

GENERAL BACTERIOLOGY

1. Bacterial cell (morphology, staining reactions, classification of bacteria)

The protoplast is bounded peripherally has a very thin, elastic and semi-permeable cytoplasmic membrane (a conventional phospholipid bilayer). Outside, and closely covering this, lies the rigid, supporting cell wall, which is porous and relatively permeable.

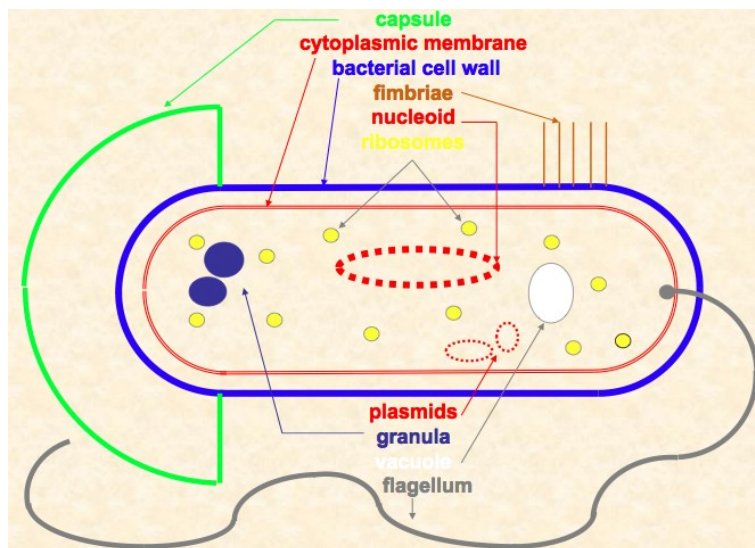
The structures associated with the cell wall and the cytoplasmic membrane (collectively the cell envelope) combine to produce the cell morphology and characteristic patterns of cell arrangement.

Bacterial cells may have two basic shapes: spherical (coccus) or rod-shaped (bacillus); the rod-shaped bacteria show variants that are comma-shaped (vibrio), spiral (spirillum and spirochetes) or filamentous.

The cytoplasm, or main part of the protoplasm, is a predominantly aqueous environment packed with ribosomes and numerous other protein and nucleotide-protein complexes. Some larger structures such as pores or inclusion granules of storage products occur in some species under specific growth conditions.

Outside the cell wall there may be a protective gelatinous covering layer called a capsule.

Some bacteria bear, protruding outwards from the cell wall, one or more kinds of filamentous appendages: flagella, which are organs of locomotion, fimbriae, which appear to be organs of adhesion, and pili, which are involved in the transfer of genetic material. Because they are exposed to contact and interaction with the cells and humoral substances of the body of the host, the surface structures of bacteria are the structures most likely to have special roles in the processes of infection.



Shape: this can be of 3 main types:

round (cocci)

- regular (staphylococci)
- flattened (meningococci)
- lancet shaped (pneumococci)

elongated (rods)

- straight (majority of them are like this; e.g. E.coli)
- short (coccobacilli; e.g. acinetobacters)
- long (fibres) – these are mainly found in OLD cultures
- slender – mycobacterium tuberculosis
- robust – lactobacilli, bacillus
- with split ends - bifidobacteria
- branching - nocardiae, actinomycetes
- curved - campylobacters
- with flat ends – bacillus anthracis
- spindle-shaped - fusobacteria
- club-shaped - corynebacteria
- pleomorphic – haemophili
- spiral – helicobacter, spirillum

Spiral bacteria (spirochetes): these are different to the spiral bacteria mentioned above! They are tightly coiled bacteria.

- Thick: Spirillum
- Uneven: borrelia
- Delicate, regular: Treponema
- Slender with bent ends: Leptospira

STAINING

Two basic methods provide foundations for differential staining and detection of bacteria: the Gram stain and acid-fast stains.

Bacteria are generally studied when fixed and stained. Smears or films of bacterial cultures and clinical specimens are usually fixed by heat, the slide being first thoroughly dried in air and then heated gently in a flame. Vegetative bacteria are thereby killed, attached to the surface of the slide and preserve from undergoing autolytic changes.

During staining, the coloured, positively charged cation of basic dyes such as methylene blue combines with negatively charged groups in the cell protoplasm, specially with the phosphate groups in the abundant nucleic acids. Acid dyes, having coloured anions, do not stain bacteria strongly except at very acid pH values, and thus can be used for “negative staining”.

Negative or background staining is of value as a rapid method for the simple morphological study of bacteria and yeasts.

In the case of bacteria, Gram’s stain has the widest application, distinguishing them as “Gram-positive” or “Gram-negative”, according to whether or not they resist decoloration with acetone, alcohol or aniline oil after staining with a triphenyl methane dye, such as methyl violet, and subsequent treatment with iodine. The Gram-positive bacteria resist decoloration and remain stained a dark purple colour. The Gram-negative bacteria are decolorized, and are then counterstained light pink by the subsequent application of safranin, neutral red or dilute carbol fuschin.

Gram reactivity appears to reflect a fundamental aspect of cell structure and is correlated with many other biological properties.

Gram-positive bacteria are more susceptible than Gram-negative bacteria to the antibacterial actions of penicillin, acids, iodine, basic dyes, detergents and lysozyme, and less susceptible to alkalis, azide, tellurite, proteolytic enzymes, lysis by antibody and complement, and plasmolysis in solutes of high osmotic pressure. The probable mechanism of the Gram stain is attributed to differences in the permeability of the two essential cell wall types. After staining with methyl violet and treatment with iodine, a dye-iodine complex is formed within the cell; this is insoluble in water but moderately soluble and dissociable in the acetone or alcohol use as the decolorizer. Under the action of the decolorizer, the dye and iodine diffuse freely out of the Gram-negative cell, but not from the Gram-positive cell, presumably because the cell wall of the latter is less permeable. Gram-positive bacteria become Gram-negative when their cell wall is ruptured or removed.

		G+	G-
1. Fixation by flame	3 times		
2. Gram stain	20 s		
3. Lugol solution	20 s		
4. Alcohol	máx. 20 s		
5. Aqua fontis	rinse		
6. Safranin	1 min		
7. Aqua fontis	rinse		
8. Drying			

2. Anatomy of the bacterial cell I – contents of cytoplasm, cytoplasmic membrane

The cytoplasm, or main part of the protoplasm, is a predominantly aqueous environment packed with ribosomes and numerous other protein and nucleotide - protein complexes.

Bacterial nucleoid

The genetic information of bacterial cell is mostly contained in a single, long molecule of double-stranded deoxyribonucleic acid (DNA). The cell solves the problem of packaging this enormous macromolecule by condensing and looping it into a supercoiled state.

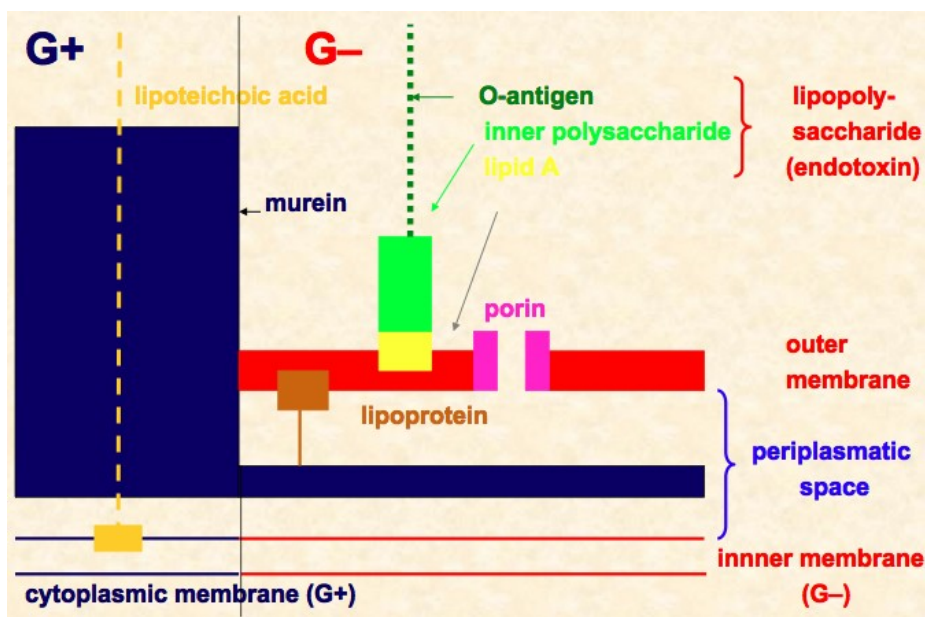
The bacterial nucleoid lies within the cytoplasm. This means that as DNA-dependent RNA polymerase makes RNA, ribosomes may attach and initiate protein synthesis on the still attached (nascent) messenger RNA. Synthesis of mRNA and protein are therefore seen to be directly coupled in bacteria.

Cytoplasmic membrane

The bacterial protoplasm is limited externally by a thin, elastic cytoplasmic membrane which is 5-10nm thick and consists mainly of phospholipids and proteins.

Integral, transmembrane and peripheral or anchored proteins occur in abundance and perform similar functions to those described in eukaryotes (e.g. transport and signal transduction).

Prokaryotic cell membranes are relatively protein-rich, allowing relatively little space for phospholipids.



3. Anatomy of the bacterial cell II – cell wall, capsule, flagella, fimbriae, pili

Cell wall

The cell wall encases the protoplast and lies immediately external to the cytoplasmic membrane. It is 10-25nm thick, strong and relatively rigid, tough with some elasticity, and openly porous, being freely permeable to small molecules. It supports the weak cytoplasmic membrane against the high internal osmotic pressure of the protoplasm and maintains the characteristic shape of the bacterium in its cocci, bacilli, filamentous or spiral form.

Capsule

Many bacteria have been demonstrated to possess a more or less continuous but relatively amorphous layer external to the Gram-negative and Gram-positive envelopes.

This layer is called a capsule. The capsular gel consists largely of water and has only a small content of solids. In most species, the solid material is a complex polysaccharide, though in some species its main constituent is polypeptide.

The capsule has some role in interactions with the external environment. In some cases capsules have been shown to protect against phagocytosis, the lytic action of complement and bacteriophage invasion.

Capsules also appear to have a role in protecting cells against desiccation. The production of extracellular polysaccharides in general provides a matrix within which biofilm formation can take place.

Flagella

Motile bacteria possess filamentous appendages known as flagella, which act as organs of locomotion. The flagellum is a long, thin filament, twisted spirally in an open regular wave form. It originates in the bacterial

protoplasm and the structure projects through the cell envelope. According to the species, there may be one, or up to 20 flagella per cell.

The external portion of a flagellum is essentially a polymer of a single protein, flagellin, while the basal region inserted into the cytoplasmic membrane comprises multiple subunits which anchor and power the organ.

Motility is clearly important to many bacteria and probably serves mainly to place the cell in environments favourable to growth and free from noxious influences.

In some cases possession of flagella is thought to contribute to the pathogenesis of disease.

Fimbriae and pili

Many bacteria possess filamentous appendages termed fimbriae or pili. These terms are often used interchangeably, although the latter was originally reserved for structures involved in genetic exchange between bacteria (sex pili). Fimbriae are far more numerous than flagella and are much shorter and only about half as thick.

In contrast, sex pili are structurally similar to other fimbriae but are longer and confer the ability to attach specifically to other bacteria that lack these appendages. Sex pili initiate the process of conjugation; they also act as receptor sites for certain bacteriophages.

4. Anatomy of the bacterial cell III – bacterial spores

Some bacteria develop a highly resistant resting phase or endospore, whereby the organism can survive in a dormant state through a long period of starvation or other adverse environmental conditions. The process does not involve multiplication: in sporulation, each vegetative cell forms only one spore, and in subsequent germination each spore gives rise to a single vegetative cell.

In the face of sporulation stimuli, classically starvation or transition from growth to stationary phase, a programme of sequential expression of specific genes is triggered. The end result is a morphologically distinct structure, the endospore, within the mother cell.

Spores are much more resistant than the vegetative forms to exposure to disinfectants, drying and heating.

In the dry state, or in moist conditions unfavourable to growth, spores may remain viable for many years. The marked resistance of spores has been attributed to several factors in which they differ from vegetative cells: the impermeability of their cortex and outer coat, their high content of calcium and dipicolinic acid, their low content of water, and their very low metabolic and enzymic activity.

Reactivation of the spore is termed germination and it should be noted that this is not just a reversal of the process by which the spore was formed. Germination of the spore occurs in response to specific stimuli that are generally related to external conditions favourable to growth. It is irreversible and involves rapid degradative changes. The spore successively loses its heat resistance.

In the process of germination, the spore swells, its cortex desintegrates, its coat is broken open and a single vegetative cell emerges.

The initiation of germination is called activation. Activation is distinct from germination and is reversible if germination does not proceed.

Following germination, cell growth leading up to the formation of the first vegetative cell and prior to the first cell division is referred to as outgrowth. The conditions required for successful outgrowth may differ markedly from those that allow germination.

5. Microbial growth, incl. conditions required

Bacteria reproduce by binary fission, which is a form of asexual reproduction and cell division.

- Period I (initiation): the cell grows and proteins that start the next step accumulate inside it.
- Period C (chromosome replication): begins in one spot and diverges out in opposite directions
- Period D (division): a copy of macromolecules is formed. The cytoplasmic membrane inserts between the replicated chromosomes and separates them. The cell wall grows into the cell at a particular spot and forms a septum that ultimately divides the maternal cell into two daughter cells.

Division of cocci can occur in one plane e.g. streptococci or in different planes e.g. staphylococci. Division of rods can occur in the transverse plane e.g. majority of chain rods or in the vertical plane, e.g. corynebacteria, mycobacteria.

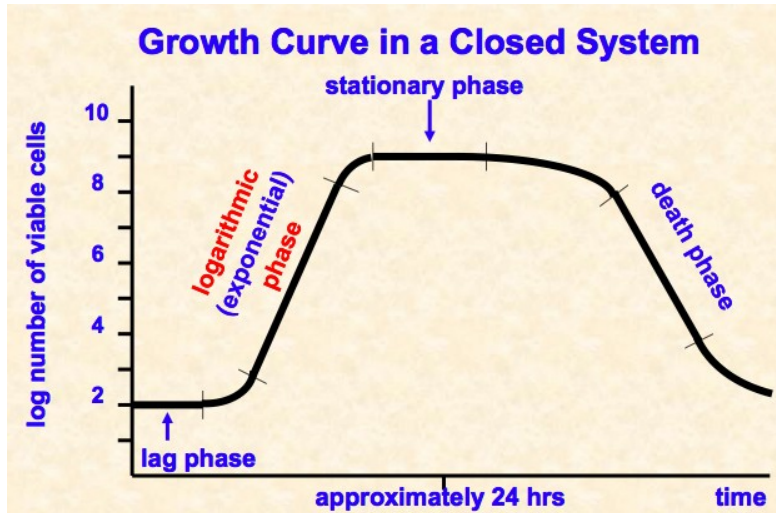
The generation time is the time taken for the number of bacteria to double or duration of the growth cycle. On average it is usually about 30 minutes.

Since the number of bacteria double generation time.

E.g. If the generation time is 30 min, after 24 hours theoretically one cell gives origin to $2^{48} = 2,8 \times 10^{14}$ cells. However the actual amount of cells produced, is approximately 5 orders less (i.e. around 10^9 cells). This

amount of cells can be seen by the naked eye and in a liquid broth it appears cloudy or sedimentation occurs at the bottom or a pellicle is seen at the top. In a solid medium (agar), a bacterial colony is formed. The result 10^9 cells/24 hours applies for stationary cultures, in which nutrients are consumed and metabolites accumulate. The speed of multiplication changes depending on time and the growth of the bacterium can be illustrated by the use of a growth curve.

A growth curve depicts the number of viable cell in the logarithmic scale, depending on the age of culture. There are 4 growth phases, and they gradually change from one to the other:



- Lag - microbes are growing but not dividing. During this phase, bacterial growth cycle, synthesis of RNA, enzymes and other molecules occurs.
- Log (exponential) - cells are dividing at a constant speed. The relation between the number of living cells and time is exponential. There are more than enough nutrients to allow the cells to grow.
- Stationary - the number of cells is stable - the number of cells being produced - number of cells dying. Here the growth rate slows down due to the lack of nutrients and the accumulation of metabolites.
- Death - number of cells dying > number of cells being produced. Here the bacteria have run out of nutrients and die.

Growth conditions:

- 1) Temperature: most bacteria grow optimally at human body temperatures (37°C). e.g. E.coli, which is part of the normal human intestinal microflora. Some can survive at higher/lower temperatures than the human body temperature.
- 2) pH: optimal conditions are between 6,7-7,5. However some other bacteria, like vibrio cholera can survive in pH conditions as high as 9,0 and gastric helicobacter can survive in acidic pH.
- 3) NaCl concentration: should be about 0.9% (physiologic saline). Staphylococci can multiply on sweaty skin, where the concentrations are about 10% (used on BA -> selective media).
- 4) Nutrients: need to be in the correct balance of carbon, nitrogen, hydrogen, sulphur, iron, etc, for synthesis of specific bacterial compounds. Some bacteria also require "growth factors" i.e. organic compounds they are unable to synthesise themselves.
- 5) Osmotic pressure: bacteria are about 80-90% water; they require moisture to grow because they obtain most of their nutrients from their aqueous environment.
- 6) Anaerobes/aerobes: oxygen may or may not be needed, depending on the species of bacteria and the type of metabolism used to extract energy from food. In all cases, the initial breakdown of glucose to pyruvic acid occurs during glycolysis, which produces a net gain of two molecules of ATP

6. Microbial metabolism

This is the way in which a microbe obtains energy and nutrients it requires; to survive and reproduce. The processes can be anabolic (synthesis of compounds and the consumption of energy) or catabolic (break down of substrates to gain energy). Although some bacteria are able to obtain their resources for growth in many ways. The basic details of glycolysis, the tricarboxylic acid cycle, oxidative phosphorylation, ATP biosynthesis and amino acid metabolism are constant.

Human pathogenic bacteria are always chemosynthetic, organotrophic bacteria (or chemoorganotrophs).

Catabolic reactions:

- Digestion - bacterial exoenzymes split the nutrient substrates into smaller molecules outside the cell.
- Uptake - nutrients are taken up by passive diffusion, or more usually active transport through the membrane.
- Preparation for oxidation - phosphorylation, etc
- Oxidation - removal of electrons and H^+ ions. The H_2 atoms are then transferred to the hydrogen acceptor.

There are 3 types of catabolism:

- 1) Fermentation - breakdown of nutrients without the need for oxygen. Only small amounts of energy are produced. Products are lactate, ethanol, etc.
- 2) Aerobic respiration - uses oxygen and a small amount of nutrient provides a large amount of energy. Products are CO_2 and H_2O .
- 3) Anaerobic respiration - another electron acceptor

The type of catabolism is related to oxygen consumption:

- Facultative anaerobes: they oxidise nutrients by respiration and fermentation. They can grow in all conditions.
- Obligate (strict) aerobes: they can only reproduce in the presence of oxygen.
- Obligate (strict) anaerobes: they die in the presence of oxygen. Their vital enzymes are inhibited by oxygen.
- Micro-aerophilic bacteria - grow in conditions with traces of oxygen
- Aerotolerant anaerobes - they don't utilise oxygen for growth but can survive in its presence.
- Capnophilic bacteria - they need higher amounts of CO_2 .

7. Media for microbial growth - examples

The objectives of early medium design were to grow pathogenic bacteria, separate them from the other organisms present in samples and, ultimately, differentiate their phenotypic properties so that they could be identified.

Microbes can be grown on a solid medium or in a liquid medium, usually called a liquid broth. There are 2 types of liquid media.

- Multiplying media: the most common and universal one, e.g. broth for aerobic culture and VL broth (Viande-Levure)
- Selectively multiplying media: only some bacteria can grow in this, e.g. selenite broth for salmonella.

Liquid broths are used when there is only a small amount of microbes in the specimen and when they multiply quickly.

SOLID MEDIA: there are several types of this.

- Selective solid media: similar to the liquid broth, only certain bacteria can grow in this, from a mixture of several bacteria, e.g. blood agar with 10% NaCl is used for staphylococci.
- Diagnostic media: they don't selectively grow any bacteria, since they don't suppress the growth of any microbe. Their contents allow microbes to grow and they can be differentiated according to some properties, e.g. blood agar to observe haemolytic properties.

Special kinds of diagnostic media are chromogenic and fluorogenic media.

- Chromogenic media: they contain a dye with bound specific substrate. The dye loses colour and is no longer a dye but a chromogen. Bacteria are able to break down this specific substrate and change the specific substrate to the original dye. The media can contain more chromogens, depending on the number of species you want to differentiate. It is used for yeast cultures.
- Fluorogenic media: works in the same way but uses a fluorescent dye instead.

BLOOD AGAR: on this you can see haemolysis of RBCs, whether it is absent, partial or total. It is also possible to see viridation (goes green). Total haemolysis shows up due to β -hemolytic activity, whereas partial haemolysis is due to α -hemolysis activity and it appears green. γ -hemolysis refers to the lack of haemolytic activity.

BA is an enriched medium, although it can also be classified as a diagnostic medium, therefore it can be classified as both.

ENDO AGAR: it is a selective and diagnostic medium, used to grow some G- bacteria. The growing bacteria can be divided into lactose positive or lactose negative. Lactose positive bacteria are usually milder pathogens than lactose negative ones.

OTHER SELECTIVE MEDIA: McConkey media, XLD and MAL - these ones are selective and diagnostic media used to grow Salmonella.

HAJNA MEDIUM: is grown in test tubes, even though it is a solid medium. This is because it is used for biochemical testing and to see the difference between the lower part (no oxygen access) and the upper part (surface of the medium).

Name	Class	Colour	Type	For
Broth	Liquid media	yellowish	multiplying	aerobes
VL-broth		darker		anaerobes
Selenite broth		pinkish	selective multiplying	Salmonella
Sabouraud Agar	solid media in test tubes!	white	selective (only with antibiotics)	Fungi
Lowenstein-Jensen		green	enriched	TBC
Blood Agar	Solid media on Petri Dish	red	enriched + diagnostic	majority of bacteria
Endo Agar		pink	selective diagnostic	mostly enterobacteria
Müller-Hinton (MH) agar		white	special	antibiotic susceptibility
NaCl		brown	selective	staphylococci
VL-Agar		red	enriched + diagnostic	anaerobes
XLD (and MAL)		orange	selective diagnostic	Salmonella
Chocolate Agar		brown	enriched	Haemophili, Niesseriae
Levinthal Agar		yellowish	enriched	Haemophili
Slanetz-Bartley		pink	selective diagnostic	Enterococci

8. Sterilization

Sterilization means the foreign of an article from all living organisms, including viruses, bacteria and their spores, and fungi and their spores. In practise, all processes of sterilization have a finite probability of failure. An article may be regarded as sterile if it can be demonstrated that there is a probability of less than 1 in a million of there being viable microorganism on it.

Uses:

Sterilization is required for instruments and materials used in procedures that involve penetration into normally sterile parts of the body, e.g. in surgical operations, intravenous infusions, hypodermic injections and diagnostic aspirations. It is also required for media, reagents and equipment used in laboratory practice.

METHODS:

- Heat - the only method of sterilization that is both reliable and widely applicable is by heating under carefully controlled conditions at temperatures above 100°C to ensure that bacterial spores are killed.
- Ionizing irradiation - both β (electrons) irradiation and γ (photons) irradiation are employed industrially for the sterilization of single-use disposable items such as needles and syringes, latex catheters and surgical gloves, and in the food industry to reduce spoilage and remove pathogens.
- Filtration - filters are used to remove bacteria and all larger microorganisms from liquids that are liable to be spoiled by heating, e.g. blood serum.
- Sterilant gases - ethylene oxide is used mainly by industry for the sterilization of plastics and other thermolabile materials that cannot withstand heating. Formaldehyde in combination with subatmospheric steam is more commonly used in hospitals for reprocessing thermolabile equipment.
- Sterilant liquids - use of liquids such as glutaraldehyde is generally the least effective and the most unreliable method, only to be applied when no other sterilization method is available.

The method to use for sterilization depends on the resistance of the materials to various temperatures, humidity, chemicals, etc. Also, the temperature and time has to be sufficient enough to kill the microbes.

To check how effect the sterilization was, indicators that change colour can be used at certain temperatures. This is the chemical method. There is a biological one as well, which consists of using resistant strains of Bacillus genus. Since they are resistant, their survival is assessed at the end of the sterilization cycle.

9. Disinfection

Disinfection, the freeing of an article from some or all of its burden of contaminating microorganisms, is a relative term embracing a wide range of efficacy against particular viruses, vegetative bacteria and fungi, but not usually including bacterial spores.

Uses:

Disinfection is applied in circumstances in which sterility is unnecessary or sterilizing procedures are impracticable.

METHODS:

Disinfection is achieved by means of processes similar to, although less severe than, those used for sterilization.

- Heat - various methods are available using steam or water; some incorporate a cleaning stage within an automatic controlled process.
- Ultraviolet radiation - this has limited application for the disinfection of surfaces and some piped-water supplies, but lacks penetrative power for more widespread application.
- Gases - formaldehyde is used as a fumigant in laboratory environments.
- Filtration - are supplied to operating theatres and other critical environments is filtered to removed potentially hazardous microorganisms.
- Chemicals - various chemicals with antimicrobial properties are used as disinfectants. They are all liable to be inactivated by excessive dilution and contact with organic materials. Nevertheless, they may provide a convenient method for environmental disinfections and other specific applications.
- The effectivity of various disinfectants to various organisms, can be found in booklets where they are divided into different groups, which are alphabetically arranged, e.g. A = effective to yeast and bacteria, B = effective against viruses, C = bacterial endospores, T = TB mycobacterium, M = atypical mycobacterium and V = filamentous fungi

10. Mechanisms of antimicrobial drug action

Bactericidal: kills all the cells. It is an irreversible process.

Bacteriostatic: stops bacterial cell growth. It is a reversible process. It is used mainly in people with a functional/normal immune system.

Ex: chloramphenicol inhibits growth of E.coli (bacteriostatic) and kills haemophilus influenzae (bactericidal).

Types:

- Antiparasital
- Antimycotics
- Antivirotic
- Antituberculotics
- Antibiotics
- Antiseptics

Primary resistance bacteria have innate resistance, all strains of a given species are resistant.

Secondary resistance: some bacteria have acquired resistance - non-susceptible mutants arise and due to selection pressure - their number increases.

Mechanism of resistance:

- 1) Block entrance of antibiotics into the cell: by alteration of target site e.g.: alteration of PBP and MRSA.
- 2) Active efflux of an antibiotic from a cell
- 3) A false receptor is offered to the antibiotic
- 4) Alteration of metabolic pathway: e.g. enzymes that modify/destroy the antibiotic -> β -lactamic antibiotic.

Sites of action and mechanisms of antimicrobial drugs.

- Prevention of cell wall synthesis (β -lactams)
- Prevention of protein synthesis (microlides, tetracyclines)
- Alteration of metabolic pathways (sulfonamid)
- Destruction of nucleic acid (quinolones)
- Destruction of cytoplasmic membrane

11. Inhibitors of bacterial cell wall synthesis

Since most bacteria possess a rigid cell wall that is lacking in mammalian cells, this structure is a prime target for agents that exhibit selective toxicity, the ability to inhibit or destroy the microbe without harming the host. However, the bacterial cell wall can also prevent access of agents that would otherwise be effective. Inhibitors of bacterial cell wall synthesis act on the formation of the peptidoglycan layer.

β -lactam agents

Penicillins, cephalosporins and other compounds that feature a β -lactam ring in their structure fall into this group. All these compounds bind to proteins situated at the cell wall - cell membrane interface. These penicillin-binding proteins are involved in cell wall construction.

Opening of the β -lactam ring by hydrolytic enzymes, collectively called β -lactamases, abolishes antibacterial activity.

Penicillins: Benzylpenicillins (penicillin G) exhibits unrivalled activity against staphylococci, streptococci, neisseriae, spirochetes and certain other organisms.

Benzylpenicillin revolutionized the treatment of infection caused by some of the most virulent bacterial pathogens, but it also suffers from several shortcomings.

- breakdown by gastric acidity when given orally
- very rapid excretion by the kidney
- susceptibility to penicillinase (β -lactamase)
- a restricted spectrum of activity

Methicillin-resistant *Staphylococcus aureus* (MRSA) and other staphylococci that owe their resistance to alterations in the target penicillin-binding proteins are resistant to all penicillins and to all other β -lactam antibiotics.

Cephalosporins

Cephalosporins are generally stable to staphylococcal penicillase but they lack activity against enterococci. They exhibit a broader spectrum than most penicillins and are less prone to cause hypersensitivity reactions.

Other β -lactam agents

Monobactams - are monocyclic compounds with a spectrum that is restricted to aerobic Gram-negative bacteria.

Carbapenems - have an unusually broad spectrum of activity, embracing most Gram-positive and Gram-negative aerobic and anaerobic bacteria.

Oxo-cephems - are broad-spectrum β -lactamase - stable compounds

The clavam, clavulanic acid, exhibits poor antibacterial activity, but has proved useful as a β -lactamase inhibitor when used in combination with β -lactamase - susceptible compounds.

The sulphones also act as β -lactamase inhibitors and are marketed combined with ampicillin.

Glycopeptides

Vancomycin and teicoplanin are large molecules that are unable to penetrate the outer membrane of Gram-negative bacteria, and the spectrum is consequently restricted to Gram-positive organisms. Their chief importance resides in their action against Gram-positive cocci with multiple resistance to other drugs.

12. Inhibitors of bacterial protein synthesis

Bacterial ribosomes are sufficiently different from those mammalian cells to allow selective inhibition of protein synthesis.

Tetracyclines

These are broad-spectrum agents with important activity against chlamydiae, rickettsiae, mycoplasmas and, surprisingly, malaria parasites, as well as most conventional Gram-positive and Gram-negative bacteria. They prevent binding of amino-acyl transfer RNA (tRNA) to the ribosome and inhibit, but do not kill, susceptible bacteria.

Chloramphenicol

This compound also possesses a very broad antibacterial spectrum. They act by blocking the growth of the peptide chain. Use of chloramphenicol has been limited to typhoid fever, meningitis and a few other clinical indications because of the occurrence of a rare but fatal side-effect, aplastic anaemia.

Aminoglycosides

Streptomycin is predominantly active against enterobacteria and *M. tuberculosis*. Like other members of the aminoglycoside family it has no useful activity against streptococci, anaerobes or intracellular bacteria. The group also has a common tendency to damage the eighth cranial nerve (ototoxicity) and the kidney

(nephrotoxicity). They inhibit formation of the ribosomal initiation complex and also cause misreading of messenger RNA (mRNA).

Such compounds have been widely used, with which they interact synergically.

Macrolides

They act by interfering with the translocation of mRNA on the bacterial ribosome. They are mainly used as antistaphylococcal and antistreptococcal agents, though some have wider applications. They have no useful activity against enteric Gram-negative bacilli.

Lincosamides

Its spectrum includes staphylococci, streptococci and most anaerobic bacteria against which clindamycin has been tempered by an association with the occasional development of severe diarrhoea, which sometimes progresses to a life-threatening pseudo-membranous colitis.

Lincosamides bind to the 50S ribosomal subunit at a site closely related to that at which macrolides act.

Fusidic acid

It blocks factor G, which is involved in peptide elongation. Fusidic acid has an unusual spectrum of activity that includes corynebacteria, nocardia and *M. tuberculosis*, but the antibiotic is usually regarded simply as an antistaphylococcal agent. It penetrates well into bone and has been widely used (generally in combination with a β -lactam antibiotic to prevent the selection of resistant variants) in the treatment of staphylococcal osteomyelitis.

Oxazolidinones

These compounds prevent the formation of the ribosomal initiation complex and are narrow-spectrum anti-Gram-positive agents.

Streptogramins

The combination exhibits bactericidal activity against most Gram-positive cocci, but has poor activity against *Enterococcus faecalis*.

Mupirocin

It blocks the incorporation of isoleucine into proteins. Its useful activity is restricted to staphylococci and streptococci.

13. Inhibitors of bacterial nucleic acid synthesis

A number of important antibacterial agents act directly or indirectly on DNA or RNA synthesis.

Sulphonamides and diaminopyrimidines

These agents affect DNA synthesis because of their role in folic acid metabolism. Sulphonamides are analogues of para-aminobenzoic acid, and prevent the condensation of this compound with dihydropteridine during the formation of folic acid. Diaminopyrimidines prevent the reduction of dihydrofolate to tetrahydrofolate.

Sulphonamides are broad-spectrum antibacterial agents, but resistance is common and the group also suffers from problems of toxicity.

Quinolones

These drugs act on the α subunits of DNA gyrase. Their use is virtually restricted to urinary tract infection, although they have also been used in enteric infections and, in the case of acroloxacin, in gonorrhoea.

Quinolones are quite well absorbed when given orally and are widely distributed throughout the body.

Nitroimidazoles

Azole derivatives feature prominently among antifungal, antiprotozoal and anthelmintic agents.

The representative of the group most commonly used clinically is metronidazole.

Nitrofurans

The most familiar nitrofuran derivative is nitrofurantoin, an agent used exclusively in urinary tract infection.

Novobiocin

This compound acts on the β subunit of DNA gyrase. It was once widely used as a reserve antistaphylococcal agent, but is no longer favoured because of problems of resistance and toxicity.

Rifamycins

This group of antibiotics is characterized by excellent activity against mycobacteria, although other bacteria are also susceptible; staphylococci in particular are exquisitely sensitive. These compounds act by inhibiting transcription of RNA from DNA. Rifampicin, the best known member of the group, is used in tuberculosis and leprosy.

14. The strategy of antimicrobial chemotherapy

Antimicrobial chemotherapy is the use of chemical agents for antimicrobial action, which work by either killing it (bactericidal) or stopping its growth (bacteriostatic). The strategy of the antimicrobial agents is to have a target site exclusively present on the microbe and not on the host cell -> SELECTIVE TOXICITY

Selective toxicity with desired properties of a new antimicrobial agent:

- Selectivity for microbes rather than mammalian cells
- Bactericidal activity
- Slow emergence of resistance
- Narrow spectrum of activity
- Non-toxic side effects to the host
- Long plasma half-life (so, low dosage is possible)
- Good body distribution -> to reach harder places, i.e. adipose tissue
- Decrease in plasma protein-binding
- No interference with other drugs
- Easy administration

The antimicrobial agents target cell wall, protein, and nucleic acid synthesis; metabolic pathways, and cell membrane functions.

Triangle relationship
Antimicrobial agents

Human host

Microorganism

(Interfering with one side of the triangle will affect the other parts).

15. Bacterial genetics

Genetic organization and regulation of the bacterial cell

All properties of a bacterial cell are determined ultimately by the genetic information contained within the cell genome. This information is normally encoded by the DNA of the cell.

Most of the bacterial cell is arranged in the form of a single circular double-stranded chromosomes.

The DNA is not associated with protein or histone molecules as it is in eukaryotic cell chromosomes.

In addition to the single main chromosome, bacterial cells may also carry one or more small circular extrachromosomal elements termed plasmids. Although dispensable, they often carry supplementary genetic information coding for beneficial properties (e.g. resistance to antibiotics) that enable the host cell to survive under a particular set of environmental conditions.

Processes leading to protein synthesis

The DNA acts as a template for the transcription of RNA by RNA polymerase for subsequent protein production within the cell.

Gene regulation

During normal bacterial life, some polypeptides will be required only at particular stages, while others will be needed only when the cell is provided with a new or unusual growth substrate, or is confronted with a new challenge (e.g. antibiotic).

Protein production is an energy-intensive process, and therefore the expression of many genes is controlled actively within the cell to prevent wasteful energy consumption.

In prokaryotic bacteria the process of gene expression is regulated mainly at the transcriptional level.

This is achieved by means of regularly elements that either inhibit or enhance the rate of RNA chain initiation and termination for a particular gene. Related genes involved in a common regulatory system are often clustered on the bacterial chromosome. Such functional clusters are known as operons.

For transcription to occur as the first stage in protein synthesis, RNA polymerase has to attach to DNA at a specific promoter region. This process can be switched off by the attachment of a repressor molecule to a specific region of the DNA. Known as operator.

The repressor is often an allosteric molecule with two active sites. One recognizes the operator region so that the repressor can bind to it to prevent transcription. The other recognizes an inducer molecule. When the inducer is present it binds to the repressor and alters simultaneously the binding specificity at the other side, so that the repressor no longer binds to the operator and transcription can resume.

MUTATION

Occasional rare inaccuracies produce a slightly altered nucleotide sequence in one of the progeny cells. Such a mutation is heritable and will be passed on stably to subsequent generations.

In a small proportion of cases an enzyme with altered specificity for substrates, inhibitors or regulatory molecules may be produced. This is the kind of mutation that is most likely to be of evolutionary value to an organism.

Other mutations may alter a gene so that a non-functional protein is formed; if this protein is essential to the cell then the mutation will be lethal.

- Phenotypic variation

Phenotypic variation occurs when the expression of genes is changed in response to the environment. This is reversible, being dependent on environmental conditions and altering when these change.

- Types of mutation

Mutations can be divided conveniently into multisite mutations, involving extensive chromosomal rearrangements such as inversions, duplications and deletions, and point mutations, which are defined as only affecting one, or very few, nucleotides. The structure of DNA is such that point mutations can be divided into one of 3 basic types:

- the substitution of one nucleotide for another
- the deletion of one or more nucleotides
- the insertion of one or more nucleotides

GENE TRANSFER

A change in the genome of a bacterial cell may be caused either by a mutation in the DNA of the cell or result from the acquisition of additional DNA from an external source. DNA may be transferred between bacteria by 3 mechanisms:

- transformation
- conjugation
- transduction

- Transformation

Bacteria in some genera have been shown to be capable of taking up DNA either extracted artificially or released by lysis from cells of another strain.

Once a piece of DNA has entered the cell by transformation, it has to become incorporated into the existing chromosome of the cell by a process of recombination in order to survive.

- Conjugation

Conjugation is a process in which one cell, the donor or male cell, makes contact with another, the recipient or female cell, and DNA is transferred directly from the donor into the recipient. Certain types of plasmids carry the genetic information necessary for conjugation to occur. Only cells that contain such a plasmid can act as donors; those lacking a corresponding plasmid act as recipients.

Plasmids capable of mediating conjugation carry genes coding for a pilus, on the surface of the donor cell. The tip of the pilus attaches to the surface of a recipient cell and holds the two cells together so that DNA can then pass into the recipient cell.

The DNA is replicated during transfer so that each cell receives a copy. As donor ability is dependent upon having a copy of the plasmid, the recipient strain becomes converted into a donor, able to conjugate with further recipients and convert them in turn. In this way a plasmid may spread rapidly through a whole population of recipient cells, this process is sometimes described as infectious spread of a plasmid.

- Transduction

The third known mechanism of gene transfer in bacteria involves the transfer of DNA between cells by bacteriophages.

THE GENETIC BASIS OF ANTIBIOTIC RESISTANCE

With regard to antibiotic resistance it is important to distinguish between intrinsic and acquired resistance. Intrinsic resistance is dependent upon the natural insusceptibility of an organism.

- Intrinsic resistance

The most obvious determinant of bacterial response to an antibiotic is the presence or absence of the target for the action of the drug.

- In some cases the loss of sensitivity may be slight, but often organisms become resistant to clinically achievable concentrations of a drug. Once resistance has appeared, the continuing presence of an antibiotic

exerts a selective pressure in favour of the resistant organisms. Three main factors affect the frequency of acquired resistance:

- the amount of antibiotic which is being used
- the frequency with which bacteria can undergo spontaneous mutations to resistance.
- the prevalence of plasmids able to transfer resistance from one bacterium to another.

Chromosomal mutations

Random spontaneous mutations occur continuously at a low frequency in all bacterial populations, and some mutations may confer resistance to a particular antibiotic.

With single large-step mutations the drug target is altered by mutation so that it is totally unable to bind a drug, although it can still carry out its normal biological functions sufficiently well to permit the continued survival of the cell.

Once a slightly resistant organism has been produced, additional mutational events - each conferring an additional small degree of resistance - can eventually lead to the production of organisms that are highly resistant that is called the multistep pattern resistance.

16. Pathogenicity and virulence

Pathogenicity: requires the attributes of transmissibility or communicability from one host or reservoir to a fresh host, survival in the new host, infectivity or the ability to breach the new host's defences, and virulence, a variable that is multifactorial and denotes the capacity of a pathogen to harm the host. Virulence is a sense of a parasite-host relationship in which the capacity of the organism to cause disease is considered in relation to the resistance of the host.

Virulence determination: both opportunistic and primary pathogens possess virulence determinants or aggressins that facilitate pathogenesis. Possession of a single virulence determinant is rarely sufficient to allow the initiation and production of pathology. Many bacteria possess several virulence determinants, all of which play some part of various stages of the disease process.

!!! Different strains or species of bacteria may produce different types of fimbriae which can be identified on the basis of antigenic composition, morphology and receptor specificity.

Adhesion induces structural and functional changes in mucosal cells -> contributes to disease. Cell invasion confers the ability to avoid humoral host defence mechanisms and potentially provides a niche rich in nutrients and devoid of competition from other bacteria.

Other kind of invasion is penetration through or between epithelial cells.

Uptake into host cells:

- bacteria inside membrane-bound vesicle. Haemolysin/listeriolysin destroy the membrane -> bacteria is free in cell's cytoplasm and inhibits the host protein synthesis
- bacteria inside membrane-bound vesicle. Several vesicles coalesce to form large intracellular vacuoles.

Avoidance of host defence mechanisms: Lysozyme is active primarily against G⁺ bacteria but potentiates the activity of complement against G⁺ organisms. Transferrin and lactoferrin chelate iron in body fluids, and reduce the amount of free iron to levels that necessary for bacterial growth.

CAPSULES:

1. the hydrophobic nature of the capsule may hinder uptake by phagocytes;
2. capsules prevent efficient opsonization
3. it makes more immunogenic surface components and reduce interactions with both complement and antibody

IM protein:

binds fibrinogen and fibrin and covers the staph's surface.

ANTIGENIC VARIATION: variation in surface antigen composition during the course of infection provides a mechanism of avoidance of specific immune responses. Another interesting mechanism of antigen variation is the genetic rearrangements demonstrated in the fimbriae.

IRON ACQUISITION: Some staphylococci produce extracellular called siderophores and receptors for transferrin and lactoferrin.

Infectious process can be divided into several stages:

1. entry into host, evasion host primary defenses
2. adhesions of microorganism to host cells
3. propagation of the organism
4. damage of host cells by bacterial toxins or inflammatory response of the host.

5. evasion of host secondary defenses

Virulence: how many organisms are required to cause disease in 50% of those exposed to the pathogen.

Virulence factor: characteristic of bacteria that enhances its pathogenicity.

1. entry into host: microorganism can enter host by the respiratory tract, GIT, urogenital tract or skin that has been burned, cut or punctured. Once entered the pathogen has to overcome host defenses like phagocytosis, acidic environment of stomach and urogenital tract, hydrolytic + proteolytic enzymes formed in saliva, stomach and small intestine. Bacteria with outer polysaccharide capsule - more chance of surviving
2. Adherence to host cell: can either be due to pili (groups A strep, Neisseria) adhesion molecules (interactions between specific receptors on the mammalian cell membrane - usually carbohydrates, and ligands - usually proteins, on the bacterial surface) or particularly hydrophobic cell walls, surface charge. Adherence enhances virulence by preventing bacteria from being carried away by peristalsis, mucus, saliva, urine... Flagella can act as adhesin.
3. Invasiveness: invasiveness is facilitated by several enzymes including collagenase, hyaluronidase... - liquefies tissue and facilitates invasion after - inflammation. Toxins, other aggressins and induction of intracellular signalling pathways mediated tissue damage at local or distant sites.
4. Bacterial toxins: exotoxins: proteins secreted by Gram+ and Gram- bacteria.
endotoxins: lipopolysaccharides and are integral part of Gram- cells.

Endotoxins cause septic shock (fever, shock, hypotension, thrombosis)

Primary pathogens: cause disease even in healthy people. Different strains of any bacterial species can vary in their genetic make up and virulence. Conversely, people vary in their genetic make up and susceptibility to invading bacteria.

Opportunistic pathogen: cause disease under certain conditions (decreased immunity of individuals or when it reaches other places in body) - normal members of flora. Rarely cause disease in individuals with intact immunological and anatomical defenses. Only when such defenses are impaired or compromised are these bacteria able to cause disease. Also, normal human flora, when introduced into anatomical sites in which they are not normally found may allow their localized multiplication and subsequent development of disease.

17. Colonization and Invasion

Colonization means the colonization of a nonpathogenic micro (or by a pathogen which does not cause pathological symptoms at that area) on a bodily surface. It is the establishment of a stable population of bacteria on the host.

Invasion is when the microbe enters the host, and it is usually through the mucosae. It is also commonly preceded by colonization (overcoming the possibility of commensalism). For successful entry of the microbe it needs to adhere to the epithelium by means of adherence factors, and penetrate through the epithelium by means of penetration factors.

Adherence factors:

- 1) Fimbriae (pili) – their end reacts with a receptor on the epithelial surface.
- 2) Nonfimbrial adhesions – hemagglutinins of yersinae, bordetellae, F protein of streptococcus pyogenes.
- 3) They can envelope projections (hemagglutinin) of influenza virus. Glycoprotein gp120 of HIV is also an adherence factor.
- 4) Parasites – suck themselves to the mucosae.
- 5) Micromycetes – glucans and mannans of yeasts and keratophilia of dermatophytes (skin moulds).

Penetration factors:

- Direct penetration:
 - Small cracks in skin
 - Small cracks in mucosa
 - Animal bite
 - Arthropod bite
 - Enzymes
- Forced penetration:
 - Changing cellular framework (invasiveness)
 - Ruffling (trouble) of epithelial surface (e.g. Salmonella)

- Unknown mechanisms.

Multiplication:

- Intracellular multiplication – better because of good nutrient supply, and defense against host immunity. Examples of intracellular parasites are mycobacteria, rickettsiae, chlamydiae, uesteriae, salmonellae.
- Extracellular multiplication – stopped by antibacterial substances (e.g. complement, lysozyme, antibodies) but mostly because of shortage of free iron (lactoferrin, transferrin) and also high temperature.

Spreading of the microbe in the body:

- Localized infections (common cold, salmonellosis, gonorrhoea).
- Systemic infections (influenza, meningitis).
- Generalized infections (morbili, typhoid fever, and even localized and systemic infections).
- Way of spreading:
 - lymph
 - blood
 - per continuitatem along nerves

Invasion by transmission:

- The way of transmission – exit point of the body, and entry point of the host.
- Microbe tenacity – degree of resistance to the external environment. E.g. Clostridium tetani, Giardia lamblia, and Helminthes eggs (Taenia saginata) are all spore forming, so, that they can survive in the external environment.
- Minimum infectious dose – amount of microbes required to start infection. An immune person has a high infectious dose Coxiella burnetii, which causes Q fever, has an extremely low infectious dose.
- Behavior of the host - immune defense reflexes (cough, sneezing, diarrhea).
- Way of elimination - every biological substance is infectious.
- Amount of eliminated substance
- Portal of entry - better penetration through mucosa than skin. Some requires direct transmission (sexual contact, e.g. streptococci, treponemae), biological vector (tick, mosquitos -> arboviruses, borreliae), and transmission by water (leptospirae, shigellae).

18. Avoidance of host defense mechanism

They can overcome innate immunity:

- Antigenic variation
- Resisting complement (seroresistant) - “O” side-chains in their LPS
 - inhibiting its activation
 - protecting their own surface (capsules, M protein)
- Resisting phagocytosis
 - avoiding being engulfed (inhibitors of chemotaxis - leukocytins, lecithinase), capsule
 - surviving inside phagocyte (blockage of phagolysosome; resistant to O₂ species - by production of catalase)
- Interfering with cytokine function

They can overcome acquired immunity

- Attempts to avoid antibodies or immune lymphocytes
 - acquired reproduction
 - attempts to deceive immune system
 1. by hiding (in neural ganglions; intracellular membranes; infectious focuses in privileged sites)
 2. changing ones own antigens (antigen mimicry, antigen camouflage, antigen variability)
 3. induce tolerance (CNV, Rubella...)

- Attempts to suppress immune reaction
 - Immunoglobulin A proteases

Host defences: Bacteria are surrounded by a cytoplasmic membrane and a peptidoglycan cell wall. Associated with these basic structures there can be a variety of other components such as proteins, capsules, lipopolysaccharides or teichoic acids. There are also structures involved in motility or adherence to the cells of the host. These are some of the components to which the immune system directs its response. Specific antibodies can bind to flagella or fimbriae, affecting their ability to function properly and can inactivate various bacterial enzymes and toxins.

19. Bacterial Toxins and aggressins

Endotoxins: G- lipopolysaccharide, also called LPS is a component of the outer membrane of G- bacteria, and is released from the bacterial surface via outer membrane vesicles. LPS is anchored into the bacterial outer membrane through a unique molecule termed lipid A.

Endotoxin is a potent activator of macrophages, resulting in the induction of a range of cytokines which are involved in the regulation of immune and inflammatory responses.

Exotoxins: are diffusible proteins secreted into the external medium by the pathogen. Most pathogens secrete various proteins molecules that facilitate adhesion to, or invasion, of, the host. Many others cause damage to host cells.

Exotoxins vary in their molecular structure, biological function, mechanism of secretion and immunological properties.

- type I toxins bind surface receptors and stimulate transmembrane signals
- type II toxins act directly on membranes, forming pores or disrupting lipid bilayers
- type III toxins translocate an active enzymatic component into the cell which modifies an intracellular target molecule.
- spreading factors (DNase, hyaluronidase, collagenase)
- cytolysins (lecithinase, hemolysins)
- inhibitors of proteosynthesins (diphtheria toxin)
- neurotoxins (botulinism, tetanus toxin)
- superantigens (staphylococcal enterotoxin + exfoliatin + strep. pyogenes toxin)

Aggressins

Any substance produced in the body by a pathogenic bacteria that enhances virulence of bacteria by paralyzing host defence mechanism (e.g. RBC, ureases, metalloprotease, mucinases, phospholipases, hyaluronidases).

20. Antigens including antigen recognition and bacterial antigens

Antigens are substances that are recognized by the immune system as foreigner and they trigger an immune response (they need to have a high molecular weight and be chemically complex).

In innate immunity antigens are attached non specifically by macrophages and killed by phagocytosis.

In acquired immunity, in cell mediated one you have Antigen presenting cell (APC)

APC - it includes macrophages, B cells and dendritic cells. These cells are equipped with specialized cell membrane proteins called Major Histocompatibility Complex (MHC).

APCs first phagocytize and partially degrade foreign molecules then display a frequent peptide antigen that it is bound by MHC and triggers cytotoxic T cell response (presence of MHC reassures T cells that the antigen is non self).

MHC in humans = HLA - human leucocyte antigen

MHC:

type I - presents peptide from proteins inside cell (e.g. viral infection). All nucleated cells present them

type II - peptides from extracellular and intravesicular proteins that enter cell by phagocytosis. Presented by macrophages, dendritic cells, B cells.

Antigen recognition: an antibody is a glycoprotein produced by plasma cells and circulates in the blood and other body fluids. Antibody molecules will recognize free native antigen.

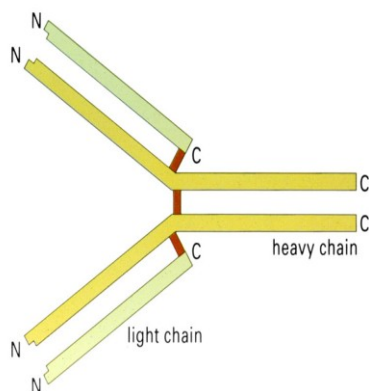
This contrasts dramatically with the situation in cell-mediated immunity; the T lymphocyte antigen receptor will only bind the fragments of antigen that are associated with products of the major histocompatibility complex (MHC). T cell recognition of antigen is said to be MHC-restricted.

T cells express surface receptors that allow them to recognize unique, antigen derived peptide sequences (generally composed of α/β polypeptide chains joined by disulfide bond).

E.g of bacterial antigens: O, H, K in Enterobacteria

21. Immunoglobulins

The basic chain structure of immunoglobulins



From: L. Kolonel, Slidecasts of immunology, 3rd edition

Soluble, globular proteins that are important components of acquired immunity. They are able to recognize a lot of epitopes (1 Ab \rightarrow 1 epitope).

5 subtypes: IgM, IgE, IgD, IgG and IgA

IgM - first immunoglobulin to appear after exposure to Ag has pentamer structure. Normally restricted to intravascular space, they have high molecular weight (but inflammation due to increase capillary permeability may go to ISF). It cannot cross the placenta.

IgD - major surface component of mature naive B cells

IgG - predominant Ig in blood, lymph and peritoneal fluid.

IgG can cross placenta thus conferring mothers humoral immunity to infection to fetus and neonate. IgG can aid NK cells finding their targets (binds Fab to antigen Fc to NK).

IgA - primary Ig found in external secretions (tears, mucous, saliva...). Its role is to prevent microbial pathogens from attaching to and penetrating epithel (e.g. in breast milk provides newborn with protection against infection). It can cause agglutination and prevent viruses from entering cells.

IgE - important in allergies (type I hypersensitivity) + parasitic infection

Antibody structure: All antibody molecules have the same basic 4 chain structure composed of 2 light chains and 2 heavy chains. The heavy chains: α , δ , ϵ and μ . In light chains, one end of the chain is identical in all members of the same isotype, and is termed the constant region of the light chain, C2. The other end shows variation, and is known as the variable region, VL.

The heavy chains also have VH and CH. The tertiary structure generated by the combination of the VL and VH regions determines the shape of the antigen-combining site or paratope.

- Activation of complement system (IgG, IgM)
- Opsonization (particularly IgG)
- Neutralization of antigens (IgG, IgA, IgM)
- Adherence interference (IgA, IgG)
- Antibody dependent cellular cytotoxicity (ADCC)
- Agglutination, precipitation (IgG, IgM)
- Mast cells degranulation (IgE)
- Transport through placenta (IgG)
- Immunoregulation (mainly IgG)

22. Antibody function in infection

1. Neutralization of toxins

bacterial toxin can damage or kill susceptible host cell -> specific antibodies bind and neutralize bacterial toxins. If the antibody is directed against surface antigens of particular material such as microorganisms or erythrocytes then agglutination will occur. This results in a clump or aggregate that will isolate the potential pathogen, stop its dissemination and stimulate its removal by other mechanisms - Ag-Ab complex is recognized as foreign by phagocytes which scavenge and degrade toxins.

If the antigen is soluble then the size of the complex will determine its physical state. Small complexes will remain soluble while large complexes will form precipitates).

2. Opsonization of bacteria

specific antibodies coat surface of bacteria making it a more recognizable target for ingestion by macrophages -> a specific conformation on the Fc receptors on the surface of the phagocyte. The important residues are in the CH2 domain near the hinge region.

3. Activation of complement

Bacterium in plasma is coated with Antibodies -> The Fc portion of certain isotypes once antigen has been bound, will activate complement -> bound antibodies form a receptor for the 1st protein of the complement system -> This requires that C1q, a subunit of the 1st complement component cross-links 2 antibody Fc portions. -> complement can form a lethal pore in cell membrane of some bacteria releasing cell contents -> complement binding also favours uptake and destruction of bacteria by phagocytes.

23. Immune system

Interacting set of specialized cells and proteins that are designed to identify and destroy foreign invaders or abnormal substances before they can destroy the body.

Most common intruders are viruses, bacteria, parasites, fungi.

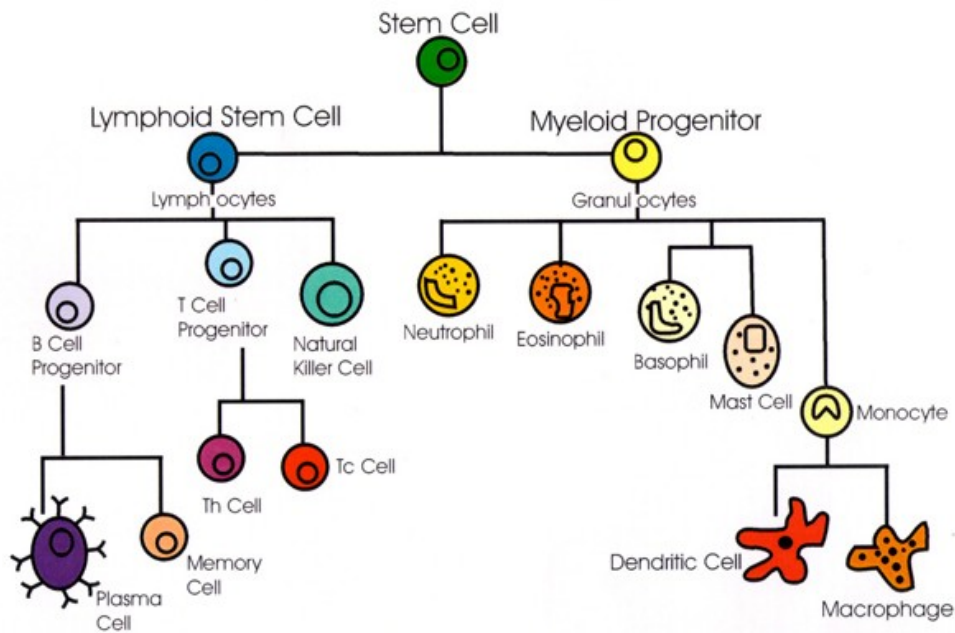
For the immune system to mount a defense against foreign invaders, it must be able to differentiate "self" from "nonself". This discrimination between self and nonself and subsequent destruction and removal of foreign bodies is accomplished by the 2 types of the immune system: innate and adaptive immune system.

- Innate immunity is fully operative the first time the body is in contact with foreign substance, it is present in some ways in all vertebrates and some invertebrates repeated exposure does not enhance it and response is non specific.
- Adaptive immunity needs days to develop, the response is highly specific and will be enhanced or repeated exposure to pathogen.

Although it is convenient to define the immune system in terms of these 2 components in reality their responsibility overlaps.

The immune system consists of a number of organs and several different cell types.

Cells of the Immune System



LYMPHOID CELLS

Lymphocytes make up about 20% of the white blood cells present in the adult circulation. The mononuclear cells comprise the T and B cell populations. The large cells are referred to as large granular lymphocytes because they contain cytoplasmic granules. Cells within this population are able to kill certain tumour and virally infected cells (nature killing) and destroy cells coated with immunoglobulin (antibody-dependent cell-mediated cytotoxicity).

MYELOID CELLS

-Mononuclear phagocytes: the common myeloid progenitor in the bone marrow gives rise to monocytes that circulate in the blood and migrate into organs and tissues to become macrophages. This actively phagocytic cell has a ruffled membrane and many cytoplasmic granules. These lysosomes contain enzymes and molecules that are involved in the killing of microorganisms.

Their activities can be enhanced by molecules produced by T lymphocytes, called lymphokines.

Macrophages and monocytes are capable of producing various complement components, prostaglandins, interferons and monokines such as interleukin (IL)-1 and tumor necrosis factor (TNF).

- Polymorphonuclear leucocytes: neutrophils constitute 60-70% of the leucocytes, but also migrate into tissues in response to injury or infection.

- Neutrophils: their granules contain numerous microbicidal molecules and the cells enter the tissues when a chemotatic factor is produced, as the result of infection or injury.
- Eosinophils: they are present in low numbers in a healthy normal individual (1-2%), but their number raise in certain allergic conditions. The granule contents can be released by the appropriate signal, and the cytotoxic molecules can then kill parasites that are too large to be phagocytosed.
- Basophils: less that 0.2%. They release molecules that stimulate an inflammatory response.

24. Innate Immunity - characteristics and humoral mechanisms

Characteristics:

- action is immediate
- non specific response
- response is not enhanced on repeated exposure to pathogen

The innate system is active at the time of infection, restraining the microorganisms until the lymphocytes of the adoptive immune system can eliminate the pathogen.

The innate immune system consists of protective cellular and chemical components found on body surface as well as white blood cells and their derivatives that often subsurface protection.

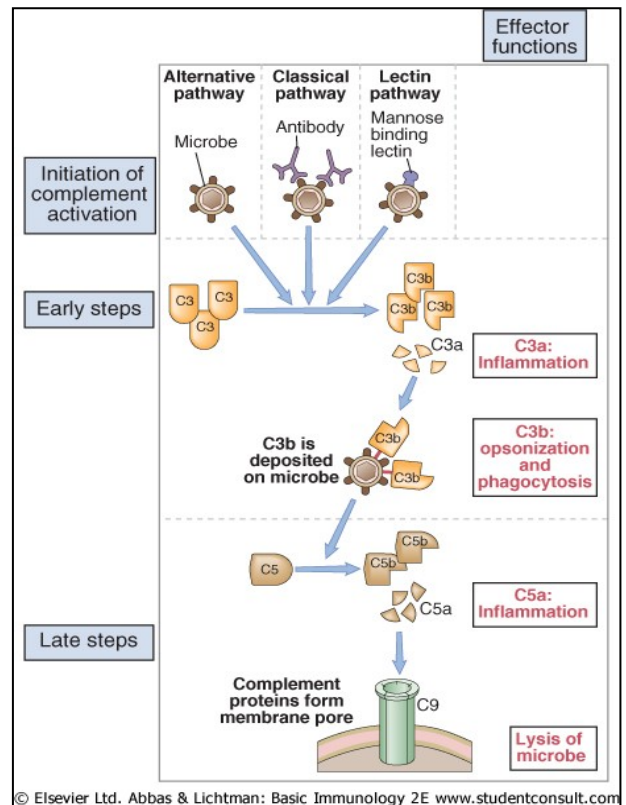
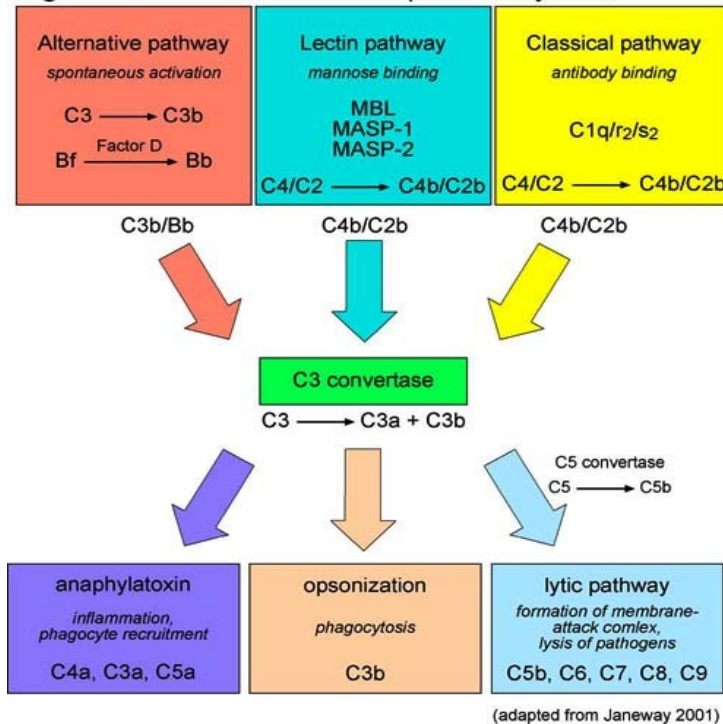
Response of innate immune system:

- Non inflammatory reaction: body's static defences (e.g. skin, gastric pH, lysozyme in specialized body fluids).
- Acute response: local inflammation (migration of phagocytes and plasma proteins to infected tissue)

Humoral Mechanism

One of the mechanisms by which the immune innate system defends body is by inflammatory reaction which is accompanied by increased concentration of serum proteins called acute phase proteins (e.g. CRP is produced by liver in response to tissue damage. It binds to cell walls of bacteria and fungi and activates the complement system.

Figure 7: Overview of the complement system



*Lysozyme - found high concentrations in most tissue fluids, functioning as a mucolytic enzyme, splitting sugars of cell wall of many G⁺ bacteria causing their lysis.

25. Innate Immunity - barriers and cell mediated mechanically

Barriers: skin: sheets of dry cornified epithel that are highly impermeable to bacteria + viruses unless damaged (because keratin is indigestible by most microorganisms).

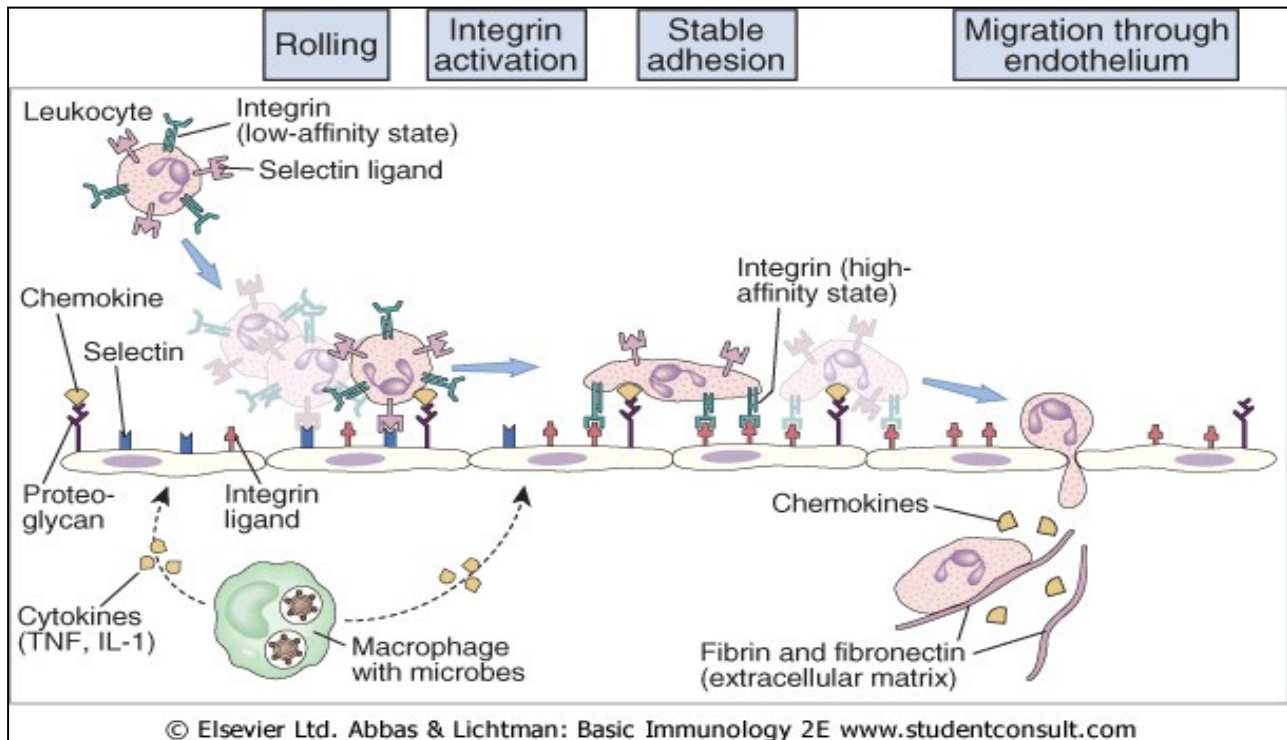
The only place where the bacteria/viruses could enter in intact skin are hair follicles and sebaceous glands. Respiratory tract: mucus traps the organism whilst cilia transport the trapped microorganism back to the external openings. Cough propels organisms away from lungs.

GIT: digestive enzymes have antimicrobial action and gastric acid pH provides a chemical barriers against microbes.

Genitourinary tract: normal flow of urine may carry microbes away by low vaginal pH also provides an inhospitable environment for pathogens colonization.

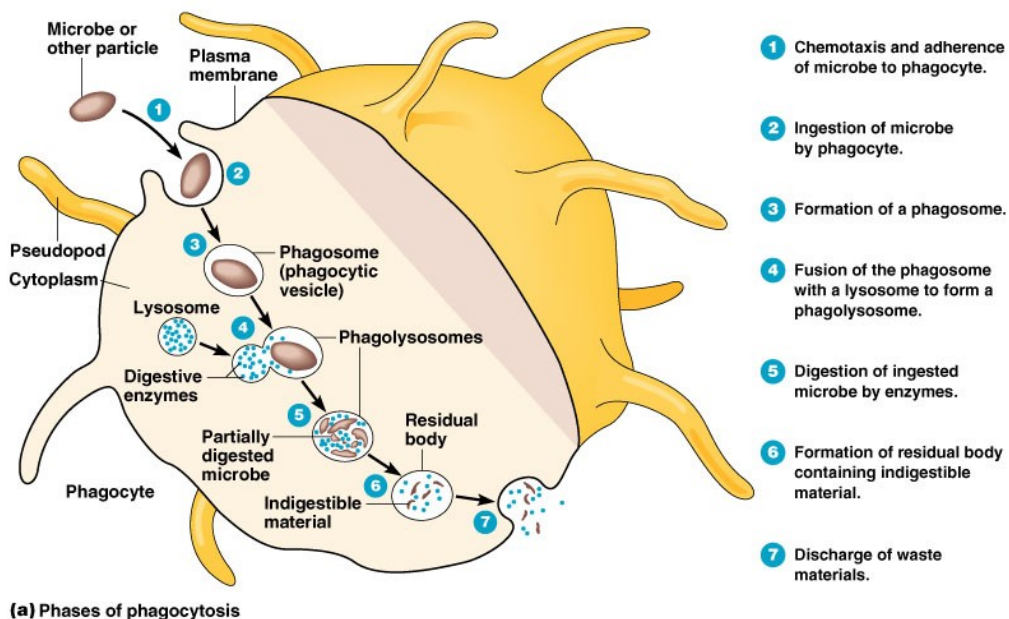
Tears, saliva, mucous: contain lysozyme which protect against gram + infectious commensal organisms: the non pathogenic microorganisms that constitute our normal microflora are beneficial because they compete with potential pathogens and produce by-products that can inhibit the growth of other mechanisms.

CELL MEDIATED MECHANISMS



1. Vasodilation: increased blood flow to injured area provides, increased delivery of plasma proteins, neutrophils and phagocytes.
2. Increased permeability: protein rich exudate with Igs + complement moves to injured area
3. Emigration of leucocytes
4. Chemotaxis - for phagocytic cells to be effective they must be attracted to the site of infection. Once they have passed through the capillary walls they move through the tissues in response to a concentration gradient of molecules produced at the site of damage:
 - products of injured tissue
 - factors from the blood (C5a)
 - leukotrienes, histamine (neutrophils and mast cells)
 - bacterial products

Some of these cells destroy the invading microorganism by phagocytosis followed by intracellular digestion (-> phagocytes whilst others limit the infection by releasing compounds toxic to microorganisms -> K, natural killer cells).



Natural killer cells

They destroy abnormal host cells such as virus-infected cells or neoplastic by released of compounds that are highly cytotoxic causing the formation of pores in the membrane of a target cell resulting in osmotic lysis or triggering apoptosis.

They can also cause apoptosis by surface contact with the target cell. They are stimulated by IFN and IL-2.

Eosinophils are no efficient phagocytic cells but their granules contain molecules that are toxic to parasites (worms and others that cannot be phagocytosed). This release of molecules must be controlled so that tissue damage is avoid.

26. Acquired immunity - general characteristics

Main characteristics:

- actions requires days to develop
- response is specific
- response is enhanced on repeated exposure to pathogen
- exhibits memory

It can be divided into humoral and cell mediated response.

Humoral immunity results from actions of soluble molecules such as antibodies in body fluids while cell mediated immunity involves specialized lymphocytes and antigen presenting cell (APCs).

27. Acquired Immunity - humoral immunity

B cells - they produce Abs that recognize epitopes and binds to these unprocessed Ags (with that need of APCs). Important to mention B cell as APC tht might cause MHC II complex.

MHC II - antigen are bound to T helper cell -> cloning of this helper T cell -> cytokines activate killing of intracellular or organism ->(Th2 cells; IL-4,5,6) B cell division and differentiation -> differentiation to plasma cells -> memory B cells

then switching to IgG might occur) -> complement activation

-> opsonization

-> neutralization of toxins

T cell dependent activation of B cells.

Multivalent antigens cross-link B cell surface receptors which results in B cell clones -> Plasma cells -> Antibody production (no class switching + affinity mutations - no Th2)

Antibodies acquired by either immunization or previous infection or given passively as antiserum are able to neutralize bacterial toxins.

Many bacterial exotoxins are enzymes, and protective antibody can prevent interaction of the enzyme with its substrate.

Antibody may also act by stopping activation of a zymogen into an active enzyme.

The direct binding of antibody to a bacterium can interfere with its normal functioning in numerous ways.

Antibody can kill bacteria or its own or in conjunction with host factors and alls.

Antibodies that affect the activity of specific transport system will deprive that bacteria of their supply and other essential chemicals.

Invasion can also be inhibited by antibody that attaches to the flagella of the microorganism in such a way as to affect their motility. Antibodies can agglutinate bacteria, and formation of the aggregate will impede the spread of the organism.

In certain circumstances, antibodies in conjunction with other bactericidal molecules lead to more efficient bacterial destruction.

28. Acquired immunity - cell mediated immunity

T cells

cytotoxic T-cell identifies and kills cells infected with viruses, its receptor only recognizes peptides when they are present on surface of nucleated cell in association with a class I MHC molecule.

Helper T cell assists the activation of killer T cell and signals B cells to start secreting antibody in response to presence of foreign antigen. They also activate phagocytes.

1. Antigen is recognized by an antigen presenting cell that will partially degrade this antigen and present a fragment peptide of the antigen on the MHC molecule on the membrane.
- 2.1 Specific T cell receptors of activated CD8+ T cells bind to class I MHC molecule complex with antigenic peptide fragment resulting in antigen driven clonal expansion of specific T cell and ultimately killing of the specific presenting cell.
- 2.2 Class II MHC peptide fragment complex is presented on surface of APC (macrophage dendritic cells, B cells) and the complex is recognized by appropriate T cell receptor of CD4+ T cells. Antigen driven clonal expansion of specific T cell occurs resulting in cytokine mediated helper T cell functions.

CD4 T cells activation and action

Macrophage engulphs and partially digests foreign antigen -> Macrophage displays antigen fragments bound to MHC II on cell surface -> Antigen specific CD4+ T-helper cells bind to antigen MHC-II complex and initiates cytokine secretion -> T cells that bind to complex are induced to propagate creating T cell clone with same receptor specific -> cytokines activate the killing of intracellular organisms in macrophage -> (IL-2 released by Th1) NK cells

-> (IL-4,5,6 released by

Th2) B cell division and differentiation

CD8 T cells activation and action

Virus infected cells synthesizes viral proteins using viral mRNA -> hydrolyzed viral peptides are transported to ER there they are loaded to binding groove of MHC class I molecule to cell surface -> MHC I interacts with CD molecules thereby restricting T cell receptor recognition to antigens displayed in groove of class I MHC -> cytotoxic CD8+ T cells proliferate forming a clone of cells with specific antiviral peptide receptors -> cytotoxic T cells kill virus infected cells -> some T cells become memory cell

29. Immunodeficiency

Can be:

- primary - genetic, inborn disorders
- secondary - acquired, due to presence of another disease (e.g. malnutrition)

Causes:

- defects in B cells or antibody production
- defects in T cells with or without concurrent B cells defect (Severe Combined Immunodeficiency = SCID)
- phagocyte and/or NK cell defect (chronic granulomatous disease)
- deficiencies in complement, cytokines or another mediator

This might lead to really severe infections.

The deficiency states seen are due either to defects in one of the components of the system itself, or are secondary to some other diseases process affecting the normal functioning of some part of the lymphoid tissues.

The compromised host is prone to infectious diseases that the normal individual would easily eradicate or not succumb to in the first place.

- Defective innate defense mechanisms:

Defects in phagocyte function takes 2 forms:

1. Where there is a quantitative deficiency of neutrophils which may be congenital (e.g. infantile agranulocytosis) or acquired as a result of replacement of bone marrow by tumor cell or the toxic effects of drugs or chemicals.
2. Where there is a qualitative deficiency in the functioning of neutrophils which, while ingesting bacteria normally, fail, because of an enzymatic defect, to digest them.

Characteristic of these diseases is a susceptibility to bacterial and fungal, but not viral or protozoan infections.

The complement system can also suffer from certain defects in function leading to increased susceptibility to infection.

- Defective acquired immune defense mechanisms

■ Primary immunodeficiencies: arise through failure of any of the developmental processes from stem cell to functional end cell.

There are several types of B cell defect that give rise to hypogammaglobulinaemias, i.e. low levels of γ -globulins (antibodies) in the blood.

In patients with dysgammaglobulinaemia there is a deficiency in only one antibody class.

Individuals with T cell defects tend to have more severe and persistent infections than those with antibody levels since TH cells are involved in the generation and control of humoral immunity.

■ Secondary immunodeficiency acquired deficiencies can occur secondarily to a number of disease states or after exposure to drugs and chemicals.

30. Immunity in bacterial infections

Steps of bacterial infection:

1. attachment of bacteria to host tissue
2. invasion to deeper host tissues and toxin production
3. inflammation at site of invasion
 - a) antibodies bind to bacteria
 - b) complement activation
 - c) wound healing mechanisms

Immunity to extracellular bacteria by antibodies

Gram -

1. Opsonization
2. Antibodies activate classical complement pathway which will induce lysis of bacteria
3. ABCC: Antibody dependent cytotoxicity is activated by antibodies and will evoke granulocytes which will degrade bacteria.

Gram+

1. Opsonization by antibodies and complement
2. No activation of complement system due to the thick cell wall
3. ABCC

Immunity to intracellular bacteria by cell-mediated immunity

1. Presentation of bacterial antigen to cytotoxic T cells on MHC I
2. Bacterial antigens present in endosomes of infected host cells processed via exogenous pathway and presented to helper T cell
3. Bacterial antigens can be present on the surface of the infected host cell and targeted for killing by NK cells

Microorganism



Attachment ← Innate defense mechanisms,
Antibody to adhesins



Local proliferation ← Phagocytes
Complement lysis
Block metabolite transport



- Invasion - Antibody to aggressins, antibody to organism, complement lysis, phagocytes
- Toxin production (toxigenic bacteria) - Antibody to toxin
- Intracellular growth (chronic bacterial infections) - cell-mediated immunity

31. Passive Immunization

Antibodies, which may be antitoxic, antibacterial or antiviral, in preparations of human or animal serum are injected to give temporary protection. Human preparations are injected to give temporary protection. Human preparations are referred to as homologous, and are much less likely to give rise to the adverse reactions.

An additional advantage is that, although they do not confer durable protection, their effect may present for 3-6 months, whereas the protection afforded by a heterologous serum is likely to last for only a few weeks.

Types of immunoglobulins used to give passive immunization:

- Non-specific standard Igs: mixture of plasma proteins containing broad spectrum of antibodies (IgG predominant). They contain mixture of Abs reflect previous exposures of plasma donors to various) either by natural infection or immunization. Prevention or attenuation of illness (e.g. HepA)
- Hyperimmune human immunoglobulin: high concentration of antibodies directed to specific pathogen or toxin (e.g. varicella or diphtheria)

Mother gives passive immunity to baby through IgG and IgA from mother's milk.

Adverse effects: the recipient might mount an adverse reaction to antigenic determinants of foreign Abs potentially leading to systemic anaphylaxis (more true for non human source).

32. Active Immunization

Active immunization requires several days to months to become effective. It leads to prolonged immunity.

- Toxoids: if the signs and symptoms of a disease can be attributed essentially to the effects of a single toxin, a modified form of the toxin that preserves its antigenicity but has lost its toxicity (a toxoid) provides the key to successful active immunization against the disease.
- Inactivated vaccines: it may be possible to stimulate the production of protective antibodies by using the killed (inactivated) organisms.
- Attenuated vaccines: another approach is to use suspensions of living organisms that are reduced in their virulence (attenuated) but still immunogenic.
- Microbial extracts: instead of using whole organisms, vaccines are composed only the antigen molecules. The more effective vaccines are those that the vaccine antigen is present in all the organism strains (pneumococcal vaccine has 23 polysaccharides comprising the Ags produced by the most common strains).
- Vaccine conjugates: vaccines can produce humoral immunity through B cell proliferation leading to Ab production which may or may not involve T helper cells. Hemophilus polysaccharide was conjugated to a protein Ag such as diphtheria toxoid protein.

SPECIAL BACTERIOLOGY

1. Staphylococcus aureus

Staph. aureus is a Gram-positive coccus. The cocci are mainly arranged in grape-like clusters, but some may occur as a single cell or pairs of cells.

Pathogenesis: Staph. aureus is present in the nose of 30% of healthy people and may be found on the skin. It causes infection most commonly at sites of lowered host resistance, e.g. damaged skin or mucous membranes.

Virulence factors: there are plenty of virulence factors but only some of them are present in nearly 100% of strains; others are produced just by one strain among one thousand. Examples: hemolysin, coagulase, hyaluronidase, protein A (antiphagocytic effect), fibronectin, cytolytic exotoxin, TSST-1.

Staphylococcal toxins:

- ENTEROTOXINS: enterotoxins, types A-E, G, H, I and J are commonly produced by up to 65% of strains of Staph. aureus. When ingested as preformed toxins in contaminated food, microram amounts of toxin can induce within a few hours the symptoms of staphylococcal food poisoning: nausea, vomiting and diarrhoea.
- TOXIC SHOCK SYNDROME TOXIN (TSST-1): a multisystem disease caused by staphylococcal TSST-1 or enterotoxin, or both -> established with the use of highly absorbent tampons. TSST-1 and the enterotoxins are now recognized as superantigens, i.e. they are potent activators of T lymphocytes resulting in the liberation of cytokines such as tumour necrosis factor.
- EPIDERMOLYTIC TOXINS: two kinds of epidermolytic toxins (A and B) -> cause blistering diseases.

Epidemiology: the sources of infection are infected lesions -> large numbers of staphylococci are disseminated in pus and dried exudate discharged from large infected wounds, burns, secondarily infected skin lesions, and in sputum coughed from the lung of a patient with bronchopneumonia. Direct contact is the most important mode of spread.

Diseases:

- | | |
|--------------------------------------|--------------------------|
| - <u>in skin</u> | - <u>musculoskeletal</u> |
| furuncle | osteomyelitis |
| carbuncle | arthritis |
| impetigo | - <u>genitourinary</u> |
| cellulitis | renal carbuncle |
| wound infection | UTI of the lower part |
| superficial abscesses (folliculitis) | - <u>cardiovascular</u> |
| | endocarditis |

Treatment

In staphylococci the drug of choice is Oxacilin.

- in UTI - cefalosporins of first generation
 - allergic persons - macrolids
 - locomotor system - lincosamids
 - aminoglycosides in combination only
 - glycopeptidic antibiotics (vankomycin and teikoplanin) are a reserve. Used in MRSA and MRSKN
- In strains resistant even to glycopeptides, or in patients that have contraindications newer antibiotic linezolid can be used.

MRSA -> methicilin resistant staphylococci are epidemiologically important strains, often causing serious hospital infections. They are caused by change of so named membrane penicillin binding proteins (PBP).

2. Coagulase negative staphylococci

Are commonly found on the surface of healthy persons in whom they are rarely the cause of infection.

Staphylococcus epidermidis accounts for about 75% of all clinical isolates, probably reflecting its preponderance in the normal skin flora. Other species include Staph. haemolyticus, Staph. capitis and Staph. saprophyticus.

Coagulase-negative staphylococci are morphologically similar to Staph. aureus. Coagulase-negative staphylococci are opportunistic pathogens that cause infection in debilitated or compromised patients, often by colonizing biomedical devices such as prostheses, implants and intravascular lines.

Pathogenesis

Production of an exopolysaccharide, allowing adherence and subsequent formation of a multi-layered biofilm, appears to be essential for the pathogenesis of device related Staph. epidermidis infection. Attachment is enhanced by the presence of matrix proteins, such as fibronectin and fibrinogen. The subsequent incorporation of teichoic acid appears to provide the biofilm with stability.

Staphylococcus epidermidis are sensitive to vancomycin and novobiocin.

Staphylococcus saprophyticus are sensitive to G-penicillin but are resistant to novobiocin

3. Streptococcus pyogenes

This species, which consists of Lancefield group A streptococci, is among the most prevalent of human bacterial pathogens. It causes a wide range of suppurative infections in the respiratory tract and skin, life-threatening soft tissue infections, and certain types of toxin-associated reactions.

Pathogenesis

VIRULENCE FACTORS: strains of Str. pyogenes express a large arsenal of virulence factors and, hence, their pathogenicity and the clinical signs that they induce are very diverse. The virulence factors are involved in adherence, evasion of host immunity and tissue damage.

ADHESION: interaction with host fibronectin, a matrix protein on eukaryotic cells, is considered the principal mechanism by which Str. pyogenes binds to epithelial cells of the pharynx and skin.

M PROTEINS: the ability of Str. pyogenes to resist phagocytosis is to a high degree due to the cell surface-exposed M protein.

CAPSULE: like other bacterial capsules it has an antiphagocytic effect.

C5a PEPTIDASE: it specifically cleaves, and thereby inactivates, human C5a, one of the principal chemoattractants of phagocytic cells.

STREPTOLYSINS: Str. pyogenes produces two distinct haemolysins - streptolysins O (oxygen-labile) and S (serum-soluble), both of which lyse erythrocytes, polymorphonuclear leucocytes and platelets.

PYROGENIC EXOTOXINS: most strains of Str. pyogenes exotoxins because of their ability to induce fever.

HYALURONIDASE: streptococci use a secreted hyaluronidase to degrade hyaluronic the ground substance of host connective tissue. This property may facilitate the spread of infection along fascial planes.

STREPTOKINASE: streptokinase, also known as fibrinolysin, is another spreading factor. Once host plasminogen is bound to the bacterial surface, it is activated to plasmin by streptokinase. As a result, soft tissue infections due to Str. pyogenes are more diffuse, and often rapidly spreading, in contrast to the well localized abscesses that typify staphylococcal infections.

DEOXYRIBONUCLEASES (DNAases): the enzymes hydrolyse nucleic acids and may play a role as spreading factors by liquefying viscous exudates.

Clinical features

The most common route of entry of Str. pyogenes is the upper respiratory tract. Spread from person to person is by respiratory droplets or by direct contact with infected wounds or sores on the skin.

- Non-invasive streptococcal diseases: the most common infections caused by Str. pyogenes are relatively mild and non-invasive infections of the upper respiratory tract (pharyngitis) and skin (impetigo).

Pharyngitis: clinical signs such as abrupt onset of sore throat, fever, malaise and headache generally develop 2-4 days after exposure to the pathogen. The posterior pharynx is usually diffusely reddened, with enlarged tonsils that may show patches of gray-white exudate on their surface and, sometimes, accumulations of pus in the crypts. The local inflammation results in swelling of cervical lymph nodes.

Scarlet fever: pharyngitis caused by certain pyrogenic exotoxin-producing strains of Str. pyogenes may be associated with a diffuse erythematous rash of the skin and mucous membranes.

Skin infections: Str. pyogenes may cause several types of skin infection, sometimes in association with Str. aureus. It primarily affects exposed areas on the face, arms or legs. The skin becomes colonized after contact with an infected person and the bacteria enter the skin through small defects. Initially, clear vesicles develop, which within a few days become pus-filled.

- Invasive soft tissue infections

Necrotizing fasciitis: this infection progresses very rapidly, destroying fat and fascia. Systemic shock and general deterioration occur very quickly.

Streptococcal toxic shock syndrome: patients with invasive and bacteraemic. *Str. pyogenes* infections, and in particular necrotizing fasciitis, may develop streptococcal toxic shock syndrome. A striking feature of this acute fulminating disease is severe pain at the site of initial infection, usually the soft tissues. The additional clinical signs resemble those of staphylococcal toxic shock syndrome and include fever, malaise, nausea, vomiting and diarrhoea, dizziness, confusion, and a flat rash over large parts of the body.

Rheumatic fever: this manifest as an inflammation of the joints (arthritis), heart (carditis), central nervous system (chorea), skin (erythema marginatum), and/or subcutaneous nodules. Polyarticular arthritis is the most common manifestation, whereas carditis is the most serious as it leads to permanent damage, particularly of the heart valves. The disease is autoimmune in nature and is believed to result from the production of autoreactive antibodies and T-lymphocytes induced by cross-reactive components of the bacteria and host tissue.

Acute post-streptococcal glomerulonephritis: the clinical manifestations include:

- coffee-coloured urine caused by haematuria
- oedema of the face and extremities
- circulatory congestion caused by renal impairment

Treatment

- penicillin (either G-penicillin for parenteral use or V-penicillin for oral use)
- macrolids - in penicillin allergic persons only
- vancomycin - is a reserve, 100% effective
- RESISTANT TO AMINOGLYCOSIDES (used as selective medium)

4. β -hemolytic streptococci other than *Streptococcus pyogenes*

Streptococcus agalactiae

Str. agalactiae is a primary habitant of human colon. It may be carried in the throat and importantly, 10-40% of women intermittently carry in the vagina.

Str. agalactiae has become the leading cause of neonatal infections in industrialized countries and is also an important cause of morbidity among peripartum women and non-pregnant adults with chronic medical conditions. Among β -hemolytic streptococci, *Str. agalactiae* is the most frequent isolate from blood culture.

Virulence factors:

Str. agalactiae produces several virulence factors, including haemolysins, capsule polysaccharide, C5a peptidase, hyaluronidase and various surface proteins that bind humans IgA and serve as adhesins.

Clinical features

- Infection in the neonate: two different entities are recognized:

- early-onset disease, most cases of which present at or within 12h of birth.
- late-onset disease, presenting more than 7 days and up to 3 months after birth.

EARLY ONSET: this results from ascending spread of *Str. agalactiae* from the vagina into the amniotic fluid, which is then aspirated by the infant, and results in septicaemia in the infant or the mother or both. Infants borne by mothers carrying *Str. agalactiae* may also become colonized during passage through the vagina.

The clinical symptoms include lethargy, cyanosis and apnoea, when septicaemia progresses, shock ensues and death will occur if treatment is not quickly instituted. Meningitis and pulmonary infection may be associated.

Risk factors for neonatal colonization and infection are:

- premature rupture of membranes
- prolonged labour
- premature delivery
- low birth weight
- intrapartum fever

LATE-ONSET DISEASE: purulent meningitis is the most common manifestation, but septic arthritis, osteomyelitis, conjunctivitis, sinusitis, otitis media, endocarditis and peritonitis also occurs.

Many cases are acquired in hospital. Mastitis in the mother has also been described as a source of infection.

- Infections in the adult: disease may manifest as sepsis, pneumonia, soft tissue infections such as cellulitis and arthritis, and urinary tract infections complicated by bacteraemia. The risk factors in these patients are diabetes mellitus, liver cirrhosis, renal failure, stroke and cancer.

non A non B streptococci

Cause pharyngitis but can also be normal people's throat.

Treatment

G+ penicillin and ampicillin sensitive

5. Streptococcus pneumoniae

Str. pneumoniae, commonly called the pneumococcus, is a member of the oropharyngeal flora of 5-70% of the population. They are G+, encapsulated cocci, viridating and facultative anaerobes. Str. pneumoniae generally occurs as characteristic diplococci. Str. pneumoniae is an important pathogen, which is largely ascribed to its capsular polysaccharide. It primarily causes disease of the middle ear, paranasal sinuses, mastoids and the lung parenchyma, but may spread to other sites, such as the joints, peritoneum, endocardium and biliary tract and, in particular, the meninges.

Pathogenesis

Virulence factors:

CAPSULE: the capsule is antiphagocytic, inhibiting complement deposition and phagocytosis, where type-specific opsonic antibody is absent.

IgA1 PROTEASE: pneumococci produce an extracellular protease that specifically cleaves human IgA1 in the hinge region. This protease enables these pathogens to evade the protective functions of the principal immunoglobulin isotype of the upper respiratory tract.

PNEUMOLYSIN: pneumococci produce an intracellular membrane-damaging toxin known as pneumolysin, which is released by autolysis. Pneumolysin inhibits:

- neutrophil chemotaxis
- phagocytosis and the respiratory burst
- lymphocyte proliferation and immunoglobulin synthesis

AUTOLYSIN: autolysis enables the release of pneumolysin and, in addition, large amounts of cell wall fragments. The massive inflammatory response to these peptidoglycan fragments is an important component of the pathogenesis of pneumococcal pneumonia and meningitis.

Clinical features: person-to-person spread is common.

Pneumonia results from aspiration of pneumococci contained in upper airway secretions into the lower respiratory tract, for example, when the normal mechanisms of mucous entrapment and expulsion by an intact glottic reflex and mucociliary escalator are impaired. Human immunodeficiency virus (HIV) infection carries an increased risk of bacterial infections, including those caused by the pneumococcus.

PNEUMONIA: Str. pneumoniae is the most frequent cause of pneumonia. Pneumococcal pneumonia follows aspiration with subsequent migration through the bronchial mucosa to involve the peribronchial lymphatics. Contiguous spread commonly results in inflammatory involvement of pleura. Pericarditis is another uncommon but well recognized complication.

OTITIS MEDIA: middle ear infections: approximately 1/3 of cases are caused by Str. pneumoniae. Disease occurs after acquisition of a new strain to which there is no pre-existing immunity.

MENINGITIS: Str. pneumoniae is among the leading causes of bacterial meningitis. It is assumed that invasion arises from the pharynx to the meninges via the bloodstream.

Treatment

Penicillin. When resistance to penicillin is developed, vancomycin is used

Vaccines: PPV (pneumococcal polysaccharide vaccine): risk individuals > 2 years old

PCV (pneumococcal conjugated vaccine): babies and toddlers

6. Enterococci & alpha-haemolytic streptococci other than Streptococcus pneumoniae

They are G+ cocci, occur in pairs or chains, the catalase test is negative, and they grow in BE + 6.5% NaCl.

Viridans streptococci

The viridans streptococci are dominant members of the resident flora of the oral cavity and pharynx in all age groups. They play an important role by inhibiting the colonization of many pathogens, including pyogenic streptococci. This is achieved by 2 different mechanisms:

- production of bacteriocins
- production of hydrogen peroxide (also responsible from α -hemolysin)

Most strains secrete bacteriocins.

- Mitis group: colonize tooth surfaces as well as mucosal membranes. Because of their presence in the bacterial deposits (dental plaque) on tooth surfaces, these species may enter the bloodstream during dental procedures such as tooth extraction or vigorous tooth cleaning, particularly if the gingival tissue is inflamed. They are recognized as cause of often fatal septicaemias in immunocompromised patients.
- Mutans group: colonize both enamel and do not occur until tooth eruption. Their proportions in dental plaque are closely related to sugar consumption. Like most other plaque streptococci they may cause subacute bacterial endocarditis.

Enterococcus species

Enterococcus have their natural habitat in the human intestines. The species most commonly associated with human disease are *E. faecalis* and *E. faecium*. The diseases with which they are associated are:

- urinary tract infection
- infective endocarditis
- biliary tract infections
- suppurative abdominal lesions
- peritonitis

E. faecalis and *E. faecium* are important causes of wound and urinary tract infection in hospital.

Treatment

They are susceptible to penicillin in combination with aminoglycosides. They are resistant to vancomycin.

7. Corynebacterium

The term coryneform is used to describe aerobic, non-sporing and irregularly shaped Gram + rods.

CORYNEBACTERIUM DIPHTHERIAE: the major disease caused by *C. diphtheriae* is diphtheria, an infection of the local tissue of the upper respiratory tract with the production of a toxin that causes systemic effects, notably in the heart and peripheral nerves.

C. diphtheriae are non-motile, non-spore forming, straight or slightly curved rods with tapered ends.

Snapping division produces groups of cells in angular and palisade arrangements.

C. diphtheriae is aerobic and facultative anaerobic.

To cause disease *C. diphtheriae* must:

- invade, colonize and proliferate in local tissues
- be lysogenized by a special β -phage, enabling it to produce toxin.

The diphtheria toxin possibly assists colonization of the throat or skin by killing epithelial cells or neutrophils.

The exotoxin is produced locally and is spread by the bloodstream to distant organs, with a special affinity for heart muscle, the peripheral nervous system and the adrenal glands.

The diphtheria toxin binds to a specific receptor on susceptible cells and enters by receptor-mediated endocytosis.

Inhibition of protein synthesis is probably responsible for both necrotic and neurotoxic effects of the toxin.

Production of toxin is enhanced considerably when the bacteria are grown in low iron conditions.

Nontoxigenic strains of *C. diphtheriae* may cause pharyngitis and cutaneous abscesses.

Diphtheria antitoxin (hyperimmune horse serum) is given, since antibiotics have no effect on preformed toxin which rapidly diffuses from the local lesions and soon becomes irreversibly bound to tissue cells.

Treatment

Treatment with parenteral penicillin or oral erythromycin eradicates the organism and terminates toxin production.

High population immunity achieved through mass immunization.

Other medically important corynebacteria

They comprise strictly aerobic bacteria as well as facultative or preferentially anaerobic bacteria which are commensals of the skin and mucous membranes.

CORYNEBACTERIUM ULCERANS: like *C. diphtheriae* and *C. pseudotuberculosis*, *C. ulcerans* can produce diphtheria toxin. In man, *C. ulcerans* is almost exclusively seen in cases of exudative pharyngitis, but occasional soft tissue infections occur.

Infection usually takes the form of acute pharyngitis with pseudomembranes, and cardiac or neurological complications can occur. Therapy involves administration of appropriate anti-biotics such as penicillins or erythromycin and administration of diphtheria antitoxin.

CORYNEBACTERIUM PSEUDOTUBERCULOSIS: human infections mainly occur in patients with animal contact.

CORYNEBACTERIUM JEIKEIUM: *C. jeikeium* is part of the normal skin flora, particularly in inguinal, axillary and rectal areas.

Most infections are associated with skin damaged by wounds or invasive devices.

C. jeikeium are highly resistant to penicillins and cephalosporins in vitro. Even with susceptible isolates, penicillin is incompletely bactericidal. *C. jeikeium* is sensitive to glycopeptides and these antibiotics are bactericidal.

CORYNEBACTERIUM UREALYTICUM: *C. urealyticum* is a frequent skin colonizer, mainly in hospital patients. The groin is the most frequent skin site of colonization, followed by the abdominal wall and axilla. This microorganism is associated with urinary tract infections.

Like *C. jeikeium*, *C. urealyticum* is usually highly resistant to most antimicrobial agents, except glycopeptides.

8. *Listeria monocytogenes*

They are non-sporing Gram-positive bacilli, found usually in pairs or short chains. They have a positive catalase test and can grow even in low temperatures and increased NaCl levels.

Listeria is phagocytosed by macrophages -> phagolysosome is not formed because of the action of Lysosteinolysin O -> *Listeria* escapes and multiplies -> bacteria on surface of macrophages -> pseudopod extension -> *Listeria* infects other macrophage

The disease chiefly affects pregnant women, unborn or newly delivered infants, the immunosuppressed and elderly. It is predominantly transmitted by the consumption of contaminated food.

The bacilli are non-motile at 37°C, but exhibit characteristic “tumbling” motility when tested at 25°C.

Most cases of human listeriosis are caused by serovars 4b, 1/2a and 1/2b.

It grows in a wide range of foods having relatively high water activities and over a wide range of temperatures. Growth at refrigeration temperatures is relatively slow.

Pathogenesis

L. monocytogenes is an intracellular parasite, and it is in this environment that the pathogen gains protection and evades some of the host's defences.

Listerial surface protein, internalin is involved with the initial stages of invasion on all cell types. After internalization, *L. monocytogenes* becomes encapsulated in a membrane-bound compartment.

The ability to polymerize actin by listeria all surface protein subverts the host cell's cytoskeleton and confers intracellular motility to the bacterium.

L. monocytogenes principally causes intra-uterine infection, meningitis and septicaemia.

Pregnant women often have very mild symptoms (chills, fever, back pain, sore throat and headache, sometimes with conjunctivitis, diarrhoea or drowsiness) but may be asymptomatic until the delivery of an infected infant.

Cultures from high vaginal swabs, stool and midstream urine samples, together with pre- or postnatal antibody tests, are of little help in diagnosis.

While the outcome of infection for the mother is usually benign, the outcome for the infant is more variable. Abortion, stillbirth and early-onset neonatal disease are common.

Early neonatal listeriosis is predominantly a septicaemic illness, contracted in utero.

Early-onset disease represents a spectrum of mild to severe infection which can be correlated with the microbiological findings. The neonates who die of infection usually do so within a few days of birth and have pneumonia, hepatosplenomegaly, petechiae, abscesses in the liver or brain, peritonitis and enterocolitis.

Treatment

ampicillin + sulfamethoxazole

9. *Mycobacterium tuberculosis*

M. tuberculosis are non-motile, non-sporing, non-capsulated, straight or slightly curved rods.

Tubercle bacilli are able to grow on a wide range of enriched culture media, but Lowenstein-Jensen (LJ) medium is the most widely used in clinical practice.

All mycobacteria are obligate aerobes.

Tubercle bacilli survive in milk and in other organic materials and on pasture land so long as they are not exposed to ultraviolet light, to which they are very sensitive. They are also heat-sensitive, and are destroyed by pasteurization. Mycobacteria are susceptible to alcohol, formaldehyde.

The tubercle bacillus owes its virulence to its ability to survive within the macrophage rather than to the production of a toxic substance. The nature of the immune responses following infection changes with time so that human tuberculosis is divisible into the primary and post-primary forms with quite different pathological features.

-Primary tuberculosis

These bacilli are engulfed by alveolar macrophages. Some bacilli are carried to the hilar lymph nodes where additional foci of infection develop.

Within about 10 days of infection, clones of antigen-specific T lymphocytes are produced. These released cytokines, notably interferon- γ , which activate macrophages and cause them to form a compact cluster, or granuloma, around the foci of infection.

Granuloma formation is usually sufficient to limit the primary infection: the lesions become quiescent and surrounding fibroblasts produce dense scar tissue, which may become calcified. Not all bacilli are destroyed: some remain in a poorly understood dormant form which, when reactivated, causes post-primary disease.

- Post-primary tuberculosis

Endogenous reactivation may occur spontaneously or after an intercurrent illness or other condition that lowers the host's immune responsiveness.

CD4+ \rightarrow IFN- γ \rightarrow activation of macrophages \rightarrow macrophages engulf M. tb but aren't able to destroy it \rightarrow epithelioid cells + Langerhans giant cells \rightarrow granulomas with central caseous necrosis are formed \rightarrow lesions may arrest and become fibrotic and calcified

\rightarrow they may rupture leading to propagation of Tb to the rest of respiratory system + blood

Tuberculin test is positive

Interferon- γ release test positive

Treatment

takes 6 months or more and uses a combination of drugs (ethambutol, streptomycin, pyrazinamide, isoniazid = RESPI)

BCG (bacille Calmette Guérin) vaccine is used in individuals under heavy sustained risk of infection.

10. Mycobacterium leprae

Leprosy bacilli resemble tubercle bacilli in their general morphology, but they are not so strongly acid-fast. They are transmitted through human to human contact. They are typically found within macrophages in dense clumps. They have unlimited source of recombinant protein antigens. The principal target cell for the leprosy bacillus is the Schwann cell. The resulting nerve damage is responsible for the main clinical features of leprosy: anaesthesia and muscle paralysis. Repeated injury to, and infections of the anaesthetic extremities leads to their gradual destruction. Infiltration of the skin and cutaneous nerves by bacilli leads to the formation of visible lesions, often with pigmentary changes.

Bacilli are disseminated from the nasal secretions of patients with lepromatous leprosy. In addition, the blood of patients with lepromatous leprosy contains enough bacilli to render transmission by blood-sucking insects a definite, though unproven, possibility.

Leprosy often commences during childhood or early adult life but, as the incubation period is usually 3-5 years, it is rare in children aged less than 5 years.

- Tuberculoid leprosy: low infectivity, cell mediated immunity. The lesion is a large maculae in organs such as testis, skin or superficial nerve endings \rightarrow trophic changes of muscles

Treatment: dapsone + rifampin

- Lepromatous leprosy: high infectivity, decreased immunity. There are extensive erythematous macules, papule or nodules \rightarrow extensive skin lesion: destruction of nasal bones, uveitis, corneal infections.

Treatment: clofazimine

Vaccine with BCG has some protective effect.

Lepronin and leprosin are skin tests made for diagnosis.

11. Atypical mycobacteria

Termed environmental (or non-tuberculous) mycobacteria, some species occasionally cause opportunistic disease in animals and man.

4 groups of mycobacteria associated with human disease according to their production of yellow or orange pigment and their rate of growth.

- Photochromogens, which are colourless when incubated in the dark
- Scotochromogens, which produce pigmented colonies even when grown in the dark
- Non-chromogens, which are unpigmented
- Rapid growers

PHOTOCHROMOGENS: *Mycobacterium kansasii*, *M. simiae*, *M. marinum*

SCOTOCHROMOGENS: *M. goodii*, *M. scrofulaceum*, *M. szulgai*

NON-CHROMOGENS: *M. avium*, *M. intracellulare*

In man, they are responsible for lymphadenitis, pulmonary lesions and disseminated disease, notably in patients with the acquired immunodeficiency syndrome (AIDS).

RAPID GROWERS: *M. chelonae*, *M. fortuitum*

They occasionally cause pulmonary or disseminated disease but are principally responsible for post-injection abscesses and wound infections, including corneal ulcers.

Environmental mycobacteria are of low virulence and, although man is frequently infected, overt disease is very uncommon except in those who are profoundly immunosuppressed. Four main types of opportunistic mycobacterial disease.

- Lymphadenitis

In most cases a single node, usually tonsillar, is involved, and most patients are children aged less than 5 years.

Lymphadenitis occasionally occurs as part of a more disseminated infection, particularly in individuals with AIDS.

- Post-injection abscesses

Abscesses occur sporadically or in small epidemics when batches of injectable materials are contaminated by these bacteria. They are painful, may become quite large - up to 8 or 10 cm in diameter - and may persist for many months.

- Swimming pool granuloma

Those affected are mostly users of swimming pools, keepers of tropical fish and others involved in aquatic hobbies. The bacilli enter scratches and abrasions and cause warty lesions similar to those seen in skin tuberculosis.

- Buruli ulcer

The 1st manifestation of the disease is a hard cutaneous nodule, which is often itchy. This enlarges and develops central softening and fluctuation owing to necrosis of the underlying adipose subcutaneous tissues caused by a toxin. The overlying skin becomes anoxic and breaks down, the liquefied necrotic contents of the lesions are discharged and one or more ulcers with deeply undermined edges are thereby formed.

- Pulmonary disease

This is the most frequently seen in middle-aged or elderly men with lung damage caused by smoking or exposure to industrial dusts.

- Disseminated disease

Up to a half of all persons dying of AIDS in the USA has disseminated mycobacterial disease.

Treatment

Most environmental mycobacteria are resistant to many antituberculosis drugs in vitro although infections often respond to various combinations of these drugs.

Rifampicin + Isoniazid + ethambutol -> given for up to 18 months

12. Actinomyces & Nocardia

Actinomyces

A. israelii and *A. propionica* are part of normal microflora of intestines and oral cavity. They are facultative anaerobes (but grow better in anaerobic condition), slight gram + bacilli, slight acid-fast.

They cause the following diseases:

- abscesses leading to scar and disfigurement

- cases associated to poor dental health or tooth extraction
- mycetoma - hard, red swelling filled with liquid, ruptures to surface discharging pus.

Actinomycosis - is a chronic disease characterized by multiple abscesses and granulomata, tissue destruction, extensive fibrosis and the formation of sinuses.

Lab ID

Sulphur granules in pus.

Treatment

Penicillin G for weeks or months - surgical debridement is done

If the patient is allergic - tetracyclines

Nocardia

Nocardia asteroides and *Nocardia brasiliensis* are opportunistic human infection, inhaled or acquired by contamination of skin wounds. Cutaneous nocardiosis starts with traumatic implantation - cellulite with swelling. In most cases there are multiple confluent abscesses with little or no surrounding fibrous reaction and local spread may result in empyema.

They are strict aerobe, grow in BA.

They cause chronic pneumonia which is predisposed by lymphomas, drugs or immunocompromised patients.

Treatment

Sulfonamides with or without trimethoprim.

13. Bacillus

Are Gram+ rods which form endospores (central), are encapsulated, facultative or strictly aerobic.

B. anthracis is a non-motile straight, sporing bacillus.

Spores are never found in the tissues. The spores are resistant to chemical disinfectants and heat.

Contracted by infected animals products, contaminated dust inhalation or by subcutaneous inoculation due to skin abrasion.

Pathogenic factor:

- capsule: antiphagocytic
- 3 exotoxins that are plasmid coded: protective antigen, edema factor, lethal toxin.

CUTANEOUS ANTHRAX: the resulting lesion is often described as a malignant pustule.

RESPIRATORY ANTHRAX: this condition carries a high mortality due to the intense inflammation, hemorrhage and septicaemia which result from the multiplication of organisms in bronchi and spread to the lungs, lymphatics and bloodstream.

INTESTINAL ANTHRAX: an individual may suffer after a day or so from haemorrhagic diarrhoea, and dies rapidly from septicaemia.

B. cereus are Gram + bacillus, motile. The organism is found in most of raw foods, specially cereals like rice.

- Vomiting within 6h: preformed toxin
- Diarrhoea 8-24h after ingestion: enterotoxin formed in the intestine

Treatment

Penicillin, erythromycin, doxycycline and ciprofloxacin

Vaccine: cell free vaccine available for high risk occupations.

14. Clostridium botulinum

Are Gram+ rod, motile, strictly anaerobe grows in VL blood agar, spore forming.

Their spores frequently infect vegetables, meat or fish.

Pathogenic factor: botulinum absorbed in intestine from where it is going to pass through circulation to nerve ending, it is going to dissociate into a heavy and light chain preventing acetylcholine from being released from presynaptic nerve leading so to flaccid paralysis.

Diseases:

- Classic botulinism: difficulty focusing vision then swallowing then other cranial nerve function until patient succumbs with respiratory paralysis.

- Floppy child syndrome (infant botulism): colonization of infants large bowel -> constipation , feeding problem, poor muscle tone...
- wound botulism

Treatment

Antitoxin (trivalent A, B, E) and inward botulism - penicillin

15. Clostridium tetani

They are straight, motile, slender rods with rounded ends capable of forming spores (terminal). They are obligate anaerobes which are commonly common in backyard, garden and other soils generally acquired through puncture wound (e.g.: splinter).

Pathogenesis:

Infected locus -> retrograde neural flow (from peripheral to central, bacteria stays there and toxin is absorbed by blood and enters nerves) -> the heavy chain mediates attachment to gangliosides and the toxin is internalized -> Light chain blocks neurotransmitter release at inhibitory synapses (GABA) -> prolonged spasm

This microorganism grows in VL BA:

- haemolysin (tetanolysin)
- neurotoxin (tetanospasmin)

Disease:

- Tetanus: 4 days to several weeks incubation, muscle spasm firstly involving the site of infection then propagate to other muscles until reaching chest muscles.

Prevention:

Active immunization with tetanus toxoid in DPT vaccine and boosters every 10 years

Treatment:

Penicillin or metronidazole + antitoxin + debridement of wound

16. Clostridia other than Clostridium botulinum & Clostridium tetani

Clostridium Difficile

The organism is acquired from an exogenous source by a patient whose intestinal colonization resistance has been compromised by antibiotic exposure.

They generally appear as result of appliance of broad spectrum antibiotics which will make the intestinal flora disappear and make this species proliferate.

Pathogenic factors:

- toxin A: increased secretion but also stimulation of inflammatory response
- toxin B: disrupts protein synthesis and causes disorganization of cytoskeleton

Diseases:

- AAD (antibiotic associated diarrhea)
- Pseudomembranous colitis (seen better in endoscopy)

Both cause by cephalosporins, ampicilin + clindamycin

Treatment:

Discontinuance of drugs used + fluid replacement

Vancomycin or metranidazole added

Clostrisium Perfringens

They are G+ bacillus with blunt ends capsulated and non-motile. They are part of the normal flora of vagina and GIT.

Pathogenic factors:

- exotoxins (at lest 12) from which the most important α toxin, lecithinase which degrades mammalian cell membranes causing lysis of endothelial cells, WBC, RBC and platelets – cytotoxic and necrotic effect.
- enterotoxin: acts in loer portmon of small intestine by disrupting ion transport and leasing to loss of fluid and intracellular proteins.
- degradative enzymes: proteases, DNAses, collagenases which liquefy tissue and promote spread of infection

Diseases:

Gas gangrene: the disease is characterized by rapidly spreading oedema, myositis, necrosis of tissues, gas production and profound toxæmia occurring as a complication of wound infection.

The main source of the organisms is animal and human excreta, and spores of the causative clostridia are distributed widely.

It may be indirectly derived from dirty clothing, street dust, and even the air of an operating theatre if the ventilating system is poorly designed or improperly maintained. The skin often bears spores of *C. perfringens*, especially in areas of the body that may be contaminated with intestinal organisms.

Treatment: penicillin + metronidazole + aminoglycoside

Food poisoning:

The *C. perfringens* enterotoxin (CPE) mediating the disease is heat-labile (inactivated at 74°C) and can be detected in poorly prepared meat and poultry.

17. *Neisseria gonorrhoeae*

They are G- diplococci, piliated, aerobic, and they grow on ChA and split glucose.

Pathogenic factors:

- pili: confer resistance to phagocytosis, enhances attachment of organism to host epithelial cells and mucosal cell surfaces; they are also antigenic (>20 gonococcal genes code for Pili)
- IgA protease which cleaves IgA1 helping pathogen to invade.

Diseases: classical venereal disease, being almost exclusively spread by sexual contact.

- it most often colonizes mucous membrane of genitourinary tract or rectum
- genitourinary tract infection:

in males: yellow purulent urethral discharge and painful urination (acute urethritis)

in females: infection in endocervix to urethra and vagina, greenish yellowish cervical discharge and intermenstrual bleeding -> uterus -> pelvic inflammatory disease -> fibrosis. Acute salpingitis -> can lead to sterility

Rectal infections: most common in gay people -> constipation painful defecation, purulent discharge

Pharyngitis: purulent exudate -> sore throat

Ophthalmia neonatorum: infection of conjunctival sac, may lead to blindness

Lab ID:

Neutrophils that contain diplococci

Treatment:

Penicillin, 3rd generation cephalosporins

Erythromycin also treats chlamydia and is less toxic

18. *Neisseria meningitidis*

Many serogroups A, B and C: epidemic and outbreak cerebrospinal meningitis

They are non-motile, G- diplococci, piliated (allow attachment to nasopharynx mucosa where it is harbored in carriers and in those with meningococcal disease).

Pathogenic factors:

- polysaccharide capsule – antiphagocytic + antigenic:
 - LOS – 14 capsular polysaccharide types = serogroups
 - Outer membrane protein = serotype + LOS

19. *Salmonella typhi*

Among the host-adapted serotypes, Typhi and Paratyphi A, B and C are primarily pathogens of humans. All strains are placed under *S. enterica*, being *Salmonella Typhi*, one of the subtypes.

S. typhi is a facultative anaerobe, G- rod with H₂A red +, oxidase -, lactose - on endo agar.

Salmonella enters intestinal cell by endocytosis -> pass through endothelial cell to submucosa where they are taken up by macrophages -> macrophages carry *Salmonella* to reticulo-endothelial system where bacteria multiply -> Lymphoid hyperplasia and hypertrophy -> reenters bowel via liver and gallbladder

Salmonella antigens:

- Long-chain lipopolysaccharide (LPS) comprises O antigens

- Salmonellae are usually highly motile when growing in laboratory media and flagellar protein subunits contain the epitopes that form the basis of the flagella-based serotyping scheme generally known as the H antigens.

Certain serotypes of *S. enterica* express a surface polysaccharide, of which the Vi (virulence) antigen of *S. Typhi* is the most important example. Since the polysaccharide may encapsulate the entire bacterium, antibodies designed to recognize the LPS antigens may be prevented from binding, which can occasionally make detection of the O antigens difficult.

Diseases:

- Enteric fever: fever + abdominal symptoms (nonspecific symptoms: chills, sweats, headache, anorexia, weakness, sore throat and diarrhoea/constipation) incubation 5-21 days.
Complications: intestinal, hemorrhages and focal infections and endocarditis
- Paratyphoid fever: transmitted by ingestion of focal or water contaminated by human feces.

Treatment

β lactams and fluoroquinolones. Prevention is done by accomplishing proper sewage disposal, correct handling of food and good personal hygiene.

vaccination: Typhi, Paratyphi A and Paratyphi B (TAB) - for endemic level.

20. Salmonellae other than Salmonella Typhi

It comprises *S. enteritidis* and *typhimurium*. Their mechanism is the same mechanism as *S. typhi*.

Diseases:

- Enterocolitis: also called salmonellosis. It is characterized by nausea vomiting and diarrhoea usually non bloody which develops within 48h after ingesting contaminated food or water. In uncompromised patients, disease is generally self limiting (48-72h).
It may be acquired by eggs and poultry.

Treatment

Antibiotic are not needed and may prolong the convalescent carrier state.

21. Shigella

This is a G- rod, with Hajna +, oxidase - and lactose - on endo agar. They are non-motile and non-capsulated pathogens.

It is spread from person to person with contaminated stools as major source of organisms (flies and contaminated food also transmit the disease) - low infectious dose (because *Shigella* has innate tolerance to low pH).

40 serotypes of *Shigella* organized into 4 groups (A, B, C and D). Group D is *Shigella sonnei*.

Shigella enters intestinal cells by endocytosis through Payer's patches -> *Shigella* escapes from endocytic vesicles and multiplies inside cell protected from macrophages -> *Shigella* invades neighboring cells (lamina propria) -> mucosal abscess forms as cells die causing diarrhea with blood mucous and painful abdomen (patches of necrotic epithelium are sloughed and ulcers form).

Shigella invades and destroys mucosa of large intestine -> infection rarely penetrates deeper layers and does not lead to bacteriemia.

Pathogenic factor:

- exotoxin (Shigatoxin) - enterotoxin and cytotoxic effect (secondary role development of intestinal lesions)
- LPS - causes localized cytokine release and the resultant inflammatory response and cellular disruption enables bacteria to enter

Disease:

- bacillary dysentery (diarrhoea with blood, mucous and painful abdominal cramping)

Treatment:

Antibiotics like ciprofloxacin and ampicillin might be used to diminish duration of disease and period of shedding organism but use controversial.

Protection of water and flood supply and personal hygiene crucial!

Maintenance of hydration!

22. Escherichia coli

G- rod, facultative pathogen which grows in endo agar, with Hajna red +, oxidase -, lactose + on endo agar. Some strains are hemolytic.

They are part of the normal flora of colon but can be pathogenous both inside and outside the GIT. Serotyping based on 3 structural antigens: O (cell wall Ag), H (flagella Ag, only flagellated bacteria have it) and K (capsule + fimbria Ag).

E. coli pathogenic factors:

- Polysaccharides O and K protect the organism from the bactericidal effect of complement and phagocytosis in the absence of specific Ab.
- Many strains express haemolysin -> pathogenic mechanism for releasing essential ferric ions bound to hemoglobin.
- Expression of the aerobactin-mediated iron uptake system.

They are transmitted by oral-fecal route (indicator of fecal pollution of water sources).

- ETEC (Enterotoxigenic E. coli) - causes travelers' diarrhea
person to person, water or food contaminated by waste
colonizes small intestine and its enterotoxins cause Cl⁻ hypersecretion and water by intestinal mucosal cells and inhibits reabsorption of Na⁺ -> diarrhea
it has a heat-labile enterotoxin and a heat-stable enterotoxin (treatment with fluoroquinolones)
- EPEC (Enteropathogenic E. coli) -> cause of diarrhea in infants (infantile enteritis)
locations with poor sanitation and newborn infection during birth
EPEC attaches to the mucosa of intestines, destroys microvilli and develops characteristic lesion (Shiga-like toxin) - watery diarrhea
- EHEC (Enterohemorrhagic E. coli)
binds to intestines where it produces an exotoxin causing a severe form of copious bloody diarrhea without invasion or inflammation (serotype O157:H7)
potential fatal renal failure (in children - hemolytic uremic syndrome)
- EAaggEC (Enteraggregative E. coli)
chronic diarrheal disease in some developing countries
- EIEC (Enteroinvasive E. coli)
similar to Shigella infection

Extraintestinal diseases:

- UTIs: more common in female due to short urethra. High incidence in pregnant due to impairment of urine flow. In male, prostatic enlargement is a predisposing factor. They are caused by uropathogenic strain of E. coli (non-complicated UTI -> mainly cause ascending manner)
- neonatal meningitis: in 1st month of life
- nosocomial infections: sepsis, bacteremia...

Can be prevented by care in selection, preparation and consumption of food and water.

Treatment:

Extraintestinal diseases require antibiotics but susceptibility test has to be performed: ampicillin, cephalosporins, tetracyclines, quinolones...

23. Facultative pathogenic Enterobacteriaceae (other than Escherichia coli)

They are G- rods which grow on endo agar, Hajna red +, oxidase - and lactate + in endo agar. They are facultative pathogens.

They include: E. coli, Proteus, Serratia, Klebsiella

They generally don't cause primary disease in humans but are common nosocomial infections, specially in association with antibiotic treatment in dwelling catheters or invasive procedures.

Klebsiella

They are facultative anaerobic, non-motile but express fimbriae. They also possess K and O antigens and a large luxurious capsule (-> mucoid appearance).

Klebsiella pneumonia and Klebsiella oxytoca cause necrotizing lobar pneumonia in individuals compromised by alcoholism, diabetes or COPD. Klebsiella pneumonia might also cause UTIs and bacteremia particularly in hospitalized patients.

They grow through enterobactin-mediated iron-sequestering

Treatment

ampicillin, amoxicillin + β -lactamase inhibition

Serratia

Is a motile pathogen.

Serratia marcescens might infect humans and might cause lower respiratory infection, urinary tract infections, meningitis, septicemia, endocarditis.

Proteus

HAS RAUS PHENOMENON IN AGAR. They are motile pathogens and they cause nosocomial UTIs, wound infections, pneumonias and septicemias in compromised patients.

Urea is splitted (by urease) into ammonia and the resulting alkaline environment promotes precipitation of struvite stones.

24. *Pseudomonas aeruginosa*

They are G- rods glucose non fermenters, which grow endo agar, Hajona red -, oxidase + and produce green pigments. They are obligatory aerobic, non-capsulated and motile pathogens with a characteristic grape-like odour.

They are opportunistic pathogens and the major cause of nosocomial infections (pneumonia, UTIs, surgical site infections, infections of severe burns and patients undergoing chemotherapy or antibiotic. Worsens greatly the status of patients with cystic fibrosis).

In the community they may cause corneal infections due to contaminated lense, and jacuzzi rashes.

Pilli mediated adherence to media and glycocalyx capsule reduces effectiveness of cleaning mechanisms.

Host tissue damage facilitates this process. Toxins that promote local invasion and dissemination:

- exotoxin A
- exo-enzyme S
- proteases, phospholipases

Infections:

- localized: eye, ear, skin (wound sepsis), UTI (specially in catheterized patients), respiratory tract (pneumonia, congestive heart failure), GIT (diarrhoea), CNS (meningitis, brain abscess) - never disseminates
- systemic (mainly in immunocompromised patients): bacteriemia (in immunocompromised patients), secondary pneumonia, bone and joint infections, endocarditis, CNS and skin infections.

Treatment

Difficult to find antibiotics against due to its rapid development of resistance mutation. Aminoglycoside and β -lactam

25. *Pseudomonas mallei* & *Pseudomonas pseudomallei*

Pseudomonas mallei

They differ from genus because it's nonmotile.

Etiologic agent of glanders - serious inflammation that affects primarily horses, donkeys and mules - rarely humans.

In humans, clinical presentation depends on route of transmission. It may be:

- 1) Localize suppurative infection leading to mucopurulent discharge. Common: eye, lips, nose
- 2) Acute pulmonary infection (lung abscesses, lobar pneumonia)
- 3) acute septicemic infection

Pseudomonas pseudomallei

It is a normal inhabitant of soil and water. The transmission is done through wounds in skin, ingestion, and inhalation.

It causes melioidosis with varied presentations like *P. mallei*

26. *Campylobacter*

They are G- rod, not growing on endo agar, curved or spiral S shaped with a single polar flagellum grows in microaerophilic conditions. They grow on BA with antibiotics (to inhibit growth other fecal flora). It has O, H and K antigens.

They adapted to colonize mucous membranes and are able to penetrate mucous with facility.

They are transmitted via oral-fecal route or direct contact with contaminated water

Campylobacter jejunii:

- acute enteritis appears after 7 days incubation in healthy people and it is self-limiting. Symptoms may be either systemic (fever, headache and myalgia) and intestinal (cramps and diarrhea with or without blood)
- causes also travelers' diarrhea and pseudoappendicitis
- bacteremia may occur in children and elderly people

The jejunum and ileum are the 1st to become colonized, but the infection extends distally to affect the terminal ileum and usually the colon and rectum.

They are invasive, similar to Salmonella, Shigella or Yersinia infections.

Diarrhea should be treated with fluid and electrolyte replacement unless for patients with severe symptoms (->erythromycin)

Complications:

- reactive arthritis
- Guillain-Barre

27. Helicobacter pylori

They are G- rod, not growing on endo agar. They grow in microaerophilic environment and are transmitted from person to person.

H. pylori penetrates the mucous layer lining the stomach attracted to the chemotactic substances hemin + urea -> H. pylori recruits and activates inflammatory cells -> releases powerful urease: urea - ammonia which neutralizes acid secretion -> exposes underlying C. T. to stomach acid

- Patients taking antacid are more at risk
- Acute gastritis (may lead to gastric ulcers + duodenal)
- Untreated may become chronic, lifelong or even develop to gastric carcinoma and gastric B-cell lymphoma.

Lab ID

urea breath test +

urea splitting +

Treatment

2 or more antibiotics are applied due to rapid resistance of H. pylori, generally tetracyclin + metranidazole + bismuth salts.

28. Vibrio

They are G- which grow on endo agar with Hajna red +, oxidase +. They are motile by means of single flagellum, has O and H antigens but only O is important distinguishing strains that cause epidemics, facultative anaerobe, which the growth is stimulated by NaCl.

Vibrio cholerae is transmitted by contaminated food or water, there are 2 biotypes of other species: Classic and El Tor (hemolysin, higher carriage rates and the ability to survive in water for long time).

Vibrio cholerae infects the small intestine. The organism is non invasive and causes disease through the action of an enterotoxin.

Cholera toxin binds to a ganglioside receptor -> A subunit enters the cell membrane, activates Gs which will activate adenylate cyclase -> Adenylate cyclase produces elevated cAMP -> cAMP causes active secretion of ions and water. Inhibition of uptake of Na⁺ and Cl⁻ and hypersecretion of Cl⁻ and HCO₃⁻.

Diseases:

-Cholera: characterized by massive loss of fluid and electrolytes (vomiting and watery diarrhoea) from body incubation of hours to days is followed by a profuse watery diarrhoea. If untreated -> death from severe dehydration followed by hypovolemic shock will ensue.

Replacement of fluids and electrolytes is crucial in preventing shock.

Treatment

Doxyxylin

Vibrio parahaemolyticum will not grow in the absence of NaCl. Outbreaks of GIT disease (explosive diarrhoea, abdominal pain, nausea and vomiting) that results from ingestion of inadequately cooked seafood disease is self limiting and antibiotics don't alter cause of infection.

29. Haemophilus

They are G- rod, not growing on endo agar (Pasteurellaceae grows on chocolate agar).

H. ducrey: sexually transmitted disease, chancroid (painful penile ulcers). X factor only in BA (H. parainfluenza: V factor).

H. influenza: may be encapsulated (6 capsular types) or unencapsulated. Satellite phenomena is visible in BA with Staph. aureus. When cultured on ChA with factor V and X, if growing on both.

It has an important virulence factor -> serious invasive H. influenzae disease is associated with capsular type b.

Hib is particularly important in young children. Nonencapsulated strains can also cause pneumonia among elderly people and people with chronic lung disease..

Normal component of upper respiratory tract flora, may colonize conjunctiva and genital tracts - humans only natural hosts.

Pathogenic factors:

- IgA protease - degrades secretory IgA, facilitating colonization of upper respiratory tract mucosa -> H. influenza enters the blood stream and disseminates to distant sites.
- Fimbriae which assists the attachment to epithelial cells - outer membrane components.

Diseases:

- Otitis media, sinusitis, epiglottitis, bronchopneumonia (contagious spread from its site of colonization in respiratory tracts).
- Meningitis, septic arthritis, cellulitis (invasion of blood stream)

Treatment

- vaccine against Hemophilus influenza type b, administered to infants has helped decrease the frequency of bacterial meningitis (appeared with otitis media)
- 3rd generation cephalosporins - ceftriaxone, for meningitis and epiglottitis
- amoxicillin for sinusitis, otitis media and upper RTIs

30. Bordetella

They are G- rods which grows on BG (Bordet-Gengou) media, are encapsulated that grows singly or in pairs. They are aerobic pathogens, transmitted by droplets via coughing but the organism survives only briefly outside RTI.

Bordetella pertussis

pathogenic factors:

- pertussis toxin: lymphocytosis, sensitization to histamine, activation of insulin production - hypoglycemia.
- agglutinogens: promote attachment of bacteria to host cells
- dermonecrotic toxin: vasoconstriction and ischemic necrosis
- tracheal cytotoxin: inhibits cilia movement and regeneration of damaged cells.
- adenylate cyclase: decreases chemotaxis and phagocytosis of bacteria

Treatment

- Erythromycin
- DPT vaccine

Bordetella parapertussis

Grows on nutrient agar.

Pertussis incubation period is 2-3 weeks divided into 2 phases:

- catarrhal: rhinorrhea, mild conjunctival infection, malaise -> dry non productive cough
- paroxysmal: paroxysms of coughing followed by a whoop as patient inspired rapidly, causes leucocytosis

Convalescence requires at least 2-3 weeks where 2nd infections might occur.

31. Legionella

They are G- rods which grow on BCYE (Buffered Charcoal Yeast Extract), aerobic, facultative intracellular bacteria that causes respiratory tract infection.

Their natural habitat is soil and water (including cooling towers and water distribution system).

Legionella Pneumophila most important subtype, may be acquired by inhalation of aerosolized organisms but may also be due to swimming in contaminated water. It is not transmitted from person to person. They are chlorine resistant.

Organism enters upper RT by aspiration of water or inhalation of aerosol - failure to clear organism permits them to reach lungs alveolar macrophages in lung bed are an important line of defense - phagocytize L. pneumophila but phagosome doesn't fuse with lysosome - organism multiplies in phagosome until cell ruptures - new crop of bacteria.

Diseases:

- RTI
- Legionnaires disease: incubation 2-10 days. Atypical acute lobar pneumonia that develop in 1-5% of people exposed to common source. Many LD patients have symptoms: fever, malaise, confusion, hallucinations, myalgia, respiratory distress.
- Pontiac fever: influenza like that infects healthy people generally 90% of people exposed to same source are infected. 1 week - healed people, no therapy needed.

Treatment

macrolides, erythromycin, azithromycin

32. Brucella

B. abortus - cattle B. mellitensis - goat + sheep B. suis - suine B. canis - dog

All of them may cause disease in humans. Infections arise through direct contact with infected animals.

They are G- rods, aerobic, intracellular parasites that can survive and multiply within host phagocytes.

Caused by contact with cow, pig or goat or by consumption of dairy products. Brucella typically enters body by cuts and abrasions in skin or through GIT and eventually inhalation of aerosols among abattoir workers. Once organisms enter; they are transported via lymphatic system to reticulo-endothelial system and lymph nodes, spleen, bone marrow, liver, kidneys.

Disease

Brucellosis: incubation varies from 5 days to several months. Symptoms are nonspecific and flu like - malaise, sweats, fever, anorexia, GI symptoms, headache.

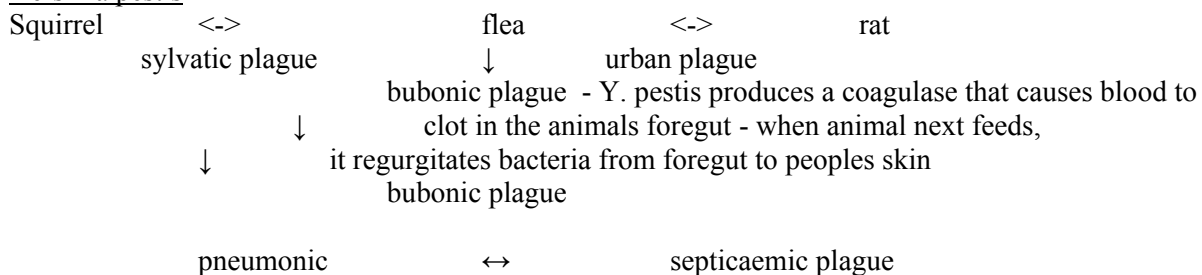
Treatment:

Doxycycline + gentamicin (6 weeks treatment necessary)

33. Yersinia

They belong to Enterobacteriaceae and are non-sporing, non-motile, facultative anaerobe, G- rods which grow on endo agar, lactose -, H₂S red +, oxidase -.

Yersinia pestis



The organisms are carried by lymphatic system to regional lymph nodes, where they are ingested by phagocytes. Organisms are resistant to killing by phagocytes so multiply inside of these cells. Bacteria released from lysed phagocyte has a new envelope antigen that confers increased resistance to phagocytosis. Affected lymph nodes - hemorrhagic necrosis

Diseases:

- Bubonic plague = pestis minor - 2-8 days incubation
lymph nodes become affected results in adenitis - typically localized in groin
but may spread to all parts of the body.
high fever + chills + headache + myalgia + weakness
Complications: bronchopneumonia, septicaemia, meningitis
- Pneumonic plague: purulent pneumonia that if untreated is fatal and might cause be contagious
- Plague meningitis: may occur following inadequately treated bubonic plague. Purpura may develop in the skin ("Black death") and disseminated intravascular coagulation .

Treatment

Intramuscular streptomycin and chloramphenicol for plague meningitis. NOT PENICILLIN.
Formalin killed vaccine should be given for people at high risk.

Yersinia Enterocolitica and pseudotuberculosis

Infection via ingestion of food that was contaminated - raw meat. It causes enterocolitis which results in ulcerative lesions in terminal ileum, necrotic lesions in Payers patches and enlargement of mesenteric lymph nodes. The symptoms are fever, abdominal pain and diarrhoea.

Treatment

Limiting potential contamination of meat.

Antibiotic therapy (ciprofloxacin) is essential for systemic diseases but questionable for endocarditis.

34. Francisella tularensis

It is a non-motile, non-sporing G- rods, strict aerobes with lipid rich capsule, intracellular facultative pathogens that can infect macrophages.

Wild, domestic mammals, birds and house pets can be infected. Biting or blood sucking arthropods can serve as vectors. Human infection occurs after contact with infected animal or bite of an infected arthropod.

Tularemia is an occupational risk for vets, hunters, trappers, domestic livestock workers and meat handlers.

It is a very infectious pathogenic bacteria (biologic weapon)

cutaneous inoculation -> papules that ulcerates after several days -> regional lymphatic nodes become larger -> skin, lungs, liver, spleen, kidney, CNS

Diseases:

- ulceroglandular tularemia
- glandular tularemia: no ulcer visible but lymphadenopathy
- pharyngeal tularemia: organism enters in pharynx causing sore throat
- oculoglandular tularemia: organism enters through conjunctiva - pneumonic tularemia

Treatment

Streptomycin

35. Bacteroides & other non-sporing anaerobes

They obligate anaerobes, non-sporing G- rods, rounded ends, predominant anaerobe in human colon, polysaccharide capsule (antiphagocytic effect).

They are transmitted from colon to blood or peritoneum after abdominal trauma (endogenous transmission).

B. fragilis is major disease causing bacteroides:

- if to blood: bacteremia occurs
- if to abdominal cavity: peritonitis and/or abdominal abscess

Treatment

Metronidazole, surgical drainage of abscess is essential! Cefoxitin might be administered before surgery to prevent bacteroidesinfection.

Prevotella meranigenicus

It is an anaerobe. Normal gingival flora, respiratory and alimentary up tract. They provoke oral abscessi, dental and sinus infection, pulmonary tract infection and abscess. brain abscess.

Heparinase leads to clotting in brain.

Treatment

Penicillin

Fusobacterium

They are G- rods, spindle shaped growing in mouth, female genital tract and colon. They cause peritonitis, abdominal abscesses, pelvic inflammatory disease, emphysema, necrotizing aspiration pneumonia, periodontal disease and Vincent's angina.

36. Treponema pallidum

Doesn't Gram stain because the wall is too thin - dark field fluorescence microscopy.

It secretes hyaluronidase (a substance that disrupts ground substance), It is transmitted wither by sexual contact or transplacentally (neonates may be infected during passage through the infected birth canal).

3 stages of syphilis:

1) 1st stage (3 weeks after infection): *T. pallidum* enters tissues by penetration of intact mucosae or through abraded skin. Hard chancre appears in the place where treponema entered the organism (typically external genitalia, cervix, peri-anal area or mouth) and spread to lymph nodes: formation of a primary complex. Hard chancre heals spontaneously.

asymptomatic period of 2-12 weeks

2) 2nd stage: there is hematogenous spread and a non specific rash appears in the body. You might have systemic involvement causing meningitis, glomerulonephritis or hepatitis. (highly infectious)

Latent period that can last many years (3-30 days) - no clinical manifestations are evident

3) 3rd stage: gummatous syphilis (granulomatous lesions of the skeleton, skin or mucocutaneous tissue) and neurosyphilis

Congenital syphilis: after 10-15 weeks of pregnancy, the fetus might be infected and might die or be aborted. Those who live have secondary syphilis.

Lab ID

	Screening tests	Confirmation
treponema test - antigen from treponema	MHA-TP - agglutination on carrier	ELISA + Western Blotting
non treponema test (with cardiolipin Ag)	RRR - rapid reagin test	

Treatment

Penicillin single treatment for primary + secondary syphilis

37. *Borrelia*

They have a linear rather than circular plasmid and chromosomal DNA.

Transmitted by Ixodes tick - becomes infected while feeding on infected animals and then bites man.

Borrelia Burgdoferi

Lyme disease is transmitted by a bite a Ixodes tick that needs to be attached at least for 24 hours before infecting reason. Primary reservoirs are deers and rodents.

1) stage: 3-32 days after bite, a characteristic red circular lesion with clear centre appears (erythema migrans appears). Flu like symptoms accompany this erythema. The organism spreads via lymph or blood to musculoskeletal sites, skin or heart.

2) stage: arthritis, arthralgia, cardiac and neurologic complications

weeks to months later

3) chronic arthritis and CNS disease

Rarely fatal but might cause poor level of life if untreated.

Lab ID:

IgG and IgM determination using ELISA and confirmation by W. blotting and PCR.

Treatment:

Tetracyclines, cephalosporines, penicillin

Borrelia Recurrentis

Responsible for disease relapsing fever which is characterized by several cycles of recovery (development of specific anti-spirochetes Ab) followed by relapse. The natural hosts for these organisms include rodents and other small mammals on which the ticks normally feed.

B. recurrentis is able to change its surface protein antigens (with each relapse a new antigenic variant appears).

Transmitted from human to human by body lice.

Lab ID

Diagnosis is made by appearance in Giemsa stain.

Treatment

tetracyclins, erythromycin and penicillin

38. Leptospira

They have an envelope composed of 3-5 layers of protein, polysaccharide and lipid covers the bacteria and is the main target for the host immune response.

Leptospira interrogans: Sensitive to drying and a broad range of disinfectants. Leptospirosis is primarily an animal disease that is transmitted to humans primarily by water or food contaminated with animal urine. The leptospira can also enter the body via small skin abrasions or the conjunctiva, mucous membrane.

1-2 weeks after infection fever occurs at which time spirochets appear in blood. After 1 week symptoms decrease. However in cases of biphasic diseases: spirochets reappear accompanied by invasion of liver, kidneys and CNS (results in jaundice, hemorrhage, tissue necrosis and or aseptic meningitis).

Vasculitis resulting in damage to the endothelial cells of small blood vessels is probably the main underlying pathology.

Lab ID

Diagnosis based on serologic agglutination test.

Penicillin + doxycyclin at first stage (24-48h) but later ineffective

39. Chlamydia

They are non-motile, small bacteria, which are obligate intracellular parasite depending on host cell for energy (ATP). They grow in cytoplasmic vacuoles. The genus is divided into: *Chlamydia Trachomatis* (genitourinary tract and eye diseases), *Chlamydia passitaci* and *C. pneumonia* cause infection in various levels of RT.

Treatment

Macrolide and tetracyclin sensitive

Extracellular infections form - elementary body (is the only infectious stage of the chlamydil developmental cycle), can survive extracellular cell to cell passage and initiate infection.

Growth cycle: elementary body taken up by phagocytosis into susceptible host cells (facilitated by proteins in chlamydial cell envelope that function as adhesins) -> elementary body prevents fusion of phagosome with lysosome - particle reorganizes in the next 8h into larger non infectious reticulate body - metabolically active non-infectious and will divide repeatedly by binary fission within cytoplasm of host cell -> inclusion body -> after 48h multiplication ceases and reticulate body condenses to become new elementary body -> elementary bodies are released from cells by cytoplasmic ending in host cell death.

Chlamydia trachomatis

Major casual agent of non gonococcal urethritis and can also cause eye infections with symptoms ranging from irritation to blindness, ectopic pregnancy or infertility in pelvic inflammatory disease.

NGU (non gonococcal urethritis) serotypes D-K - young sexually active individuals, in males the urethra is the principal site of infections while in females, cervicitis and urethritis are more common.

Cervicitis - fallopian tubes infection - pelvic inflammatory disease

Same symptoms as gonorrhea but longer incubation, more mucoid and less purulent reaction. This progresses to epididymitis or prostatitis.

LGV (lymphogranuloma venereum) - more invasive sexually transmitted disease transient papules in external genitalia -> (1-2 months) painful swelling of inguinal and perirectal lymph nodes -> affected lymph nodes suppurate and chronic inflammation and fibrosis lead to ulceration and blockage of lymph drainage - peroscrotal elephantiasis

Trachoma

Chronic keratoconjunctivitis that often results in blindness. It is transmitted by personal contact.

Neonatal conjunctivitis: acquired while passing through birth canal.

Lab ID:

direct test by microscopic examination using direct fluorescent Ab that reveal cellular cytoplasmic inclusions.

Treatment

azithromycin and tetracyclin

40. Rickettsia, Coxiella & Ehrlichia

3 things in common:

- grow only inside living host cells (obligatory intracellular pathogen)
- most are transmitted by infected arthropods
- rickettsial diseases are generalized infections with rash sometimes being a predominant factor.

Rickettsia

Small, coccobacillary, double layered G- cell wall - poor staining so generally visualized with giemsa or macchiavelo.

The plasma membrane is leaky - permeable to host cell nutrients and coenzymes. Following the bite of an arthropod (rat, fleas, ticks, lice...), the organisms are disseminated by blood and are taken into cells by induced phagocytosis where the organism multiplies in nucleus and cytoplasm of host cells. They exit the cells like Listeria (by mobilizing host cell actins) - host cells are killed - rickettsia spreads via blood stream or lymphatics where focal thrombi may form, even in skin, small hemorrhages and hemodynamic disturbances.

Rocky Mountain Spotted fever: caused by Rickettsia Rickettsii

- caused by infected wood, dog or tick generally during warm months
- symptoms: 7 days after, high fever, malaise, severe headache, myalgia, anorexia, followed by first macular then petechial rash (on extremities palms or soles -> whole body).
- if untreated, vascular disturbances, myocardial or renal failure may occur.

Other Spotted fevers: they vary in severity. A very specific one is rickettsial pox caused by Rickettsia akari for which the vector is a mite. At the site of biting an eschar is formed -> scattered papulovesicles during few days.

Typhus:

- EPIDEMIC TYPHUS: initial symptoms (headache and fever) start 6-15 days after being exposed to R. prowazellii. A macular rash, often noted 4-7 days after patients become ill, first appears on the trunk and axillary folds and then spreads to the extremities but in more severe cases it may last much longer and become haemorrhagic. The mental state of the patient may progress from dullness to stupor and, in very severe cases, even coma.
- SCRUB TYPHUS: human infection with scrub typhus rickettsiae may be mild or fatal. Symptoms develop 6-18 days after being bitten by infected mite larvae (chiggers). An eschar is often apparent at the site of the bite with enlargement of focal lymph nodes. Progression of the disease may be accompanied by interstitial pneumonitis, generalized lymphadenopathy, splenomegaly and rash. Death may result from encephalitis, respiratory failure and circulatory failure.

Treatment:

Doxycyclin except for pregnant women (chloramphenicol)

Ehrlichia

They are G- bacilli which parasitize leucocytes and grow in cytoplasmic vacuoles, creating morulae (inclusions).

2 of its tick born forms are known:

- human monocytic ehrlichiosis (HME) caused by E. chaffeensis
- human granulocytic anaplasmosis (HGA) caused by E. equi

They cause acute fever, myalgia, moderate to severe leucopenia and thrombocytopenia.

Treatment

Doxycyclin

Coxiella

They are obligatory intracellular, G- bacilli

Coxiella burnetii - causes Q fever

- 1) it can resist hot degradative enzymes within cytoplasmic vacuoles
- 2) they are resistant to heat and drying and also can persist outside the host for a long time
- 3) human infection is caused by inhalation of infected dust in barnyards, slaughterhouse
- 4) It reproduces in respiratory tract

Treatment

Doxycyclin

41. Mycoplasma pneumoniae

It lacks cell wall, can't be classified as cocci or rod, smallest of known living things.

They are facultative anaerobes which are transmitted by respiratory droplets and causes primary atypical (nonpurulent) pneumonia and milder infections such as bronchitis, pharyngitis and non purulent otitis media.

More common in older children and young adults (6-20 years)

Pathogenic factors:

- P1 (citoladhesin): in specialized organelle which binds sialic acid rich glycolipids found in some host cell membranes (e.g. ciliated bronchial epithelial cells) - organism grows attached to host cell luminal surface and inhibit ciliary action - patches of affected mucosa desquamate - inflammatory mucosa with mononuclear infiltrate starts.

Extrapulmonary manifestations include:

- arthralgia
- meningitis or encephalitis
- haemolytic anaemia
- myocarditis
- pericarditis

Main damages are created by host cells, not by the mycoplasma.

Primary atypical pneumonia: on onset: headache, fever, chills, malaise - dry cough and earach.

Without immune problems disease permits after 3-10 days.

Treatment

Doxycyclin or azythromycin - may persist in coalescence upper RT for week

42. Urogenital mycoplasmas

Mycoplasma Hominis, Ureoplasma ureolyticum, Mycoplasma genitalium are common in urogenital tract.

They can be distinguished by their carbon utilization patterns.

M. hominis - arginine Ureoplasma ureolyticum - urea

M. hominis: found in the vagina of women who have bacterial vaginosis; it may lead to pelvic inflammatory disease. It causes postpartum or postabortal fever - resistant to erythromycin but tetracyclin sensitive.

U. ureolyticum: urethritis when neither gonococcus nor chlamydia can be demonstrated in males.

Endometritis and vaginal secretions of women who undergo premature labor or deliver low birth weight babies.

VIROLOGY

1. Composition of viruses

Viruses are complexes consisting of only proteins and nucleic acids (DNA or RNA).

They lack cellular structures and independent metabolic processes - therefore they have no metabolic system and so depend on synthetic mechanism of a living cell.

Viruses deliver their nucleic acid into a host cell and proceed to produce components of new viruses in accordance with the genetic information they contains. They can infect bacteria (so called bacteriophages) plants, animals and humans.

Many viruses have an envelope composed of a protein containing lipid bilayer, whose presence or absence further distinguishes on virus group from another.

A mature virus particle is also known as a virion.

It consists of:

- Genome of DNA or RNA
- Capsid - virus coded proteins enclosing the nucleic acid of the virus, determining its antigenicity. The capsid can have a cubic (rotational), helical or complex symmetry and is made of subunits called capsomers. In some cases an envelope surrounds the capsid, and it is always delivered from cellular membrane.
- Enzymes - e.g. neurominidase, required for invasion and release of myxoviruses; DNA polymerase in small pox virus.

There are NA and DNA viruses. The nucleic acid of DNA virus is usually double-stranded and linear or circular depending on the family. The nucleic acid of RNA viruses is usually single stranded with the exception of reoviruses.

Viruses with ssRNA are divided into 2 groups:

1. Sense/ positive strand virus: if genome RNA has same polarity as viral mRNA and can thus function directly as mRNA then it is called positive strand or sense strand.
2. Antisense/negative strand virus: if genome RNA has polarity opposite to that of mRNA they have first to be transcribed into a complementary strand, before it is translated into proteins.
3. The primary characteristic of virus is that replication is obligatorily intracellular

2. Virus-cell interactions

A virus-cell interaction is a very quick process involving fusion of the viral envelope with cell membranes.

Productive interactions: viruses that infect and replicate within cells causing cells to lyse when the progeny virions are released (this is known as the cytolitic cycle).

Non-productive interactions: viruses that infect cells but do not complete the replication cycle.

The host cell is termed permissive or non-permissive depending on the outcome of the virus-cell interaction.

Interaction of viruses with cells can result in:

- production of new virus particles with or without lysis of host cells
- abortive infection (a non-productive infection)
- latency, where the virus exists with limited expression of viral genes

Cytolitic growth cycle - when virus particles come into contact with the host cells, part of the capsid binds to a specific receptor to initiate entry into the host cell. The virion then enters or penetrates into the host cell and is partially uncoated to reveal the viral genome, and so macromolecular synthesis of virus components can take place.

Early mRNA is first transcribed and translated into proteins. The newly formed virus particles are assembled and released during cell lysis.

3. Antiviral Agents

Drugs	Site of action
Amantadine	Inhibition of early events (entry or uncoating of the virus)
Aciclovir, Ganciclovir, Ribavirin	Inhibition of viral nucleic acid synthesis

Drugs	Site of action
Saguinavir, Indinavir	Cleavage of precursor polypeptides
Interferons (naturally occurring antiviral compounds)	Inhibition of protein synthesis

Inhibitors of herpes virus DNA Polymerases

- Aciclovir, established record in treatment of HSV and V3V disease
- Ganciclovir, works against cytomegalovirus
- Ribavirin, for respiratory syncytial virus infection

note: action exerted mainly during acute phase of viral infections

Inhibitors of viral uncoating

- Amantadine, blocks ion channel formed by integral membrane protein

Inhibitors of neuraminidase

- Sanguinavir, blocks action of influenza virus neuraminidase

4. Interferons

Type I: IFN α , IFN β : produced by the virus infected cells (fibroblasts, macrophages). In the target cells they inhibit viral replication by blocking translation of viral protein.

Type II „Immune“: IFN γ : produced by activated T_H 1 cells in response to a specific antigenic signal, causes activation of macrophages.

IFN- α and IFN- β are produced in response to the presence of viruses and certain intracellular bacteria. Double-stranded RNA may be the important inducer.

To exert their biological effects these molecules must interact with cell surface receptors. IFN- α and IFN- β share a common receptor while IFN- γ binds to its own specific receptor. After binding to the cell surface receptors, interferons act by rapidly and transiently inducing or up-regulating some cellular genes and down-regulating others. The overall effect is to inhibit viral replication and activate host defence mechanisms.

They can inhibit many stages of the virus life cycle - attachment and uncoating, early viral transcription, viral translation, protein synthesis and budding.

Some viral proteins can inhibit the interferon response

5. Humoral immunity to viral infections

Antibodies cannot enter cells, and therefore are ineffective against latent viruses and those that spread directly from cell to cell. They will, however, bind to extracellular viral epitopes. These epitopes can be on intact virions or on the surface of infected cells. The binding of antibody to free virus can inhibit a number of processes essential to virus replication. Antibodies can block binding to the host cell membrane, and thus stop attachment and penetration.

Antibody can also work at stages after penetration. Uncoating, with the release of viral nucleic acid into the cytoplasm, can be inhibited if the virion is covered by antibody.

Antibody can also cause aggregation of virus particles, thus limiting the spread of the infectious particles and forming a complex that is readily phagocytosed.

In some infections, viral proteins remain on the surface of the cell after entry or become associated with the cell membrane during replication. Antibodies against these molecules can cause cell lysis by the classical pathway but an intact alternative pathway is necessary to amplify the initial triggering by the antibody-dependent pathway.

In many situations, viruses seem to be able to escape the humoral defence mechanisms. Some viruses become latent, e.g. herpesviruses, and are reactivated despite the presence of circulating antibody, as they can pass directly from cell to cell. Other escape mechanisms include antigenic variation in which the

antigenic structure of the virus (e.g. influenza type A) changes so that antibodies formed to the previous strain are no longer effective.

In viral infections the efficiency of antibody depends largely on whether the virus passes through the blood-stream outside host cells to reach its target organ.

In comparison, in viral diseases such as influenza and the common cold, the viruses do not pass through the bloodstream. In this type of infections a high level of antibody in the blood will be relatively ineffective in comparison with its effect on blood-borne viruses. In this case the antibody must be present in the mucous secretions at the time of infection.

Humoral immunity does play a major protective role in polio and a number of other viral infections, and is probably the predominant form of immunity responsible for protection from reinfection.

6. Cell-mediated immunity to viral infections

The destruction of virus-infected cells is an important mechanism in the eradication of virus from the host. The destruction of an infected cell before progeny particles are released is an effective way of terminating a viral infection. For this process to occur the immune system must recognize the infected cell.

As viral proteins are synthesized within the cell some of these molecules are processed into small peptides. These endogenously produced antigen fragments become associated with MHC class I molecules, and this complex is then transported to the cell surface where it acts as the recognition unit for cytotoxic T (T_C) lymphocytes. Once these T_C cells have bound to the infected cell they release molecules that induce apoptosis.

$CD4^+$ and $CD8^+$ T cells can produce various lymphokines when stimulated by antigen. These will include molecules that are active in the elimination of virus, eg. IFN- γ and TNF and others that generally increase the effectiveness of the immune system by attracting cells to the site of infection, stimulating the production of more cells and supporting their growth. Macrophages will be activated, and this will lead to enhanced microbicidal activities and the production of monokines.

7. Immunopathology in viral infections

Viruses have evolved a multitude of mechanisms for exploiting weaknesses in the host immune system and avoiding, and sometimes actually subverting, immune mechanisms.

One of the most important strategies developed by viruses is to infect cells of the immune system itself. Many common human viruses, including rubella, mumps, measles and herpes viruses, infect cells of the immune system, as does the human immunodeficiency virus (HIV). The consequences of viral infection of cells of the immune system have been categorized in two ways:

1. Infections that cause temporary immune deficiency to unrelated antigens and sometimes to the antigens of the infecting virus.
2. Permanent depression of immunity to unrelated antigens and occasionally to antigens of the infecting virus.

Viruses have also developed other mechanisms to avoid the immune system. These include:

- Antigenic variation
- Release of antigens
- The production of antigens at sites that are inaccessible to the immune system

A virus will remain relatively safe from immune destruction if it remains within the cell and allows only very low or no viral antigen expression on the infected cell membrane.

Viruses that move from cell to cell without entering the extracellular fluids will also escape the action of antibodies, as will those passed from one cell to another by cell division.

A number of infections continually shed virus into external secretions, such as saliva, milk or urine. As long as the infected cell only forms virus on the luminal surface of the mucosa then cells of the immune system and antibody will be unable to destroy the infected cell. IgA present in the secretions may neutralize the virus, but this class of antibody does not activate complement efficiently so the cell will not be lysed.

Susceptibility to infection is generally greater in very young and very old people because of a weaker immune response. However, the immunopathology tends to be less severe.

Physical and physiological differences may also contribute to age-related disease susceptibility. Certain viral infections produce a milder disease in children than in adults e.g. varicella.

Antigenic variation

A micro-organism can avoid the acquired immune response by periodically changing the structure of molecules that are recognized by the host immune system. The micro-organism will only be able to change a component in a way that does not alter the functioning of the molecule.

Antigenic variation is likely to be an important viral adaptation for overcoming host immunity in long-lived species such as humans where there is a need for multiple re-infection of the same individual if the virus is to survive and the virus is unable to become latent.

Persistence of virus

Certain viruses give rise to a persistent infection, which is held in check as long as the immune system remains intact. In other persistent infections, the immune system contributes to the pathology of the disease.

8. Adenoviruses

Are non-enveloped DNA viruses, with icosahedral nucleocapsid and a single piece of double-stranded DNA. They belong to the Adenoviridae family, which consists of:

- Mastadenovirus – infects mammals
- Aviadenovirus – infects avian species

Epidemiology:

- The site of clinical syndrome is generally related to the mode of virus transmission. Most adenoviruses are primary agents of respiratory diseases and asymptomatic diseases of the intestine and can be isolated from stool of both healthy and people after they had a respiratory problem. Two adenovirus subtypes are associated with GI disease and are transmitted by fecal-oral route.
- Ocular infections are transmitted by direct inoculation of the eye by virus-contaminated hands, ophthalmic instruments.

Replication:

1. Virus attaches to host cell receptors via knobs on the tips of viral fibres → entry into cell by cell-mediated endocytosis → viral genome is uncoated whilst being transported to nucleus → in nucleus there is transcription of viral genes, genome replication and assembly occurs. The adenovirus encodes for early proteins such as:
 - DNA polymerase and others that affect transcription and replication of viral genome.
 - The ones that inactivate p53 and pRb that prevents progression towards the cell cycle
2. In the end of the productive cycle, the virus kills the host cell as cellular DNA, RNA and protein synthesis are all shut off during infection.

Respiratory tract diseases:

- Infants and young children → acute febrile pharyngitis (symptoms: cough, fever, sore throat, nasal congestion)
- Pharyngoconjunctival fever → same and conjunctivitis (can progress to viral pneumonia)
- Acute respiratory syndrome → common among military recruits

Adenovirus infections are said to mimic whooping cough in some patients and dual infections with *Bordetella pertussis* have been found.

Ocular diseases (usually associated with respiratory symptoms)

- Follicular conjunctivitis → self-limiting, no permanent sequelae
- Epidemic keratoconjunctivitis → corneal epithel also involved and may be followed by corneal opacity

GI diseases

- Infantile gastroenteritis → viral diarrhoeal disease (serotypes 40 and 41)

UT disease

- Hemorrhagic cystitis → hematuria and virus can usually be identified in urine

Lab ID

- Not done generally but may be done in epidemics or nosocomial outbreaks
- GIT type: ELISA direct test of stool specimen

- Serotype can be identified by agglutination inhibition using type specific antisera

Treatment

- Live attenuated adenovirus vaccine is used for protection of military population
- No antibiotics

9. Herpes simplex virus

Latent infection - a type of persistent infection in which the viral genome is present but infectious virus is not produced except during intermittent episodes of reactivation.

Reactivation – reactivation from the latent state may be restricted to asymptomatic virus shedding.

Recurrence or recrudescence – when reactivated virus produces clinically obvious disease.

Outside the capsid in mature particles is an amorphous proteinaceous layer, the tegument, surrounded by a lipid envelope derived from cell membranes.

The genome of herpes virions is linear double-stranded DNA.

The family Herpesviridae comprises three broad groups:

- Alphaherpesviruses, e.g. HSV, VZV; rapid growth, latency in sensory ganglia
- Betaherpesviruses – CMV; slow growth, restricted host range
- Gammaherpesviruses, e.g. EBV; growth in lymphoblastoid cells

Replication

After attachment to receptors, the envelope of herpes virions fuses with the cell membrane. The nucleocapsids cross the cytoplasm to the nuclear membrane; replication of viral DNA and assembly of capsids take place within the nucleus. With HSV, it is known that tegument protein transactivates expression of the first set of genes.

These proteins are of 3 types:

1. Immediate early (α) – mainly regulatory functions
2. Early (β) – includes many enzymes involved in DNA replication.
3. Late (γ) – structural proteins of capsid, glycoproteins

Herpes Simplex Virus

There are two distinct types of HSV, named type 1 (HSV-1) and type 2 (HSV-2). These two types are generally associated with different sites of infection in patients; type 1 strains are associated primarily with the mouth, the eye and the central nervous system, while type 2 strains are found most often in the genital tract.

Pathogenesis

Primary infection

The typical lesion produced by HSV is the vesicle. The underlying layer of basal epithelium is usually intact, as vesicles only occasionally penetrate the subepithelial layer. The roof of the vesicle breaks down, and an ulcer forms: this happens rapidly on mucous membranes and non-keratinizing epithelia; on the skin the ulcer crusts over, forming a scab, and then heals.

After resorption or loss of the vesicle fluid the damaged epithelium is regenerated. Natural killer cells play a significant role in early defense by recognizing and destroying HSV-infected cells.

During replication phase at the site of entry in the epithelium, virus particles enter through the sensory nerve endings and are transported along the axon to the nerve body in the sensory (dorsal root) ganglion by retrograde axonal flow. In some ganglion cells a latent infection is established in which the neurons survive but continue to harbor the viral genome.

Latent infection

In latency, viral DNA exists as free circular episomes. Very few virus genes are expressed in the latent state.

HSV-1 is regularly detected in:

- The trigeminal ganglion
- Other sensory and autonomic ganglion (e.g. vagus)
- Adrenal tissue and in the brain

HSV-2 latency in the sacral ganglia has been demonstrated. Either type may become latent in other ganglia.

Reactivation and recrudescence

Herpes viruses' DNA passes along the nerve axon back to the nerve ending where infection of the epithelial cells may occur.

The interval between the stimulus and the appearance of clinically obvious lesions is 2-5 days.

Oral infection

Classically, the first infection presents as an acute, febrile gingivostomatitis in children. Vesicular lesions ulcerate rapidly and are present in the front of the mouth and on the tongue. Gingivitis is usually present. Vesicles may also develop on the lips and skin around the mouth and cervical lymphadenopathy occurs. There may be an associated mononucleosis in the older patient; pharyngitis is also notable.

Skin infection

Herpetic whitlow: Hand infections with HSV are not uncommon. Three presentations may be seen:

- The classical primary lesion on the fingers or thumb of the toddler with herpetic stomatitis, due to autoinoculation
- Accidental inoculation in health care workers.
- Associated with HSV-2 and genital herpes and seen in young adults

Eczema herpeticum: a severe form of cutaneous herpes. Extensive ulceration results in protein loss and dehydration, and viremia can lead to disseminated disease with severe, even fatal, consequences.

Eye infection

There may be conjunctivitis, or keratoconjunctivitis associated with corneal ulceration. The presence of typical herpes vesicles on eyelid margins is a useful clinical guide but is not always seen. The majority of eye infections are HSV-1.

Central nervous system infection

HSV may reach the brain in several ways. Viremia has been detected during primary herpetic stomatitis, and infection may be carried within cells into the brain and meningitis.

HSV encephalitis (HSVE): this is a rare condition, but is the commonest sporadic fatal encephalitis recognized in developed countries. Fever and malaise is followed by headache and behavioral change sometimes associated with a sudden focal episode such as seizure, or paralysis; coma usually precedes death.

Cerebrospinal fluid (CSF) collected in the acute stages of HSVE should be sent to a laboratory.

Genital tract infection

Genital infection may be acquired by auto-inoculation from lesions elsewhere on the body, but most often results from intimate sexual contact, including oralgenital contact. The lesions are vesicular at first but rapidly ulcerate:

- In the male, the glans and shaft of the penis are the most frequent sites of infection
- In the female, the labia and vagina or cervix may be involved

Neonatal herpes

HSV-2 is acquired by passage through an infected genital tract at birth. Virus dissemination to internal organs is the most serious complication, in which the infant shows signs of general sepsis, including fever, poor feeding and irritability. Pneumonia and jaundice develop, with or without signs of meningitis or encephalitis.

Lab ID:

Inoculation of human cell tissue culture with a sample of vesicle fluid or genital swab is the method to demonstrate presence of HSV.

Infected cells can be detected within 24h by use of immunofluorescence or immunoperoxidase with Ab against viral early proteins.

Treatment:

Acyclovir (or famciclovir, and topical penciclovir)

10. Varicella-zoster virus

Infection with VZV presents in two forms:

- The primary infection varicella (or chickenpox) is a generalized eruption
- The reactivated infection zoster (or shingles) is localized to one or a few dermatomes

The enveloped virions released from the nucleus remain closely attached to microvilli along the cell surface and the infection being passed from cell to cell.

Pathogenesis

Varicella

This is a disease predominantly of children. characterized by a vesicular skin eruption. Virus is thought to enter through the upper respiratory tract, or conjunctivae, and multiply in local lymph tissue for a few days before entering the blood and being distributed throughout the body. Following replication in reticulo-endothelial sites, a second viraemic stage precedes the appearance of the skin and mucosal lesions.

The clearance of virus-infected cells is dependent on functional cell-mediated immune mechanisms, cytotoxic T cells and antibody-dependent cell cytotoxicity in particular.

Clinical features: the incubation period averages 14-15 days. The patient is infectious for 2 days before and up to 5 days after onset, while new vesicles are appearing. Initially macular, the rash rapidly evolves through papules to the characteristic clear vesicles. Secondary bacterial infection of skin lesions is the commonest complication.

A variety of organs may be affected, producing myocarditis, arthritis, glomerulonephritis and appendicitis. The two most frequent problems are related to the lungs and the central nervous system.

PNEUMONIA: viral pneumonia is a most serious complication. Cough, dyspnoea, tachypnoea and chest pain begin a few days after the rash.

CENTRAL NERVOUS SYSTEM: neurological complications include the common but benign cerebellar ataxia syndrome.

VARICELLA IN PREGNANCY: varicella virus can cross the placenta following viraemia in the pregnant woman, and infect the fetus. Two types of intra-uterine infection are noted:

1. The fetal varicella syndrome is a consequence of fetal infections with VZV in the first half of pregnancy.
2. Neonatal (congenital) varicella occurs when varicella develops within the first 2 weeks of life, following maternal varicella in late pregnancy.

Zoster

The latent virus is found in neurones and in satellite cells in sensory ganglia, and more than one region of the genome is transcribed.

It seems likely that virus reaches the ganglion from the periphery by travelling up nerve axons, as HSV does, but there is also the possibility that during viraemia some virus enters ganglion cells.

Reactivation of VZV manifested as zoster can occur at any age in a person who has experienced a primary infection.

More than one episode of zoster is uncommon in any individual. The stimulus to reactivation is not known, nor the details, but virus does appear to travel from sensory ganglia to the peripheral site. The zoster is usually limited to one dermatome.

Clinical features: prodromal paraesthesia and pain in the area supplied by the affected sensory nerve are common before the skin lesions develop. The evolution of the rash is similar, with some new vesicles appearing while the earliest ones are crusting.

POSTHERPETIC NEURALGIA: this is the most common complication of zoster. It is defined as intractable pain persisting for 1 month or more after the skin rash. Constant pain at the site, or stabbing pains or paresthesiae may continue over 1 year or much longer.

OPHTHALMIC ZOSTER: involvement of the ophthalmic division of the trigeminal nerve occurs in up to one-quarter of zoster episodes, with ocular complications. Corneal ulceration, stromal keratitis and anterior uveitis may result in permanent scarring, so this complication may threaten sight when the nasociliary branch is involved. Ramsay-Hunt syndrome (facial palsy with auricular zoster vesicles) suggest that motor neurones can also be involved.

Lab ID:

Reacting epithelial cells scraped from base of vesicles with the strains described in HSV or hybridization with specific VZV DNA probes.

Treatment:

IV administration of acyclovir

Vaccine: live attenuated vaccine for children older than 1 or non immune adults at risk of being exposed

11. Epstein-Barr virus

EBV primary infection is:

- Most often asymptomatic and occurs early in childhood
- The classical infectious mononucleosis (glandular fever) of adolescents in the developed world

This virus cannot be grown in human fibroblast or epithelial cell lines. This lymphotropic virus is classified as a gammaherpesvirus.

The full replication cycle of EBV is now known to take place in certain differentiated epithelial cells. EBV receptors are expressed on mature resting B lymphocytes and similar receptors are present on cells of stratified squamous epithelium – in the oropharynx, salivary glands and ectocervix.

The latent state of EBV infection is maintained in a subset of resting memory B lymphocytes, and perhaps in certain epithelial cells. Specific EBV early RNA species are found in all cells infected with the virus. A variable number of EBV genes are expressed in the latent state.

Pathogenesis:

Infection of oropharyngeal epithelial cells occurs initially, then infection of B lymphocytes, which disseminate through the circulation, with the potential to enter a productive phase and release virus elsewhere in the body. Most shedding of virus, however, takes place in the oral cavity.

A proportion of infected B lymphocytes undergoes transformation and continues to proliferate. Activated B lymphocytes secrete immunoglobulin, and EBV is a potent polyclonal activator of antibody production by B cells.

Recovery from primary EBV infection is associated with humoral and cellular response. Thus, large initial infective doses result in high number of circulating infected B lymphocytes.

The cytotoxic elements carry the ability to kill EBV-infected cells.

Infectious mononucleosis:

The disease known as infectious mononucleosis or glandular fever is a primary EBV infection seen predominantly in the 15-25 year age group. The onset is abrupt with a sore throat, cervical lymphadenopathy and fever, accompanied by malaise, headache, sweating and gastrointestinal discomfort. Pharyngitis may be severe, accompanied by a greyish-white membrane and gross tonsillar enlargement.

Complications of glandular fever:

Acute airway obstruction, splenic rupture (rare) and neurological complications include meningitis, encephalitis and the Guillain-Barre syndrome.

Other EBV-associated disease, tumors and immunodeficiency:

EBV is associated with an increasing number of diseases, including malignant tumors.

Lab ID:

Atypical lymphocytes may be observed in the blood smear of a person with IM.

Paul-Bennell test based upon the fact that polyclonal stimulation of B cells by EBV results in non-specific elevation of all Ig (agglutination test).

Treatment:

Acyclovir, but only inhibits replication of EBV.

12 Cytomegalovirus

It is a member of the β -herpesviridae family. The full name for the virus infecting humans is human cytomegalovirus (HCMV). The name was chosen on account of the swollen state of infected cells as seen in culture and in tissues. The replication cycle is significantly larger and the nuclei of productively infected cells contain a large inclusion body, giving a typical appearance.

In vivo the virus replicates in epithelial cells in salivary glands, the kidney and in the respiratory tract.

Pathogenesis

Generally it is acquired during childhood (majority of people have Abs against it by adulthood).

- Usually asymptomatic infection and children shed viruses by body fluids like tears or saliva, and infection occurs by intimate contact with these fluids.
- In adults, the virus can be also transmitted by semen and vaginal secretions, by organ transplants (specially of Ab+ donors, because the presence of Abs implies the presence of persistent virus), or by blood donors and breast milk.
- HCMV can also cross the placenta and infect the fetus in utero.

- CMV persists in the host for life. Recurrent infections may follow reactivation of latent (endogenous) virus, or re-infection with another (exogenous) strain. Latency is probably established in monocytes and macrophages but also in kidneys and liver.

MONONUCLEOSIS: primary infection as an adult is infectious mononucleosis (hepatitis, fever and lymphocytosis but pharyngitis and lymphadenopathy are unusual) The difference would be the absence of heterophile Abs. Can be associated with HIV infection.

CYTOMEGALIC INCLUSION DISEASE: HCMV is the most common intrauterine virus infection. Of infants born from women experiencing their first CMV infection during pregnancy, 35-50% will become infected. This problem ranges from fetal death to various degrees of liver, spleen, blood forming organs or CNS damages (retardation, teinites, encephalitis...).

INFECTION IN THE COMPROMISED PATIENT: the complications of CMV in cellular immunodeficiency include: pneumonitis, encephalitis, retinitis, oesophagitis/colitis, hepatitis, pancreatitis or adenitis.

Host responses

The host response to primary CMV includes IgM, IgG and T cell responses. CMV early genes transactivate other viral and cellular genes and this may be an important interaction with HIV, leading to the production of HIV from latently infected cells. Because CMV infects mononuclear cells, there is a degree of immunosuppression associated with the acute infection.

from recurrent infection by IgG seroconversion or HCMV specific IgM.

Lab ID

ELISA will distinguish primarily

Treatment:

Inhibitors of HCMV DNA polymerase: ganciclovir (generally), cidofovir (retinitis).

13. Poxviruses

It belongs to the family Poxviridae. Poxviruses are the largest animal viruses. The DNA is enveloped and their virions are big enough to be seen as dots by light microscopy. Its replication occurs in cytoplasm.

It caused variola or smallpox, which is the first infectious disease to be declared as eradicated from the world (due to availability of very effective attenuated vaccine, stable antigenic structure of variola, no asymptomatic cases...)

Clinical features:

SMALL POX virus spreads from person to person by the respiratory route. After infecting mucosa cells in the upper respiratory tract without producing symptoms it spreads to the regional lymph nodes and, after transient viraemia, infected cells throughout the body. Multiplication of viruses in these cells led to a second and more intense viraemia which heralded the onset of clinical illness. During the first few days of fever the virus multiplied in skin epithelial cells, leading to the development of focal lesions and the characteristic rash. Macules progressed to papules, particularly on the face. There are two kinds of smallpox: variola major and variola minor (very mild).

Infected upper RT -> regional LN and small vessels of skin -> first viraemia -> cells of all body -> second viraemia -> rash, bleeding, cardiovascular collapse

MOLLUSCUM CONTAGIOSUM: the lesions of this mild disease are small copper-coloured warty papules that occur in the trunk, buttocks, arms and face. It is spread by direct contact or fomites. The lesion consists of a mass of hypertrophied epidermis that extends into the dermis. When material from the lesion is crushed, some of the inclusions burst open, and from them large numbers of virions escape. Lesions can persist for as long as 2 years, and re-infection is common.

Lab ID

Observation of DNA containing intracytoplasmic inclusion bodies in cells scraped from lesion.

Immunization: Vaccinia vaccine (attenuated cowpox) is given nowadays only in military and laboratory workers.

14. Papillomaviruses

Papillomavirus belongs to the Papovaviridae family. They are non envelopped and have icosahedral DNA. They infect the squamous epithelia and mucous membranes and are responsible for many varieties of warts and fibropapillomata. Although the lesions are usually benign, their association with tumours is documented.

Gene organization and replication (correlates to appearance of malignancy)

Integration of viral DNA into the host chromosomes -> collapse of the cellular cytoskeleton -> transforming activity in some papillomavirus types -> E7 binds to tumour suppressor gene product, Rb, which is required for terminal differentiation of keratinocytes -> E6 binds to the tumour suppressor gene product p53 and causes its rapid degradation -> the cell can divide even with DNA damages.

Clinical features

CUTANEOUS WARTS: cutaneous warts commonly infect the keratinized epithelium of the hands and feet, frequently seen in young children and adolescents.

They usually disappear spontaneously but occasionally may be resistant to treatment.

ANOGENITAL WARTS: these lesions are commonest in sexually active adults. In women they are found on the vulva, within the vagina or on the cervix.

In men the most common sites for lesions are the shaft of the penis, peri-anal skin or the anal canal.

Subclinical and latent infections of the genital tract are common.

OROLARYNGEAL LESIONS:

Recurrent respiratory papillomatosis: this is a rare condition characterized by the presence of benign squamous papillomata on the mucosa of the respiratory tract. Peaks of incidence are in children under 5 years of age and adults after the age of 15 years. Children acquire the disease by passage through an infected birth canal, while adults acquire the disease from orogenital contact with an infected sexual partner. The disease presents with hoarseness of voice or, in children, with an abnormal cry. As the lesions grow they may cause stridor and upper airway obstruction which can be life-threatening. Malignant conversion of laryngeal papillomas has been described.

Oral papillomatosis: a variety of papillomata and benign lesions associated with HPV occur in the oral mucosa and tongue. Multiple lesions may develop on the buccal mucosa. It is acquired during orogenital contact with an infected sexual partner.

HPV and cancer

Premalignant lesions of the genital tract: Malignant disease of the cervix is preceded by neoplastic change in the surface epithelium, a condition known as cervical intra-epithelial neoplasia (CIN).

Untreated CIN II/III can progress to invasive cancer in a large percentage of affected individuals while CIN I lesions are less likely to progress.

Squamous cell carcinoma: the association of wart viruses with invasive cancers of the skin, larynx and genital tract is well documented.

The commonest association with invasive cancer, however, is with tumour of the anogenital tract.

Lab ID

Diagnosis of cutaneous warts done by inspection

Immunoassay for viral antigens or DNA hybridization – important to determine if it is benign or malignant.

Treatment:

Warts generally are either surgically removed or destroyed by liquid N₂. Laser vaporization or cytotoxic chemicals.

Cidofovir – applied topically, inhibits DNA synthesis

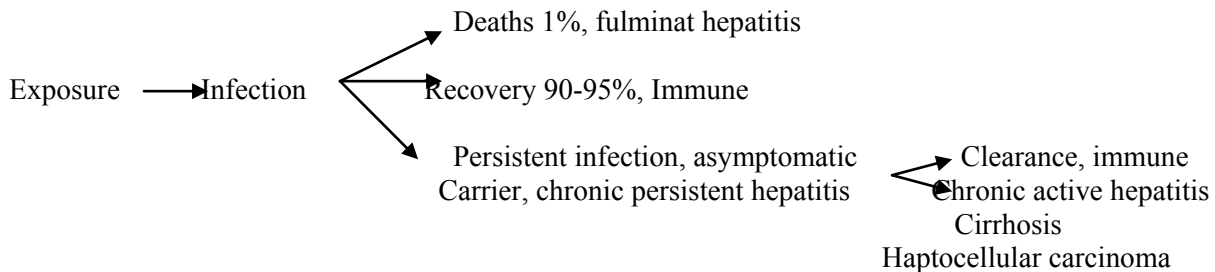
Interferon – laryngeal papillomas

Vaccine is present

15. Hepatitis B virus

HBV is a major cause of chronic liver disease and hepatocellular carcinoma. The predominant form is a small, spherical particle. Filaments are also present. Both types of particle are composed of lipid, protein and carbohydrate; they are not infectious and consist solely of surplus virion envelope. The third type of particle, the virion or Dane particle is enclosed within the envelope, which contains the viral DNA. HBV can be classified into at least six genotypes, A-F. Genotypes B and C are found in the Far East.

Replication of viral nucleic acid starts within the hepatocyte nucleus where the viral DNA can be free-, extrachromosomal, or integrated at various sites within the host chromosomes.



The incubation period varies widely, from 40 days to 6 months, but is often about 2-3 months. A prodromal illness occurs in some patients, who complain of malaise and anorexia accompanied by weakness and myalgia. Arthralgia also occurs and may be accompanied by an related to circulating immune complexes containing HBsAg.

In the acute stage there are signs of inflammation in the portal triads: the infiltrate is mainly lymphocytic.

In chronic hepatitis, damage extends out from the portal tracts, giving the piecemeal necrosis appearance. As the disease progresses fibrosis develops and, eventually, cirrhosis.

Infectious HBV is present in all body fluids of infected person, so all of them can be a source of infection

ACUTE DISEASE: HBV replicates in the hepatocytes. During replication HBcAg and HBeAg are also present at the cytoplasmic membrane. These antigens induce both B and T cell responses; damage to the hepatocyte can result from antibody-dependent, NK and cytotoxic T cell action.

PERSISTENCE OF HBV: persistence of HBV is indicated by the continued presence of HBsAg and HBV DNA in the blood for more than 6 months.

In the neonate, infection occurs in the presence of maternal IgG anti-HBc and tolerance to HBeAg which can cross the placenta. This will have the effect of masking HBcAg on hepatocyte membranes and thus will prevent its recognition by cytotoxic T cells and other immune mechanisms. Carriers may continue to replicate virus to high levels without evidence of liver damage. It ends with the disappearance of HBeAg and the appearance of anti-HBe. The change happens at a variable time after infection, but each 5-20% of patients go through this transition, usually associated with a period of liver cell damage. This is often, but not always, accompanied by the disappearance of HBV DNA from the blood, signalling a transition from high to low infectivity carrier status. A carrier may undergo several episodes of hepatitis. Eventually, -hbv may disappear in 1-2% of carriers each year.

CHRONIC LIVER DISEASE AND HEPATO CELLULAR CARCINOMA: chronic liver damage results from continuing, immune-mediated destruction of hepatocytes expressing viral antigens.

Hepatocellular carcinoma (HCC) is one of the 10 most frequent tumours in the world, and there is considerable evidence that 80% are caused by chronic infection with HBV. There may be an interval of 30-40 years between infection and tumour development.

The mechanism of carcinogenesis is not yet clear, although it is usually associated with cirrhosis.

The rate of progression to cirrhosis and HCC varies according to the age of infection and stage, the state of the patient's immune system, geographic factors and genetic factors.

Lab ID

Antibodies against HBs, Hbe and HBc

Treatment

Prolonged treatment with interferons

Prevention

Active immunization with HBsAg – children up 12 years old

Passive immunization with Hepatitis B immunoglobulin – contaminated people

16. Non-A, non-B hepatitis viruses

Hepatitis D virus

- Found in nature only as coinfection with HBV.
- Circular RNA with negative polarity that code for δ antigen (HDAg)
- Enveloped which has HBV coded HBsAg -> HBV is thus a helper virus for HDV.

Transmission: same as HBV but less sexually transmitted

Diseases: simultaneous primary coinfection with HBV that will cause acute hepatitis similar to HBV one but with more risk of developing fulminant hepatitis and the likelihood of its progression to chronic coinfection is greater and risk for HCC and cirrhosis are increased as well.

Primary coinfection of HDV of chronically HBV infected individuals

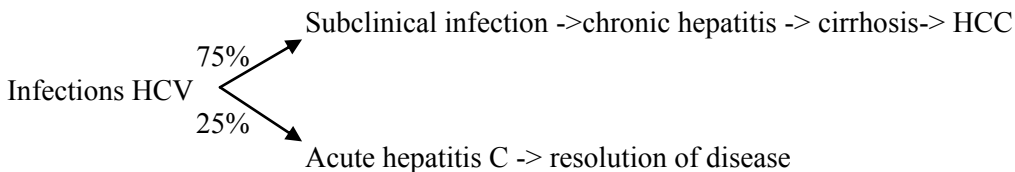
HBV -> incubation -> chronic HBV infection -> + HDV -> severe acute hepatitis -> chronic disease.

Lab ID

Determining δ antigen or antibodies against it.

Hepatitis C virus

Enveloped virus with 3 structural proteins. Capsid protein, viral RNA, 2 other proteins are envelope associated.



Most common posttransfusion, intravenous drug users or on patients that are hemodialysed (also sexually transmitted and from mother to child)

Treatment: α -IFN + ribavirin

Lab ID

ELISA detection of IgM and IgG

Hepatitis E virus (calicivirus)

Nonenveloped RNA

Most common cause for fecal-oral transmitted, waterborne hepatitis. It is frequent in young adults and it is specially severe in pregnant women.

No progression to chronic hepatitis seen. The signs and symptoms are similar to other hepatitis

Lab ID

ELISA detection of IgM and IgG

17. Parvoviruses

Are icosahedral and lack envelope. This family is divided into whether the virus is able of independent replication (autonomous parvovirus) or requires coinfection with helper DNA virus (adenoassociated viruses).

Replication

The virus attaches and penetrates into the host cell -> DNA released into the nucleus -> synthesis of non-structural proteins -> synthesis of structural proteins and viral DNA -> assembly of virus + lysis of host cell
Replication of parvoviruses requires host cells in which DNA synthesis is in progress (the damage is limited to specific tissues that are mitotically active, so it is not surprising that disease of haemopoietic system and the fetus frequently feature).

Dependoviruses

Adenoassociated viruses (AAV) were isolated from children with mild disease, usually in association with adenoviruses of different serotypes. It was first thought that only adenoviruses could supply the necessary helper functions but more recently herpesviruses, human papillomaviruses (HPV) and vaccinia have all been shown to be able to provide helper functions for AAV replication.

There is as yet no evidence of an association with an acute disease.

AAV is highly prevalent in the female genital tract. Infectious virions have been found in cervical epithelium and HPV was also frequently present. AAV DNA has also been demonstrated in broncho-alveolar lavage samples, again frequently together with HPV DNA. The interaction with the helper virus is beneficial to AAV but usually inhibits the replication of the helper. AAV may therefore be beneficial to the host.

Erythroviruses (B19)

The virus is infectious when given in the form of nasal drops. One week later there is an intense viraemia and virus is excreted in the nasal secretions. The viraemia lasts for only a few days before there is a brisk antibody response, initially of the IgM class but followed rapidly by the appearance of IgG antibody.

Erythroid precursors are absent from the bone marrow and there is consequent disappearance of reticulocytes from the peripheral blood and a small fall in the hemoglobin level.

The rash and arthralgia associated with B19 infection occur during the third week after inoculation. They follow the disappearance of the viraemia and occur at a time when there is an easily detectable immune response it is assumed that the rash and arthralgia are immune mediated.

In infected fetuses there appears to be a persistent infection with damage to hematopoietic cells, leading to anaemia, which is one of the factors responsible for hydrops fetalis. The reason is that such individuals produce only small amounts of antibody in response to infection and none of it is capable of neutralizing the virus.

18. Poliovirus

Are small, non enveloped, icosahedral virus with single-stranded RNA with positive polarity.

The polioviruses. Coxsackieviruses and echoviruses are described as enteroviruses because they are all found in the intestines and are excreted in the faeces.

Specific neutralizing antibodies are considered to be the major mechanism of protection against infection. Enteroviruses have a number of features in common:

- They attach to cells in the intestinal tract by specific receptor sites and replicate in cells of the intestinal tract.
- They commonly cause asymptomatic immunizing infections, which protect against future infections with the same virus.
- They can give rise to viraemia
- They occasionally cause infection of the central nervous system and other target organs.
- They are commoner in children than in adults

Polioviruses have affinity for the nervous tissue. There are 3 types of poliovirus infection:

1. Asymptomatic infection or a mild, transient „influenza-like“ illness. The virus is excreted in the faeces for a limited time, and an immunological response develops which protects against re-infection with the same strain.
2. Infections with the same symptoms as above and evidence of the involvement of the central nervous system with headache, neck stiffness and back pain (meningitis).
3. Paralytic poliomyelitis in which the patient develops paralysis. The paralysis is usually flaccid due to the destruction of lower motor neurones, although invasion of the brain stem by the virus can lead to inco-ordination of muscle groups and painful spasms

Pregnant women in the third trimester of pregnancy can have severe disease, but there is no firm evidence of congenital defects in infants born to mothers with poliomyelitis. Maternal infection acquired late in pregnancy may lead to perinatal infection and disease of the newborn.

Treatment: symptomatic

Prevention

Live attenuated (Sabin) or attenuated (Salk) poliovaccines

19. Enteroviruses other than poliovirus

Echoviruses: most echovirus infections cause few or no clinical symptoms. Infection can be widespread in a community, although only a few suffer from clinical illness. Symptoms occur following a short incubation period of 3-5 days (simple fever, upper respiratory symptoms or diarrhoea). Non-specific rashes of fleeting duration have been reported.

The onset the meningitis is abrupt, with severe headache and vomiting. Symptoms are self-limiting, and after a variable convalescent period a full recovery is made, although rare cases of paralysis have been recorded. Certain types cause haemagglutination of human group O erythrocytes. The virus reacts with a receptor present on group O cells. Temperature, pH and age of the red blood cell donor influence this property.

Coxsackieviruses:

Groups A – these viruses give rise to a number of different illnesses. Aseptic meningitis, indistinguishable clinically from that caused by other enteroviruses. Herpangina is an acute feverish disease, usually in young children, characterized by lesion in the mouth consisting of papules on the anterior pillars of the fauces.

Hand, foot and mouth disease presents as a painful stomatitis with a vesicular rash on the hands and feet.

Group B – Epidemic myalgia is characterized by fever and the sudden onset of agonizing stich-like pains in the muscles of the chest, epigastrium and hypochondrium.

20. Hepatitis A virus

Hepatitis A (HAV) is the causative agent of infectious hepatitis. It is a non-enveloped virus, containing linear, single-stranded RNA.

Clinical features

Transmission by fecal-oral route. Although the incidence has fallen in the last decade, hepatitis A is still responsible for almost 60% of acute viral hepatitis. The illness is usually mild, and occurs after an incubation period of 14-45 days. There is a prodrome of malaise, muscle pain and headache, and there may be a low-grade fever. The symptoms usually improve and disappear as jaundice develops. Fulminating hepatitis and liver failure can also occur. There are no carriers of the virus. Infection is mildest in young children. Arthritis and aplastic anaemia are rare complications.

Pathogenesis

Like the enteroviruses, HAV probably infects cells in the gut initially and then spreads to the liver via the blood. The histopathology is similar to that of hepatitis B, with periportal necrosis and infiltration of mononuclear cells. Viral antigens are seen in the cytoplasm of the hepatocytes. Virus is excreted via the bile into the gut 1-2 weeks before the onset of jaundice, and excretion then declines rapidly over the next 5-7 days. Virus is also present in the urine of clinical and subclinical cases during the same period.

Lab ID

Anti-HAV IgM + IgG using ELISA or IgM and total Ig.

Treatment

Vaccination available and recommended in people travelling to mediterranean countries

21. Orthomyxoviruses

Enveloped RNA with its own RNA-dependent RNA transcriptase that synthesizes viral mRNA from RNA. It has an helical nucleocapsid from which the segment contains not only viral RNA but also 4 proteins: NP (nucleocapsid protein) and 3 proteins involved in the synthesis and replication of viral DNA). Its replication occurs in the nucleus.

The viruses are divided into Influenza A, B and C but only A and B have medical importance.

They have haemagglutinin (H protein) and neuraminidase (N protein) as the 2 spikes proteins on the surface.

Pathogenesis

The virus is transmitted by respiratory droplets. The destruction of respiratory epithelial cells is attributed to host immune response, specifically cytotoxic T-cells. The symptoms are chills, high fever, muscle aches and extreme drowsiness. The disease lasts 4-5 days after which there is a gradual recovery. There is a risk of development of pneumonitis in elderly people, young people or people with chronic cardiac or pulmonary problems.

Types and subtypes

Influenza virus is classified as type A, B and C depending on their M and NP proteins. Thus all type A viruses share common internal antigens different from those shared by B.

Classification into subtypes depends on antigens associated with H and N proteins. Human influenza viruses are therefore classified into H1N1, H2N2, H3N2...

Antigenic drift: minor antigenic changes in H and N proteins that occur each year

Antigenic shift: involve change of subtype H1N1 -> H3N2 (occurs each 10-20 years)

The consequences of this antigenic variation is that people become immunologically unprotected which might give rise to an epidemics or even pandemics.

If there is antigenic shift a new vaccine needs to be taken. antibodies made against H protein are neutralizing.

Treatment

Given before disease or early stages: Amantadine and Rimantadine - they stop viral uncoating by inhibition of M2 membrane protein -> INFLUENZA A

For uncomplicated acute illness in adults and adolescents 12 years or more. Zanamivir (inhalation) and oseltamivir (orally) are given. -> INFLUENZA A + B

Prevention

Formalin inactivated Influenza virus vaccine is recommended for the elderly people in high risk groups or patients with chronic pulmonary or cardiac disease.

22. Respiratory syncytial viruses and parainfluenza virus

Respiratory syncytial virus

RS virus is placed in the genus Pneumovirus because of a lack of a haemagglutinin, a haemolysin or a neuraminidase. The nucleic acid is negative-sense single-stranded RNA . RS virus has no haemagglutinin but has a G glycoprotein instead. It is a receptor for cell attachment but not red blood cells, and differs in chemical composition from the HN protein of other paramyxoviruses. There are fusion (F), matrix (M), polymerase and nucleocapsid proteins. The F proteins is probably responsible for both virus penetration and spread in the host.

There are two subtypes, A and B.

The most serious illness caused by RS virus is bronchiolitis in young babies leading to hyperinflation of the lungs but it is also associated with minor upper tract infections. This infection is potentially life-threatening, particularly in those who are immunosuppressed or immunodeficient.

Recovery is apparently complete, although it has been suggested that the infection predisposes to chronic respiratory tract disease (asthma, bronchiectasis, etc).

Parainfluenza virus

Parainfluenza is a negatively-stained virions. The outer surface of the virion is a pleomorphic envelope consisting of a lipoprotein membrane derived from host cell membrane and covered by projections. Paramyxovirus particles are easily deformed by external forces, may assume a variety of shapes and break up more easily than orthomyxoviruses.

The envelope surface projections are of two kinds:

- The HN, with haemagglutinin (H) and neuraminidase (N) functions.
- F glycoproteins, which cause cell membranes to fuse

There are also matrix proteins, M which line the inner surface of the envelope. All the paramyxoviruses carry an RNA-dependent RNA polymerase within the virion.

Within the enveloped virion is a genome of single-stranded negative-sense RNA complexed with protein to form a helical nucleocapsid.

There are four types of parainfluenza viruses (1-4) with antigenically distinct epitopes.

Parainfluenza viruses attach via the haemagglutinin to sialic acid containing receptors on the cell surface. The F protein then fuses the viral envelope with the cell membrane. RNA-dependent RNA polymerase carried within the virion is required to produce subgenomic-sized mRNA transcripts, which are translated to produce some of the early virus-specific polypeptides.

The viral components are assembled beneath the cell membrane and the surface HN and F proteins are incorporated into a stretch of membrane, converting it to viral envelope. This evaginates and buds off, enclosing a nucleocapsid.

The parainfluenza viruses are mostly associated with:

- Croup, a harsh brassy cough in children. It is due to a combination of tracheitis and laryngitis
- Minor upper respiratory tract illness
- Some cases of bronchiolitis and “failure to thrive”

The incubation period is from 3 to 6 days, during which the virus spreads locally within the respiratory tract.

Both viruses are transmitted by respiratory droplets or contaminated hands carrying the viruses to nose or mouth.

Treatment

Ribovirin administered by aerosol.

23. Mumps virus

It is an enveloped virus, with negative polarity RNA, which belongs to the paramyxoviridae family. The spikes on the envelope carry either a combined haemagglutinin and neuraminidase (HN) or a fusion (F) protein. The envelope also contains a matrix (M).

Infection is probably by droplet into the respiratory tract. The incubation period is 14-18 days and is followed by generalized illness with localization in the salivary gland, usually the parotids. The generalized phase is the usual “flu-like” illness with fever and malaise, followed by developing pain in the parotid glands, which then swell rapidly. Much of the swelling is due to the blockage of the efferent duct.

Neurological involvement is common in mumps though the majority of cases are not clinically apparent. However, clinical meningitis remains the most common serious complication of mumps.

Mumps meningitis is rarely fatal and complete recovery is usual. Meningo-encephalitis has been described, but is much rarer.

The best known complication, in postpubertal males, is orchitis. This, though painful and causing softening and atrophy of the affected testicle, is usually unilateral and rarely causes sterility. Oophoritis also occurs in girls.

Prevention

Live attenuated vaccine present (MMR)

24. Measles virus

Measles is an enveloped virus with single stranded RNA from the genus morbillivirus.

It has some important functional differentents. The virion structure includes:

- spikes, carrying a haemagglutinin but not a neuraminidase function
- an F protein that is also a haemolysin
- a matrix protein, M, below an envelope lipid bilayer

There is only one serotype of measles virus.

Measles is an acute febrile illness, mostly in childhood, after an incubation period of 10-12 days. The onset is the "flu-like", with high fever, cough and conjunctivitis. Koplik's spots (red spots with a bluish-white centre on the buccal mucosa) may be present at this stage. After 1-2 days the acute symptoms decline, with the appearance of a widespread maculopapular rash.

Over the next 10-14 days, recovery is usually complete as the rash fades, with considerable desquamation. Complications include:

- giant cell pneumonia, more common in adults
- otitis media
- post-measles encephalitis

The pneumonia is due to direct invasion with virus, but the role of virus in the other two is uncertain. Measles encephalitis can cause severe and permanent mental impairment in those it does not kill. It is rare but disastrous.

One further complication of measles is subacute sclerosing panencephalitis (SSPE), which occurs in children or early adolescents who have had measles early in life, usually under 2 years of age. It is a progressive and inevitably fatal degenerative disease. Within infected cells is a defective form of measles virus, which, because it is unable to induce the production of a functional M protein, is not released as complete virus from the cells. Patients deteriorate over several years, losing intellectual capacity before motor activities.

Treatment

Life attenuated vaccine (MMR)

25. Arboviruses: alphaviruses

Alphaviruses are an enveloped, RNA virus. It has 3 structural proteins: C protein, E1 and E2 proteins (glycoproteins that form hemagglutinin containing spikes that project from lipid bilayer).

Alphaviruses enter cells by receptor-mediated endocytosis. The nucleocapsid is then released into the cytoplasm where it binds to ribosomes, and the non-structural proteins are translated directly from the genomic RNA.

Infection by arboviruses is initiated when mosquitoes or other arthropods deposit saliva in extravascular tissues while blood-feeding. The initial site of replication is the Langerhans cell. Alphavirus replication appears to stimulate:

- the migratory response of the Langerhans cell to the lymph nodes
- the accumulation of leucocytes in the draining lymph node, where local replication produces viraemia

Arboviruses induce high titres of viraemia. 1-4 days after parenteral inoculation or following bites by infected arthropods.

Inoculation of α -viruses by mosquito -> patient has viraemia -> viruses may be seeded in various target organs (e.g. CNS in encephalitis virus)

Invasion of the central nervous system (CNS) via the olfactory nervous tract may ensue in some infections while other viruses cross the blood-brain barrier. In alphaviral infections accompanied by rash and arthritis, virus replication and necrosis occurs in the epidermis and possibly the muscles, tendons and connective

tissue. Infection of macrophages may mediate musculoskeletal pathology via suppression of cytokine induction.

Antibodies are first detected when the fever subsides, usually within 5-10 days after infection, and may persist for many years. Antibodies are of the IgM class for 1-7 weeks after incubation; subsequently they are of the IgG class.

Arboviruses capable of producing encephalitis typically cause a spectrum of disease:

- in apparent infection
- acute encephalitis

Within the central nervous system, arboviruses multiply in and induce necrosis of neurons, which in turn become surrounded by microglia, forming glial knots.

Perivascular cuffing with mononuclear cells affects many cerebral blood vessels. Usually there is concomitant meningitis with accumulation of mononuclear cells in the subarachnoid space and hyperemia of adjacent capillaries.

The tissue tropism of arboviruses can be divided into three categories:

- infections of the CNS (e.g. encephalitis, aseptic meningitis)
- infections of the visceral organs (e.g. hepatitis and hemorrhagic fevers)
- febrile infections

Treatment

Venezuelan equine encephalitis vaccine

26. Arboviruses: flaviviruses

Morphologically, flaviviruses are similar to alphaviruses but are smaller. The molecular biology of flaviviruses is different from the alphaviruses and has resulted in the viruses being classified into different virus families, the Flaviviridae and Togaviridae, respectively. Flavivirus virions have three structural proteins; the viral RNA genome is encapsulated by a small core protein and there are two proteins, termed the membrane (M) and envelope (E), on the outside of the virus particles.

The virus genome is one single-stranded, positive-sense RNA molecule. Flaviviruses replicate in the cytoplasm of cells.

Pathogenesis

YELLOW FEVER: yellow fever is caused by a mosquito-borne flavivirus. The disease is characterized by the sudden onset of headache and fever with temperatures exceeding 39°C, and is accompanied by generalized myalgia, nausea and vomiting, after an incubation period of 3-6 days. Jaundice may appear by the third day of illness, but frequently this is mild or absent. Hematemesis and melena may occur from bleeding into the gastro-intestinal tract and epistaxis and bleeding gums may also be noted.

DENGUE: dengue is caused by four serologically related flaviviruses called dengue-1, dengue-2, dengue-3 and dengue-4.

The disease dengue presents as an acute febrile illness with chills, headache, retro-ocular pain, body aches and arthralgia in more than 90% of apparent cases, accompanied by nausea or vomiting and a maculopapular rash. Illness usually persists for 7 days followed with fever remitting after 3-5 days followed by relapse ("saddleback fever"), and pains in the bones, muscles and joints sufficiently severe to earn the epithet "breakbone fever".

DENGUE HAEMORRHAGIC FEVER: this is a less manifestation of dengue, mainly affecting children, is occasionally accompanied by a shock syndrome, known as dengue shock syndrome. These two severe forms of dengue are observed in patients who undergo successive infection with two different dengue viruses. After an acute onset, fever of 40°C, accompanied by vomiting and anorexia, enlarged liver and petechiae persists for 5-10 days. This is followed by a complete recovery unless shock supervenes.

Treatment

Live attenuated vaccine yellow fever

Formalin inactivated vaccine

27. Arboviruses: bunyaviruses

Bunyaviridae are icosahedral enveloped viruses. The genetic material of the virus is divided between three pieces of single-stranded RNA termed large (L), medium (M) and small (S) segments.

Unlike alphaviruses and flaviviruses, the bunyaviruses have a negative-sense RNA genome.

Arthropodes serve as vectors for the diseases transmitted to humans.

Pathogenesis:

Initial infection by the virus and primary spread of the virus causes the onset of non-specific symptoms such as headache and fever. Secondary spread and the multiplication of the virus in the CNS (central nervous system) causes symptoms such as stiff neck, lethargy and seizures. It then can result in encephalitis, when inflammation of the brain, produced by infection by the virus, damages nerve cells, which affects signaling of the brain to the body.

After the virus enters the body via a mosquito bite, the virus undergoes local replication at the skin site where virus entered the body. A primary spread of virus occurs, with seeding of the reticuloendothelial system, mainly in the liver, spleen, and lymph nodes. With the ongoing replication of the virus a secondary spread occurs, with the seeding of the CNS. Not all the cases reach this stage, depending on the efficiency of viral replication at the different stages and the degree of virus spread. The California encephalitis virus invades the CNS through either the cerebral capillary endothelial cells or the choroid plexus.

28. Rubella virus

Rubella is a single-stranded RNA virus with an envelope and is the only member of the genus Rubivirus. It has an icosahedral capsid and there are 3 major virion glycoproteins: C and the envelope glycoproteins E1 and E2.

Only one antigenic type of rubella virus is recognized.

POSTNATAL RUBELLA: the incubation period for postnatal primary rubella is 12-21 days. Virus may be excreted in the throat for up to a week before and after the rash. The characteristic clinical features are:

- A macular rash, which usually appears first on the face and then spreads to the trunk and limbs
- General features such as minor pyrexia, malaise and lymphadenopathy also occur, with the suboccipital nodes being those most commonly enlarged and tender
- Arthralgia is uncommon in children but may occur in up to 60% of adult females. The joints commonly involved are the fingers, wrists, ankles and knees
- Encephalitis and thrombocytopenia are rare complications of rubella and usually recovery is complete

Rubella appears to present little danger to the immunocompromised patient, in whom the clinical features are similar to those seen in normal individuals.

CONGENITAL RUBELLA: in the fetus is infected during a primary Maternal infection a wide spectrum of abnormalities may occur. The classical congenital rubella syndrome (CRS) triad consists of abnormalities of the eyes, ears and heart.

Abnormalities of the eyes include cataracts, micro-ophthalmia, glaucoma and pigmentary retinopathy, which may result in blindness.

Bilateral or unilateral sensorineural deafness may be present at birth.

There are many possible heart defects, with a patent ductus arteriosus, pulmonary artery and valvular stenosis, and ventricular septal defect being the most common

Rarely, a persistent infection of the central nervous system occurs called progressive rubella subacute panencephalitis, which is similar to the subacute sclerosing encephalitis caused by measles

Pathogenesis

Rubella virus is transmitted by the air-borne route. Infection is established in the upper respiratory tract, and, towards the end of the incubation period, a viraemia occurs and seeds the target organs such as the skin and joints. Most of the clinical features are probably a consequence of the host's immune response to the virus.

During the viraemia the virus is able to infect the differentiating cells of the fetus.

Treatment

Live attenuated rubella vaccine given routinely during childhood

29. Arenaviruses

Members of the family Arenaviridae have a single-stranded RNA genome. The genome has two segments: L (large) and S (small). The virions are spherical enveloped particles. The genome is encapsid in a helical nucleocapsid. The lipid envelope is derived from the plasma membrane.

The virions contain not only virus genome but also host ribosomes.

The arenaviruses that affect humans are grouped as New World and Old World viruses.

Replication

Arenaviruses can replicate in a number of mammalian hosts and in most tissues. Growth is restricted in terminally differentiated cells such as lymphocytes or macrophages.

Clinical features and pathogenesis

LYMPHOCYTIC CHORIOMENINGITIS VIRUS: LCM virus has a worldwide distribution. Most human infections are acquired by contact with laboratory mice or hamsters. LCM is a rare illness but may present as:

- an undifferentiated febrile illness
- aseptic meningitis
- encephalitis

The incubation period is 1-2 weeks and the illness is of short duration.

LASSA FEVER: this has an incubation period of 1-3 weeks with a gradual onset of fever, headache and muscle and joint pain. Pharyngitis with a non-productive cough is a common feature. In severe cases there is vomiting, diarrhoea and a raised hematocrit. Within a few days the patient will become increasingly febrile and complain of abdominal and retrosternal pain. The patient is lethargic, with oedema of the face and neck and enlarged lymph nodes. Oedema and bleeding may occur together or independently. Recovery takes 1-3 weeks.

SOUTHAMERICAN HAEMORRHAGIC FEVER:

Argentinian - Junin virus

Bolivian - Machupo virus

Venezuelan - Guanarito virus

Brazilian - Sabia virus

The incubation is 1-2 weeks and illness begins with a "flu-like" prodrome. In severe disease petechiae develop and there can be bleeding from the gastro-intestinal tract. There is fluid leak through damaged vascular endothelium, leading to hypotension, oliguria and hypovolaemic shock.

Treatment

Ribavirin benefits both lassa fever and southamerican haemorrhagic fever.

30. Filoviruses

Marburg and Ebola, the two members of the Filoviridae, are enveloped viruses with a single-stranded, unsegmented, helical negative sense RNA genome.

Infection was related directly or indirectly to blood or tissues of vervet monkeys.

Replication

Filoviruses can be grown in a variety of cell lines. The mode of entry is not known but is presumed to involve membrane fusion. Filovirus replication takes place in the cytoplasm and the large inclusions are formed. Mature virus is released as nucleocapsids bud through areas of plasma membrane.

Clinical features and pathogenesis

After an incubation period of 4-10 days there is a rapid onset of:

- fever
- malaise
- malaise
- severe frontal headaches

Bradycardia and conjunctivitis occur early in the disease. It progresses rapidly with:

- Nausea, vomiting, abdominal pain and diarrhoea to haematemesis and melaena
- Frank haemorrhagic manifestations, including petechiae, ecchymoses, and uncontrollable bleeding from venepuncture sites, within 5-7 days of onset.

Often maculopapular rash appears around day 5, which is followed by desquamation. Death to shock usually occurs 6-9 days after onset. Infection of pregnant women usually results in abortion with fatal infection of

the neonate. Recovery of survivors is slow, with weight loss, prostration and amnesia for the period of acute infection.

Lab ID

made by ELISA of antiviral antibodies

Treatment

Supportive treatment is all that can be offered to patients.

31. Reoviruses & rotaviruses

Four out of the nine genera of the Reoviridae family infect humans:

- Orthoreovirus
- Orbivirus and Coltivirus
- Rotavirus

All reoviruses have a double-shelled capsid, no envelope double-stranded RNA. All reoviruses replicate in the cytoplasm of infected cells and form intracytoplasmic inclusion bodies.

Reoviridae were made from both respiratory secretions and faeces but could not be associated with disease - hence the name reoviruses (respiratory enteric orphan).

Rotaviruses

Rotavirus infections are usually mild to moderately severe in developed countries but can become very severe and cause high mortality in developing countries. Rotavirus also cause diarrhoea.

Transmitted by fecal-oral route.

Rotavirus activated by mild proteolysis in GIT -> infect epithelial cells of small intestine where they replicate in cytoplasm -> causes shortening and atrophy of villi + ↓production of digestive enzymes -> (-) RNA strand used to produce RNAs that can serve both as mRNA and genomic RNA precursors -> structural proteins + viral enzymes + (+) strandRNA form core-like structure -> the (+) RNA is transcribed to produce (-)RNA → cisRNA -> get out of the cell

Pathogenesis

Rotaviruses replicate exclusively in the differentiated epithelial cells at the tips of the villi of the small intestine. Progeny virus is released in large numbers into the intestinal lumen ready to infect other cells. Biopsies show atrophy of the villi with reactive crypt hyperplasia and lymphocytic infiltrates in the lamina propria. The cellular damage leads to malabsorption of nutrients, electrolytes and water, and the crypt hyperplasia to hypersecretion.

The infection is followed by a local, humoral and cell-mediated immune response and is normally overcome within a week. Rotavirus-specific IgA enteric antibodies, which are secreted into the gut, are the best known correlate of protection.

Clinical features

The onset of symptoms is abrupt after a short incubation period of 1-2 days. Diarrhoea and vomiting are seen in the majority of infected children and last for 2-6 days. Although symptoms of respiratory tract infection are frequently observed at the time of rotavirus infections.

Rotavirus infections can be life-threatening if children are already malnourished.

Lab ID

ELISA for antiviral antibodies

Treatment

Rapid and efficient replacement of fluids and electrolytes IV.

32. Human immunodeficiency virus & other retroviruses

All retroviruses have an outer envelope consisting of lipid and viral proteins; the envelope encloses the core, made of other viral proteins, within which lie two molecules of viral RNA and the enzyme reverse transcriptase.

Viral stability. HIV is inactivated by:

- * heat, in the autoclave or hot air oven
- * glutaraldehyde 2%
- * hypochlorite; 1 in 10 dilution of domestic bleach
- * other disinfectants, including alcohols.

Replication

Retroviruses differ from other RNA viruses in that they replicate and produce viral RNA from DNA copy of the virion RNA. The best studied method of attachment of HIV to cells is by the interaction of the external envelope glycoprotein gp120 with part of the CD4 molecule of T helper lymphocytes and other cells.

After this, entry of the virus occurs by fusion of the viral envelope with the cellular membrane. Once the RNA is released into the cytoplasm, the reverse transcriptase acts to form the double-stranded DNA copy, which is circularized, enters the nucleus and is spliced into the host cell DNA. Once inserted into the host DNA, infection with HIV is permanent. The virus may stay latent or enter a productive cycle.

Clinical features

HTLV-1 infection

It is associated with adult T cell leukemia/lymphoma. The disease is an acute T cell proliferative malignancy; clinically, the features are leukemia, generalized lymphadenopathy and hepatosplenomegaly with bone marrow and skin involvement. Also a non-Hodgkin T cell lymphoma is associated with HTLV-1. HTLV-1 is the cause of a neurological disease, tropical spastic paraparesis.

HIV and AIDS

The acute seroconversion illness resembles glandular fever, with adenopathy and flu-like symptoms. Persistent generalized lymphadenopathy (PGL) is present in 25-30% of patients who are otherwise asymptomatic.

The acquired immune deficiency syndrome (AIDS) presents in many ways, all due to the underlying severe loss of the ability to respond to infectious agents and to control tumours. Constitutional symptoms of fever, weight loss and diarrhoea and minor opportunistic infections. Without treatment, such patients will progress rapidly to AIDS.

Oral hairy leucoplakia appears to be unique to HIV-infected patients. An association with Epstein-Barr virus and papilloma viruses has been proposed.

Kaposi's sarcoma was one of the earliest diseases used to define AIDS.

The tumours arise from endothelial cells of blood vessels, causing bluish-purple, raised irregular lesions. The aetiological agent is thought to be human herpes-virus 8.

Pneumocystis carinii pneumonia was another presentation found in many of the first patients recognized.

Toxoplasma gondii infections can manifest at various sites, but are always associated with compromised patients. The brain is an important site.

HIV dementia develops in 25% of patients with AIDS and is marked by a gradual loss of cognition, progressing to overt dementia.

Pathogenesis of HIV infection and AIDS

The incubation period in the acute stage is from 1 to 2 months. This is preceded by a period of intense, unrestrained viral replication.

The effectiveness of the immune system in controlling virus replication at this time forecasts when the virus will escape control and symptoms appear.

Patients with AIDS are profoundly immunosuppressed.

The proportion of infected CD4+ cells and the level of circulating virus rise as the infection progresses.

Lab ID:

Detection of antibodies to the virus: tests for anti-HIV

Detection of the virus itself: PCR, tests for p24 antigen, virus isolation

Treatment

- HTLV-1

Interferon and inhibitors of reverse transcriptase may have a role.

- HIV

Therapy: combination of antiretrovirals (HAART = highly active antiretroviral treatment) zidovudin, lamivudin, nevirapin, saquinavir etc.

33. Caliciviruses, astroviruses & "small round structured" viruses

Based on differences in their genomic organization, human caliciviruses have been characterized within two genera of the Caliciviridae:

- Sapporo-like viruses
- Norwalk-like viruses

There are eight strains of astrovirus that have been identified with specific antisera. The use of a monoclonal antibody shown that all serotypes share a group antigen,

Caliciviruses and astroviruses have shown that some adults become infected after they had been challenged with faecal filtrates containing virus.

Astroviruses can be readily propagated in a human intestinal cell line provided trypsin is incorporated in the medium. Replication occurs within the cytoplasm.

Pathogenesis

Replication occurs in the jejunum. The villi in the proximal part of the small intestine were broadened and blunted, and the enterocytes covering the damaged villi were cuboidal and vacuolated. At the same time the numbers of intra-epithelial lymphocytes and neutrophils were increased. Epithelial cells remained intact but the microvilli were disarranged and reduced in length.

The symptoms are similar between calicivirus and astrovirus, but vomiting, sometimes projectile, is more frequently reported in calicivirus infection involving adults the illness resembles "gastric flu", i.e. diarrhoea, headache, fever, aching limbs and malaise.

Transmission

Fecal-oral route following ingestion of contaminated food or water (cold foods, shellfish, water, etc)

Lab ID

antiviral antibodies by ELISA

Treatment

No specific treatment for these infections.

34. Coronaviruses

The most studied coronaviruses are IBV (infectious bronchitis virus) and MHV (mouse hepatitis virus). The particles are pleomorphic and enveloped.

The genome is encoded in non-segmented single-stranded positive-sense RNA.

It is now accepted that there two species of human coronavirus (HCoV) causing respiratory disease. Human enteric coronavirus (HECV) is a likely third species.

The confirmed species of coronavirus may readily be distinguished from each other by their limited host range. Serological studies of the N, M and S proteins have revealed antigenic relationships.

Coronavirus attach to either protein or carbohydrate on host cells. The viruses replicate in the cytoplasm with a growth cycle. They bud not from the plasma membrane but from the rough endoplasmic reticulum into intracytoplasmic vesicles. These are transported via the Golgi apparatus to the plasma membrane through which they are released by exocytosis.

Clinical features

Coronavirus show:

- marked species specificity
- strong tissue tropism

They usually infect via the gut and/or respiratory tract. Infections of the liver and CNS are well recognized.

Coronaviruses can also infect neural cells, in which they may persist, and macrophages.

HUMAN CORONAVIRUS: the only significant condition known to follow HCoV infection is upper respiratory tract disease, and it is estimated that coronaviruses cause up to 30% of "common colds".

Coronaviruses may cause severe lower respiratory tract infection in the very old and very young, including premature infants.

There is some evidence that they may cause pneumonia in immunocompromised patients. The incubation period is from 2 to 4 days. Symptoms outlast virus shedding and typically persist for a week, probably as a result of secondary-bacterial infection.

- CNS INVOLVEMENT: these viruses are undoubtedly neuro-invasive and they may have a role in the aetiology of multiple sclerosis, but whether their presence is causative or incidental is presently unclear.

Treatment

Symptomatic only

35. Rhabdovirus

Members of the family must be enveloped, single-stranded RNA viruses. Two genera are known whose members have important roles in animal or human disease.

This genus has rabies virus as its prototype. Lyssavirus has rabies virus as its prototype. Vesiculovirus, whose members are associated with the disease vesicular stomatitis.

Transmission: raccoons, skunks, squirrels, foxes and bats work as reservoirs. Bite of an animal infects humans.

Rabies is a distressing disease that develops rapidly into an acute encephalomyelitis, often frenzied initially, then subsiding into delirium, coma and death. A prominent feature is hydrophobia.

Rabies virus typically is bullet-shaped. Its inner nucleoprotein core of single-stranded RNA enclosed in nucleoprotein (N) with helical symmetry provides the group antigen for the genus.

The viral membrane or matrix (M) protein lies between the core and the outer lipoprotein envelope.

Extraction of the outer envelope releases a glycoprotein (G) that can induce the formation of neutralizing antibody.

Virus attaches via the glycoprotein of the envelope. In neural tissue virus attachment occurs at neuromuscular junctions via the acetylcholine receptors. Entry is by endocytosis. Replication occurs in cytoplasm.

Accumulation of cytoplasmic viral protein inclusions (Negri bodies) may be visible by light microscopy after appropriate staining. This has long been a useful diagnostic feature.

Clinical features

Rabies may present as a:

- Predominantly encephalitic disease – furious rabies
- Paralytic illness – dumb rabies

In humans, about two-thirds suffer the encephalitic form and die within 7 days, the rest initially present as paralytic the develop encephalitis, and death may not occur for 2-3 weeks. Survival is exceedingly rare.

The incubation period in humans can be very variable.

The virus spreads to gain access to the nerves via the motor end plates. Once within the nerve fibers it is out of reach of any circulating antibody as it travels along the axons towards the central nervous system. The manifestations of illness are:

- Initially, fever, malaise and headache
- Then, symptoms related to the wound site e.g. tingling, pain, lumbar weakness and ascending paralysis after leg bites, and numbness, hyperesthesia and pain with increasing shoulder weakness after hand or arm bites.

Diagnosis

Postmortem – Negri bodies

Viral nucleic acid by PCR

Prevention

Killed rabies virus vaccine

Post exposure: thorough cleaning of wound + passive immunization with antirabies immunoglobulins

36. Agents of spongiform encephalopathies (prions)

The transmissible spongiform encephalopathies, or prion diseases, are a unique group of fatal neurodegenerative disorders occurring in humans and animals that take their name from two major characteristics:

1. One hypothesis concerning these agents suggests that they are composed entirely of protein, without any nucleic acid, for which the term prion. No evidence of a conventional host immune reaction has been found in these diseases.
2. The diseases caused by these agents are characterized in all species by spongiform change in the central nervous system (CNS). This consists of numerous small vacuoles that are formed within neuronal cell bodies and their processes.

A noninfectious form of PrP (prion protein) is present in normal mammalian brains on surface of neurons and glial cells. PrP is host protein. Infectious (β sheets) \neq noninfectious (α helical)

Diseases:

- Bovine spongiform encephalopathy (BSE or mad cow disease) occurred due to feeding of bovines with processed animal parts from diseased sheep and cattle. Infection animal to human might be possible.
- Kuru: human-to-human transmission. Infectious agents acquired by an individual's exposure to diseased brain tissue in a ritualistic cannibalism among members of tribe in New Guinea.
- Creutzfeldt-Jacobs disease: iatrogenic transmission of CDJ by use of prion contaminated human pituitary derived GH. 15% inherited as mutation in PrP gene.

Pathology

Extracerebral exposure to prions results in their multiplication in lymphoreticular and other peripheral tissues after infection but infection of CNS results in typical clinical effects. Accumulation of PrP in form of amyloid fibrils (cytoplasmic vesicles in neurons, extracellular amyloid plaque) -> extensive vacuolation, neuronal loss and microglial proliferation.

Symptoms: CDJ: rapidly progressive dementia, behavioral disturbances ending in death within a year.

Prevention:

Decontamination of CDJ brain specimen, animals showing signs of illness are killed.

MYCOLOGY / PARASITOLOGY

1. Fungi and fungal disease of men

Fungi are saprophytes in the soil and on decaying plant material. They are eukaryotic, with a range of internal membrane systems, membrane-bound organelles, and a well-defined cell wall which is composed largely of polysaccharides

- **MOULDS** (filamentous fungi): moulds reproduce by means of spores produced by asexual cell division or as a result of sexual reproduction.
- **YEASTS** : which are predominantly unicellular. Most reproduce by an asexual process called budding in which the cell develops a protuberance, which enlarges and eventually separates from the parent cell.
- **DIMORPHIC FUNGI**: which are capable of changing their growth to either a mycelial or yeast phase, depending on the growth conditions.

Fungal diseases in man:

Some fungi can establish an infection in all exposed individuals. Others, such as *Candida* and *Aspergillus* species, are opportunist pathogens which ordinarily cause disease only in a compromised host. In some mycoses the form and the severity of the infection depend on the degree of exposure to the fungus, the site and the method of entry into the body, and the level of immunocompetence of the host.

Some fungi may cause serious, occasionally fatal, toxic effects in man, either following ingestion of poisonous toadstools or consumption of mouldy food that contains toxic secondary metabolites (mycotoxins). Allergic disease of the airways may result from inhalation of fungal spores.

Some yeasts are commensals of man and cause endogenous infections when there is some imbalance in the host. Only ringworm (dermatophyte) infections are truly contagious.

Types of infection

SUPERFICIAL MYCOSES: diseases of the skin, hair, nail and mucous membranes are the most common of all fungal infections.

- Ringworm is a complex of diseases affecting the keratinous tissues of hair, nail and the horny layer of the skin; it is caused by a group of closely related mould fungi called dermatophytes which can colonize and digest keratin.
- Yeast infections affect the skin, nail and mucous membranes of the mouth and vagina, and are usually caused by commensal *Candida* species. Infection is generally endogenous in origin but genital infection can be transmitted sexually.

SUBCUTANEOUS MYCOSES: they result from the traumatic inoculation of saprophytic fungi from soil or decaying vegetation into the subcutaneous mycoses are mycetoma, chromomycosis and sporotrichosis

SYSTEMIC MYCOSES: deep-seated fungal infections generally result from the inhalation of air-borne spores produced by the casual moulds, present as saprophytes in soil and on plant material. They are mostly caused by dimorphic fungi.

- Coccidioidomycosis
- Blastomycosis
- Histoplasmosis
- Paracoccidioidomycosis

These infections are being seen with increasing frequency in patients compromised by disease or drug treatment. In transplant patients, for example, these fungi are among the most frequent causes of mortality due to infection.

Pathogenesis

It is clear that infection most often arises due to deficiencies in the host rather than because of any inherent pathogenic properties of the fungus.

Antigenic variation on the surface of *Candida* cells may help the organism to avoid host defenses. Cellular immunity is suppressed by cell wall mannan, the capsular mucopolysaccharide and melanin.

2. Dermatophytes and ringworm infections

Ringworm infections are common diseases of the stratum corneum of the skin, hair and nail; they are also referred to as dermatophytosis or tinea.

Ringworm infections are caused by about 20 species of dermatophyte fungi which are grouped into three genera: Trichophyton, Microsporum and Epidermophyton.

Most ringworm infections in Europe are caused by Trichophyton rubrum, Epidermophyton floccosum and Microsporum canis.

Infections are spread by direct or indirect contact with an infected individual or animal. The infective particle is usually a fragment of keratin containing viable fungus. Indirect transfer may occur via the floors of swimming pools and showers or on brushes, combs, towels and animal grooming implements.

In addition to exposure to the fungus, some abnormality of the epidermis, such as slight peeling or minor trauma, is probably necessary for the establishment of infection.

Dermatophytes invade keratin by enzymatic digestion and mechanical pressure.

Many dermatophyte species produce two types of asexual spore: macroconidia and microconidia.

Pathogenesis

Sometimes there is only dry scaling or hyperkeratosis, but more commonly there is irritation, erythema, edema and some vesiculation. More inflammatory lesions with weeping vesicles, pustules and ulceration are usually caused by zoophilic species.

- Tinea pedis (athlete's foot): Trichophyton rubrum, Trichophyton mentagrophytes and Epidermophyton floccosum.
Infected tissue is generally between the toes but can spread to nails which become yellow and brittle. Skin fissures can lead to secondary bacterial infections with consequent id reaction; skin lesions develop at sites distant from infected area.
- Tinea corporis (ringworm): epidermophyton floccosum, several species of Trichophyton and Microsporum.
Lesions look like advancing annular rings with scaly centers periphery of the ring which is the site of active fungal growth is usually inflamed and vesiculated (lesions generally occur in non-hairy areas of the body).
- Tinea capitis (scalp ringworm): species of Trichophyton and Microspores
Small scaling patches to envelopment of entire scalp with extensive hair loss.
- Tinea cruris (jock itch): Epidermophyton floccosum and Trichophyton rubrum.
Lesions occur in the moist groin area where they spread from upper thighs to genitals
- Tinea unguium: most often Trichophyton rubrum.
Nails are thickened, discolored and brittle. 3-4 months treatment

Treatment

Removal of infected skin, topical application of antifungal antibiotics such as miconazole or clotrimazole.

Oral antifungals are required to treat infections of the nail and scalp.

Superficial candidosis

Superficial Candida infections involving the skin, nails and the mucous membranes of the mouth and vagina are very common. Candida albicans accounts for 80-90% of cases, but other species, notably C. tropicalis, C. krusei, C. glabrata, C. parapsilosis, C. guilliermondii and C. lusitaniae may occur. Candida species, usually C. albicans, are found in small numbers in the commensal flora.

Yeast overgrowth and infection occur when the normal microbial flora of the body is altered or when host resistance to infection is lowered by disease.

Pathogenesis

Mucosal infection: this is the commonest form of superficial candidosis. Discrete white patches develop on the mucosal surface.

In oral candidosis white flecks appear on the buccal mucosa and the hard palate and the surrounding mucosa is red and sore. Infection may spread to the tongue.

Vaginal infection: itching, soreness and non-homogeneous white discharge accompany typical white lesions on the epithelial surfaces of the vulva, vagina and cervix. The perivulvar skin may become sore and small satellite pustules may appear around the perineum and natal cleft. Vaginal candidosis is common, especially during pregnancy.

Chronic oropharyngeal candidosis: may extend to give esophageal infection, is very common in AIDS patients.

Skin and nail infection: Candida infections of the skin almost invariably occur at moist sites such as the axillae, groin, perineum, submammary folds and occasionally the toe clefts.

Chronic mucocutaneous candidosis: this is a rare form of candidosis. Takes the form of a persistent, sometimes granulomatous, infection of the mouth, skin and nails.

Lab ID

Candida species grow well on Sabouraud medium or on blood agar at 25-37°C.

Treatment

Most superficial infections respond well to topical therapy with an imidazole. In oral candidosis, miconazole is used.

3. Superficial mycoses other than ringworm & candidosis

Pityriasis versicolor

This is a mild, chronic infection of the stratum corneum which produces a patchy discoloration of the skin. The yeasts are common members of the normal skin flora. Disease is probably related to host or environmental factors.

On normal skin and in conditions such as dandruff and seborrhoeic dermatitis.

Pathogenesis

Small, well demarcated, non-inflammatory, scaling macules are usually present on the upper trunk or neck; these may appear hypopigmented or hyperpigmented.

Treatment

Pityriasis versicolor responds well to selenium sulphide or azoles.

OTHER SUPERFICIAL INFECTIONS

Skin and nail

Certain non-dermatophyte moulds may cause infection of skin and nail. They are often resistant to the agents used to treat ringworm and superficial candidosis. Scopulariopsis brevicaulis is the most common cause.

Non-dermatophyte mould infections do not respond to existing antifungal agents.

Tinea nigra: This is a superficial, asymptomatic skin disease characterized by pigmented macules of variable size, usually on the palms and soles. It is caused by a black mould, Exophiala werneckii.

Tinea nigra responds well to treatment with keratolytic agents such as Whitfield's ointment.

Hair

White piedra: this disease, caused by the yeast Trichosporon beigellii, results in soft, white greyish or light-brown nodules on the hair shafts, mainly in the axillae. The hair often breaks at the point of infection, leaving hairs with a clubbed or swollen end. Shaving of the affected area is usually sufficient to effect a cure.

Black piedra: caused by Piedraia hortae, is characterized by the presence of black, hard nodules up to 1 mm in diameter. Shaving to remove infected hairs is a satisfactory treatment.

Otomycosis

About 10-20% of chronic ear infections are due to fungi. The commonest causes are species of Aspergillus, in particular A. niger. Treatment with topical antifungals is usually successful, although relapse is common.

Mycotic keratitis

Fungal infections of the cornea are secondary to injury, bacterial infection and treatment with antibacterial agents and steroids. They are caused by Aspergillus and Fusarium species. Treatment is with topical antifungal agents, in particular natamycin.

4. Subcutaneous mycoses

Mycetoma

Mycetoma is a chronic, granulomatous infection of the skin, subcutaneous tissues, fascia and bone, which most often affects the foot or the hand. It may be caused by one of a number of different actinomycetes

(actinomycetoma) or moulds (eumycetoma). Infection follows traumatic inoculation of the organism into the subcutaneous tissue from soil or vegetable sources, usually on thorns or splinters.

A large number of organisms have been implicated in this disease, including species of *Madurella*, *Exophiala*, *Acremonium*, *Pseudallescheria*, *Actinomyces*, *Nocardia* and *Streptomyces*. Within host tissues the organisms develop to form compacted colonies.

Pathogenesis

Localized swollen lesions, which develop multiple draining sinuses, are usually found on the limbs.

There is often a long period between the initial infection and formation of the characteristic lesions; spread from the site of origin is unusual but may occur.

Treatment

Actinomycetoma responds well to rifampicin in combination with sulphonamides or co-trimoxazole. In eumycetoma, chemotherapy is ineffective and radical surgery is usually necessary.

Chromoblastomycosis

Is a chronic, localized disease of the skin and subcutaneous tissues, characterized by crusted, warty lesions usually involving the limbs. The principal causes are *Fonsecaea pedrosoi*, *F. compacta*, *Phialophora verrucosa* and *Cladosporium carrionii*.

Treatment

Promising results have been obtained with terbinafine and with itraconazole either alone or in combination with flucytosine.

Phaeohyphomycosis

Non-specific solitary subcutaneous lesions caused by any black fungus. Diagnosis is often made at surgery, and treatment is by excision.

Sporotrichosis

Is a chronic, pyogenic granulomatous infection of the skin and subcutaneous tissues which may remain localized or show lymphatic spread. It is caused by *Sporothrix schenckii*.

Pathogenesis

Most frequent presents as a nodular, ulcerating disease of the skin and subcutaneous tissues, with spread along local lymphatic channels. Typically, the primary lesion is on the hand with secondary lesions extending up the arm. The primary lesion may remain localized or disseminated to involve the bones, joints, lungs and, in rare cases, the CNS. Disseminated disease usually occurs in debilitated or immunosuppressed individuals.

Treatment

Prolonged therapy is usually required. For the cutaneous form, treatment with potassium iodide or itraconazole is satisfactory. In disseminated disease, intravenous amphotericin B is required.

Other subcutaneous mycoses

Rhinosporidiosis is a chronic, granulomatous disease of the mucocutaneous tissues, with the appearance of large polyps or wart-like lesions in the nose or conjunctiva. The organism responsible is a primitive aquatic bacterium.

Loboa lobi, *Basidiobolus haptosporus* and *Conidiobolus coronatus*, occasionally cause subcutaneous infections. Surgical excision is often curative in rhinosporidiosis and *L. lobi* infections; antifungal therapy may be used for the other infections.

5. Coccidioidomycosis, histoplasmosis, blastomycosis & paracoccidioidomycosis

Coccidioidomycosis

This is primarily an infection of the lungs caused by *Coccidioides immitis*. Recovery usually confers lifetime immunity.

Pathogenesis

C. immitis usually causes an asymptomatic or self-limiting pulmonary illness, but a progressive and sometimes fatal secondary disease occasionally develops. Primary pulmonary coccidioidomycosis develops 7-28 days after infection. Skin rashes develop in up to 20% of those with the primary disease and indicate a good prognosis. In some cases primary infection may result in a chronic, cavitating, pulmonary infection which may resolve after several years, or may progress to the disseminated form.

Treatment

Intravenous amphotericin B is the standard therapy, but oral fluconazole, itraconazole or ketoconazole are also used.

Histoplasmosis

H. capsulatum is found in soil enriched with the droppings of birds and bats, and infection results from the inhalation of spores.

Pathogenesis

Most infections are asymptomatic. Sometimes an acute influenza-like illness develops with fever and a non-productive cough. These infections are usually self-limiting, but patients are frequently left with discrete, calcified lesions in the lung.

6. Cryptococcosis, aspergillosis, systemic candidosis & zygomycosis

Cryptococcosis

Cryptococcosis, caused by the capsulate yeast *Cryptococcus neoformans*, is most frequently recognized as a disease of the CNS, although the primary site of infection is the lungs.

There are four serotypes of *C. neoformans* (A, B, C, D) which represent two varieties of the organism, namely, *C. neoformans* var. *neoformans*, which is commonly found in the excreta of wild and domesticated birds.

Pathogenesis

Infection follows inhalation of the cells or basidiospores of *C. neoformans* which, in nature, are thought to be small, allowing the organism to enter deep into the lung. The disease is more common in the men than women.

A mild, self-limiting pulmonary infection is believed to be the commonest form of cryptococcosis. Lesions may take the form of small discrete nodules, which may heal with a residual scar or may become enlarged, encapsulated and chronic (cryptococcoma form).

The meningeal form of cryptococcosis can occur in apparently healthy individuals, but occurs most frequently in patients with abnormalities of T lymphocyte function.

Treatment

In immunocompetent individuals, cryptococcosis may be treated with oral fluconazole or itraconazole. Intravenous amphotericin B in combination with flucytosine is usually the treatment of choice for immunocompromised individuals.

Aspergillosis

The most important are *A. fumigatus*, *A. niger*, *A. flavus*, *A. terreus* and *A. nidulans*

Pathogenesis

ALLERGIC ASPERGILLOSIS: Asthma with eosinophilia is a more chronic form, which manifests as episodes of lung consolidation and fleeting shadows on chest radiography; the fungus grows in the airways to produce plugs of fungal mycelium which may block off segments of lung tissue and which, when coughed up, are a diagnostic feature.

ASPERGILLOMA: The fungus colonizes preexisting (often tuberculous) cavities in the lung and forms a compact ball of mycelium, eventually surrounded by a dense fibrous wall.

Patients are either asymptomatic or have only a moderate cough and sputum production. Surgical resection is most often used to treat this condition.

INVASIVE ASPERGILLOSIS: this form occurs in severely immunocompromised individuals who have serious underlying illness. Neutropenia is the most common predisposing factor and *A. fumigatus* is the species most frequently involved.

The lung is the sole site of infection in 70% of patients, but dissemination of infection to other organs occurs in many cases. Fungus invades blood vessels, causing thrombosis; septic emboli may spread the infection to other organs, especially the kidneys, heart and brain.

PARANASAL GRANULOMA: *A. flavus* and *A. fumigatus* may colonize and invade the paranasal sinuses and the infection may spread through the bone to the orbit of the eye and brain.

Treatment

Allergic forms of aspergillosis are treated with corticosteroids. Aspergilloma is treated by surgical excision. In invasive aspergillosis, the treatment of choice is intravenous amphotericin B.

Systemic candidosis

An iatrogenic infection encountered among certain groups of hospital patients, who carry more yeasts in the mouth and gastro-intestinal tract than the normal population.

Infection may be localized, e.g. in the urinary tract, liver, heart valves (endocarditis), meningitis or peritoneal cavity, or may be widely disseminated and associated with a septicaemia (candidaemia).

Candidaemia is seen mainly in postoperative or immunosuppressed patients; in some patients it clears spontaneously, or disappears when contaminated intravenous catheters are removed. However, some patients with candidaemia, notably those treated with cytotoxic drugs or corticosteroids, develop generalized or localized deep-seated infection.

Common sites of involvement in disseminated infection include the kidney, liver, spleen, brain and gastro-intestinal tract; pulmonary infections are rare. One common sign of deep-seated candidosis is the presence of white lesions within the eye (Candida endophthalmitis). Candida endocarditis usually follows surgery for valve replacement.

C. albicans accounts for most cases of systemic candidosis.

Treatment

The treatment of choice for most forms of systemic candidosis are:

- intravenous amphotericin B (conventional or liposomal)
- intravenous or oral fluconazole

Zygomycosis

Is a relatively rare, opportunistic infection caused by saprophytic mould fungi, notably species of *Rhizopus*, *Mucor* and *Absidia*.

The best known form of the disease is rhinocerebral zygomycosis, a rapidly fulminating infection which is almost invariably associated with acute diabetes mellitus, or with debilitating diseases such as leukaemia or lymphoma. There is extensive cellulitis with rapid tissue destruction, most commonly spreading from the nasal mucosa to the turbinate bones, paranasal sinuses, orbit and brain. The condition is rapidly fatal if untreated.

Pulmonary and disseminated infections can occur in severely immunocompromised individuals.

Treatment

High doses of intravenous amphotericin B control of any diabetes and aggressive surgical intervention.

7. Antifungal drugs

- Amphotericin B and Nystatin bind to ergosterol present in cell membranes of fungal cell. There they form pores that disrupt membrane function, resulting in cell death.
- Imidazole antifungal drugs (= clotrimazole) and triazole antifungal drugs (fluconazole) block demethylation of lanosterol to ergosterol

* amphotericin B + flucytosine - broad spectrum antimycotics

* nystatin, clotrimazole - local antimycotic

8. Candida

Superficial candidosis

Superficial Candida infections involving the skin, nails and the mucous membranes of the mouth and vagina are very common. *Candida albicans* accounts for 80-90% of cases, but other species, notably *C. tropicalis*, *C. krusei*, *C. glabrata*, *C. parapsilosis*, *C. guilliermondii* and *C. lusitaniae* may occur. Candida species, usually *C. albicans*, are found in small numbers in the commensal flora.

Yeast overgrowth and infection occur when the normal microbial flora of the body is altered or when host resistance to infection is lowered by disease.

Pathogenesis

Mucosal infection: this is the commonest form of superficial candidosis. Discrete white patches develop on the mucosal surface.

In oral candidosis white flecks appear on the buccal mucosa and the hard palate and the surrounding mucosa is red and sore. Infection may spread to the tongue.

Vaginal infection: itching, soreness and non-homogeneous white discharge accompany typical white lesions on the epithelial surfaces of the vulva, vagina and cervix. The perivulvar skin may become sore and small satellite pustules may appear around the perineum and natal cleft. Vaginal candidosis is common, especially during pregnancy.

Chronic oropharyngeal candidosis: may extend to give esophageal infection, is very common in AIDS patients.

Skin and nail infection: Candida infections of the skin almost invariably occur at moist sites such as the axillae, groin, perineum, submammary folds and occasionally the toe clefts.

Chronic mucocutaneous candidosis: this is a rare form of candidosis. Takes the form of a persistent, sometimes granulomatous, infection of the mouth, skin and nails.

Lab ID

Candida species grow well on Sabouraud medium or on blood agar at 25-37°C.

Treatment

Most superficial infections respond well to topical therapy with an imidazole. In oral candidosis, miconazole is used.

Systemic candidosis

An iatrogenic infection encountered among certain groups of hospital patients, who carry more yeasts in the mouth and gastro-intestinal tract than the normal population.

Infection may be localized, e.g. in the urinary tract, liver, heart valves (endocarditis), meningitis or peritoneal cavity, or may be widely disseminated and associated with a septicaemia (candidaemia).

Candidaemia is seen mainly in postoperative or immunosuppressed patients; in some patients it clears spontaneously, or disappears when contaminated intravenous catheters are removed. However, some patients with candidaemia, notably those treated with cytotoxic drugs or corticosteroids, develop generalized or localized deep-seated infection.

Common sites of involvement in disseminated infection include the kidney, liver, spleen, brain and gastro-intestinal tract; pulmonary infections are rare. One common sign of deep-seated candidosis is the presence of white lesions within the eye (*Candida endophthalmitis*). *Candida endocarditis* usually follows surgery for valve replacement.

C. albicans accounts for most cases of systemic candidosis.

Treatment

The treatment of choice for most forms of systemic candidosis are:

- intravenous amphotericin B (conventional or liposomal)
- intravenous or oral fluconazole

9. *Pneumocystis carinii*

Pneumocystis carinii is a fungus, but its morphology, behaviour and response to antimicrobial agents are more typical of a protozoan.

The organism was originally described as a cause of atypical pneumonia in malnourished infants and it is a common cause of pneumonia, which is fatal in patients with AIDS.

Around 10-40% of HIV-negative patients undergoing immunosuppressant treatments for malignancy, connective tissue disease or organ transplantation also develop *P. carinii* pneumonia, with mortality rates of 40-50%.

In addition to pneumonia, other as yet unrecognized forms of *Pneumocystis* infection may exist.

Broncho-alveolar lavage or biopsy may be needed to establish the diagnosis.

P. carinii is sensitive to co-trimoxazole.

10. Malaria parasites

Four species are encountered in human disease: *Plasmodium falciparum*, which is responsible for most fatalities; *P. vivax* and *P. ovale*, both of which cause benign tertian malaria (febrile episodes typically occurring at 48h intervals); and *P. malariae*, which causes quartan malaria (febrile episodes typically occurring at 72h intervals).

Life cycle: when an infected mosquito bites, sporozoites present in the salivary glands enter the bloodstream and are carried to the liver, where they invade liver parenchyma cells. They undergo a process of multiple nuclear division, followed by cytoplasmic division and, when this is complete, the liver cell ruptures, releasing several thousand individual parasites into the bloodstream. The merozoites penetrate red blood cell.

In the case of *P. vivax* and *P. ovale*, some parasites in the liver remain dormant, and the cycle is complete only after a long delay.

In the bloodstream, the young ring develop and start to undergo nuclear division. The red cell ruptures to release the individual merozoites, which then infect fresh red blood cells.

Pathogenesis

Malaria is characterized by severe chills, high fever and sweating, often accompanied by headache, muscle pains and vomiting. *Falciparum* malaria, unlike the other forms, may progress (especially in primary infections) to coma, convulsions and death. This condition, cerebral malaria, is associated with the adherence of parasitized red blood cells to the endothelium of brain capillaries.

Lab ID

Thick blood stream with Giemsa stain

Thin blood smear is used when more detail is needed to determine the species involved

Treatment

The standard treatment for acute malaria was chloroquine. However, resistance to that drug in *P. falciparum* is now widespread. The most reliable alternative to chloroquine is quinine (or quinidine).

11. *Toxoplasma gondii* & *Cryptosporidium parvum*

Toxoplasma gondii

It is a unicellular spore forming coccidian protozoa important in nosocomial infections. 2 species life cycle – live in one, reproduce in another. Only reproduce in cats – often infected by rats. Human infected by undercooked meat (ingestion of mature oocysts) or by handling cat feces. Mature oocyst contains 2 sporocysts, tachyzoites develop from this.

Mature oocyst excreted by cat – ingested by human/other mammal – pass to blood & lymph – invade macrophages – multiply inside macrophages – develop bradyzoites with slow metabolism.

It is usually asymptomatic but affects immunocompromised people – fever maculopapulous exanthema, generalized lymphadenitis, hepatosplenomegaly, toxoplasma retinitis.

Both oocysts and tissue cysts transform into tachyzoites shortly after ingestion. Tachyzoites localize in neural and muscle tissue and develop into tissue cyst bradyzooids. If a pregnant women becomes infected, tachyzoites can infect the fetus via bloodstream.

LAB ID

Acid fast – ZN stain, difficult to find

Complement fixing test (CFT)

Serological test – Abs to *T. gondii* (ELISA, one specific feature is that instead of IgM + IgG we mostly search for IgA + IgG antibodies. IgA antibodies are typical for recent infections, IgG for “status after” an infection.

Treatment

Spiramycin – during pregnancy

Clindamycin – Cerebral toxoplasmosis

Cryptosporidium parvum

From animals or water (fecal-oral route)

Severe diarrhea if immunocompromised patients (acute, watery, non-bloody)

It can also cause Cryptosporidiosis – parasitic disease of intestinal tract (anorexia, nausea, vomiting, abdominal pain)

In healthy people is usually mild, self-limiting disease.

LAB ID

See stool for oocysts – ZN method

Treatment

Nitazoxanide and fluid replacement

12. Entamoeba histolytica

This is the most important amoebic parasite of humans. The amoebae invade the colonic mucosa, producing characteristic ulcerative lesions and a profuse bloody diarrhoea (amoebic dysentery). Systemic infection may arise, leading to abscess formation in internal organs, notably the liver.

Transmission

ingestion of cysts -> formation of trophozoites -> penetration of cell wall -> multiplication of trophozoites within colon wall -> may invade epithelia -> ulceration -> systemic invasion (liver abscess) -> cysts discarded with feces

Lab ID

examination of stool sample

Treatment

Not all strains of *E. histolytica* are invasive. Nevertheless, it is not possible readily to distinguish pathogenic from non-pathogenic strains in asymptomatic cyst excretors.

Acute amoebiasis is usually effectively treated with metronidazole or tinidazole. Chloquine is also useful in amoebic liver abscess.

13. Giardia lamblia

This intestinal parasite lives attached to the mucosal surface of the upper small intestine. Vast numbers may be present, and their presence may lead to malabsorption of fat and chronic diarrhoea. Young infants may be particularly severely affected. Infection is usually water-borne.

Transmission

Ingested cyst (water) -> trophozooid formation in duodenum where they attach to wall but don't invade

Lab ID

fecal examination; ELISA test to measure giardia Ag

Treatment

Giardiasis can be treated with 5-nitroimidazoles such as metonidazole or, on rare occasions when this fails, with mepacrine.

14. Trichomonas vaginalis

T. vaginalis is a flagellate protozoon with four anterior flagella and one lateral flagellum which is attached to the surface of the parasite to form an undulating membrane. There is no cyst form; the parasite is transmitted by sexual intercourse.

T. vaginalis is predominantly a vaginal parasite, although urethritis may occur in the male consorts of infected women. The best pH for the pathogen is 6 so abnormal alkalinity favours acquisition of the disease.

Lab ID

Either there is a slide for Giemsa staining or a CAT swab (medium is cultured overnight and microscopied as wet mount).

Treatment

The organism is responsible for a mild vaginitis, with discharge, which ordinarily responds to treatment with metronidazole or tinidazole.

15. Trypanosoma

Trypanosomes have a complex life cycle involving an insect vector. The diseases that are caused in humans, African trypanosomiasis (sleeping disease) and South American trypanosomiasis (Chaga's disease), are restricted in distribution according to the habitat of the insect host.

African trypanosomiasis

Tsetse flies act as the insect vector. Cattle and wild antelope act as reservoirs of infection.

Pathogenesis

Following the bite of an infected tsetse fly, a localized trypanosomal chancre may appear transiently, but invasion of the bloodstream rapidly occurs. The parasites multiply in blood.

Swollen lymph glands in the posterior triangle of the neck are often present. In untreated, the disease inexorably progresses to involve the central nervous system (CNS) with the classic signs of sleeping sickness and, ultimately, death.

South American trypanosomiasis

In Chaga's disease the trypanosomes are not transmitted by the bite, but are present in the bug's faeces, which the unwitting sleeper rubs into the bite wound. The trypanosomes enter the bloodstream, but do not multiply there; instead, they invade the cells of the reticulo-endothelial system and muscle, where they lose their flagellum and associated undulating membrane and adopt a more rounded shape. They multiply in the muscle and are liberated from ruptured cells which disseminate the infection and provide the parasitaemia needed to infect fresh bugs when they next feed.

Pathogenesis

Extensive cardiomyopathy, sometimes with gross distension of other organs (mega-oesophagus and mega colon). Death is usually from heart failure.

Lab ID

Detection of motile trypanosomes in Giemsa staining smears of body fluids, CSF, blood and lymph nodes aspiration and generally specific serological tests are used as confirmation.

Treatment

African sleep disease: early stage -> suramin or pentamidine

late stage -> melarsoprol

South American Chaga's disease: nitrofurans has been used with modest success.

16. Leishmania

Leishmania species are intracellular parasites of the reticulo-endothelial system. There are only two morphological forms: amastigotes (non-flagellate forms), which occur in the infected lesion, and promastigotes (flagellate forms that lack an undulating membrane), which occur in the insect vector.

The parasites are transmitted by sandflies.

Pathogenesis

Cutaneous leishmaniasis (oriental sore) causing a boil-like swelling on the face or other exposed part of the body. The central part of the lesion may become secondarily infected with bacteria, but the leishmania organisms reside in the raised, indurated edge of the lesion. With some species a more severe disseminated cutaneous leishmaniasis may occur. Parasites of the *Leishmania mexicana* complex may cause a destructive lesion of the outer ear (Chiclero's ulcer).

In mucocutaneous leishmaniasis (espundia), which is associated with the *L. braziliensis* complex, disfiguring lesions of the mouth and nose may be caused. The most serious form of leishmaniasis is visceral leishmaniasis which is a life-threatening disease involving the whole of the reticulo-endothelial system.

Lab ID

Examination of Giemsa stained tissue and fluid samples for amastigote. Cutaneous and mucocutaneous disease can be diagnosed from tissue samples.

Treatment

Difficult because available drugs have high toxicity and failure rates. Prevalent antimonials are conventional therapy.

17. *Ascaris lumbricoides* & other intestinal nematodes

Infection with intestinal roundworms is generally associated with conditions of poor hygiene. Low worm burdens are generally asymptomatic, but heavy infections may cause problems, especially in young children where they have been associated with impaired development.

Ascaris lumbricoides

This is the common roundworm.

In warm, moist conditions, infective larvae develop within fertile eggs, but do not hatch. Such eggs can survive for long periods in soil. If ingested, the eggs hatch in the duodenum and the larvae penetrate the gut mucosa to reach the bloodstream. They are carried to the pulmonary circulation, where they gain access to the lung and undergo two moults before migrating via the trachea to the intestinal tract. Having completed their round-trip, they mature in the gut lumen and live for several years.

Pneumonic symptoms may accompany the migratory phase and the adult worms may invade the biliary and pancreatic ducts. Moreover, heavy infection with these large worms can cause intestinal obstruction. Allergy is also sometimes a problem.

The dog ascarid, *Toxocara canis*, may accidentally infect man. Larvae hatch in the small intestine and penetrate the gut wall, but they are unable to complete their migratory phase. Instead, they find their way to remote parts of the body, a condition known as visceral larva migrans.

Trichuris trichuria

This is the common whipworm. Like those of *Ascaris*, they develop infective larvae in warm, moist conditions, but the ova do not hatch outside the body. However, after ingestion and hatching, there is no migratory phase and adult worms develop directly in the large intestine.

Infection is usually trivial, though massive infections can cause rectal prolapse in young children, and a form of dysentery is described.

Hookworm

The two species produce indistinguishable thin-walled eggs which hatch in soil. The larvae undergo several moults before infective larvae are produced. These are capable of penetrating unbroken skin, and in this way they gain access to the bloodstream to begin a migratory phase similar to that of *Ascaris*. When they reach the gut they attach by their mouthparts to the mucosa of the small intestine.

Hookworms ingest blood and, moreover, move from site to site in the gut mucosa, leaving behind small bleeding lesions. These two facts are responsible for the chief pathological manifestation of heavy infection with hookworms: iron deficiency anaemia.

Strongyloides stercoralis

Human infections arise after penetration of infective larvae through skin and there is a migratory phase involving the lungs. However, human infection appears to be restricted to female worms, which attach to the gut mucosa and produce eggs that contain fully developed larvae; these hatch within the intestinal lumen so that larvae, not eggs, are found in faecal samples.

Symptoms are usually benign.

Enterobius vermicularis

This is the common threadworm, which infects children. Adults live in the large intestine and are occasionally found in the appendix. Mature, gravid females crawl through the anus at night and lay their eggs in the peri-anal area. These eggs contain fully developed larvae. Ingestion of these eggs initiates a fresh infection. Symptoms are restricted to itching (pruritus ani) associated with the deposition of eggs.

Since eggs are not discharged by the worm into faeces, faecal examination is not appropriate in the laboratory diagnosis of the threadworm infection.

Treatment

Most effective are the benzimidazole derivatives, especially albendazole and mebendazole.

18. Tissue nematodes

This group includes the filarial worms, the Guinea worm (*Dracunculus medinensis*) and *Trichinella spiralis*.

Wuchereria bancrofti

This filarial worm is transmitted by the bite of various species of mosquito throughout the tropical belt of the world. The larvae invade the lymphatics, usually of the lower limbs, where they develop into adult worms. Presence of the adult worms causes lymphatic blockage and gross lymphoedema, which sometimes leads to the bizarre deformities associated with bancroftian filariasis, elephantiasis.

Embryonic forms (microfilariae) are liberated into the bloodstream. Microfilariae remain in the pulmonary circulation during the day, emerging into the peripheral circulation only at night, to coincide with the biting habits of the insect vector.

Loa loa

It is transmitted by biting flies (*Chrysops* species). The adult worms live in subcutaneous tissue and wander round the body, provoking localized reactions known as Calabar swellings and sometimes migrating across the front of the eye.

The sheath microfilariae of *Loa loa* exhibit diurnal periodicity, so that, unlike those of *W. bancrofti*, they appear in peripheral blood only during the day.

Brugia malayi

Can cause elephantiasis. Microfilaraemia usually shows a nocturnal periodicity.

Onchocerca volvulus

It is transmitted by species of black-fly. Adult worms develop in subcutaneous and connective tissue, and often become encapsulated in nodules, which form on bony parts of the body, such as the hip, elbow and the head. The microfilariae are not found in blood, but live in the superficial layers of the skin causing itching and, in heavy chronic infections, gross thickening of the skin. The eye is commonly invaded by microfilariae, which may cause corneal and retinal lesions that lead to blindness. The condition is known as river blindness.

Mansonella species

They are transmitted by biting midges. The unshathed microfilariae appear in the bloodstream and exhibit no periodicity. They are generally regarded as non-pathogenic.

M. streptocera causes skin infections similar to those of *O. volvulus*.

Treatment

Diethylcarbamazine (DEC) has been used for many years for the treatment of all forms of filariasis. It effectively kills microfilariae, but is not reliably lethal to adult worms.

Suramin kills the adult worms, but is much more toxic than DEC.

Dracunculus medinensis

This is the Guinea worm. The infective larvae develop within water fleas and human infection is normally acquired through infected drinking water. The larvae penetrate the gut mucosa and grows to maturity in connective tissue.

After fertilization, the female worm incubates the larvae to maturity and, when ready to give birth, emerges to the skin surface to provoke an intensely irritating blister.

Trichinella spiralis

Trichinella spiralis has an extremely wide host range. Human infections are usually acquired by eating undercooked pork products. The infected larvae lie dormant in skeletal muscle and are released when the meat is digested. Male and female worms develop to maturity attached to the mucosa of the small intestine. The larvae penetrate the gut wall and migrate to skeletal muscle, where they enter the quiescent phase. Most of the symptoms of trichinosis, which can be severe, even life-threatening, are associated with the migration of larvae.

19. Trematodes

The flukes are a diverse group of worms that share a similar life cycle involving a snail host and, often, a second intermediate host that provides the vehicle for the transmission of infection.

Life cycle

When excreted, trematode eggs often contain a fully developed ciliated organism called a miracidium. In water, the miracidium escapes, either through a lid-like operculum in the egg shell, or by osmotic rupture of the egg. The miracidium penetrates the appropriate species of snail and undergoes several stages of asexual reproduction before emerging as a free-swimming body called a cercaria. The cercariae encyst in the muscle of fish, crabs. Men become infected by ingesting the encysted metacercariae.

Clonorchis sinensis

Infection is acquired from uncooked freshwater fish, notably carp. The metacercariae excyst in the small intestine and pass into the bile ducts, where they mature.

Infection is commonly asymptomatic, but fibrosis of the bile ducts with impairment of liver function may occur in heavy, chronic infections.

Fasciola hepatica

Human infections have usually been associated with eating wild watercress from infected sheep pastures. The adult worm can cause biliary fibrosis and obstructive jaundice.

Unlike other trematode infections, fascioliasis does not reliably respond to praziquantel, and treatment with antihelminthic triclabendazole or the more toxic chlorophenol derivative bithionol may be required.

Paragonimus westermani

Human infection follows ingestion of raw, infected muscle of freshwater crabs and crayfish. The metacercariae penetrate through the gut wall and diaphragm to reach the lung, where they develop to maturity. Occasionally the larvae find their way to the brain. Pulmonary infection usually provokes the production of sputum, in which the characteristic large eggs can be found, often associated with flecks of altered blood. Praziquantel is used for treatment.

Intestinal flukes

Infection is often acquired by the habit of opening water chestnuts with the teeth. The adult flukes live attached to the wall of the small intestine, and produce a large number of eggs that resemble those of *F. hepatica*.

Infection is usually asymptomatic unless the worm burden is large.

Schistosoma species

Human infection follows exposure to cercaria in water harbouring infected snails. The cercariae penetrate the skin, often causing a transient dermatitis, called swimmer's itch. Once in the bloodstream, the schistosomula migrate to the liver, where they develop into mature male and female worms.

The mature worms migrate to the small veins of the rectum or the bladder. Eggs, which contain a fully developed miracidium, are passed through the bladder wall into the urine.

Pathogenesis

Most of the serious manifestations of schistosomiasis are associated with the deposition of eggs, with the formation of granuloma and fibrotic lesions of the liver, bladder or other organs. Such effects may herald malignant changes.

Treatment

Praziquantel is effective against all the human schistosomes and is the drug of choice.

20. Cestodes

Taenia species

Taenia saginata, the beef tapeworm, is much more prevalent than the related *T. solium*, the pork tapeworm. Human infection is acquired by eating raw and undercooked beef or pork containing the encysted larval stage, the cysticercus. The larvae hatch in the small intestine, and attach to the mucosal surface. The worm grows backwards from the head. Eggs start to be produced in the uterine canal. This becomes grossly distended as more eggs are produced, so that the fully gravid segments at the end of the worm become nothing more than bags full of eggs.

Eggs are not laid. They are retained within the proglottids, which become detached from the end of the worm and are passed with the faeces. Animals become infected by ingesting the eggs.

Considering the size of the worm, infection, is usually remarkably asymptomatic. However, in the case of *T. solium*, eggs may hatch in the human host and form cysticerci. When they lodge in the brain, they may cause a serious epileptiform disease, cerebral cysticercosis.

Diphyllobothrium latum

This is the fish tapeworm. The mature adult worm lays numerous operculate eggs within which a ciliated body called a coracidium develops. This hatches in water and is ingested by the water flea. After a period of development, the larva awaits ingestion by a freshwater fish in which it invades the muscle.

Human infection is usually asymptomatic, although a form of pernicious anaemia caused by competition for dietary vitamin B12 has been described.

Treatment

Niclosamide or praziquantel is used for treatment.

Hymenolepis nana

Hymenolepis nana is only 2-4 cm long - dwarf tapeworm. It has a very simple life cycle with no known intermediate host.

Treatment

Infection is usually asymptomatic; heavy infections can be treated with praziquantel or niclosamide.

Echinococcus granulosus

This is the tapeworm of the dog and other canine species and usually, humans are an intermediate host.

Sheep are the usual intermediate host.

After ingestion of the eggs, larvae hatch in the small intestine, penetrate the gut mucosa and are carried by the bloodstream to various organs (commonly the liver), where they are filtered out by the capillaries. The larva starts to grow, eventually forming a cystic cavity.

The cyst may die and calcify, but it often continues to grow inexorably, eventually seriously compromising the function of the organ in which it is situated.

Treatment

Cysts of *E. granulosus* can often be removed surgically. Some success has been obtained with benzimidazole derivatives, notably albendazole, and with praziquantel.

CLINICAL MICROBIOLOGY

1. Etiology and laboratory diagnosis of upper respiratory tract infections

Upper respiratory tract infections are infections that occur in the nose, nasopharynx, oropharynx including tonsils, paranasal sinuses, middle ear and conjunctiva.

PHYSIOLOGICAL FLORA:

- nasal cavity: staphylococcus epidermidis it can also be sterile and rarely contains coryneform rods, Staphylococcus aureus and pneumococcus
- pharynx: always Neisseria and Streptococci (viridans group), usual hemophilus.

Rhinitis & Nasopharyngitis

- virus: the most common is the “common cold” virus or rhinovirus being followed by the coronavirus and then other respiratory viruses.
- bacteria: acute infections: usually secondary Staph. aureus, Hemophilus influenza, Streptococcus pneumonia, Moraxella catarrhalis.

chronic infections: Klebsiella ozaenae, Kl. rhinoscleromatis

Treatment: generally no antibiotic is required and even no bacteriological exam if necessary (pus full of polymorphonuclears, ↑CRP level - bacterial infection).

Acute sinusitis + otitis media

usually started by respiratory viruses or S. pneumoniae. Secondary pyogenic infections are due to S. pneumoniae, H. influenza type B, Moraxella catarrhalis, Staph. aureus, Strep. pyogenes and even anaerobes such as Bacteroides, Prevotella, Porphyromonas.

!Complications: mastoiditis, purulent meningitis

Chronic sinusitis + otitis media and externa

Sinusitis maxillaris or frontalis chronica: Staph. aureus and genus peptostreptococcus

Otitis media chronica: Pseudomonas aeruginosa, Proteus mirabilis

Otitis externa acuta: Staph. aureus

Treatment: not recommended to perform bacteriological exam in otitis media and sinusitis except when a relevant specimen is available. aminopenicilin or 1st generation cephalosporins

Conjunctivitis

usually of viral origin and accompanies acute upper respiratory tract infection

- in adenovirus: follicular conjunctivitis, pharyngoconjunctival fever, epidemic hematoconjunctivitis
- it can have other origins such as herpetic keratoconjunctivitis or hemorrhagic conjunctivitis (enterovirus)

Treatment: local

bacterial origin:

- acute: suppurative such as with Strep. pneumoniae. S. aureus and in children other causes might be possible while inclusion C. trachomatis.
- chronic: Staph. aureus, C. trachomatis
- allergic

Oropharyngeal infections

acute tonsillitis and pharyngitis:

- usually viral (rhinovirus, coronavirus, adenovirus, EBV and coxsackievirus - herpangina)
- bacterial: acute tonsillitis or tonsillopharyngitis due to Strep. pyogenes, rare but significant: N. gonorrhoeae + C. diptheria

Treatment: bacteriological exam is recommended in all cases including “typical tonsillitis”. When Streptococcus pyogenes are found - penicilin G

!azithromycin + erythromycin should only be used in allergic people. besides bacteriological exam, CRP should also be determined.

2. Etiology and laboratory diagnosis of pneumonias

Physiological flora: generally sterile but materials from these sites are often contaminated with upper respiratory tract flora.

1. acute community acquired pneumonia - in original healthy people.

adults: there is bronchopneumonia and lobar pneumonia, for which the causative agents can be Streptococcus pneumoniae Staph. aureus and Hemophilus influenzae type B

There is also atypical pneumonia: Mycoplasma pneumoniae, Chlamydia pneumoniae and Influenza A.

children: bronchopneumonia might be caused by Hemophilus influenzae, Strep. pneumoniae or Moraxella catarrhalis and in case of newborns Strep. agalactiae or enterobacter. Atypical pneumonia is caused by respiratory viruses (RSV, adenoviruses) but can also be caused by bacteriae (Mycoplasma pneumoniae or Chlamydia pneumoniae)

In debilitated individuals: pneumococci, staphylococci, hemophilus, klebsiella pneumoniae or legionella pneumophila.

In more serious immunodeficiencies: pneumocystis jirovecii, CMV, atypical mycobacteria, nocardia asteroides, aspergillus or candida.

After contact with an animal: bronchopneumonia: Pasteurella multocida, Francisella tularensis

atypical pneumonia: Chlamydia psittaci and Coxiella burnetii (Q fever)

2. Acute nosocomial - ventilator associated pneumonia

early (up to 4th day of hospitalization) is due to community strains of respiratory agents.

Late (>5th day on) is due to resistant hospital strains

3. Subacute and chronic

aspiration pneumonia and lung abscesses due to Prevotella melanogenica, Bacteroides fragilis or Peptostreptococci. Lung TB or mycobacteriosis due to Mycobacter TB, bovis or atypical.

3. Etiology and laboratory diagnosis of ear infections

check question 1

4. Etiology and laboratory diagnosis of eye infections

check question 1

5. Etiology and laboratory diagnosis of gastro-intestinal infections

Normal colonic flora: 99% anaerobes (Bacteroides, Fusobacter, Clostridium, Peptostreptococci) + 1% Enterobacter (mainly E.coli) and Enterococci

Normal mouth flora: Streptococci viridans, oral Neisseria or Hemophilus of very low pathogenicity such as parainfluenza.

Stomach infection: generally stomach works as sterilization chamber killing most of swallowed microbes by means of HCl but doesn't kill Helicobacter pylori that causes chronic gastritis and peptic ulcers.

Enteric fever - Salmonella Typhi, Salmonella Paratyphi A, B and C.

Listeriosis - Listeria Monocytogenes

Peritonitis - generally colonic flora after appendicitis or injury

Small + large intestine infections . after surgery, during depressed peristalsis bacteria may grow in the intestines causing steatorrhea, diarrhoea and malabsorption of vitamins.

Diarrhoea: increase in daily amount of stool water

Disentery: acute inflammation of colon - abdominal pain and small volume of stool with blood, pus or mucous (Shigella or amoeba).

1. Diarrheal diseases: - infectious (bacteriae, viruses, parasitosis...)
- noninfectious (food poisoning)

6. Etiology and laboratory diagnosis of food poisoning

1. Intoxication due to a toxin preformed in food: Cl. perfringens, Bacillus cereus, Cl. botulinum (heat labile neurotoxin)
2. Intoxication due to infection with an invasive microorganism: Salmonella gastroenteritis, ETEC/EHEC, Listeria monocytogenes

7. Etiology and laboratory diagnosis of liver infections

1. Acute cholecystitis: obstruction due to gall stones
Etiology: intestinal bacteria (E.coli)
Complication: ascending cholangitis
2. Chronic cholecystitis
the most dangerous agent is Salmonella Typhi

3. Granulomatous Hepatitis

Q fever, Tbc, brucellosis

4. Parasitic infections of liver

Amoebiasis (liver abscesses), Malaria (life cycle of plasmodia), Leishmaniasis (L. donovani), Schistosomiasis (eggs of Schistosoma japonicum)

5. Viral hepatitis

HAV, HBV, HCV, HDV, HEV

8. Etiology and laboratory diagnosis of acute purulent meningitis

Purulent is nearly always of bacterial origin. Generally the doctor has to take a good anamnesis, clinical disease and the laboratory is suspecting for acute purulent meningitis needs to examine CSF, cytology (appearance and number of cells), biochemistry (glc + proteins) and microbiology (microscopy, Ags and culture).

In purulent meningitis cells have an increased number, proteins as well but glucose level is decreased.

Newborns: group B streptococci and Listeria monocytogenes

Infants: H. influenza b, Neisseria meningitidis and Strep. pneumoniae

Young adults: N. meningitidis, Strep. pneumoniae

Adults: Strep. pneumoniae, N. meningitidis

Elderly: Strep. pneumoniae, Listeria Monocytogenes

9. Etiology and laboratory diagnosis of acute aseptic meningitis

Aseptic is nearly always of virus origin. In aseptic meningitis, the cells are in increased numbers, so are proteins but glucose stays at the same level.

The causative agents can be:

- mumps virus - more common before vaccination
- enterovirus (echovirus + coxsackievirus)
- tick borne encephalitis - highly common in Czech Rep.
- rarely some bacteria can also cause it

West Nile virus might also be found.

Other European pathogenic arboviruses might be imported to Czech Rep.

10. Etiology and laboratory diagnosis of chronic meningitis & brain abscesses

Chronic meningitis:

bacteria - M. tuberculosis

Moulds and yeasts - Aspergillus or Cryptococcus neoformans

Encephalitis (acute of viral origin):

Tick borne encephalitis

HSV

Acute brain abscesses (only bacterial):

Mixed aerobic + anaerobic flora

Staphylococcus

Streptococcus

Chronic brain abscesses:

bacteria: M. TB, Nocardia Asteroides

parasite: Cysticercus cellulosae (T. solium)

! Puncture or excision for bacteriology, mycology and histology

11. Etiology and laboratory diagnosis of urinary tract infections

It is the 2nd most common type of infections (after respiratory) -> in adults it is the most common specially in females due to shorter urethra.

Cystitis

- most common UTI, generally caused by intestinal microflora, ascendent infection.
- symptoms: dysuria, pollakiuria (urgent need to pee but peeing just a little bit)

Pyelonephritis

- can be ascendent or hematogenous
- EPEK = E. coli, Proteus, Enterobacter and Klebsiella

Urethritis -> STDs

UTIs can be:

- non-complicated: generally caused by *E. coli* (80%), *Enterococcus* (15%), *Proteus mirabilis* and the rest is other *Enterobacter*.
- accompanying structural abnormalities (e.g.: prostate hypertrophy, urinary stones, pregnancy, catheter)
- accompanying functional disorders (vesicouretral reflux, DM...)

- complicated UTIs are 80% of the tissue caused by EPPEK, *enterococcus*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and the *E. coli* while the rest is caused by other *Enterobacter*, *Candida*...

How to take a urine sample: 1. through cleaning of genitalia

2. middle stream of urine only

3. use sterile vessel

4. pour urine into a sterile tube

5. if not possible to process it within 2h -> place specimen into 4°C for 18h.

Microbiologists are interested in the kind of microbes present in urine sample but also the amount of microbes present (increased number = UTI; decreased number = contamination)

- urine is inoculated on culture media by means of a calibrated loop usually taking 1 µl of urine.

CFU = colony forming unit = 1 bacterial /yeast cell

Type of specimen, symptoms	Type of microbe	Significant number (CFU/ml)
Middle stream, symptoms present	Primary urine pathogen	10 ³
	Dubious urine pathogen	10 ⁵
Middle stream, no symptoms	Any	10 ⁵
Suprapubic puncture	Any	10 ¹

Generally for urine exam you use blood agar but chromogenic media may also be used (oriented for the most frequent urinary pathogens and their colonies have different colors) and according to requirements further media might be used.

Primary urine pathogens: *E. coli* + other *Enterobacter*, *Enterococci*, *S. agalactiae*, *Staphylococci*, yeasts (mainly *Candida*) and *Pseudomonas aeruginosa*.

12. Etiology and laboratory diagnosis of traditional venereal diseases

Gonorrhea: caused by *Neisseria Gonorrhoeae*

They can either be infections of lower parts of urogenital tract, or upper parts of urogenital tract or even localized infections or, rarely, disseminated infections.

- infections of lower UGT:

females: cervicitis (most common)

males: urethritis

- infection of upper UGT:

females: salpingitis up to adnexitis (Pelvic inflammatory disease -> sterility)

males: epididymitis

- other localized infections:

females and males: proctitis/pharyngitis/ blennorrhoea neonatorum

females: peritonitis/perinepatitis

- disseminated infections
female and male: skin postulae, joints (purulent arthritis, septic arthritis)

Lab: generally samples are taken from urethra (males and females) and cervix (in females) and microscopy or culture is performed.

2 swabs are made, 1 to inoculate directly onto the warmed culture media and another is used to make a film on the slide.

Microscopy: it is important specially in acute gonorrhoea in males and symptomatic gonorrhoea in females.

Media: combine ChA + medium with antibiotics

Biochemistry: oxidase +, glucose+, maltose-

Therapy: many strains are now resistant to penicilin and tetracyclines so ceftriaxone is used.

Syphilis

- early syphilis: primary . secondary - latent - tertiary

Treatment: penicillin (one dose of benzathin penicillin in 1ry syphilis, 3 times after a week in 3rd syphilis of benzathin penicillin)

Lab: direct detection: darkfield microscopy from exudative lesions (ulcus durum)

indirect detection: nontreponema test (cardiolipin Ag) - RRR (fast, cheap, early positive, reflects activity) or treponema test - MHA-TP, ELISA, WB (sensitive, more expensive more specific)

Hemophilus Ducreyi

- causes ulcus molle, more common in tropical countries
- it causes genital ulcerations (more easy to catch HIV) and purulent lymphadenitis
- cultured on enriched media (ChA + supplements)

Chlamydia Trachomatis

- causes lymphogranuloma venereum, more common in tropical + subtropical carriers.
- purulent lymphadenitis with fistulae + scarring devastating pelvic region in females
- serology - CFT (complement fixation test) with Chlamydia antigen

13. Etiology and laboratory diagnosis of non-specific sexually transmitted diseases

Papillomavirus

- causes urogenital warts in both males and females
- genotypes 16, 18 and 32 causes infection of the cervix in women leading to carcinogen
- cultivation is impossible and diagnosis is performed mollecular methods

Chlamydia Trachomatis, serotypes D to K

- males: nongonococcal or post gonococcal urethritis
- females: cervicitis ->ophtalmia neonatorum
- treatment: macrolides + tetracyclines
- laboratory: detection of Ag or DNA

Yeasts - Candida albicans

- female: vaginal mycosis
- treatment: clotrimazole or fluconazole
- laboratory: microscopy and cultivation (Soboraud agar)

Trichomonas vaginalis

- females: vaginitis
- treatment: metronidazole (both partners must be treated)
- lab diagnosis: microscopy (wey mount + giemsa)
culture on special media

Mycoplasmas - Mycoplasma Hominis + Ureoplasma ureolyticum

- male and female: urethritis
- female: postpartum fever
- treatment: macrolides
- laboratory: direct only

HSV-2

- female and male: herpes genital that can be primary or recurrent
- treatment: acyclovir
- laboratory: detection of DNA on PCR
isolation on cell culture

HCV

- ♂ & ♀: viral hepatitis C, acute and chronic
- Therapy: pegylated interferon + ribavirin Lab. dg: detection of viral RNA detection of antibodies (anti-HCV)

HIV

- laboratory: detection of antibodies, antigens and viral load
- treatment: HAART

14. Etiology and laboratory diagnosis of wound infections

Common superficial injuries - Staphylococcus aureus, Streptococcus pyogenes, β hemolytic streptococci
! in case there are foreign bodies in the wound or deeper stab wounds consider Cl. Tetanii

Severe contused wounds

- agents of Clostridium myonecrosis (Cl. perfringens, Cl. histolyticum)
- gas gangrene or malignant edema
- Clostridium tetani, Staphylococcus aureus, Strep. pyogenes

Wounds sustained in water

- fresh water: Pseudomonas aeruginosa and Aeromonas aerophilus
- salt water: Vibrio parahaemolyticus, Mycobacter marinum (also in tanks + aquariums)

Injuries sustained on tropics (mainly feet)

- soil Nocardia (Dermatophilus congolensis, Rhodococcus equi)
- Atypical mycobacter (Mycobacter ulcerans, Mycobacterium haemophilus)
- Micromycetes (Sporothrix schenckii, Paracoccidioides brasiliensis)

Surgical wounds

- Staphylococcus aureus, Staphylococcus epidermiditis

Burns

- Pseudomonas aeruginosa, Staphylococcus aureus

Man inflicted bites

- members of oral microflora like anaerobes, S. aureus, oral Streptococci

Animal bites

- Pasteurella multocida (dogs + cats), Staphylococcus aureus (any animal)

Other injury by animals

- Francisella tularensis, Bacillus anthracis, Burkholderia mallei

15. Etiology and laboratory diagnosis of sepsis

Bacteremia x Sepsis

Presence of bacteria in blood Suspect or proved infection + systemic inflammatory response syndrome (SIRS) -> interaction of microbial products with

macrophages releases a lot of cytokines (fever, accelerates

pulse

and breathing, leukocytosis)

Severe sepsis: sepsis + organ dysfunction (metabolic acidosis, confusion...)

Septic shock: severe sepsis + hypotension (despite adequate of fluids)

Wound originated sepsis: Staphylococcus aureus, Streptococcus pyogenes

Urosepsis: mainly E.coli but also Proteus mirabilis

Abdominal sepsis: Anaerobes + facultative anaerobes (E.coli + P.mirabilis)

Fulminant sepsis: Neisseria meningitidis (death in 24h) also S. pyogenes, Yersinia pestis

Treatment: At ICU only!

- control of infection :

1. antibiotics (first broad spectrum -> oriented)
 2. removal of all infected tissues or devices
- support of breathing and hemodynamics (artificial ventilation of O2, fluids...)

16. Etiology and laboratory diagnosis of bone & joint infections

OSTEOMYELITIS

It is the name given to infections of the bone. It may be either acute or chronic in nature.

Acute osteomyelitis...

Over 90% of acute osteomyelitis cases are caused by *Staphylococcus aureus* but *Streptococcus pyogenes* and *Haemophilus influenzae* may also cause acute infection of the bone although infection with *Haemophilus influenzae* is rare due to the widespread use of Hib vaccine.

Bone is infected by bacteria circulating in the blood stream, which seed the infection. Typically, acute osteomyelitis affects the growing points of long bones since blood flow is sluggish through these regions. This allows opportunity for bacteria in the circulation to settle and to set up an infection at these sites. Although rare, it is most often seen in children and adolescents who are growing rapidly. Sometimes it may be caused by spread of infection from adjacent tissues and in these cases the infection is likely to be polymicrobial.

Chronic osteomyelitis..

The bacterium *Mycobacterium tuberculosis* is the most common cause of chronic osteomyelitis. This condition results from the secondary spread from a pulmonary infection. Unlike acute osteomyelitis, chronic osteomyelitis caused by *mycobacterium tuberculosis* is most likely to affect the vertebrae. Other bones, including those of the hip and knee and bones of the hands and feet may be involved in chronic osteomyelitis. Pressure on the spinal cord caused by chronic osteomyelitis may be sufficient to cause paralysis below the affected region.

Other bacteria causing chronic osteomyelitis include salmonellas and other coliform bacteria, *pseudomonas aeruginosa* and spirochaete *Treponema pallidum*. *T. Pallidum* may cause bone lesions in children suffering from congenital syphilis.

Lab ID

In Acute osteomyelitis bone culture is the definitive test. Open bone biopsy has the highest detection rate but culture can be performed on samples obtained by needle aspiration. Any pus obtained should be collected in a sterile syringe or container and sent to the lab for urgent Gram staining. Joint fluid can also be collected for culture. In biochemical tests the ESR and CRP are elevated and the white cell count is increased but the count rarely rises above 15,000/mm³.

In chronic osteomyelitis bone biopsy performed through non-infected soft tissue provides the best specimen. Blood cultures are rarely positive. Raised ESR/CRP may be the only alteration in lab values in chronic osteomyelitis.

Supporting evidence may be obtained from x-rays and various other radiological techniques.

ARTHRITIS

In arthritis, the affected joint becomes swollen, painful and red. This may be a direct consequence of infection of the joint or because of an immunological reaction. In septic arthritis, joints become infected by bacteria that spread either through the bloodstream or through direct inoculation of the joint, for example through trauma.

The most common cause of septic arthritis in otherwise healthy joints is *Staphylococcus aureus*. *Salmonellas* and *Haemophilus influenzae* may cause septic arthritis in children. Septic arthritis is also a rare complication of gonorrhoea and is most often seen in women who otherwise have an asymptomatic *Neisseria gonorrhoeae* infection. Although septic arthritis typically affects one joint, a monoarthritis, in gonococcal arthritis several joints may be affected simultaneously; it is polyarthritis. Arthritis may occasionally be caused by *Mycobacterium tuberculosis*. This chronic condition typically affects the hip or the knee joints.

Joints may become inflamed because of an immunological reaction to infection elsewhere in the body. A number of viruses cause reactive arthritis through generation of circulating immune complexes. Hepatitis B virus and rubella virus are notable causes of reactive polyarthritis. Rheumatic fever is a rare complication of infection caused by *streptococcus pyogenes*. It is an immunological disease affecting joints and endothelial tissues. Streptococcal antigens that cross-react with host tissues provoke an immune response following infection. These initiate the damage seen in rheumatic fever.

Lab ID

Several lab tests are used to diagnose infectious arthritis. The definitive test involves culturing the fluid from the involved joint after aspiration or incision and drainage. Gram stains are often unreliable, although they provide initial clues. Synovial fluid analysis usually reveals a turbid fluid with leukocyte counts greater than 100,000 mm³ in 30-50% of cases. In bacterial arthritis, the level of polymorphonuclear leukocytes often approaches 90%. Low joint fluid glucose levels and high lactate levels are indicative of septic arthritis, but are non specific. Peripheral blood leukocyte counts are usually elevated in children, but are often within normal limits in adults. Radiography may show joint space widening and soft tissue swelling in infections more than 2 weeks old.

17. Etiology and laboratory diagnosis of skin infections

Primary skin infection

- acute bacterial

acne vulgaris, carbunculus nuchae, erysipelas, folliculitis, furuncles, impetigo, paronychia

- chronic bacteria

actinomycosis, skin granulomas, leprosy, lupus vulgaris, chronic subcutaneous abscess

Secondary skin infection

decubitus, trophic ulcer, infected atheroma, infected intertrigo, infected wounds

Systemic bacterial infections

Roseola, disseminated gonorrhoea, erythema migrans, infected endocarditis, meningococemia, scarlet fever, SSSS (Staph. Skin Scalded Syndrome), toxic shock syndrome, syphilis

Superficial mycosis, mucocutaneous mycosis, cutaneous mycoses, subcutaneous mycoses, suppurative skin mycoses

Skin symptoms in viral diseases: morbillivirus, rubellavirus, erythema infectiosum

18. Etiology and laboratory diagnosis of immunodeficiencies & opportune infections

Immunodeficiency disorders involve malfunction of the immune system, resulting in infections that develop and recur more frequently, are more severe, and last longer than usual.

□ Immunodeficiency disorders usually result from use of a drug or from a long-lasting serious disorder (such as cancer) but occasionally are inherited. □ People usually have frequent, unusual, or unusually severe infections. □ Doctors suspect immunodeficiency based on symptoms and do blood tests to identify the particular disorder. □ People are given antibiotics to prevent and treat infections. □ Immune globulin may be given if antibodies (immunoglobulins) are missing. □ If the disorder is severe, stem cell transplantation may be done.

Immunodeficiency disorders impair the immune system's ability to defend the body against foreign or abnormal cells that invade or attack it (such as bacteria, viruses, fungi, and cancer cells). As a result, unusual bacterial, viral, or fungal infections and rare cancers may develop.

There are two types of immunodeficiency disorders:

□ Congenital (primary): These disorders are present at birth and are usually hereditary. They typically become evident during infancy or childhood. All are relatively rare.

□ Acquired (secondary): These disorders develop later in life and often result from use of a drug or from another disorder, such as diabetes or human immunodeficiency virus (HIV) infection. They are more common than congenital immunodeficiency disorders.

Some immunodeficiency disorders shorten life span. Others persist throughout life but do not affect life span, and a few resolve with or without treatment.

Causes

Congenital immunodeficiency: These disorders are caused by a genetic abnormality (often X-linked-only boys are affected). As a result, about 60% of people with congenital immunodeficiency disorders are male.

Congenital immunodeficiency disorders are classified by which part of the immune system is affected:

□ B cells (lymphocytes), a type of white blood cell that produces antibodies (immunoglobulins) □ T cells (lymphocytes), a type of white blood cell that helps identify and destroy foreign or abnormal cells □ B and T cells □ Phagocytes (cells that ingest and kill microorganisms) □ Complement proteins (proteins with various immune functions, such as killing bacteria and other foreign cells and making foreign cells easier for other immune cells to identify and ingest. The affected component of the immune system may be missing, reduced in number, or abnormal and malfunctioning. Problems with B cells are the most common congenital immunodeficiency disorders, accounting for more than half.

Acquired immunodeficiency disorders: These most commonly result from drugs (mainly immunosuppressants, which are used to treat serious disorders). Immunosuppressants are used to intentionally suppress the immune system. For example, some are used to prevent rejection of a transplanted organ or corticosteroids, a type of immunosuppressant, are used to suppress inflammation due to various disorders, such as rheumatoid arthritis. However, immunosuppressants also suppress the body's ability to fight infections and perhaps to destroy cancer cells. Chemotherapy and radiation therapy can also suppress the immune system, sometimes leading to immunodeficiency disorders.

Immunodeficiency disorders may result from almost any prolonged serious disorder. For example, diabetes can result in an immunodeficiency disorder because white blood cells do not function well when the blood sugar level is high. Human immunodeficiency virus (HIV) infection results in acquired immunodeficiency syndrome (AIDS), the most common severe acquired immunodeficiency disorder.

Undernutrition—whether of all nutrients or only one—can impair the immune system. When undernutrition causes weight to decrease to less than 80% of recommended weight, the immune system is often impaired. A decrease to less than 70% usually results in severe impairment.

Symptoms

People with an immunodeficiency disorder tend to have one infection after another. Usually, respiratory infections develop first and recur often. Most people eventually develop severe bacterial infections that persist, recur, or lead to complications. For example, sore throats and head colds may progress to pneumonia. However, having many colds does not suggest an immunodeficiency disorder.

Infections of the skin and the membranes lining the mouth, eyes, and digestive tract are common. Thrush, a fungal infection of the mouth, may be an early sign of an immunodeficiency disorder. Sores may form in the mouth. Ear infections and skin infections by bacteria or viruses are also common. Bacterial infections (for example, with staphylococci) may cause pus-filled sores to form (pyoderma). Warts (caused by viruses) may form.

Many people lose weight.

Infants or young children may have chronic diarrhea and may not grow and develop as expected (called failure to thrive). The earlier symptoms begin in children, the more severe the immunodeficiency.

Other symptoms vary depending on the severity and duration of the infections.

Diagnosis

Doctors must first suspect that an immunodeficiency exists. Then they do tests to identify the specific immune system abnormality.

Doctors suspect immunodeficiency when a severe or an unusual infection recurs often or when an organism that normally does not cause severe infection (such as Pneumocystis or cytomegalovirus) causes severe infection. The results of a physical examination may also suggest immunodeficiency. Rashes, hair loss, chronic cough, weight loss, and an enlarged liver and spleen are often present. Lymph nodes and tonsils may be extremely small in some forms of immunodeficiency, whereas in other types the lymph nodes may be swollen. Certain symptoms may suggest a particular disorder to doctors.

To help identify the type of immunodeficiency disorder, doctors ask at what age the person began to have recurring or unusual infections. Infections in infants younger than 6 months usually indicate an abnormality in T cells. Infections in older children usually indicate an abnormality in B cells and antibody production. The type of infection may also help doctors identify the type of immunodeficiency disorder.

Doctors ask the person about risk factors, such as diabetes, use of certain drugs, exposure to toxic substances, and the possibility of having close relatives with immunodeficiency disorders (family history).

The person is asked about past and current sexual activity and use of intravenous drugs to determine whether HIV infection could be the cause.

Tests: Laboratory tests are needed to confirm the diagnosis of immunodeficiency and to identify the type of immunodeficiency disorder. A blood sample is taken and analyzed to determine the total number of white blood cells and the percentages of each main type of white blood cell. The white blood cells are examined under a microscope for abnormalities. Antibody levels, the number of red blood cells and platelets, and the levels of complement proteins are determined. If any results are abnormal, additional tests are usually done. Skin tests may be done if the immunodeficiency is thought to be due to a T-cell abnormality. The skin test resembles the tuberculin skin test, which is used to screen for tuberculosis. Small amounts of proteins from common infectious organisms such as yeast are injected under the skin. If a reaction (redness, warmth, and swelling) occurs within 48 hours, the T cells are functioning normally. No reaction suggests a T-cell abnormality.

People whose families are known to carry a gene for a hereditary immunodeficiency disorder may wish to have genetic testing to learn whether they carry the gene for the disorder and what the chances of having an affected child are. Talking with a genetic counselor before testing is helpful. Several immunodeficiency disorders, such as X-linked agammaglobulinemia, Wiskott-Aldrich syndrome, severe combined immunodeficiency, and chronic granulomatous disease, can be detected in a fetus by testing a sample of the fluid around the fetus (amniotic fluid) or the fetus's blood (prenatal testing). Such testing may be recommended for people with a family history of an immunodeficiency disorder when the mutation has been identified in the family.

Opportunistic infections: Infections that take advantage of the debility of the immune system and in normal individuals do not have a clinical manifestation.

Candidiasis

Herpes Zoster – Generally constitutes one of the first clinical associations to HIV infection. The clinical diagnosis is done by the observation of cutaneous lesions over the nerve tracks (which can or not be accompanied by pain or hemorrhagic process).

Pneumocystis carinii – see question D9

TB (pulmonary) – Pulmonary tuberculosis (TB) is a contagious bacterial infection that mainly involves the lungs, but may spread to other organs. It is caused by the bacteria *Mycobacterium tuberculosis* (M. tuberculosis). You can get tuberculosis by breathing in air droplets from a cough or sneeze of an infected person. The primary stage of the disease usually doesn't have symptoms.

When symptoms do occur, they may include:

Cough (sometimes producing phlegm) Coughing up blood Excessive sweating, especially at night Fatigue Fever Unintentional weight loss

Cerebral toxoplasmosis - Toxoplasmosis is an infectious disease caused by the one-celled protozoan parasite *Toxoplasma gondii*. Can be fatal. Cats, the primary carriers of the organism, become infected by eating rodents and birds infected with the organism. When symptoms do occur, they may include:

Enlarged lymph nodes Muscle pains Intermittent fever General sick feeling

Visceral leishmaniose - also known as kala-azar, black fever, and Dumdum fever, is the most severe form of leishmaniasis. Leishmaniasis is a disease caused by protozoan parasites of the *Leishmania* genus. The clinical symptoms are fever, asthenia, weight loss, anemia, change in hepatic functions.

19. Etiology and laboratory diagnosis of congenital & neonatal infections

Congenital infections are infections that occur prenatally and neonatally.

Infections can either be perinatal (closely before and during delivery) and postnatal (up to 4 weeks of life).

These are generally caused by agents unusual in older children.

Fetuses are generally protected by the placenta and amnion, the maternal IgG (actively transported through placenta, IgG against capsular polysaccharides are active only up to 3 months after delivery and against viruses up to 12-15 months) and colostrum IgA.

Gravity of the affliction is generally the highest in 1st trimester when being able to cause abortion!

Agent	Trimester			Congenital defects	Postnatal persistence
	1	2	3		
T. pallidum	-	✓	✓	✓	✓
L. monocytogenes	-	-	✓	-	-
Rubellavirus	✓✓	✓	-	✓	✓
CMV	✓	✓	✓	✓	✓
Parvovirus	✓/ X	✓	✓/ X	-	-
VZV	✓	-	-	✓/ X	✓
HSV				-	✓
Toxoplasma gondi	✓/ X	✓	✓✓	✓	✓

Diagnosis: prenatal infection

mother should be examined for syphilis and toxoplasmosis. Newborns should have their IgM examined (antibodies aren't their mother's since they cannot pass through placenta) or if possible direct detection should be done (CMV in urine).

Treatment: for the mother: penicillin for syphilis and spiramycin in toxoplasmosis

Prevention: healthy mother

Infections proceeding more severely in pregnancy

- Malaria, Virus Hepatitis, Influenza (during pandemics), Polyomyelitis, UTIs (pressure of ureter), Candidosis and Listeriosis.
- Agents that might activate themselves in pregnancy: CMV, JC and BK virus, HSV-2, EBV

Perinatal Infections

- during delivery
- Chl. trachomatis D-K, E.coli and other enteric rods, N. gonorrhoea, Listeria monocytogenes, H. influenza, Mycoplasma hominis, Candida albicans, HSV-2 -> Agents originating in vagina, cervix or rectum

Postnatal infections

from mother (group β Streptococci, Staph. aureus, Mycobacter Tb, CMV and HIV)

20. Etiology and laboratory diagnosis of nosocomial (hospital) infections

Nosocomial = hospital acquired = NIs 5% of patients gets this in connection with stay in medical institution

Exogenous Source – other patients, environment, personnel (mostly neglect of washing hands)

Endogenous - From the patient

40% higher death rate Longer hospitalization More medicines 1/3 can be avoided

1. UTIs – from catheters about 40% 2. RTIs – about 20 %

Early respirator-associated pneumonia (early VAP) Late respiratory-associated pneumonia: aspiration pneumonia, other respiratory infections

3. Purulent infections in surgical wounds – about 20 %

4. Blood-stream infections – sepsis from iv- catheters 15%

Etiology: E. coli 25% , other enteric bac 20%, Enterococci 15%, P. aeruginosa 10%, Other G(-) non-fermenting rods 10%, Yeasts 5%

Etiology of respiratory: early VAP S. aureus 25%, Str. Pneumoniae 20%, Haemophilus influenzae 15%, Enteric bacis 10%, other aerobic 5%, Anaerobes 1%,

Monomicrobial etiology: agents originate in community

Etiology of respiratory: late VAP G- non-fermenting rods 40%, Enteric bacteria 30%, Staphs, mainly aureus 20%, Yeasts 5%

Polymicrobial origin – hospital origin

Etiology Aspiration: pneumonia same as in VAP but more G- rods, non-fermenting rather than enteric Pneumonia in febrile neutropenia

Initial days: G+ cocci(staphs and pneumococci) occur 2x more than G-rods(enteric bacteria & pseudomonads)

Later: fewer G+ cocci, more Candida and aspergilli Allogenic transplantation of BM: mainly CMV, also Candida

Surgical wounds (depend on type of surgery) S.aureus Coagulase(-) staphs Str. pyogenes Enteric bacteria E.coli Bacteroids, prevotellae, peptostreptococci G- non fermenting rods Clostridium perfringens □ gas gangrene

Etiology of sepsis by i.v. catheter Coagulase (-) staphylococci > 50% Because of biofilm Enterococci – because of cephalosporins S. aureus

Enteric bacis E.coli, Klebsiella Pseudomonas aeruginosa Acinetobacter spp. Candida spp.

Etiology of nosocomial VIRAL infections: Influenza virus – infants and elderly; RSV – newborns and suckling infants; Adenoviruses – ophthalmic wards; CMV – after cytotoxic treatment; Rubella virus – children (vaccination available); Rotaviruses – children mainly; VHB – risk elevated if longer hospitalization; HIV – in developing countries mostly

Risk factors: Age – older & newborns; Treatment – cytotoxic drugs, steroids, antibiotics Underlying disease: Hepatic, DM, Cancer, Renal failure, Skin disorders, Neutropenia, Trauma – including surgery

4 main strategies to prevent NIs

1. Elimination of sources of infection: Sterile instruments, dressings, medicaments and iv-fluids, screen blood for infection, clean linen, uncontaminated food, avoid contact with infected staff – carriers and acutely ill

2. Break chain of infection – disinfection, hygiene, isolation

Facilities: ventilation systems & air flow (legionella in air condition, aspergilli in building work). Water systems – hot water – legionella. Isolation of patients – keep susceptible from contaminated people: aseptic behaviour of staff, wash hands

3. Improve host resistance:

Immunization: influenza in elderly, Pneumococcal infections before transplant or splenectomy, VHB if seronegative before haemodialysis, Varicella

ATB prophylaxis: “dirty” surgery, “super clean surgery” orthopaedics and neurosurgery.

Reduce postoperative risk of infections: Correct operating technique, care of invasive devices and iv-fluids, correct nursing techniques, pressure sores, physiotherapy

4. Investigating hospital infection

Surveillance: monitoring allows early recognition, investigation of outbreaks, epidemiological and microbiological, establish procedures for this.