tRNA from the donor site.

Resistance
May be either mutational or plasmid mediated. The chromosomal change which imparts resistance is due to a conformational change in one of the ribosomal proteins resulting in a decrease in drug binding. Plasmid-mediated resistance is due to methylation of an adenine residue in the 23S subunit, which reduces its affinity for the antibiotic. The modified ribosomes are cross-resistant to lincomycin and clindamycin, suggesting that these two non-macrolide antibiotics have a similar site of action to that of erythromycin.

Clinical use
This bacteristatic antibiotic is used as the primary drug for M. pneumoniae infections and for Legionella as well. It is also used against streptococci in patients allergic to penicillin.

GRISEOFULVIN

This is a fungistatic agent which is active against mycotic agents which have chitin in their cell walls. It is used primarily in the treatment of dermatophyte infections. Griseofulvin inhibits the assembly of proteins, thus it inhibits cell division by blocking the assembly of microtubules from tubulin (micro-tubule formation is essential for chromosome movement during mitosis).

Chloramphenicol is a bactericidal/static agent.

Erythromycin is bactericidal/static.

Answers to protein synthesis antibiotics questions

A=rifamycin
B=tetracycline
C=aminoglycosides
D=chloramphenicol

INTERFERENCE WITH RNA SYNTHESIS

Rifamycins

Activity
Rifamycins are bactericidal for many species of Gram positive bacteria, Gram negative bacteria and Mycobacterium tuberculosis. Rifamycins cause RNA synthesis to decrease. They inhibit the action of DNA-dependent RNA polymerase by binding to RNA polymerase which makes the enzyme inactive. They inhibit initiation of RNA synthesis.

Resistance
Resistance to rifamycins is due to an altered β-subunit of RNA polymerase with a decreased ability to bind rfampicin.

Clinical use
Rifampicin is the most widely used of the rifamycins since, unlike the other rifamycins, it is readily absorbed from the gastrointestinal. Its principal use is in the treatment of tuberculosis.

REVIEW QUESTIONS

Antibiotics which interfere with protein synthesis include:

1. _____
2. _____
3. _____
4. _____

The major bacteristatic antibiotics include:

1. _____
2. _____
3. _____

REVIEW

	Antibiotic	Mechanism of Resistance (R factor mediated)
1.	Erythromycin Clindamycin	Methylation of ribosomal RNA keeps antibiotic from binding to ribosome.
2.	Sulfonamides Tetracycline	Alteration of cell membrane decreases permeability to the antibiotic.
3.	Chloramphenicol	Acetylation of the antibiotic inactivates it.
4.	β-Lactams	β-lactamase break down antibiotic to inactive form.
5.	Aminoglycosides	Modifying enzymes cause a)acetylation b)adenylation c)phosphorylation

Antibiotic Actions

Antibiotic	Synthetic Step Blocked	Level of Blockade
Streptomycin	Protein Synthesis	30S ribosomal subunit
Amikacin		-blocks initiation
Kanamycin		
Neomycin		
Gentamicin		
Tetracycline		-blocks binding of aminoacyl tRNA
Chloramphenicol		50S ribosome: inhibits
Chindamycin		peptidyl transferase
Erythromycin		
Bacitracin	Cell Wall Synthesis	inhibits hydrolysis of lipid pyrophosphate
Penicillin		inhibits transpeptidation
Cephalosporin		
Cycloserine		competes with D-alanine
Rifampicin	RNA Synthesis	DNA-dependent RNA polymerase

REVIEW STATEMENTS

These should be used to confirm your understanding of the subject. If you are uncertain about the veracity of a statement, please "check it out".

The flagellar filament is composed of an elastic protein, flagellin.

Flagella are the organelles responsible for motility of bacteria. Their direction of rotation controls movement.

Pili are short hair-like protein structures which occur on the surface of numerous strains of gram negative bacteria. Some, for example, those that occur on the gonococcus, are thought to be associated with the adherence of the organism to host cells. The sex pilus, which is found only in male strains of bacteria is involved in conjugation.

Capsules of most species of bacteria are composed of carbohydrate, with the exception of Bacillus anthracis, which has a capsule composed of poly D glutamic acid. In many pathogenic microorganisms, the capsule inhibits phagocytosis.

The rigid layer of the cell wall of gram negative and positive bacteria is similar and is composed of mucopeptide (peptidoglycan). Gram positive bacteria contain a great deal more peptidoglycan in their cell walls than do gram negatives. In the former the mucopeptide is three dimensional, as opposed to a two dimensional layer on gram negative organisms.

Three kinds of proteins associated with the cell membrane of bacteria include biosynthetic enzymes, transport enzymes and cytochrome enzymes, which serve the following functions in the cell: synthesis of external layers of the cell, transport of water-soluble materials into the cytoplasm, and electron transport activities, respectively.

The nucleus of bacteria exists as a single circular chromosome, usually attached to the mesosome. There is no nuclear membrane, nor histones.

Cytoplasmic inclusions such as ribosomes, lipid inclusions and metachromatic granules occur in bacteria.

Spores are highly resistant to deleterious agents in the environment, probably because of the content of keratin proteins in the spore coat, and their low metabolic rate.

Heterotrophs are microorganisms that require the major portion of their carbon in the form of organic compounds, although they still require some CO_2. Autotrophs can obtain all of their carbon from CO_2.

Mesophilic bacteria have an optimal growth temperature between 20 and 40C. Strict anaerobes are unable to grow in the presence of O_2, they lack a functional electron transport system, and are unable to produce energy by oxidative phosphorylation.

Anaerobic bacteria lack superoxide dismutase and catalase.

The enzyme which penicillin inhibits is a transpeptidase.

The cytochrome system of bacteria is associated with the cell membrane.

Bacteria reproduce by binary fission. Each division is a generation and results in a doubling of cell number. Ten generations of bacterial growth would result in a 1,000-fold increase in cell numbers. In another ten generations, the total bacterial population would be 1,000,000-fold the original inoculum. Assuming a generation time of 30 minutes, this entire process would have taken 10 hours. The culture inoculated at noon on one day should reach a total cell mass of one million million by 8 am the next morning. This does not happen! The organism stops growing (goes into the stationary phase of growth) due to either depletion of an essential nutrient or accumulation of a toxic metabolite.

Flagella and axial filaments are composed of protein which is low in cysteine and aromatic amino acids.

Flagella and axial filaments are probably organelles of motility.

Pili of N. gonorrhoeae function in the virulence of the agent by facilitating adherence to the mucosal surfaces.

Erythromycin is used in legionella and mycoplasma pneumonias.

The cell wall of gram negatives is composed of the outer layer, a middle protein layer and peptidoglycan (mucopeptide).

Salmonella mutants without the O antigen do not agglutinate in serospecific antisera.

Loss of mucopeptide results in an osmotically sensitive spherical cell (a protoplast or a spheroplast).

The periplasmic space and outer layer are unique to the gram negative bacteria, as are porin channels in the outer membrane.

Ribitol teichoic acids are unique to gram positives.

The cell membrane is responsible for active transport.

The function of the mesosome may be involved in cell division.

Tetracyclines are the drug of choice for mycoplasma, Rickettsiae and Chlamydiae.

The bacterial nucleus is composed of double-stranded DNA, without histones.

Pinocytosis does not occur in bacteria.

Nutrients are transported into the cell with kinetics described by the Michaelis-Menton equation.

Substrates are transported from the medium into the bacterial cell by one of three processes; active transport, facilitated diffusion, and group translocation.

Bacterial resistance to antibiotics is usually passed by plasmids (R factors) which induce production of enzymes which modify the antibiotic.

A complication of clindamycin therapy is enterocolitis caused by Clostridium difficile.

Cationic detergents are good bactericides but their action is inhibited by soaps.

Gentamicin is used for gram negative bacteria (e.g., Pseudomonas).

Chloramphenicol is useful in the treatment of typhoid fever and H. influenzae meningitis.

Group translocation occurs only with sugars and is effected by the phosphotransferase system.

Bacteria usually transport amino acids by active transport; one system may be responsible for more than one amino acid.

Transport systems for sugars are usually inducible with the exception of glucose.

Fermentation is the anaerobic metabolism of substrate. Energy is mobilized during fermentation in phosphoryl groups on molecules such as ATP and PEP.

Aerobic metabolism is a much more efficient energy-yielding process than is fermentation.

The electron transport system of bacteria is located in the cell membrane.

Cytochrome enzymes have an iron porphyrin active site.

Penicillin and cephalosporin inhibit transpeptidation, the final reaction of mucopeptide synthesis.

Bacterial resistance to these two antibiotics is usually the result of a β-lactamase, whose production is governed by a plasmid.

Penicillin-destroying enzymes from gram positive bacteria are relatively inactive against cephalosporins.

Cycloserine is an analog to D-alanine; vancomycin inhibits the transfer of the disaccharide - pentapeptide from the phospholipid carrier to the cell wall; bacitracin inhibits hydrolysis of lipid pyrophosphate.

Four aminoglycoside antibiotics which inhibit protein synthesis by binding to the 30S ribosome are streptomycin, kanamycin, gentamicin, and amikacin.

Antibiotics which inhibit protein synthesis by binding to the 50S ribosome include chloramphenicol and erythromycin.

Tetracyclines are bacteristatic to both gram + and - organisms, inhibiting binding of amino acyl tRNA to the 30S ribosome.

Rifamycins inhibit the action of DNA dependent RNA polymerase by binding to the RNA polymerase.

Sulfonamides are bacteristatic drugs which inhibit the condensation of pteridine pyrophosphate with PABA.

The resistance of sulfonamides seen in enteric bacteria is usually associated with the presence of an R factor.

Another analog of PABA which has a similar mechanism of action is para-amino salicylic acid, which is widely used in the treatment of tuberculosis.

Enterochelins are siderophores which allow enteric bacteria to accumulate iron essential for growth.

Sulfonamides and para-aminosalicylic acid are both bacteristatic inhibitors of folic acid synthesis which are ineffective in the presence of para-aminobenzoic acid.

The cell wall of gram negative bacteria is composed of the outer layer, periplasmic space and mucopeptide.

Mutants of Salmonella that have lost the O-antigen lose most of their ability to agglutinate the antiserum against the serotype of the parent.

Loss of mucopeptide in a gram positive organism results in a protoplast which will lyse in media of ordinary osmotic pressure.

The periplasmic space and outer layer are found only in gram negative bacteria.

The function of mesosomes is secretion and participation in cell division.

The cell membrane is the site of active transport.

When Clostridium tetani is inoculated into tryptone-yeast extract broth with glucose as the energy source and incubated anaerobically, it will produce approximately 2 ATP per mole of glucose fermented.

Sulfanilamide is a bacteristatic drug which is relatively non-toxic for man because man is unable to synthesize folic acid.

Streptomycin, kanamycin, neomycin and gentamicin are all bactericidal aminoglycosides which bind to the 30S ribosome of bacteria and may cause damage to the eighth cranial nerve.

Streptomycin inhibits initiation of protein synthesis.

Tetracyclines are broad spectrum antibiotics which bind to the 30S ribosome and inhibit binding of aminoacyl tRNA.

Sterilization of metallic instruments and glassware can be achieved with the autoclave or ethylene oxide.

Membrane filters will retain bacteria but allow the passage of viruses.

Antibiotic resistance in staphylococci can be transferred to other staphylococci by bacteriophage and is associated with penicillinase production.

An organism which lacks alanine racemase would be unable to make a complete peptidoglycan.

Drugs which affect the 30S ribosome are the aminoglycosides and tetracyclines.

Resistance to streptomycin is due to enzymes that phosphorylate or adenylate streptomycin.

Chloramphenicol and the tetracyclines are both broad spectrum antibiotics which inhibit protein synthesis; they differ in that chloramphenicol attaches to the 50S ribosomal subunit while tetracyclines attach to the 30S subunit.

Electron transport enzymes and enzyme II of the phosphotransferase system are both located in the cell membrane.

Short, hair-like structures occurring on the surface of gram negative bacteria are called pili.

Bacteria reproduce by binary fission. Nine generations of growth would result in 512-fold increase in cell numbers.

An intermediate in the biosynthesis of the cell wall is N-acetylmuramic acid pentapeptide-phospholipid.

Bacteria which can use CO_2 as a sole source of carbon are called autotrophs.

Cessation of bacterial growth as noted in a growth curve is caused by toxic metabolic end products, unfavorable pH, and/or exhaustion of nutrients.

Transport of sugars by bacteria takes place through the cell membrane, frequently involves an inducible transport system, and usually involves a steriospecific discrimination of the sugar.

The action of sulfanilamide is bacteristatic since it is reversed by p-aminobenzoic acid.

The antibacterial action of sulfanilamide is due to the inhibition of folic acid synthesis.

Penicillin owes its antibacterial action to inhibition of cross-linking between adjacent peptide chains and mucopeptide.

One action of penicillin on bacteria is to interfere with transpeptidation.

Rifamycins are bactericidal due to their interference with RNA synthesis.

Chloramphenicol interferes with protein synthesis by binding to the 50S ribosome and inhibiting peptide bond formation (peptidyl transferase).

Flagella and axial filaments are composed of protein which is low in cysteine and aromatic amino acids.

Flagella and axial filaments are probably responsible for motility in bacteria and spirochetes respectively.

Cell wall teichoic acids of St. pneumoniae act as a positive effector of this organism's autolysin.

Mutants of Streptococcus pneumoniae which lose their capsule are not virulent.

Capsules of bacteria are either polysaccharide or polypeptide.

Methicillin and cloxicillin are used for penicillin-resistant strains of bacteria.

Lipid A, core and O antigen are all parts of lipopolysaccharide.

D-Cycloserine, vancomycin and bacitracin are all inhibitors of peptidoglycan synthesis.

MICROBIAL PHYSIOLOGY PROFICIENCY TEST

These questions were used in a fall, 1985 course to sophomore Medical students. The mean on this test was 85 percent. I would suggest that any score below 70 percent is indicative of a deficiency in this area.

1. Translocation of glucose from the medium into the cell by Escherichia coli requires

   A. flagella.
   B. pili.
   C. energy.
   D. light.
   E. mesosomes.

2. Staphylococcus aureus isolated from hospitals is usually penicillin resistant whereas S. aureus isolated from the community at large is usually penicillin sensitive. The reason for this difference is

   A. penicillin-sensitive strains are eliminated from hospitals by treatment with antibiotics.
   B. hospital strains mutate forming penicillin-resistant strains which survive better than sensitive strains.
   C. hospital strains acquire a plasmid which carries the structural gene for penicillinase.
   D. non-hospital strains do not form β-lactamase because it is an inducible enzyme which is only formed in the presence of penicillin.
   E. (A) and (C) above.

3. One difference between Bacillus and Staphylococcus is that Bacillus species are

   A. cocci, while Staphylococcus are rods.
   B. rods, while Staphylococus are cocci.
   C. Gram positive, while Staphylococcus are Gram negative.
   D. Gram negative, while Staphylococcus are Gram positive.
   E. anaerobic, while Staphylococcus are facultative.

4. Cycloserine is a water soluble antibiotic with a molecular weight of 102. You would predict that

   A. it would arrive in the periplasmic space by diffusion through the pores (porins) of the outer membrane.
   B. it would arrive in the periplasmic space by diffusion through the phopholipid rigion of the outer membrane.
   C. it would be transported through the cell membrane by the phosphotransferase system.
   D. it would pass through the cell membrane by simple diffusion.
   E. it would probably not pass through the cell membrane.

5. Cell wall teichoic acids

   A. occur in Gram negative bacteria only.
   B. of Streptococcus pneumoniae act as a positive effector of the autolysin of this organism.
   C. are responsible for the rigidity of the cell wall.
   D. act as molecular sieves.
   E. are responsible for the endotoxin activity of lipopolysaccharide.

6. When bacteria with rigid cell walls are treated with lysozyme,

   A. a linkage between N-acetylmuramic acid and N-acetylglycosamine is hydrolyzed.
   B. the cell becomes a spheroplast.
   C. the cell becomes osmotically fragile.
   D. the peptidoglycan layer disappears.
   E. all of the above.

7. Tetracyclines are useful drugs which

   A. prevent RNA synthesis.
   B. are bactericidal.
   C. prevent binding of aminoacyl tRNA to the 30S ribosome.
   D. bind to L12 of 50S ribosome.
   E. inhibit DNA gyrase.

8. Chloramphenicol is more effective against prokaryotes than eukaryotes because it

   A. inhibits prokaryotic DNA-directed RNA polymerase.
   B. inhibits protein synthesis by 70S, but not by 80S ribosomes.
   C. activates a cell wall autolysin.
   D. causes misreading of UUU codon in mRNA.
   E. inhibits DNA synthesis by prokaryotes.

9. Streptomycin

   A. inhibits peptidyl transferase.
   B. prevents initiation of protein synthesis by ribosomes of prokaryotes.
   C. disrupts cell membranes which contain sterols.
   D. binds to 50S ribosome such that it inhibits peptide bond formation.
   E. prevents binding of mRNA to the ribosome.

10. Antibiotics with antibacterial activity similar to kanamycin include

   A. cycloserine, vancomycin and cephalosporin.
   B. chloramphenicol and tetracycline.
   C. colistin and polymyxin B.
   D. streptomycin, neomycin and gentamicin.
   E. lincomycin and clindamycin.

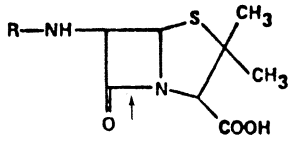


FIGURE 1

11. What is the molecule shown in Figure 1?

    A. Sulfanilamide
    B. Cephalothin
    C. Tetracycline
    D. Penicillin
    E. Cycloserine

12. The family of the antibiotics shown in Figure 1

    A. inhibits protein synthesis.
    B. inhibits peptidoglycan synthesis.
    C. inhibits DNA-directed RNA polymerase.
    D. inhibits reverse transcriptase.
    E. destroys the cell membrane.

13. The point indicated by the arrow in Figure 1

    A. is where β-lactamases attack.
    B. is where the phosphotransferase attacks.
    C. opens to produce the active form of the antibiotic.
    D. is where acyl groups are added to produce semisynthetic antibiotics resistant to acid and β-lactamases.
    E. is where lysozyme attacks.

14. One important reason why trimethoprim and sulfamethoxazole together are more effective against bacteria than the sum of their individual activities is because

    A. both inhibit dihydropteroic acid synthetase.
    B. both inhibit dihydrofolate reductase.
    C. trimethoprim is bacteristatic and sulfamethaxazole is bactricidal.
    D. trimethoprim inhibits cell wall synthesis and sulfamethoxazole inhibits folic acid synthesis.
    E. both inhibit folic acid synthesis, but inhibit different steps.

For each of the incomplete statements, one or more of the completions given is correct. The correct answer is

A. if only (1), (2) and (3) are correct.
B. if only (1) and (3) are correct.
C. if only (2) and (4) are correct.
D. if only (4) is correct.
E. if all are correct.

15. The phosphotransferase system of bacteria

    1. concentrates transported substrates.
    2. produces a phosphorylated product.
    3. is used to transport sugars.
    4. is used to transport amino acids.

16. A structure which contains lipid A, core polysaccharide and O antigen would occur in an organism

    1. with a periplasmic space.
    2. with ribitol teichoic acid in its cell wall.
    3. which is Gram negative.
    4. which is Gram positive.

17. Bacterial chemotaxis

    1. occurs only in bacteria with flagella.
    2. results in the movement of bacteria away from a repellant.
    3. results in the movement of bacteria toward a nutrient.
    4. occurs only in bacteria which possess fimbriae.

18. Sulfadiazine, p-aminosalicylic acid and trimethoprim are all

    1. inhibitors of protein synthesis.
    2. antimetabolites.
    3. inhibitors of RNA polymerase.
    4. inhibitors of folic acid synthesis.

19. The effect of penicillin G on Streptococcus pneumoniae

    1. is most pronounced when added during the exponential phase of growth.
    2. results in death of the streptococci.
    3. is to prevent formation of peptidoglycan cross links.
    4. depends on the presence of an autolysin.

20. Antibiotics which inhibit protein synthesis in bacteria

    1. include the tetracyclines and chloramphenicol.
    2. attach to the 30S or 50S ribosome.
    3. in many cases owe their selective antibacterial activity to a greater inhibition of protein synthesis by 70S ribosomes than by 80S ribosomes.
    4. include cephalothin and vancomycin.

For each of the incomplete statements, one or more of the completions given is correct. The correct answer is

A. if only (1), (2) and (3) are correct.
B. if only (1) and (3) are correct.
C. if only (2) and (4) are correct.
D. if only (4) is correct.
E. if all are correct.

21. Peptidoglycan

    1. is a unique polymer found in Prokaryotae.
    2. protects the cell against osmotic shock.
    3. contains muramic acid.
    4. contains D-alanine.

22. The polymyxin antibiotics

    1. attach to cell membranes lacking sterols.
    2. are drugs of choice for fungal infections.
    3. are useful for septicemias caused by Gram negative bacteria.
    4. attach to cell membranes possessing sterols.

23. The bacterial cell membrane

    1. surrounds the protoplast.
    2. is the site of active transport.
    3. is the site of oxidative phosphorylation.
    4. is the site where passive diffusion takes place through water-filled pores (porins).

24. Superoxide dismutase is an enzyme which

    1. catalyzes the conversion of superoxide anion to hydrogen peroxide.
    2. catalyzes the conversion of hydrogen peroxide to water and oxygen.
    3. is usually present in aerobic and facultative bacteria.
    4. is usually present in anaerobic and facultative bacteria.

1. C	9. B	17. A
2. E	10. D	18. C
3. B	11. D	19. E
4. A	12. B	20. A
5. B	13. A	21. E
6. E	14. E	22. B
7. C	15. A	23. A
8. B	16. B	24. B

REVIEW OF MICROBIAL GENETICS

DEFINITIONS

Anticodon - the nucleotide triplet in a tRNA that associates by complementary base pairing with the codon in the mRNA during translation.

Cistron - A DNA fragment that codes for a particular polypeptide.

Complementation - the production of a phenotype similar to wild type when two mutations are brought together.

Conjugation - transfer of DNA from one bacterial cell to another which is dependent upon cell to cell contact.

Complementary DNA (cDNA) - DNA that is complementary to messenger RNA; used for cloning or as a specific probe in hybridization.

Episome - A genetic element that replicates either free or as part of the normal cellular chromosome.

Exon - portion of DNA that codes for the final mRNA.

Integration and excision - integration = a recombination in which a genetic element is inserted; excision = reverse of integration.

Intron - intervening sequence in DNA; a portion of a gene that does not appear in the final mRNA transcript.

Nonsense mutation - a mutation that results in the termination of a polypeptide chain.

Suppressor gene - a gene that can reverse the effect of a specific mutation in another gene.

Transcription - formation of RNA from the DNA template.

Transduction - the transfer of genetic material from one cell to another by means of a viral vector.

Transformation - the introduction of exogenous DNA into a competent cell.

Transposable element (transposon) - a segment of DNA that can move from one position in the genome to another.

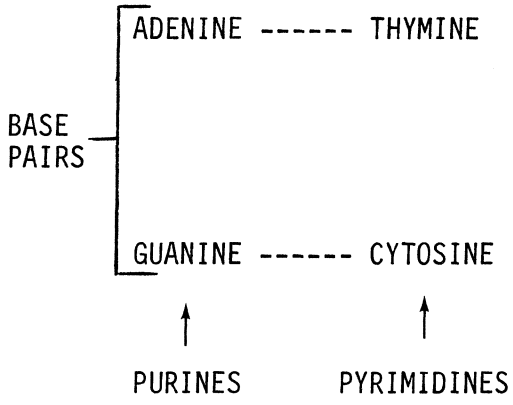
Vector - A DNA molecule known to autonomously replicate in a cell to which another DNA segment may be attached to bring about the replication of the attached segment.

MUTATIONS

The term mutation refers to an abrupt and usually stably inherited change in properties of an organism. Mutations can occur either spontaneously, or may be induced by a mutagen.

Mutation in Populations

The proportion of mutants in a given population of cells is called the mutant frequency. The probability that a mutation will occur during a particular time interval, such as the generation time, is the mutation rate, and is expressed as a mutation per cell, per division.



Selection of Mutants

Wild type is the designation given to strains as they are found in nature, or to certain laboratory standard strains. Mutants, called auxotrophs, have an additional nutritional requirement. Markers are other detectable mutations with a distinctive observable effect on the organism serving to mark the chromosome at the locus at which it occurs. Such markers include colony morphology, fermentation patterns and antibiotic resistance.

Point Mutations

Point mutations may occur as a result of a base pair substitution in the specific nucleotide sequence of a gene. When one purine on a chain replaces another purine, and when the pyrimidine on the other chain is replaced by a different pyrimidine, the substitution is called a transition.

Transversions occur when a purine in a DNA chain is replaced by a pyrimidine, and when the pyrimidine on the other chain is replaced by a purine.

DEFINE THE FOLLOWING POINT MUTATIONS:
(Answers on next page)

1. GC changes to AT
2. GC changes to TA
3. CG changes to AT
4. TA changes to GC

Transitions may be produced by agents such as the pyrimidine analog 5-bromouracil or the purine analog 2-aminopurine. Alkylating agents such as ethylethanesulfonate or sulfur mustards possessing a single reactive group also cause transitions. Nitrous acid oxidatively deaminates the amino-substituted bases; adenine, quanine, and cytosine.

A missense mutation occurs when the triplet code is altered such that a different amino acid is inserted into the protein. The product may be inactive or only partially active.

A nonsense mutation occurs when the triplet code is altered such that a chain termination codon (UAG, UAA, or UGA) appears.

Frameshift mutations. Mutations which result in an addition or deletion of a nucleotide into a sequence of m RNA cause a reading frameshift of the trinucleotide sequences. Frameshift mutations may result in an addition or deletion of one, two, four or five nucleotides, all of which result in a shift of the reading frame. Frameshift mutations are known to be caused by a group of polycyclic compounds called acridines (e.g., proflavin). Acridines are capable of strong binding to DNA by intercalation between adjacent base pairs.

Deletions

Mutations resulting in the loss of large segments of DNA, covering from one to several genes, are referred to as deletions. Deletions can occur spontaneously, or may be induced by X-rays, UV Light, or treatment with nitrous acid.

UV light (and ionizing radiation) can also damage DNA by causing the formation of thymidine dimers. Bacteria contain enzymes which can repair this damage; they delete the ssDNA segment in which the dimer occurs (via endonuclease), and resynthesize it under the direction of the complementary DNA strand (via DNA polymerase).

Define the following mutations, identify a suitable mutagen, and describe the consequences of the mutation. Remember, nonsense codons are UAG, UAA, UGA.

(answers on next page)

Wild type gene=AUG-ACC-UGG-UCA-CCA-TTT-AAT-

Auxotroph #1=AUG-ACT-UGG-UCA-CCA-TTT-AAT-

Auxotroph #2=AUG-ACC-UGA-GUC-ACC-ATT-TAA-T

Auxotroph #3=AUG-ACC-UUC-ACC-ATT-TAA-T

Auxotroph #4=UAG-ATT

Answers to point mutations question

1 = transition
2 = transversion
3 = transversion
4 = transversion

RECOMBINATION

Recombination is the formation of a new genotype by reassortment of genes following a genetic cross. It involves a structural change due to the crossing over, or exchange of genetic material between two different, but homologous chromosomes. Only a portion of the donor chromosome is added to the recipient to form a partial diploid. Once this has occurred, recombination can take place.

Model of Recombination: The Breakage and Reunion model of recombination proposes that the chromosomes break and are reunited such that each progeny contains genetic material from both parents. This model is supported by the observation that recombination can occur without DNA synthesis. Reciprocal recombination is the rule, however, on occasion, the recombinant is unidirectional (progeny contains genes from one parent but the 2nd reunion does not occur).

COMPLEMENTATION

Complementation is the process by which two recessive mutations can supply each other's deficiency to produce a wild type phenotype. It is another method of genetic analysis used to determine whether two mutants, apparently defective in the same way, are defective in the same gene. It should not be confused with recombination which deals with structural changes between genes. Two types of complementation are known to occur.

Intergenic complementation occurs with genes specifying proteins consisting of two or more nonidentical polypeptides.

Intragenic complementation occurs with genes specifying multimeric proteins consisting of two or more identical polypeptides.

Transferred chromosomal fragments cannot replicate unless they are integrated into the recipient DNA (e.g. in transformation and some conjugation events).

Types of Recombination

A. Legitimate (Generalized)

Host cell enzymes that are involved include:

- Rec A = Proteolytic and ATPase activities
- Rec BC = Endonuclease, exonuclease
- Nucleases
- DNA polymerase
- DNA ligase

B. Nonhomologous (or illegitimate)

Independent of Rec A gene function
e.g. = Transposon insertion, and certain phage mediated events.

Answers to Auxotroph questions

1= Transition; 5-bromouracil; missense mutation-protein may or may not lose function.
2= Frameshift by insertion; acrindine orange or proflavin; nonsense mutation-protein loses function due to premature chain termination.
3= Frameshift by deletion; acridine dyes; missense mutation-usually loss of protein function
4= Deletion of larger segment of DNA:irradiation, nitrous acid or bifunctional alkylating agent; protein loses function.

SUPPRESSION

The effects of a harmful mutation in an organism may be reversed to yield the wild type phenotype. When this occurs there may be a true back mutation to the original genotype, or the genetic code may be misread leaving the original mutation unchanged. When the effects of a primary mutation are eliminated by altering the translation process, the phenomenon is called suppression. Two types of suppression are known: genotype suppression in which a second mutation results in permanent alteration of the translation process; and phenotype suppression in which added substances allow temporary nonheritable alterations in the interactions of translation components to occur.

Genetic suppression occurs when _____

_____

_____.

Genotype suppression

When the effects of a primary mutation are eliminated by a secondary mutation the latter is called a suppression mutation. Such suppressor mutations are classified as intragenic suppressors if they are located in the same gene as the original mutation; or extragenic suppressors if they are located in a different gene, or even a different chromosome, than the original mutation.

Genotype/phenotype suppression occurs when a second mutation "corrects" the harmful effects of an earlier mutation.

In intragenic suppression the secondary mutation is found in the same gene and cancels the deleterious effect of the primary mutation. Several examples can be cited: (1) a missense mutation followed by another missense mutation; (2) a frameshift mutation followed by another frameshift mutation of opposite sign; and (3) a nonsense codon which reverts not back to the original codon, but to another codon which allows a functional protein to be made.

Extragenic (or intergenic) suppression occurs when the suppressor is located on a gene other than the one containing the primary mutation. These suppressors must, therefore, influence the expression

of the primary mutation by virtue of an alteration in a second functional unit of the translation process. The biochemical basis of the type of extragenic suppression that has been described in most detail involves synthesis of specific species of tRNA's.

Examples of intragenic suppression

1. _____

_____

2. _____

_____

3. _____

_____

Phenotype Suppression

Streptomycin and other antibiotics belonging to the aminoglycoside family of antibiotics are known to cause "ambiguities" in amino acid incorporation; i.e., codon recognition of other amino acids at low frequencies. A translation error of this sort is sufficient to allow insertion of a proper, or at least compatible, amino acid at a position that is otherwise mutant. This sort of change results in a translation error which allows the synthesis of a small amount of active protein.

Effects of Some Common Mutagens on DNA

Mutagen	Induced DNA Alteration
Chemical Agents	
5-Bromouracil	T → C transition
2-Aminopurine	A → G transition
Nitrous acid	A → G transition C → T transition
Ethyl ethanesulfonate	Transition or transversion
Magnesium	Transition or transversion
Acridine dyes	Frame-shift
Physical Agents	
Ultraviolet irradiation	Frame-shift, transversion C → T transition, deletion
Visible light	Transition, transversion, frame-shift
Heat	Transition or transversion

GENE TRANSFER

Three types of gene transfer take place in bacteria. All involve an unidirectional transfer of genetic material from donor to recipient cells.

TRANSFORMATION

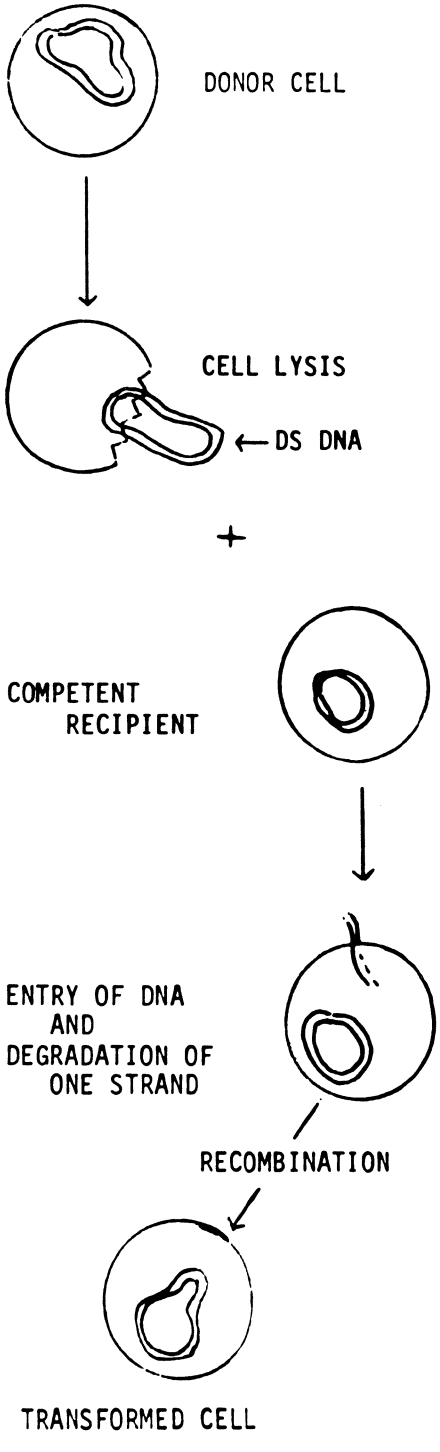
Transformation is the DNA-mediated transfer of a limited amount of genetic information from a disrupted to an intact cell. DNA is obtained from the donor cell either naturally by cell lysis, or artificially by a chemical extraction procedure, and is added to the recipient cells. Once DNA is taken up by the recipient cells, recombination of any marker can take place, and the cell is said to be transformed. Native double stranded DNA is the form most effective in transformation.

The Recipient

For successful transformation the recipient cells must be in a particular physiological state, called competence. The duration of the state is restricted to the late logarithmic phase of growth. Only competent cells can trap or bind the donor DNA to the recipient cells. Once the donor DNA is taken into the cell, integration of the donor DNA is accomplished by recombination. In transformation, the donor DNA is incorporated as a single strand, and the other strand is degraded.

Once donor DNA is taken into the competent recipient cell, integration occurs by _____. Enzymes involved include (refer to "recombination" for answer).

1. _____
2. _____
3. _____
4. _____
5. _____



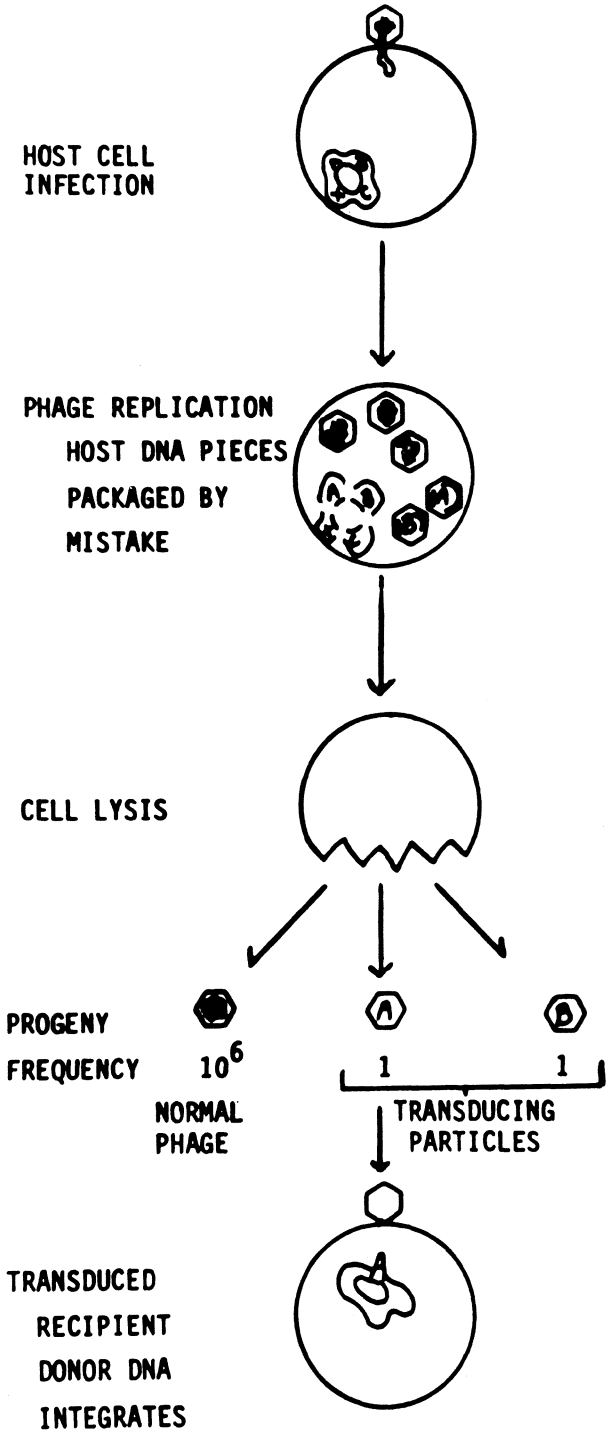
TRANSDUCTION

Transduction is the type of gene transfer mediated by a bacteriophage, and involves the transfer of a limited amount of genetic information from a lysed donor cell to an intact recipient cell.

Generalized transduction occurs when a bacteriophage has the capacity to transfer any of the genes of the bacterial chromosome. Following infection of phage into bacteria, the virus particles multiply, making new enzymes, DNA, and coat protein (the assembly process is called encapsidation). Occasionally, a mistake is made during the assembly of the phage and a piece of bacterial DNA is packaged into the phage coat protein. This is the transducing particle, and since it contains little or no phage DNA, it cannot replicate further. Upon lysis of the bacteria, the phage particles are released, with the transducing particles making up only a small percentage of the total virus particles. A generalized transducing phage can pick up genes from any region of the bacterial chromosome.

Specialized transduction occurs when a particular phage strain can transduce only a few restricted genetic markers. These bacteriophages are temperate phages which during lysogeny integrate into a specific site on the bacterial chromosome. Upon induction of the prophage into the lytic cycle, genes adjacent to the prophage insertion point are occasionally carried along with the phage chromosome.

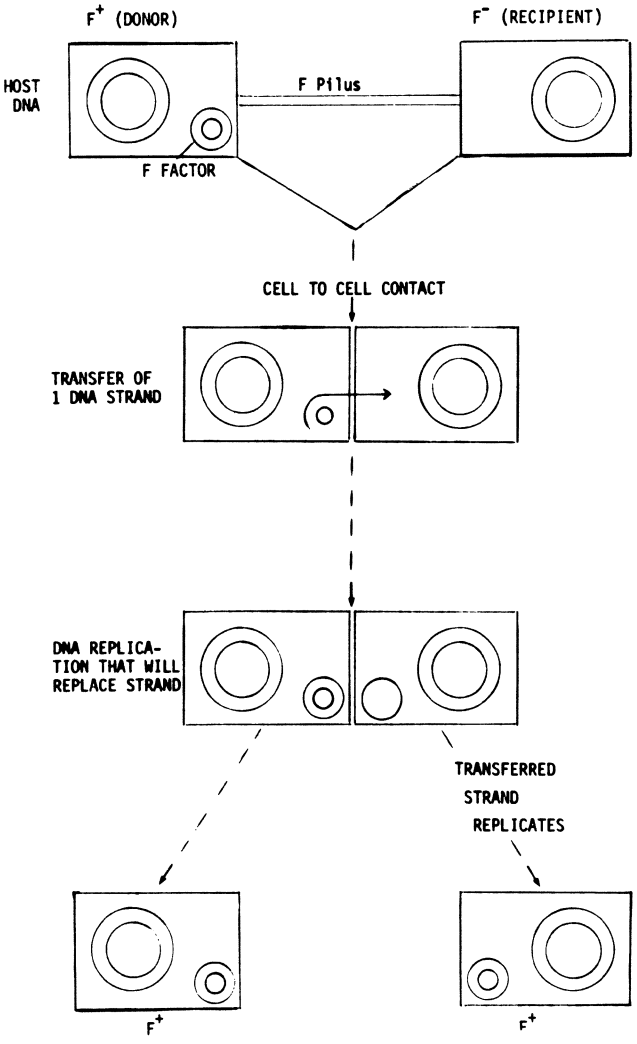
Abortive transduction occurs when the added piece of chromosome from a transducing phage fails to recombine or replicate, but still functions.



CONJUGATION

Conjugation is a process of genetic exchange between two bacterial strains which is dependent upon cell to cell contact.

In conjugation there are two mating types; the male is the donor and contains an F (for "fertility") factor, and is referred to as F+; the female is the recipient and because it lacks an F factor is referred to as an F-. When strains of opposite mating type are allowed to grow together for a short period of time, and undergo conjugation, the F factor of the male replicates, transferring one of its copies to the female recipient. The recipient cell is converted to an F+ cell, now being capable of serving as a donor. Thus, as long as growth continues, the conjugation process can continue in an infectious manner.



The F factor is a circular piece of DNA of molecular weight 50 X 10^6 and codes for approximately 40-60 proteins. Certain clones of F+ cells are capable of transferring chromosomal genes with increased efficiency, resulting in a high frequency of recombination (Hfr). These cells are called Hfr's and arise from F+ cells in which the F factor becomes integrated into the bacterial chromosome. The F factor DNA contains a region of homology with the bacterial chromosome and a recombination event takes place between the two DNA's such that the F factor is inserted linearly into the bacterial chromosome.

F' formation

The integration of the F+ factor into the chromosome to form an Hfr is a reversible process. In some instances, the F factor brings along with it some of the chromosomal genes, and in this state is termed an F'. The chromosomal genes incorporated into the circular F factor are passed on during conjugation. This process is called sexduction and results

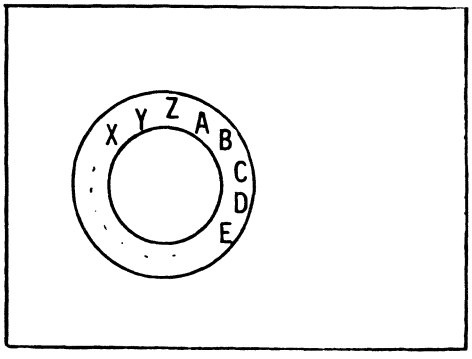
in a high frequency transfer of the F factor and linked chromosomal genes. The recipient cell in sexduction now possesses the same properties as the donor; i.e., it is F+ and contains the added genes attached to the F factor.

Physiology of Conjugation

Male strains have a small tubular appendage which forms a bridge between male and female strains. This appendage is called the F pilus and is synthesized under the control of F, with up to five pili per cell. The function of the F pilus is to form a specific attachment between the male and female that will allow conjugation to proceed.

Cell-to-cell contact, which is essential for conjugation is accomplished via a specialized appendage called the _____ _____.

IDENTIFY THE CELLS BELOW (F-, Hfr, F' and F+)



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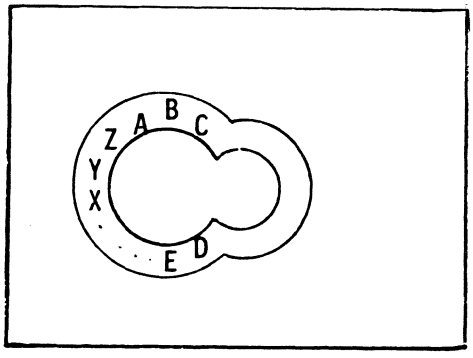

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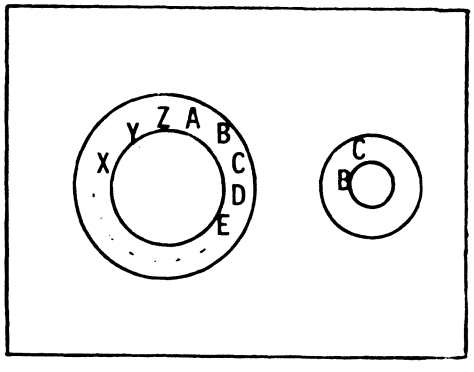

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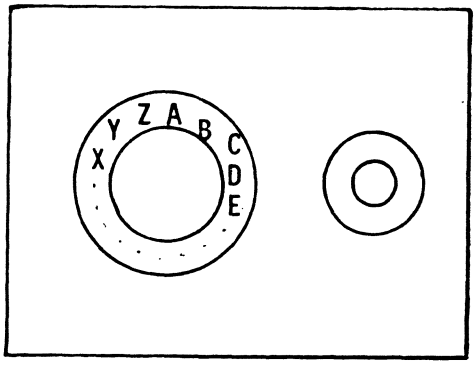


_____

EXTRACHROMOSOMAL GENETIC ELEMENTS

Extrachromosomal genetic elements are often found in bacteria in addition to the normal chromosomal DNA. They are referred to as plasmids and are capable of autonomous replication in the cytoplasm. When an extrachromosomal element is capable of replicating either autonomously or integrated into the bacterial chromosome it is called an episome. Thus, the F factor which can alternatively exist as an F+ or an Hfr, is an example of an episome.

Phenotype functions mediated by plasmids include resistance to antibiotics, heavy metals, ultraviolet light, and specific phages, as well as production of antibiotics, bacteriocins, some toxins and other virulence enhancing factors (such as hemolysins, coagulase, etc.)

Bacteriocinogenic factors are extrachromosomal elements which produce bactericidal substances called bacteriocins. Bacteriocins differ from antibiotics in that they are proteins which act on only the same or closely related species of bacteria. Organisms which produce the bacteriocin are resistant to its action, whereas sensitive cells readily absorb the bacteriocin. Once absorbed, the bacteriocin initiates a highly specific action which leads to the death of the cell. This action differs for each type of bacteriocin, and may involve inhibition of oxidative phosphorylation, or cessation of DNA, RNA, or protein synthesis.

Resistance Transfer Factors

Resistance to antibiotics and other chemotherapeutic agents has been found to exist in a variety of microorganisms. In the Enterobacteriaceae, individual strains may show resistance to several antibiotics. Such multiple drug resistance is specified by an extrachromosomal element, called a resistance factor or R factor.

Plasmids which are capable of integration into the host genome are called

_____.

Two examples of episomes would be

_____ and

_____.

REVIEW STATEMENTS

For successful transformation the recipient cell must be in a particular physiologic state, called

_____.

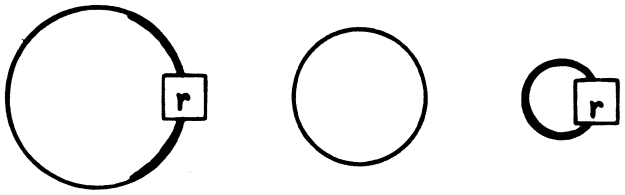
The difference between generalized and specialized transduction is that in the former/latter the temperate bacteriophage integrates into the host genome at a specific site and, upon induction, genes adjacent to the prophage insertion site are carried along with the phage chromosome.

An R factor can be transferred by conjugation from one cell to another, and is often referred to as a resistance transfer factor (RTF). Genetic studies have shown that R factors consist of two distinct components; a transfer factor (RTF) and a resistance determinant (r determinant). The RTF is thought to be similar in function to an F factor, being responsible for both its own autonomous replication and conjugal transfer. The r determinant contains genes which specify resistance to various antibiotics. These two elements may exist independently, or associated together as an RTF:r determinant complex, i.e., R factor (perhaps similar to an F').

TRANSPOSONS - These are linear pieces of DNA, often containing r determinants, which promote their own transfer from 1 piece of DNA to another. For example, a transposon contained in the chromosome of a bacterium could transfer to a plasmid in the same cell; this complex may then be transferred to another bacteria by conjugation. The transposon might then disociate itself from the plasmid and incorporate into the genome of the host cell. It can inactivate host genes during insertion.

The transposon has a particular set of nucleotides on either side of the genes to be transferred which are mirror images of each other (they are palindromes). These are called INSERTION SEQUENCES which permit recognition of the correct area in the host genome, and insertion. The transposon has an endonuclease which will only "nick" DNA after a certain nucleotide sequence has occurred.

Transposon effects include mutations, and insertion of antibiotic resistance genes. Mutations can occur due to deletions or insertions, depending upon whether the transposon is coming or going.



R factor = RTF + r determinant

TRANSPOSON

I	GENES TO BE TRANSFERRED	S
S	e.g. r dtmt.	I

Match the processes below with the components required for these processes to occur (answers on next page).

1. Sexduction _____
2. Transduction _____
3. Conjugation _____
4. Transformation _____

COMPONENTS:
- A) Bacteriophage
- B) Cell-to-cell contact
- C) F' cell
- D) Competent cell
- E) Hfr cell
- F) F- cell
- G) F pilus
- H) Host bacterium

Which properties are common to

5. Plasmids and episomes?
6. Episomes and transposons?

- A) Autonomous replication
- B) Integration into host DNA
- C) Both
- D) Neither

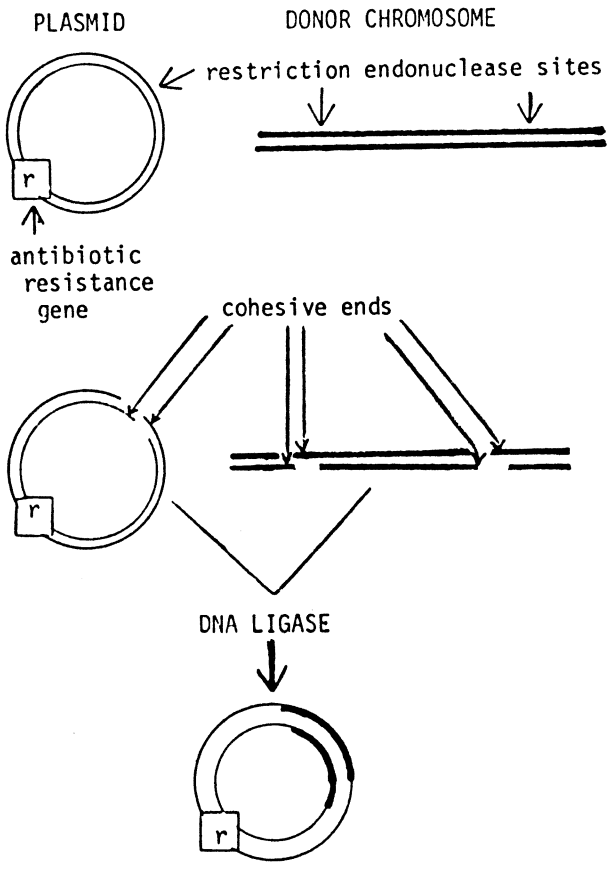
RECOMBINANT DNA

The essential features in the construction and transfer of plasmid recombinant DNA molecules include: a method of specifically cleaving and then joining together DNA molecules from different sources; a source of carrier DNA capable of replicating itself and any foreign DNA joined to it; a method of transferring the compositive DNA molecule to recipient bacterial cells; and finally, a method of detecting whether the recipient cells contain the recombinant DNA molecule.

Several specific enzymes are involved in the construction of plasmid recombinant DNA molecules. The first step involves a cleavage of a circular plasmid DNA molecule into a linear open form. One way of producing such a molecule is with E. coli R factor 1 restriction endonuclease (Eco R1), which makes staggered nicks in complementary strands of DNA at sites separated by several nucleotides. Different endonucleases recognize specific nucleotide sequences hence the DNA can be split at different sites. The resulting single-strand ends contain complementary sequences (cohesive ends) capable of hydrogen bonding again to form a circular molecule. Hydrogen bonding may also occur with single-strand regions of another plasmid or segment of foreign DNA cleaved by Eco R1 endonuclease. In this case, a recombinant DNA molecule is formed which is made up of the original plasmid and the segment of foreign DNA. The final step, sealing of the DNA molecules, is accomplished by DNA ligase, which catalyzes phosphodiester bonds to re-unite the DNA strands.

Summary of essential features for gene cloning:

1. method of specifically splitting and then re-joining DNA's
2. a source of carrier DNA (e.g., plasmids)
3. a transfer method (e.g., conjugation)
4. a method of selecting the recombinant (e.g., antibiotic resistance)
5. a method of detecting the product of the cloned gene (e.g., ELISA)



Answers to genetic transfer questions

1. B, C, E, F, G, H
2. A, H
3. B, F, G, H
4. D, H
5. A
6. B

BACTERIOPHAGES

Viruses that specifically infect bacteria are called bacteriophages, or simply phages. The life cycle of a virulent bacteriophage consists of infection, intracellular development and assembly into a complete phage, and lytic release of new progeny phage; this is called the lytic cycle. Bacteriophages called temperate phages can provoke one of two responses upon infection into a host cell; the lytic cycle (as above) or lysogeny. In lysogeny, the phage chromosome is integrated and replicates in concert with the host chromosome. The integrated phage chromosome is called a prophage, and the bacterial cell containing the prophage is called a lysogen. Lysogenic bacteria continue cell division indefinitely, and may lose the prophage in one of two ways; by the return of the prophage to the lytic cycle, or by spontaneous loss of the prophage. In the former case, bacteria are lysed and progeny phage are released, whereas in the latter case, bacteria retain their viability.

The Lytic Cycle

The lytic cycle of phage multiplication consists of the following steps:

1) absorption
2) penetration
3) intracellular development and
4) maturation
5) lysis

1) Absorption. This process involves the presence of both an absorption organ on the phage and a highly specific receptor site on the bacteria.

2) Penetration. The attachment of the tail fibers and pins to the cell surface triggers a contraction of the tail sheath. This results not only in the penetration of the tail core into the cell wall, but also a syringe-like action discharging the DNA into the host cell.

3) Intracellular Development. Intracelluar development begins immediately after infection, with transcription of the phage DNA by host RNA polymerase. The phage mRNA is translated by the protein synthesizing machinery of the host and forms a number of new proteins called "early" proteins.

The next step is replication of nucleic acid. In dsDNA phages replication proceeds by the general mechanism of DNA synthesis. In ssDNA phages a complementary strand of DNA is synthesized the dsDNA replicative form which serves as a template for the synthesis of both mRNA and new phage DNA.

Shortly after replication begins, the "late" proteins begin to appear. Among the late proteins synthesized are subunits for phage components, as well as the enzyme lysozyme. This enzyme attacks the mucopeptide of the host cell wall and is primarily responsible for lysis and release of progeny phage.

4) Maturation. Once all the structural components are synthesized, maturation begins. The initial step involves the condensation of DNA, possibly with the aid of positively charged proteins, called internal proteins. The capsid subunits assemble around the condensed DNA to form the head. The tail and tail fibers are also formed independently and assemble to form the complete phage.

5) Lysis. The final step in phage multiplication is the lytic release of progeny phage. Lysis involved two or more gene products; at least one involved with an action on the membrane, and the other the action of lysozyme on the mucopeptide portion of the cell wall.

LYSOGENY

Definition

The stable (integrated) association of a temperate phage chromosome, in the prophage state, with the bacterial chromosome is called lysogeny. The prophage contains all the genetic information of the phage; however these genes are repressed.

Lysogen Formation

Following temperate phage infection, there is a critical period in which the viral particle either enters the lytic cycle or the lysogenic state. If lysogeny is to occur, this will be initiated by the synthesis of an mRNA which codes for a repressor protein. The repressor binds to the phage DNA in a region called the immunity region. This binding results in repression of virtually all phage directed syntheses and prevents the phage from entering the lytic cycle. The repressed phage chromosome becomes integrated in the host chromosome and remains in the lysogenic state.

INTEGRATION AND EXCISION

The next step is the attachment of the phage chromosome to the bacterial DNA. The phage chromosome circularizes by joining single stranded cohesive ends, which are linked together by DNA ligase. Reciprocal recombination occurs between the phage and bacterial DNA's resulting in a linear integration of the phage chromosome. Excision occurs by a reversal of the steps of integration.

PHAGE CONVERSION

Lysogeny often results in the expression of new characteristics by the bacterial population. These may be due to
1) expression of phage genes, or
2) the induction of previously silent bacterial genes.

A medically significant phage conversion system involves the relationship of C. diphtheria exotoxin (tox+). The gene responsible for the production of the diphtheria toxin is located on the chromosome of phage beta.

Phage conversion involving toxin production occurs in several other organisms. For example, erythrogenic toxin, the toxin responsible for the rash of scarlet fever, is produced by lysogenic strains of Streptococcus pyogenes. In addition, types C and D botulinum toxins of Clostridium botulinum are produced following infection of specific phages.

REVIEW STATEMENTS

These should be used to confirm your understanding of the subject. If you are uncertain about the veracity of a statement, please "check it out".

The number of mutants in a total population of cells is referred to as the mutant frequency.

Mutants which have additional nutritional requirements are auxotrophs.

A transversion occurs when a purine in DNA is replaced by a pyrimidine.

Acridines cause frameshift mutants.

Recombination occurs by breakage and reunion of DNA molecules.

A genetic test of function which depends on the interaction of gene productions is complementation.

A secondary mutation which eliminates the effects of a primary mutation is a suppressor.

General recombination requires extensive DNA sequence homology.

Ultraviolet light can cause thymidine dimers. Repair enzymes can correct this error.

Host cell enzymes involved in legitimate recombination include RecA, RecBC, nucleases, DNA polymerase and DNA ligase.

Polypeptide chain termination is caused by nonsense codons.

Temperate bacteriophages can enter either the lytic cycle or the lysogenic state.

Bacteriophages are made of two molecular components, nucleic acid and protein.

Virulent phages form clear plaques following infection into bacteria.

DNA ligase is a phosphodiesterase.

Abortive transduction occurs in general transduction when the donated DNA fails to recombine or replicate, but still functions. A term that describes this event would be complementation.

Cell lysis is promoted by the enzyme lysozyme.

Only lysogenic *C. diphtheriae* produce diphtheria toxin.

The type of gene transfer mediated by chemically isolated DNA is transformation.

Competence is a physiological state of recipient cells which is required for DNA binding in transformation.

Bacteriophage mediated transfer of genetic material is called transduction.

A phage chromosome that has integrated into the bacterial DNA is a prophage.

Chemotherapeutic agents that exert their bactericidal effect by inhibiting cell wall synthesis include penicillin, cephalosporin, cycloserine and bacitracin.

Genetic complementation tests are used to determine whether two different mutants carry mutations in the same cistron.

A secondary mutation which eliminates the phenotypic consequence of a primary mutation is called a suppressor.

The transfer of genetic determinants in a cell-free, phage-free, DNA preparation is transformation.

Competence is a physiological state of recipient cells required for successful transformation.

The bacteriophage mediated transfer of genetic information from one bacteria to another is termed transduction.

The conjugation bridge in bacteria is formed by F-pili.

The R-factor of gram negative enteric bacteria carries genes for resistance to several antibiotics.

Episomal transfer of resistance to antibiotics (RTF) occurs by the genetic mechanism called conjugation.

Following injection into a bacterial cell, the life cycle of a virulent bacteriophage consists of multiplication, packaging of nucleic acid, and cell lysis (release).

A bacterium is said to be lysogenic when it contains a bacteriophage chromosome integrated into its own chromosome.

Lysogeny is the stable association of the DNA of a temperate bacteriophage with the DNA of a bacterial host.

Following infection of sensitive bacteria by certain bacteriophages, the phage DNA becomes integrated in the host cell DNA and may influence or convert the recipient cell to produce new antigens or new toxins.

Repressors that have been characterized are proteins.

A codon is a trinucleotide complex that reacts with tRNA to code for a particular amino acid.

In a F' suc x F- cross the frequency of F factor transfer is high, as is the frequency of lac recombination.

A cryptic plasmid contains no known phenotypic traits.

Genetic exchange between two bacterial strains which is dependent on cell to cell contact is conjugation.

The male fertility factor in bacteria is an F-factor.

An F-factor integrated in the bacterial chromosome is an Hfr.

Phenotypic suppression is a temporary non-heritable alteration in translation and occurs only in the presence of added substances.

The transfer of hereditary characteristics via cell-free DNA to competent recipient bacteria is termed transformation; DNAse interferes with this process.

A generalized transducing particle of E. coli containing host DNA can transfer any of the bacterial genes to a sensitive bacterial cell.

F factors of E. coli are examples of episomes since they can replicate either autonomously or in an integrated state as an Hfr.

A mutation of DNA resulting in a transition results in one purine replacing another which may be caused by the analog 5-bromouracil.

Excision repair involves the removal of thymidine dimers from ssDNA leaving a gap, and then utilizes the complementary strand as a template to resynthesize a new portion of DNA, which is sealed by ligase.

Drug resistance (R) plasmids may also carry genes which promote conjugal transfer.

A missense mutation occurs when 1 base pair in a codon is replaced by another (with resultant replacement of 1 amino acid in the protein).

Nonsense codons cause polypeptide chain termination, due to a nucleotide change in the code which results in a termination signal (e.g., UGA).

Extrachromosomal, autonomously replicating circular DNA segments are called plasmids.

Episomes are plasmids that have the ability to also replicate as a part of the cell's own DNA.

Recombination occurs due to breakage and reunion of chromosome strands.

Genotypic suppression occurs when a second mutation "corrects" for a primary mutation.

Transformation is the DNA-mediated transfer of a limited amount of genetic information from a disrupted cell to an intact one.

For successful transformation the recipient cell must be in a particular physiologic state called competence.

Transduction is the type of gene transfer mediated by a bacteriophage.

Transduction involves the transfer of a limited amount of DNA from the lysed donor cell to an intact one.

Conjugation is a process of genetic exchange between two bacterial cells which is dependent upon cell-to-cell contact.

The F+ cell is the male "donor"; F- female cells are the recipients, which are converted to F+ as a consequence of conjugation.

Certain F+ cells can transfer DNA with high efficiently; these are called Hfr strains.

In Hfr strains the DNA integrates into the chromosome of the host cell.

F' cells are those which have a piece of donor chromosome DNA that has been transferred along with the F factor during conjugation. This process is called sexduction.

The cell appendage which is essential for conjugation is the F pilus.

Multiple drug resistance can be passed between bacterial strains by resistance transfer factors, RTFs, which are episomes.

Transposons are linear pieces of DNA, often containing r determinants, which promote their own transfer from one piece of DNA to another.

Transposons are flanked by insertion sequences, which are the unique nucleotide sequences which permit recognition of the correct area of the host genome and insertion of the genes into the chromosome.

The enzyme that splits the plasmid chromosome for recombination is a restriction endonuclease. DNA annealling of the "new" DNA into the plasmid is accomplished by DNA ligase.

MICROBIAL GENETICS PROFICIENCY TEST

These questions were used in a fall, 1985 course to sophomore Medical students. The mean on this test was 88 percent. I would suggest that any score below 70 percent is indicative of a deficiency in this area.

1. A prophage is

   A. induced from the lysogenic state by interference with DNA replication.
   B. replicated during cell division as part of the chromosomal DNA.
   C. one of the possible states of a virulent bacteriophage.
   D. all of the above.
   E. only A and B above.

2. In lysogenic conversion

   A. the phage contains the gene for the synthesis of the new toxins.
   B. the phage may effect or turn on the synthesis of several new antigens by the host.
   C. the host remains phenotypically converted, even after the loss of the phage.
   D. all of the above.
   E. only A and B above.

3. The repressor synthesized immediately after infection of bacteriophage lambda into E. coli

   A. turns off early and late phage protein synthesis.
   B. is a stable mRNA molecule.
   C. can repress functions of any heterologous or different phage.
   D. all of the above.
   E. only A and B above.

4. An auxotroph

   A. requires additional growth requirements and will not grow in minimal medium.
   B. is not affected by penicillin in minimal growth medium.
   C. grows normally in a nutritionally enriched medium.
   D. all of the above.
   E. only A and B above.

5. Shifts in the reading frame of a nucleotide sequence result in mutations because

   A. a nonsense mutation may be generated which causes chain termination.
   B. missense mutations are generated which cause incorporation of different amino acids and form a different protein.
   C. bacteria are prokaryotes and mammals are eukaryotes.
   D. all of the above.
   E. only A and B above.

6. Point mutations or base pair substitutions are caused by all of the following EXCEPT

   A. alkylating agents.
   B. nitrous acid.
   C. pyrimidine anologs.
   D. acridines.
   E. sulfur mustards.

7. Thymidine dimers are formed in DNA due to the effect of

   A. ionizing radiation.
   B. ultraviolet radiation.
   C. alkylating agents.
   D. photoreactivation.
   E. all of the above.

8. If DNA is damaged by an alkylating agent causing interstrand links, the most effective type of repair would be

   A. direct repair.
   B. photoreactivation.
   C. excision repair.
   D. postreplication repair.
   E. suppression.

9. Competence factor

   A. allows chromosomal DNA to be packaged in transducing phages.
   B. is present on the tips of F pili and is necessary for effective pair formation.
   C. is the protein which promotes genetic complementation.
   D. binds DNA to the cell surface of recipient cells during transformation.
   E. promotes induction of prophages from bacterial lysogens.

10. An F factor

   A. promotes transfer of chromosomal DNA from a Hfr to a F- cell.
   B. is a circular DNA containing the genes for F pili synthesis.
   C. is found in all female E. coli cells.
   D. all of the above.
   E. only A and B above.

11. Transformation of Streptococcus pneumoniae

   A. occurs by the uptake of double stranded DNA.
   B. occurs by calcium ion induced changes in cell wall and membrane permeability.
   C. requires specialized phage induced by lysogens.
   D. all of the above.
   E. only A and B above.

12. Naturally occurring genetic recombination involves all of the following EXCEPT

A. recA protein.
B. recBC protein.
C. a structural change in the chromosome.
D. restriction enzymes.
E. DNA ligase.

13. A +1 frameshift mutation in a gene can be corrected by

A. a -1 frameshift mutation near the primary mutation in the same gene.
B. a +1 frameshift mutation in the anticodon region of a tRNA gene.
C. a nonsense mutation in a tRNA gene.
D. all of the above.
E. only A and B above.

14. All of the following are genetic processes which enable cells to overcome a mutation EXCEPT

A. repair.
B. recombination.
C. suppression.
D. complementation.
E. lysogeny.

15. The term that most appropriately describes abortive transduction is

A. suppression.
B. lysogeny.
C. complementation.
D. recombination.
E. competence.

16. In a F' lac x F- cross

A. the frequency of F factor transfer is high and the frequency of general recombination is high.
B. the frequency of F factor transfer is high and the frequency of lac recombination is high.
C. the frequency of F factor transfer is low and the frequency of general recombination is high.
D. the F' lac is rarely transferred because it is integrated in the chromosome.
E. recombination of lac or any other gene will occur only if the recipient cells are competent.

17. Specialized transducing phage are produced by

A. induction of an integrated prophage out of the bacterial host chromosome.
B. treatment of the F+ cell with acridine orange to obtain cured cells.
C. infection of sensitive bacteria with a bacteriophage.
D. mutagenesis of bacteriophage in the lytic cycle.
E. genetic complementation.

18. A genetic element capable of moving from one chromosome to another that is independent of recA function is a

    A. transvertant.
    B. transition.
    C. transposon.
    D. transductant.
    E. transformant.

19. Antibiotic resistance genes responsible for most clinical types of resistance are found predominantly in

    A. plasmid DNA.
    B. chromosomal DNA.
    C. both.
    D. neither.

20. Which of the following statements concerning plasmids is <u>false</u>?

    A. A cryptic plasmid contains no known or described phenotypic traits.
    B. Plasmids are replicons capable of autonomous replication.
    C. Plasmids contain genes which specify enterotoxins and hemolysins involved with disease.
    D. Plasmid copy numbers may be higher than the number of chromosomes.
    E. Plasmids can be specifically cleaved by recA associated proteases.

21. Restriction endonucleases are enzymes found in bacteria that

    A. degrade DNA sequentially beginning at the 5' end.
    B. cleave DNA at sequence-specific sites to yield either sticky (single stranded) or blunt ends.
    C. form phophodiester bonds at the site of a single strand break in DNA.
    D. are an integral part of genetic recombination.
    E. cleave a portion of the gene that is transcribed but do not appear in the final mRNA transcript.

22. DNA vectors used in recombinant DNA technology should have all of the following properties EXCEPT

    A. a selectable phenotype.
    B. single sites for restriction enzymes.
    C. autonomous replication.
    D. ability to transfer genes to viable cells.
    E. intervening sequences.

For each of the incomplete statements, one or more of the completions given is correct. On the answer sheet fill in the space under

A. if only (1), (2) and (3) are correct.
B. if only (1) and (3) are correct.
C. if only (2) and (4) are correct.
D. if only (4) is correct.
E. if all are correct.

23. Bacterial R factors

1. are extrachromosomal pieces of circular DNA.
2. are carriers of the genetic loci for resistance to aminoglycosides.
3. are carriers of the genetic loci for resistance to penicillins.
4. are transferable to the same or closely related species of bacteria.

24. Genetic transfer in bacteria occurs by

1. transformation.
2. conjugation.
3. transduction.
4. complementation.

ANSWERS

1. E
2. E
3. A
4. D
5. E
6. D
7. B
8. D
9. D
10. E
11. A
12. D
13. E
14. E
15. C
16. B
17. A
18. C
19. A
20. E
21. B
22. E
23. E
24. A

REVIEW OF IMMUNOLOGY

DEFINITIONS

Adjuvant - A substance that increases the production of antibodies, prolongs the productive phase of the immune response, or directs the response to a particular compartment.

Allergen - Substances which have a propensity to induce an allergic state.

Allotype - Genetically determined, polymorphic antigenic variations in a given plasma protein which occur within a species.

Anamnesis - The heightened immune response resulting from a second exposure to an antigen some time after the primary exposure.

Anaphylatoxins - Mediators of inflammation produced during the activation of complement.

Arthus Reaction - Local immune complex injury characterized by erythema, edema, hemorrhage, and necrosis.

B Cell - Lymphocyte derived from the bursa of Fabricius (or bursa equivalent) precursor of the antibody-forming plasma cell.

Blocking Antibody - Antibody of the IgG class which combines with allergen and prevents it from reacting with cell-fixed IgE.

Coombs Test - Use of antiglobulin antiserum produced in a heterologous species to detect nonagglutinating antibodies on red blood cell surfaces.

Epitope - The part of an antigen that directs specificity. Antibodies produced in response to antigen will react specifically with each individual determinant group, or epitope.

Domain - Segments (loops) on H and L chains formed by intrachain disulfide bridges.

Endotoxin - Toxic lipopolysaccharides from the cell wall of Gram-negative bacteria. A mitogen for B cells.

Heterophile Antigen - Antigen which occurs in tissues of many different species and is therefore highly cross-reactive.

HLA - Human Leukocyte Antigen; the major histocompatibility (MHC) locus in man.

Idiotype - Unique antigenic determinant on the antigen-binding region of an immunoglobulin molecule.

Immunosuppression - General reduction in immune responsiveness.

Interferon - Protein(s) elaborated by virus-infected cells capable of inducing protection from viral infections in noninfected cells. There are three types of interferons; alpha, beta and gamma.

Isohemagglutinin - Antibodies to red blood cells that agglutinate red cells of other individuals of the same species.

J Chain - Glycoprotein found in polymeric forms of immunoglobulins such as IgA and IgM (J = joining).

Leukotriene B4 - a lipoxygenase metabolite of arachidonic acid, has chemotactic properties. Leukotrienes C4, D4 and E4 are vasoactive and cause an increase in capillary permeability.

Lymphokines - Hormones secreted by lymphocytes which cause maturation and proliferation of various cells in the body.

Lysozyme - A mucopeptidase found in a variety of body fluids, e.g., tears, saliva, and serum.

Memory Cell - Lymphocyte which mediates immunological memory (anamnesis).

Monoclonal Gammopathy - Overproduction of immunoglobulins or their fragments by a single clone of plasma cells.

Null Cell - Lymphocyte lacking surface characteristics to identify it as either a B cell or T cell.

Oncofetal Antigen - Antigen found in both fetal and tumor tissue.

Prostaglandins - cyclooxygenase metabolites of arachidonic acid which cause smooth muscle contraction and increase vascular permeability.

Rhogam - Rh (D antigen) immune globulin used for the prevention of erythroblastosis fetalis.

Secretory Component - Glycoprotein synthesized by epithelial cells and apparently essential for the release of the dimeric secretory IgA.

Serum Sickness - Systemic immune complex injury characterized by fever, rash, splenomegaly, lymphadenopathy, arthritis, glomerulonephritis.

Suppressor T Cell - T cell involved in suppression of the immune response.

Titer - The highest dilution of a given substance (e.g., antibody) that will still produce a reaction with another substance (e.g., antigen).

Valence - Number of reactive (combining) sites on an antigen or antibody.

INNATE IMMUNITY

Immunity (resistance to, or assistance with, an infectious disease) can be acquired by various means which are summarized below.

Role of Recipient	Method	Examples	Duration*
Passive	Natural	IgG across placenta; IgA in colostrum**	months days
	Artificial	Horse antitoxin Human gamma globulin	days weeks
Active	Natural	Recovery from infection -clinical or subclinical	months to years
	Artificial	Vaccination with agent*** or product	months to years

*duration of immunity will vary with:

1. Amount and class of gamma globulin received,
2. Foreignness of gamma globulin to recipient,
3. Nature of infectious agent (i.e., there is no post-infection immunity with some agents; e.g., Staphylococcus and Herpes), and
4. Type of vaccine (attenuated are better than killed)

** gut immunity only; sIgA does not get into the circulation.

***stimulates production of specific, protective antibody which will enhance phagocytosis or neutralize the toxin, virus, etc., dependent upon the nature and composition of the vaccine.

The immunoglobulin which crosses the human placental barrier is _____.

Transplacental immunity has a duration of 4 - 6 (days/weeks/months/years) in humans.

Recovery from measles should induce an _____ immunity which will persist for (days/weeks/months/years).

Secretory IgA in colostrum is an example of _____ immunity. It is effective in protecting the (gut/central nervous system).

Pooled human gamma globulin will provide a passive immunity of longer/shorter duration than would horse antitoxin.

The major phagocytic cells of the body are the _____ and the _____. (see next page for answer).

CELLULAR DEFENSE MECHANISMS

1. Principal cells are polymorphonuclear leukocytes (PMN's) and monocytes in the blood, and tissue macrophages. While these are not the only cells which phagocytize, they are most important.
 A. All are capable of ameboid movement - that is, they can move in and out of blood vessels.
 B. Chemotaxis - they move because they are attracted by certain chemicals, i.e., tissue components, and complement components C5a and C5b67, as well as leukotriene B4 and endotoxin (LPS).
 C. Once close enough to the target, they engulf it.
 D.They digest, or attempt to digest, particles engulfed.

2. Phagocytosis - First a phagosome is formed by the phagocyte when it engulfs the particle and surrounds it with a part of its cell membrane. This pinches off and moves into the cell's cytoplasm. In the cytoplasm are lysosomes, or membrane-bound bags of proteolytic enzymes, which fuse with the phagosome (phagolysosome). Phagocytosis is accompanied by an increase in lactic acid production, O_2 uptake and hexose monophosphate shunt activity.

3. Enhancement - Certain factors increase the efficiency of phagocytosis. Surfaces, such as the vascular wall and fibrin clots, provide a support upon which the phagocytic cell operates. Antibody and complement opsonize by reacting with Fc and C3b receptors in the phagocytic cell's membrane. In addition, certain microbial products, such as endotoxin, increase the efficiency of the reticuloendothelial system (RES).

4. Natural Killer (NK) cells are lymphocytes which are present prior to Ag stimulation; their numbers do not increase with immunization. They react wtih foreign tissues, tumors and virus-infected cells and rid the body of them. They are neither T nor B cells, but have some membrane markers of each. They also have a monocyte marker.

The essential steps in phagocytosis include:

1. _____

2. _____

3. _____

Chemotactic factors which are generated during complement activation include _____ and _____.

The energy required for efficient phagocytosis is generated by the _____.

Opsonization, the facilitation of the engulfment process, is enhanced by

1. the Fc portion of the antibody.
2. C3b molecules that adhere to the surface of the particle.
3. Both
4. Neither

(Answer on next page.)

5. Killing occurs via the contents of the lysosomes, which include:

A. Myeloperoxidase + H_2O_2 + Halide (Cl- or I): kills via halogenation of the organism, also toxic hypochlorite is generated, as are toxic aldehydes. H_2O_2 (as well as singlet oxygen, superoxide anion and hydroxyl ion) also come from HMP shunt.

B. Lysozyme is another antimicrobial substance found in lysosomes, as well as in tears, saliva, and most body fluids. This enzyme attacks the bacterial cell wall and lyses it, both in gram+ and gram- bacteria. It is a mucopeptidase.

C. Fatty Acids - are antimicrobial.

D. Toxic Proteins - basic proteins are found in lysosomes. They are very effective antimicrobial agents and seem to be simple polypeptides, as (Arg)n and (Lys)n.

E. Phagocytin - kills microorganisms and is more active at low pH.

F. Acid Hydrolases - phosphatases, glucuronidase, cathepsin, etc.

6. Cellular defense mechanisms are also enhanced by substances released from monocytes (monokines) which effect other aspects of resistance.

A. LAF = lymphocyte activating factor (interleukin I)

B. Prostaglandins which increase vascular permeability

Antimicrobial contents of the lysosome include:

1. lactoferrin, an Fe binding protein

and

2. _____

3. _____

4. _____

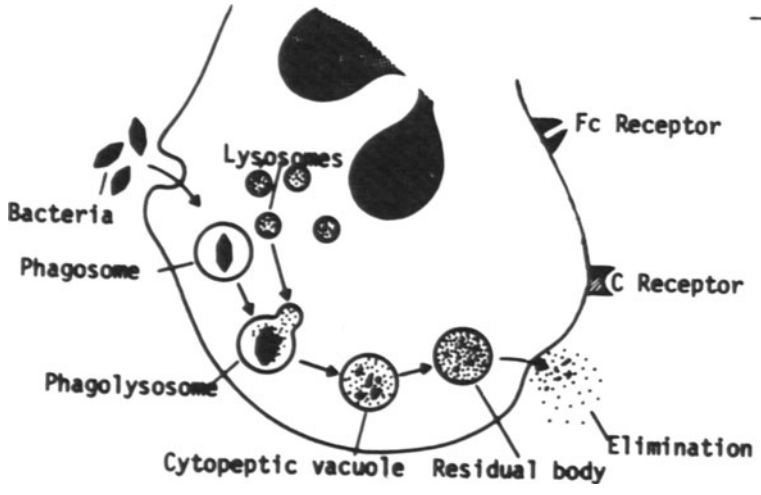
5. _____

6. _____

7. _____

_____ kills bacteria by halogenation of essential microbial components such as transport enzymes, etc.

The mucopeptidase which strips the cell wall off many bacteria is called _____.



Answer = 3

ANTIBODY STRUCTURE AND FUNCTION

Antibodies are divided into 5 classes on the basis of their antigenically distinct heavy chains. Any one antibody molecule will have 2 identical heavy chains per unit. There are two types of light chains called kappa and lambda, but in any one antibody molecule the light chains are both of the same type. There are subtle differences in the constant region of the heavy chains and light chains which are called allotypes, and they are analogous to the differences in blood type, i.e., they impart unique antigenic specificity to the molecule. The allotypes are designated Gm for gamma chain, Am for alpha chain, and Km for kappa chain. Heavy chain class is controlled by isotypic determinants also found in the constant region. Unique determinant groups are found in the variable region, associated with the antigen-binding (Fab) capability of the molecule. These are called idiotypes. Anti-idiotype Ab will resemble the original antigenic determinant group.

Antigen binding occurs at the

variable/constant portion of the H/L/both

chain(s).

CHARACTERISTICS OF ANTIBODY MOLECULES

Characteristic	IgG	IgA	IgM	IgD	IgE
Molecular Wt.	150,000	150-400,000	900,000	180,000	190,000
Serum level (mg%)	1,200	200	100	5	5µg
Saliva level (mg%)	5	30	1	-	-
Colostrum level (mg%)	10	1,200	50	-	-
Half life	21 days	5	5	3	1(?)
Valence	2	2-8	10	2	2
J chain	-	+	+	-	-
Secretory piece	-	+	-	-	-
Heavy chain	gamma	alpha	mu	delta	epsilon
Heavy chain subclasses	4	2	2	0	0

CHEMISTRY

Figure 1 is the structure of the IgG molecule, and this structure is also the same basic structure for all five classes. The molecule is composed of four polypeptide chains: two heavy and two light chains joined by disulfide bonds. Enzymatic treatment of this molecule with papain splits it into three fragments. The parts containing the heavy and ilght chains are called fragment antigen binding, or Fab, and this end of the molecule is the one that binds to the antigen. Each Fab molecule has a valence of 1. Pepsin splits below the disulfide interchain bond, producing a divalent molecule, $F(ab')_2$.

The fragment containing only heavy chains is called fragment crytstallizing, or Fc, and this portion binds serum complement, contains some carbohydrate, and is the constant region of the molecule. Fd refers to the heavy chain component of Fab. Since the immunoglobulin is a polypeptide, there must be a N terminal end and a C terminal end. The N terminus binds to the antigen while the C terminus dictates whether or not the molecule will pass through the placenta, fix complement, attach to mast cells, etc.

The variable and constant regions of each chain are divided into domains, which are compact, globular loops of the polypeptide stablized by S-S bonds. The light chains have 2 domains, V, and C, gamma and alpha chains have 4, mu and epsilon, 5.

Papain treatment of an immunoglobulin molecule splits it into the following fragments:

_____ and

_____.

The Fc portion of the immunoglobulin molecule has several biological functions and/or chemical characteristics, among which are

_____,

_____,

_____,

_____, and

_____.

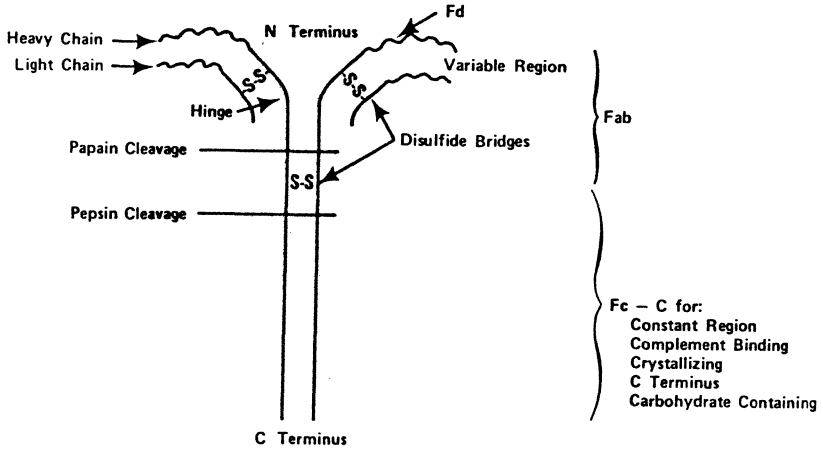


Figure 1. Component parts of an immunoglobulin G molecule.

FUNCTIONS

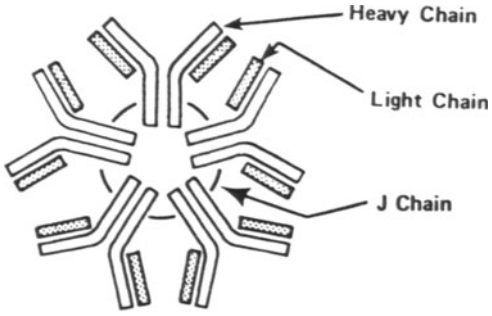
IgG is the only immunoglobulin that can cross the placenta. It has gamma type heavy chains. It is the most abundant antibody in serum.

IgA is a variable molecule because it can exist as a dimer in secretions or a monomer in serum. H chains are α type. A type of IgA is called secretory IgA, which is two IgA molecules joined together by a secretory component which enables the molecules to leave the secretory epithelial cell. The "J" or joining chain also aids in stabilizing the dimer. This antibody is responsible for the immune reactions involving such secretions as saliva, colostrum, tears, bronchial mucous, intestinal tract and urinary tract secretions. It is responsible for local (mucosal) immunity.

IgM is a macroglobulin that exists as a pentamer, held together by S-S bonds and a single J chain. Its heavy chains are mu type. IgM is the most efficient Ab in C-mediated lytic reactions and agglutination. IgM is the first antibody synthesized after antigen challenge and is the primary antibody synthesized in utero.

IgD may constitute a primitive antigen receptor on lymphoid cells. It occurs on fetal and leukemic lymphocytes. Heavy chains are delta type.

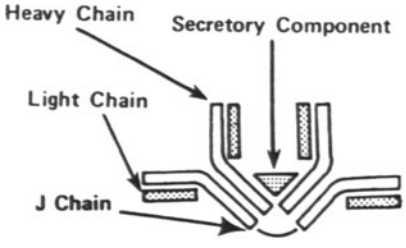
IgE is responsible for the immediate types of hypersensitivity (atopy and anaphylaxis). It is involved in allergies such as asthma, hay fever and reactions to foods, as well as in parasitic infections. It appears to be involved in immunity to certain parasitic disease, e.g., ascariasis. Epsilon heavy chains are found in IgE. The molecule has a high affinity for mast cells and basophils.



IgM Globulin

The IgM IgG switch occurs in a committed Ig producing cell. It is accompanied by a change in the constant heavy gene (change from $C_{H}\mu$ to $C_{H}\gamma$). There is no change in the variable domain genes.

The blocking antibodies induced in atopic allergies are IgG.



Secretory IgA Globulin (sIgA)

IMMUNOPROLIFERATIVE DISEASES (MONOCLONAL GAMMOPATHIES) involve an overproduction of immunoglobulins or their fragments by a single clone of plasma cells;

Multiple myeloma - most cases involve IgG. Bence-Jones protein (dimer of light chains) may be found in urine.

Waldenstrom's macroglobulinemia involves IgM overproduction.

Heavy chain disease involves production of incomplete heavy chains. The most common is alpha heavy chain (IgA) disease characterized by intestinal lymphoma.

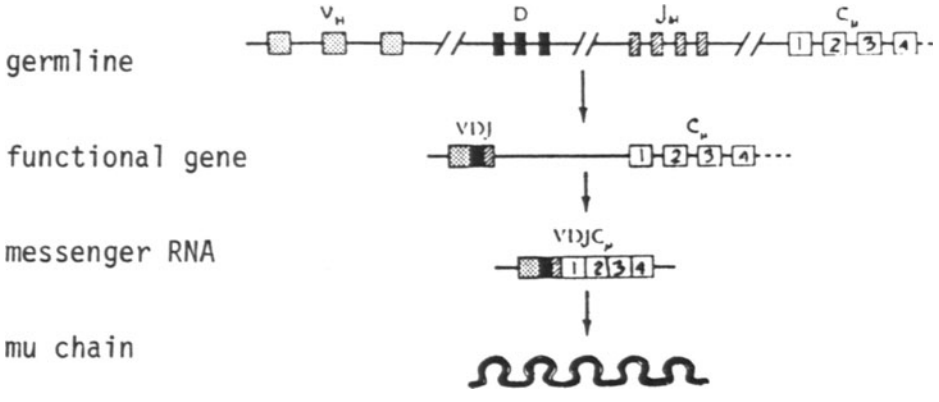
Amyloidosis in some patients is due to deposition of variable domain fragments of light chains in various organs (e.g kidney).

Cryoglobulinemia and pyroglobulinemia, commonly associated with multiple myeloma, indicate presence of, usually, abnormal IgM molecules in the blood which precipitate at low temperature (cryoglobulins) or high temperature (pyroglobulins).

Antibody diversity is due to rearrangement in the DNA. This occurs before contact with the antigen.

Review of the Genetics of Immunoglobulin Synthesis

On line 1 the germline chromosomal segment(s) for the mu heavy chain are diagrammed. One each of the many V region genes is selected and linked through rearrangement to selected D and J region genes. This trigenic complex codes for the variable portion of the mu chain. It is transcribed, along with the appropriate C region genes to form the mRNA, which serves as the template for the mu chain polypeptide.

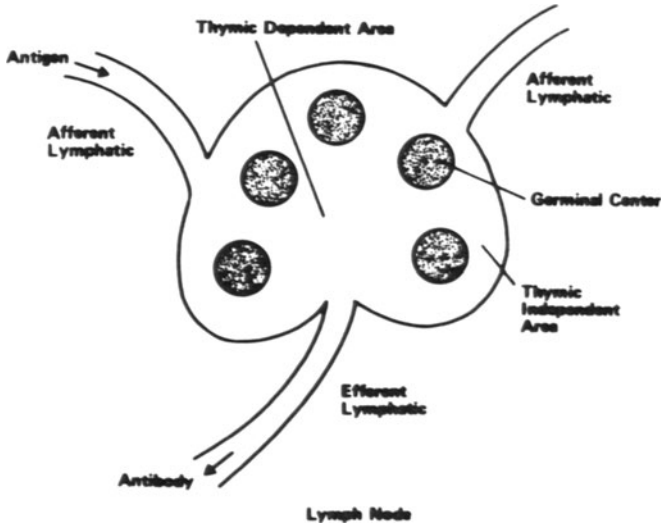


Light chain genes have a similar structure but are on a different chromosome and lack the D region.

IMMUNE RESPONSE

TERMS TO KNOW

Thymus dependent areas of lymph node - Juxtamedullary (paracortical) areas involved in cell mediated immunity.
Thymus independent areas - germinal centers involved in Ab synthesis.
Primary immune response - first exposure to Ag - mainly IgM Ab produced.
Secondary immune response - second exposure - mainly IgG
Booster - anamnesis or memory, much more rapid rise in Aby level
Adjuvant - substance which greatly enhance the immune response

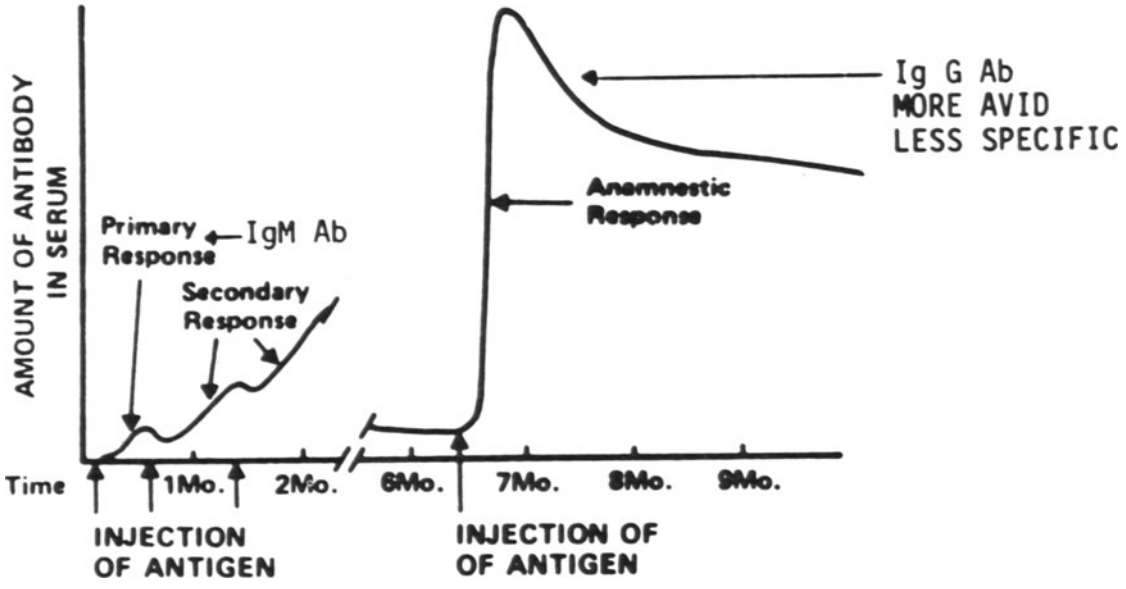


ANAMNESIS

The first response to antigen is mainly IgM; there is a feedback to IgM and its production is shut down. The cell then switches to production of IgG. With the booster antigen challenge, antibody goes to a higher level and stays there longer. The challenge boosts antibody production. Immune memory depends on both B and T cells, although T cells seem to be most important. It takes far less Ag to produce an anamnestic response than to initially immunize.

IMMUNOGENICITY = SIZE + FOREIGNNESS + CHEMICAL COMPLEXITY

PHASES OF THE IMMUNE RESPONSE

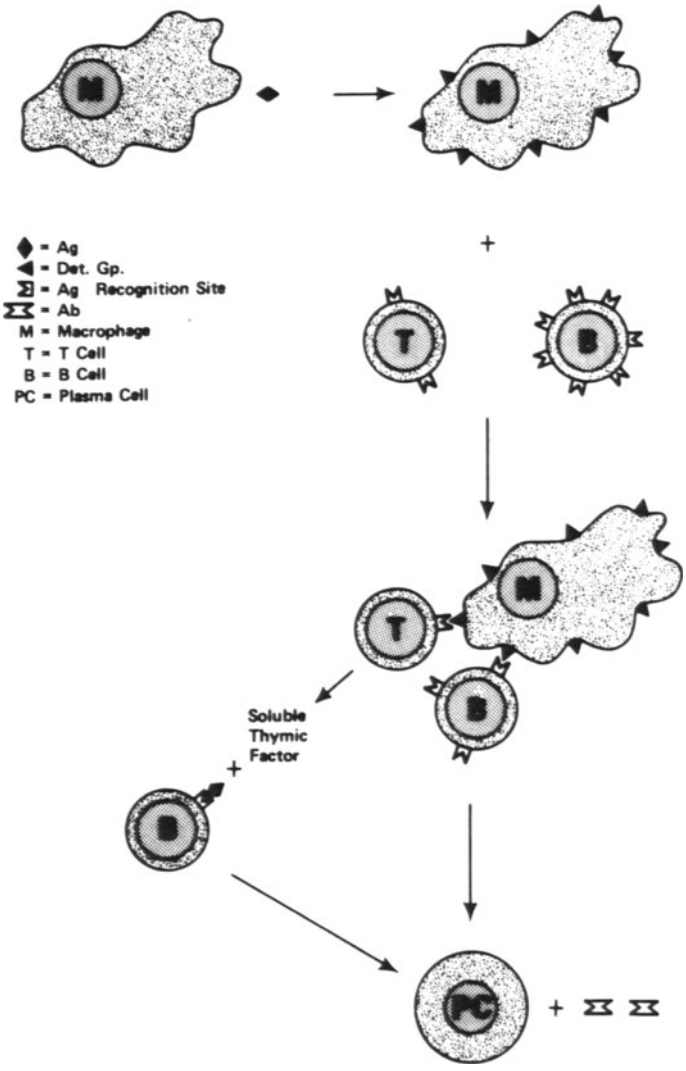


THE CELLULAR EVENTS OF THE IMMUNE RESPONSE

When an antigen enters a node (or the spleen), it first encounters a macrophage. It is processed and presented on the cell membrane. A lymphocyte with a membrane-bound IgM homologous to the antigen then reacts. The macrophage releases a monokine, lymphocyte activating factor (LAF or Interleukin I) which stimulates the lymphocyte to divide and differentiate. In most immune responses there are 2 lymphocytes; a T helper cell, and a B cell (the plasma cell precursor). The T helper cell releases a lymphokine called Interleukin II or T cell growth factor, which aids in the T cell proliferation events. The interaction between the T and B lymphocytes is called collaboration. Both humoral and cellular immunities are produced to some degree, depending on the antigen and how it is administered.

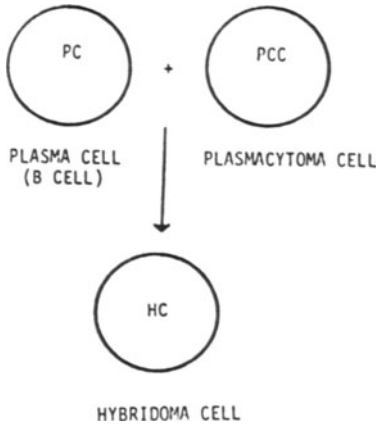
Most immune responses are T dependent: all cell-mediated responses are, and most of the humoral ones also require a T helper cell. The few that do not are called T independent responses. The antigens which are responsible (T independent antigens) are usually composed of monotonously repeating units (e.g., CHO, LPS). The responses are IgM in nature and do not show an anamnestic response. Some T independent antigens are polyclonal B cell activators.

Macrophages are essential for immune responses. However, they do not produce antibody and, in contrast to B and T cells, do not demonstrate any selectivity in the antigens with which they react. The in utero maturation of B and T cells is antigen- independent.



HYBRIDOMAS

Hybridomas are artificially created cells that produce pure or "monoclonal" antibodies. Having a constant and uniform source of pure antibody, instead of the usual mixture produced by the immune system, not only affords a powerful research tool but can be expected to provide quicker and more accurate diagnosis of viruses, bacteria, and cancer cells. The long-range promise of monoclonal antibodies is that they will be therapeutically useful as vaccine replacement and in the treatment of cancers.



GENETICS OF THE IMMUNE RESPONSE

There are three genes involved in synthesis of a light chain, 1 each for the variable, constant and joining reginos (V, C, and J respectively). These are selected from a large "library" of genes linked on the same chromosome. There are four genes involved in the synthesis of a heavy chain, V, C, J, and D (for diversity). These also are selected from a large gene pool and re-arranged to serve as an mRNA template.

ONTOGENY OF THE IMMUNE RESPONSE

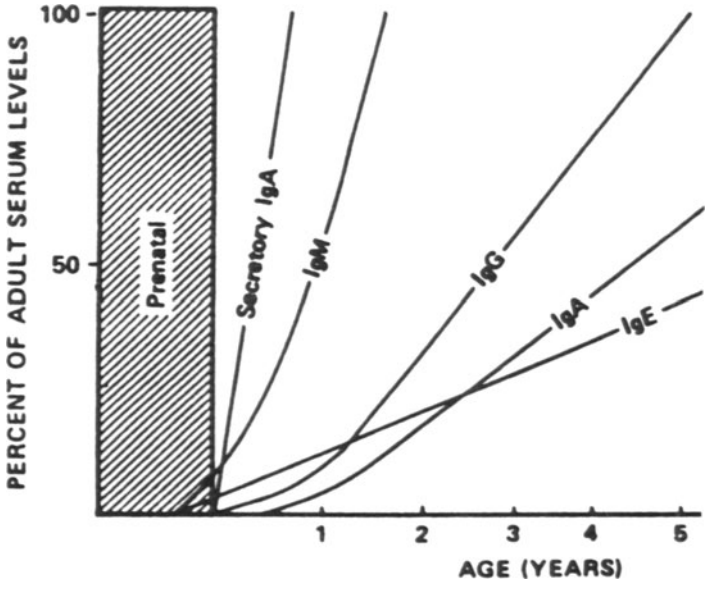
IgM is the first immunoglobulin to be produced by the fetus (ca. 3 months). IgG is the major immunoglobulin in fetal serum, due to the large amount which is acquired via the placenta (IgG of maternal allotype). Maternal IgG in newborn prevents optimal priming immediately after birth for the corresponding Ag. Elevated levels of IgM in cord blood may signify congenital infection (e.g., rubella or syphilis). IgA is not produced in utero: production beginning shortly after birth. Adult levels of these immunoglobulins are attained at approximately 1, 4 and 8 years, respectively. Adult IgE levels are not seen until 14-16 years of age. Both IgM and IgD are found on lymphocyte membranes in utero.

Haptens - These materials cannot induce Ab formation unless conjugated to a material which increases their size and the density of haptenic groups per molecule (e.g., carrier molecules). They will, however, react with the antibody induced, even in the absence of a carrier molecule. Most substances used to impart immunogenicity to haptens are antigenic themselves; in fact, in certain systems, they must be immunogenic to the recipient. The Ab induced to the hapten- carrier complex will be heterogenous; i.e., Ab vs. carrier, Ab vs. hapten and perhaps Ab vs. area of conjugation. The carrier effect demonstrates the importance of the carrier molecule in the anamnestic response to the hapten. A secondary response to the hapten will not occur if the carrier used in the booster injection is different from that employed to prime the animal.

Congenital infections will cause an increase in fetal IgG/IgM levels in cord blood.

The null period is the time of greatest risk for an infant. It is when the maternal Ab is waning and the infant is just beginning to synthesis its own IgG.

Adult levels of most of the immunoglobulins are reached by the age of _____ years.



However, if the animal was immunized to the second carrier (without attached hapten) previously, then a normal anamnestic response to the hapten will occur. Thus priming in both the B (hapten specific) and T (carrier specific) components of the immune response must occur to demonstrate the booster phenomenon.

The secondary response to the injection of an immunogen is called the _____ response.

CELLS INVOLVED IN THE IMMUNE RESPONSE

Characteristic	B Cells	T Cells
Receptor for E.B. Virus	+	-
Binding of Specific Antigen	+	+
Increased in Secondary Response (Memory cells)	+	+
Rosette with sheep RBC	-	+ (E rosette)
Complement receptor (EAC rosette)	+	-
Site of gamma globulin synthesis	+	-
Phytohemagglutinin (PHA) receptor	-	+
Concanavalin A receptor	-	+
Pokeweed receptor	+	+
LPS	+	-

The lymphocyte that has a membrane receptor for PHA is the T/B cell.

Receptors for complement components are found in the membranes of

1. B cells.
2. T cells.
3. Macrophages and PMNs.
4. All of the above.
5. 1 and 3 only.

(Answer on next page)

SURFACE ANTIGENS ON THYMOCYTE (T CELL) SUBCLASSES

	Cell	Memory	Helper	Cytotoxic	Suppressor	Cell-mediated Immunity
Antigen	T4	+	+	-	-	+
	T8	-	-	+	+	-

T1, T3 and T11 are on all peripheral T cells; T10 is found on activated, peripheralized T cells.

IMMUNE SUPPRESSION AND TOLERANCE

Unresponsiveness - the absence of an immune response to a substance that under ordinary conditions would be antigenic. The substance has all the features necessary for antigenicity, but there is no immune response to it. Unresponsiveness can be divided into two broad categories, (1) tolerance, and (2) immunosuppression. The first is antigen specific; in the second, immunosuppression, the host is in a state of general immunologic impairment, i.e., limited or absent response to many antigens.

Natural or autotolerance is the inability to mount an immune response to one's own antigens. Immune tolerance is an artificially induced state of immunologic unresponsiveness. Factors which influence the establishment of this state include:

1. Age - the younger the animal, the more easily it is rendered tolerant.
2. Immune status - Obviously correlated with (1) above. However, adults can be rendered tolerant if first immunosuppressed by X-ray or drugs.
3. Amount of Antigen - Less antigen is required to induce T cell tolerance than B cell tolerance.
4. Nature of Antigen - the simpler the antigen, the more efficient it is as a tolerogen. Haptens alone are often good tolerogens; polysaccharides with only a few determinant groups are also effective. Viruses and bacterial cells are very poor tolerogenic materials, as are complex proteins (particularly in aggregated form) due primarily to their antigenic omplexity and ready interaction with phagocytic cells.

The loss of the capacity to make an immune response might be due to deletion of Ag-reactive B cells or T cells or to generation of suppressor cells.

General immunosuppression is encountered in many natural states, including malignancy, senility, developmental difficulty (e.g., di George syndrome) and certain

Cancer patients who are receiving chemotherapy are often susceptible to opportunistic infections. This is due to a state of General/Specific immune suppression.

The state of antigen (epitope) specific immune tolerance is most easily induced in T/B cells?

Autoimmune diseases occur because the body has bypassed the natural state of _____.

Molecules which interfere with DNA synthesis should be GOOD/BAD general immunosuppressants.

General immunosuppression is encountered in the following natural conditions:

1. _____
2. _____
3. _____
4. _____

Answer to C receptor question = 5

infectious diseases such as measles, where a trasient loss of cell mediated immunity may be observed. In addition, perinatal removal of the central lymphoid organs (thymus and bursa or its equivalent in mammals - GALT = gut associated lymphoid tissues) produces deficits in cellular and humoral immunity, respectively.

Immunosuppression may also be induced by chemical or biological treatment regimens, unfortunately often similar to those used in the treatment of autoimmune diseases and cancer.

IMMUNOSUPPRESSIVE AGENTS

A. Lymphotoxic agents - include (1) alkylating agents such as nitrogen mustard and Cytoxan, (2) steroids such as hydrocortisone and prednisone, and (3) X-irradiation. All these agents destroy lymphocytes by damaging their DNA so that their replication is inhibited. (Corticosteroids are thought to have an additional anti-inflammatory action by stabilizing lysosomal membranes.)

B. Anti-metabolites - include such things as purine analogs, pyrimidine analogs and folic acid antagonists. They damage the DNA of lymphoid cells so that they can't divide and become Ab-forming cells. The specificity of all these pharmaceutical agents and X-rays is not directed toward lymphoid cells per se, simply toward rapidly dividing, DNA-synthesizing cells.

C. Antibiotics - not often used clinically for immunosuppression, they include Actinomycin D, Mitomycin C, and Chloramphenicol. Cyclosporin A has recently been shown to be a highly potent immunosuppressive agent.

Answers to questions at top right side of page:

1 = B
2 = A, C
3 = A, C
4 = A, C
5 = A, C

MATCHING

Match the immunosuppressive drug with its mechanism of action.

DRUG 1. Anti-thymocyte serum

2. Alkylating agents

3. Ionizing radiation

4. Cytoxan

5. Steroids

ACTION A. Lymphotoxic

B. Lympholytic

C. Damage to DNA

D. Anti-metabolites

(Answers at bottom of this page, left hand side

D. Antibodies - used to inhibit immune responses in two ways. The first kind of antibodies are Ab that react with lymphoid cells, such as antilymphocyte globulin or antilymphocyte serum. This is the means of attacking peripheral lymphoid tissue to produce immunosuppression. Anti-lymphocyte serum, particularly anti-thymocyte serum, is most useful in trans-plantation patients. It is lympholytic.

There is another use of Ab in immune suppression. If a preformed Ab is injected into an animal, followed by injection of that particular Ag, Ab formation in the host will be blocked. The injected Ab binds the injected Ag, and prevents access of lymphoid tissue to that injected Ag. This is the principle through which Rhogam was developed to combat the Rh incompatibility problem. Ab against the immunogen (Rh° antigen) will neutralize the Ag through some mechanism, either by neutralizing the Ag or by coating it in such a way that its cleared very rapidly. Thus there is a very short time of access of Ag to the lymphoid tissue, which would respond were it not for the injected Ab. Maternal antibody in a new born prevents optimal priming immediately after birth for the corresponding antigen.

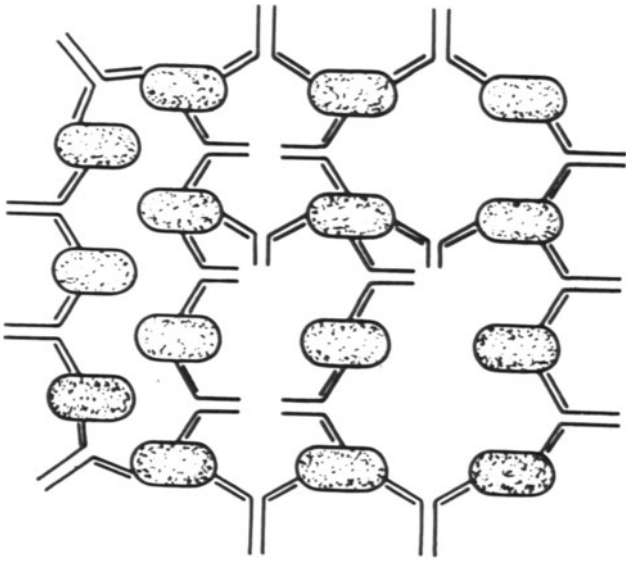
In the table at the bottom of this sheet, why was there no serious consequence of the Rh- infant born as a result of the second pregnancy? (answer on next page)

Rh ANTIGEN PROFILE

PREGNANCY	MOTHER	INFANT	TREATMENT	CONSEQUENCES
FIRST	Rh+	Rh-	NONE	NONE
	Rh-	Rh+	NONE	NONE, but mother may become sensitized to Rh antigen durir birth process
	Rh-	Rh+	RhoGAM	NONE, and Rh antigen sensitization will NOT occur.
SECOND (et seq.)	Rh-	Rh+	NONE	Infant may develop hemolytic disease (erythroblastosis fetalis), due to maternal IgG destroying Rh+ RBCs of the child.
	Rh-	Rh-	NONE	NONE
	Rh-	Rh+	RhoGAM after 1st birth	NONE

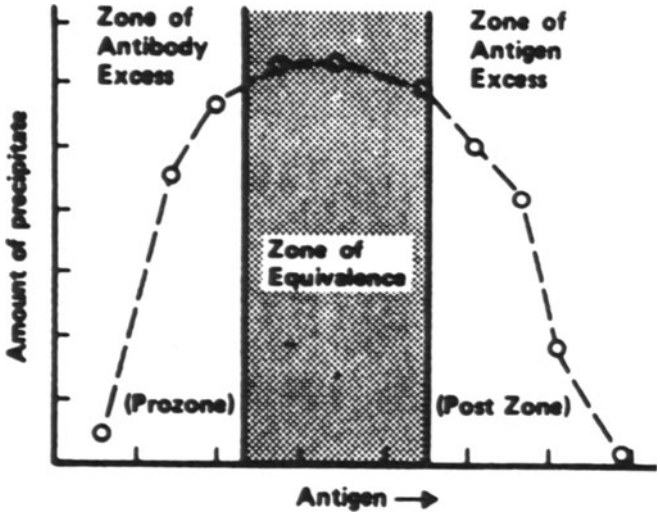
ANTIGEN:ANTIBODY REACTIONS

1. The antigen - antibody complex is not bound very firmly together: They even dissociate spontaneously, although the association constant is very high (e.g., 1×10^9 for insulin:anti-insulin interaction).
2. Two main electrostatic forces act to hold the complex together:
 A. Van der Waals forces (act due to spatial fit).
 B. Coulombic forces: electrostatic interactions between positive and negative charges.



Lattice structure of antigen-antibody reactions.

If one plots on a graph the antibody-antigen precipitate versus the amount of antigen added to a fixed amount of antibody, one notices the following:



Where the antigen concentration is very low with a relative superabundance of antibody (Zone of Ab excess), formation of complexes occurs but residual antibody will remain in the supernatant. This area is known as a prozone. As more antigen is added, the antibody (AB) - antigen (Ag) complex forms a precipitate due to the production of a lattice of molecules which forms a large network (Zone of Equivalence) But instead of reaching a plateau this curve comes back down to zero with increasing amounts of antigen (Zone of Ag excess).

Answer to question from preceding page- The infant's RBCs do not contain the Rh Ag in their membranes therefore, they will not be sensitized to complement-mediated lysis or erythrophagocytosis by the anti-Rh IgG antibodies that have passively crossed the placenta.

When a zone of Ab excess occurs in vivo the precipitates are usually removed from the circulation very rapidly (A process known as immune elimination). The antigen usually remains in the body long enough to effect a booster to the pre-existing immune system. However, in instances of passively acquired antibody, no priming or immunologic memory may result (e.g., Rhogam intervention of Rh sensitization, pooled gamma globulin prophylaxis for rubella and hepatitis).

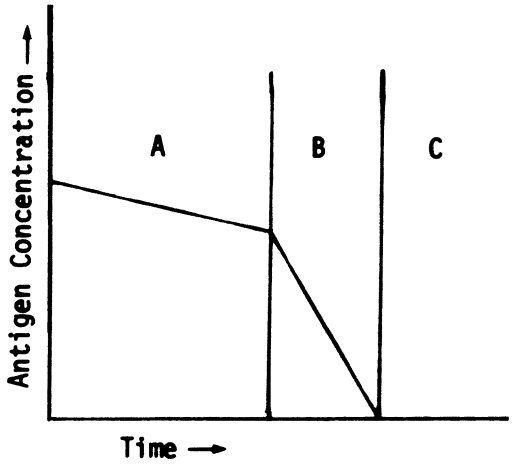
When Ag excess occurs in vivo it may result in a disease called serum sickness. An example of this would be the administration of diphtheria antitoxin contained in horse serum, which to a human is a foreign protein. Some persons develop serum sickness. The soluble Ag-Ab complex causes the release of various mediators with resultant vasculitis, urticaria, etc. Immune complexes probably account for most glomerulonephritis in man.

Serum sickness occurs when

_____

_____

_____.



A = Normal catabolism phase of antigen clearance.
B = Immune clearance phase; Ag:Ab complexes form here.
C = phase of detectable antibody.

REACTIONS OF VARIOUS CLASSES OF IMMUNOGLOBULINS

Reaction	IgG	IgA	IgM
Agglutination	+	+	++
Precipitation	+	+	+
Virus neutralization	+	+	+
C fixation	+++	-	+
C dependent lysis	+	-	+++
Immune Complex	+	-	+

The immunoglobulin class which is the most efficient at "fixing" complement is IgG/IgM. The IgG/IgM excells at sensitizing cells for C dependent lysis.

RADIOIMMUNOASSAY

Radioimmunoassay (RIA) is an extremely sensitive method that can be used for the quantitation of any substance that (1) is antigenic or haptenic and (2) can be labeled with a radioactive isotope, for example, I^{125}. The method is capable of measuring picogram quantities or less, depending on the substance being assayed. Basically, the method depends upon the competition between labeled (known) and unlabeled (unknown) antigen for the same antibody. A known amount of labeled antigen, a known amount of specific antibody, and an unknown amount of unlabeled antigen are allowed to react together. The antigen-antibody complexes that form are then separated out, and their radioactivity is determined. By measuring the radioactivity still remaining in the supernatant (unbound, labeled antigen), one can calculate the percentage of labeled antigen bound to the antibody. The concentration of an unknown (unlabeled) antigen can be determined by reference to a standard curve constructed from data obtained by allowing varying amounts of unlabeled antigen to compete.

Relative sensitivity of serological procedures to detect antibody

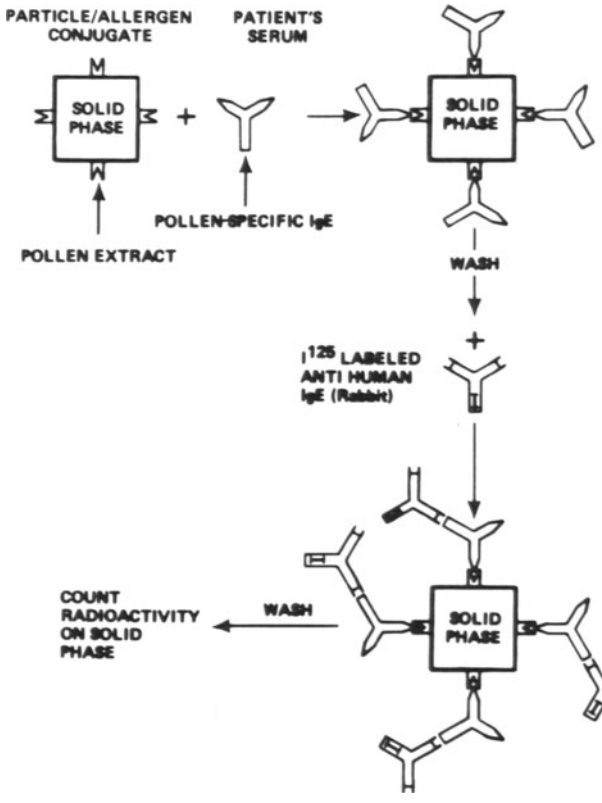
most sensitive = RIA

↓ ELISA

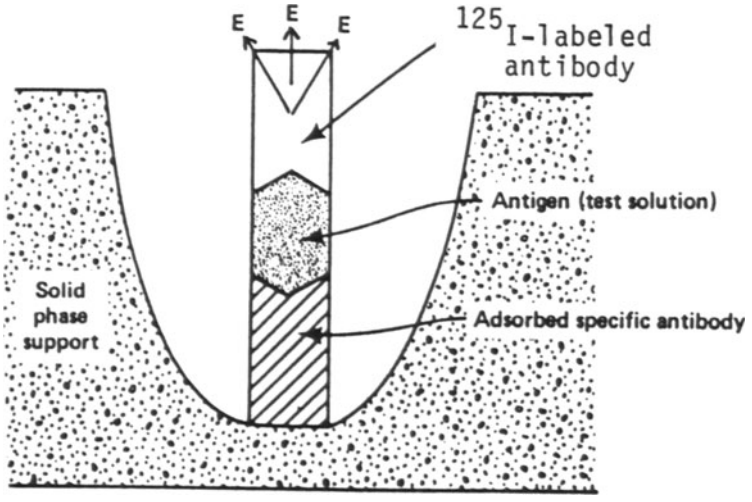
↓ AGGLUTINATION

↓ FLOCCULATION

least sensitive = PRECIPITATION



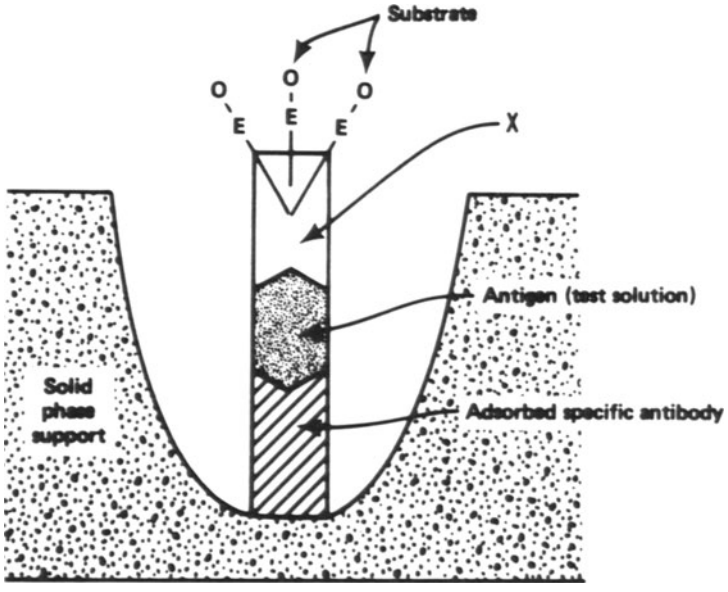
Energy (E) emitted from labeled Ab is detected by scintillation counter.



The above flow diagram depicts a paper radioimmunosorbent test, the PRIST assay, used to quantitate the amount of allergen-specific IgE in an atopic person's serum.

ENZYME-LINKED IMMUNOSORBENT ASSAY

Enzyme-linked Immunosorbent Assay (ELISA) has virtually the same sensitivity as radioimmunoassay without the latter's potential risk and requirement for expensive equipment. It can be used to assay both antigens and antibodies. The requisites are (1) that antigen or antibody can be attached to a solid phase support and still retain its immunological activity and (2) that either antigen or antibody can be linked to an enzyme and both immunological and enzymatic activity are retained by the antigen- or antibody-enzyme complex. Solid phase support systems used include paper disks, plastic surfaces, etc.; enzymes used include horseradish peroxidase and alkaline phosphatase. The application of one variant of ELISA, the double antibody sandwich for the assay of an antigen, is performed as follows. Antibody specific for the antigen being assayed is coated on a plastic surface (polystyrene plate). The solution being tested for antigen is applied to the surface, and any unreacted material is removed by washing. Enzyme-labeled specific antibody is then applied , and any excess conjugate is removed by washing. Finally, the enzyme substrate is added. The rate of substrate degradation is determined by the amount of enzyme-labeled antibody bound, which is determined by the amount of antigen in the solution being tested. A substrate that will give a color change on degradation is chosen. The color change can be measured quantitatively in a spectrophotometer.



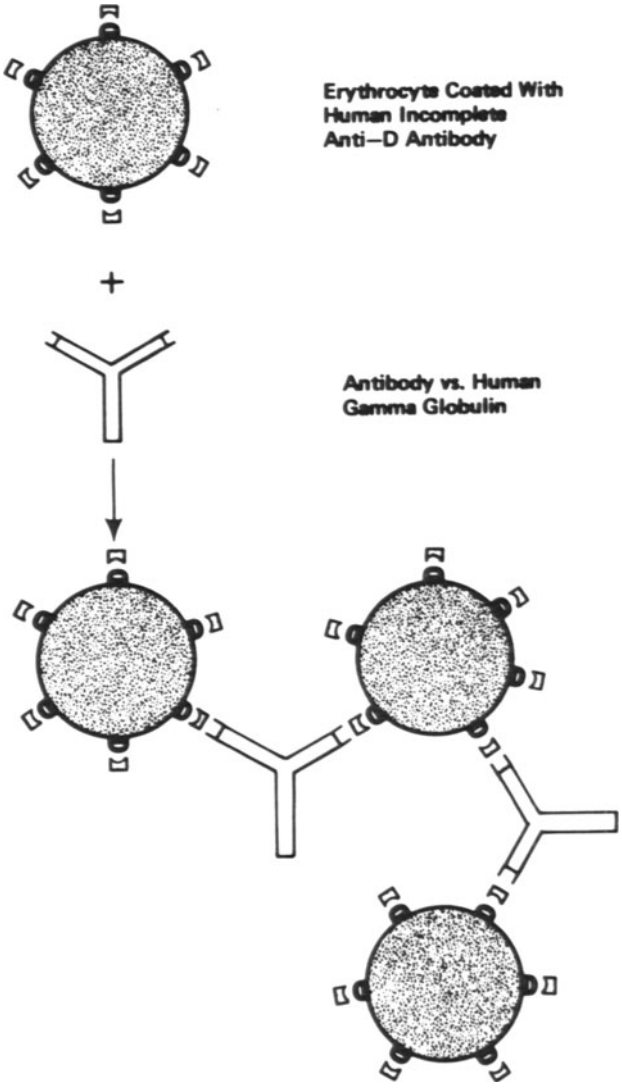
In the figure above what is the substance labeled "X"?

(answer at bottom of page)

X = Enzyme-labeled specific antibody

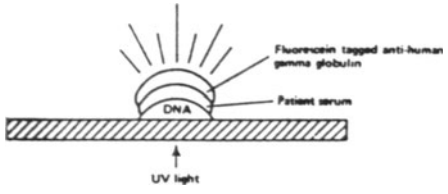
THE COOMBS TEST

Various factors are involved in the detection of antibodies against the Rh antigens. These antibodies, commonly seen in erythroblastosis fetalis, are able to cause agglutination in the presence of high concentrations of proteins, or if the erythrocyte has previously been stripped of certain cell membrane constituents by mild enzyme treatment. If such conditions are not fulfilled, the antibodies will not agglutinate the red blood cells, but will still attach to them. This adsorbed gamma globulin can be detected by the addition of an antiserum against human gamma globulin prepared in a non-human mammal (Coombs serum). The Coombs test can be used to detect hemolytic disease of the newborn, hemolytic transfusion reactions, idiopathic acquired hemolytic anemia, and autoimmune hemolytic anemia.



In the assay to the right, if the antibody were tagged with fluorescein it could also be used in:

1. SLE to detect Ab vs DNA.



2. syphilis to detect Ab vs T. pallidum (FTA test)

COMPLEMENT

The complement system is made up of nine serum proteins (complement components) and several regulatory factors which control the interactions of the complement components. The divalent cations Ca++ and Mg++ are also involved in function of the complement system. Macrophages produce many C proteins.

Many of the complement components are produced in _____.

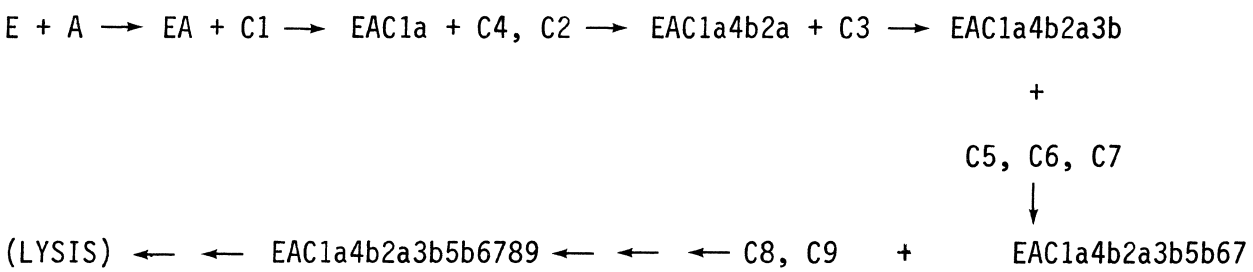
FUNCTIONS OF COMPLEMENT

1. Immune cytolysis. This is the antibody and complement mediated destruction of cell membrane integrity.
2. Acceleration of acute inflammation. When complement reacts with antigen-antibody complexes or sensitized cells, peptides are enzymatically split from some of the complement components. These peptides accelerate acute inflamation in several ways. One of these (C5a) enhances the migration of polymorphonuclear leukocytes, thus it is called a chemotactic factor. C3b causes opsonization (allowing cells which have the innate ability to phagocytize to do so more efficiently). C3a and C5a are also called anaphylatoxins and cause mast cells to release histamine. In turn, histamine alters vascular permeability and smooth muscle tone.
3. Immune adherence. Membranes bearing certain activated complement components, e.g. C3b, behave as though they were sticky. This phenomenon may be important in vivo in aggregation of bacteria, leukocytes, platelets, etc. and play a role in complement acceleration of blood clotting and phagocytosis.

Functions of complement include:

1. _____
2. _____
3. _____
4. _____
5. _____
6. _____

SUMMARY OF COMPLEMENT'S SEQUENTIAL REACTION CASCADE



COMPLEMENT NOMENCLATURE

The complement components are distinct serum proteins, all of which acting together have the cytolytic activity of complement. Other proteins are involved with complement as regulators, activators, inactivators, etc.

Classically, EA is used to designate the immune complex; E is the antigen (erythrocyte in the original work) and A is the antibody. The C components bind to EA to form a very large macromolecular complex, written EAC142356789. The numbers indicate the order in which components bind to the complex. As complement components are activated, they may bind to a growing complex or in the immediate vicinity of the sensitizing antibody.

Match the following: (see previous page)

1. Anaphylotoxins
2. Chemotactic factor
3. Opsonin
4. Immune adherence
5. Histamine release

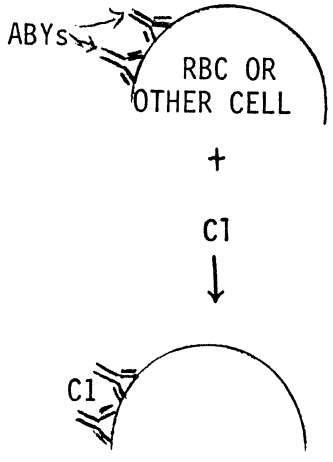
A. C1a
B. C3b
C. C3a
D. C5a
E. C5b67

MECHANISMS OF COMPLEMENT SYSTEM ACTIVATION

THE CLASSICAL PATHWAY

When IgM or IgG (except IgG-4) antibody reacts with an antigen, there is a rearrangement in the structure of the Fc portion of the antibody, forming a C1 binding site.

C1 is a complex macromolecule made up of 3 polypeptides. The C1 molecule contains a calcium ion (necessary for activity) and 3 polypeptides C1q, C1r, and C1s. C1q is the peptide which binds to the modified Fc portion of an immune complex. Binding of the C1q causes a change in the structure of the C1q which in turn leads to rearrangement of structure in the C1r and C1s peptides. The C1s peptide acquires an enzyme activity as a result of this arrangement. Activated C1s becomes an esterase (C1a). Under physiological conditions, C1q, C1r, and C1s are always bound to each other and to the CA^{++} ion.



EAC1a is the first complex containing an activated complement component. C1 esterase (C1a) converts native C4 and C2 into activated states. Activated C4 binds to EAC1a to form EAC1a4b. Activated C4 binds to receptor sites on membranes, usually (but not necessarily) the same membrane to which the C1a is bound. C2 binds to activated C4 forming EAC1a4b2a. The C2a portion of this complex is enzymatically active. C4b2a has the descriptive name "C3 convertase."

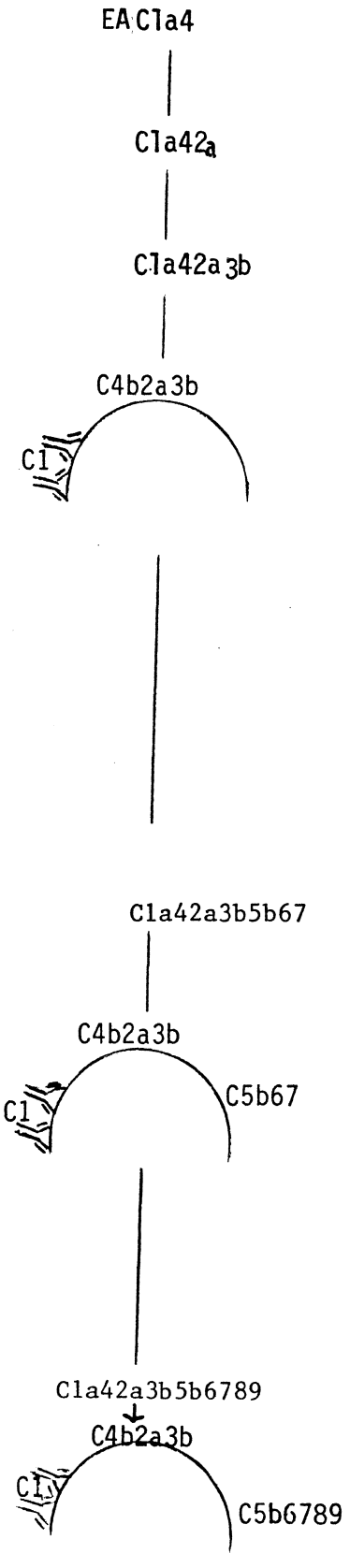
Through the action of C3 convertase, C3 is split into active peptides. One of these, C3b, binds to the membrane forming EAC1a42a3b.

The C3 fragments formed in the classical pathway are listed below. C3a and C3b may also be produced from C3 by other proteolytic enzymes (e.g., trypsin, plasmin, Hageman factor, etc.). Other fragments are produced.

1. Opsonic factor (C3b) - Associated with increased activity of polymorphonuclear leukocytes; when membranes are opsonized they are more susceptible to the phagocytic activity of PMN.
2. Anaphylotoxins (C3a and C5a) Cause mast cells and basophils to release their histamine (histamine in turn causes increased capillary permeability and vasodilitation). C5a also attracts polymorphonuclear leukocytes (chemotaxis).

Those complexes containing both C3 convertase and C3b activate C5, 6 and 7. C5 is split into fragments, C5a and C5b; C5b binds to C6 and C7 forming C5b67 which is also bound to membranes. These steps also lead to the formation of biologically active factors, C5a or anaphylotoxin and the C5b67 chemotactic factor.

C8 then adds to the growing complex to form membrane bound C5b678, and slow dissolution of the membrane begins. (This is the effector step in the "attack sequence" of the complement cascade). The rate of membrane destruction is enhanced by the addition of C9 to form the completed complex.



THE ALTERNATE PATHWAY (PROPERDIN PATHWAY)

The alternate (non-antibody dependent) pathway of complement activation involves three additional serum proteins, factor B, factor D and Properdin. C3 undergoes a natural decay process in the body and C3b is produced at a low level. This is usually inactivated by complexing with Factor B (beta 1 H globulin) and C3b inactivator protein. If the C3b is bound to a protective surface such as a bacterial cell, LPS, zymosan, complex CHO's, or certain immune complexes then inactivation does not occur and the complement cascade distal to the C142 participation steps ensues. Bound C3b, in the presence of factor D and Mg++, cleaves factor B releasing a small peptide (Ba) and forming the alternate pathway C3 convertase, C3bBb. Properdin stabilizes the C3 convertase, forming C3bBbP which then is able to cleave C3 and C5 and complete the cascade to the membrane attack complex, C5b6789.

C3b + B + C3bina → inactive C3b

\+

AC (activating surface)

\+

B

↓ D, Mg++ cofactors

AC-C3bBb (C3 convertase)

\+

P (properdin)

↓

AC-C3bBbP (stabilized complex)

\+

C567, C89

↓

LYSIS

CLINICAL IMPLICATIONS

With the addition of C1 to form EAC1a, an alternate pathway with decay of the complex to form EA + free C1a may occur. The free C1a is enzymatically active, and it may cause trouble. Free C1a can activate subsequent components, and if it does this in a fluid phase (as opposed to a membrane-bound phase), symptoms of systemic vasodilitation and inflammation occur. This is normally prevented by a protein that inhibits C1a called C1 esterase inhibitor. C1 esterase inhibitor slows generation of free C1a and blocks its enzymatic activity competitively. Hereditary angioneurotic edema (HAE) is a disease in which occasional spontaneous edema occurs. These individuals have a deficiency in C1 esterase inhibitor.

Congenital absence of C1 esterase inhibitor results in _____ _____ _____.

Hereditary deficiency of certain complement components is associated with recurrent bacterial infections. The most severe forms are the deficiencies of those components which are involved in opsonization and chemotaxis (C3 and C5). Absence of early C proteins is associated with a Systemic lupus erythematosus-like disease.

A protein has been discovered in the serum of patients with chronic membranoproliferative glomerulonephritis which is able to stabilize the C3bBb:C3 convertase complex in a manner similar to that of Properdin. It has been suggested that this factor, called C3 nephritic factor (C3NeF), is an IgG immunoglobulin that is directed against a component of the C3bBb complex. This may explain the continuous alternate C pathway activation that accompanies this disease.

Complement levels are often decreased in autoimmune diseases and occasionally also in acute infectious diseases. These changes are due to C utilization. A serious C consumption can occur during septic disease and is associated with disseminated intravascular coagulopathy.

Inherited deficiencies of certain complement components have been associated with increased incidence of infectious diseases. The deficiencies are usually those involving C components from C3 through the final membrane attack molecule, C9. Deficiencies of C6-8 have been associated with gram negative coccal infections.

There are several reasons why complement levels may be depressed, including such things as

_____,

_____,

_____, and

_____.

Complement Fixation Test

1. Useful in diagnosis of viral infections and syphilis

2. Comprised of 2 stages
   a. Test system
      Patient's serum
        +
      Known Antigen
        +
      Complement

   b. Indicator system
      Sheep RBCs
        +
      Hemolysin

3. If C is "fixed" (consumed) in 2a due to the Ag:Ab complexes, then there will be no lysis when step 2b is performed, i.e., a positive test.

If the complement is "fixed", the erythrocytes will/will not lyse; this means there was/was not an antigen:antibody reaction in the test system.

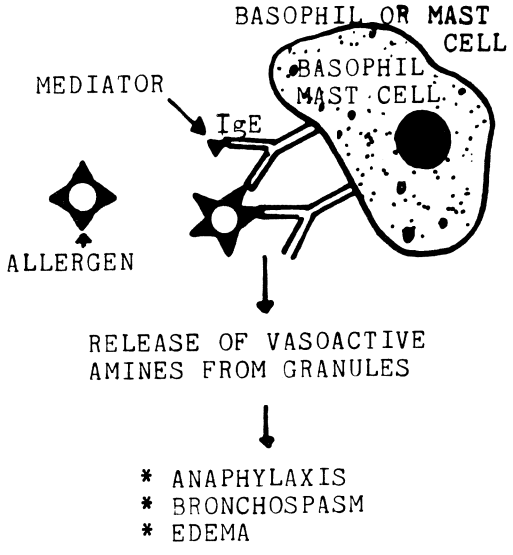
IMMEDIATE HYPERSENSITIVITY

The manifestations of immediate hypersensitivity are not protective, but rather are deleterious. There is genetic predisposition toward atopic (Type I, Gell and Coombs) hypersensitivity. The Type I hypersensitivities manifest themselves in many different ways, depending on the target organ. Some of these manifestations are:

1. Anaphylaxis - an immediate hypersensitivity reaction which causes bronchoconstriction in humans. The main symptom is the inability to breathe; there is complete vasomotor collapse and bronchoconstriction.
2. Allergic asthma (extrinsic asthma) - characterized mainly by bronchospasm. Eosinophilia in blood and sputum may be seen.
3. Allergic rhinitis (hay fever) - characterized mainly by watery nasal discharge, sneezing, etc. Eosinophilia may be present in nasal secretions.
4. Allergic urticaria (hives) - characterized by wheals (dermal edema), erythema (reddening), angioedema (severe local swelling) around the face and sometimes respiratory obstruction due to edema in the larynx and pharynx.
5. Atopic dermatitis (eczema) - characterized by pruritis (itching), usually seen in infants or young children.

Some of the manifestations of ATOPIC disease are:

1. _____
2. _____
3. _____
4. _____
5. _____



COMPLETE THE TABLE BELOW (It will be necessary to read ahead)

Gell and Coombs Classification	Immune Reactivity	Mechanism of Tissue Damage
TYPE I		
TYPE II		
TYPE III		
TYPE IV		

The primary class of antibodies that cause allergic responses in man is IgE. IgE is a noncomplement fixing, non-placental passing antibody that has a remarkable tissue affinity (homocytotropic). It binds in tissue to the mast cell and to the basophil in blood, via the Fc fragment. The small amount of IgE in the serum is of major importance in that it is the basis for a passive cutaneous test for the detection of allergies (Prausnitz--Kustner or P-K test) and the very sensitive RIA tests (RAST & PRIST).

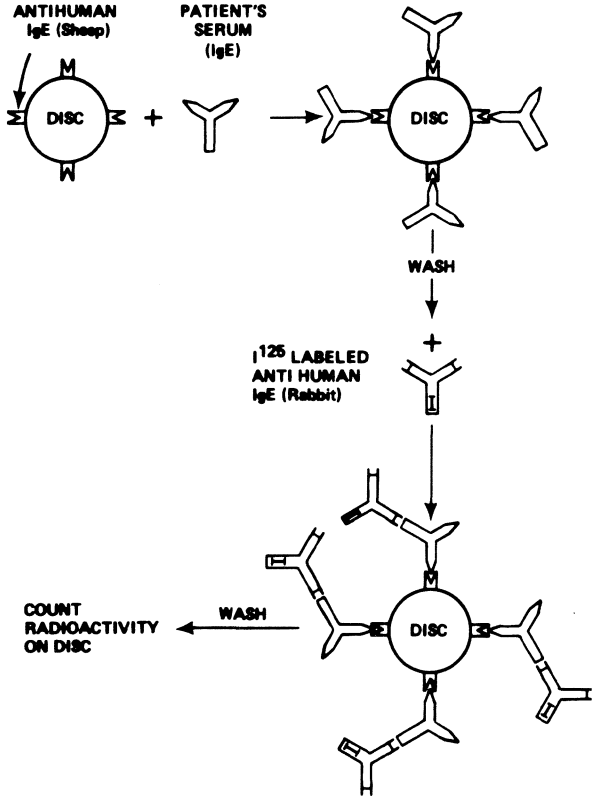
When an allergen attached to the combining sites of the antibody, the mast cell or basophil is triggered to degranulate and release a number of different biologically active materials. This process is energy-requiring, requires the bridging of two cell-bound IgE molecules by allergen, and does not involve cell death. The mateials are pharmacologically active mediators and are the cause of the symptoms of immediate hypersensitivity. Some of these substances are; 1) histamine, 2) slow reacting substance of anaphylaxis or SRS-A, 3) bradykinin, 4) serotonin, and 5) eosinophil chemotactic factor of anaphylaxis. The first four cause contraction of smooth muscle, and increased capillary permeability. Complement is not involved in this reaction.

The second type of allergic disease classified by Gell and Coombs (type II) is due to cytotoxic antibodies. The antigen on the cell surface combines with antibody and sensitizes the cell to lysis or phagocytosis. In the presence of complement the C cascade ensues, with destruction of the membrane and generation of chemotactic factors, etc. An example of this type of injury is erythroblastosis fetalis, wherein the mother has produced antibodies to an antigen on the fetal RBC (usually Rh°, although other antigens may be involved, e.g. A or B). Ab's cross the placenta and sensitize the infant's erythrocytes, resulting in a hemolytic disease which may occur during the pregnancy or shortly postpartum. Other examples of type II hypersensitivity are autoimmune hemolytic disease and Goodpasture Syndrome.

Allergen reactions with mast cell-bound IgE causes the following sequence of events:

1) Influx of CA^{++} into cell
2) protease activation
3) decrease in cAMP
4) granule margination and fusion with cell membrane
5) exocytosis of granule content

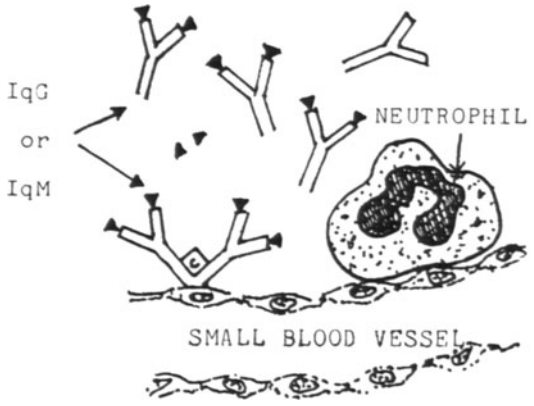
Cromolyn sodium blocks step (1).

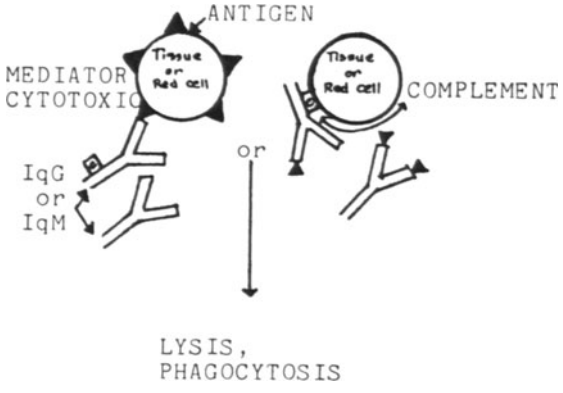


IMMUNE-COMPLEX DISEASE (TYPE III REACTION OF GELL AND COOMBS)

In experimental animals there is an induced disease called the Arthus reaction. It is similar to serum sickness; they both have the same trigger, i.e., administration of foreign serum or drugs. The symptoms are fever, lymph node enlargement, a rash, and arthritis. The mechanism is as follows: Antibody of the IgM or IgG type is produced against the foreign protein. When the level of Ab is significant enough to form Ag-Ab complexes, there is still an Ag excess. The complexes at this point are soluble and cause the ensuing process to occur. The Ag-Ab complex attracts the complement system of the host and this Ag-Ab-C complex is deposited primarily in the basement membrane of the glomerulus of the kidney. Polymorphonuclear leukocytes are chemotactically attracted to the site of deposition of the complex, where they proceed to release their lysosomal enzymes into the extracellular space. The proteolytic enzymes destroy the basement membrane, and the resulting symptoms are those of kidney failure. A similar process occurs in the other tissues (synovial membranes, vascular endothelium). Serum complement levels may be decreased. A hallmark of the disease is the "lumpy-bumpy" deposit of complexes on the basement membrane revealed by immunofluorescense.

Serum sickness is not the only example of immune-complex injury. Any time there is an antigen that triggers production of soluble antigen-antibody-complement complexes that ends up in the kidney, there will be a case of immune-complex disease. Two examples are: 1) post-streptococcal glomerulonephritis and 2) nephritis of systemic lupus erythematosus.





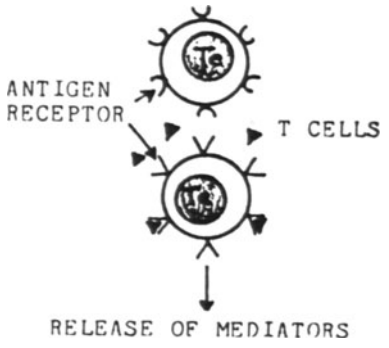
What type of immunologic injury is depicted in the illustration above?

(answer on next page)

Immune complex deposition in the glomeruli would be evidenced by a (lumpy-bumpy/linear) pattern of immunofluorescence along the basement membrane if the kidney is stained with a fluorescein-tagged antibody specific for human (gamma globulin/complement/either).

DELAYED HYPERSENSITIVITY

Cellular immunity (delayed hypersensitivity) is a reaction involving the T lymphocyte. It has nothing to do with circulating antibodies. The histologic hallmark of delayed hypersensitivity is an accumulation of mononuclear cells (lymphocytes and monocytes) at the site of the reaction causing induration.



Delayed hypersensitivity is especially characteristic of many chronic microbial infections, and in particular those that are intracellular. Tuberculosis, brucellosis and typhoid fever are examples where the immunity is based on cellular events. Protection from fungal infections (histoplasmosis, blastomycosis), and virtually all viral infections is due to cellular immunity. Specifically-sensitized lymphocytes can destroy virus-infected cells, as can antibody plus complement. Some disease manifestations of fungi are due to delayed hypersensitivity and, while circulating antibodies are also demonstrable in viral infections, recovery from viral infections is primarily cellular whereas resistance to reinfection is usually antibody-mediated. Graft rejection, tumor immunity and some autoimmune diseases also have a strong cell-mediated immunity component.

Cell-mediated immunity is a common feature of infections by

1. _____
2. _____
3. _____
4. _____
5. _____

The T cell antigen receptor (that part of the membrane which imparts immunologic specificity to the cell) is structurally very similar to an Fab molecule, although chemically quite different. The antigen receptor has 2 polypeptide chains, alpha and beta, both of which contain constant and variable domains. The chains also contain short J and D regions, further mirroring the structure of the antigen binding portion of antibody molecules.

Answer: The illustration is of Gell and Coombs Type II immune injury by the action of cytotoxic antibodies.

In Goodpasture's disease, antibody deposition in the glomeruli would be evidenced by (lumpy-bumpy/linear) pattern of immunofluorescence along the basement membrane if the kidney is stained with a fluorescein tagged antibody specific for human gamma globulin/complement/either.

The activated T cell involved in cell-mediated immunity has the following characteristics.

1. It is cytotoxic. Following a homograft, the host makes sensitized lymphocytes which destroy the graft in the absence of immunosuppressant therapy. The suppression of the sensitized T cell is a must for successful transplantation.
2. It produces a substance known as Transfer Factor, or TF, which, like T cells, can transfer passively this type of reactivity to another host or to neighboring cells. TF can cause the activation of new T cells.
3. It produces many soluble factors called lyphokines, macrophage migration inhibitory factor (MIF), macrophage activating factor (MAF), macrophage chemotactic factor, mitogenic factor for lymphocytes, lymphotoxin, leukocyte inhibitory factor, etc.

Other forms of Type IV reactivity include contact (e.g., poison ivy, DNCB sensitivity) and certain drug allergies. Here, the inducing agents act as haptens and become antigenic via coupling to host proteins.

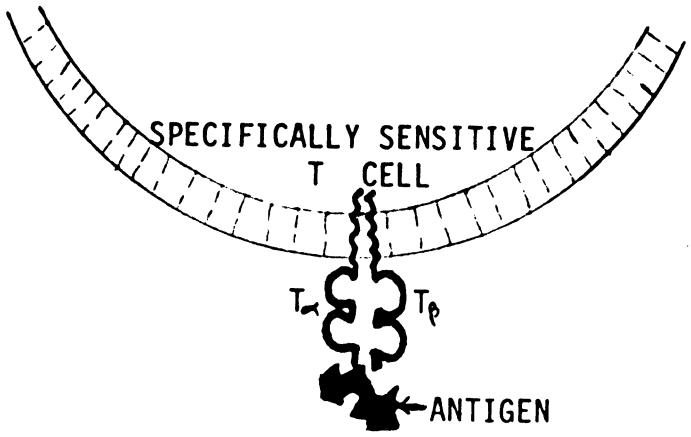
Products of Activated Lymphocytes

Mediators affecting macrophages
- Migration inhibitory factor (MIF)
- Macrophage activating factor (MAF)
- Chemotactic factor for macrophages (MCF)

Mediators affecting polymorphonuclear leukocytes
- Leukocyte inhibitory factor (LIF)
- Chemotactic factors for neutrophils, eosinophils, and basophils

Mediators affecting lymphocytes
- Mitogenic factors
- Immunoregulatory factors
- Transfer factor (TF)

Factors affecting other cell types
- Lymphotoxins
- Growth inhibitory factors
- Osteoclast-activating factor
- Interferon
- Colony stimulating activity



The reaction illustrated above will result in the release of what lymphokines?

_____

_____

_____

_____

_____

_____

_____

Biological effects of Interleukin-1
- Induces IL-2 production by T helper cells
- Causes T cells to proliferate and mature
- Enhanced PMN activity

Biological effects of Interleukin-2
- Induces proliferation of other T lymphocytes (e.g., helper, suppressor and cytotoxic)
- Generates new cytotoxic cells
- Enhances natural killer cells

Biological effects of Interleukin-3
- Promiscuous cell mitogen

AUTOIMMUNE DISEASES

Autoimmune diseases are defined as the occurrence of an immune response resulting in the production of either antibody and/or sensitized lymphoid cells capable of reacting with normal body constituents.

There are several hypothesized mechanisms for the development of autoimmune disease.

1. Release of a sequestered antigen, an antigen that ordinarily doesn't encounter antibody-forming cells.

2. Self antigens may be slightly altered, and the body makes antibodies to these self antigens.

3. Cross-reacting (heterophile) or closely related antigen. For example, the streptococci and human heart tissue share a common antigen.

4. The spontaneous emergence of clones of cells ("forbidden clones") capable of making an immune response to one's own tissues.

5. Deficiency of suppressor T cells (an immunodeficiency disease that results in an autoimmunity).

There are several general signs of autoimmune disease. These include:

1. Increase in the amount of serum gamma globulin.

2. Occurence of different autoantibodies.

3. Decreased concentration of complement in serum.

4. Presence of immune complexes in serum.

5. Absence of T8 lymphocytes (this is a membrane marker of T suppressor cells).

6. Lumpy-bumpy immunoglobulin and C deposits seen in immune complex diseases in vascular walls and basement membranes.

7. Linear deposit of immunoglobulin and complement in anti-glomerular basement membrane diseases such as Goodpastures.

General features of autoimmune diseases include:

1. _____
2. _____
3. _____
4. _____
5. _____
6. _____
7. _____

POST-VACCINAL ENCEPHALOMYELITIS

Post-vaccinal encephalomyelitis (PVE), first seen in an occasional individual given rabies vaccine, follows the injection of nervous tissue. It may occur following immunization with embryo-derived vaccines. In man, the signs and symptoms usually appear in one week to 10 days following subcutaneous injection and include:

1. Irritation at the site of injection with induration, inflammation and sometimes pain, progressing to headache and backache.
2. Paralysis and general weakness in the extremities. There is demyelination and accumulations of cells around blood vessels, a perivascular "cuffing." These cells are lymphocytes, and this is a cellular type response.

This disease, with all its signs and symptoms, has been reproduced in animals where it is called experimental allergic encephalomelitis. A highly purified basic protein (made up of basic amino acids), a histone-like protein, has been identified as the responsible antigen in nervous tissue.

There are several other human diseases similar to post-vaccinal encephalomyelitis. One of them occurs following measles (subacute sclerosing panencephalitis or SSPE) and is a virus-induced disease whose mechanism is unknown. Another is multiple sclerosis.

THYROIDITIS

This disease involves the antigen, thyroglobulin. Several kinds of antibodies are made in response to this antigen. In the target organ, the thyroid, there is an accumulation of lymphoid cells which somehow have the ability to disrupt the colloid of the gland causing fibrosis.

Although thyroglobulin is the major antigen identified with this disease, two other antigens have been found within thyroid tissue. Thyroiditis has been passively transferred to normal hosts by sensitized lymphocytes and serum.

The absence of T suppressor cells in certain patients with autoimmune disease is detected by enumerating the number of cells bearing the (T4/T8) membrane marker.

Three central nervous system diseases which resemble post-vaccinal encephalitis are:

1. _____
2. _____
3. _____

SYSTEMIC LUPUS ERYTHEMATOSUS

Systemic lupus erythematosus (SLE), is a generalized autoimmune disease; virtually all organs in the body appear to be affected, though most fatalities in this disease appear to have the kidney as the target organ. This disease predominates in women, 10-40 years of age.

The hallmark of SLE is the LE cell, or lupus erythematosus cell, which is a polymorphonuclear leukocyte which has phagocytized some nuclear material. It may appear as an inclusion body with the cell's own nucleus pushed to the side.

The conclusion from this observation is that SLE involves an immune response against nuclear protein or nuclear material, causing its extrusion from the cell. However, antibodies are directed against several targets:

1. nucleoprotein
2. nucleoli
3. DNA
4. histone
5. RBC and platelets
6. Gamma globulins
7. mitochondria
8. clotting factors

The renal failure that leads to death seems to be due to immune complex disease. The renal damage is attributable to immune complexes consisting of DNA plus the antibody, plus complement; these complexes are deposited in the glomeruli causing destruction or dissolution of the basement membrane. Serum complement levels are often depressed.

Several patterns of nuclear staining are seen in this disease. Homogenous staining is due to anti-DNA antibodies; the outline pattern will be produced by anti-ds-DNA antibodies or antibodies against soluble nucleoprotein. Both these patterns are characteristic of ACTIVE SLE disease. The speckled pattern reflects the presence of antibodies to non-DNA nuclear components. High levels of these antibodies is indicative of mixed connective tissue disease. The nucleolar pattern of staining is most often seen in sceroderma and polymyositis. Numerous other autoimmune diseases may present with anti-nuclear staining reactions (e.g., Rheumatoid arthritis, Sjogren's).

SLE is one of the classic autoimmune diseases, affecting approximately 1 per 1,000 in the U.S. Many diverse autoantibodies are seen in these patients, including antibodies against

1. _____
2. _____
3. _____
4. _____
5. _____
6. _____
7. _____
8. _____

The importance of complement in SLE glomerulonephritis is as a chemotactic stimulus. A significant infiltration of PMNs is seen and these cells are responsible for most of the tissue damage due to the release of hydrolytic enzyme and other mediator molecules which cause local damage.

AUTOIMMUNE HEMOLYTIC DISEASE

Autoimmune hemolytic disease (AHD) involves the destruction of one's own RBC's. Some of the antibodies involved are incomplete or non-agglutinating and can be detected only with Coomb's serum (Ab against human gamma globulin). Two types of antibodies are found in AHD: warm antibodies or incomplete antibody and cold antibodies. These antibodies are not exclusively found with AHD; for example, cold antibodies have been identified with mycoplasmal pneumonia. The mechanism of this disease is unknown but involves destruction of RBC's.

The following is a list of diseases which are also thought to have an autoimmune etiology:

1. Idiopathic Addison's disease in which antibody is formed to the adrenal gland.
2. Rheumatoid arthritis involves rheumatoid factor (RF) which is mainly IgM antibody. This antibody is formed against another immunoglobulin, IgG. These people are making an immune response to their own IgG.
3. Guillain-Barré (acute idiopathic polyneuritis) in which sensitized lymphocytes are formed against peripheral nervous tissue - peripheral neuritis.
4. Idiopathic thrombocytopenic purpura in which antibodies are made against blood platelets.
5. Myasthenia gravis - antibody and sensitized lymphocytes against acetyl choline receptors.
6. Goodpasture's syndrome - antibody which cross reacts with lung and kidney basement membrane.
7. Pernicious anemia - antibody against parietal cells and/or intrinsic factor.

Match the autoimmune disease with the antigen.

1. Addison's disease
2. Guillain Barre
3. Rheumatoid arthritis
4. Myasthenia gravis
5. Goodpasture's syndrome
6. Systemic lupus erythematosus
7. Pernicious anemia

A. DNA

B. Adrenal gland

C. Basement membrane

D. Peripheral nervous tissue

E. IgG

F. Intrinsic factor

G. Acetyl choline receptors

(answers below, left)

1 = B
2 = D
3 = E
4 = G
5 = C
6 = A
7 = F

IMMUNE DEFICIENCY DISEASES

TESTS TO DETERMINE IMMUNE FUNCTION

Phagocytic cells = Phagocytosis, intracellular killing, chemotaxis, nitroblue tetrazolium reduction
B cells = Ig levels, isohemagglutinins, typhoid agglutinins, Schick test, allergy scratch test, EAC rosettes
T cells = PHA stimulation, SRBC rosettes, DNCB sensitization, skin test with common antigens, e.g. Candida, trichophytin, streptokinase- streptodornase (varidase)

THE MOST IMPORTANT HALLMARK OF IMMUNODEFICIENCY IS THE OCCURRENCE OF REPEATED INFECTIONS, OFTEN BY ORGANISMS CONSIDERED TO BE OF LOW VIRULENCE.

DEFICIENCIES IN NON-SPECIFIC RESISTANCE

Two inherited diseases which cause decreased production of effective phagocytic cells at the bone marrow level are: (1) The deVaal syndrome (or reticular dysgenesis) affects all stem cells by drastically diminishing their production. (2) The Fanconi syndrome (or congenital pancytopenia) has as its signs the suppression of erythroid blood elements (the patient has an anemia), and suppression of the myeloid series (polymorphonuclear leukocytes or PMN's).

Also important are diseases which affect the functions of phagocytic cells instead of affecting the numbers of these cells.

Chronic Granulomatous Disease is characterized by recurrent, primarily bacterial, infections. The mechanism is a defect in the oxidized NAD - related activities, (deficiency of NADH or NADPH oxidase) in the PMN's.

Neutrophils from patients with Chediak-Higashi syndrome have deficiencies in chemotaxis and intracellular killing.

Complement deficiencies may also predispose an individual to recurrent bacterial infections. The most severe are deficiencies of C3 and C5, which are the complement components most closely associated with native immune mechanisms (opsonization and chemotaxis).

The major immunologic features of chronic granulomatous disease are:

1. Occurrence of recurrent infections with organisms of low virulence, e.g., S. epidermidis, Aspergillus, Serratia.
2. X-linked inheritance (a female variant does occur rarely).
3. Onset of infections occurs by 2 years of age.
4. Diseases = pneumonia, osteomyelitis, abscesses
5. Diagnosis established by nitroblue tetrazolium test or intracellular killing assay of peripheral blood neutrophils.

DEFICIENCIES IN SPECIFIC RESISTANCE

Thymic-independent areas. This refers to the bursa equivalent, or a defect in the B cell, the plasma cell, or the production of the five classes of immunoglobulins, e.g., Bruton's congenital hypogammaglobulinemia. The patient has recurrent bacterial infections. There may be no detectable immunoglobulins in the serum. Their cellular immune system is intact and therefore, the patient can reject tissue grafts, become tuberculin positive and resist fungal and viral infection (with the possible exception of polio). They have no germinal centers in their lymph nodes, and no plasma cells in the lymph nodes, spleen, or marrow. Tonsillar and Peyer's Patch tissues are either hypoplastic or absent. There are normal levels of circulating lymphocytes due to the fact that most of these are T cells. The treatment is administration of pooled gamma globulin.

Selective Immunoglobulin Deficiency is a syndrome in which there are isolated deficiencies of any one or two classes of immunoglublins. The most common is IgA. These patients may have repeated infections of the lungs or respiratory system.

Thymic dependent areas. The Di George syndrome (thymic hypoplasia) is the absence of the thymus due to the faulty development of the third and fourth pharyngeal pouches in the embryo. The patient has no cellular immunity, but has normal levels of plasma cells and circulating antibodies.

Nezelof Syndrome is a deficiency in thymus accompanied by selective immunoglobulin deficiency. These patients are unable to respond to immunogenic stimuli.

Chronic mucocutaneous candidiasis is a T cell abnormality with no skin test response to Candida. The patients usually expire of endocrinopathy.

Bruton's disease is an X-linked condition, which means it is seen in <u>males/females</u>.

The major immunologic features of Bruton's X-linked hypogammaglobulinemia are:

1. recurrent pyogenic infections beginning at 5-6 mo.
2. Absence of B cells in peripheral blood.
3. Absence of germinal centers.
4. IgG less than 200 mg%.
5. Absence of other immunoglobulins.
6. Good clinical response to pooled gamma globulins.

The most common immunoglobulin deficiency seen in the "selective" category is a deficiency in _____.

T lymphocyte numbers can be estimated by the following tests:

1. Sheep RBC rosettes
2. Membrane marker stains such as fluorescein-labelled anti-T3 or T11.
3. Peripheral blood lymphocyte count (as most are T cells).

T lymphocyte function can be estimated by the following tests:

1. _____
2. _____
3. _____

More serious is the severe combined immunodeficiency (SCID) which involves thymic independent and thymic dependent mechanisms. This is also known as Swiss type agammaglobulinemia. The defect, extremely severe, is in both cellular and humoral immune mechanisms. There is a depression of lymphocytes, plasma cells, circulating antibodies, and delayed hypersensitivity; there are no germinal centers in the lymph nodes; there is little thymus. This disease is usually fatal within the first year or two of life. A biochemical defect (deficiency of the enzyme adenosine deaminase, ADA) has been identified in about 50% of SCID patients with the autosomal recessive form of the disease.

Another purine catabolism enzyme, purine nucleoside phosphorylase, is defective in some infants with T cell deficiencies.

Other disease syndromes are accompanied by deficits in the immune response. One of these is the Wiskott-Aldrich syndrome in which the patients have recurrent infections. They have a general depression in the number of lymphocytes and thrombocytes, and a defect in their cellular immunity and delayed hypersensitivity (because of the low number of lymphocytes). They also have a defect in their ability to produce antibodies to polysaccharide antigens. They do not make normal levels of antibodies, especially IgM.

There is a disease syndrome called telangiectasia characterized by immune deficiency. These patients have recurrent infections (usually pulmonary), ataxia, general depression of cellular and humoral immunity, with decreased IgA and IgE.

Sarcoidosis, Hodgkin's disease and lepromatous leprosy involve suppression of lymphocytes, which are mandatory for cellular immunity. Other tumors may also cause defects in cellular immunity, as evidenced by lack of reactivity to a battery of commonly encountered Ag's such as PPD, mumps, Candida, streptokinase and streptodornase.

Acquired and secondary immunodeficiency

I. Acquired Immune Deficiency Syndrome

Major features include:

a. Depressed T4 (helper) lymphocytes which cause inversion of T4-T8 ratio.
b. Reduce lymphocyte response to PHA.
c. Elevated immunoblobulin levels.
d. Increased circulating immune complexes.
e. Reduced NK cell activity.
f. Anti-HTLV-III antibodies in serum.
g. Infections with bizarre opportunists such as Pneumocystis carinii, and other organisms (e.g., cryptococcus, cytomegavirus, candida, atypical mycobacteria).

II. Secondary Immune Deficiency

a. Due to infections, usually transient and non-specific
   1. Measles
   2. Rubella
   3. Other viral disease
   4. Leprosy*
   5. Tuberculosis*
   6. Coccidioidomycosis*

   *These are usually specific to the agent and last for the duration of the illness.

b. Due to malignancies ataxia
   1. Hodgkin's
   2. Leukemia

c. Autoimmune diseases-may be due to therapy
   1. SLE
   2. Rheumatoid arthritis

d. Other conditions
   1. Diabetes
   2. Alcoholism
   3. Sarcoidosis

TRANSPLANTATION IMMUNOLOGY

TERMINOLOGY USED IN TRANSPLANTATION

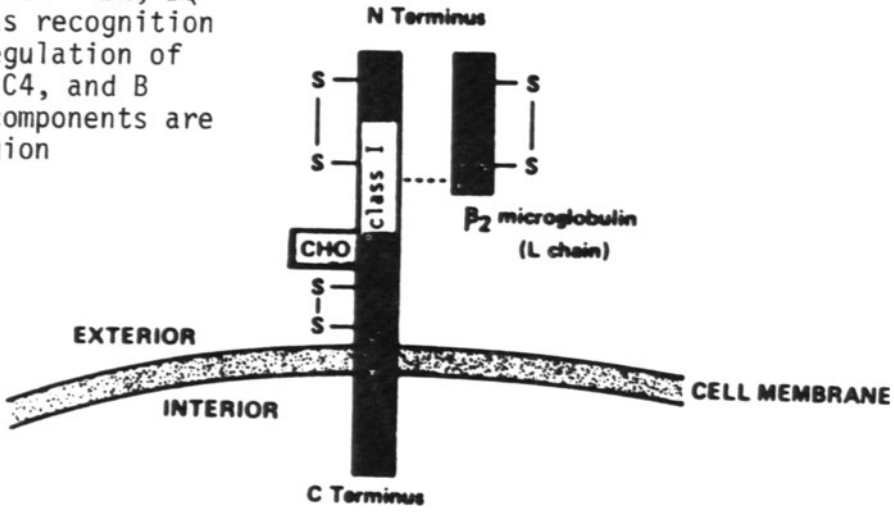
Autograft - graft from one part of the body to another (e.g. skin)
Allograft (homograft) - graft from one member of a species to another
Xenograft - graft from one species to another Terms used to describe antigenic relationship between donor and recipient
Autologous - same individual: in inbred animals the term is syngeneic
Heterologous - different individuals: allogeneic antigen source or xenogeneic antigen source

A kidney graft between identical twins would be <u>allogeneic/syngeneic</u>.

In the human, the major histocompatability locus is called the HLA locus (human leucocyte antigen). The alleles are expressed codominately.

Nature of histocompatibility antigens

1. Products of genes of the MHC (major histocompatibility complex)
2. Glycoprotein component of cell membrane
3. Occurs in lymphocytes in high concentrations; also present on other nucleated cells of the body (e.g., macrophage, hepatocytes)
4. Are divided into 2 classes
   a. Class I = coded for by HLA A, B, and C genes; Function as target antigens for immune recognition and killing
   b. Class II = coded for by HLA - DR, DQ and DP genes; function as recognition molecules in cellular regulation of immune interaction, C2, C4, and B
   c. Class III = complement components are coded for in the MHC region



HOST RESPONSE TO TRANSPLANT

First of all, one can make antibodies to the histocompatibility antigens. One can also get a T-cell activation or cellular immune response. Both of these responses can be mounted following a transplant.

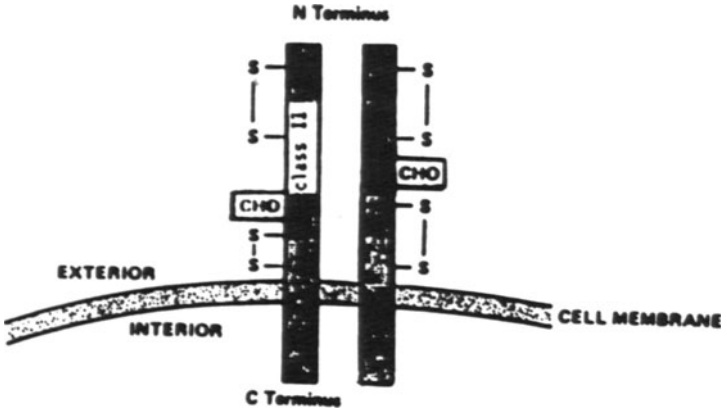
There are at least three types of rejection reactions that can take place following a transplant.

1. Hyperacute rejection - performed ANTIBODIES attack the organ. There is a rapid vascular spasm and vascular occlusion and the organ is not perfused by recipient's blood.
Hyperacute rejection occurs within hours or days.
2. Acute or Accelerated rejection - this is believed to be due to sensitized T LYMPHOCYTES. This is a type of rejection reaction that one sees 10-30 days after a transplant. Since the patient has not been previously sensitized it takes a while to develop sensitized immune lymphocytes which then increase in number and attack the graft. Here we see the typical picture of a cell-mediated immune response. We see infiltration into the graft (especially around small blood vessels) of small lymphocytes and mononuclear cells along with some granulocytes which causes destruction of the graft transplant.
3. Chronic rejection - in a kidney transplant with this kind of rejection one sees a slow loss of kidney function over a period of months or years. It may be a cellular immune response, an antibody response, or a combination of the two.

Class I histocompatibility antigens are coded for by the HLA genes _____ _____. They function as _____ _____.

Class II histocompatibility antigens are coded for by the HLA genes _____ _____. They function as _____ _____.

Class III histocompatibility genes code for _____ _____.



POSTGRAFT PATIENT MANAGEMENT

All transplants, except between identical twins, require immunosuppression. Drugs or chemical or physical manipulations are used to reduce the immune and inflammatory responses.

One class of drugs used is the corticosteroids. Other drugs that are used antimetabolites or alkylating agents such as Azothioprine and cyclophosphimide. Cyclosporin A is a fungal product that inhibits helper T cells. Anti-lymphocyte globulin or antithymocyte globulin is also used in the management of the recipient.

With the use of such high doses of immunosuppressive drugs, one often gets into trouble with infection and/or malignancy. About 25% of the deaths that occur in kidney transplants are due to sepsis.

Complete the following table.

Type of Rejection	Time of Onset	Immunologic Basis
Hyperacute		
Acute		
Chronic		

GRAFT VS HOST DISEASE

This is not a problem with heart or kidney transplants but is a problem in bone marrow transplants. The problem occurs whenever there is an antigen difference between donor and recipient, such as is the case when parental cells (e.g., AA) are injected into the F_1 (AB) offspring. If the bone marrow (since it produces immunologically competent cells such as lymphocytes) can produce lymphocytes which can become sensitized to the recipient's antigens. Thus, the graft tissue can mount an immunological attack on the recipient. This is graft rejection of the host. The graft vs host reaction in humans is characterized by liver abnormalities, by a skin rash that looks like measles, and by diarrhea, wasting, and death.

Graft vs. host disease occurs when

_____

_____

_____.

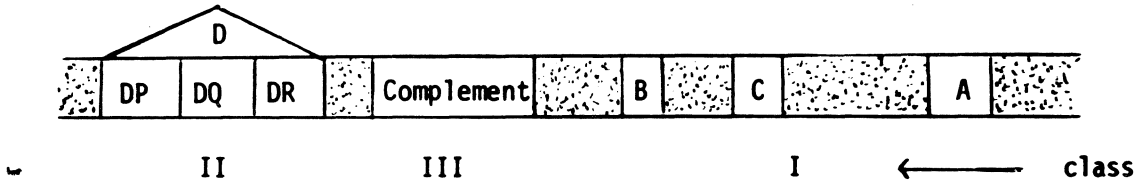
Symptoms of this phenomenon in humans include

1. _____
2. _____
3. _____
4. _____
5. _____

IMMUNE RESPONSE (IR) GENE

The Ir sublocus on the HLA locus is involved in both humoral and cell mediated immune responses. Genetics of the mouse indicated that the ability of the immune system to make antibodies to particular antigens is genetically determined. The gene locus for this seems to be linked to the H2 locus - the major histocompatibility locus of the mouse. This has also been observed in the guinea pig. Humans with ankylosing spondylitis or Reiter's syndrome are usually HLA-B27 histotype.

The major histocompatibility complex in humans occurs on the 6th chromosome. It can be diagrammed as follows.



A, B and C = Serologically defined antigens - their presence is detected by cytotoxic of specific antisera plus complement.

D = lymphocyte defined (LD) antigens-antigens detected by mixed lymphocyte reactions. DR, DQ and DP (D related antigens) also code here.

Mixed lymphocyte reaction detects blastogenesis of T lymphocytes in response to histocompatibility antigens foreign on the membrane of the stimulating cell (donor cell: usually a lymphocyte as these cells are particularly rich in these antigens). Donor lymphocytes are poisoned (mitomycin) so they can not divide in response to recipient lymphocytes.

I = genes involved in the recognition and destruction of virally infected activity cells. May also serve as targets in graft rejection.

II = genes involved in immune responses, T & B cell interactions, etc.

III = genes for some of the complement proteins.

HUMAN TUMOR IMMUNOLOGY

Some human tumors have characteristic specific antigens, much like the virus induced tumors in animals, such as neuroblastoma and Wilm's tumor, while other tumors have antigens unique to the individual tumor in the individual host (e.g., certain melanoma antigens).

Examples of human tumor specific antigens (TSA) include:

1. CEA (carcinoembryonic) antigen and alpha fetoprotein
2. Neuroblastoma
3. Wilm's tumor, lung carcinoma, melanoma.
4. MA - membrane antigen of Burkitt lymphoma.

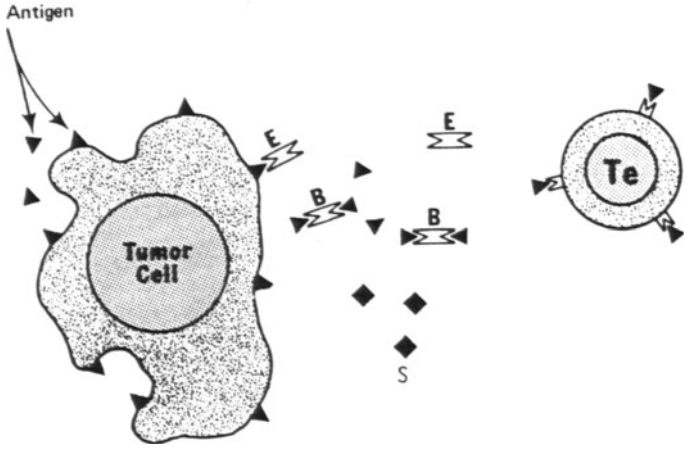
There are many malignancies which synthesize embryonic antigens, for example:

Alpha fetoprotein: normally only made by the fetal liver, but also seen in hepatoma, gastric CA, prostate CA. But no all hepatomas make alpha fetoglobulin, so if this were used as a screening test, there would be false negatives.

CEA: a glycoprotein found in the glycocalyx of cells derived from endoderm and present in the GI carcinomas, especially CA of the colon. It is not completely absent in normal individuals. It is used to follow a patient under therapy, as after surgical removal of CA of the colon.

Tumor specific transplantation antigens (TSTAs) are those against which an immune response could effect irradiation of the tumor. They must be expressed on the cell membrane to be accessible to the protective antibodies and regulatory cells of the host. These are the "candidate" vaccines that are being sought.

Tumor Products Which Aid Growth



E = Enhancing antibody

B = Blocking factor

S = Suppressor molecules

IMMUNE RESPONSE TO TUMORS

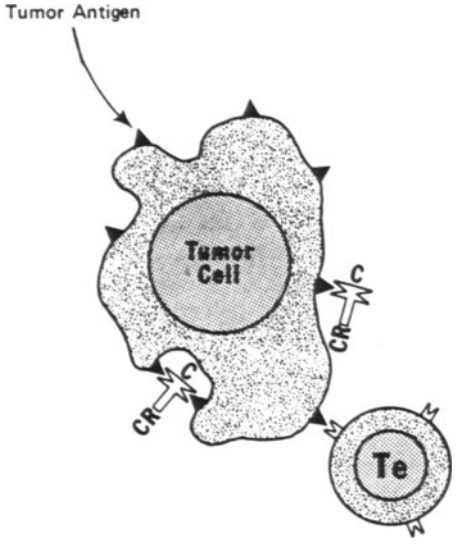
A. Protective to host:

1. Cytotoxic T cells - contact killing - vs tumor antigens.
2. Antibody-dependent cell - mediated cytotoxicity (ADCC) via K cells with receptors for Fc of Ig - contact killing.
3. Natural killer (NK) cells - contact killing.
4. "Activated" macrophages - e.g. via lymphokine (MAF) - "non-specific" contact killing.
5. Cytotoxic antibody (plus complement).

B. Deleterious to host:

1. "Enhancing" antibody - non cytotoxic = combines with tumor target, preventing interaction with cytotoxic T cell.
2. "Blocking" factors, e.g.
   a. Soluble ("Shed") tumor antigen
   b. Antigen (tumor) + antibody complexes combined with cytotoxic T cells or K cells preventing interaction with tumor target.
3. Suppressor (Ts) cells, or their soluble products, interfere with:
   a. cytotoxic T cells
   b. NK cells
   c. antibody (protective) formation

Host Factors Which Limit Tumor Growth



C = Cytotoxic antibody

CR = Complement receptor

Te = T effector lymphocytes (cytotoxic cell, etc.)

Immunology Review Statements

These should be used to confirm your understanding of the subject. If you are uncertain about the veracity of a statement, please "check it out".

A graft exchanged between brother and sister is defined as an allograft.

Adjuvants are nonspecific, mildly irritating substances which are sometimes used to enhance antibody responses.

A graft vs. host reaction occurs when immunocompetent cells are put into an immuno-incompetent host.

Serum sickness may ocur when there are immune complexes in the blood.

A positive delayed type hypersensitivity skin reaction involves a rather complex interaction of antigen, antigen-sensitive lymphocytes and monocytes/macrophages.

In the third and fourth pharyngeal pouch syndrome (DiGeorge syndrome), the patient's thymus is absent, one might, therefore, predict that thymus-dependent areas of lymph nodes would be sparsely populated.

Complement activation is involved in production of anaphylotoxin, immune hemolysis, and enhanced phagocytosis.

Lymphokines play an essential role in delayed hypersensitivity reactions.

One of the current theories concerning the etiology of autoimmune diseases suggests that they might actually represent an immune deficit in suppressor T-cells.

Examples of cancer-associated antigens which probably arise from tissue de-differentiation include carcinoembryonic antigen and alpha fetoprotein.

Cell mediated immunity (delayed hypersensitivity) is suppressed by cortisone.

A T cell response to BP (basic protein) appears to be responsible for the pathogenesis of experimental allergic encephalomyelitis.

Leukotriene B4 is chemotactic for neutrophils.

Lymphocytes sensitized to acetylcholine receptor appear to be involved in the pathogenesis of myasthenia gravis.

Reticular dysgenesis (deVaal syndrome) is characterized by defective development of T-cells, B-cells and PMN's.

Recurrent infections, defective processing of polysaccharide antigens, T-cell deficit, elevated serum IgA, and depressed serum IgM are characteristic of Wiskott-Aldrich syndrome.

A serious complication of the use of immunosuppressive agents is the increased susceptibility to opportunistic infections and malignancy.

In general, immune suppressive measures are most effective when given just prior to antigen exposure.

Monomeric IgA molecules acquire secretory piece while passing through epithelial cells.

In a C fixation test, too much C would cause a false negative and anti-complementary antigen would cause a false positive.

Anti-light chain antiserum would precipitate the Fab fragment of immunoglobulins of all classes.

T cells are more susceptible to tolerance induction than are B cells.

T cells tolerance lasts longer than B cell tolerance.

T cells and B cells are both needed for most humoral immune responses (macrophages also participate as "presentors" of the antigen).

Con A and PHA are mitogenic for T cells.

T cells form rosettes wtih sheep RBC.

B cells from rosettes with antibody and complement-coated sheep RBC.

Low doses of antigen induce T lymphocyte tolerance, high doses induce tolerance in both T and B cells.

Thyroglobulin tolerance in humans is a type of low dose tolerance.

Animals in low dose tolerance will have normal B lymphocytes.

Prostaglandins are vasoactive compounds which are formed in the body as a result of cyclooxygenase action on arachidonic acid.

Parental cells into F 1 hybrids produce graft vs. host reactions.

In Bruton's hypogammaglobulinemia there are; no plasma cells, recurrent bacterial infections, and normal responses to most viral and fungal agents.

Diseases with depressed CMI include Hodgkins disease, sarcoidosis, and lepromatous leprosy.

Evidence for a 3 gene code for L chains includes the V, J and C regions in the molecule; H chains have an addition gene, the D gene.

Serum sickness is often accompanied by decreased complement levels.

Cell mediated immunity is passively transferred by lymphocytes.

B cells are in germinal centers, are precursors of plasma cells, and have a short life span.

PMN degranulation is due to lysosome fusion with phagosome.

T cell functions are evaluated in humans by skin tests with the following antigens: Candida, DNCB, mumps, PPD and/or SK-SD.

IgE attached to basophils and mast cells is essential for atopic allergy.

Allergy and eosinophilia often go hand-in-hand.

The vaccine component which prevents pneumococcal disease is the capsule which induces opsonins.

Immunodeficiency resulting in susceptibility to viral infections is due to a deficiency in T cells.

Impaired cellular immunity is frequently associated with Hodgkin's disease and sarcoidosis.

The target tissues in Goodpasture's syndrome are alveolar and glomerular basement membranes.

An autoimmune disease with a characteristic IgM antibody response to autologous IgG is rheumatoid arthritis.

A 4 year-old girl suffering from repeated infections with staphylococci and streptococci was found to have normal phagocytic function and delayed hypersensitivity responses. Lymph node biopsy would probably reveal absence of germinal centers.

Transplantation of fetal thymus would be beneficial in an immunodeficient neonate with di George syndrome.

Immunodeficiency resulting in susceptibility to acute pyogenic bacterial infections is due to a deficiency in macrophages or B cells.

In systemic lupus erythematosus, death from renal failure is probably due to glomerular deposition of antigen-antibody-complement complexes.

Rheumatoid factor is an antibody directed against determinants on the gamma chain.

Graft vs. host reaction may occur when viable lymphoid cells are present in the graft.

Anti-thymocyte serum is effective in suppressing allograft rejection because of its ability to suppress cell-mediated immunity.

Immunologically based chronic graft rejection may be caused by specifically sensitized lymphocytes or humoral antibodies.

Hyperacute graft rejection may be caused by blood group incompatibility between donor and recipient, or previous sensitization of female recipients as a result of multiparity.

"HLA" antigens are human leukocyte antigens found primarily at the cell surface.

Agammaglobulinemic patients lack plasma cells.

In order to classify a tumor antigen as an oncofetal antigen it must be shown to be present in regenerating cells or in fetal or embryonic cells.

Most polysaccharides are thymus independent antigens, i.e. they interact directly with B cells to trigger antibody synthesis without the participation of T helper cells and macrophages.

Only IgG and IgM activate C by the classical pathway.

Clinically, assays for carcinoembryonic antigen (CEA) show their greatest promise in evaluation of completeness of surgical excision of the malignancy, and follow-up for recurrence.

The strongest evidence for the role of the immune system in preventing the establishment of tumors ("immune surveillance") is the marked increased incidence of malignancies in persons with congenital or acquired immune deficiencies.

T cells are involved in production of lymphokines.

Chronic granulomatous disease is due to a deficit in oxidized NAD.

Toxoids are antigenic and nontoxic.

The immunosuppressive effect of cortisone is attributed primarily to its ability to produce a lymphopenia.

Anti-thymocyte serum is effective in suppressing homograft rejection because of its ability to suppress delayed hypersensitivity.

Anaphylatoxin is a peptide split product of C5 (C5a).

Erythroblastosis fetalis is a hemolytic disease in the newborn infant caused by maternal IgG antibody against fetal red cell antigens.

In humans, anaphylactic reactions result from the combination of allergens with homocytotropic antibodies of the immunoglobulin class IgE.

Interferon stimulates the production of translational inhibitory proteins, which are the actual antiviral materials.

IgE has affinity for mast cells and basophils.

Lesions seen in serum sickness result from complexes formed by antigen, complement, and IgG or IgM.

The fundamental lesion in immune complex-induced glomerulonephritis requires neutrophils and complement.

Immune complexes appear to be involved in the pathogenesis of post-streptococcal glomerulonephritis, the Arthus reaction, and glomerulonephritis of systemic lupus erythematosus.

The class of immunoglobulin capable of passing through the human placenta from mother to fetus is IgG.

An Fab fragment of IgG immunoglobulin consists of an entire light chain and part of a heavy chain.

Erythroblastosis fetalis can be prevented if the mother is injected, at parturition, with an anti-Rh° antibody called Rhogam.

Fab fragments can neither precipitate nor agglutinate an antigen, but they can block these reactions which could be caused by complete antibody molecules.

Specific immunologic tolerance is most easily induced in T cells.

The complement components which react with the classical pathway but not in the C3 bypass are C1, C4 and C2.

Individuals with hereditary angioneurotic edema have a deficiency in C1 esterase inhibitor.

Innate immunity can be defined as a complex, naturally-occuring system of defense mechanisms which protect the individual from infectious agents.

Administration of corticosteroids will heighten susceptibility to infectious diseases.

Chemotaxis is directed movement of phagocytic cells (usually toward) in response to foreign materials in the body.

The mucopeptidase which attacks the bacterial cell wall is lysozyme.

Normal serum enhances phagocytosis; this process is called opsonization.

Antimicrobial products of the NAD system include superoxide anions.

Interferon induces host cells to produce anti-viral proteins which interfer with translation of viral mRNA.

The predominant antibody in saliva is sIgA.

In addition to immunogenicity, antigens have the property of specific reactivity.

Substances which are antigenic only when coupled to a protein carrier are called haptens.

An antigen which occurs irregularly in various tissues of different species is referred to as heterogenetic.

Haptens have reactability but no immunogenicity.

Immunoelectrophoresis of serum from a patient with Bruton's hypogammaglobulinemia would most likely reveal no gammaglobulin bands.

A negative complement fixation test is indicated by hemolysis.

The rapid rise, elevated level, and prolonged production of antibody which follows a second exposure to antigen is called anamnesis, and is mainly IgG.

T cell membranes contain receptors for phytohemagglutinin (PHA).

B cells form rosettes with sheep RBC + Ab + C.

Immunocompetent B cells have surface immunoglobulin of the IgM class.

IgM is the major antibody that a fetus can make. If the IgM level is elevated at birth it may signify in utero infection.

Bordetella pertussis adjuvant will induce an increase in the IgE response.

The thymic lymphocytes which recognize an antigen and interfere with the development of a humoral immune response are called suppressor cells.

IgG is the most efficient Ig at fixation of complement.

Mu and epsilon heavy chains have 5 domains; gamma and alpha heavy chains have 4.

All light chains have 2 domains (1 variable and 1 constant).

T lymphocytes are important in viral immunity; one of the lymphokines they release is interferon.

Anti-histamines are useful in the treatment of atopic disease.

Histamine is one of the mediators released from mast cells and basophils.

The immunologic problem in kidney transplants is host vs. graft disease (acute rejection, etc.).

In bone marrow transplants, it is graft vs. host disease because the recipient is immunodepressed.

Cytotoxic T lymphocytes are involved in killing tumor cells and transplanted tissues, but are not active in killing bacteria.

The radioallergosorbent test (RAST) measures IgG specific for a given allergen, while the paper radioimmunosorbent test (PRIST) quantitates the total serum IgE.

Aryl sulfatase, an ezyme of eosinophil origin, inactivates SRS-A, slow reacting substance of anaphylaxis.

The IgG antibodies which are produced after allergen injection protect the allergic individual by competing with cell-bound IgE for the triggering antigen.

The most common immunoglobulin deficiency disease involves absence of IgA.

Patients with deficiencies of C6-8 have an increased occurrence of infections caused by the gonococci and the meningococci.

IMMUNOLOGY PROFICIENCY TEST

When this examination was taken by sophomore Medical students in the fall of 1985, the mean was 84 percent. A score of less than 70 percent would suggest that more study in immunology is indicated.

1. All of the following are indices of B cell activity EXCEPT

   A. Radial immunodiffusion.
   B. Quellung reaction.
   C. Mumps complement fixation test.
   D. Enumeration of E rosettes.
   E. Polio plague reduction assay.

2. Infectious agents associated with acquired immune deficiency disease include

   A. Pneumocystis carinii.
   B. cytomegalovirus.
   C. Human T cell lymphotropic virus (HTLV-III).
   D. All of the above.
   E. A and C only.

3. Rho-specific immune globulin (RhoGAM) therapeutic preparations are correctly described as composed of

   A. Anti-inflammatory agents.
   B. blocking antibodies.
   C. anti-lymphocyte antibodies.
   D. anti-allergen antibodies.
   E. enhancing antibodies.

4. Antibody against autologous IgG would be synthesized in

   A. central lymphoid organs.
   B. peripheral lymphoid organs.
   C. thymic tissue.
   D. macrophages.
   E. phagosomes.

5. A person of blood group AB has

   A. anti-A and anti-B isohemagglutinins in their plasma.
   B. A and B antigens (hemagglutininogens) on their erythrocytes.
   C. both.
   D. neither.

6. The mucopeptidase which attacks the bacterial cell wall is

   A. glucuronidase.
   B. lysozyme.
   C. myeloperoxidase.
   D. cathepsin.
   E. trypsin.

7. Immunogenicity is related to

   A. molecular size.
   B. degree of foreignness.
   C. chemical composition.
   D. all of the above.
   E. A and C only.

8. Maximum precipitation occurs in the

   A. Prozone.
   B. zone of equivalence.
   C. postzone.
   D. demilitarized zone.
   E. end zone.

9. The indicator system of a complement fixation test includes

   A. specific antibody and complement.
   B. specific antigen and complement.
   C. erythrocytes and hemolysin.
   D. heat-inactivated patient's serum.
   E. erythrocytes and complement.

10. In the FTA test used to diagnose syphilis, the final reagent added to a slide is

    A. fluorescein-tagged antibody to Treponema pallidum.
    B. fluorescein-tagged antibody to human gamma globulin.
    C. cardiolipin and carbon particles.
    D. cardiolipin alone.
    E. fluorescein-tagged cardiolipin.

11. The absence of agglutination when patient's serum is mixed with influenza virus and added to a suspension of erythrocytes indicates

    A. the patient has an antibody specific for erythrocytes.
    B. the patient has an antibody specific for influenza virus.
    C. the serum was heated to 56° for 30 minutes.
    D. the virus shares an antigen with the erythrocyte.
    E. the patient has an immunodeficiency.

12. Which of the following tests is the most sensitive measure of antibody?

    A. Precipitation
    B. Agglutination
    C. Radial immunodiffusion
    D. Radioimmunoassay
    E. Flocculation

13. Functional assessment of T lymphocytes includes

    A. E rosette assay.
    B. sIG evaluation.
    C. transforamtion of lymphocytes by PHA.
    D. serum immunoglobulin determination.
    E. enumeration of theta antigen bearing cells.

14. The OKT8 monoclonal antibody reacts with the T cell subpopulation responsible for

A. Helper cell activity.
B. Suppressor cell activity.
C. Natural killer cell activity.
D. All of the above.

15. The mixed lymphocyte reaction is a measure of

A. B cell function.
B. granulocyte function.
C. natural killer cell function.
D. T cell function.
E. macrophage function.

16. Bruton-type agammaglobulinemia is a disease in which

A. a failure of development of the thymus is noted.
B. a failure of development of the B cell system occurs.
C. severe lymphopenia occurs.
D. monocytes do not present antigen to the lymphoid system properly.

17. Severe combined immunodeficiency can be caused by

A. infection with HTLV-III.
B. a deficiency of the enzyme adenosine deaminase.
C. a deficiency of the enzyme purine nucleotide phosphorylase.
D. All of the above.
E. A and C only.

18. Tumors which are induced by viruses may have

A. TSTA associated with the membrane of the tumor.
B. TSA in the cytoplasm of the tumor cell.
C. TSA in the nucleus of the tumor cell.
D. All of the above.

19. Measurement of increased alpha fetoprotein levels is useful in

A. diagnosis of primary liver cancer.
B. diagnosis of tumors of the GI tract.
C. monitoring the course of therapy of primary liver carcinoma.
D. all of the above.
E. A and C only.

20. The most effective serum blocking factors consist of

A. free tumor antigens.
B. anti-tumor antibodies.
C. anti-granulocyte antibodies.
D. antigen-antibody complexes containing tumor antigen and anti-tumor antibody.

21. Which of the following is not effective in suppressing tumor growth?

A. Cytotoxic T cells
B. Natural killer cells
C. Suppressor cells
D. Antibody dependent cytotoxic cells

22. Natural killer cells have which of the following characteristics?

A. Large granular lymphocytes
B. Non-T, non-B lymphocytes
C. Do not require presensitization for activity
D. All of the above
E. A and C only

23. In Nezelof's syndrome there is

A. an inability to make immunoglobulin of any class.
B. an inability to mount an anamnestic response upon antigenic challenge.
C. a profound depletion of B lymphocytes.
D. a negative NBT test.

24. Which of the following assays would you consider as one of the first to choose when evaluating a patient for possible immunodeficiency?

A. Helper cell assay
B. Suppressor cell assay
C. Natural killer cell assay
D. Quantitative immunoglobulin levels

25. The goal of immunotherapy of asthma is to increase

A. the total IgE level.
B. the allergen-specific IgE.
C. the total IgG level.
D. the allergen-specific IgG level.

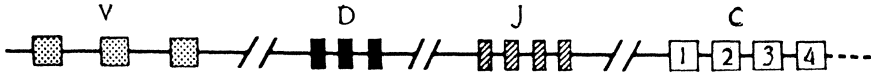
26. The drug of choice for the prevention of an asthmatic attack is

A. atropine.
B. benedryl.
C. cromolyn sodium.
D. dexedrene.
E. epinephrine.

27. An asthmatic attack can be precipitated by

A. sinusitis.
B. exercise.
C. rhinitis.
D. all of the above.
E. A and C only.

28. This diagram represents



A. a germline gene.
B. an active gene.
C. an immunoglobulin light chain.
D. an immunoglobulin heavy chain.

29. The structural genes for immunoglobulin light chains and heavy chains

A. are genetically linked.
B. are present on different chromosomes.
C. regulate rates of formation of the immunoglobulins.
D. also code for tranplantation antigens and complement components.

30. Which of the following statements best describes the function of properdin in the alternative complement pathway?

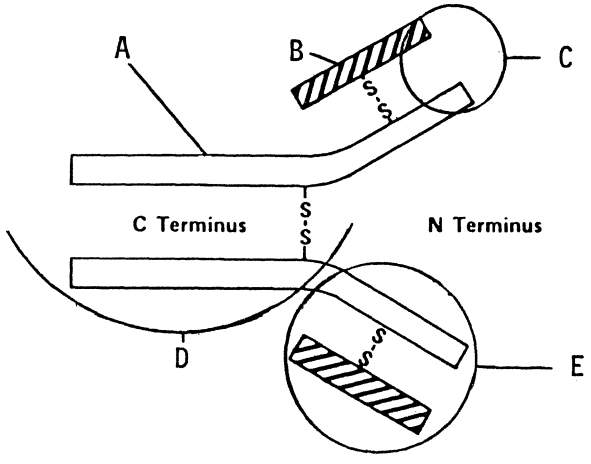
A. It is the recognition protein of the alternative pathway.
B. It interacts directly with cell membranes.
C. It stabilizes the C3 convertase of the alternative pathway.
D. One of its cleavage fragments is chemotactic.
E. It is opsonic.

31. Idiotypic determinants of immunoglobulin molecules, the CDR's, are associated with

A. the Fc portion.
B. constant regions of both H and L chains.
C. hypervariable regions of L chain only.
D. hypervariable regions of both H and L chains.
E. hypervariable regions of H chain and Fc portion.

Questions 32-34

For each of the components described below, choose the letter on the diagram that represents the portion of the immunoglobulin molecule with which it is associated.



32. Light chain

33. Fab fragment

34. Fc fragment

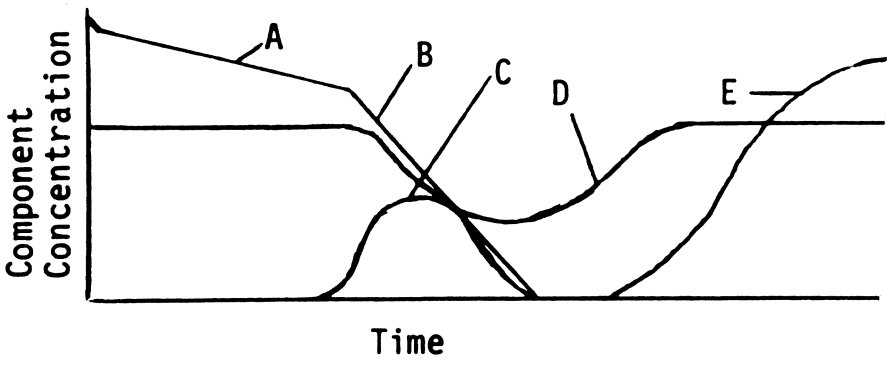
35. Anamnestic reaction refers to

A. gradually rising antibody titers.
B. true immunologic paralysis.
C. the prompt production of antibodies following secondary injection of antigen.
D. species specific antibodies.
E. the lag period in antibody production following a single primary injection of antigen.

36. The mediators of anaphylaxis

A. include histamine, ECF-A, and SRS-A.
B. are released with increasing cellular levels of cAMP.
C. result in smooth muscle contraction, increased vascular permeability and increased mucous secretion.
D. all of the above.
E. only A and C.

Various immunologic factors play a role in serum sickness. For each term below, choose the letter in the diagram that identifies the corresponding component of this disease process.



37. Phase of immune clearance

38. Immune Complexes

39. Complement level

40. Antibodies against acetylcholine neural receptors are involved in the pathogenesis of

A. myasthenia gravis.
B. multiple sclerosis.
C. acute idiopathic polyneuritis.
D. Guillain-Barre syndrome.
E. postpericardiotomy syndrome.

41. Lesions seen in serum sickness result from complexes formed by antigen, complement, and

A. IgA.
B. IgD.
C. IgG.
D. IgE.

42. In delayed hypersensitivity

A. reactivity can be passively transferred with polymorphonuclear leukocytes.
B. reactivity can be passively transferred with lymphocytes.
C. the cellular infiltrate at a positive skin test site (48 hours) consists primarily of macrophages, eosinophils and basophils.
D. the inducing agents are always of bacterial origin.

43. "HLA" antigens are

A. human leukocyte antigens found primarily intranuclearly.
B. genetically determined antigens found on the surface of human leukocytes.
C. the antigens which mark horse lymphocytes as foreign to humans.
D. assayed in vitro in order to detect rejection when it begins.

44. β2 microglobulin is associated with

A. HLA Class I antigens.
B. HLA Class II antigens.
C. HLA Class III antigens.
D. delayed hypersensitivity.

For each of the incomplete statements, one or more of the completions given is correct. The answer is

A. if only (1), (2) and (3) are correct.
B. if only (1) and (3) are correct.
C. if only (2) and (4) are correct.
D. if only (4) is correct.
E. if all are correct.

45. Infections are a significant cause of death in patients with

1. Bruton's hypogammaglobulinemia.
2. Di George syndrome.
3. chronic granulomatous disease.
4. systemic lupus erythematosus.

46. Receptors for C3b are present in the membranes of

1. macrophages.
2. B lymphocytes.
3. neutrophils.
4. T lymphocytes.

47. Serodiagnosis of autoimmune disease usually involves the detection of which of the following in a person's blood?

1. Elevated levels of γ-globulin.
2. Depressed levels of complement.
3. Immune complexes.
4. Depressed numbers of T8-bearing lymphocytes.

48. A preparation of pooled human IgM injected into a rabbit may stimulate production of antibodies reactive with

1. λ light chain.
2. μ chain.
3. κ light chain.
4. J chain.

49. The major histocompatibility complex includes genetic loci for

1. transplantation antigens.
2. complement components.
3. complement product receptors.
4. immune responsiveness.

50. In the DiGeorge syndrome (complete form) there is

1. a depletion of lymphocytes in the paracortical areas of lymph nodes.
2. depression of PHA responsiveness.
3. depressed mixed lymphocyte reaction.
4. absence of parathyroid glands.

ANSWER SHEET

1.	D	31.	D
2.	D	32.	B
3.	B	33.	E
4.	B	34.	D
5.	C	35.	C
6.	B	36.	E
7.	D	37.	B
8.	B	38.	C
9.	C	39.	D
10.	B	40.	A
11.	B	41.	C
12.	D	42.	B
13.	C	43.	B
14.	B	44.	A
15.	D	45.	E
16.	B	46.	A
17.	D	47.	E
18.	D	48.	E
19.	C	49.	E
20.	D	50.	E
21.	C		
22.	D		
23.	B		
24.	D		
25.	D		
26.	C		
27.	D		
28.	B		
29.	B		
30.	C		

REVIEW OF PATHOGENIC MICROBIOLOGY

DEFINITIONS

Endogenous Infection: The source of infection is from within; the etiologic agent of the disease is often a member of the normal indigenous flora.

Exogenous Infection: The source of infection is from without; usually, from another infected host or directly from the environment.

Healthy Carrier: A healthy human (or animal) host which is infected with a potential pathogen in a body region from which the pathogen can escape and be transmitted to other susceptible hosts.

Host: The larger organism (plant or animal) which is parasitized.

Infection: The entrance, growth (colonization), and multiplication of a parasite in a living host organism, regardless of the effect on the host.

Infectious Disease: The result of multiplication and growth of a parasite within a susceptible host in which the host is altered or damaged; i.e., the development of clinical signs and symptoms.

Invasiveness: The ability of a parasite to spread through host tissues.

Normal Flora: Parasitic microrganisms which are normal residents of mucosal surfaces of practically all healthy individuals. Synonyms - resident flora, indigenous flora, autochthonous flora.

Opportunistic Pathogen: A parasitic or saprophytic (free-living) organism which ordinarily does not cause disease, but, under appropriate conditions, may produce damage in a compromised host.

Parasite: A small organism which lives within or upon a larger organism of a different species, usually at the expense of the larger organism.

Pathogenicity: The ability of a parasite to cause disease in a susceptible host.

Persisting Latent Infection: A form of inapparent infection following a clinical case in which the pathogen is not completely eliminated, but remains viable in the tissues after the host recovers. The host may be infectious.

Virulence: The degree of disease-producing power of a pathogen. A quantitative measure of pathogenicity.

GRAM STAIN

The Gram stain aids in the identification of bacteria according to their size, shape and grouping by rendering them more visible. It also helps identify them by dividing the eubacteria into Gram positive or negative organisms. The cell wall composition of Gram negative bacteria differs from that of Gram positive. Since Gram negative bacteria have a high lipid content of their cell walls, one theory holds that the decolorizer (acetone or ethanol) solubilizes the cell wall thus reaching the crystal violet. In the cell wall of Gram positive organisms, the decolorizer is unable to act as a solvent thus the crystal violet remains.

Step	Reagent
1.	Crystal violet (c.v.)
2.	Sodium bicarbonate
3.	Gram's iodine
4.	Acetone
5.	Sofranin

Appearance of organisms, step by step:

	Gram Positive	Gram Negative
1.	purple	purple
2.	purple;alkalinized	purple;alkalinized
3.	purple;fixes c.v. in cell	purple;fixes c.v. in cell
4.	purple	colorless; c.v. washes out because cell wall (lipid) soluble in acetone -thus the organisms are unstained again
5.	purple	red/pink

Mycoplasma do not have a cell wall.

Their gram reaction would be

_____.

Age can cause conversion from Gram positive to negative. What technical errors in the staining procedure could do the same?

1. _____
2. _____
3. _____

MORPHOLOGY AND GRAM REACTION OF SOME BACTERIA OF MEDICAL IMPORTANCE

Gram +	Gram -
COCCI	
Staphylococcus	Neisseria
Streptococcus	Veillonella(2)
BACILLI	
Bacillus(1)	Escherichia
Clostridium(1,2)	Klebsiella
Corynebacterium	Proteus
Listeria	Pseudomonas
Mycobacterium	Salmonella
	Shigella
	Brucella
	Francisella
	Yersinia
	Haemophilus
	Bordetella
	Bacteroides(2)
SPIROCHETES	
	Borelia(2)
	Leptospira
	Treponema(2)

1. only sporeforming pathogens
2. only obligate anaerobic pathogens in list

Generalities That Can Be Draw From Gram Reaction Table

Generality	Exceptions
All cocci are Gram positive	Neisseria, Veillonella
All rods are Gram negative	Bacillus, Clostridia, Corynebacteria, Listeria, Mycobacteria
All pathogens are facultative anaerobes	Clostridia, Bacteroides (obligate anaerobes)
Only bacilli have flagella	None

SIGNIFICANT CHARACTERISTICS OF SELECTED HUMAN PATHOGENS

ORGANISM	DISEASE(S)	CHARACTERISTIC
GRAM POSITIVE BACILLI		
Corynebacterium diphtheriae	Diphtheria	Natural host man. Noninvasive disease due to toxin.
Bacillus anthracis	Anthrax	Pathogen of herbivorous animals who ingest spores; occasional human infection.
Clostridia	Tetanus, gas gangrene, botulism	Widely distributed in soil and intestines.
GRAM NEGATIVE BACILLI		
Escherichia coli	Urinary tract infections Gastroenteritis	Normal intestinal inhabitant (man and animals). Many antigenic types.
Salmonella sp.	Enteric Fever, food poisoning	S. typhi-natural host man. Invasive. Other Salmonella mainly animal pathogens.
Shigella sp.	Bacillary dysentery	Obligate parasites of man. Local invasion only.
Proteus sp.	Urinary tract and wound infection	Common in soil, feces. Occasionally pathogenic.
Klebsiella sp.	Urinary tract and wound infection, otitis, meningitis, pneumonia	Present in vegetation, soil, sometimes feces. Pathogenic when host resistance lowered.
Pseudomonas aeruginosa	Urinary tract and wound infection	Resists many antibiotics.
Haemophilus influenzae	Pneumonia, meningitis	Human commensal. Invades damaged lung.
Bordetella pertussis	Whooping cough	Human respiratory parasite.
Yersinia pestis	Plague	Pathogen of rodents; Flea transfer to man.
Yersinia enterocolitica	Gastroenteritis	invade intestinal mucosa.
Brucella sp.	Undulant fever	Pathogens of goats, cattle, pigs with secondary human infection.
Bacteroides sp.	Pulmonary abscesses, Pelvic inflammatory disease	Normal oral and intestinal flora.

SIGNIFICANT CHARACTERISTICS OF SELECTED HUMAN PATHOGENS (CONTINUED)

ORGANISM	DISEASE(S)	CHARACTERISTIC
ACID FAST BACILLI		
Mycobacterium tuberculosis	Tuberculosis	Chronic respiratory infection.
Mycobacterium leprae	Leprosy	Obligate parasite of man. Attacks skin and nerves.
GRAM-POSITIVE COCCI		
Staphylococcus aureus	Boils, septicemia, food poisoning.	Common skin flora. Phage typing identifies virulent strains. Enterotoxin caused food poisoning.
Streptococcus pyogenes	Tonsillitis, scarlet fever, erysipelas, septicemia	Glomerulonephritis, and rheumatic fever, with immuno-pathological basis.
Viridans streptococci	Vegetative endocarditis	Harmless oral flora. Settles on abnormal heart valves during bacteremia.
Streptococcus pneumoniae	Pneumonia, otitis, meningitis	Normal upper respiratory tract flora, can spread to damaged lungs.
GRAM-NEGATIVE COCCI		
Neisseria gonorrhoeae	Gonorrhoea	Obligate human parasite.
Neisseria meningitidis	Meningitis	Obligate human parasite;
MISCELLANEOUS		
Vibrio cholerae	Cholera	Noninvasive intestinal infection.
Treponema pallidum	Syphilis	Obligate human parasite.
Actinomyces israeli	Actinomycosis	Normal inhabitant human mouth; obligate anaerobe.
Leptospira interogans	Leptospirosis	Human infection from rat urine.
Campylobacter jejuni	diarrhea	Microaerophilic, gram negative rod
Camphylobacter fetus	septicemia and meningitis	Microaerophilic, gram negative rod

GRAM POSITIVE COCCI

STREPTOCOCCUS GENUS

I. General Characteristics

A. Some have capsules (Group A = hyaluronic acid)
B. Production of hemolysis
   1. α-hemolytic (viridans)
   2. β-hemolytic (complete)
   3. γ-hemolytic (none)
C. Catalase negative
D. Penicillin = Drug of Choice

The drug of choice for most streptococcal infections is _____.

STREPTOCOCCUS PNEUMONIAE

I. General Characteristics

A. bile, optochin, detergents lyse colonies
B. alpha hemolytic; optochin sensitive
C. Drug of choice = penicillin

II. Antigenic Structure

A. Capsular polysaccharide = 75-80 serological types
   1. SSS - specific soluble substance = quellung rx (capsule swelling)
   2. protective antibody = opsonin
   3. Vaccine is composed of SSS from the most prevalent types (20+)

Artificial, actively acquired immunity against pneumococcal pneumonia is readily induced by a vaccine composed of _____.

III. Distribution

A. Normal flora; common cause of lobar pneumonia in alcoholics, and as secondary invader following viral infections (e.g., influenza) Hematogenous spread may result in meningitis in debilitated adults.

The pneumococcus is alpha/beta/gamma hemolytic.

IV. Bacterial Factors Involved in Pathogenicity

A. Prime Virulence factor = Capsule - antiphagocytic - content of vaccine (Pneumovac) - confers opsonic immunity

β-HEMOLYTIC STREPTOCOCCI

I. Antigens used for Identification (bacterial cell wall antigens)

A. Group-specific C Antigens
   1. carbohydrate
   2. divide organisms into groups A-O; A is sensitive to bacitracin

B. Type-specific M Antigens (protein)
   1. subdivide group A into 50 + types
   2. antiphagocytic - virulence factor
   3. immunity is type specific

II. Other Surface Components

A. Hyaluronic acid capsule = anti-phagocytic CHO; not antigenic
B. Lipoteichoic acid = important in adherence to epithelial cells

III. Extracellular Products

A. Erythrogenic toxin (pyrogenic exotoxin) - produced by lysogenic strains of group A
   1. erythema and edema
   2. three immunological types
   3. positive Dick test indicates susceptibility to Scarlet fever

B. Streptolysin O (oxygen labile; sulfhydryl activated)
   1. antigenic - used to detect antibody resulting from infection (ASO)
   2. leukotoxic - cytolytic

C. DNAse
   1. 4 immunologic types (A,B,C,D) - type B used to measure antibody resulting from infection

D. Fibrinolysin - converts plasminogen to plasmin

Antigens of importance in the serologic identification of beta hemolytic streptococci include

A. group specific C carbohydrate

B. type specific M protein

C. both

D. neither

(answer at bottom of page, left side)

Antiphagocytic surface components of group A streptococci include

A. hyaluronic acid

B. M protein

C. both

D. neither

(answer at bottom of page, left side)

(answer to both questions = C)

IV. Types of Diseases

A. Group A (S. pyogenes) acute infections
   1. streptococcal pharyngitis (with toxemia = scarlet fever)
   2. pneumonia
   3. puerperal sepsis
   4. erysipelas

B. Group A post-infection sequelae
   1. acute glomerulonephritis - associated with skin or upper respiratory tract infections
      a) one + weeks after infection
      b) nephritogenic strains (e.g., types 4, 12, 49)
      c) may be antigen-antibody reaction resulting in binding of complement ("lumpy-bumpy" deposition of Ig in basement membrane)
   2. acute rheumatic fever - associated with upper respiratory tract infection only: strep cross reacts with heart tissue.

C. Non-group A Diseases
   1. Caries - plaque caused by adherent dextrans of S. mutans
   2. Endocarditis (oral and enteric strep): neonatal CNS (group B)

Non-supportive sequalae of group A streptococcal infections include

A. Scarlet fever

B. erysipelas

C. puerperal fever

D. caries

E. Rheumatic fever

(answer at bottom of page, left side)

PEPTOCOCCI, PEPTOSTREPTOCOCCI

I. Obligate anaerobes

II. Mixed Infections

III. Pulmonary Abscesses

(answer is E)

Two streptococcal organisms are commonly associated with meningitis. Complete the table below.

	Patient Group	Streptococcal species
example-	kids with caries	S. mutans
	neonates	
	debilitated adults	

STAPHYLOCOCCUS AUREUS

I. General Characteristics
 A. beta hemolytic
 B. golden pigment
 C. coagulase produced

II. Surface Antigens
 A. capsular antigen - antiphagocytic,
 B. protein A-antiphagocytic, reacts with Fc portion of Ig

III. Extracellular substances
 A. coagulase
 B. alpha toxin - Lethal, dermonecrotic
 C. enterotoxin - exotoxin
  1. resistant to boiling for 30 min
  2. resistant to proteolytic enzymes
  3. incubation period 2-6 hrs for symptoms of food poisoning
  4. toxin ingested preformed
  5. causes nausea, vomiting, diarrhea
 D. β-lactamase (penicillinase)

IV. Types of Infection
 A. Most common - pimples, boils, carbuncles, furuncles
 B. Most dangerous - septicemia, endocarditis, meningitis, pneumonia
 C. Other diseases - food poisoning (2-4 hr incubation), pseudomembranous enterocolitis, scalded skin syndrome in neonates (exotoxin = exfoliatin).

V. Diagnosis
 A. Organism isolated on selective media such as Mannitol Salt Agar
 B. Demonstrate beta hemolysis
 C. Test for production of coagulase and/or DNAse
 D. If concerned with hospital epidemic, do bacteriophage typing

VI. Therapy
 A. Penicillin would be drug of choice but because of high incidence of beta lactamase producers,
 B. start with methicillin or another lactamase-resistant drug until lab reports sensitivity.

Staphylococcal protein A acts as an anti-opsonic by reacting with antibody molecules at the Fab/Fc portion.

Staphylococcal food poisoning is due to ingestion of pre-formed enterotoxin, thus the disease has a short/long incubation period.

GRAM NEGATIVE COCCI
NEISSERIA

I. General Characteristics of Genus

A. Most species are normal human flora (NOT gonococcus)
B. Pathogens occur in vivo inside PMNs
C. Pathogens (fastidious) - grow on chocolate, Thayer-Martin Media, 37C, 10% CO_2, produce indophenol oxidase which is useful in identification of colonies. Produce IgA1 protease.
D. Killed rapidly by drying, sunlight, UV, moist heat at 55 C, phenol
E. Penicillin sensitive: a few strains have plasmid directed β lactamase

NEISSERIA MENINGITIDIS

I. Antigenic Structure

A. There are 4 type-specific capsular polysaccharides (A-D)
   1. Type A majority of epidemics
   2. Anti-phagocytic
B. Endotoxin involved in disease (Waterhouse-Frederichsen syndrome)

II. Epidemiology

A. Man is only reservoir - up to 30% carriers
B. Disease appears sporadically or in epidemics in military personnel

III. Pathogenesis

A. gain access to nasopharynx
B. Pili affect adherence
C. local inflammatory rx - organism produces IgA1 protease
D. bacteremia → meningitis or meningococcemia
E. result in metastatic lesions in skin (causes purpuric rash), joints, ears, lungs, adrenals
F. fulminating cases - acute adrenal insufficiency (Waterhouse-Friderichsen syndrome) associated with hemorrhagic necrosis of both adrenal glands

(answer = D)

The drug of choice for neisserial infections is _____.

The major virulence factors of the meningococcus are

A. endotoxin
B. IgA protease
C. capsular carbohydrate
D. all of the above
E. A and C only

(answer at bottom, left)

Meningococci adhere to host cells by means of pili/capsule.

NESSISERIA GONORRHOEAE

I. Antigenic Structure

A. K antigen - cell wall polysaccharide - lost on subculture I → IV related to virulence (I and II are virulent, contain pili and are leukocyte-associated).

II. Pathogenicity

A. Enter through mucous membrane of genitourinary tract
B. Penetrates between columnar epithelial cells. In subepithelial tissue causes acute inflammatory response resulting in purulent yellow urethral or vaginal discharge.
C. Pili responsible for adherence to cell membranes.
D. Organism elaborates a protease which cleaves IgA1 at the hinge region of the H chain.

III. Symptomatology

A. Male
1. incubation period - 2-8 days
2. frequent, urgent, painful urination
3. mucopurulent discharge
B. Female
1. commonly asymptomatic
2. urethritis
3. may involve fallopian tubes; may cause pelvic inflammatory disease, salpingitis.
C. Culture is required to confirm diagnosis in both males and females

IV. Diseases Other Than Gonorrhea

A. Arthritis via hematogenous dissemination
B. Ophthalmia neonatorum acquired passing through birth canal

V. Therapy

A. Penicillin = drug of choice
B. Spectinomycin for PPNG (Penicillinase producing Neisseria gonorrhoeae)

Gonococci adhere to host cells by means of pili/capsules.

One of the most serious complications of gonorrhea is PID, which stands for _____ _____.

The drug of choice for penicillinase producing N. gonorrhoeae is _____.

GRAM POSITIVE RODS

BACILLUS ANTHRACIS

I. Pathogenicity of Anthrax
 A. Spore is infectious particle
 B. Penicillin = drug of choice
 C. Three routes of infection
  1. cutaneous (puncture); disease = malignant pustule
  2. ingestion
  3. inhalation (woolsorter's disease)
 D. Virulence
  1. d-polyglutamic acid capsule (anti-phagocytic)
  2. Tri-molecular toxin
   a. Edema factor = adenylate cyclase
   b. Protective antigen - binds to host cell membrane
   c. Lethal factor

The antiphagocytic capsule of B. anthracis is composed of _____.

CLOSTRIDIUM GENUS

I. Gas Gangrene: complex infection by anaerobic bacteria

 A. Organisms involved
  1. Clostridium perfringens type, A,D,F
  2. Clostridium novyi
  3. Clostridium septicum

 B. Pathogenicity
  1. Any wound contaminated with dirt has potential for gas gangrene infection due to low redox potential of traumatized tissue.
  2. Toxins and enzymes
   a. alpha toxin - lecithinase C
   b. collagenase, hyaluronidase
   c. Hemolysin
   d. Enterotoxin = food poisoning

 C. Treatment
  1. antiserum
  2. debridement of wound
  3. hypochlorite or H_2O_2
  4. hyperbaric oxygen
  5. penicillin, tetracycline

The spore is the infectious particle in all of the following diseases except:

A. tetanus

B. anthrax

C. gas gangrene

D. botulism

(answer to question above is D)

II. Tetanus - organism involved - Clostridium tetani

A. Toxin
   1. Tetanus toxin - H chain binds to cell; L chain is the toxic moeity.
      a. acts at synaptic junction of specific interneurons to block inhibitory pathways in anterior horn cells.
   2. Toxoid=prophylaxis; antitoxin=-therapy; single antigenic type

B. Pathogenesis
   1. Deep wound and inflammatory response
   2. Anaerobic conditions
   3. Very limited infection: disease is an intoxication
   4. Toxin production locally; spread through body via neurons.
   5. Causes spastic paralysis; opisthotonus.

Tetanus toxin interferes with _____ _____ _____.

III. Botulism-organism involved - Clostridium botulinum

A. Pathogenicity
   1. Potent neurotoxin-H chain binds to cell; L chain is toxic moiety.
      a. 8 serologic types-A, B, and E most common in man.
      b. protein, heat labile, resistant to gastric acidity and proteolysis.
      c. mechanism of action - interferes with release of acetylcholine in the efferent autonomic nervous system and prevents the transmission of nerve impulses across the myoneural junction.

Botlinum toxin inteferes with _____ _____ _____.

IV. Pseudo-membranous enterocolitis
Organism involved = Clostridium difficile
   A. Clindamycin oral therapy is involved as a precipitating factor. It depresses anaerobic gut flora which allows C. difficile to grow.
   B. Vancomycin is drug of choice.

The drug of choice for C. difficile enterocolitis is _____.

CORYNEBACTERIA

I. Diphtheria Causative agent - Corynebacterium diphtheriae
diphtheroids = normal flora

A. Diphtheria toxin
1. Protein of 65-70,000 daltons
2. Production of toxin
   a. requires the presence of β phage, and low iron content
   b. toxin is a phage-coded protein (lysogenic conversion)
3. Mechanism of action
   a. Fragment B - attaches to cell membrane
   b. Fragment A - inhibits protein biosynthesis by inhibiting the transferase II enzyme, Elongation Factor 2, by ADP ribosylation.

Diphtheria toxin inhibits protein synthesis by _____ _____ _____.

B. Disease is due to toxemia. No bacteria are found in the blood, therefore, the therapy of choice is antitoxin, not antibiotics. Prophylactic toxoid stimulates the production of antitoxin.

Therapy of choice for diphtheria is _____ _____.

C. Pseudomembrane that forms in the throat is composed of fibrin, PMNs, dead tissue cells and bacteria. It may break free from the underlying epithelium and close off the airway, causing suffocation.

D. Diagnosis
1. Observed Gram + pleomorphic rods in palisade arrangement in stain of pseudomembrane swab.
2. Culture organism on Blood agar or Loeffler's or tellurite agars.
3. Demonstrate metachromatic nature of organism.
4. Prove Toxigenicity of organism. This is the definitive step; must use antitoxin as specificity control.

ACID FAST BACILLI

I. Mycobacterioses

A. Organisms
   1. Mycobacterium tuberculosis (facultative intracellular parasite)
   2. Mycobacterium leprae (obligate intracellular parasite)
   3. Atypical mycobacteria e.g., M, kansasii M. intracellulare
   4. BCG = bacille Calmette Guerin;
      a. attenuated M. tuberculosis (bovine strain)
      b. vaccine vs. tuberculosis

B. Morphology - acid fast rods-high lipid content (mycolic acids)

C. Growth - most are slow growers (M. tuberculosis = 3-6 weeks)

D. Toxic product = Cord Factor (trehalose 6,6 dimycolate); ↓ oxidative phosphorylation

E. Cell wall is rich in lipids, e.g., wax D

F. Epidemiology

   1. M. tuberculosis
      a. Man to man or animal to man transfer by ingestion, contact, aerosol
   2. Unclassified mycobacteria
      a. No man to man transfer
      b. Probable source in soil and water
      c. Portal of entry is upper respiratory tract
   3. M. leprae
      a. Man is major host; agent has also been cultured in armadillo
      b. Transmission by prolonged contact

G. Diagnosis

   1. Observe acid fast rods in tissue
   2. Culture (except M. leprae)
   3. Niacin test = M. tb = positive, atypicals = negative

(answer = C)
(answer = A)

M. tuberculosis is

A. Acid fast

B. Gram positive

C. Both

D. Neither

(Answer at bottom, left)

Niacin production is a valuable laboratory test in the identification of

A. M. tuberculosis

B. M. leprae

C. Both

D. Neither

(answer at bottom, left)

H. Treatment

1. First Line Drugs (atypicals may be resistant)
   a. INH and PAS
   b. Streptomycin and PAS
   c. Rifampicin and Ethambutol
   d. INH alone for skin test converters

2. Second Line Drugs
   a. Ethionamide
   b. Cycloserine
   c. Kanamycin

First line drugs for tuberculosis include

1. _____
2. _____
3. _____
4. _____
5. _____

Leprosy

1. Etiologic agent: M. leprae (Hansen's bacillus)

2. Two types of disease
   a. lepromatous (nodular skin lesions with abundant acid fast bacilli; lepromin negative)
   b. tuberculoid (anaesthetic macular skin lesions with very few acid fast bacilli; lepromin positive)

3. Transmission is by direct contact

4. Skin testing - lepromin - extract of lepromatous nodules

5. Chemotherapy with DAPSONE

Drug of choice for Hansen's bacillus is

_____.

II. Actinomyces israelii

A. weakly acid fast

B. obligate anaerobe

C. lumpy jaw; cervico facial abscess with "sulfur granule" exudate

D. therapy = penicillin or tetracyclines

Drug of choice for actinomycosis is

_____.

GRAM NEGATIVE RODS

I. Common Characteristics of Enterics

A. Motility is variable - all Shigella are non-motile (no "H" antigens)
B. All contain endotoxin
   1. component of cell wall; lipopolysaccharide (lipid A = toxic part)
   2. pyrogenic and induces release of endogenous pyrogen (interleukin 1) which acts on hypothalamus
   3. B cell mitogen; weakly antigenic
   4. Measured by the Limulus test
   5. activation of alternate pathway of complement
   6. may trigger disseminated intravascular coagulation

The cell wall of gram negatives contains a potent toxin LPS, which stands for _____.

The toxic moiety is _____.

ESCHERICHIA COLI

I. Diseases

A. Probably most common cause of urinary tract infections (cystitis, pyelonephritis of pregnancy)
B. Neonatal meningitis: especially during 1st 2 months (with Gp. B strep); ascending infection occurs in utero or organisms are acquired during birth process
C. Epidemic infantile gastroenteritis (under age 2)
D. Traveler's diarrhea

Diseases of *E. coli* include

1. _____
2. _____
3. _____
4. _____

II. Immunologic Considerations
A. O Antigens - (somatic)
B. H Antigens - (flagellar)
C. K Antigens - (capsule)

III. Products Associated with Disease
A. Endotoxin
B. 2 Enterotoxins, 1 heat stable, 1 heat labile (adenyl cyclase)

IV. Used as index of fecal contamination of drinking water

V. 10^5/ml urine suggests etiology of UTI

IV. Therapy
A. Systemic diseases = aminoglycosides
B. Cystitis = sulfonamide
C. Diarrhea = trimethoprim + sulfa

KLEBSIELLA

I. Diseases Caused by K. pneumoniae

A. Normal flora in 5% of population
B. Important in elderly. compromised by major surgical or medical problems - alcoholics
C. Pneumonia - bronchitis, bronchiectasis. Necrosis accompanied by cavitation and fibrosis
D. Virulence factor = anti-phagocytic capsular polysaccharide

SALMONELLA

I. General Characteristics

A. Non-lactose fermenters, Acid and gas from glucose
B. All motile, several species H_2S+

II. Immunology

A. O somatic antigen and H flagellar antigen
B. Vi (virulence) antigen--found in S. typhi
C. LPS=endotoxin=major virulence factor
D. Iron binding siderophores (enterochelins) = virulence factors too

III. Types of Diseases (Salmonelloses)

A. Enteric fevers
B. Septicemia --suppurative lesions; prototype = typhoid fever
C. Gastroenteritis--contaminated food-poultry --infection, not intoxication --incubation period=12-24 hrs
D. Treatment=chloramphenicol, ampicillin

IV. Three species = typhi, cholerae-suis and enteritidis

A. Typhi is restricted to humans, the others are zoonotic
B. Just one organism in each of the first two
C. All of the "old" species are now serotypes of enteritidis

Septicemia occurs commonly in infection with

A. S. typhi
B. S. cholera-suis
C. S. enteritidis
D. All of the above
E. A and C only

(answer at bottom of page)

(answer = A)

SHIGELLA

I. General Characteristics

A. Non-lactose fermenter
B. Differentiated from Salmonella by:
   1. acid only from carbohydrates
   2. no H_2S
   3. non-motile
D. Disease = high fever, bloody mucoid diarrhea

II. Antigenic Structure

A. Grouping is based on major cell wall antigen (O); no H antigens

III. Toxic Metabolites

A. All have endotoxin
B. Sh. dysenteriae produces an exotoxin
   1. heat labile
   2. Neurotoxic in mice - paralysis of limbs, diarrhea and death -
   3. enterotoxin in man - effects the colon, not the ileum (see cholera below)

VIBRIO CHOLERAE

I. General Characteristics

A. Gram negative, comma shaped
B. Motile
C. Easily destroyed by heat and disinfectants

II. Pathogenicity due to

A. Enterotoxin (choleragen) which stimulates adenyl cyclase and increases the secretion of Cl, HCO_3 and H_2O. This acts in ileum, not colon.
B. Adherence to the gut epithelium in the jejunum and ileum.
C. Motility of the organism, which appears to be related to adherence; motile strains adhere, non-motile strains do not.

Complete the following table:

	Salmonella	Shigella
acid from lactose	_____	_____
acid from glucose	_____	_____
gas from glucose	_____	_____
H_2S	_____	_____
motile	_____	_____

III. Disease

A. Found in man only; fecal-oral route of transmission
B. Nausea, vomiting, diarrhea, abdominal cramps.
C. Rice-water stools and mucus - 20 liters of liquid lost per day
D. Local infection - no bacteremia.

Choleragen acts _____

_____

_____.

IV. Immunity

A. Due to Aby to choleragen-choleragenoid vaccine experimental.

PSEUDOMONAS

I. P. aeruginosa produces blue-green pus in wound and burn infections due to production of water soluble pigments (pyocyanin). It is a non-fermenter.

II. Clinical significance (as an opportunist)

A. Hospital acquired infections
   1. Wounds, burns, etc.
   2. Urinary tract via catheter
   3. Respiratory via nebulizers, etc.
   4. High rate of multiple drug resistance
   5. Treatment = gentamicin + carbenicillin
B. Produces enterotoxin which causes cholera-like symptoms
C. Produces exotoxin similar to diphtheria (i.e., A and B fragments; interferes with protein synthesis by adenosine ribosylating elongation factor II)

Drug(s) of choice for pseudomonas infection include _____

_____.

BACTEROIDES

I. Gram negative, non-sporeforming, anaerobic rods
II. The predominant organism in the bowel
III. Virulence factors
   A. Capsule
   B. Endotoxin is of minor importance
IV. Diseases = abscesses, septicemia
V. Treatment = clindamycin

GRAM NEGATIVE RESPIRATORY PATHOGENS

HAEMOPHILUS INFLUENZAE

I. Growth factors - requires both:

A. x factor - hematin
B. v factor - NAD

II. Virulence factors

A. Polysaccharide capsule
   1. serotypes a-f (b most common; causes 90% of human disease)
   2. antiphagocytic
B. Endotoxin
C. IgA protease

III. Types of infection

A. Upper respiratory tract-life threatening epiglottitis in infants
B. Lower respiratory tract - pneumonia
C. Meningitis in young children (2-60 months)

IV. Culture

Satellite phenomenon; capsular swelling to serotype.

V. Therapy

Chloramphenicol and ampicillin

Most cases of H. influenzae meningitis in young children are due to capsular type _____.

The drugs of choice for H. influenzae meningitis include _____ _____.

BORDETELLA PERTUSSIS

I. Growth

A. Very fragile and survive only a few hours outside the body
B. Require special media - Bordet-Gengou agar
C. Colonizes ciliated epithelial cells of Respiratory tract; non-invading

II. Epidemiology of whooping cough

A. Disease of man only: highly contagious
   1. 50% of cases are under 4 years of age
   2. 67% of deaths are under 1 year
B. Antibodies to B. pertussis do not cross the placental barrier so newborns are completely unprotected.

III. Pathogenicity

A. Three stages of whooping cough
   1. Catarrhal stage
   2. Spasmodic (Paroxysmal) stage
   3. Convalescent stage

B. Toxic Products
   1. Carbohydrate capsule; major vaccine component.
   2. Pilus; adherence organelle.
   3. Lipopolysaccharide endotoxin.
   4. Pertussis exotoxin; responsible for lymphocytosis and histamine sensitivity.
   5. Adenylate cyclase.

C. Localized Infection

IV. Immunity

A. single antigenic type
B. commercial vaccine available
C. anti-capsule Ab seems to be most important
D. excellent convalescent immunity

Vaccine induced immunity in whooping cough is antitoxic/opsonic.

LEGIONELLA

I. Cause of pneumonia (nosocomial infection acquired from the environment, e.g., water cooled, air conditioning units)
II. Fastidious organism, gram negative

COMMON CAUSES OF PURULENT MENINGITIS

AGE	MICROBIAL ETIOLOGY	INCIDENCE
less than 2 mo.	E. coli	40%
	Gp. B streptococci	30
2 to 60 mo.	H. influenzae	60
	N. meningitidis	20
5 to 40 yr.	N. meningitidis	50
over 40 yr.	S. pneumoniae	50

MYCOPLASMA

I. Similar to Protoplasts, Spheroplasts and L-forms of Bacteria

A. Protoplasts and spheroplasts are laboratory-induced forms of bacteria which contain little or no cell wall: induced by penicillin or lysozyme
B. Drug of choice = tetracyclines or erythromycin
C. Pathogenesis = organisms attach to host cell membrane's sialic acid residues via a neuraminidase-like receptor. There is no tissue invasion, but the production of toxic metabolites such as H_2O_2 cause damage locally.

Mycoplasma, protoplasts and spheroplasts all lack _____ _____ and are resistant to _____ antibiotics.

II. Morphology and Growth

A. Gram-negative cells of varying morphology and size; filterable
B. Require cholesterol; genus Acholeplasma do not

III. Diseases

A. Mycoplasma pneumoniae - primary atypical pneumonia (PAP)

IV. Characteristics of PAP

A. Non-productive cough
B. Minimal physical findings; may be myalgia, no pleuritic chest pain
C. Usually normal white blood cell count; few polymorphonuclear leukocytes in sputum
D. X-ray findings show pulmonary involvement out of proportion to physical findings
E. Etiologic agents; M. pneumoniae, psittacosis (chlamydia), C. burnetti, adenovirus, respiratory syncytical virus, influenza virus, parainfluenza virus
F. Disease caused by Mycoplasma, psittacosis and Q fever can be treated with tetracycline. Other etiologies of PAP will not respond to antibiotic therapy

Etiologics of primary atypical pneumonia include:

1. _____
2. _____
3. _____
4. _____
5. _____

V. Laboratory Diagnosis of M. pneumoniae
 A. Cultures
    1. Special media required for isolation; colonies are beta hemolytic
    2. Isolation requires 1-2 weeks
 B. Serology
    1. Non-specific
       a. Cold hemagllutinins
       b. Streptococcus MG agglutinins
    2. Specific
       a. Dye reduction inhibition test
       b. Immunofluorescence
       c. Complement fixation

UREAPLASMA

I. Very similar to mycoplasma, but hydrolyze urea

II. Grow extremely tiny colonies (less than 20 microns) hence also called T strains (T=tiny)

III. Etiology implicated in nongonococcal urethritis

OPPORTUNISTIC PATHOGENS

Source	Microorganism
Normal Flora(1)	Candida
	Staphylococcus
	Pneumocystis
Environment	Klebsiella
	Enterobacter
	Serratia
	Pseudomonas
	Legionella
	Aspergillus
	Phycomyes

1. Other normal flora may be pathogenic when introduced into normally sterile areas ex. PID with Bacteriodes, aspiration pneumonia with oral flora, peritonitis with gut flora

GENERALIZED GRAM NEGATIVE PATHOGENS

ALL 3 ARE: FACULTATIVE INTRACELLULAR PARASITES
: ZOONOSES
: TREATMENT = STREPTOMYCIN

YERSINIA PESTIS

I. Epidemiology of plague

A. Disease of animals (rodents) transmitted to man by
   1. rat fleas (bubonic plague)
   2. direct contact with infected animals
B. Man to man transmission by
   1. human fleas (bubonic)
   2. direct contact - droplet infection (pneumonic)
C. Most cases occur in rural populations in contact with wild animals (sylvatic)

II. V and W antigens - associated with virulence, as is capsular polysaccharide (F I)

Y. pestis is

A. facultative intracellular parasite.

B. zoonotic agent transmitted to may by rat fleas.

C. all of the above

D. A and C only.

(answer at bottom, left)

FRANCISELLA TULARENSIS

I. Epidemiology of tularemia

A. Disease of rodents (rabbits and squirrels)
B. Transmitted to man by contact with infected animal; by tick bites; and by ingestion of contaminated meat (e.g., rabbit), or water

II. Two types of disease

A. Ulceroglandular
B. Typhoidal (must be distinguished from typhoid fever)

F. tularensis is

A. transmitted to man by infected ticks.

B. an obligate intracellular parasite.

C. susceptible to streptocmycin.

D. all of the above.

E. A and C only.

(answer at left)

(answer = D)
(answer = E)

BRUCELLA

I. Introduction

A. Three species
1. Brucella suis - swine
2. Brucella melitensis - goats, sheep
3. Brucella abortus - cattle

II. Tissue trophism - B. abortus

A. Infection in animals usually limited to placenta due to high concentration of erythritol
1. Abortion
2. Infection of supramammary lymph nodes and spillage of organisms into milk
B. In man infection is usually generalized

B. abortus is a cause of abortion in cattle due to _____

_____

_____.

III. Clinical types of Brucella infections

A. Intermittent-high fever rising to 101-104, night sweat
B. Chronic-CNS abnormalities seen
C. Undulant-step wise increases in temperature over a period of days
D. Malignant - sustained high temperature, extreme hyperpyrexia before death

Treatment of brucellosis involves _____

_____

_____

_____.

IV. Pathogenicity

A. Organisms are continually phagocytosed by the reticuloendothelial system, then released into the blood stream
B. Virulent organisms resist intracellular killing

V. Treatment

A. Combination of Streptomycin and tetracycline recommended
B. Chronic nature of the infection may necessitate prolonged period of therapy

POTENT BACTERIAL PROTEIN EXOTOXINS

TOXIN	MECHANISM OF ACTION	NOTES ON ANTIGENICITY	MOLECULAR COMPOSITION
Anthrax	-synergistic; causes increased vascular permeability and capillary thrombosis	-II=immunogen	3 molecules I=adenylate cyclase II=membrane adherence III=lethal factor
Botulism	-pre-synaptic block of acetyl choline release in peripheral nervous system (not in CNS)	-8 types -treatment via anti-toxin (AT)	H chain binds L chain is toxic
Diphtheria*	-stops protein synthesis by inactivating amino acyl transferase (EF-2)	-1 only -excellent Toxoid & AT	A = enzyme which inactivates EF-2 by adding adenosine diphosphoribose from NAD (ADP ribosylation) B = responsible for cell membrane adsorption of the toxin; A then splits off and enters cell
Tetanospasmin	-blocks synaptic inhibitory systems; both in CNS & PNS	-1 only -excellent Toxoid & AT	H chain binds L chain is toxic
Perfringens Alpha Toxin	lecithinase	-1 only	single peptide molecule
Erythrogenic Toxin	-increases vascular permeability	-3 types	Heat stable portion induces hypersensitivity; heat labile portion is responsible for toxicity
Cholera Enterotoxin**	activates adenyl cyclase; causes hypersecretion of Cl, HCO_3, and H_2O	-1 only	A activates adenyl cyclase B is responsible for membrane binding

*Certain Pseudomonas strains produce a similar toxin
** Ceratin enterotoxic E. coli strains produce a similar toxin

Acute Bacterial Diarrheas and "Food Poisoning"

Organism	Incubation Period	Vomiting	Diarrhea	Ingestion of Toxin	Pathogenesis
Staphylococcus aureus	1-8 hrs	+++	+	+	Enterotoxin acts on receptors in gut that transmit impulse to medulla
Bacillus cereus	2-16 hrs	+++	++	+/-	Enterotoxins formed in food or in gut from growth of B. cereus
Clostridium perfringens	8-16 hrs	±	+++	-	Enterotoxin causes hypersecretion.
Clostridium botulinum	24-96 hrs	±	±	+	Toxin blocks acetylcholine at neuromuscular junction.
Escherichia coli (some strains)	24-72 hrs	±	++	-	Toxin* causes hypersecretion in small intestine ("traveler's diarrhea")
Vibrio cholerae (mild cases)	24-72 hrs	+	+++	-	Toxin* causes hypersecretion in small intestine
Shigella sp (mild cases)	24-72 hrs	±	++	-	Organisms invade epithelial cells, blood, mucus, and PMNs in stools
Salmonella sp (not typhi)	8-48 hrs	±	++	-	Superficial infection of gut, little invasion
Clostridium difficile	?	-	+++	-	Toxin causes epithelial necrosis in colon; pseudomembranous colitis
Campylobacter jejuni	2-10 days	-	+++	-	Invasion of mucous membrane
Yersinia enterocolitica	?	±	++	-	Gastroenteritis. Occasional bacteremia. Toxin produced (+/-)

*Toxin stimulates adenylate cyclase activity and increases cAMP concentration in gut; this increases secretion of chloride and water and reduces reabsorption of sodium.

SPIROCHETES

I. Introduction

A. Organisms
1. Genus Borrelia
2. Genus Leptospira
3. Genus Treponema

B. Morphology and Growth (See Table 1)

Table I

Genus	Morphology	Oxygen Requirements
Borrelia	Long with loose spirals	anaerobes
Leptospira	Fine, tight spirals with hooked ends	aerobes
Treponema	Short with tight spirals	anaerobes

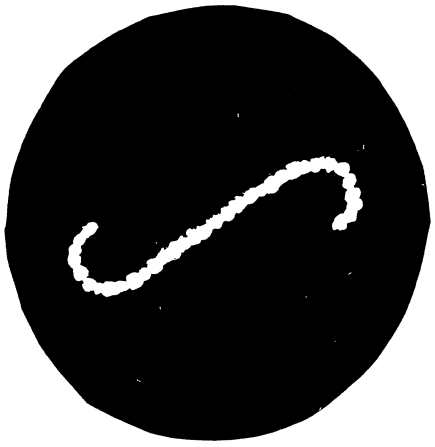
LEPTOSPIRA

A. Organism involved - L. interrogans
B. Description of Disease Process
1. Fever with jaundice
2. Infection of kidney, death from acute renal failure
C. Epidemiology
1. Parasites of wild and domesticated animals
2. Infection by contact with urine from infected animals or water contaminated with urine

BORRELIA

A. Organism involved
1. B. recurrentis - relapsing fever
B. Reservoir and Transmission
1. Organism is perpetuated by tick-animal cycle
2. Maintained in tick by transovarial transmission
3. Wound created by tick bite is contaminated with secretions and excretions of tick
4. Endemic disease
5. Epidemics are created by body louse

(answer = C)



The organism shown above most likely

A. can grow in the presence of oxygen.

B. causes relapsing fever.

C. causes sexually transmitted diseases.

D. causes Lyme disease.

E. causes acute necrotizing ulcerative gingivitis.

(answer below, left)

C. Description of Relapsing Fever
   1. abrupt onset with chills, fever (3-10 days), generalized pain, prostration and delirium
   2. free interval of 1-3 weeks followed by several relapses; relapses due to emergence of new antigenic types

D. Borrelia also seen in Vincent's angina, fuso-spirochetal stomatitis; acute necrotizing ulcerative gingivitis

TREPONEMA PALLIDUM

Stages of syphilis

1. Primary stage
   a. lesion usually appears 10-30 days after infection
   b. hunterian (hard chancre) - indolent, indurated ulcer, usually single, painless-loaded with spirochetes

2. Secondary stage
   a. usually occurs 6-12 weeks after chancre
   b. invasion of skin, eyes, blood stream, cerebrospinal fluid
   c. ulcerating, necrotic lesions of skin (rash), alopecia, numerous spirochetes

3. Latent stage
   a. gummata of skin, bones, nervous system, no organisms seen
   b. cardiovascular and neurosyphilis

4. Congenital syphilis
   a. primary and secondary stages occur in utero
   b. child has latent stage upon birth

1. VDRL and RPR
   a. antibody measured (reagin) is not specific antibody for T. pallidum

2. FTA-abs (Fluorescent Treponemal Antibody-Absorption Test)
   a. Patient's sera contains antibody to T. pallidum
   b. Fluorescent labelled antiglobulin reacts with antibody fixed to surface of T. pallidum

The drug of choice for syphilis is penicillin.

A NEW (?) DISEASE

Lyme disease is a condition of spirochetal etiology which is characterized by a migrating erythematous rash that is usually followed a few weeks later by migratory polyarthritis. Thought to be spread by the bite of ticks. First described as epidemic in Lyme, Conn.

BACTERIA, RICKETTSIAE, CHLAMYDIAE AND VIRUSES:

CHARACTERISTICS IN COMPARISON

	Characteristic	Bacteria	Rickettsiae	Chlamydiae	Viruses
1.	Obligate intracellular parasite	-	+	+	+
2.	Growth on lifeless media	+	-	-	-
3.	Contain both DNA and RNA	+	+	+	-
4.	Multiple by fission	Binary	Binary	Unequal	Subunit Assembly
5.	Visible with light microscope	+	+	-	-
6.	Contains muramic acid in cell wall	+	+	+	-
7.	Independent metabolic activity	+	+	+	-
8.	Possess ribosomes	+	+	+	-
9.	Synthesize ATP	+	+	-	-
10.	Susceptible to antibacterial antibiotics	+	+	+	-

RICKETTSIAE AND CHLAMYDIAE

Properties of the two groups:

1. Considered to be bacteria rather than viruses because they:

   a. Contain both DNA and RNA.
   b. Multiply by binary fission, chlamydia also multiply by unequal fission involving elementary and reticulate bodies.
   c. Contain some metabolically active enzymes.
   d. Contain ribosomes.
   e. Resemble gram-negative bacteria in that they possess a cell wall with mucopeptides containing muramic acid.
   f. Are inhibited by antibiotics, e.g., tetracycline, etc.
   g. Are obligate intracellular microorganisms, intermediate in size between large viruses and large bacteria.
   h. Each group has toxic properties associated with the cell. This endotoxin differs from lipopolysaccharide in that it can be detoxified, and is neutralized by type-specific antisera.

2. The two groups differ, in general as follows:

   a. Rickettsiae (R) have an arthropod vector; Chlamydiae (C) do not.
   b. R are transmitted by the bite of the vector; C by droplet or contact.
   c. R diseases involve endothelial lining of blood vessels C are localized infectious processes (e.g., lungs, eyes)
   d. Tetracycline is the drug of choice for both. Sulfa is contraindicated for R and for psittacosis.
   e. Serodiagnosis of R is by Weil Felix agglutination and C fixation. C diseases are diagnosed by C fixation and neutralization.
   f. The Frei skin test for lymphogranuloma venereum employs lygranum and depends upon cell mediated immunity.

ETIOLOGY, EPIDEMIOLOGY AND SEROLOGY OF RICKETTSIAL DISEASE

RICKETTSIAL GROUPS AND DISEASES[1]	AGENTS	ARTHROPOD[2] VECTORS	RESERVOIR	SPREAD TO MAN	WEIL-FELIX REACTION
Louse-borne Typhus Epidemic typhus	R. prowazeki	human louse	Human and louse	Infected louse feces into bite wound	OX-19
Brill's relapsing typhus	R. prowazeki	None	Human only	Recurrent infection of original attack of epidemic typhus	Negative
Flea-borne Typhus Endemic or murine typhus	R. typhi (mooseri)	rat flea	Rats, small rodents	Infected flea feces into bite wound	OX-19
Mite-borne Typhus Tsutsugamushi fever (Scrub typhus)	R. tsutsugamushi	chigger mite	Rats, voles, shrew	Mite bite	OX-K
Tick-borne Typhus Rocky Mountain Spotted Fever (RMSF)	R. rickettsi	dog and wood tick	Dogs, wild rodents, etc.	Tick bite	OX-19 and OX-2
Rickettsialpox	R. akari	mouse mite	House and wild mice	Mite bite	Negative
Q Fever	Coxiella burneti	Ticks among animals	Cattle, sheep, goats, rodents	Inhalation of dried infectious material, ingestion of contaminated milk	Negative

[1]Treatment; chloramphenicol, erythromycin, tetracyclines.
[2]All vectors are infected for life; some (louse & flea) die of disease.

CHLAMYDIAL AGENTS

Agent	Disease	Mode of Transmission	Reservoir	Laboratory Diagnosis
Group A:				
Chlamydia trachomatis	Trachoma	Contact with infected human or fresh fomites	Human	Intracytoplasmic inclusions
	Inclusion conjunctivitis	Passage out birth canal contact	Human	Specific serology
	Infant pneumonia	Passage out birth canal contact	Human	Specific serology
	Genital trachoma	Sex mediated	Human	
	lymphogranuloma venereum	Sex mediated	Human	Frei skin test with lygranum. Specific serology
Group B:				
Chlamydia psittaci	Psittacosis	Contact with sick birds and their infectious excreta; inhalation of infectious material from birds.	Bird	Four-fold ABY rise with absorbed sera Intracytoplasmic inclusion

[1]Treatment: Group A: Sulfonamides and/or tetracyclines
Group B: tetracyclines

MEDICAL MYCOLOGY

The following table summarized the material most appropriate for review.

Agent and Infectious* Disease	Particle	Geographic Distribution	APPEARANCE in vivo and 37°C	APPEARANCE room temperature
Cryptococcus neoformans meningitis	yeast	world-wide; found in bird excreta	encapsulated yeast	encapsulated yeast
Candida albicans Vulvovaginitis, thrush	yeast	world-wide; (endogenous infection)	yeast and pseudohyphae	yeast, pseudohyphae and chlamydospore
Sporothrix schenckii lymphadenitis	microconidia	world-wide	cigar-shaped yeast**	hyphae: spores in "daisy" clusters
Blastomyces dermatididis lung & skin	microconidia	Eastern United States	yeast** broadbased bud	hyphae: microconidia
Histoplasma capsulatum*** lung and RES	microconidia	Eastern United States	intracellular** yeast	hyphae: pyriform tuberculate macroconidia
Coccidioides immitis*** lung and brain	arthrospore	Southwestern United States	spherules**	hyphae: arthrospores
Mucor & Rhizopus Phycomycosis	spores	world-wide	coenocytic hyphae	non-septate hyphae
Aspergillus systemic	spores	world-wide	septate hyphae	septate hyphae and spores

NOTA BENE: AMPHOTERICIN B is the drug of choice for all agents listed except Sporothrix=KI and, perhaps, Candida=nystatin GRISEOFULVIN is the drug of choice for dermatophytes; KETACONAZOLE is good for blastomycosis, coccidioidomycosis, and Candida

*THE ONLY AGENTS THAT ARE COMMUNICABLE FROM MAN TO MAN ARE THE DERMATOPHYTES M. audouini & T. tonsurans

**DIMORPHIC FUNGI

EPIDEMIOLOGY AND PATHOGENESIS OF PARASITIC INFECTIONS

TRANSMITTED VIA INGESTION OF OVA

Parasite	Pathogenesis
Enterobius vermicularis	Adults in rectum and colon; Most common parasite of children; anal pruritis.
Ascaris lumbricoides	Adults in small intestine; light infections asymptomatic; occasional intestinal obstructions or abnormal migrations of adult in heavy infection.
Toxocara canis	Larvae invade various organs; mark eosinophilia; ↑IgE hepatosplenomegaly occasionally retinal granuloma due to larval migration.
Trichuris trichiura	Adults in colon and rectum; light infections asymptomatic; heavy infections may cause diarrhea, tenesmus and rectal prolapse.
Taenia solium	Larvae in all tissues; CNS damage may be serious.
Echinococcus granulosus	Growth of cyst damages liver or lung.

TRANSMISSION VIA INGESTION OF CYST

Entamoeba histolytica	Primary ulcers in large intestine; secondary abscess in liver.
Giardia lamblia	Asymptomatic to protracted diarrhea.
Toxoplasma gondii	Usually asymptomatic in adults; serious CNS damage to fetus if mother infected during pregnancy. Obligate intracellular parasite.

TRANSMISSION VIA INGESTION OF LARVAE

Trichinella spiralis	Adults cause GI disturbances; larvae cause muscle pains, ocular edema, eosinophilia.
Taenia saginata	Vague GI disturbances
Taenia solium	Vague GI disturbances
Diphyllobothrium latum	Vague GI disturbances; rarely pernicious anemia.

TRANSMISSION VIA LARVAL PENETRATION OF SKIN

Necator americanus or Ancylostoma duodenale	Adults in small intestines; light infections are asymptomatic; heavy infection plus malnutrition causes anemia and hypoproteinemia.
Strongyloides stercoralis	Adults in small intestinal mucosa; symptoms vary, i.e. asymptomatic, mucoid diarrhea with malabsorption potentially fatal in immunological comprised host due to autoinfection.

TRANSMISSION VIA CERCARIAL PENETRATION OF SKIN

Schistosoma mansoni or *S. japonicum*	Granulomatous reactions to eggs deposited in intestinal venules or those trapped in liver or other organs.

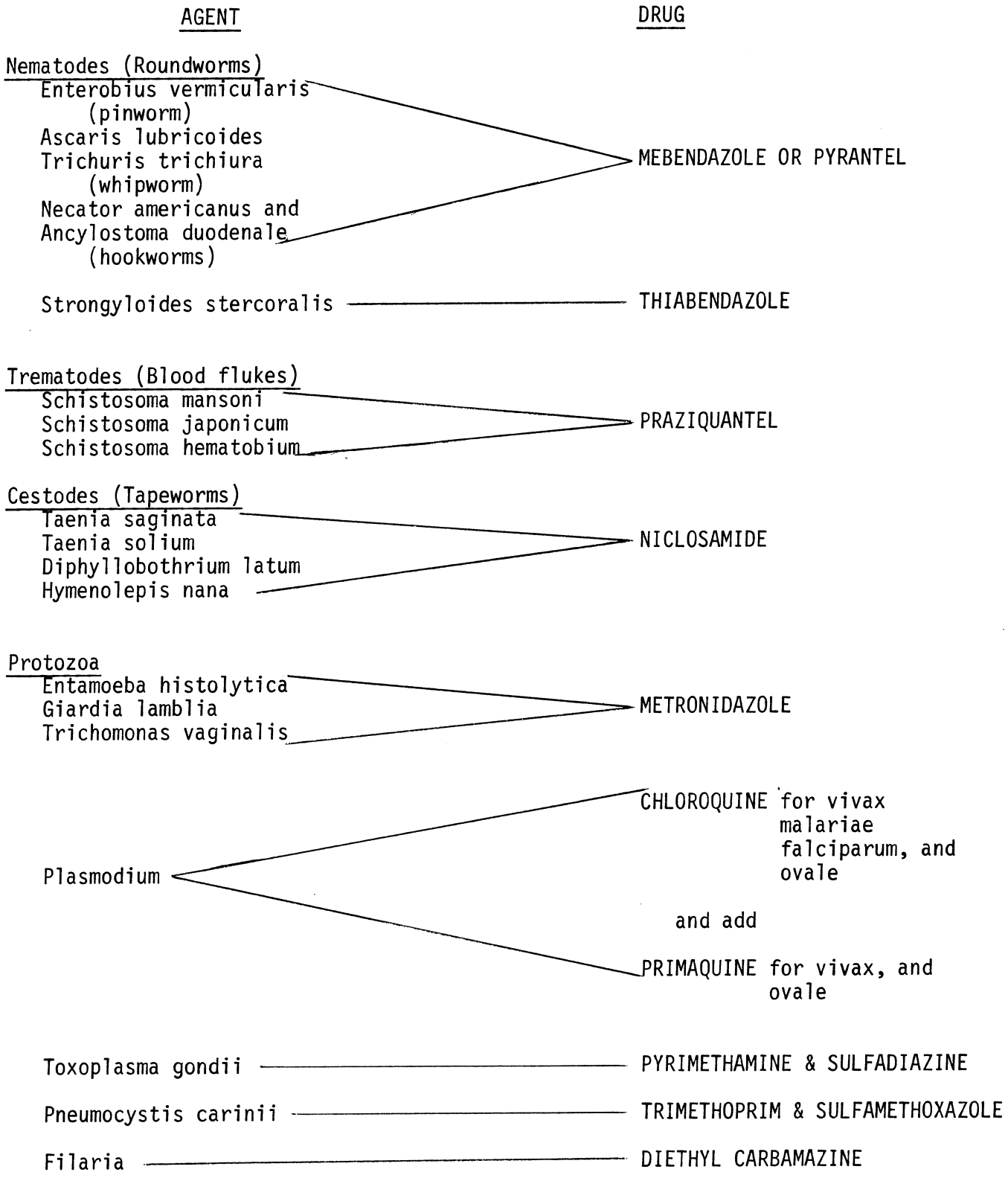
TRANSMISSION VIA BITE OF ARTHROPOD VECTOR

Plasmodium vivax malariae *P. falciparum* *P. falciparum*	Fever, musculoskeletal pains, severe headache, diarrhea; capillary occlusions are especially dangerous.
Onchocerca volvulus	larvae develop into adult worms in subcutaneous tissue; cause formation of tumor-like nodules. Microfilarial forms migrate through eye and may cause blindness.
Wuchereria bancrofti	Larva develop into adults in lymphatics. Host's allergic response to these causes lymphadenitis which may develop to elephantiasis.

TRANSMISSION VIA DIRECT CONTACT AND/OR INVASION

Trichimonas vaginalis	Local, non-fatal disease; usually symptomatic in females as vaginitis.
Naegleria fowleri	Travel up olfactory to brain; cause amebic meningoencephalitis.

PARASITE CHEMOTHERAPY



REVIEW STATEMENTS

Pneumococci are highly sensitive to chemical disinfectants and detergents.

The major virulence factor of the pneumococci is the capsular carbohydrate.

The capsule of the pneumococcus aids in the invasiveness of the agent by virtue of its antiphagocytic action.

Pneumococcal vaccine is composed of capsular antigens from the most common types.

Streptococcus pneumoniae causes alpha hemolysis on Blood agar.

The pneumococci are differentiated from the green (viridans) streptococci by their sensitivity to optochin.

Pneumococcal pneumonia occurs in the adult in the lobar form.

The antibiotic of choice for pneumococcal pneumonia is Penicillin.

Group A beta hemolytic streptococci are differentiated from other beta hemolytic streptococci by their sensitivity to Bacitracin.

Two antiphagocytic surface components of Group A streptococci are the M protein and hyaluronic acid. The latter is not antigenic.

Erythrogenic toxin is produced only by lysogenic strains of streptococci.

A positive Dick test indicates susceptibility to scarlet fever.

The extracellular product of streptococci which converts plasminogen to plasmin is fibrinolysin (streptokinase).

There are four immunologic types of streptococcal DNAse (A - D). Type B is used to measure antibody response to infection.

Acute glomerulonephritis is associated with infections of either skin or upper respiratory tract whereas rheumatic fever occurs only after infection of the upper respiratory tract.

Staphylococcal enterotoxin is the most heat and enzyme resistant bacterial exotoxin.

Staphylococci are resistant to penicillin by virtue of a plasmid-conferred enzyme, beta lactamase.

Staphylococcal carriers are identified in epidemiologic studies (e.g., hospital nurseries) by bacteriophage sensitivity.

Penicillinase attacks the beta lactam ring of the molecule.

Surface components of the staphylococcus which are anti-phagocytic include capsule and protein A.

Streptococcus pneumoniae is a common cause of pneumonia in debilitated patients and in alcoholics.

Aerobic gram negative diplococci belong to the genus Neisseria.

Neisseria are sensitive to killing by physical and chemical agents.

N. meningitidis is divided into four serologic types on the basis of antigenic difference of the capsular carbohydrate.

The majority of meningococcal epidemics are caused by type A.

The acute adrenal insufficiency seen in fulminating cases of meningococcemia is called the Waterhouse Friderichsen syndrome.

The surface components of the meningococcus which are involved in its pathogenicity include the capsule and endotoxin.

Pili (flagella-like structures on the surface of the gonococcus) are responsible for the organism's interaction with host cell membranes.

Pili are found only on the virulent strains of gonococci, types I and II.

Gonorrhea may be an asymptomatic infection, particularly in females.

Gonorrhea can be diagnosed in males (only) by the observation of gram negative diplococci inside PMN's.

The pathogenic neisseria are cultured on Chocolate Agar or Thayer Martin media in the presence of 10% CO_2.

In addition to "A flow of seed" the gonococcus also causes arthritis, and ophthalmia neonatorum.

The treatment of choice for neisserial infections is penicillin.

Protoplasts and spheroplasts are laboratory-induced (usually with penicillin) bacterial forms which lack a cell wall.

Mycoplasmal particles are able to pass through bacteria-retaining filters.

Primary atypical pneumonia (as a disease entity) may be caused by various chlamydial and viral agents, however, it is most closely associated with Mycoplasma pneumoniae.

Penicillins and cephalosporins are not indicated in the treatment of mycoplasmal infections because these are inhibitors of cell wall synthesis and mycoplasma do not have a cell wall.

Diseases caused by mycoplasma can be treated with tetracyclines.

The most common serologic tests for the identification of Mycoplasma pneumoniae (although they rely on heterophile antigens) are cold agglutinins and Strep MG agglutinins.

The two genera of gram positive rods which form spores are Clostridium and Bacillus; the former will only grow anaerobically.

The two major virulence factors of the anthrax bacillus are polypeptide capsule and anthrax toxin.

The anthrax toxin is somewhat unique in biology in that its lethal effect on the host is due to the synergistic action of 3 proteins.

Although gas gangrene can be caused by several clostridia, the species most commonly associated with this disease entity is perfringens.

Three toxic enzymes which are of particular importance in gas gangrene are lecithinase, collagenase and hyaluronidase.

A common feature in the treatment of clostridial infections is the use of specific antitoxin.

Gas gangrene and tetanus are both contracted via soil contamination of wounds; their clinical pictures differ markedly in that gangrene is an invasive process whereas tetanus is a very localized infection the symptoms of which are due to toxemia.

The etiologic agent of Hansen's disease has not been grown in vitro.

Leprosy is thought to be transmitted to man via direct contact.

Leprosy can be diagnosed by skin testing with an extract of lepromatous nodules referred to as lepromin.

Botulism is an intoxication caused by an organism which elaborates a potent heat labile neurotoxin which acts on the autonomic nervous system and interferes with transmission of nerve impulses at the myoneural junction.

As is true of the other diseases caused by gram positive rods, diphtheria is most effectively treated by the administration of specific antitoxin; antibiotics are insufficient alone because most of the symptomatology is due to the organism's excretion or toxin.

Enteric bacilli stain gram negative and contain endotoxin.

Three classical enteric diseases are typhoid, dysentery, and cholera, caused by Salmonella, Shigella, and Vibrio, respectively.

The chemical composition of endotoxin is lipopolysaccharide.

Escherichia coli is probably the most important cause of urinary tract infection.

Capsular carbohydrate is a major virulence factor of K. pneumoniae, and is referred to as K-antigen.

Three types of antigens associated with Salmonella typhi are O, H, and Vi.

An important virulence factor of Salmonella which is pyrogenic and lethal is endotoxin.

Shigella may be differentiated from Salmonella by motility and H_2S production, they are negative in both tests.

Shigella dysenteriae produces a potent exotoxin which acts as an enterotoxin in man.

The pathogenesis of Vibrio cholerae is dependent upon choleragen, an enterotoxin which increases adenyl cyclase activity.

Rice-water stools, fluid and electrolyte loss, and eventual hypovolemic shock is characteristic of asiatic cholera.

The drug of choice for pseudomonas infection is gentamicin.

Salmonella are divided into groups on the basis of the O antigens; they are speciated by differences in their H antigens.

Of all the enteric bacilli Salmonella typhi is the most likely to be isolated from blood.

Haemophilus influenzae requires X and V factors for growth.

An antiphagocytic polysaccharide capsule similar to that of St. pneumoniae is a virulence factor of Haemophilus.

The etiologic agent of whooping cough is Bordetella pertussis.

Heat-killed Bordetella pertussis is employed in the DPT vaccine.

Yersinia pestis may be transmitted by rat fleas and human lice.

Three major species of Brucella are abortus, melitensis and suis.

Brucella abortus demonstrates a tissue tropism in cattle since the organisms localize in the placenta which contains a high concentration of erythritol.

The virulence of Brucella is attributed to their ability to resist intracellular killing.

There are three genera of spirochetes that cause human disease; Leptospira, Treponema and Borrelia. Two are anaerobes, the third, Leptospira have been cultured in vitro. Two genera are zoonotic in nature (i.e., infect animals primarily with man as an incidental host); Leptospira and Borrelia. The primary organ affected in leptospirosis is the liver. The agents causing the other spirochetal diseases are more diverse in their distribution throughout the body. Leptospirosis is contracted via exposure to contaminated water; relapsing fever occurs after tick bites.

A microorganism which usually causes infectious disease when introduced into a susceptible host in reasonable numbers is called a Pathogen.

Streptococci of the viridans group are differentiated from Streptococcus pneumoniae by the fact that pneumococci are optochin-sensitive.

Scarlet fever is a local infection with toxemia.

The serological grouping of streptococci depends upon a cellular carbohydrate antigen.

A rise in antistreptolysin-O titer is an indication of recent infection with Streptococcus pyogenes.

Man is the only natural host for Neisseria meningitidis. Persons with meningococcal pharyngitis may fail to develop meningitis. Meningococcal petechial hemorrhages often contain Neisseria meningitidis.

The meningococcus and gonococcus are distinctly different from the nonpathogenic Neisseria of the normal flora in that only Neisseria of the normal flora can be cultured on nutrient agar at room temperature.

Spore-forming bacilli are divided into two genera according to whether or not they can grow in anaerobic cultures.

Virulent strains of Corynebacterium diphtheriae may be identical to avirulent strains except in toxin production.

In the routine diagnosis of diphtheria, positive identification of Corynebacterium diphtheriae is made by demonstration of its ability to produce diphtheria toxin.

BCG, which is used for active immunization against tuberculosis, consists of attentuated bovine tubercle bacilli.

The most commonly encountered species in uncomplicated urinary infection is Escherichia coli.

The production of a water soluble, blue-green pigment in a culture indicates the presence of Pseudomonas aeruginosa.

The principal virulence factor of Salmonella typhi is its potent endotoxin.

The etiologic agent of cholera is transmitted chiefly by ingestion of contaminated food or water.

The common invertebrate vector of Yersinia pestis is the tick.

The medium of choice for the isolation of Bordetella pertussis is Bordet-Gengou agar.

The spirochetes associated with fusospirochetal infections, as well as the etiologic agents of relapsing fever, belong to the genus Borrelia.

In a patient with diagnosed histoplasmosis, a rising antibody titer, as demonstrated by the complement fixation test, probably means the patient has active disease which is becoming disseminated.

Malarial relapses are due primarily to emergence of persisting exoerythrocytic merozoites.

Fatal malaria most often results from infection with P. falciparum.

Botulinum toxin resists proteolysis, therefore, it is effective where ingested.

H. influenzae causes life-threatening epiglottis in neonates.

One virulence factor of Mycobacterium tuberculosis is the cord factor (6,6' trehalose dimycolate).

During oral antibiotic therapy, a patient may develop pseudomembranous enterocolitis caused by Staphylococcus aureus or clostridium difficile.

Escherichia coli is commonly used as an index of fecal contamination of water.

The tetanus toxin, like all protein exotoxins from gram positive organisms is easily toxoided. This fact has been helpful in vaccine development.

Enterobius is the most common helminth parasite infection of children in the United States.

Enterobius female worms lay their eggs on the perianal skin. Anal impression smears can be made to detect these eggs.

After ingestion of Ascaris eggs, the hatched larvae migrate to the lungs, up the respiratory tree and back to the small intestines where they become adults.

Light Ascaris infections are well tolerated. Occasional intestinal obstructions or abnormal migrations of the adults occur in heavy infections.

Hookworms (Nector americanus or Ancylostoma duodenale) attach to the small intestinal mucosa and suck blood.

The potential for autoinfection makes Strongyloides a dangerous infection, especially in the immunologically compromised host.

Muscle pains, ocular edema and eosinophilia are symptoms associated with symptomatic trichinosis.

Schistosome adults live in venules and deposit eggs that elicit granulomatous reactions.

Adult tapeworm infections are acquired by ingesting animal tissue that harbors the larvae stage, e.g., T. solium in pork and T. saginata in beef.

Enterobius infections (pinworm) are treated with mebendazole.

Cysticerosis may occur if man ingests Taenia solium eggs.

Diphyllobothrium latum competes with the host for Vitamin B_{12} and this competition occasionally results in pernicious anemia.

Entamoeba histolytica may cause abscesses in the large bowel with secondary lesions in other organs, especially the liver.

Giardiasis is the most common intestinal protozoan infection of man in the United States.

Resistance to phagocytosis and intracellular killing are important attributes of virulence of pathogenic microbes.

Numerous pathogens secrete an IgA protease which aids in infectivity; e.g., H. influenzae, Neisseria gonorrhoeae, N. meningitidis, streptococci (pneumoniae, mitis, and sanguis), and Bacteroides.

Candida albicans is a part of the normal flora.

Fungi are resistant to most antibacterial chemotherapeutic agents.

Listeria, an occasional cause of meningitis, are differentiated from Corynebacteria on the basis of their motility in young cultures.

Clinical malaria is best treated with chloroquine.

Primaquine is necessary to eradicate persisting exoerythrocytic liver stages of Plasmodium vivax.

Metronidazole is an effective drug for treating Trichomonas vaginalis. Sexual partner(s) must also be treated to prevent reinfection.

Leishmania donovani, the etiologic agent of Kala Azar, is an intracellular parasite of reticuloendothelial cells, especially of the liver, spleen, lymph nodes and bone marrow.

Pneumocystis carinii is an opportunistic parasite that causes pneumonia in patients whose immunocompetence has been compromised.

Trypanosoma gambiense and Trypanosoma rhodesiene, the etiologic agents of African sleeping sickness, are transmitted via the bite of the tse tse (Glossina) fly.

Trypanosoma gambiense and T. rhodesiense effectively circumvent the host immune response by forming new antigenic variants.

Bacteroides are the predominant flora of the gut.

Actinomycosis is characterized by endogenous origin of anaerobic infectious agent, and sulfur granules in exudate of lesion which are really masses of Gram positive rods.

C. neoformans is the ONLY encapsulated yeast that is pathogenic for man. It is identified by India ink preparations of spinal fluid.

North American blastomycosis has an exogenous saprophytic source of infection.

Lipopolysaccharide endotoxin is located in the cell wall of Gram negative bacteria; exotoxins (proteins) are excreted from the cell.

Mycoplasma pneumoniae is resistant to penicillin because it does not have a cell wall.

Diagnosis of gonorrhea in the male is accomplished by observing Gram negative diplococci inside PMN's. In the female, culture and identification are necessary for an etiologic diagnosis.

PATHOGENIC MICROBIOLOGY PROFICIENCY EXAMINATION

This set of 50 questions was used in an examination of 1985 given to sophomore Medical students. Their mean score was 83 percent. A score much below 65 percent would suggest that the examinee is in need of more review in the areas of Pathogenic Microbiology covered in this test.

1. Acute rheumatic fever may follow an infection with an organism which is

   A. bile soluble.
   B. gram negative.
   C. coagulase positive.
   D. bacitracin sensitive.
   E. optochin sensitive.

2. A gram positive, catalase negative coccus, sensitive to optochin is probably:

   A. group A Streptococcus pyogenes.
   B. Staphylococcus aureus.
   C. alpha hemolytic Streptococcus faecalis.
   D. Streptococcus pneumoniae.
   E. Peptostreptococcus spp.

3. Streptococcus pyogenes (group A)

   A. is often associated with scarlet fever.
   B. possesses an antiphagocytic M protein.
   C. produces glomerulonephritis by extensive replication in the glomeruli and production of hyaluronic acid.
   D. All of the above are true.
   E. Only A and B above are true.

4. Corynebacterium diphtheriae will grow on

   A. tellurite agar.
   B. blood agar.
   C. Loeffler's serum agar.
   D. All of the above.
   E. A and C only.

5. Hospital acquired Staphylococcus aureus infections are "traced to source" by

   A. fermentation pattern.
   B. phage susceptibility.
   C. cell wall antigenic composition.
   D. all of the above are useful.
   E. only A and C are useful.

6. Potential virulence factors of Bordetella pertussis include all of the following except

   A. carbohydrate capsule.
   B. pilus.
   C. endotoxin.
   D. exotoxin.
   E. IgA protease.

7. Potential virulence factors of Neisseria meningitidis include all of the following except

   A. carbohydrate capsule.
   B. pilus.
   C. endotoxin.
   D. exotoxin.
   E. IgA protease.

8. A two year old boy with symptoms of bacterial meningitis had a total white blood cell count in his cerebrospinal fluid of 4,280 cells per cu. mm. The predominating cell type most likely would be

   A. monocyte.
   B. plasma cell.
   C. eosinophil.
   D. polymorphonuclear neutrophil.
   E. lymphocyte.

9. A Gram negative bacterium was isolated from the cerebrospinal fluid of the patient above. It would grow on enriched chocolate agar but would not grow on blood agar except adjacent to a streak of staphylococci. The organism is

   A. Neisseria meningitidis.
   B. Neisseria gonorrhoeae.
   C. Haemophilus influenzae.
   D. Streptococcus pneumoniae.
   E. Listeria monocytogenes.

10. A fungus which produces yeast at 37°C and hyphae at room temperature is

   A. dimorphic.
   B. a mixed culture.
   C. coenocytic.
   D. usually a laboratory contaminant.
   E. a true yeast.

11. Sporothrix schenckii, grown at 37°C, produces

   A. daisy-like spores.
   B. yeast cells.
   C. coenocytic hyphae.
   D. septate hyphae.
   E. hyphae and spores.

12. Yeast reproduce

   A. with septate hyphae.
   B. with coenocytic hyphae.
   C. by budding.
   D. with hyphae and spores.
   E. by spores only.

13. The drug of choice for intestinal tapeworm infections is

A. mebendazole.
B. quinine.
C. niclosamide.
D. chloroquine.
E. hetrazan.

14. In schistosomiasis the major long term pathogenic effects are caused by

A. adult worms that destroy tissue during migration.
B. cercaeiae during penetration of the skin.
C. schistosomula migrating from the lungs to the liver.
D. host reactions against the eggs.
E. host reactions against the adults.

15. The drug of choice for most trematode infections is

A. mebendazole.
B. praziquantel.
C. niclosamide.
D. chloroquine.
E. hetrazan.

16. Salmonella typhi produces

A. typhus.
B. dysentery.
C. acute diarrhea.
D. septicemia.
E. food poisoning.

17. All of the following zoonotic diseases have rodents as a common reservoir except

A. leptospirosis.
B. salmonellosis.
C. plaque.
D. brucellosis.
E. endemic typhus.

18. Botulinum toxin

A. is transported intraaxonally.
B. is destroyed by heating at 37°C for 5 min.
C. inhibits protein synthesis.
D. is produced by dried spores.
E. inhibits synaptic transmission at neuromuscular junctions.

19. Factors important in gas gangrene include all of the following except

A. devitalized, anoxic tissue.
B. plasmid-mediated drug resistance.
C. low redox state.
D. mixture of obligate anaerobes and facultative anaerobes.
E. predominance of Clostridium perfringens.

20. *Pseudomonas aeruginosa*

A. is coagulase positive.
B. ferments glucose to form acetic acid.
C. produces anaerobic infections.
D. causes acute pharyngitis.
E. synthesizes a toxin similar to diphtheria toxin.

21. Morbidity rate refers to

A. number of deaths occurring per unit of population.
B. clinical appearance of shock patients.
C. the incidence of disease.
D. a killing curve of disinfectants.
E. number of morticians applying for licensure.

22. *Shigella dysenteriae*

A. is a major cause of endocarditis.
B. produces cholera.
C. invades colonic mucosal epithelium.
D. is not found in the U.S.
E. colonizes the upper bowel.

23. The vector of bubonic plague is

A. rat louse.
B. rat flea.
C. dog tick.
D. chigger mite.
E. dog flea.

Questions 24-25

An 18 year-old high school student developed a sore throat and fever, which lasted for about 3 days. Ten days later, he developed hematuria, puffy eyelids, and swollen ankles. He went to a physician, who noted an inflamed pharynx, enlarged red tonsils, and palpable cervical lymph nodes. The patient had a blood pressure of 165/105 mm Hg; and the urine contained 4+ protein, with a few red blood casts also present. Serological findings indicated a decreased C3 complement level, an elevated antistreptolysin O titer, and increased blood urea nitrogen.

24. The patient most likely had an infection by

A. *Streptococcus pneumoniae*.
B. *Streptococcus faecalis*.
C. *Streptococcus agalactiae*.
D. *Streptococcus pyogenes*.
E. *Staphylococcus aureus*.

25. The disease the patient had was

A. Scarlet fever.
B. Rheumatic fever.
C. Acute glomerulonephritis.
D. Lupus erythrematosus.
E. Erysipelas.

26. The outstanding feature of Histoplasma capsulatum cultured at room temperature is the

A. small yeast cells.
B. tuberculated macroconioiospores.
C. encapsulated yeast cells.
D. "cigar bodies".
E. pyriform microconidia.

27. Which of the following is an intracellular parasite?

A. Candida albicans
B. Actinomyces israelii
C. Histoplasma capsulatum
D. Mycobacterium tuberculosis
E. Neisseria meningitidis

A if 1, 2 and 3 are correct.
B if 1 and 3 are correct.
C if 2 and 4 are correct.
D if 4 is correct.
E if 1, 2, 3 and 4 are correct.

28. The presence of an antiphagocytic capsule is important in the infectivity of

(1) Bordetella pertussis.
(2) Neisseria meningitidis.
(3) Haemophilus influenzae.
(4) Klebsiella pneumoniae.

29. Rocky Mountain spotted fever is an acute infectious disease that

(1) is caused by Rickettsia typhi.
(2) stimulates the production of Proteus agglutinins.
(3) involves a rash that spreads from the trunk to the extremities.
(4) is acquired by the bite of an arthropod.

30. Common examples of indigenous human flora are organisms found in the genus

(1) Streptococcus.
(2) Escherichia.
(3) Corynebacterium.
(4) Neisseria.

31. Common signs of disseminated intravascular coagulopathy include

(1) elevated blood urea nitrogen level.
(2) decreased urine output.
(3) hypotension.
(4) increased prothrombin level.

32. Aspects of host-parasite relationships include

(1) toxicity.
(2) host and tissue specificity.
(3) avoiding the host response.
(4) transmission of disease.

33. Possible result(s) following exposure to a microorganism is (are)

(1) carrier state.
(2) inapparent infection.
(3) latent infection.
(4) clinical disease.

34. Epidemiological surveillance includes

(1) statistical analysis of data.
(2) dissemination of information.
(3) collection of data by routine reporting.
(4) inoculating volunteers with test vaccines.

A if 1, 2 and 3 are correct.
B if 1 and 3 are correct.
C if 2 and 4 are correct.
D if 4 is correct.
E if 1, 2, 3 and 4 are correct.

35. Infection may be spread by

(1) food, milk or water.
(2) aerosol.
(3) arthropod vectors.
(4) direct or indirect contact.

36. The function(s) of the indigenous flora of the body surfaces may include:

(1) essential vitamin synthesis.
(2) production of skin pigmentation.
(3) antigenic "priming" of the immune response.
(4) lubrication.

37. Which of the following statements is (are) correct regarding Salmonella food poisoning?

(1) It is an enteric disease.
(2) The organism is passed only from man to man.
(3) Many serotypes are implicated.
(4) Blood cultures are frequently positive.

38. The general mechanism of action of enterotoxins (e.g., choleragen) involves

(1) attachment of a binding subunit to the mucosal cell membrane.
(2) NAD ribosylation of EF-2.
(3) efflux of Na^+, CL^-, K^+ and HCO_3^- ions from the serosal side to the luminal side of the intestine, followed by water.
(4) inhibition of protein synthesis in the mucosal epithelium.

39. Tetanospasmin is

(1) responsible for tetanus.
(2) transported by the blood stream.
(3) a potent protein neurotoxin.
(4) heat stable and found in improperly canned vegetables.

40. Which of the following should be routinely cultured for anaerobes?

(1) aspirated pus.
(2) normally sterile tissue.
(3) blood.
(4) throat swabs.

A if 1, 2 and 3 are correct.
B if 1 and 3 are correct.
C if 2 and 4 are correct.
D if 4 is correct.
E if 1, 2, 3 and 4 are correct.

41. *Escherichia coli* may produce

(1) traveler's diarrhea.
(2) a heat-labile enterotoxin (LT).
(3) a heat-stable enterotoxin (ST).
(4) neonatal meningitis.

42. *Bacteroides fragilis* is pathogenic by virtue of a

(1) leukotoxin.
(2) potent neurotoxin.
(3) cytotoxin active in the colon.
(4) capsular polysaccharide.

43. Examples of the normal indigenous flora include

(1) *Staphylococcus epidermidis*.
(2) *Candida albicans*.
(3) *Streptococcus faecalis*.
(4) *Yersinia pestis*.

44. Bacteriological and clinical hint(s) of anaerobic infection include

(1) growth of cultures only in well aerated conditions.
(2) difficulty of antibiotic therapy.
(3) the lack of involvement by indigenous flora.
(4) putrid smelling discharge.

45. Bronchopulmonary defense mechanisms include

(1) aerodynamic design of upper airway.
(2) mucocilliary escalator.
(3) alveolar macrophages.
(4) sIgA.

46. Vaccines are available for prevention of lower respiratory infection due to

(1) *Bordetella pertussis*.
(2) *Streptococcus pneumoniae*.
(3) influenza virus.
(4) *H. influenzae*.

Select the single BEST answer for each of the next four questions.

47. A 4 year old child with a history of recurrent pulmonary infections has been brought to the emergency room in obvious respiratory distress. Gram stain of he sputum reveals numerous leukocytes and gram positive cocci in grape-like clusters. The drug of choice to be employed until the antibiotic sensitivity report is received from the laboratory is

    A. penicillin.
    B. methicillin.
    C. streptomycin.
    D. gentamicin.
    E. chloramphenicol.

48. A laboratory test used to identify the organism above is based on the clotting of plasma. The microbial product which is responsible for this activity is

    A. coagulase reactive factor.
    B. prostaphylocoagulase.
    C. prothrombin.
    D. thrombin.
    E. thromboplastin.

49. A petechial rash on the palms of the hands is commonly seen in

    A. Rocky Mountain spotted fever.
    B. scarlet fever.
    C. typhoid fever.
    D. valley fever.
    E. rheumatic fever.

50. Which of the following zoonotic diseases is usually transmitted to humans by the bite of an arthropod vector?

    A. Anthrax.
    B. Brucellosis.
    C. Salmonellosis.
    D. Plague.
    E. Leptospirosis.

ANSWER SHEET

1.	D	26.	B
2.	D	27.	C,D
3.	E	28.	E
4.	D	29.	C
5.	B	30.	E
6.	E	31.	A
7.	D	32.	E
8.	D	33.	E
9.	C	34.	A
10.	A	35.	E
11.	B	36.	B
12.	C	37.	B
13.	C	38.	B
14.	D	39.	B
15.	B	40.	A
16.	D	41.	E
17.	D	42.	D
18.	E	43.	A
19.	B	44.	C
20.	E	45.	E
21.	C	46.	A,E
22.	C	47.	B
23.	B	48.	B
24.	D	49.	A
25.	C	50.	D

REVIEW OF VIROLOGY

Definitions

Attentuated: Made less virulent, usually by passage through an abnormal host.

Capsid: The outer-most component of a virion, the protein coat which protects the nucleic acid from the environment.

Capsomere: The structural protein subunits of the viral capsid.

Envelope: The structure which some viruses acquire as they leave the host cell. It consists of viral protein components (usually glycoproteins) and host-cell derived lipids and lipoproteins of the cell membrane. Lipid solvents react with the envelope and inactivate the infectivity of the virus.

Genome: The nucleic acid core of the virus, either DNA or RNA. It contains the genetic information of the virus. Some genomes are segmented, others are polycistronic. They may be single-stranded, as is the case with most RNA viruses, or double stranded. The RNA viruses are + stranded in the case of polio, WEE and the togaviruses.

Icosahedron: A 20-sided polygon.

Latent infection: A form of inapparent infection following a clinical case in which the pathogen is not completely eliminated, but remains viable in the tissues after the host recovers. The host may be infectious.

Nucleoprotein: Proteins associated with the nucleic acid of the viral genome. These are internal proteins and may be structural, or enzymes such as RNA-dependent DNA polymerase.

Prion: An infectious proteinaceous particle; virus-like but with no nucleic acid content.

Provirus: Naked viral nucleic acid intracellularly located. It may remain dormant in the host cell or it may become integrated into the host cell genome.

Uncoating: The process whereby the viral genome is freed into the internal milieu of the host cell. After this process occurs the viral genes are read and synthesis of early proteins, etc. begins.

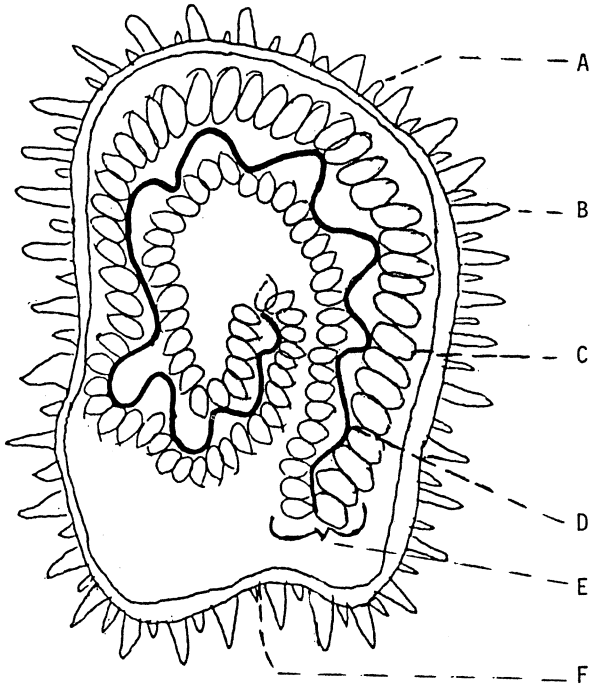
VIRUS STRUCTURE

The outermost component of a virion is the capsid, made up of protein subunits called capsomers. The capsid serves four important functions: (1) it protects the viral genome, (2) it aids in infection by attaching the virion to susceptible cells, (3) it is the stimulus for antibody production, and (4) it serves as the antigen in serologic tests.

The viral genome, the other major component of every virion, is found inside the virus particle and may be either double-stranded or single stranded DNA, or single-stranded or double-stranded RNA. Once introduced into a susceptible cell, the viral genome provides the genetic information needed for production of new virions in a cell. The cell contributes cellular structures (ribosomes), energy, and enzymes for the synthesis of viral macromolecules. Since viruses lack these essential components, they must invade and make use of living cells in order to be replicated.

Animal virions are either naked or enveloped. A naked virion consists of nulceic acid enclosed in a protein shell known as the capsid; nucleic acid and capsid together are termed nucleocapsid. An enveloped virion in turn consists of a nucleocapsid surrounded by a structure called the envelope. The envelope consists of viral protein components (usually glycoproteins), and host cell-derived lipids and lipoproteins. Lipid solvents react with the lipid-containing envelope to inactivate the infectivity of the virus. The capsid and the envelope contribute antigens useful in vaccine development and in serologic tests.

Label the components of the virus.



A. _____

B. _____

C. _____

D. _____

E. _____

F. _____

VIRAL CLASSIFICATION

All naked animal virus particles resemble icosahedra. Enveloped animal virions exhibit a large variety of shapes (symmetry). In many cases, a nucleocapsid that is distinctly icosahedral or helical, depending on the virus, is surrounded by an envelope which gives the particle the appearance of a sphere, e.g., influenza virus. Other enveloped animal viruses are shaped like a bullet, e.g., rabies virus; and still others look like bricks, e.g., poxviruses.

Taxonomic classification of viruses is based on the relatively constant physical and chemical properties of virions. Some of the criteria used for classification are (1) type of nucleic acid found in the virion (DNA or RNA) and whether the nucleic acid is single-stranded or double-stranded, (2) shape of the viral nucleocapsid (icosahedral or helical), (3) nature of the outermost viral component (naked or enveloped), and (4) antigenic properties.

Viruses are classified on the basis of

1. _____
2. _____
3. _____
4. _____

MAJOR HUMAN VIRUS GROUPS

<u>DNA viruses</u>

1. Herpes
   a. simplex I & II
   b. varicella
   c. cytomegalo
   d. Epstein-Barr
2. Hepadna (Hepatitis B)
3. Adeno
4. Pox
5. Papova
6. Parvo

RNA viruses

1. Picorna

   a. entero
      1. Polio
      2. Coxsackie
      3. ECHO
      4. Hepatitis A
   b. Rhino

2. Toga

   a. WEE, EEE, VEE
   b. SLE, Dengue, yellow fever
   c. Rubella

3. Rhabdo

4. Orthomyxo

5. Paramyxo

   a. Parainfluenza
   b. Mumps
   c. Measles
   d. Respiratory syncytial

6. Reo

   a. Reo
   b. Rota

7. Arena (LCM)

8. Bunya

9. Corona

10. Retro

   a. Oncorna
   b. HTLV

Uncertain NA type

1. Slow Viruses,
2. prions (protein only)
3. non A/non B Hepatitis

The table below is a helpful way to group the viruses. There are 6 families of DNA-containing viruses, Herpes, Hepadna, Adeno, Papova, Pox and Parvo (The HHAPPPy viruses). If one can remember these and the generalities associated with the DNA viruses, then any other virus will be the opposite. As nothing is ever that simple, the table also contains the exceptions to that rule, e.g., if all DNA viruses are Double stranded and Naked, then the RNA viruses should all be single stranded and enveloped; and they are with the exception of the REO (dsRNA) and Picorna (naked RNA) as noted in the table.

VIRUS CLASSIFICATION

Virus Type	Generalities	Exceptions
DNA Viruses	Double Stranded	Parvo
Herpes	Naked	Pox and Herpes
Hepadna		
Adeno	Nuclear site for Replication	Pox
Papova		
Pox		
Parvo	Icosahedral (cubic) symmetry	Pox (complex)
RNA Viruses	Single Stranded	REO
Orthomyxo		
Paramyxo	Enveloped	REO and Picorna
Toga		
Picorna		
Arena	Cytoplasmic site for replication	Orthomyxo and Retro (nuclear + cytoplasmic)
Bunya		
Corona		
Retro		
Rhabdo	Helical symmetry	REO, Picorna, Toga
REO		

VIRAL REPLICATION

STEPS	ACTIVITY	
1. Adsorption	-virus attaches to specific receptors on cell membrane -interaction is, at first, reversible, then becomes irreversible	The first step in viral replication is _____.
2. Penetration	-virus particle is actively taken up by cell through a process called pinocytosis or phagocytosis	Antibody to viral capsid/nucleic acid blocks this process.
3. Uncoating	-takes place at cell membrane or vesicles -viral nucleic acid is released inside of cell by cell host enzymes	
4. Intracellular replication of viral components		Early viral proteins are _____ _____ _____; late proteins are _____ _____ _____.
A. RNA viruses	-early proteins are enzymes for viral RNA synthesis or inhibitors of cellular synthetic events. -late proteins are viral structural proteins and assembly proteins synthesized in response to the viral genome	
B. DNA viruses		
1. Early protein synthesis	-synthesis of enzymes for DNA synthesis, tumor antigens, etc. -uses small part of viral genome	

2. Viral DNA synthesis
   - -many new copies of viral DNA

3. Late protein synthesis
   - -viral capsid proteins(structural)

4. Assembly:
   - -newly synthesized viral nucleic acid and protein assembled inside cell
   - -viral envelope added (usually from cell membrane)
   - -new viruses released from cell by budding or lysis

The template for early proteins in a Herpes infected cell is provided by <u>viral RNA/newly synthesized RNA</u>.

5. Effects of Viruses on Cells
   - -lytic viruses inhibit cell RNA, DNA, protein sythesis
   - -tumor viruses transform cells
   - -latent viruses (herpes) probably do not alter host cell greatly

REPLICATION OF SPECIFIC RNA VIRUSES

1. <u>Picorna and Togavirus</u>
   e.g. polio, rubella, WEE
   - -viral RNA is single stranded piece of messenger RNA (+mRNA)
   - -naked viral RNA can infect cells
   - -viral RNA (+mRNA) gets to polysomes to make new viral proteins
   - -new proteins made as one long protein, then cleaved to viral specific proteins
   - -replicase (RNA-dependent RNA polymerase), an enzyme which copies +mRNA and makes a negative strand of mRNA
   - -the -mRNA is then used as template to make new viral RNA (+mRNA)

The template for late proteins in a polio infected cell is provided for by <u>viral RNA/newly synthesized RNA</u>.

2. Orthomyxovirus
(influenza virus)
-viral RNA is fragmented into 8 pieces of (-mRNA)
-virus carries into cell a transcriptase enzyme (RNA dependent RNA polymerase)
-transcriptase copies (-mRNA) to make (+mRNA) for viral proteins
-(+mRNA) is also template for new viral (-mRNA)

3. Paramyxoviruses
(rabies, mumps, measles)
-viral RNA is single-stranded (-mRNA), not segmented
-virus carries transcriptase like influenza virus; similar replication cycle

4. Diplornavirus
(reovirus and retrovirus)
-viral RNA is double-stranded and composed of ten fragments
-virus carries transcriptase

5. Retroviruses
(RNA tumor virses)
-viral RNA is single stranded
-virus carries reverse transcriptase (RNA-dependent DNA polymerase)
-transcriptase copies viral RNA into DNA
-DNA is integrated into host genome and serves as template for new viral RNA

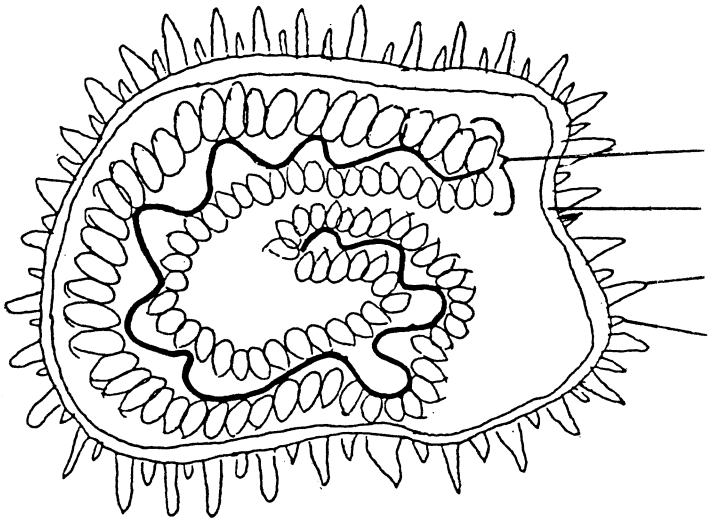
The RNA dependent RNA polymerase found in influenza virus is also called _____.

The template for mumps capsid is provided for by viral RNA/newly synthesized RNA.

REPLICATION OF SPECIFIC DNA VIRUSES

1. Herpesvirus
   - -replicates inside nucleus
   - -viral envelope obtained from nuclear membrane
2. Adenovirus
   - -replicates inside nucleus
3. Poxvirus
   - -replicates in cytoplasm; complex cycle
   - -virus carries many enzymes into cell
4. Papovavirus
   - -replicates in nucleus, circular DNA

REVIEW OF VIRAL MORPHOLOGY



To what virus group does this agent belong? Answer at bottom of page.

Terms to Know

capsid-shell which encloses the nucleic acid core.

capsomeres-protein subunits of capsid

nucleocapsid-capsid + nucleic acid

envelop-double membrane which encloses nucleocapsid of some viruses.

virion-complete, infective virus particle.

(answer = Myxoviridae)

INTERFERENCE WITH VIRAL REPLICATION

1. Interferon (IFN)
   - -a group of proteins made by cells in response to viruses, synthetic nucleotides (poly r:IC), foreign cells

   IFN species:
   leukocyte (alpha), fibroblast (beta), lymphocyte (gamma/immune)
   - -cell genome has information for IFN: if one inhibits cell metabolism with actinomycin D, no IFN produced
   - -IFN induces cells to make products which inhibit viral or foreign cell replication
   - -IFN released from cells, spreads to other cells and induces new IFN

   IFN induced products
   a. protein kinase which inactivates elongation factor-2 by phosphorylation
   b. A phosphodiesterase which inhibits peptide elongation
   c. Oligoisoadenylate which activates a cellular RNAse to degrade viral mRNA

2. Specific Antibody
   a. IgG, A, M
   b. Neutralize by interfering with
      -absorption
      -penetration
      -uncoating (rare)

Interferon produced by a macrophage would be an $\alpha/\beta/\gamma$ interferon

Interferon is effective against

A. DNA viruses

B. RNA viruses

C. Both

D. Neither

(answer at bottom of page)

Interferon inhibits viral replication by inducing cellular production of

1. _____

2. _____

3. _____

(answer = C)

3. Chemical Inhibitors

a. Amantadine
- -for influenza viruses
- -inhibits viral penetration and/or attachment

b. Methisazone
- -for poxviruses
- -inhibits late pox protein formation
- -new poxviruses are not made

c. IUDR-iododeoxyuridine-(idoxuridine)
- -inhibits DNA viruses, esp. herpes
- -inhibits DNA synthesis by inhibiting thymidylate synthetase

d. Ara-C (cytosine arabinoside, vidarabine)
- -inhibits DNA viruses (herpes and pox)
- -competitive inhibitor for DNA polymerase

e. Ara-A (adenosine arabinoside)
- -inhibits DNA viruses, esp. herpes
- -competitive inhibitor for DNA polymerase
- -Ara-ATP competes with dATP

f. Acyclovir (acycloguanosine)
- -inhibits DNA polymerase
- -herpesvirus specific thymidine kinase phosphorylates drug to active form therefore acyclovir is best vs. herpes

g. Ribavirin
- -active vs both DNA and RNA viruses
- -interferes with viral mRNA

Most chemical viral inhibitors are active against DNA/RNA viruses.

Acyclovir is relatively specific for Herpes because _____

_____

_____

_____.

VIRAL IMMUNOTHERAPY AND PROPHYLAXIS

I. Active, Artificial Immunity

A. Recommended for all persons in US

Disease	Condition of Vaccine
1. Rubella*	live attenuated
2. Measles*	live attenuated
3. Mumps*	live attenuated
4. Polio	
(Sabin)**	live attenuated
(Salk)**	inactive

Live viral vaccines are NOT recommended for immunosuppressed individuals

B. Recommended for special conditions (epidemic, military, travel, exposure)

Disease	Condition of Vaccine
1. Rabies***	inactive
2. Yellow Fever	live attenuated
3. Influenza**	inactive
4. Adenovirus**	active
5. Togavirus	inactive
6. Chickenpox****	live attenuated

*May be combined
**May be polyvalent
***Often times given with immune serum

MMR vaccine combination consists of

1. _____
2. _____
3. _____

II. PASSIVE, ARTIFICIAL IMMUNITY-use of immune serum or gamma globulin. Used only under special circumstances.

A. Rabies
B. Rubella
C. Hepatitis A and B
D. Rubeola
E. Mumps
F. Chicken Pox
G. Polio

Live viral vaccines induce immunity of long duration.

This type of treatment may be effective in disease prevention; it is of little value after onset of disease

Polyvalent viral vaccines include

1. _____
2. _____
3. _____

DNA VIRUSES

HERPESVIRUSES

Properties of the group
Enveloped viruses, which have an icosahedral nucleocapsid, contain double-stranded DNA, and replicate in the nucleus

Herpes hominis (simplex) Serotypes 1 & 2

Epidemiology
1. Man is the only known natural host
2. Most of the total population have been infected by type 1, many adults by type 2
3. Transmission occurs through close personal association

Clinical features

Primary herpes hominis
An acute illness, commonly seen in young children, characterized by fever, and small vesicular lesions of mouth, lips, face, conjunctiva, etc.
Recurrent or secondary herpes simplex (fever blisters, cold sores) A vesico-ulcerative rash, without fever, which may be triggered by trauma, emotional disturbances, menses, fever etc.

Disseminated herpes hominis (neonatal herpes): A rare illness occurring in early infancy characterized by a high fever, jaundice and encephalitis

Other diseases
Aseptic meningitis
Encephalitis
Keratoconjunctivitis
Genital herpes simplex
Particularly associated with serotype 2

Treatment

Nucleic acid analogs, such as Ara A (Vidarabine) and Ara C are used for herpetic encephalitis and ocular diseases. Acyclovir also good

Diagnosis

Culture is necessary as most people will have neutralizing antibodies

Herpes viruses

1. are DNA/RNA.
2. are single/double stranded.
3. are naked/enveloped.
4. multiply in the nucleus/cytoplasm.
5. have helical/cubic symmetry.

Drugs that are effective against herpesviruses include

1. _____
2. _____
3. _____

Varicella (chickenpox or Herpes zoster)

Serotype
There is only one serotype of the varicella-zoster virus

Epidemiology
1. Man is the natural host
2. The overall incidence of infection is very high
3. Transmission probably follows inhalation of infective aerosols, or direct skin contact

Clinical features
1. The onset is sudden, with a rash progressing from macules to papules to vesicles. Pustules do not develop; there is no scarring
2. The lesions appear in successive crops over 3 to 4 days
3. The lesions are distributed chiefly on the trunk and face

Zoster (Herpes zoster or shingles)

Epidemiology
This disease occurs chiefly in adults, most of whom give a history of having had varicella. Infection is communicable from as early as 5 days before to one week after the rash first appears

Pathogenesis
The virus probably persists in cells of the root ganglia after an attack of varicella, and is activated later with a resultant inflammatory reaction along the nerve followed by destruction of the epithelial cells served by that nerve

Clinical features
1. Erythematous maculopapular lesions develop, and on the trunk these have a band-like distribution

2. A disseminated form of disease is sometimes seen in patients who have received immunosuppressive therapy or radiotherapy

Herpes zoster causes

A. chicken pox

B. cold sores

C. shingles

D. all of the above

E. A and C only

(answer at bottom)

(answer = E)

Infectious mononucleosis

Etiologic agent (Epstein-Barr) herpesvirus

Epidemiology

1. Man is the natural host
2. Infection is most common in young adults
3. Incidence of infection is high
4. Mode of transmission; inhalation of infective aerosols during close personal association; kissing

Serodiagnosis

The heterophile agglutination test is used to detect a particular IgM antibody specific for sheep erythrocytes

Cytomegalic inclusion disease

Etiologic agent

Cytomegalovirus; multiple serotypes are known

Epidemiology

1. Man is the only natural known host
2. The virus can be transmitted across the placenta and cause congenital infection. The mode of transmission in postnatal infections has yet to be clarified, but the virus is known to be excreted in saliva, semen, breast milk and urine

Clinical features

1. Intrauterine infections may cause death of the fetus, or result in congenital disease which is frequently fatal. In those who survive, hepatosplenomegaly with jaundice, blood dyscrasias, mental retardation, microcephaly and chorioretinitis are common sequelae
2. Postnatal infections are usually symptomless in infants and children, but occasionally hepatitis, pneumonitis or acquired hemolytic anemia develop
3. In patients with malignancies, AIDS, or those receiving immunosuppressive therapy, hepatitis, pneumonitis, infectious mononucleosis-like disease (with negative hetrophile) or even generalized disease may develop, possible resulting from the activation of latent virus

Positive heterophile hemagglutinating antibodies are seen in diseases with infectious mononucleosis-like symptoms caused by

A. Cytomegalovirus

B. Epstein-Barr virus

C. Both

D. Neither

(answer at bottom)

(answer = B)

ADENOVIRUSES

Properties of the group

1. Naked viruses, which have an icosahedral nucleocapsid, contain double-stranded DNA and replicate in the nucleus
2. There are more than 30 human serotypes, some cause tumors in animals

Diseases caused by adenoviruses

A. Pharyngo-conjunctival fever

1. Particularly common in military recruits; in the general population, only about 5% of all respiratory illness is caused by adenoviruses
2. The mode of transmission is via infective aerosols or fresh fomites
3. Bronchitis and pneumonia sometimes occur, the latter usually in infants

B. Epidemic keratoconjunctivitis

1. Particularly associated with serotype 8
2. Outbreaks in certain industries are associated with minor ocular trauma resulting from dusty atmospheres, as in shipyards

C. Exanthem

Adenovirus is possibly responsible for a rubelliform rash

D. Hemorrhagic Cystitis

Particularly in children

Adenoviruses are

1. DNA/RNA.
2. single/double stranded.
3. naked/enveloped.
4. multiply in the nucleus/cytoplasm.
5. helical/cubic symmetry.

PAPOVAVIRUSES

Properties of the group

Naked viruses, which have an icosahedral nucleocapsid, contain dsDNA, and replicate in the nucleus

Papilloma virus

Only one is known which affects humans

Epidemiology

1. Man is the only known host
2. The mode of transmission is presumably by direct contact

Pathogenesis and pathology

Replication occurs in the epithelial cells of the skin; infected cells contain intranuclear inclusion bodies, virus causes a benign neoplasm

Clinical features

The common wart

Polyoma virus

Causes many different malignancies in several animal species

Vacuolating virus

A simian virus (SV40) causes tumors when inoculated into newborn animals. Also transforms cells in culture

JC virus

Has been isolated from immunocompromised individuals and from the brains of patients with progressive multifocal leukoencephalopathy. Most humans have specific antibody vs. the JC virus

The papovavirus responsible for the common wart is _____.

The papovavirus associated with progressive multifocal leukoencephalopathy is _____.

POXVIRUSES

Properties of the group

Complex ellipsoid viruses, which contain double-stranded DNA, replicate in the cytoplasm

Smallpox
Epidemiology

1. Man is the only natural host; disease has been eradicated from the planet
2. Smallpox is transmitted by inhalation of infective aerosols through personal association, or by contaminated fomites such as bedding

Treatment

Methisazone, which is of value prophylactically, is also useful in treating dermal complications following vaccination. It blocks synthesis of certain viral proteins thus inhibiting viral replication

Artificially-acquired immunity
Active (vaccination)

Immunization is carried out with live vaccinia virus

Mulluscum contagiosum
Epidemiology

Infection probably occurs through minor abrasions, and in swimming pools

Clinical features

Multiple discrete benign tumors appear on the skin anywhere except on the palms and soles; the lesions last for several months, and then disappear spontaneously

Poxviruses are

1. DNA/RNA.
2. single/double strand.
3. naked/enveloped.
4. multiply in the cytoplasm/nucleus.
5. helical/cubic symmetry.

RNA VIRUSES

ORTHOMYXOVIRUSES

Properties of the group

1. Enveloped viruses, helical nucleocapsid, contain 8 distinct segments of single-stranded RNA and replicate in both nucleus and cytoplasm.
2. Orthomyxoviruses cause influenza.

Serotypes

1. There are 3 serotypes: A, B and C.
2. Each serotype contains 2 surface antigens:

a. A hemagglutinin which enables the virion to attach to receptors on the cell surface.
b. A neuraminidase which facilitates the release of progeny virus from infected cells.

3.The 2 surface antigens of serotypes A and B undergo frequent antigenic changes resulting in antigenic 'drift'.

Serodiagnosis

Using the hemagglutination-inhibition technique, the patient's serum can be tested for antibody against a particular strain of virus.

Antiviral therapy

Amantadine hydrochloride (Symmetrel) is used as a prophylactic drug before or immediately after exposure to infection. It acts by blocking penetration of the virus into cells and also blocks uncoating of the virus.

Artificially-acquired immunity

Induced by vaccines inactivated by formalin.

Reye's syndrome (encephalopathy and fatty liver) is associated with type B, and perhaps also with other viruses (e.g. chickenpox). Salicylates may also be involved in the pathogenesis of the disease.

Influenza is an ssRNA virus whose genome is in _____ segments. It agglutinates RBCs through the action of its _____; the enzyme _____ facilitates progeny release.

PARAMYXOVIRUSES

Properties of the group

Enveloped viruses, helical nucleocapsid, contain single-stranded RNA, and replicated in the cytoplasm. The measles virus is known to replicate in both nucleus and cytoplasm.

Parainfluenzavirus infections

The viruses cause a variety of upper and lower respiratory tract illnesses - cold-like ills, pharyngitis, bronchitis, bronchiolitis and pneumonia. In young children, the viruses are the commonest cause of acute laryngotracheobronchitis (croup).

Respiratory syncytial virus is the most common cause of _____ _____.

Respiratory Syncytial Virus (RSV) Infection

1. In the infant, severe lower respiratory tract disease can occur; bacterial complications are common.
2. RSV is the most common cause of viral pneumonia in infants.
3. Reinfection occurs commonly, but is usually mild and confined to the upper respiratory tract, frequently resulting in the common cold syndrome.

Complications of mumps infection include

1. _____
2. _____

Mumps

1. Sudden onset of swelling of the parotid glands, usually bilateral.
2. Submaxillary and sublingual glands may also be involved.
3. Inflammation of the testis (orchitis) often occurs in males past puberty, but testicular atrophy or sterility is rare.
4. Meningitis is a relatively common complication.

Measles

1. Prodromal signs are fever, cough, coryza, conjunctivitis and appearance of Koplik's spots in the mouth
2. Viremia
3. After 3 days, a rash starts on the head and spreads to chest, trunk and limbs in the next day or two; the rash disappears slowly
4. Complications are fairly common, and sometimes severe: These include - Otitis media and pneumonia
5. Encephalomyelitis: rare, occurs 1 to 2 weeks after the rash, and is associated with a high mortality rate
6. Subacute sclerosing panencephalitis: May be a post-infection sequela

Prodromal signs of measles include

1. _____
2. _____
3. _____
4. _____
5. _____

ARENAVIRUSES

Properties of the group

Enveloped RNA viruses

Lymphocytic choriomeningitis

A disease usually manifest as "aseptic" meningitis or a mild influenza-like illness, rarely as a severe encephalomyelitis. The natural host of the virus is the mouse

Complications of measles include

1. _____
2. _____
3. _____
4. _____

ARTHROPOD-BORNE VIRAL DISEASES

Classification

1. The arboviruses encompass a heterogenous collection of some 400 viruses related only by the epidemiological fact that they are arthropod-borne
2. The so-called 'arboviruses' alternate between an invertebrate vector and a vertebrate reservoir
3. Arboviruses belong to several viral families: Togaviridae, Bunyaviridae, Reoviridae, Arenaviridae and others

Arthropod-borne viral diseases belong to the following viral families

1. _____
2. _____
3. _____
4. _____

Epidemiology

1. The cycle of transmission of these viruses is from arthropod to vertebrate host and back to arthropod
2. The arthropods involved are commonly mosquitoes, but sometimes ticks, sandflies and gnats act as vectors
3. The natural hosts, which act as reservoirs, include birds, reptiles, mammals and, rarely, man

BUNYAVIRIDAE

Bunyamwera viruses are enveloped, spherical viruses with helical symmetry. They are similar ecologically to the togaviruses and are arthropod-borne (arboviruses). The single-stranded RNA is composed of three segments. The pathogenesis of disease is similar to the togaviruses (encephalitis). One group of this diverse family of viruses which has been associated with encephalitis in humans is California viruses, first found in California and more recently in other parts of the USA

TOGAVIRIDAE

Arbovirus encephalitis

Most arboviruses are antigenic groups A or B; rubella belongs here. They are enveloped ssRNA viruses with (+mRNA) genome

1. These encephalitides in the USA include St. Louis, Western and Eastern equine viruses
2. The usual reservoir is birds, and the vector is the mosquito
3. Clinical findings include fever, chills, headache, widespread muscular aches, drowsiness, nuchal rigidity, convulsions, paralysis, coma and death

Yellow fever

1. The natural host is the monkey, and the vector is the Aedes mosquito; two forms of yellow fever, the urban and the jungle (sylvatic), are recognized
2. In the urban type of yellow fever, man is the main reservoir, and the transmission cycle is man-mosquito-man
3. In the jungle type, the monkey is the main reservoir, and the cycle is monkey-mosquito-monkey with man being infected occasionally
4. The outstanding feature in cases in yellow fever is the extent of damage to liver and kidney in severe cases

Dengue

1. The onset of illness is characterized by fever, chills, headache, conjunctivitis, lymphadenitis, severe pain in back, muscles and joints ('breaks-bone fever')
2. Fever often falls, then rises again within a week ('saddleback curve')

Clinical signs of viral encephalitis include

1. _____
2. _____
3. _____
4. _____
5. _____
6. _____
7. _____
8. _____
9. _____

RUBELLA VIRUS

Properties

Enveloped virus, which contain single-stranded RNA, and replicate in the cytoplasm. There is only one serotype. The rubella virus is classified with Togaviruses; however, it is not an arthropod-borne disease, but rather is droplet spread

Clinical feature of postnatal rubella

1. There is enlargement of lymph nodes with conjunctivitis, often followed by a fine macular rash; slight fever may occur

2. The main risk of this infection is that it may occur in a non-immune woman during the first trimester of pregnancy, with serious consequences for the fetus

Clinical features of prenatal rubella

1. The risk of congenital malformations is greatest when the mother is infected during the first trimester of pregnancy
2. One or more of the following features may be present
   a. Blindness
   b. Deafness
   c. Congenital heart defects
   d. Mental retardation (often with microcephaly)

Rubella virus is

1. DNA/RNA.
2. single/double stranded.
3. naked/enveloped.
4. multiplied in the nucleus/cytoplasm.

RHABDOVIRUSES

Properties of the group

1. Bullet-shaped enveloped viruses, which contain single-stranded RNA, have a helical nucleocapsid, replicate in the cytoplasm, and are released by budding.
2. The group includes the virus responsible for rabies

THE NEGRI BODY IS THE INCLUSION SEEN IN THE CYTOPLASM OF CELLS IN THE HIPPOCAMPUS AND OTHER CNS AREAS.

Rabies

Serotypes: There is only one serotype of rabies virus

Epidemiology

1. The natural hosts include many kinds of mammals and bats.
2. The usual mode of transmission is by inoculation (bite). Infection may rarely result from inhalation of infective aerosols from bat secretions

Pathogenesis

1. Virus spreads along nerves to the CNS.
2. The virus causes destruction of nerve cells and demyelination; the highest concentration is usually found in the hippocampus

Artificially-acquired immunity

Vaccines

1. Virus is grown in human diploid cells and inactivated. Weekly, SubQ injections for 4 to 6 weeks are adequate

2. Passive antibody in the form of rabies immune globulin is also available

Treatment

1. Detain animal for observation.
2. Wound must be thoroughly cleansed.
3. Inject rabies immune globulin (human origin) into the wound and I M
4. Start the vaccine immediately at another site

PICORNAVIRUSES

There are 2 groups of small (pico) RNA viruses, the enteroviruses and the rhinoviruses

ENTEROVIRUSES

Properties of the group

1. Naked viruses, icosahedral nucleocapsid, contain single- stranded RNA, and replicate in the cytoplasm
2. There are 4 subgroups: polioviruses, coxsackieviruses echoviruses, and hepatitis A

Pathology

1. Most infections are subclinical)

2. Virus multiplies first in the pharynx, small intestines and local lymph nodes

3. Viremia follows, with spread of virus to the brain and spinal cord

Polioviruses

1. A formalin-inactivated viral vaccine (Salk) containing all 3 serotypes is available for injection

2. A live attenuated viral vaccine (Sabin) containing either a single serotype or all 3 are given orally. It induces sIgA in addition to IgG and IgM and imparts immunity of long duration. May be dangerous in immunosuppressed individuals (use Salk)

3. Clinical diseases
   a. Most cases are subclinical
   b. Aseptic meningitis
   c. Poliomyelitis-an acute disease which causes flaccid paralysis. The virus replicates in many cells in the body. The target cells are the motor neurons in the CNS where destruction causes paralysis

There are 5 virus groups classified as small RNA viruses

1. _____
2. _____
3. _____
4. _____
5. _____

Coxsackieviruses

These viruses are classified as either A or B depending on their pathogenicity for mice

Clinical features

Infection may be inapparent, or result in illness ranging in severity as far as lethal disease. Several different forms of illness can develop as follows:

1. Herpangina (vesicular pharyngitis) is the commonest manifestation of infection by A-serotypes
2. "Aseptic" meningitis can be caused by some A-serotypes or any B-serotypes
3. Epidemic myalgia is a common manifestation of infection by B-serotypes
4. Myocarditis or pericarditis can occur in infants from a B-serotype infection, and B-serotypes occasionally cause a myocardiopathy in children or adults.

Echoviruses

Clinical features

1. Meningitis is commonly caused by echoviruses, but permanent paralysis is very rare
2. Skin rashes, pharyngitis and fever may occur
3. Echoviruses are a cause of a cold-like disease
4. Gastroenteritis and infantile diarrhea have been associated with echovirus infection

Hepatitis A is considered to be in this group as well. (cf section on hepatitis viruses for more information on this agent)

Match the disease with the Coxsackie virus.

serotype A

serotype B

_____ 1. vesicular pharyngitis

_____ 2. aseptic meningitis

_____ 3. myocarditis

_____ 4. pericarditis

(answers at left, bottom)

answers
1 = A
2 = A, B
3 = B
4 = B

RHINOVIRUSES

Properties of the group

Naked icosahedral viruses, contain single-stranded RNA

Serotypes

More than 100 serotypes are known.

1. These viruses are the commonest cause of the common cold (rhinitis, rhinorrea)
2. Rhinoviruses usually remain localized in the nasal mucosa

Artificially-acquired immunity

In view of the number of serotypes, the development of a vaccine is not practicable

CALICIVIRIDAE

Norwalk agent

RNA virus which is single stranded. Etiologically associated with epidemic acute gastroenteritis in children and adults

REOVIRUSES

Properties of the group

1. Naked icosahedral viruses, contain double-stranded RNA
2. Reoviruses can be isolated from feces and respiratory secretions of healthy persons, as well as from patients with a variety of illnesses, e.g., rhinitis
3. Rotaviruses cause gastroenteritis in human infants and lower animals

CORONAVIRUSES

Enveloped helical viruses, contain RNA

Coronaviruses are a common cause of a cold-like disease in adults, but they do not seem to be an important cause of acute respiratory illness in children

Match the disease with the virus

A. Norwalk agent

B. Reovirus

C. Rhinovirus

D. Rotovirus

1. Rhinitis

2. Gastroenteritis in adults

3. Gastroenteritis in infants

(answers at bottom)

Viruses with dsRNA include

1. _____

2. _____

Answers
1. B, C
2. A
D. D

HEPATITIS VIRUSES

Viral hepatitis types A and B

Three particular forms of viral hepatitis can be distinguished clinically; these are hepatitis type A (infectious hepatitis or short- incubation hepatitis) = RNA virus; hepatitis type B (serum hepatitis or long-incubation hepatitis) = DNA virus and; hepatitis non-A non-B (disease resembles that of type A)

Clinical features

Incubation period

Type A: 10 to 50 days
Type B: 50 to 180 days

Signs and symptoms

The illness is characterized by malaise, anorexia, nausea, vomiting, diarrhea, fever and also jaundice which may or may not appear between two days and three weeks after onset

Type A: In young children, infection frequently remains inapparent or develops into a mild illness without jaundice; in older age groups, infection often leads to icterus or more severe disease

Type B: Infection may remain inapparent. Many cases continue to chronic hepatitis with surface antigen carrier state. The Dane particle is the infectious particle

Laboratory Diagnosis

Serum from the patient is examined for the presence of: Hep A = Ab vs the virus; Hepatitis B = surface Ag-HBsAG, core Ag-HBcAg, and HBeAg = called "C" antigen

Which of the following viral hepatitis agents can be transmitted via transfusion?

A. Hepatitis A

B. Hepatitis B

C. Non-A Non-B

D. All of the above

E. A and C only

(answer at bottom)

The Hepadnaviruses are _____

_____.

(answer = D)

Antiviral therapy

There is no specific antiviral chemotherapy, although pooled human gamma globulin can be used to abort virus infections

Epidemiology

Type A is fecal:oral transmission, Type B is via inoculation and close contact although both viruses can be transmitted by either route. Hepatitis B appears to be associated with drug addicts and homosexuals as well as through parenteral injections

Vaccine

Formalin-treated HBsAB (particles) from carriers has proven effective

The presence of Hbs Ag in serum means

_____

_____

_____

_____.

Significance of Hepatitis B Antigens and Antibodies in Serum

Component Present in Serum			
HbsAg	Anti-HBs	Anti-HBc	Interpretation
+	-	-	Prodromal period or early acute disease. Person is considered infectious.
+	-	+	Acute disease or chronic carrier. Person is considered infectious.
-	+	+	Convalescing from the disease or immune.
-	+	-	Immune via disease or vaccination.
-	-	+	Recovered from disease and lost reactivity Antibody of IgG class; low level. or Recent disease; serum taken after HBsAg disappeared, before anti-HBs. Anti-HBc should be high. Such people are infectious.

SLOW VIRUSES

Diseases caused by viruses and virus-like agents belonging to different taxonomic groups, and linked together by the fact that they are all characterized by a long incubation period. Some of these agents may be PRIONS, small proteinaceous infectious particles

Subacute sclerosing panencephalitis SSPE
Infectious measles virus has been isolated from brain tissue and lymph nodes of affected individuals with a history of measles

Kuru

The disease seems to have resulted from cannibalism. Kuru has been found only in a single tribe in New Guinea

Progressive Multifocal leukoencephalopathy

A rare disease of the CNS. Papovavirus JC has been isolated from affected tissues

Creutzfeld-Jakob disease

A rare disease of the CNS which has been transmitted to chimpanzees by inoculation of material from the brains of patients

Multiple sclerosis

A CNS disease suspected to be of viral etiology. There is serological evidence suggesting that measles virus may be involved

Match the virus with the disease

A. measles

B. JC papovavirus

1. multiple sclerosis
2. subacute sclerosing panencephalitis
3. progressive multifocal leukoencephalopathy

(answers at bottom of page)

1=A
2=A
3=B

ONCOGENIC VIRUSES

Properties of oncogenic viruses

1. Both RNA and DNA viruses from several taxonomic groups have been shown to be oncogenic

2. Oncogenic viruses spread 2 ways:
   a) Pre-natal (vertical) transmission from one generation to the next
   b) Post-natal (horizontal) transmission

3. It appears that some or all of the genes of some oncogenic viruses may be integrated into host DNA; the RNA viruses being integrated by a RNA-dependent DNA polymerase (Reverse transcriptase)

Transformation in vitro

The properties of transformed cells include:

1. Loss of contact inhibition

2. Altered cell morphology

3. The presence of new antigens, both in the membrane (tumor specific transplantation antigens) and intracellularly

4. The ability to proliferate rapidly with concomitant high energy demand

5. Altered chromosomal morphology and/or number

6. Ability to grow in soft agar and produce tumors when injected into an appropriate host

Poxviruses

Viruses in this group include those responsible for the lesions of molluscum contagiosum in man

Herpesviruses

1. This group causes Marek's disease, a form of lymphomatosis, in chickens, as well as leukemia and reticulosarcoma in non-human primates, renal carcinoma in frogs, and lymphoma in rabbits
2. Epidemiological evidence suggests Herpes simplex type 2 may be associated with cervical carcinoma
3. EB virus is associated with Burkitt's lymphoma and nasopharyngeal carcinoma

Papillomaviruses

These cause benign papillomas (warts) in man and other mammalian species. In man, condyloma acuminatum causes a genital wart which is usually benign, but may become malignant

Polyomavirus

It induces the formation of sarcomas and carcinomas in diverse animal species

SV 40 virus and Adenoviruses

In experimental conditions, they induce malignant neoplasms in mice, and causes transformation in vitro of cells of many species

Oncornaviruses (Retroviruses)

1. These enveloped viruses contain single-stranded RNA; the virion also contains the enzyme reverse transcriptase which, together with certain other enzymes, produces double-stranded DNA homologous to the virion RNA
2. Oncornaviruses can induce leukemia and other blood malignancies, sarcomas, and mammary cancer in experimental animals
3. The oncogene hypothesis proposes that the viral genome consists of at least 2 sets of genes, one of which controls the process of oncogenesis, and the other the production of infectious virus; either, neither or both sets may be evoked by endogenous or exogenous factors
4. Retroviruses pick up host cell genes (e.g., the ONC gene)

Herpes viruses are associated with the following cancers

1. _____
2. _____
3. _____
4. _____
5. _____
6. _____
7. _____
8. _____

VIRUSES AND HUMAN CANCER

Burkitt's lymphoma

Specific antigen and nucleic acid of Epstein Barr (EB) herpesvirus is present in cells cultured from cases of Burkitt's lymphoma. This virus also appears to be responsible for infectious mononucleosis. Patients with Burkitt's lymphoma have a high incidence and high titers of antibody against EB-herpesvirus

Nasopharyngeal carcinoma

EB herpesvirus has been detected in cells obtained from cases of nasopharyngeal carcinoma, and patients show high titers of anti-EB herpesvirus antibody

Carcinoma of the uterine cervix

Seroepidemiological surveys have shown a higher incidence of specific antibody against herpes simplex virus serotype 2 in patients with this form of cancer than in those without cervical cancer

Hepatoma

Hepatitis B virus has been associated with primary carcinoma of the liver

Kaposi's Sarcoma

Human T lymphotropic Virus -III (HTLV III), a Retrovirus, has been isolated from a human T-cell lymphoma cell line. This virus resembles the bovine leukemia virus but is antigenically different from all other retroviruses. It is etiologically linked with AIDS. Individuals with AIDS suffer from infections by opportunists such as Pneumocystis and Cryptococcus. CMV infections are often activated. There will be inverted T4:T8 lymphocyte ratios (due to the T4 "target" of this virus) and normal levels of IgG, etc. The disease is particularly common in homosexuals, hemophiliacs, and intravenous drug users. High incidence of the virus is reported in various African countries.

Epstein Barr virus causes 3 human diseases. They are

1. _____
2. _____
3. _____

HTLV-III has a tropism for T4/T8 lymphocytes.

The protein coat (capsid) of true viruses functions to maintain infectivity of nucleic acid in extracellular state, serves as an antigen in vaccines, and aids in the penetration of virions into susceptible cells.

One of the first events which occurs after a virulent virus infects a cell is cessation of host cell macromolecular biosynthesis.

Interferon is a viral inhibitor produced in virus infected cells.

On recovering from infection with herpesvirus hominis, type I, the patient develops neutralizing antibodies.

IUDR (5-iodo-2'-deoxyuridine) is incorporated into viral DNA to produce faulty nucleic acid.

The etiologic agent of human warts (verrucae) is a papovavirus.

Commercially available influenza vaccine contains inactivated influenza virus, types A and B.

In general, enteroviral diseases are subclinical in nature with less than ten percent of the cases being severe.

Coxsackie A and B viruses cause herpangina and myocarditis, respectively.

Coxsackie viruses of Group B are responsible for a considerable proportion of cases of aseptic meningitis.

The hepatitis B surface antigen (HBsAG) when found in banked blood renders it undesirable for use in blood transfusions.

The incubation period of serum hepatitis usually ranges from 50-180 days.

Hepatitis B virus is more resistant to chemical and physical agents than is hepatitis A virus (HAV).

The finding of Negri bodies, cytoplasmic inclusions in neurons, is specific for the pathologic diagnosis of rabies.

Togaviruses may infect the liver to produce yellow fever or the brain to cause encephalitis.

Virus-induced cell transformation may result in the formation of new enzymes and antigens as well as altered cell morphology and tumor formation.

Cell susceptibility range of a virus is determined by surface protein units of the capsid or envelope.

The Epstein-Barr virus causes infectious mononucleosis.

The piconaviruses are single strands of (+) RNA. During replication replicative intermediates (RI = double stranded RNA: + RNA and -RNA) are formed by the enzyme replicase.

Myxoviruses and paramyxoviruses also have an RI; however, these viruses have a (-) RNA as the parental type. Myxoviruses have eight distinct (-) RNA strands; paramyxo's only have one.

Reovirus RNA is double stranded (-) RNA which exists in 10 distinct segments.

All (-) RNA viruses have a virion-bound transcriptase which synthesizes (+) RNA used as mRNA for protein synthesis.

Naked polio virus, (+) RNA, is able to penetrate mammalian cell membranes and establish an infection under appropriate laboratory conditions. Myxoviral (-) RNA can not do this because the purified RNA would not contain the transcriptase needed to make messenger RNA.

The oncornaviruses replicate through a DNA replicative intermediate. Reverse transcriptase synthesize single stranded DNA from the parental RNA template. DNA polymerase then synthesizes double stranded RNA which is transcribed to mRNA.

Measles is prevented by administration of an attentuated monovalent vaccine. It may be included with rubella and mumps vaccines to produce a polyvalent product.

Subacute sclerosing panencephalitis is thought to be caused by measles.

Complications of measles include encephalitis and pneumonia.

Four clinical signs and symptoms of measles include cough, coryza, conjunctivitis and Koplik spots.

The pathogenesis of measles and mumps include a primary infection of the respiratory tract followed by a viremia.

Mumps is a disease of secretory cells and often involves the pancreas.

Following the initial attack of mumps, long lasting protective immunity is produced.

Mumps is spread from the infected patient by infectious droplets (aerosol) or fresh fomites.

Antibodies to the glycoprotein spikes (hemagglutinins) of influenza viruses are protective.

Type A influenza viruses are known to cause pandemics while types B and C influenza viruses are less likely to do so.

Antigenic drift is brought about by minor changes in type A influenza viruses while antigenic shift involves major changes in one or more of the glycoprotein spikes.

Orthomyxoviruses contain eight pieces of single stranded RNA and an RNA-dependent RNA polymerase.

Influenza viruses are replicated in both the cytoplasm and nucleus, mature in the cytoplasm and are released by budding through the modified cell membrane.

Paramyxoviruses resemble orthomyxoviruses morphologically but are larger and the nucleoprotein is contained in one continuous strand.

Respiratory syncytial virus commonly produces a severe disease (bronchitits, bronchiolitis and croup) in infants.

Parainfluenza viruses cause bronchitis, bronchiolites and croup in children and a common cold-like disease in adults.

Togaviruses are spread by the bite of an infected arthropod.

Mammals and birds are reservoir hosts for arboviruses.

Poliovirus is spread throughout the human body via the lymphatics and viremia.

The portal of entry of enteroviruses is the oral cavity and the viruses gain entry through the oropharyngeal and intestinal mucosa.

ECHO viruses are responsible for many cases of aseptic meningitis.

Piconaviruses include the rhinoviruses which are the etiologic agents of the common cold.

Polio vaccines must be polyvalent because there are three antigenically distinct polioviruses.

The Sabin polio vaccine contains attentuated virus and is taken orally while the Salk polio vaccine is formalin-inactivated and is administered by injection.

Rhabdoviruses are single stranded, RNA-containing, bullet-shaped viruses.

Rabies virus is a rhabdovirus.

Rabies virus is usually spread by the bite or lick of a rabid animal.

Rabies vaccine of choice contains inactive (tri -n- butyl phosphate) human diploid cell produced virus.

Rabies is usually diagnosed by immunofluorescence applied to brain tissue.

The Dane particle of hepatitis virus is composed of core antigen surrounded by surface antigen and is the infectious particle.

Type A hepatitis is spread primarily by the fecal-oral route and type B hepatitis is spread primarily by injection or contact but both viruses can be spread by either route.

Non A, non B type hepatitis is also quite prevalent.

Reoviruses are double-stranded RNA-containing viruses whose nucleoproteins are contained in 10 segements.

Rotaviruses are reoviruses which are the causal agents of infantile diarrhea.

Pharyngeal-conjunctival fever is the most common syndrome associated with adenoviruses.

An eosinophilic, intracytoplasmic inclusion called the Guarnieri body is formed frequently in variola-infected cells.

The poxvirus virion contains several enzymes including a DNA-dependent RNA polymerase.

The pathogenesis of smallpox includes two viremia stages during which the virus is spread throughout the body.

The WHO claims to have eradicated variola major from the earth.

Polyoma viruses produce tumors in many organs of mice.

A slow virus requires a prolonged incubation period (months to years) before appearance of disease.

Kuru (a disease seen in New Guinea) and Creutzfeldt-Jacob diseases are transmittable spongiform encephalopathies of humans.

Subacute sclerosing panencephalitis (SSPE) is a chronic, fatal disease of humans associated with a previous case of measles.

Rubella virus belong to the togavirus family.

Lasting protective immunity is usually produced upon convalescence from rubella.

Rubella virus is known to cross the human placenta and to infect the developing fetus.

Congenital rubella is the result of fetal infection during the first trimester of pregnancy. Following birth, virus may be excreted and cause a rubella outbreak in a newborn nursery.

An attentuated viral vaccine is effective in the prevention of rubella but should not be given to pregnant humans.

The "patient's immune response" to viral disease is the most commonly used diagnostic test. A four-fold rise in antibody titer between acute and convalescent serum specimens is considered diagnostic.

Cells transformed by viruses may be characterized by having the ability to induce tumors in animal hosts and possessing virus-specific tumor antigens.

Oncogenic viruses may be transmitted naturally in a vertical fashion, from one generation to another, or horizontally transmitted from one animal to another.

The provirus of an oncornavirus is a double-stranded DNA copy of the viral genome synthesized by a virion enzyme.

RNA tumor viruses contain a reverse transcriptase and a single strand of RNA.

The Epstein-Barr virus (EBV) has been recovered from Burkitt's lymphoma tissue, from human patients with nasopharyngeal carcinomas, and from human patients with infectious mononucleosis.

Herpes simplex virus, type 1, may have produced latent infection in the majority of the human population.

Inclusion bodies in host cells infected with a herpes simplex virus are found in the nucleus.

Reactivation of a latent infection in the form of shingles (herpes zoster) occurs with the etiologic agent of varicella (chicken pox).

Herpes simplex virus may cause aseptic meningitis.

Herpes simplex virus, type 2, is associated with genital herpes (lesions of genital tract) and neonatal herpes; it has also been implicated in cervical carcinoma.

Adenosine arabinoside (Ara-A) has been used quite effectively in treating herpesvirus infections, including serious systemic infections.

Zoster (shingles) is a recurrent disease; the lesions appear unilaterally on the body.

Diagnosis of infectious mononucleosis can be done using a serological test for detecting heterophile antibody.

Non-A Non-B Hepatitis virus is the most common cause of hepatitis in the USA.

Peripheral ganglia are believed to be the sites of latency of herpes viruses.

Aspirin therapy in children with influenza or chickenpox is contra-indicated due to the possible association of these conditions with Reye syndrome.

The predominant cell in the spinal fluid in viral meningitis is the lymphocyte. This is the same cell that would predominate in the perivascular cuffing also noted in these diseases.

The polio virus genome is +mRNA. This is also true of the togaviruses and rubella.

The REOvirus RNA is double-stranded.

The poliovirus genome is polycistronic, thus the synthesized polyprotein must be cleaved after synthesis.

Male homosexuals with AIDS may have HBsAg antibody in their serum in addition to the antibody to the HTLV-III virus.

Virology Proficiency Test

This set of 40 questions was used in an examination in 1985 given to sophomore Medical students. Their mean score was 80 percent. A score below 60 percent would probably suggest that the examinee is in need of more review of the Virology section of the text.

1. The genome of papovaviruses is

   A. circular single stranded DNA.
   B. circular double stranded RNA.
   C. linear single stranded DNA.
   D. circular double stranded DNA.

2. Rotavirus infections

   A. follow the typical natural history of other enteroviruses.
   B. are treated with ribovirin and fluids.
   C. occur because of neglect in vaccination.
   D. are important causes of diarrhea in young children.

3. Inhibits viral penetration and uncoating.

   A. Acylovir
   B. Amantadine
   C. Methisazone
   D. Ara A
   E. FUDR

4. RNA-dependent-RNA-polymerase may be found in the following viruses <u>except</u>

   A. Parainfluenza.
   B. Reovirus.
   C. Influenza.
   D. Western equine encephalitis.

5. Which of the following is the preferred post-exposure therapy for rabies:

   A. Human anti-rabies immunoglobulin
   B. Duck embryo virus vaccine.
   C. Human diploid cell culture virus vaccine.
   D. All of the above.
   E. A and C only.

6. Which of the following statements about virions is <u>not</u> true?

   A. They may contain lipids.
   B. They are obligate intracellular parasites.
   C. The symmetry of the nucleocapsid is dependent on the host cell in which it is propagated.
   D. Antibodies to surface protein can neutralize infectivity.
   E. Viruses with envelopes are usually sensitive to lipid solvents.

7. Which of the following statements is <u>true</u> for the rabies virus.

   A. A live attentuated virus vaccine is available for use in man.
   B. Virus can be isolated from blood of the infected person in the last two weeks of disease.
   C. Bats are the source for most cases of rabies in the USA.
   D. The virus contains its own RNA dependent RNA polymerase.

8. Infectious RNA genome is characteristic of the following

   A. Rhabdovirus.
   B. Rubeola virus.
   C. Parainfluenza virus.
   D. Rhinovirus.

9. Interferon

   A. once produced, persists for months.
   B. requires T-lymphocytes for expression.
   C. acts indirectly by blocking specific viral receptors.
   D. synthesis can be induced in living cells exposed to viral or nonviral inducers.

10. The diagnosis of rubella infection in a newborn is accomplished by

    A. measuring IgM antibody in the mother.
    B. measuring IgA antibody in the newborn.
    C. isolation of virus from the placenta.
    D. measuring IgM antibody in the newborn.

11. In comparing the differences between the orthomyxoviruses and the paramyxoviruses, one could say that:

    A. Orthomyxovirus replication is confined to the cytoplasm.
    B. Paramyxoviruses have a primer requirement for transcription.
    C. The rate of genetic recombination is higher for paramyxoviruses.
    D. The genome of orthomyxoviruses is segmented.

12. Pandemics of influenza are due to

    A. antigenic drifting.
    B. a mutant paramyxovirus.
    C. extensive population migration.
    D. antigenic shifting.

13. The influenza A virion

    A. contains 10 segments of RNA.
    B. contains 8 segments of DNA.
    C. induces host cell polymerase activity.
    D. contains glycoproteins in the envelope.

A = 1, 2, 3, correct
B = 1, 3 correct
C = 2, 4 correct
D = only 4 is correct
E = if all are correct

14. Important factors involved in viral disease production include the

    1. nutritional state of the host.
    2. immunologic status of host.
    3. virus dosage.
    4. age of the host.

15. Which of the following is/are true of the yellow fever virus?

    1. An effective vaccine is available.
    2. Yellow fever virus is a bunyavirus.
    3. Both man and other animals may act as reservoirs.
    4. The insect vector for the virus is the sandfly.

16. Which of the following statement(s) is/are true for defective virus particles?

    1. Formed in the course of a normal infection in the animal.
    2. Interfere with the replication of wild-type virus in the mixed infection.
    3. Contain less genetic information than wild-type virus.
    4. Increase in number with high multiplicities of infection.

17. Which pairs of viruses listed below are taxonomically related?

    1. Dengue virus and yellow fever virus.
    2. Rubella virus and mumps virus.
    3. Poliovirus and rhinovirus.
    4. Respiratory syncytial virus and influenza virus.

18. Encephalitis caused by togavirus such as Eastern equine, Western equine and St. Louis encephalitis viruses

    1. occurs most frequently in the summer and early autumn in the United States.
    2. could be eliminated by vaccinating or destroying all horses.
    3. is best prevented by mosquito control program.
    4. spread directly from human to human in severe epidemics.

19. Varicella-Zoster virus

    1. causes chickenpox.
    2. has a tropism for nerve cells.
    3. is spread by respiratory secretions.
    4. can be activated to give manifestations in a dermatome distribution.

A = 1, 2, 3, correct
B = 1, 3 correct
C = 2, 4 correct
D = only 4 is correct
E = if all are correct

20. Characteristic feature(s) of the herpes virus group include(s)

1. latent infections.
2. presence of an envelope containing peplomers.
3. budding from the nuclear membrane of a cell.
4. induction of cytoplasmic inclusion bodies.

21. Which of the following statements is (are) <u>true</u> of Herpes simplex virus type 1?

1. It is antigenically related to herpes simplex type 2.
2. It can cause venereal disease.
3. The nucleocapsid contains a double strand of DNA.
4. The most common primary infection seen is gingivostomatitis.

22. Which of the following is/are characteristic of hepatitis A virus?

1. Small non-enveloped icosahedral capsid with single-stranded nucleic acid genome.
2. Previously known as "serum hepatitis".
3. May be transmitted in a manner similar to poliovirus.
4. Attenuated vaccine available.

23. Which of the following statements about viral hepatitis is/are true?

1. Hepatitis B is a DNA virus.
2. NonA-NonB hepatitis is the most frequent cause of transfusion associated hepatitis in the USA.
3. Pooled human gamma globulin can be used to passively immunize against hepatitis A.
4. Routine screening of blood bank blood for hepatitis A virus is now being done.

24. With type B hepatitis

1. the finding of anti-HBcAg in the blood denotes a noninfectious state of the disease.
2. several subtypes containing a common core antigen exist.
3. HBcAg is the major component of the available vaccine.
4. the presence of Hbs Ag antigen in the blood reflects infectivity of the blood.

A = 1, 2, 3, correct
B = 1, 3 correct
C = 2, 4 correct
D = only 4 is correct
E = if all are correct

25. What feature(s) would you expect to be altered in a herpes simplex virus mutant resistant to acyclovir?

1. RNA polymerase.
2. Thymidine kinase.
3. Neuraminidase.
4. DNA polymerase.

26. The disease called shingles is

1. caused by the same virus as the disease chickenpox.
2. caused by a virus of the family Herpesviridae.
3. due to reactivation of virus in dorsal root ganglia.
4. increased in incidence by immunosuppression.

27. Example(s) of virus(es) capable of inducing persistent viral infections include(s):

1. Measles virus.
2. Herpes simplex virus type 1.
3. Hepatitis B virus.
4. St. Louis encephalitis virus.

28. Inapparent (subclinical) viral infections

1. are important in developing life-long immunity.
2. occur more often than apparent infections.
3. can lead to latency of the virus.
4. are seldom seen in children between 2 and 8 years of age.

29. A virus can cause disease in the host by

1. producing metabolites that result in cellular injury.
2. inducing an antibody response.
3. replicating in the cerebral spinal fluid resulting in increase in pressure
4. rendering vital target cells nonfunctional.

30. Replication of pox virus is unique in comparison to other DNA viruses in that

1. the virion does not possess viral enzymes.
2. replication occurs only in the cytoplasm.
3. the virion does not contain lipid.
4. the viral DNA contains more than 100 genes.

A = 1, 2, 3, correct
B = 1, 3 correct
C = 2, 4 correct
D = only 4 is correct
E = if all are correct

31. The major complication(s) of measles infection is/are:

1. encephalomyelitis.
2. croup.
3. pneumonia.
4. loss of hair.

32. Transmission of respiratory syncytial virus (RSV) is mediated by

1. aerosols.
2. fecal contamination of food stuffs.
3. intimate contact with the infected patient.
4. dirty diapers.

33. Parainfluenza infections

1. may be clinically similar to RSV infections.
2. do not respond to acyclovir treatment.
3. may be transmitted by large droplets.
4. do not become epidemic.

34. The hemagglutinin of influenza A is

1. the target of neutralizing antibody.
2. responsible for detaching the virion from the host cell.
3. responsible for attaching the virion to the host cell.
4. the target of ribavirin activity.

35. The neuraminidase of influenza A

1. is the target of amantadine activity.
2. is a tetramer.
3. is responsible for attachment of the virion to the host cell.
4. may show antigenic drifting or shifting.

36. Early transcription of DNA virus genomes may be associated with

1. induction of viral DNA synthesis.
2. non-structural viral proteins.
3. protein(s) that binds to DNA.
4. cell transformation.

37. Currently used inactivated vaccines include:

1. Polio.
2. Influenza A.
3. Hepatitis B.
4. Rabies.

A = 1, 2, 3, correct
B = 1, 3 correct
C = 2, 4 correct
D = only 4 is correct
E = if all are correct

38. Serious complication(s) of enterovirus infections include

1. optic nerve damage.
2. diarrhea resulting in dehydration.
3. a slow infection resulting years later in brain damage.
4. aseptic meningitis.

39. Antiviral agents include

1. 2-deoxy-D-glucose (Gluconil).
2. Amantadine.
3. Ribavirin.
4. Acyclovir.

40. Acyclovir works by

1. acting as a substrate for host thymidine kinase.
2. inhibiting viral DNA polymerase.
3. preventing uncoating of herpes virions.
4. being incorporated as a DNA chain terminator.

ANSWER SHEET

1.	D	21.	E
2.	D	22.	B
3.	B	23.	A
4.	D	24.	C
5.	E	25.	C
6.	C	26.	E
7.	D	27.	A
8.	D	28.	A
9.	D	29.	C
10.	D	30.	C
11.	D	31.	B
12.	D	32.	B
13.	D	33.	A
14.	E	34.	B
15.	B	35.	C
16.	E	36.	E
17.	B	37.	B
18.	B	38.	E
19.	E	39.	E
20.	A	40.	C

COMPREHENSIVE EXAMINATION

After you have completed your review of the Microbiology and Immunology, you might find this quiz useful to judge your competence in this discipline. The examination which follows was used as a final examination for sophomore Medical students. Their mean was 80 percent. A score below 60 would probably suggest that further review in the areas of weakness is indicated.

Match the disease on the left with the vector on the right. An answer may be used more than once or not at all.

Vector

A. Flea
B. Tick
C. Mosquito/Fly
D. Louse
E. Mite

_____ 1. Bubonic plague

_____ 2. Rocky Mountain spotted fever

_____ 3. St. Louis encephalitis

_____ 4. Tularemia

_____ 5. Trachoma

_____ 6. Malaria

_____ 7. Yellow fever

Match the disease on the left with the vaccine on the right. An answer may be used more than once or not at all.

Vaccine

A. Toxoid
B. Live, attenuated organism
C. Killed, attenuated organism
D. Killed, virulent organism
E. Purified capsular carbohydrate

_____ 8. Diphtheria

_____ 9. Pertussis

_____ 10. Tetanus

_____ 11. Mumps

_____ 12. Measles

_____ 13. Rubella

_____ 14. Pneumococcal pneumonia

_____ 15. Influenza

Match the organism on the left with the drug of choice on the right. An answer may be used more than once or not at all.

Antibiotic

A. Penicillin
B. Chloramphenicol
C. Tetracycline
D. Clindamycin
E. Methicillin

_____ 16. Staphylococcus aureus

_____ 17. Streptococcus pyogenes

_____ 18. Rickettsia rickettsii

_____ 19. Chlamydia trachomatis

_____ 20. Bacteroides fragilis

_____ 21. Salmonella typhi

_____ 22. Neisseria meningitidis

_____ 23. Mycoplasma pneumoniae

_____ 24. Clostridium perfringens

Match the antibiotic on the left with the mechanism of action on the right. An answer may be used more than once or not at all.

A. Inhibits transpeptidation
B. Inhibits peptidyl transferase
C. Blocks initiation of protein synthesis
D. Inhibits DNA-dependent RNA polymerase
E. Blocks binding of aminoacyl tRNA

_____ 25. Ampicillin

_____ 26. Streptomycin

_____ 27. Gentamicin

_____ 28. Tetracycline

Match the process of genetic exchange on the left with the associated term on the right. An answer may be used more than once or not at all.

A. Mediated by bacteriophage
B. Requires competent recipient
C. Transposon-mediated
D. Intron-mediated
E. Requires F pilus

_____ 29. Generalized transduction

_____ 30. Specialized transduction

_____ 31. Conjugation

_____ 32. Transformation

Match the disease on the left with the mechanism of immunologic injury on the right. An answer may be used more than once or not at all.

Mechanism of Injury

A. IgE-mediated
B. Cytotoxic antibody
C. Immune complexes
D. Cell-mediated immunity

_____ 33. Erythroblastosis fetalis.

_____ 34. Serum sickness

_____ 35. Rheumatic fever

_____ 36. Poison ivy

_____ 37. Asthma

_____ 38. Anaphylaxis

_____ 39. Post-streptococcal glomerulonephritis

_____ 40. Systemic lupus erythematosus

Match the immunodeficiency disease on the left with the defect on the right. An answer may be used more than once or not at all.

Defect

A. Phagocytic cells
B. B cells
C. T cells
D. Both B and T cells
E. Complement

_____ 41. Severe combined immunodeficiency

_____ 42. DiGeorge Syndrome

_____ 43. Bruton's Disease

_____ 44. Chronic granulomatous disease

_____ 45. Acquired immunodeficiency syndrome

Match the disease on the left with the immune function thought to be of major importance in immunity to the agent on the right. An answer may be used more than once or not at all.

Immunity Mechanism
A. Cell-mediated
B. Antitoxic
C. Opsonic
D. Innate
E. Cytotoxic Ab

_____ 46. Pneumococcal pneumonia

_____ 47. Herpes

_____ 48. Histoplasmosis

_____ 49. Shistosomiasis

_____ 50. Tetanus

Match the microorganism on the left with the therapeutic agent on the right. An answer may be used more than once or not at all.

_____ 51. Streptococcus pneumoniae

_____ 52. Treponema pallidum

_____ 53. Mycoplasma pneumoniae

_____ 54. Chlamydia trachomatis

A. Tetracycline
B. Penicillin
C. Rifampin
D. Streptomycin
E. Chloramphenicol

_____ 55. Candida albicans (thrush)

_____ 56. Sporothrix schenckii

_____ 57. Cryptococcus neoformans

_____ 58. Aspergillus fumigatus

A. Actidione
B. Amphoterocin B
C. Nystatin
D. KI
E. Griseofulvin

Match the virus in the column on the right with the neoplasm on the left. An answer may be used more than once, or not at all.

A. Hepatitis B virus
B. Epstein Barr virus
C. HTLV-III
D. Herpes simplex
E. Molluscum contagiosum

_____ 59. Kaposi Sarcoma

_____ 60. Nasopharyngeal Carcinoma

_____ 61. Hepatoma

_____ 62. Burkitt's Lymphoma

_____ 63. Cervical Carcinoma

Match the microorganism on the left with the therapeutic agent on the right. An answer may be used more than once or not at all.

_____ 64. Varicella

_____ 65. Influenza A

_____ 66. Molluscum contagiosum

_____ 67. Cytomegalovirus

A. Amantadine
B. Acyclovir
C. Methisazone
D. Adenine arabinoside
E. Quanidine

_____ 68. Schistosoma mansoni

_____ 69. Taenia saginata

_____ 70. Ascharis lumbricoides

_____ 71. Plasmodium malariae

A. Chloroquine
B. Praziquantel
C. Primaquine
D. Niclosamide
E. Mebendazole

Match the pathogen on the left with the mode of transmission on the right. An answer may be used more than once or not at all.

_____ 72. Coxsackieviruses

_____ 73. *Streptococcus pyogenes*

_____ 74. Yellow fever virus

_____ 75. *Trypanosoma cruzi*

_____ 76. *Enterobius vermicularis*

_____ 77. *Microsporum audouinii*

_____ 78. Non-A/Non-B Hepatitis

_____ 79. *Neisseria meningitidis*

A. Fecal-oral

B. Respiratory droplet

C. Insect vector

D. Direct contact

E. Injection

Match the antigen in the column on the right with the disease listed in the left hand column. An answer may be used more than once or not at all.

_____ 80. Myasthenia gravis

_____ 81. Systemic lupus erythematosus

_____ 82. Rheumatoid arthritis

A. DNA

B. IgG

C. Acetylcholine receptors

D. Glomerular basement membrane

E. Myelin basic protein

Match the antigen used in serologic diagnosis in the right hand column with the disease listed in the left hand column. An answer may be used more than once or not at all.

_____ 83. Cryptococcal meningitis

_____ 84. Haemophilus meningitis

_____ 85. Poliomyelitis

A. Erythrocytes

B. Viable bacteria

C. Viable viruses

D. Capsular antigens

E. Attenuated protozoa

Match the cell on the right with the disease on the left. An answer may be used more than once or not at all.

_____ 86. Wiscott-Aldrich Syndrome

_____ 87. Allergic rhinitis

_____ 88. Infectious mononucleosis

_____ 89. Staphylococcal pneumonia

_____ 90. Visceral larva migrans

A. Lymphocyte

B. Neutrophil

C. Eosinophil

D. Monocyte

E. Basophil

Match the pathogen on the left with the infectious disease on the right. An answer may be used more than once or not at all.

_____ 91. Haemophilus influenzae

_____ 92. Varicella virus

_____ 93. Escherichia coli

A. Shingles

B. Relapsing fever

C. Epiglottitis

D. Ludwig's angina

E. Cystitis

Match the antibiotic on the left with the mechanism of action on the right. An answer may be used more than once or not at all.

_____ 94. Rifampin

_____ 95. Cephalosporin

_____ 96. Gentamicin

_____ 97. Tetracycline

A. Inhibits protein synthesis

B. Inhibits cell wall formation

C. Inhibits RNA synthesis

D. Inhibits DNA synthesis

E. Inhibits cell division

Match the term on the right with the statement on the left. A term may be used more than once or not at all.

_____ 98. Require competent cells

_____ 99. Inhibited by DNAse

_____100. F pilus required

A. Transformation

B. Specialized transduction

C. Conjugation

D. Generalized transduction

ANSWER SHEET

1.	A,D	31.	E	61.	A	91.	C
2.	B	32.	B	62.	B	92.	A
3.	C	33.	B	63.	D	93.	E
4.	B	34.	C	64.	B,D	94.	C
5.	C	35.	B	65.	A	95.	B
6.	C	36.	D	66.	C	96.	A
7.	C	37.	A	67.	B,D	97.	A
8.	A	38.	A	68.	B	98.	A
9.	D	39.	C	69.	D	99.	A
10.	A	40.	C	70.	E	100.	C
11.	B	41.	D	71.	A		
12.	B	42.	C	72.	A,B		
13.	B	43.	B	73.	B,D		
14.	E	44.	A	74.	C		
15.	C,D	45.	C	75.	C		
16.	E	46.	C	76.	A		
17.	A	47.	A	77.	D		
18.	C	48.	A	78.	E		
19.	C	49.	A,E	79.	B,D		
20.	B,D	50.	B	80.	C		
21.	B	51.	B	81.	A		
22.	A	52.	B	82.	B		
23.	C	53.	A,E	83.	D		
24.	A,B,D	54.	A,E	84.	D		
25.	A	55.	C	85.	C		
26.	C	56.	D	86.	A,D		
27.	C	57.	B	87.	C,E		
28.	E	58.	B	88.	A		
29.	A	59.	C	89.	B		
30.	A	60.	B	90.	C		