CHAPTER 1

Basic Bacteriology

Normal Flora

- Normal flora consists mainly of bacteria, but fungi and protozoa may be present in some individuals.

- They can provide useful nutrients (vitamin K) and release compounds with antibacterial activity against pathogenic bacteria.

- They contribute to host defense by competing with invaders for space and nutrients and maintaining conditions such as PH (Lowering the pH so that other bacteria can't grow).

- They Can cause infection if:

- Misplaced, fecal flora to urinary tract or abdominal cavity, or skin flora to catheter.
- Or, if person becomes compromised, normal flora may overgrow (oral thrush).

- They reside in the skin, mouth, nose, oropharynx, large intestine, urethra, and vagina:

A. Skin: Staphylococcus epidermidis.

B. Nose:

- Staphylococcus epidermidis.
- Colonized by Staphylococcus aureus (25% people).

C. Oropharynx: Viridans group streptococci.

D. Dental plaque: Streptococcus mutans.

E. Colon:

- Bacteroides (99% of GI tract bacteria).
- Escherichia coli.

F. Vagina: Lactobacillus which suppress pathogens by producing lactic acid.

G. In Neonates:

- GI tract of fetus is sterile.

- Neonates are rapidly colonized after birth.

- Infants delivered via cesarean section have unstable flora for longer period, because during a regular vaginal birth, infants come in contact with a rich dose of their mother's bacteria as they are pressed through the birth canal. On the other hand, C-section babies don't get this exposure, which is likely to be vital in developing the immune system and helping it to mature.

Bacterial structure

1. Shape:

- Along with other properties, shape is used to identify bacteria. It is determined by the mechanism of cell wall assembly.

- Bacterial shape usually can be determined with appropriate staining and a light microscope.

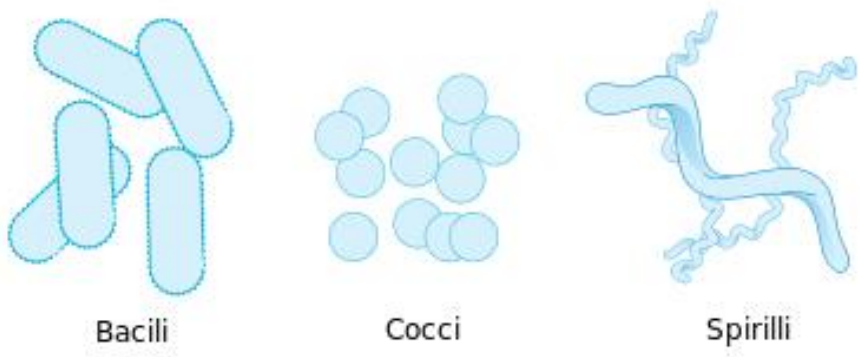
- Types:

A. Round (coccus).
B. Rod-like (bacillus).
C. Spiral.

- Cocci and bacilli often grow in doublets (diplococcic as Neisseria) or chains (streptococci). Cocci that grow in grape-like clusters are called staphylococci.

- Some bacterial species are pleomorphic, such as Bacteroides.

- Antibiotics that affect cell wall biosynthesis (penicillin) may alter a bacteria's shape.



2. Nucleus:

- In bacteria, the nucleus generally is called a nucleoid or nuclear body.

- Genetic information of a bacterial cell is contained in a single circular molecule of double-stranded DNA, which constitutes the bacterial chromosome.

- Plasmid: in many bacteria, additional genetic information is contained on plasmids which are small circular extrachromosomal DNA molecules that can replicate independently of the chromosome.

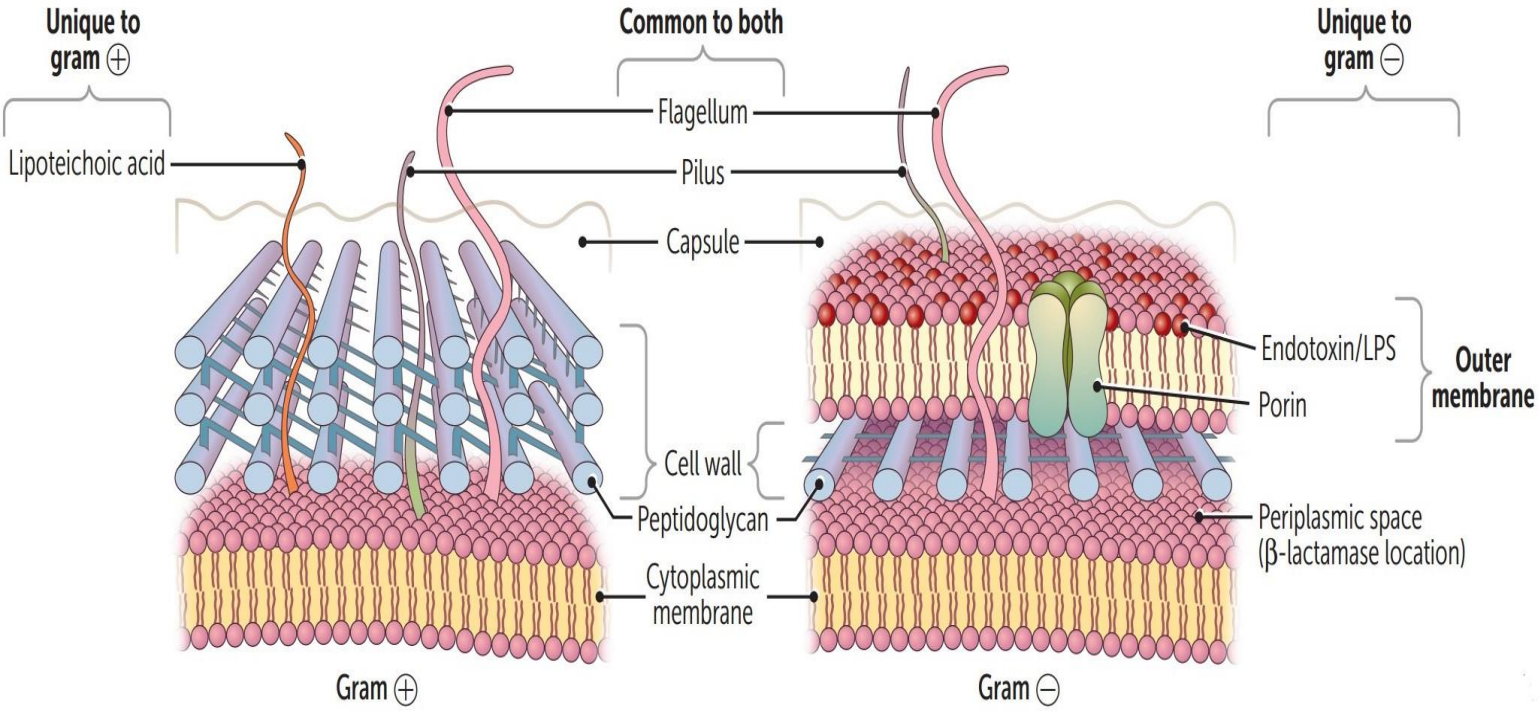
3. Cytoplasm:

- Bacterial cytoplasm contains ribosomes and various types of nutritional storage granules.

- It contains no organelles.

4. Ribosomes:

- They are the site of protein synthesis in the cell.
- Prokaryotic ribosomes have a sedimentation constant of 70S, smaller than the 80S ribosomes of eukaryotes.
- This difference makes bacterial ribosomes a selective target for antibiotic action.



5. Cell (cytoplasmic) membrane:

- In bacteria, as in other cells, the protoplast is limited externally by a thin elastic cytoplasmic membrane. It is a phospholipid protein bilayer similar to that of eukaryotic cells except that, in bacteria, it lacks sterols.
- Embedded with proteins (penicillin-binding proteins [PBPs] involved in cell wall synthesis) and other enzymes.
- It has the following functions:

A. Selective transport: in bacteria, molecules move across the cytoplasmic membrane by simple diffusion, facilitated diffusion and active transport.

B. Excretion of extracellular enzymes:

- Hydrolytic enzymes: which digest large food molecules into subunits small enough to penetrate the cytoplasmic membrane.
- Enzymes used to destroy harmful chemicals, such as antibiotics.

C. Respiration: the respiratory enzymes are located in the cytoplasmic membrane, which is thus a functional analogue of the mitochondria in eukaryotes.

D. Cell wall biosynthesis:

- The cytoplasmic membrane is the site of:
  - The enzymes of cell wall biosynthesis.
  - The lipid carrier on which the subunits of the cell wall are assembled.

E. Plasmids:

- Plasmids are small, circular, nonchromosomal, double-stranded DNA molecules that are:
- Capable of self-replication.
- Most frequently extrachromosomal but may become integrated into bacterial DNA.
- Function: contain genes that confer protective properties such as antibiotic resistance or virulence factors or their own transmissibility to other bacteria.

F. Transposons:

- Transposons are small pieces of DNA that move between the DNA of bacteria and plasmids; they do not self-replicate.
- Functions:
- Code for antibiotic resistance enzymes, metabolic enzymes, or toxins.
- May alter expression of neighboring genes or cause mutations to genes into which they are inserted.

G. Cell wall:

- The bacterial cell wall is the structure that immediately surrounds the cytoplasmic membrane.
- It is thick, strong and relatively rigid, though having some elasticity.
- Structure of the cell wall:
- The cell wall of bacteria is a complex structure.
- Its impressive strength is primarily due to peptidoglycan.
- Peptidoglycan is a complex polymer consisting of N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) that is unique to bacteria.
- Besides peptidoglycan, additional components in the cell wall divide bacteria into Gram-positive and Gram-negative.
- Gram-positive cell wall is composed of:

A. Peptidoglycan:

- There are many as 40 sheets of peptidoglycan, comprising up to 50% of the cell wall material.
- Despite the thickness of peptidoglycan, chemicals can readily pass through.

B. Lipoteichoic acids:

- They are found in the cell wall of most gram-positive bacteria.
- Lipoteichoic acids and cell wall associated proteins are the major surface antigens of the Gram-positive bacteria.

▪ Gram-negative cell wall is composed of:

A. Peptidoglycan: it is much thinner, composed of only one or two sheets comprising 5-10% of the cell wall material.

B. Outer membrane:

- It is a phospholipid protein bilayer present external to the peptidoglycan layer.
- The outer surface of the lipid bilayer is composed of molecules of lipopolysaccharides (LPS) which consist of a complex lipid called lipid A chemically linked to polysaccharides.
- Lipid A of the LPS forms the endotoxins of the Gram-negative bacteria, while polysaccharides are the outermost molecules of the cell wall and the major surface antigens of the Gram-negative bacterial cell (somatic or O antigen).

C. Periplasmic space: it is the space between the cytoplasmic and outer membranes. Contains many hydrolytic enzymes, including β-lactamases.

▪ Functions of the cell wall:

1. It maintains the characteristic shape of the bacterium.
2. It supports the weak cytoplasmic membrane against the high internal osmotic pressure of the protoplasm.
3. It plays an important role in cell division.
4. It is responsible for the staining affinity of the organism.

❖ N.B:

- Mycoplasma is the only group of bacteria that exist naturally without cell wall.
- It doesn't assume a defined recognizable shape, because they lack a rigid cell wall.
- These organisms are naturally resistant to cell wall inhibitors, such as penicillin and cephalosporins.

H. Capsule and related structures:

- Many bacteria synthesize large amount of extracellular polymer that collect outside the cell wall to form an additional surface layer.

▪ With one known exception (the polypeptide capsule of bacillus anthracis), the extracellular material is made of polysaccharides:

A. Capsule: it is such layer that adheres to the surface of the cell and forms a well defined halo when differentially stained, to be resolved with the light microscope.

B. Slime layer: it is a surface layer that is loosely distributed around the cell.

C. Glycocalyx: it is a loose meshwork of polysaccharide fibrils extending outwards from the cell.

- Functions:

1. It protects the cell wall against various kinds of antibacterial agents.

2. It protects the bacterial cell from phagocytosis. Hence, the capsule is considered an important virulence factor.

3. Some bacteria attach to the target surface by using their capsules or glycocalyx in order to establish infection. For instance, streptococcus mutans form glycocalyx, with which the bacteria stick to the tooth enamel.

I. Appendages:

- Several structures project through the cell wall of bacteria to form surface appendages. The most commonly observed are flagella and pili:

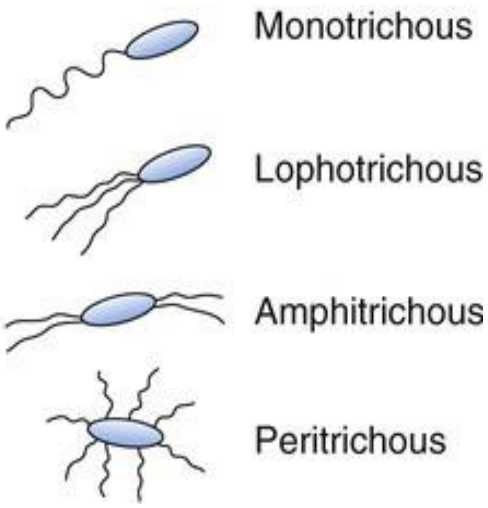
A. Flagella:

- Many genera of bacteria move by means of flagella.

- The location and number of flagella on a cell vary according to bacterial species. Organisms may be monotrichous (single polar flagellum), lophotrichous (multiple polar flagella) or peritrichous (flagella distributed over the entire cell surface).

- Flagella consist of a single type of protein called flagellin which differs in different bacterial species. The flagellins are highly antigenic (H antigen).

- Motile bacteria tend to migrate towards regions where there is a higher concentration of nutrients and solutes (a process known as chemotaxis) and away from disinfecting substances (negative chemotaxis).



B. Pili or fimbriae:

- Pili are protein tubes that extend from the cells.

- They are shorter and thinner than flagella and composed of structural protein subunits termed pilins.

- Functions:

1. Adherence:

- It is the function of the short pili (fimbriae) that occur in great numbers around the cell.
- They enable bacteria to attach to the host surfaces, thus contributing to the establishment of infection (virulence factor).
- Ex: Neisseria gonorrhea withstands the flushing action of urine by adhering to the urethral mucosa.

2. Conjugation:

- A special long pilus called the sex pilus (F or fertile pilus) is involved in the transfer of DNA between bacteria, a process known as conjugation.

J. Bacterial spores (endospores):

- Some bacteria, notably those of the genera Bacillus and clostridium, develop a highly resistant resting phase or endospore that does not grow or reproduce, and exhibits absolute dormancy.

- A single vegetative bacterium forms a single spore by a process called sporulation.

- A single vegetative bacterium emerges from a spore during germination.

❖ Sporulation:

- Sporulation is triggered by the onset of unfavorable environment conditions (depletion of nutrients, accumulation of metabolites or change in the growth requirements as moisture, temperature, pH or oxygen tension).

- The cytoplasmic membrane invaginates enclosing a section of the cytoplasm that contains the bacterial chromosome, some ribosomes and other cytoplasmic materials that will be needed for germination. It acquires a thick cortex and a thin tough outer spore coat.

❖ N.B:

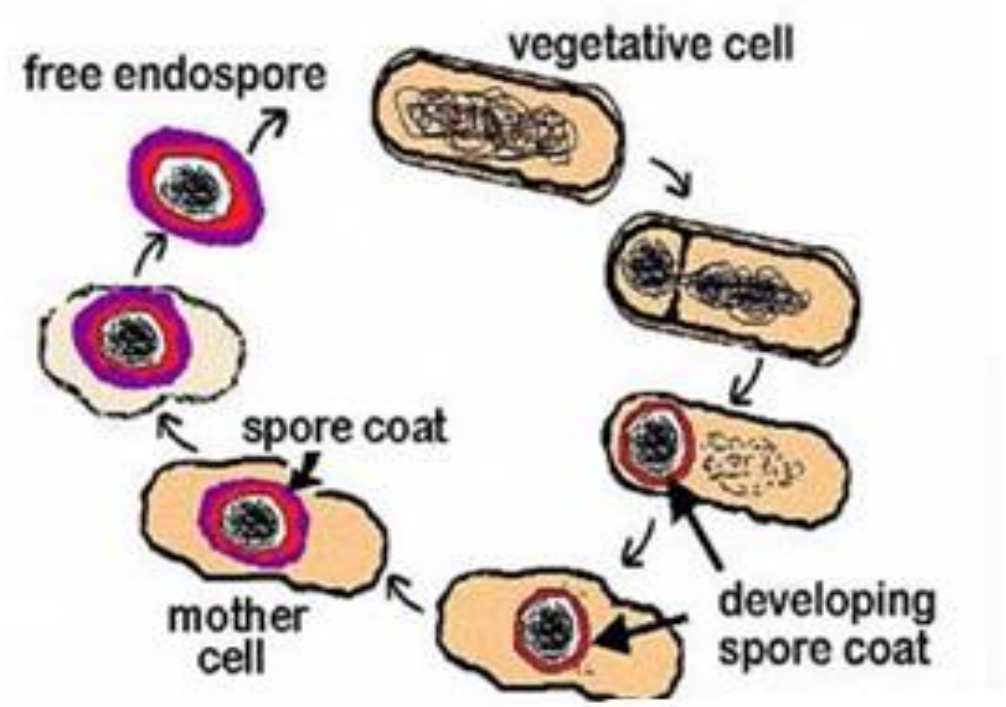
- Spores are much more resistant to disinfectants, drying and heating.
- Moist heat at 121C for 10-20 minutes is needed to kill spores while 60C is sufficient to kill vegetative forms.
- The marked resistance of the spores has been attributed to several factors:

1. Thermal resistance is provided by their high content of Ca and dipicolinic acid (a compound unique to endospores).
2. Their low content of water.
3. Their very low metabolic and enzymatic activity.

❖ Germination:

- Endospores can respond quickly to changes in the environment returning to the vegetative state within 15 min.

- In the process of germination, the spores absorb water and swell, the protective coat disintegrates, and a single vegetative cell emerges.



Structure	Function	Chemical Position
Peptidoglycan	Gives rigid support, protects against osmotic pressure.	Sugar backbone with peptide side chain cross-linked by transpeptidase.
Cell wall	Major surface antigen.	Peptidoglycan for support. Lipoteichoic acid induces TNF and IL-1.
Outer membrane (Gram – ve)	Gram ⊖ only. Site of endotoxin (lipopolysaccharide [LPS]); major surface antigen.	Lipid A induces TNF and IL-1; O polysaccharide is the antigen.
Plasma membrane	Site of oxidative and transport enzymes. PBPs involved in cell wall synthesis. Lipoteichoic acids induce TNF-α and IL-1.	Phospholipid bilayer sac with embedded proteins (penicillin-binding proteins [PBPs]) and other enzymes. Lipoteichoic acids (gram positive) only extend from membrane to exterior.
Ribosome	Protein synthesis.	50S and 30S subunits.
Periplasm	Space between the cytoplasmic membrane and outer membrane in gram-negative bacteria.	Contains many hydrolytic enzymes, including β-lactamases.
Pilus/fimbria	Mediate adherence of bacteria to cell surface; sex pilus forms attachment between 2 bacteria during conjugation.	Glycoprotein.
Flagellum	Motility.	Protein.
Spore	Gram ⊕ only. Resistant to dehydration, heat, and chemicals.	Keratin-like coat; dipicolinic acid; peptidoglycan, DNA.
Plasmid	Contains a variety of genes for antibiotic resistance, enzymes, and toxins.	DNA.
Capsule	Protects against phagocytosis.	Organized, discrete polysaccharide layer (except Bacillus anthracis, which contains d-glutamate).
Glycocalyx	Mediates adherence to surfaces, especially foreign surfaces (indwelling catheters).	Loose network of polysaccharides.

Bacterial taxonomy

Morphology	Gram-positive examples	Gram-negative examples
Spherical (coccus)	- Staphylococcus - Streptococcus	- Moraxella catarrhalis - Neisseria
Rod (bacillus)	- Bacillus - Clostridium - Corynebacterium - Gardnerella (gram variable) - Lactobacillus - Listeria - Mycobacterium (acid fast) - Propionibacterium	▪ Enterics: - Bacteroides - Campylobacter - E. coli - Enterobacter - Helicobacter - Klebsiella - Proteus - Pseudomonas - Salmonella - Serratia - Shigella - Vibrio - Yersinia ▪ Respiratory: - Bordetella - Haemophilus(pleomorphic) - Legionella (silver stain) ▪ Zoonotic: - Bartonella - Brucella - Francisella - Pasteurella
Branching filamentous	- Actinomyces - Nocardia (weakly acid fast)	
Pleomorphic		- Chlamydiae (Giemsa) - Rickettsiae (Giemsa)
Spiral		▪ Spirochetes: - Borrelia (Giemsa) - Leptospira - Treponema
No cell wall	Mycoplasma, Ureaplasma (contain sterols, which do not Gram stain)	

Microbial Pathogenicity

- The pathogenesis of a bacterium depends on its virulence properties and the capabilities of the host's defense mechanism.

- Virulence factors:

- They are chromosomal and extrachromosomal (plasmid) gene products that affect aspects related to an organism's:
  - Invasion properties.
  - Adherence and colonization.
  - Tissue damage induced by toxins, immune system reactions, and intracellular growth.
  - Eluding host defense mechanisms.
  - Antibiotic resistance.

- Normal flora may become pathogenic if they gain access to normally sterile body areas or their environmental conditions allow them to multiply to a level not controlled by the host.

- A microbe's pathogenicity is related to its:

1. Colonization:

- The first stage of microbial infection is colonization (the establishment of the pathogen at the appropriate portal of entry).

- Pathogens usually colonize host tissues that are in contact with the external environment.

- Sites of entry in human hosts include the urogenital tract, the digestive tract, the respiratory tract and the conjunctiva.

- Organisms that infect these regions have usually developed tissue adherence mechanisms and some ability to overcome or withstand the constant pressure of the host defenses at the surface.

- Adherence to cell surfaces involves:

A. Adhesins:

- A surface structure or macromolecule that binds a bacterium to a specific surface.

- Ex: pertussis toxin and hemagglutinins.

B. Pili/fimbriae:

- Filamentous proteins on the surface of bacterial cells that may behave as adhesins for specific adherence.

- Ex: primary mechanism in most gram-negative cells.

C. Lipoteichoic acids:

- Cell wall components of Gram-positive bacteria that may be involved in nonspecific or specific adherence.

- Ex: primary mechanism of gram-positive cells.

D. IgA proteases:

- IgA protease cleaves IgA, leaving the Fc region to coat the bacterium enabling it to bind to Fc receptors on epithelial/mucosal cells. Therefore, IgA protease facilitates attachment colonization of respiratory mucosa.

- Ex: S. pneumoniae, H. influenzae type B, and Neisseria (SHiN) in order to colonize respiratory mucosa.

E. Biofilm:

- Once introduced into the body, foreign bodies quickly become coated with a layer of host proteins, including fibrinogen and fibronectin. These proteins then serve as binding sites for S epidermidis.

- After attachment occurs, the bacteria multiply and communicate with one another to induce synthesis of an extracellular polysaccharide matrix (biofilm) that encases the bacteria.

- The resulting biofilm functions as a barrier to antibiotic penetration and interferes with host defenses, including opsonization, neutrophil migration, and even T lymphocyte activation.

- Once mature, these biofilms can disperse individual pathogen "seeds" (planktonic cells) into the bloodstream and surrounding areas, further disseminating the infection.

- Definitive treatment of infections caused by biofilm-producing organisms often requires removal of the foreign body.

- In vivo biofilm producing bacteria:

S. epidermidis	Catheter and prosthetic device infections
Viridans streptococci (S mutans, S sanguinis)	Dental plaques, infective endocarditis
P. aeruginosa	Respiratory tree colonization in patients with cystic fibrosis, ventilator-associated pneumonia. Contact lens-associated keratitis
Nontypeable (unencapsulated) H influenzae	Otitis media

2. Avoiding immediate destruction by host defense system:

- Anti-phagocytic surface components which inhibit phagocytic uptake:

A. Capsules:

- Capsules are usually made of polysaccharides, which are usually poor immunogens.

- The S. pyogenes capsule, for example, is made of hyaluronic acid, which mimics human connective tissue, thereby masking the bacteria and keeping them from being recognized by the immune system.

B. IgA proteases (Enzyme that cleaves IgA):

- Secreted by S. pneumoniae, H. influenzae type B, and Neisseria (SHiN) in order to colonize respiratory mucosa.

3. Antigenic Variation:

- Antigenic variation refers to the mechanism by which an infectious agent such as a protozoan, bacterium or virus alters its surface proteins in order to evade a host immune response.

- Antigenic variation not only enables immune evasion by the pathogen, but also allows the microbes to cause re-infection, as their antigens are no longer recognized by the host's immune system.

- When the body is exposed to a particular antigen (a protein on the surface of a bacterium) an immune response is stimulated and antibodies are generated to target that specific antigen.

- The immune system will then "remember" that particular antigen, and defenses aimed at that antigen become part of the immune system's acquired immune response.

- If the same pathogen tries to re-infect the same host the antibodies will act rapidly to target the pathogen for destruction.

- However, if the pathogen can alter its surface antigens, it can evade the host's acquired immune system. This will allow the pathogen to re-infect the host while the immune system generates new antibodies to target the newly identified antigen.

- Examples:

- N. gonorrhoeae-pili and outer membrane proteins.
- Enterobacteriaceae: capsular and flagellar antigens may or may not be expressed.

4. Ability to survive intracellularly:

- Evading intracellular killing by professional phagocytic cells allows intracellular growth:
- Tuberculosis survives by inhibiting phagosome-lysosome fusion.
- Listeria quickly escapes the phagosome into the cytoplasm before phagosome-lysosome fusion.

5. Type III Secretion Systems:

- Tunnel from the bacteria to the host cell (macrophage) that delivers bacterial toxins directly to the host cell. Also known as "injectisome".

- Needle-like protein appendage facilitating direct delivery of toxins from certain gram-negative bacteria (Pseudomonas, Salmonella, Shigella, E. coli) to eukaryotic host cell.

6. Inflammation or Immune-Mediated Damage:

- Cross-reaction of bacteria-induced antibodies with tissue antigens causes disease.
- Ex: Rheumatic fever.

- Delayed hypersensitivity and the granulomatous response stimulated by the presence of intracellular bacteria is responsible for neurological damage in leprosy, cavitation in tuberculosis, and fallopian tube blockage resulting in infertility from Chlamydia PID (pelvic inflammatory disease).

- Immune complexes damage the kidney in post streptococcal acute glomerulonephritis.

- Peptidoglycan-teichoic acid (large fragments) of gram-positive cells serves as:
- A structural toxin released when cells die.
- Chemotactic for neutrophils.

7. Physical Damage:

- Swelling from infection in a fixed space damages tissues.
- Ex: meningitis and cysticercosis.

- Large physical size of organism may cause problems.
- Ex: Ascaris lumbricoides blocking bile duct.

- Aggressive tissue invasion from Entamoeba histolytica causes intestinal ulceration.

Gram Stain

- Because bacteria are colorless and usually invisible to light microscopy, colorful stains have been developed to visualize them.

- The most useful is the Gram stain, which separates organisms into 2 groups: gram-positive bugs and gram-negative bugs.

- This stain also allows the clinician to determine whether the organism is round (cocci) or rod-shaped (bacilli).

- For any stain you must first smear the substance to be stained (sputum, pus, etc.) onto a slide and then heat it to fix the bacteria on the slide.

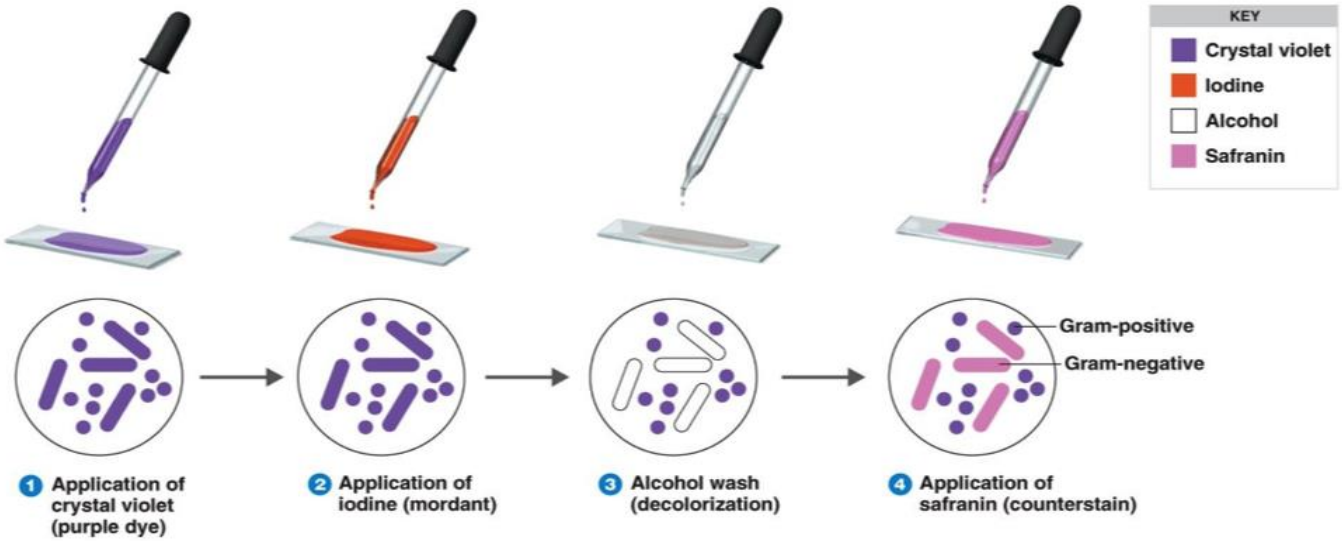
- <u>There are 4 steps to the Gram stain:</u>

- Pour on crystal violet stain (a blue dye) and wait 60 seconds.
- Wash off with water and flood with iodine solution. Wait 60 seconds.
- Wash off with water and then "decolorize" with 95% alcohol.
- Finally, counterstain with safranin (a red dye). Wait 30 seconds and wash off with water.
- When the slide is studied microscopically, cells that absorb the crystal violet and hold onto it will appear blue. These are called gram-positive organisms.
- However, if the crystal violet is washed off by the alcohol, these cells will absorb the safranin and appear red. These are called gram-negative organisms.

Gram-positive = BLUE

Gram-negative = RED

- The different stains are the result of differences in the cell walls of gram-positive and gram-negative bacteria.



❖ Gram stain limitations:

▪ These bugs do not Gram stain well:

- Treponema, Leptospira (too thin to be visualized). Dark-field microscopy and fluorescent antibody staining are used.
- Mycobacteria (high lipid content; mycolic acids in cell wall detected by carbolfuchsin in acid- fast stain).
- Mycoplasma, Ureaplasma (no cell wall).
- Legionella pneumophila (primarily intracellular). Silver stain is used.
- Legionella, Rickettsia, Chlamydia, Bartonella, Anaplasma, Ehrlichia (intracellular parasite; lacks classic peptidoglycan because of low muramic acid).
- Mnemonic: These Little Microbes May Unfortunately Lack Real Color But Are Everywhere

❖ Other Stains:

▪ Giemsa: Chlamydia, Borrelia, Rickettsia, Trypanosomes, Plasmodium.

- Mnemonic: Certain Bugs Really Try my Patience.

▪ PAS (periodic acid–Schiff): Stains glycogen, mucopolysaccharides; used to diagnose Whipple disease (Tropheryma whipplei).

- Mnemonic: PASs the sugar.

▪ Ziehl-Neelsen (carbol fuchsin): Acid-fast bacteria (Nocardia, Mycobacteria), protozoa (Cryptosporidium oocysts).

- Alternative is auramine-rhodamine stain for screening (inexpensive, more sensitive but less specific).

▪ India ink: Cryptococcus neoformans (mucicarmine can also be used to stain thick polysaccharide capsule red).

▪ Silver stain: Fungi (Pneumocystis), Legionella, Helicobacter pylori.

❖ Microbial culture:

▪ It is a method of multiplying microbial organisms by letting them reproduce in predetermined culture media under controlled laboratory conditions.

▪ Microbial cultures are used to determine the type of organism, its abundance in the sample being tested, or both.

▪ It is one of the primary diagnostic methods of microbiology and used as a tool to determine the cause of infectious disease by letting the agent multiply in a predetermined medium.

- Properties of growth media:

- The same type of media can possess both (or neither) of these properties.

A. Selective media:

- Favors the growth of particular organism while preventing growth of other organisms.
- Ex: Thayer Martin agar contains antibiotics that allow the selective growth of Neisseria by inhibiting the growth of other sensitive organisms.

B. Indicator (differential) media:

- Yields a color change in response to the metabolism of certain organisms.
- MacConkey agar contains a pH indicator; a lactose fermenter like E coli will convert lactose to acidic metabolites → color change.

- Special culture requirements:

Bug	Media used for isolation	Media content/other
H. influenza	Chocolate agar	Factors V (NAD) and X (hematin)
N. gonorrhoeae, N. meningitides	Thayer-Martin agar	Vancomycin (inhibits gram-positive organisms), Trimethoprim, Colistin (inhibits gram- negative organisms except Neisseria), and Nystatin (inhibits fungi). Mnemonic: Very Typically Cultures Neisseria
B. pertussis	Bordet-Gengou agar (Bordet for Bordetella) Regan-Lowe medium	Potato Charcoal, blood, and antibiotic
C. diphtheria	Tellurite agar, Löffler medium	
M. tuberculosis	Löwenstein-Jensen agar	
M. pneumonia	Eaton agar	Requires cholesterol
Lactose-fermenting enterics	MacConkey agar	Fermentation produces acid, causing colonies to turn pink
E. coli	Eosin–methylene blue (EMB) agar	Colonies with green metallic sheen
Brucella, Francisella, Legionella, Pasteurella	Charcoal yeast extract agar buffered with cysteine and iron	
Fungi	Sabouraud agar	"Sab's a fun guy!"

Oxygen requirements

- According to O_2 requirements, bacteria are classified into:

1. Strict or obligate aerobes:

- Require oxygen for growth; contain superoxide dismutase, which protects them from the toxic O_2.
- Use an O_2 dependent system to generate ATP.
- Example: Nocardia, Pseudomonas aeruginosa, and MycoBacterium tuberculosis.
- Reactivation of M. tuberculosis (after immunocompromise or TNF-α inhibitor use) has a predilection for the apices of the lung, which have the highest Po_2.
- Mnemonic: Nagging Pests Must Breathe.

2. Obligate anaerobes:

- They are killed by the O_2; grow maximally at a PO_2 concentration of less than 0.5% to 3%.
- Ex: include Fusobacterium, Clostridium, Bacteroides, and Actinomyces.
- Mnemonic: Anaerobes Frankly Can't Breathe Air.
- They lack catalase and/or superoxide dismutase (enzymes that destroy toxic products of oxygen metabolism) and are thus susceptible to oxidative damage.
- Instead of oxygen, they require another substance such as a hydrogen acceptor during the generation of metabolic energy and utilize fermentation pathways with distinctive metabolic products.
- Generally foul smelling (short-chain fatty acids), difficult to culture, and produce gas in tissue (CO_2 and H_2).
- Anaerobes are normal flora in GI tract, typically pathogenic elsewhere.
- AminO2glycosides are ineffective against anaerobes because these antibiotics require O2 to enter into bacterial cell.

3. Facultative anaerobes:

- Grow in the presence or absence of oxygen.
- They shift from a fermentative to a respiratory metabolism in the presence of air.
- Most pathogenic bacteria are facultative anaerobes.

4. Microaerophilic bacteria (also called aero- tolerant anaerobes):

- These bacteria use fermentation and have no electron transport system.
- They can tolerate low amounts of oxygen because they have superoxide dismutase (but they have no catalase).

Intracellular bugs

1. Obligate intracellular:

- These organisms are not capable of the metabolic pathways for ATP synthesis and thus must steal ATP from their host.
- These bacteria live in their host cell and cannot survive without the host.
- Example: Rickettsia, CHlamydia, COxiella. Rely on host ATP.
- Stay inside (cells) when it is Really CHilly and COld.

2. Facultative intracellular:

- They are capable of living and reproducing either inside or outside cells.
- Example: Salmonella, Neisseria, Brucella, Mycobacterium, Listeria, Francisella, Legionella, Yersinia pestis.
- Mnemonic: Some Nasty Bugs May Live FacultativeLY.

❖ Extracellular bacteria:

- Extracellular bacterial pathogens do not invade cells and proliferate instead in the extracellular environment which is enriched with body fluids.
- Some of extracellular bacteria even don't penetrate body tissues (V. cholerae) but adhere to epithelial surfaces and cause disease by secreting potent toxins.

Encapsulated bacteria

- Their capsules serve as an antiphagocytic virulence factor.
- Capsule + protein conjugate serves as an antigen in vaccines.
- Ex: Streptococcus pneumoniae, Haemophilus influenzae type B, Neisseria meningitidis, Escherichia coli, Salmonella, Klebsiella pneumoniae, and group B Strep.
- Mnemonic: SHiNE SKiS.

- They are opsonized, and then cleared by spleen.

- Asplenics (No Spleen Here) have ↓ opsonizing ability and thus ↑ risk for severe infections; need vaccines to protect against: (N. meningitidis, S. pneumoniae, H. influenzae)

Encapsulated bacteria vaccines

- Some vaccines containing polysaccharide capsule antigens are conjugated to a carrier protein, enhancing immunogenicity by promoting T-cell activation and subsequent class switching. A polysaccharide antigen alone cannot be presented to T cells.

- Pneumococcal vaccine: PCV (pneumococcal conjugate vaccine, Prevnar); PPSV (pneumococcal polysaccharide vaccine with no conjugated protein, Pneumovax).

- H. Influenzae type B (conjugate vaccine).

- Meningococcal vaccine (conjugate vaccine).

Toxins

- Toxins may aid in invasiveness, damage cells, inhibit cellular processes, or trigger immune response and damage.

1. Structural Toxins:

A. Endotoxin (Lipopolysaccharide = LPS):

- Endotoxins are found in the outer membrane of Gram-negative bacteria, which is composed of lipopolysaccharide (LPS).

- LPS is released during destruction of the bacterial cell wall. It can also be released during cell division.

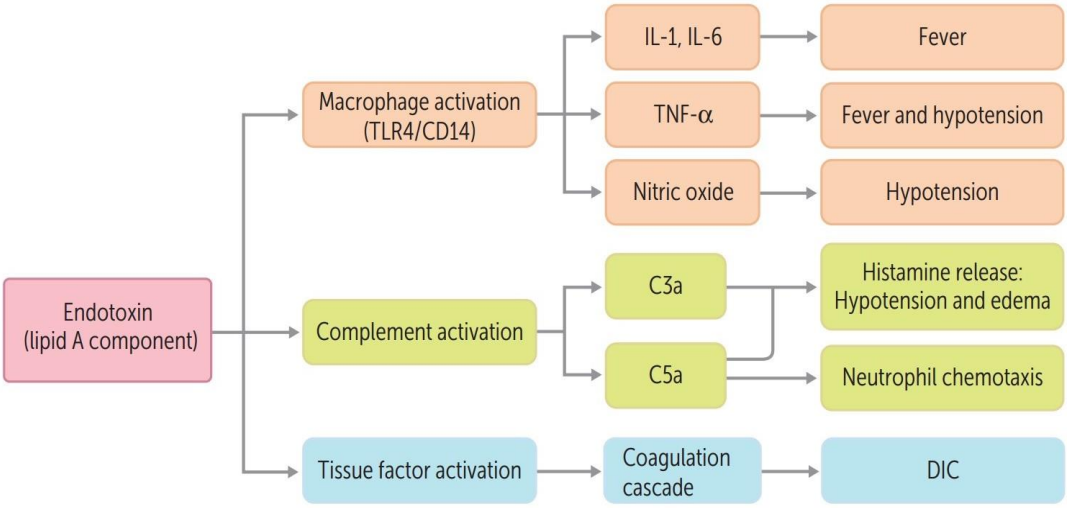
- LPS is a very long, heat-stable molecule arranged into three regions: O antigen, core polysaccharide, and lipid A.

- Lipid A is responsible for the toxic properties of LPS that lead to Gram-negative sepsis and endotoxic septic shock.

- Lipid A induces shock by activation of macrophages and granulocytes. This activation results in the synthesis of endogenous pyrogens, such as IL-1, prostaglandins, and the inflammatory mediators (tumor necrosis factor-alpha and interferon).

- These cytokines then induce a febrile response by the action of IL-1 on the hypothalamus, as well as hypotension, increased vascular permeability with third-spacing of fluids, diarrhea, disseminated intravascular coagulation, and death.

B. Peptidoglycan, lipoteichoic Acids.



2. Exotoxins:

- Are protein toxins, generally quite toxic and secreted by bacterial cells (some gram +ve, some gram -ve)

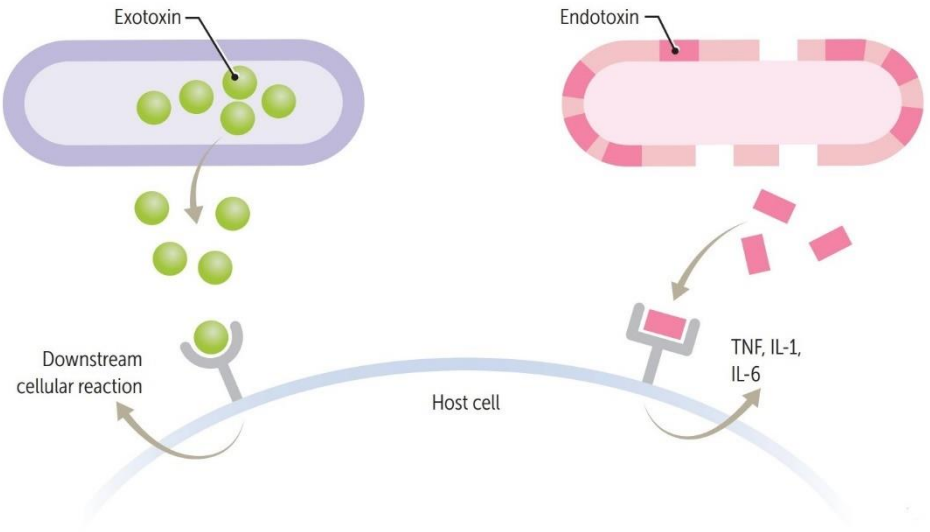
- Can be modified by chemicals or heat to produce a toxoid that still immunogenic, but no longer toxic so can be used as a vaccine.

- A-B (or "two") component protein toxins:

- B component binds to specific cell receptors to facilitate the internalization of A.
- A component is the active (toxic) component (often an enzyme such as an ADP ribosyl transferase).
- Exotoxins may be subclassed as enterotoxins, neurotoxins, or cytotoxins.

- Cytolysins:

- Lyse cells from outside by damaging membrane.
- Perfringens alpha toxin is a lecithinase.
- Staphylococcus aureus alpha toxin inserts itself to form pores in the membrane.



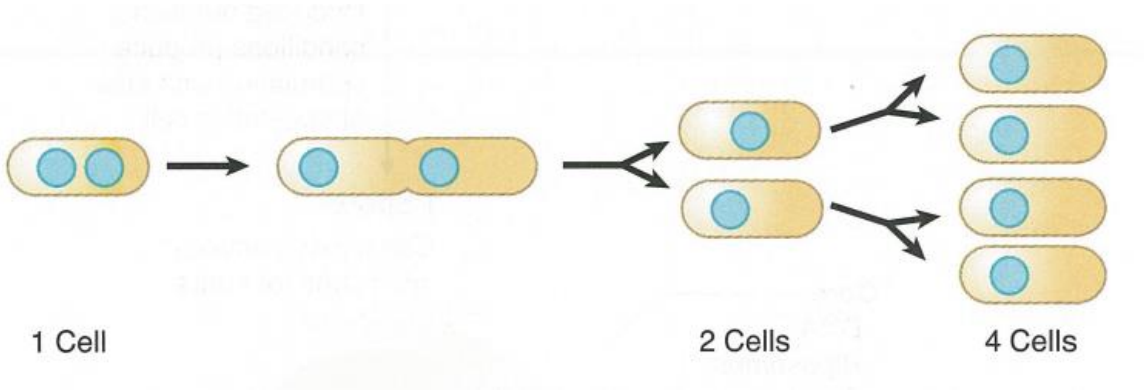
	Exotoxin	Endotoxin
Source	Certain species of gram-positive and gram- negative bacteria	Outer cell membrane of most gram-negative bacteria
Secreted from cell	Yes	No
Chemistry	Polypeptide	Lipopolysaccharide (structural part of bacteria; released when lysed)
Location of genes	Plasmid or bacteriophage	Bacterial chromosome
Toxicity	High (fatal dose on the order of 1 µg)	Low (fatal dose on the order of hundreds of micrograms)
Clinical effects	Various effects	Fever, shock (hypotension), DIC
Mode of action	Various modes	Induces TNF, IL-1, and IL-6
Antigenicity	Induces high-titer antibodies called antitoxins	Poorly antigenic
Vaccines	Toxoids used as vaccines	No toxoids formed and no vaccine available
Heat stability	Destroyed rapidly at 60°C (except staphylococcal enterotoxin and E coli heat stable toxin)	Stable at 100°C for 1 hour
Typical diseases	Tetanus, botulism, diphtheria	Meningococcemia; sepsis by gram-negative rods

Bacterial growth

- It results from bacterial reproduction due to binary fission, which may be characterized by a parameter called generation time (the average time required for cell numbers to double).

❖ Bacterial growth curve:

- If a small number of an organism is placed in a suitable fluid nutrient medium under appropriate physical and chemical conditions, then the number of viable cells per millimeter is determined periodically, and plotted, a characteristic growth curve with four phases is obtained:



1. Lag phase:

- The initial number of bacterial cells remains constant.

- During this period, the cells adapt to their new environment.

- Enzymes and intermediates are formed to permit growth.

2. Exponential (logarithmic, log) phase:

- There is marked increase in cell number and its rate is accelerated exponentially with time giving a characteristic linear plot on a logarithmic scale.

- The generation time is determined by observing the time necessary for the cells to double in number during the log phase of growth.

3. Stationary phase:

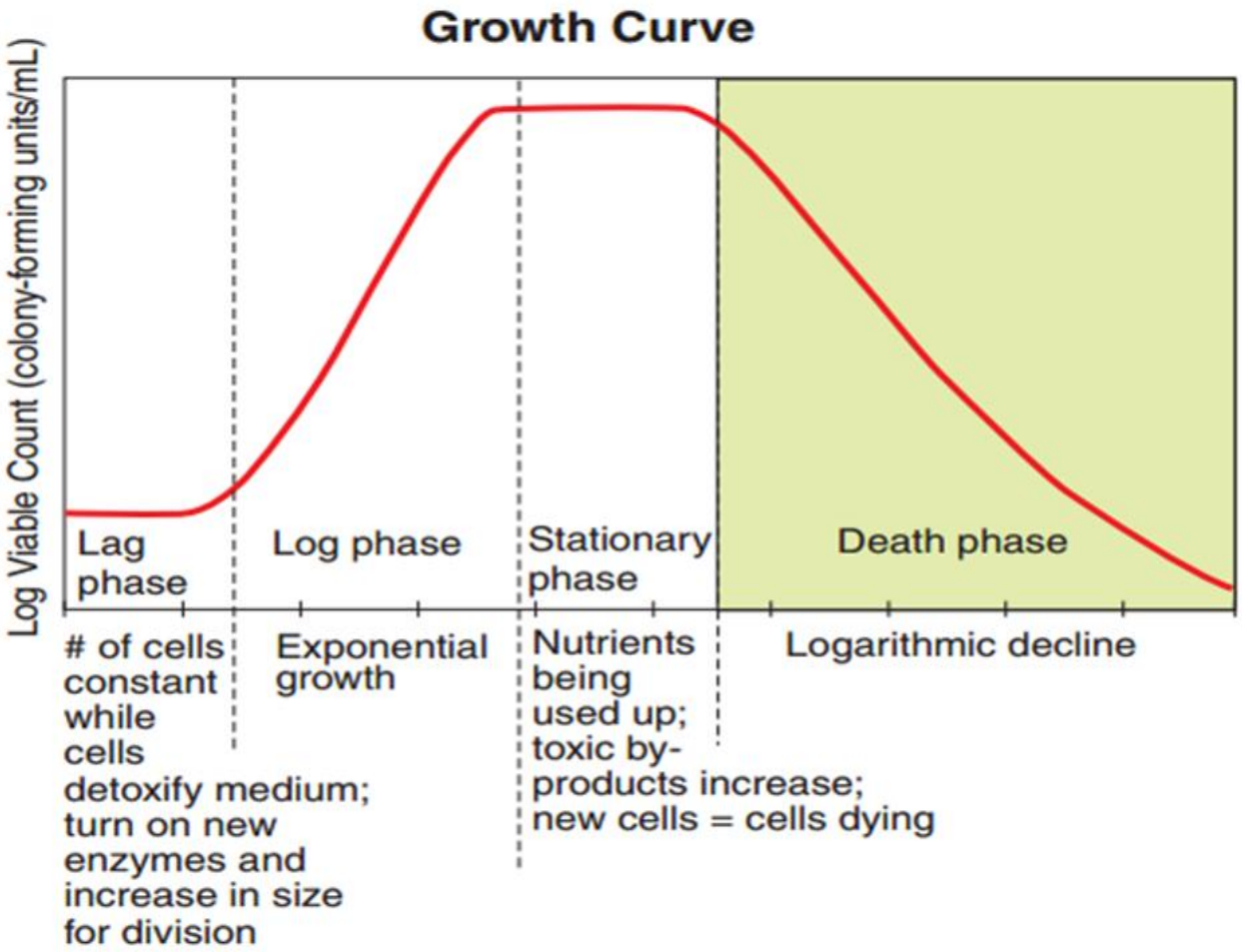
- Exhaustion of nutrients and accumulation of toxic products cause growth to decrease.

- There is slow loss of cells through death which is just balanced by formation of new cells through growth and division.

- The number of viable bacteria remains constant.

4. Decline or death phase:

- At the end of stationary phase, the death rate increases and exceeds the multiplication rate due to nutrient exhaustion and accumulation of toxic metabolic end products → The number of viable bacteria decrease.



Bacterial genetics

- Genetic variability in microbes is maintained through the exchange and recombination of allelic forms of genes.

- It can result in the acquisition of new characteristics (new virulence factors, toxins, antibiotic resistance).

- There are two processes available to stabilize "new" DNA:

A. Homologous recombination:

- Homologous recombination is a gene exchange process that may stabilize genes introduced into a cell by transformation, conjugation, or transduction.

- Imported bacterial DNA (transferred into a cell by transformation, conjugation, or transduction) is on short linear pieces of DNA called exogenotes.

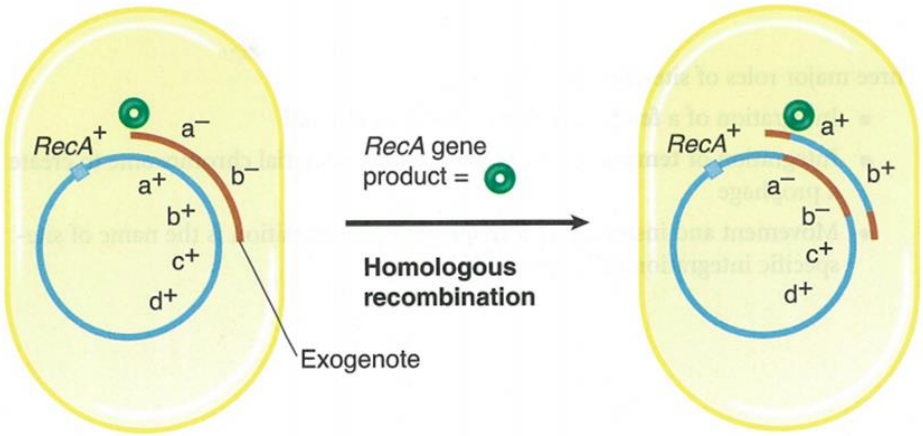
- Most linear DNA is not stable in cells because it is broken down by exonucleases.

- Homologous recombination produces an "exchange" of DNA between the linear exogenote of DNA and a homologous region on the stable (circular) bacterial chromosome.

- Homologous recombination requires:
  - Several genes worth of homology or near homology between the DNA strands.
  - A series of recombination enzymes/factors (Recombinases) coded for by the recombination genes recA, recB, recC, and recD (with recA generally an absolute requirement).

- So, Homologous Recombination:
  - Is a mechanism to incorporate short, linear pieces of DNA into the chromosome.
  - There must be some sequence homology.
  - Recombinase A is required.

- There is a one-to-one exchange of DNA.



B. Site-specific recombination:

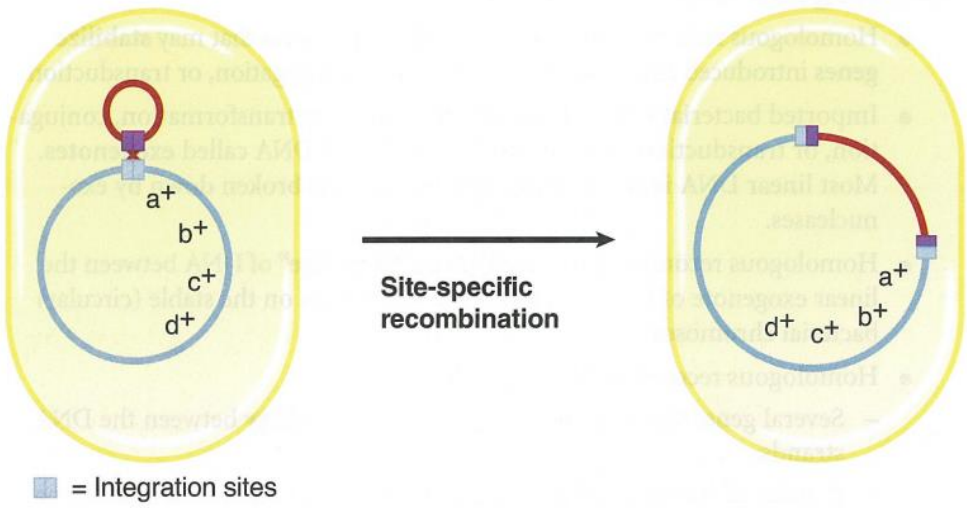
- Site-specific recombination is the integration of one DNA molecule into another DNA molecule with which it has no homology except for a small site on each DNA (called an attachment, integration, or insertion site).

- Requires restriction endonucleases and restriction endonuclease sites on each DNA.

- Because this process integrates rather than exchanges pieces of DNA, the end result is a molecule the sum of the two original molecules.

▪ So, site-specific recombination:

- Is the mechanism used to combine Circular pieces of DNA:
  - Plasmids.
  - Temperate phage.
- It requires no homology.
- No DNA is lost.
- It requires restriction endonucleases.



Bacterial viruses (Bacteriophages)

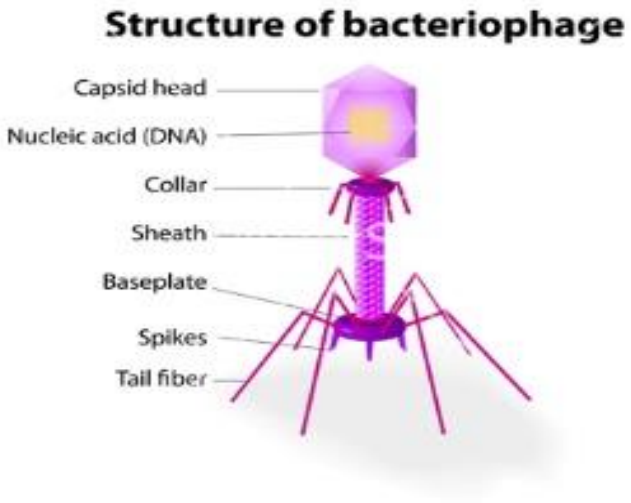
▪ Bacteriophages (or phages) are viruses that parasites bacteria (the bacteria cell serves as a host for the virus).

▪ Morphology of the bacteriophage:

- In most cases, the bacteriophage consists of:

1. Head: containing the nucleic acid core (usually DNA, rarely RNA) surrounded by a protein coat (capsid).

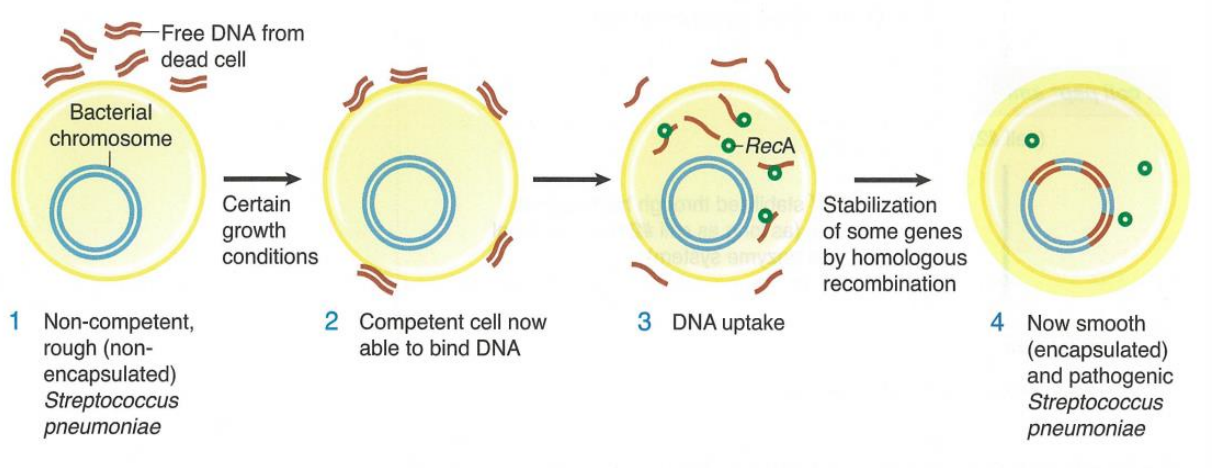
2. Tail: consists of a hollow core surrounded by a contractile sheath which ends in a base plate to which tail fibers attaches.



❖ There are 4 methods for gene transfer among bacteria:

1. Transformation:

- Transformation is the uptake of naked DNA from the environment by competent cells.
- Cells become competent (able to bind short pieces of DNA to the envelope and import them into the cell) under certain environmental conditions.
- Some bacteria are capable of natural transformation (they are naturally competent): Haemophilus infiuenzae, Streptococcus pneumoniae, Bacillus species, and Neisseria species.
- DNA (released from dead cells) is taken up.
- Newly introduced DNA is generally linear, homologous DNA a similar type of cell but perhaps one that is genetically diverse.
- The steps of transformation of a nonencapsulated Streptococcus pneumonia are shown below:



- So, transformation:

- Requires free DNA.
- Requires competent cells.
- Captured DNA is incorporated by homologous recombination.

2. Conjugation:

- Conjugation is gene transfer from one bacterial cell to another involving direct cell-to-cell contact.

- Sex pili (genes on F factor) play a role in establishing cell-to-cell contact.

- A single strand of the double helix of DNA is transferred from the donor (or male) cell to the recipient or female cell.

- Donor (male) sex:

- All have fertility plasmids known as F factors.

- F factors have a series of important plasmid "fertility" genes called the transfer or tra region, which code for Sex pili.

- Have a region called oriT (origin of transfer) where a single strand break in the DNA will be made and then oriT begins the transfer of one strand of the double helix.

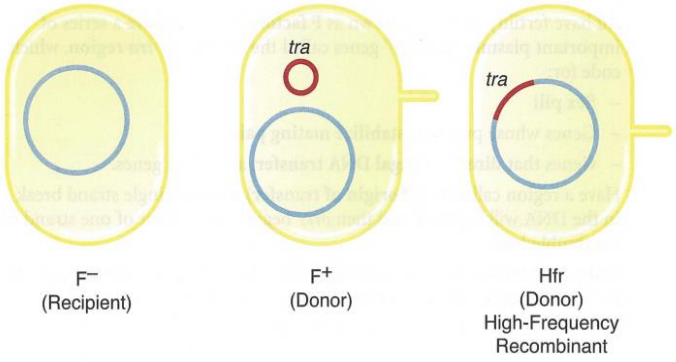
- Donor cells in which the fertility plasmid is in its free state are called F+ cells.

- Donor cells in which the fertility factor has inserted itself into the bacterial chromosome are called Hfr cells.

- Reciepient (female) cells (F- cells):

- Recipient cells lack fertility factors.

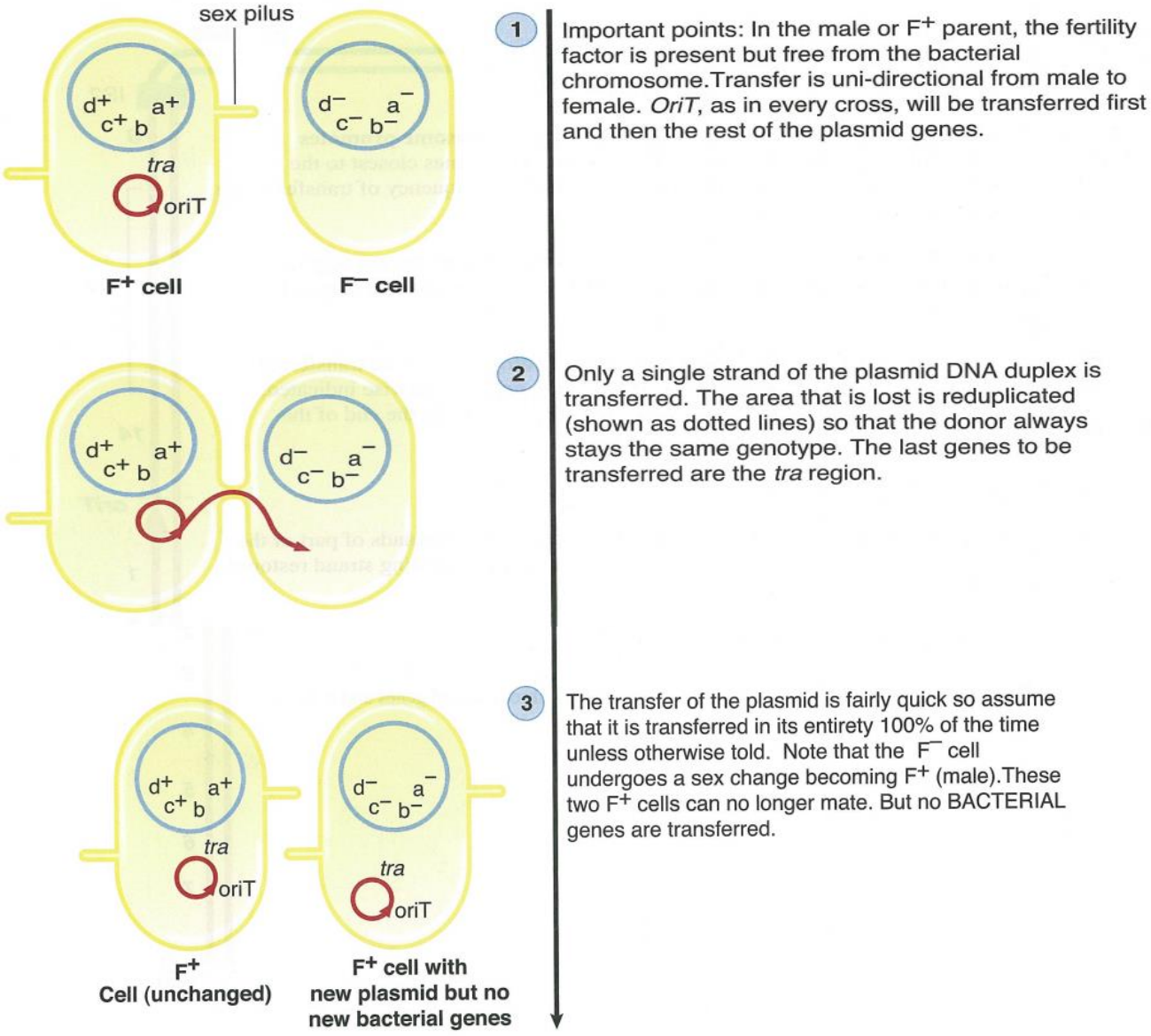
- In every cross, one cell must be an F- cell.



❖ Conjugal crosses:

- There are two major types of crosses:

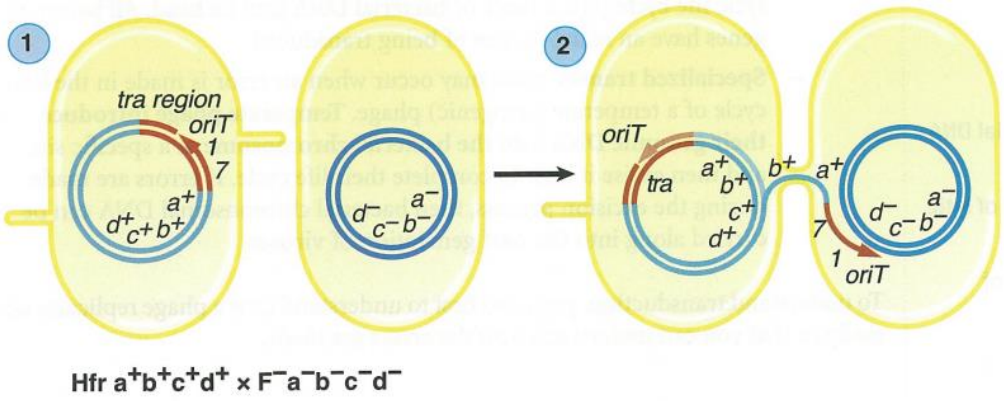
1. The F+ by F- Conjugal Cross:



- So, In the F+ x F- cross:

- One strand of the entire plasmid is transferred.
- It results in a "sex change" in the recipient.
- No transfer of chromosomal DNA.

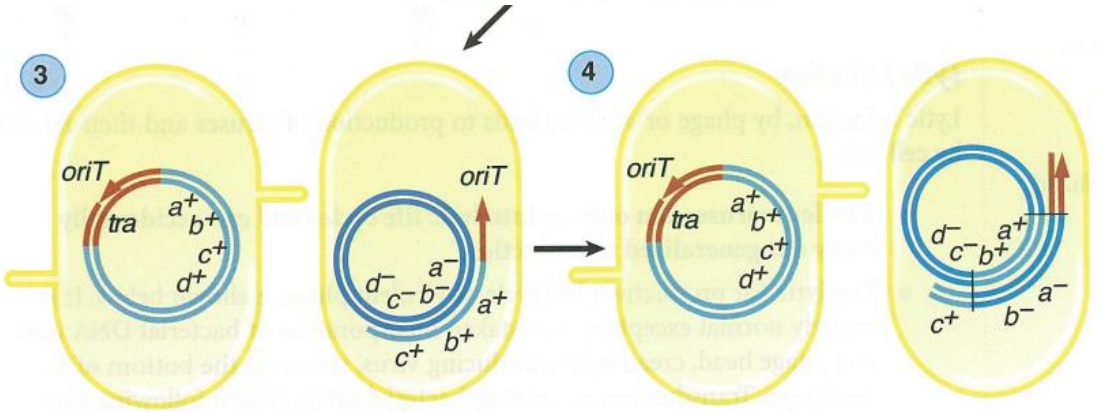
2. The Hfr x F- cross:



Hfr $a^+b^+c^+d^+ \times F^-a^-b^-c^-d^-$

Important points: Fertility factor is integrated into the bacterial chromosome. In this cross *oriT* and the first half of the fertility factor (regions 1–7 on the F factor) will be transferred first (and in that order) and then the bacterial genes in the linear order away from the plasmid.

Note, that as with the $F^+ \times F^-$ cross, only a single strand of the DNA duplex is transferred. The area that is lost is reduplicated so that the donor always stays the same genotype. The last genes to be transferred would be the *tra* region.



It takes approximately two hours for a complete transfer to occur. Because the cytoplasmic bridge and DNA strand is so fine, mating is normally interrupted before the transfer is complete. For the purpose of exam, assume that mating is interrupted and the recipient gets some new genes but does not become Hfr.

Hfr cell (unchanged)

F^- cell with new bacterial genes: a^+ and b^+ (no sex change)

- So, In the Hfr x F- cross:

- Chromosomal genes closest to oriT are transferred.
- No "sex change" occurs as the bridge does not remain long enough to transfer the tra operon.
- Recipient cell remains F- but now may have new bacterial genes.

3. Transduction:

- Transduction is the transfer of bacterial DNA by a phage vector.

- There are two types of transduction (generalized and specialized):

- Generalized transduction occurs when an error is made in the life cycle of a virulent phage. During the lytic phage cycle, the bacterial DNA is fragmented, and any fragment of DNA (whether chromosomal or plasmid) may be incorporated into the phage head. The phage particle can then transfer the incorporated bacterial DNA into another bacterial host.

- Specialized transduction may occur when an error is made in the life cycle of a temperate (lysogenic) phage. Temperate phages introduce their genomic DNA into the bacterial chromosome at a specific site and then excise it later to complete their life cycle. If errors are made during the excision process, then bacterial chromosomal DNA can be carried along into the next generation of viruses.

- To understand transduction, you need first to understand how a phage replicates normally so that you can understand how the errors are made.

❖ Phage = bacteriophage = bacterial virus

- Come in two major types:

A. Virulent phage: Infect bacterial cells, always making more virus and lysing the cells (lytic replication).

B. Temperate phage:

- Often infect without lysing the cells because they have the ability to repress active phage replication and to stably integrate their DNA into the bacterial chromosome.

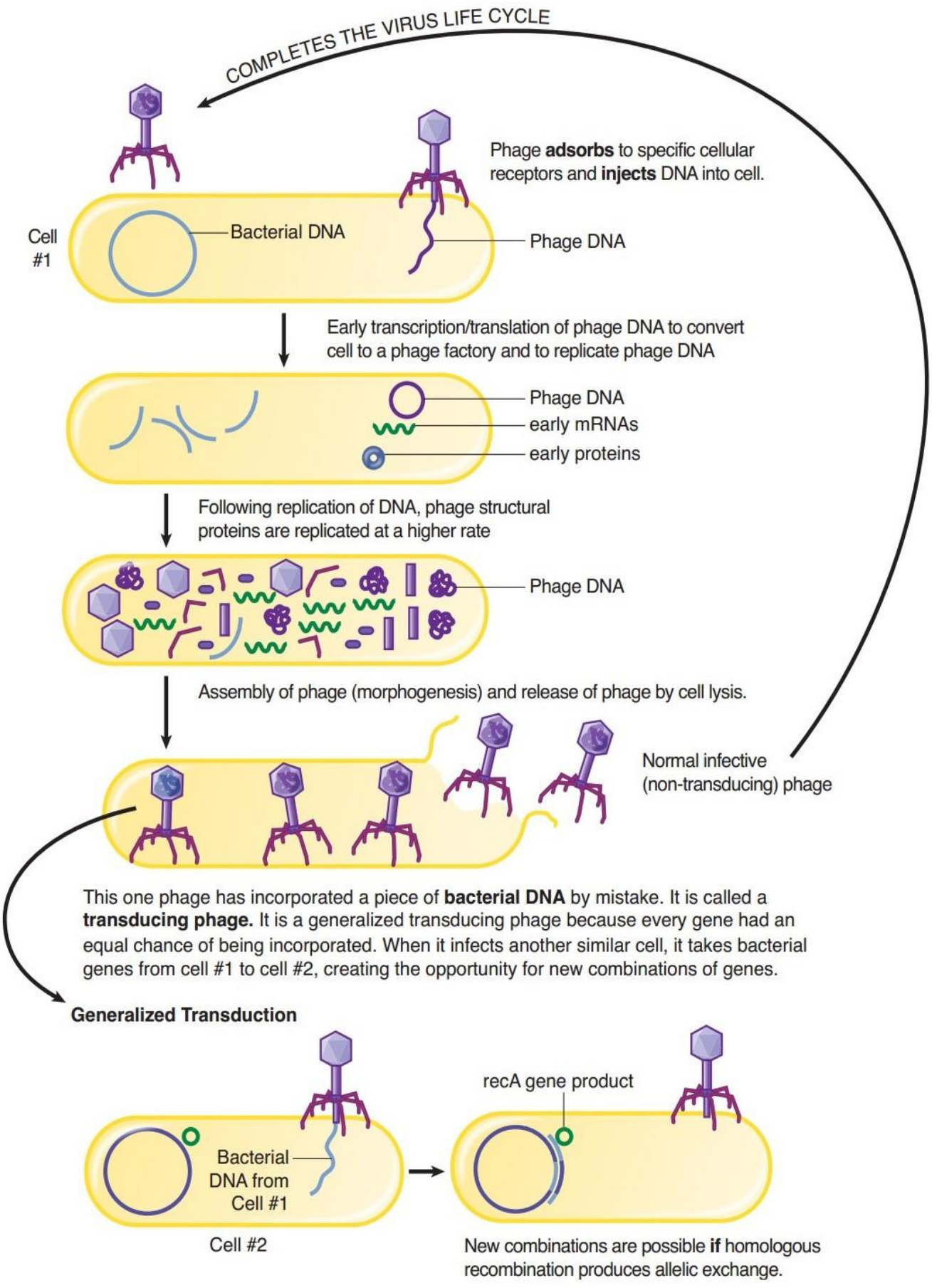
- In the absence of functional repressor protein, they also may replicate lytically.

❖ Lytic infection:

- Lytic infection, by phage or viruses, leads to production of viruses and their release by cell lysis.

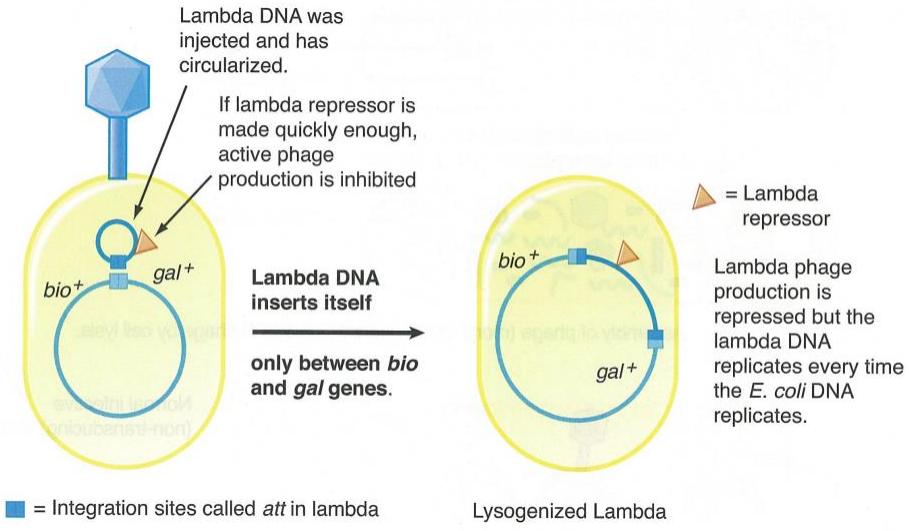
- Virulent viruses can only go into lytic life cycles and can accidentally carry out generalized transduction.

- During the lytic phage cycle, the bacterial DNA is fragmented, and any fragment of DNA (whether chromosomal or plasmid) may be incorporated into the phage head. The phage particle can then transfer the incorporated bacterial DNA into another bacterial host.


COMPLETES THE VIRUS LIFE CYCLE
Phage **adsorbs** to specific cellular receptors and **injects** DNA into cell.
Cell #1
Bacterial DNA
Phage DNA
Early transcription/translation of phage DNA to convert cell to a phage factory and to replicate phage DNA
Phage DNA
early mRNAs
early proteins
Following replication of DNA, phage structural proteins are replicated at a higher rate
Phage DNA
Assembly of phage (morphogenesis) and release of phage by cell lysis.
Normal infective (non-transducing) phage
This one phage has incorporated a piece of **bacterial DNA** by mistake. It is called a **transducing phage.** It is a generalized transducing phage because every gene had an equal chance of being incorporated. When it infects another similar cell, it takes bacterial genes from cell #1 to cell #2, creating the opportunity for new combinations of genes.
Generalized Transduction
Bacterial DNA from Cell #1
Cell #2
recA gene product
New combinations are possible **if** homologous recombination produces allelic exchange.

❖ Specialized Transduction as a Sequela to the Lysogenic Phage Life Cycle:

- Temperate phage may become prophage (DNA stably integrated) or replicate lytically.
- When repressor is made, temperate phages insert their DNA into the bacterial chromosome where it stably stays as a prophage.
- If the repressor gene gets mutated or the repressor protein gets damaged, then the prophage gets excised from the bacterial DNA and is induced into the lytic production of virus.
- Lambda phage of E. coli is the best studied. Most temperate phages have only a single insertion site.
- Lambda inserts ONLY between E. coli genes gal and bio as shown below.



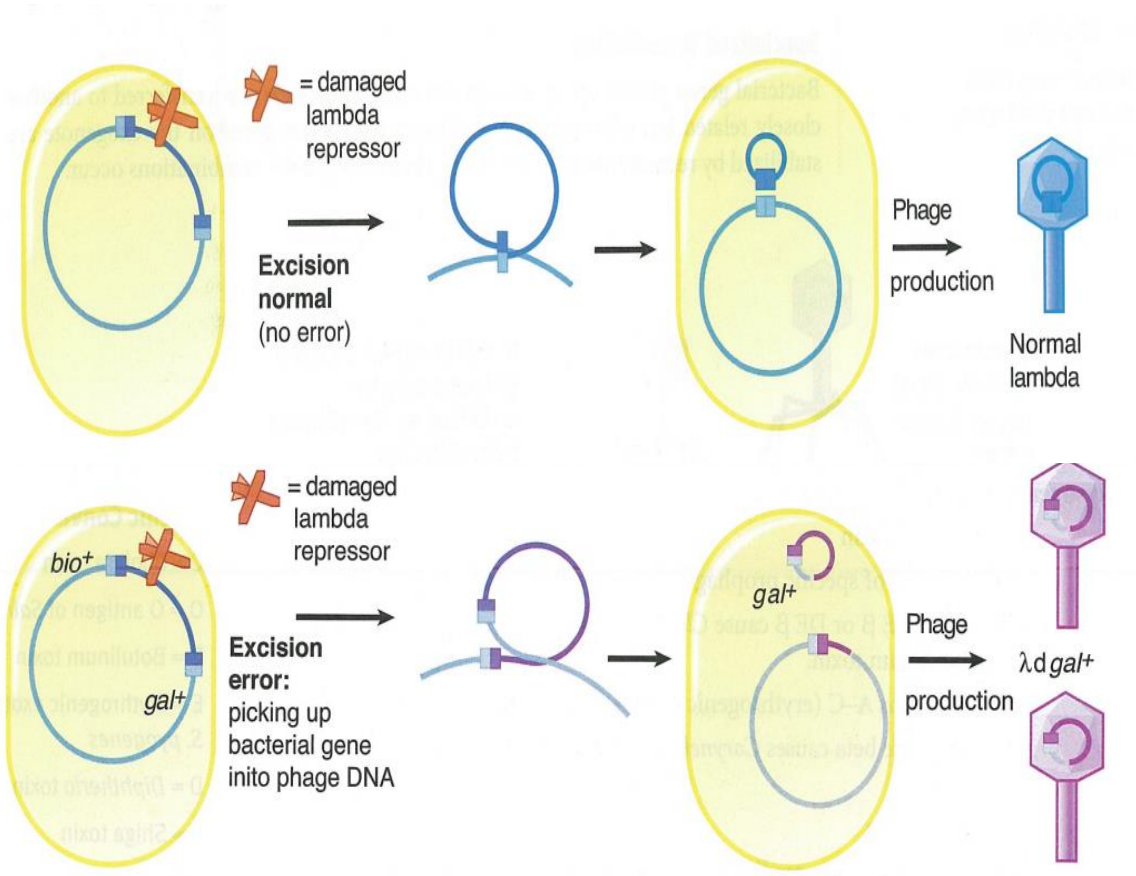
- Lysogeny is the state of a bacterial cell with a stable phage DNA (generally integrated into the bacterial DNA), not undergoing lytic replication either because it is repressed or defective.
- When the cell DNA replicates, the phage DNA also replicates and, as long as the repressor protein is not damaged, the lysogenic state continues.
- Lysogeny can confer new properties on a genus such as toxin production or antigens (lysogenic conversion).
- Phage that has both options (lytic replication or lysogeny) are called temperate phage.
- Mnemonic for phage-mediated pathogenic factors = COBEDS

- C = Cholera toxin.
- O = O antigen of Salmonella.
- B = Botulinum toxin.
- E = Erythrogenic exotoxins of S. pyogenes.
- D =Diphtheria toxin.
- S =Shiga toxin.

❖ Induction:

- If the repressor is damaged (by UV, cold, or alkylating agents), then the prophage is excised, and the cell goes into lytic replication phase. This process is called induction.

- Rarely, in the excision process, an excisional error is made and one of the bacterial genes next to the insertion site is removed attached to the lambda DNA, and a little bit of lambda DNA is left behind.



❖ Specialized Transduction:

- It takes place when a prophage contained in a lysogenized bacterial cell is induced to detach. Such prophage may carry with it the adjacent piece of the chromosomal DNA and transfer it to a nother bacterial cell.

- Only those genes next to the phage insertion site can be transduced by specialized transduction.

❖ In a Nutshell

- Transduction: transfer of bacterial DNA via phage vector.

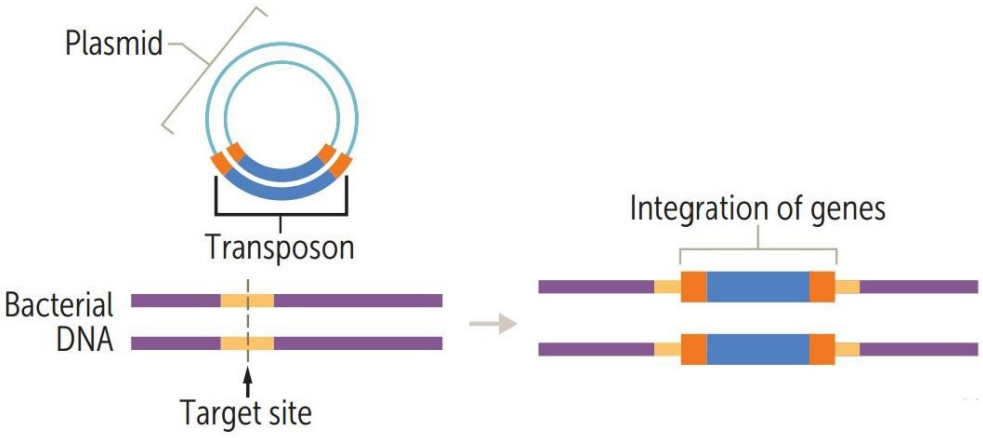
- Generalized transduction: error of lytic virus life cycle.

- Specialized transduction: error of temperate virus life cycle.

4. Transposition:

- A "jumping" process involving a transposon (specialized segment of DNA), which can copy and excise itself and then insert into the same DNA molecule or an unrelated DNA (plasmid or chromosome).

- Critical in creating plasmids with multiple drug resistance and transfer across species lines (Tn1546 with vanA from Enterococcus to S. aureus).



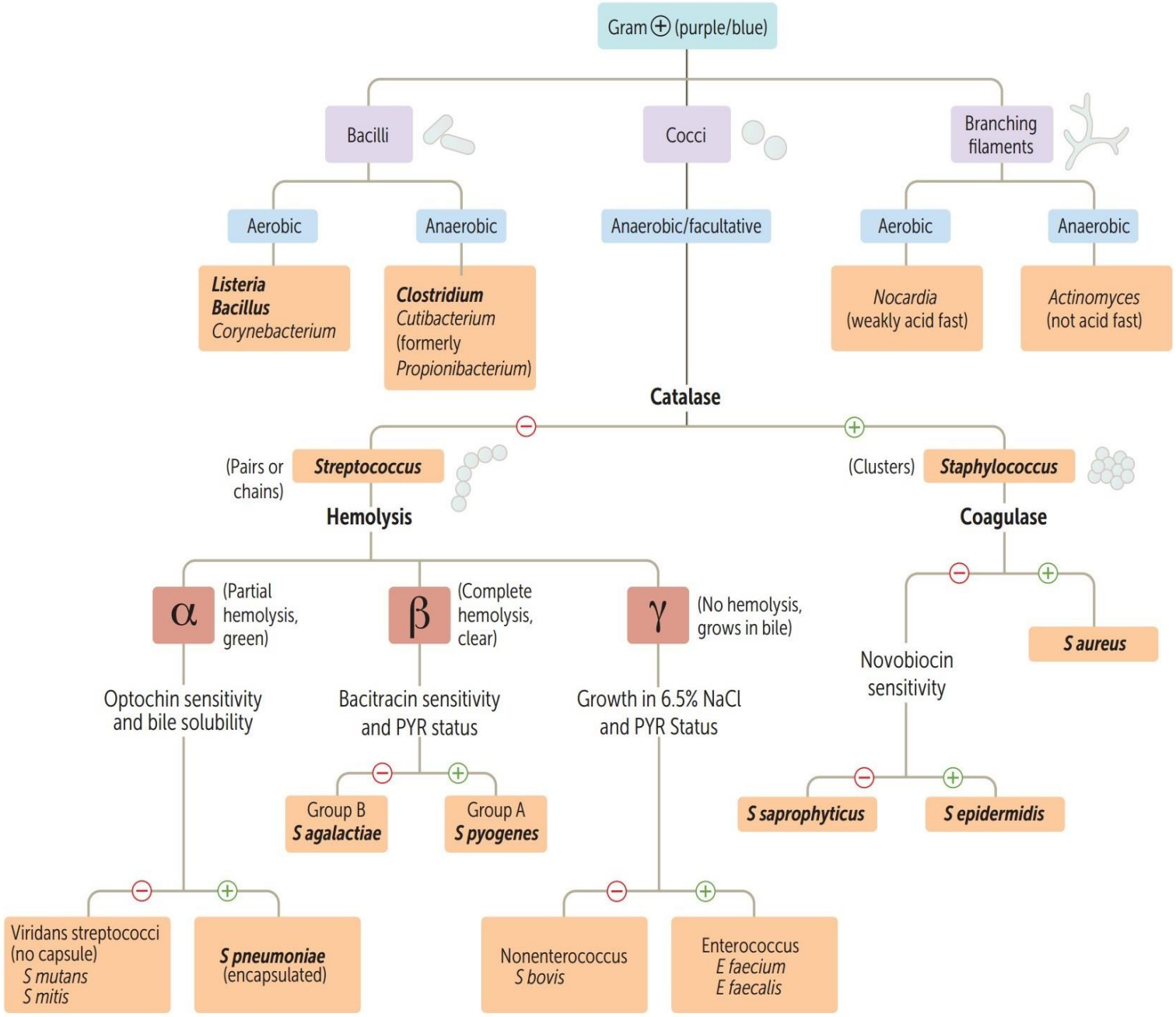
❖ Comparison of Transformation, Conjugation, and Transduction:

Requirement	Transformation	Conjugation	Transduction
Is cell-to-cell contact required?	No	Yes	No
Does it require an antecedent phage infection?	No	No	Yes
Is competency required?	Yes	No	No
Is naked (free) DNA involved?	Yes	No	No

❖ Comparison of Generalized and Specialized Transduction:

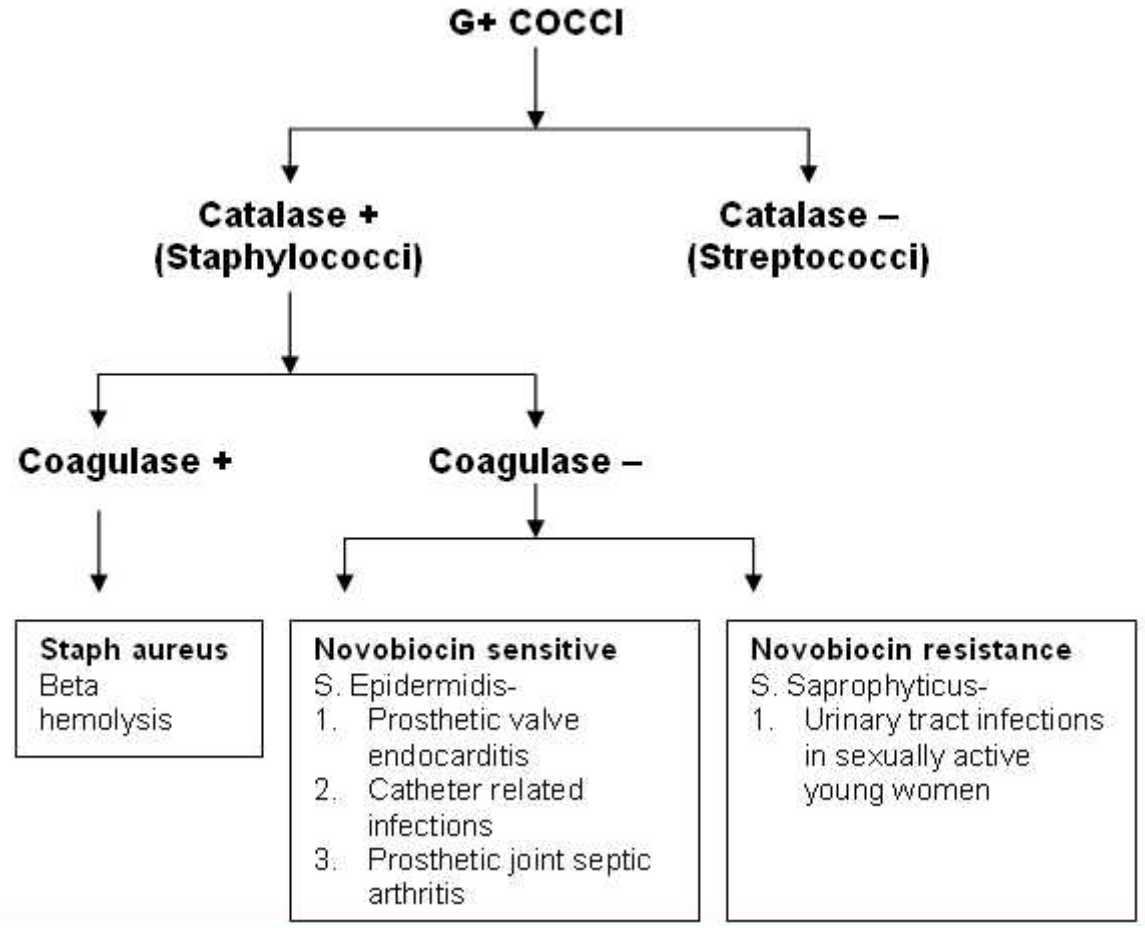
	Generalized	Specialized
Mechanism	Error in assembly "A packaging event".	Error of excision An "excision" event. Requires stable insertion of prophage DNA (lysogeny)
What genes may be transferred?	Any	Only genes next to the insertion site

Gram Positive Bacteria



Important **tests** are in **bold.** Important ***pathogens*** are in ***bold italics.***
Note: Enterococcus is either ˜ - or ° -hemolytic.

1. Gram-Positive Cocci:



Genus: Staphylococcus

- Genus Features:

- Gram-positive cocci in clusters.
- Catalase positive (streptococci are catalase negative).

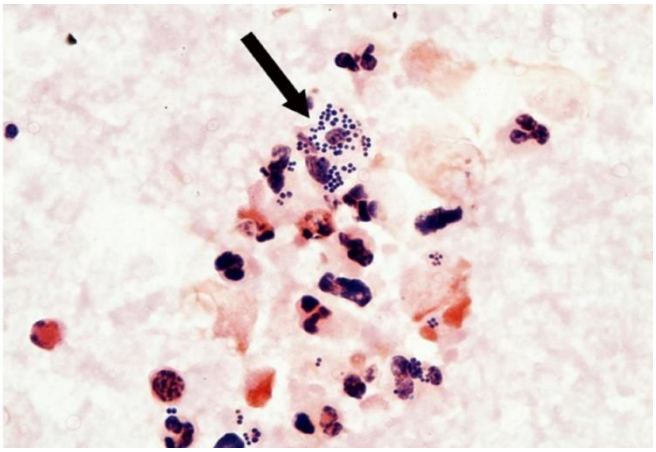
- Species of Medical Importance:

- S. aureus.
- S. epidermidis.
- S. saprophyticus.

Staphylococcus aureus

- Distinguishing Features:

- The Staphylococci are Gram-positive cocci that form clusters.

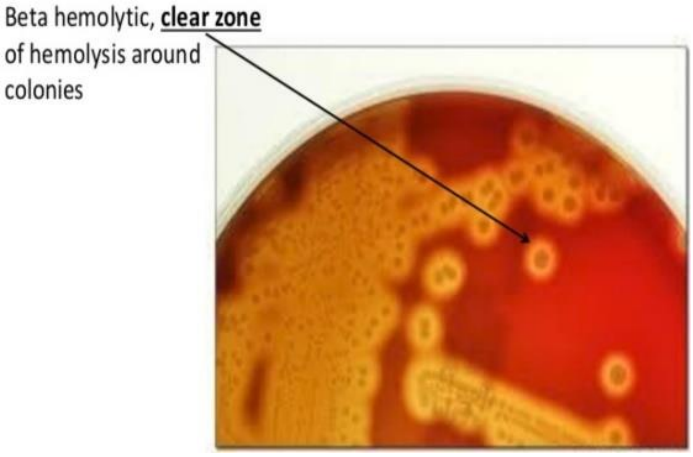


- S. aureus is a facultative anaerobe (the majority of bacteria are facultative anaerobes).

- The catalase test (with 3% hydrogen peroxide) differentiates Streptococci (catalase-negative) from Staphylococci (catalase-positive).

- The ability to clot blood plasma (produce staphylocoagulase) separates Staphylococci into two groups:
  - Coagulase-positive Staphylococci: which constitutes the most pathogenic species Staphylococcus aureus.
  - Coagulase-negative staphylococci: which constitutes S. epidermidis, S. Saprophyticus, and 30+ other species.

- It forms golden yellow colonies on blood agar surrounded with a zone of β-hemolysis (complete hemolysis) due to production of hemolysins.



- It ferments mannitol on mannitol salt agar.

- Habitats:
- Staphylococci usually inhabit the skin and mucosa.
- Nasal carriage rate for S. aureus may account to 25% of population.

- **Mode of transmission:**

- Hands (handling of food).
- Sneezing.
- Surgical wounds.
- Contaminated food.

- **Virulence factors and pathogenesis:**

1. Staphylocoagulase:

- Coagulase is an extracellular protein that has the ability to convert plasma fibrinogen to fibrin.

- By this mechanism, a fibrin barrier is formed. Thus, bacteria could protect themselves from phagocytic and immune defenses.

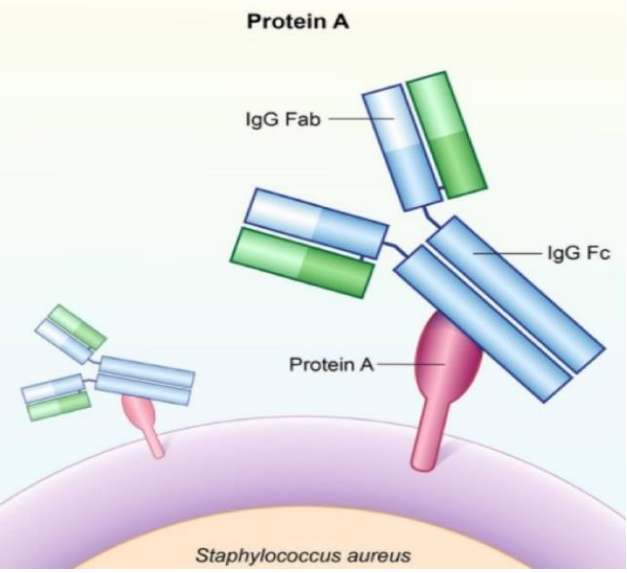
- At the same time, it leads to localization of infection. Thus, S. aureus suppuration usually has a localized form.

2. Invasins: leucocidin, staphylokinase and hyaloronidase promote bacterial spread in tissues.

3. The clumping factor (fibrinogen-binding protein): is an important adhesion expressed by S. aureus. It leads to attachment of the organism to traumatized tissue and blood clots.

4. Protein A:

- Protein A is a virulence factor that forms part of the outer peptidoglycan layer of S aureus.
- Protein A binds with the Fc portion of IgG antibodies at the complement-binding site, preventing complement activation. This results in decreased production of C3b, leading to impaired opsonization and phagocytosis.



5. Hemolysins (alpha toxin): are pore-forming toxins that lyse host cell membranes. They cause hemolysis on blood agar.

6. Exotoxin having superantigen mechanism:

a. Enterotoxins: responsible for staphylococcal food poisoning.

b. Toxic shock syndrome toxin-1 (TSST-1).

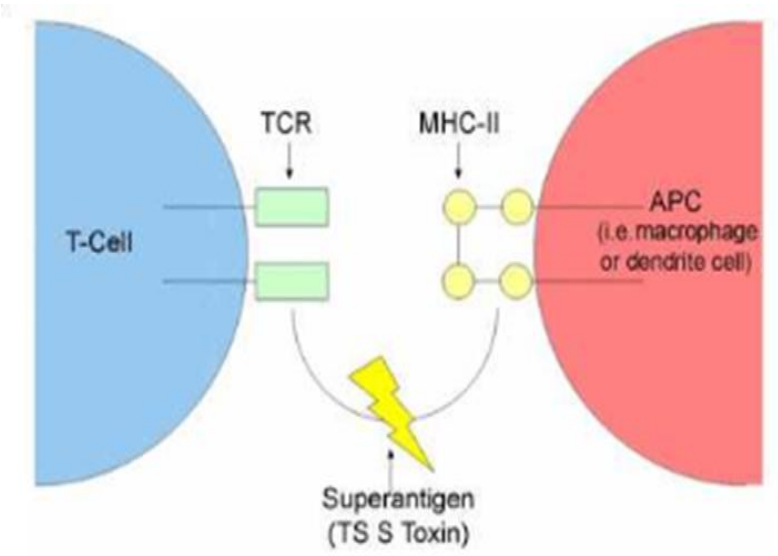
c. Epidermolytic (exfoliatin) toxins: responsible for staphylococcal scalded skin syndrome (SSSS).

- It is called a superantigen because in contrast to usual antigen, which activates few helper T cells, it activates large numbers of helper T cells.

- These toxins interact with major histocompatibility complex molecules on antigen presenting cells and the variable region of the T lymphocyte receptor to cause a nonspecific polyclonal widespread activation of T lymphocytes.

- Activation of T cells is responsible for the release of interleukin-2 (IL-2) from the T cells and IL-1 and TNF from macrophages.

- These interleukins cause capillary leakage, circulatory collapse, hypotension, shock, fever, skin findings, and multiorgan failure.



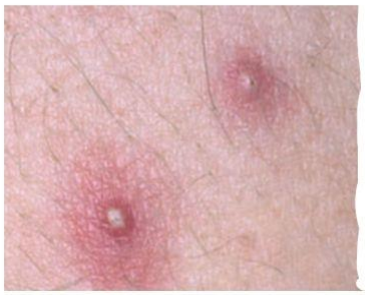
- Diseases:

1. Staphylococcus aureus pyogenic disease:

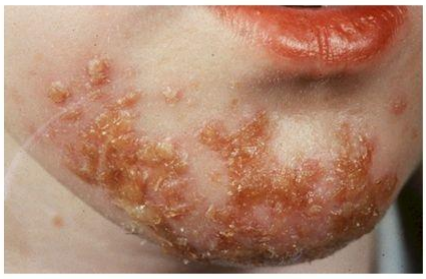
a. Localized skin infections:

- The most common staphylococcus infection.

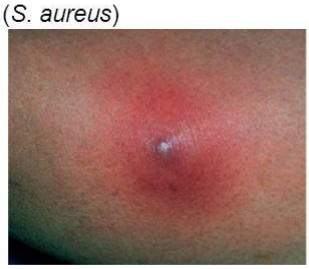
- Example:
  - Folliculitis, furuncles, carbuncles, or abscesses.
  - Postoperative surgical wound infections (hospital-acquired).
  - Traumatic wound infections following skin injury and burns.



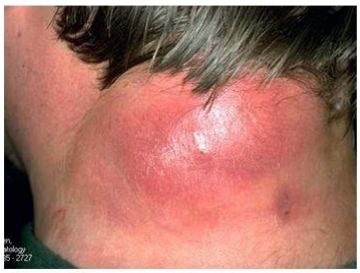
Folliculitis: infection of hair follicle (*S. aureus*)



Impetigo (*S. pyogenes, S. aureus*)



Furuncle: deep inflammatory nodule usually developing from folliculitis (*S. aureus*)



Carbuncle: more extensive than a furuncle with involvement of the subcutaneous fat (*S. aureus*)

b. Staphylococcal pneumonia: It is a frequent complication of prior viral infections (influenza).

c. Invasive conditions:

- They are more serious and usually occur in immunocompromised individuals.

- Invasion of bloodstream (bacteremia) and spread to numerous body sites lead to deep seated infections such as septic arthritis, osteomyelitis, endocarditis, and meningitis.

- Hematogenous osteomyelitis is predominantly a disease of children that most frequently affects the long bones. Staphylococcus aureus is implicated in most cases secondary to a bacteremic event. Streptococcus pyogenes (group A streptococcus) is the second most common cause of hematogenous osteomyelitis.

- A resulting septicemia may be rapidly fatal.

2. Staphylococcus aureus toxin-mediated diseases:

a. Staphylococcal food poisoning:

- It is the commonest type of bacterial food poisoning.

- S. aureus contaminates food by direct inoculation from food handlers who are frequently asymptomatic carriers.

- Subsequently, the food is allowed to sit at room temperature for an extended period of time and S. aureus is able to multiply and produce exotoxin.

- Staphylococcus aureus is capable of producing a highly heat-stable protein toxin called enterotoxin that causes staphyloenterotoxemia, a syndrome characterized by nausea, vomiting and abdominal cramps following ingestion of preformed exotoxin (exotoxin formed prior to ingestion).

- Because this is a preformed exotoxin, there is no person-to-person transmission, but outbreaks can occur with many people eating the same contaminated food. It is also the reason for the rapid onset of symptoms (usually less than 6 hours).

- Heat stable means (heating may kill the organism but doesn't destroy the toxin).

- This toxin uses a superantigen mechanism.

- S. aureus food poisoning is usually self-limited.

- Poultry and egg products, meat and meat products, egg, tuna, chicken, potato and macaroni salads, cream-filled pastries, and milk and dairy products are the foods that are frequently incriminated in staphylococcal food poisoning. The most frequently tested food item is a mayonnaise-containing food like potato or macaroni salad.

b. Toxic shock syndrome (TSS):

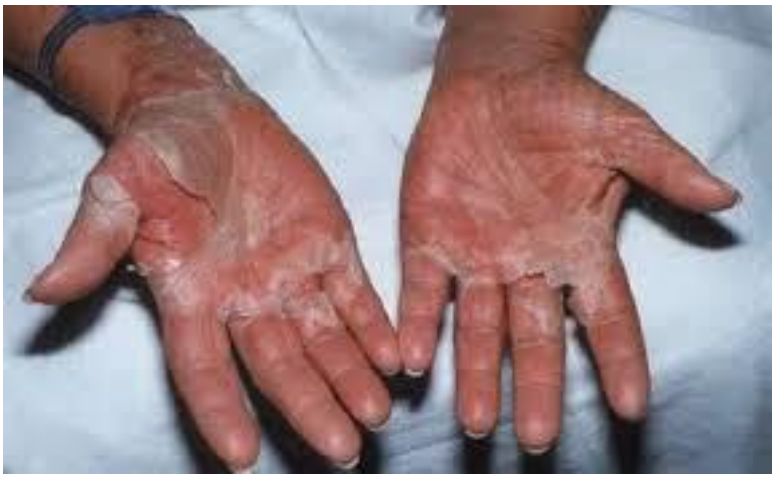
- TSS is prevalent in young menstruating females who use vaginal tampons that are left in place for extended period. The cause may be also nasal packing that is left in place for extended period.

- Staphylococcus aureus strains producing toxic shock syndrome toxin (TSST-1) are responsible for most cases of TSS. TSST acts as a superantigen.

- Fever, vomiting, diarrhea, muscle pain and erythroderma are the symptoms of Toxic Shock Syndrome (TSS).

- It can rapidly progress to severe hypotension and multisystem dysfunction.

- Desquamation, particularly on the palms and soles, can occur 1-2 weeks after the onset of illness.



c. Staphylococcal scalded skin syndrome (SSSS):

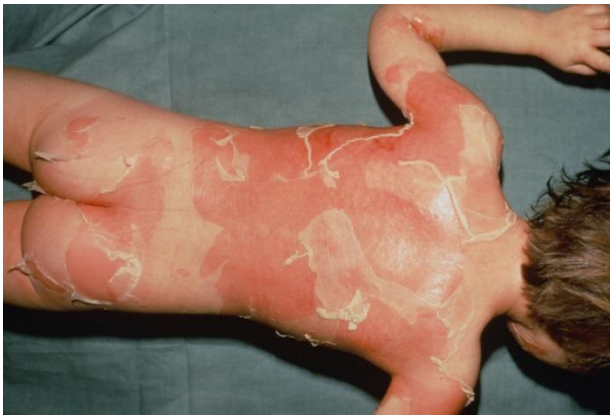
- Staphylococcal Scalded Skin Syndrome (SSSS) is caused by certain strains of Staphylococcus species that produce the exfoliatin exotoxin.

- SSSS is most common in infants and young children, and it is frequently not fatal unless the skin lesions become secondarily infected.

- Exfoliative toxins show exquisite pathologic specificity in blistering only the superficial epidermis (epidermolytic).

- Large bullae are formed under the epidermis, which rupture leaving moist, red, scalded dermis.

- Nikolsky's sign (skin slipping off with gentle pressure), epidermal necrolysis, fever and pain associated with the skin rash are the major symptoms of SSSS.



❖ N.B:

1. S. aureus is the most common cause of tricuspid endocarditis in intravenous drug users.

- These patients can develop multiple septic emboli in lungs. Pulmonary infarcts are almost always hemorrhagic due to the dual blood supply to the lungs (pulmonary and bronchial arteries).

2. Gram positive organisms have a cytoplasmic membrane composed of a phospholipid bilayer as well as a peptidoglycan cell wall outside of that cell membrane.

- The peptidoglycan cell wall provides the shape of the bacterium as well as resistance to osmotic stress.
- Under normal circumstances, Gram-positive organisms would not be destroyed by variations in tonicity within a certain range due to their intact peptidoglycan cell wall.
- So, if gram + organisms destroyed by placement in a hypotonic solution one can infer that there was damage to the peptidoglycan cell wall by the antibiotic that acts against cell wall synthesis (cefuroxime).

Staph epidermidis

- **Distinguishing Features:**

- Catalase positive.

- Coagulase negative staphylococci.

- Novobiocin sensitive (novobiocin is used to differentiate between S. epidermidis and S. saprophyticus, S. saprophyticus is novobiocin resistant).

- Does not ferment mannitol (vs S. aureus).

- **Habitats:** S. epidermidis is a part of the normal skin flora.

- **Mode of transmission:** colonization of foreign bodies.

- **Virulence factors and pathogensis:**

- S epidermidis is known for causing foreign body infections because of its ability to form biofilms.

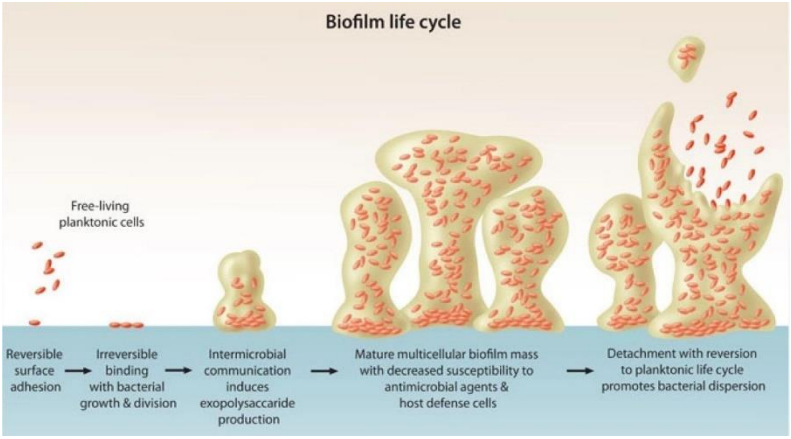
- Once introduced into the body, foreign bodies quickly become coated with a layer of host proteins, including fibrinogen and fibronectin. These proteins then serve as binding sites for S epidermidis.

- After attachment occurs, the bacteria multiply and communicate with one another to induce synthesis of an extracellular polysaccharide matrix (biofilm) that encases the bacteria.

- The resulting biofilm functions as a barrier to antibiotic penetration and interferes with host defenses, including opsonization, neutrophil migration, and even T lymphocyte activation.

- Once mature, these biofilms can disperse individual pathogen "seeds" (planktonic cells) into the bloodstream and surrounding areas, further disseminating the infection.

- Definitive treatment of infections caused by biofilm-producing organisms often requires removal of the foreign body.



- Diseases:

- Staphylococcus epidermidis have been identified as a major cause of infections in patients with predisposing factors such as indwelling catheters or implanted foreign bodies.

- These bacteria have the ability to colonize intravenous catheters and prosthetic devices (heart valves, vascular grafts, peritoneal dialysis catheters) because of their ability to produce a polysaccharide slime, which allows adherence to the prosthetic devices.

- When reported, it is initially difficult to differentiate culture contamination from real infection because S. epidermidis is a part of the normal skin flora, but recovery of organism from multiple blood cultures indicates infection.

- S. epidermidis is capable of causing an opportunistic infection associated with foreign bodies; it is the most common cause of endocarditis in patients with prosthetic valves and septic arthritis in patients with prosthetic joints.

- S. epidermidis isolates are susceptible in vitro to vancomycin and rifampin and are frequently resistant to methicillin; therefore, vancomycin, combined with rifampin or gentamicin or both, is recommended for therapy of serious infections caused by methicillin-resistant strains.

Staphylococcus saprophyticus

- Distinguishing Features:

- Catalase positive.
- Coagulase negative staphylococci.
- Novobiocin resistant.

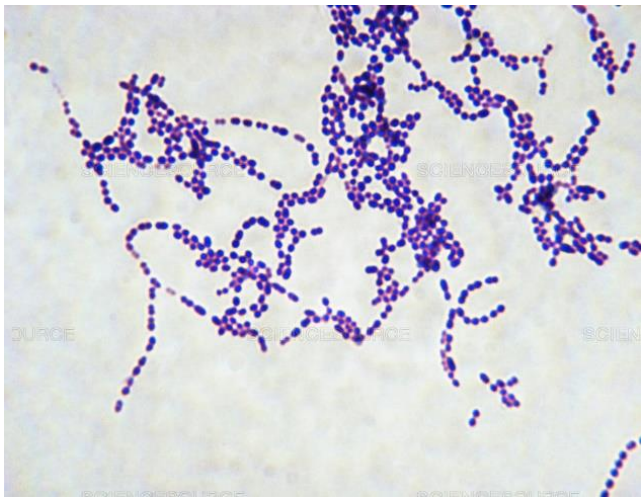
- S. saprophyticus is a common cause of urinary tract infection; it is responsible for almost half of all UTIs in sexually active young women.

- Second most common cause of uncomplicated UTI in young women (first is E. coli).

Genus: Streptococcus

- Genus Features:

- Gram-positive cocci in chains.



- Catalase negative.

- Serogrouped using known antibodies to the cell wall carbohydrates (Lancefield groups A-O).

- Hemolysis on sheep blood agar can be used for distinguishing between streptococci as follows:

1. α-hemolytic streptococci induce zones of greenish discoloration.

- Include the following organisms:

  - Streptococcus pneumoniae (catalase ⊖ and optochin sensitive)
  - Viridans streptococci (catalase ⊖ and optochin resistant)

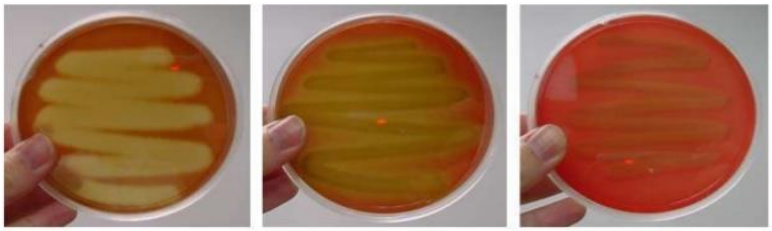
2. β-hemolytic streptococci induce zones of complete β-hemolysis (Form clear area of hemolysis on blood agar).

- Include the following organisms:

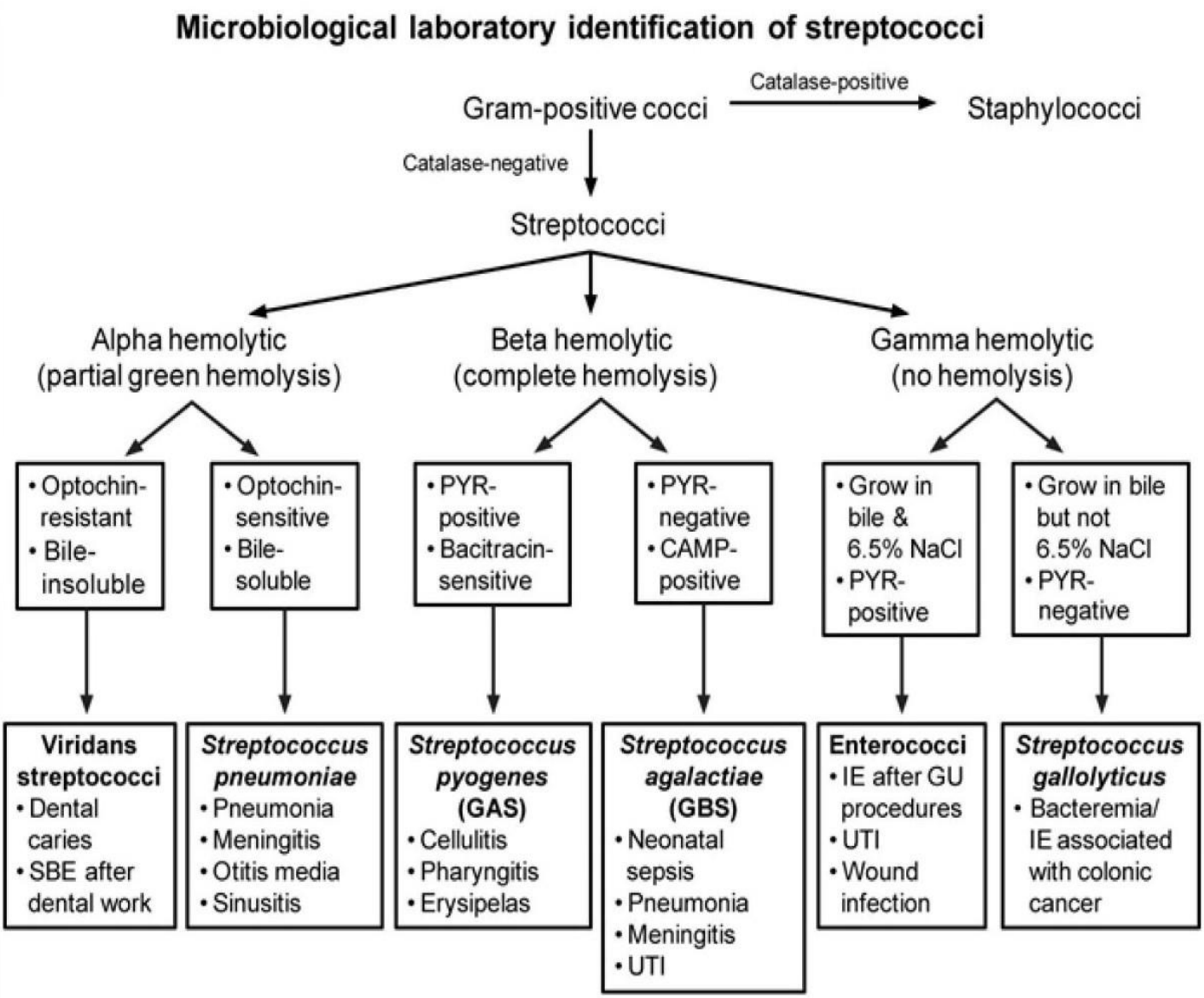
  - Streptococcus pyogenes: group A strep (catalase ⊖ and bacitracin sensitive).
  - Streptococcus agalactiae: group B strep (catalase ⊖ and bacitracin resistant).

3. γ hemolytic streptococci (Non-hemolytic streptococci) are unable to induce any change.

- Ex: Enterococci.



Beta Hemolysis Alpha Hemolysis Gamma Hemolysis



A number of species were formally reclassified from the genus *Streptococcus* to the genus *Enterococcus*, but enterococci are included here under streptococci for simplicity.

CAMP = Christie, Atkins, and Munch-Petersen test; **GAS** = group A streptococci; **GBS** = group B streptococci; **GU** = genitourinary; **IE** = infective endocarditis; **NaCl** = sodium chloride; **PYR** = pyrrolidonyl arylamidase; **SBE** = subacute bacterial endocarditis; **UTI** = urinary tract infection.

- <u>Species of Medical Importance:</u>

- S. pyogenes (group A streptococci; GAS).
- S. agalactiae (group B streptococci; GBS).
- S. pneumoniae.
- Viridians streptococci.
- Enterococcus faecalis, Enterococcus faecium.

Streptococcus pyogenes (Group A Streptococcus; GAS)

- **Distinguishing Features:**

- β-hemolytic.

- Bacitracin sensitive (used to differentiate between group A streptococcus and group B streptococcus, group B streptococcus is Bacitracin resistant).

- Because the bacitracin test is not very specific for S. pyogenes, it has been replaced in many laboratories by the Pyrrolidonyl arylamidase (PYR). S. pyogenes is PYR positive.

- **Habitats:**

- Human throat.
- Skin.

- **Mode of transmission:**

- Respiratory droplets.
- Direct contact.

- **Virulence factors and Pathogenesis:**

1. M protein:

- It is one of the cell surface proteins of S. pyogenes and represents the most important virulence factor.

- It enables the bacteria to colonize skin and inhibit phagocytosis.

- It is immunogenic and divides S. pyogenes into about 80 M serotypes.

2. Hyaluronic acid capsule:

- Acts as an immunological mask to avoid phagocytosis. Hyaluronic acid is chemically similar to that of host connective tissue. Therefore, it is not immunogenic. This allows the bacterium to hide its own antigens and used to get unrecognized by its host.

3. Invasins:

a. Streptokinase:

- It activates plasminogen of human plasma into plasmin that digests fibrin and fibrinogen.
- Streptokinase has been referred to as fibrinolysin or streptococcal spreading factor.
- It is used for emergency therapy of myocardial infarction to remove blood clots.

b. Hyaluronidase: It can digest host hyaluronic acid, thus helping spread of infection in tissues.

c. Nucleases: they depolymerize DNA.

d. Streptolysins:

- There are two pore-forming toxins that lyse host cell membranes.

- They are streptolysin O (oxygen labile) and streptolysin S (oxygen stable):
  - Streptolysin O (SLO) is a highly immunogenic protein and induces specific antibody formation (its detection is the basis for the anti-streptolysin O test). Antibodies to Streptolysin O (ASO) titer of > 200 is significant for rheumatic fever.
  - Streptolysin S (SLS) is non-immunogenic.

4. Pyrogenic exotoxins:

- Three different streptococcal pyrogenic exotoxins (SPE A, B and C) have been identified.

- These toxins use a superantigen mechanism.

- SPE A is referred to as erythrogenic toxin or scarlet fever toxin because it is responsible for the red rash characteristic of the disease.

- These toxins cause toxic shock syndrome, septicemia, and necrotizing fasciitis.

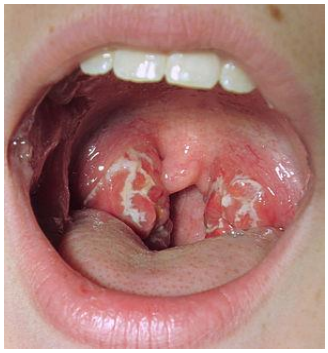
▪ Diseases:

1. Pharyngitis (sore throat, tonsillitis):

- This condition is the commonest infection caused by S. pyogenes.

- Pharyngitis is usually transmitted via respiratory droplets.

- It is characterized by pain, redness and swelling of posterior pharynx, accompanied by greyish white tonsillar exudate (membrane) and fever.



2. Scarlet fever:

- Scarlet fever occurs when pharyngitis or any other type of streptococcal infection is caused by an erythrogenic toxin-producing S. pyogenes.

- It is most often associated with streptococcal pharyngitis, which begins acutely after an incubation period of 1-5 days.

- Initial symptoms include fever, malaise, abdominal pain, and sore throat.

- The pharynx is typically erythematous, swollen, and possibly covered with gray-white exudates. In addition, the tongue can have inflamed red papillae with an appearance similar to that of a red strawberry.

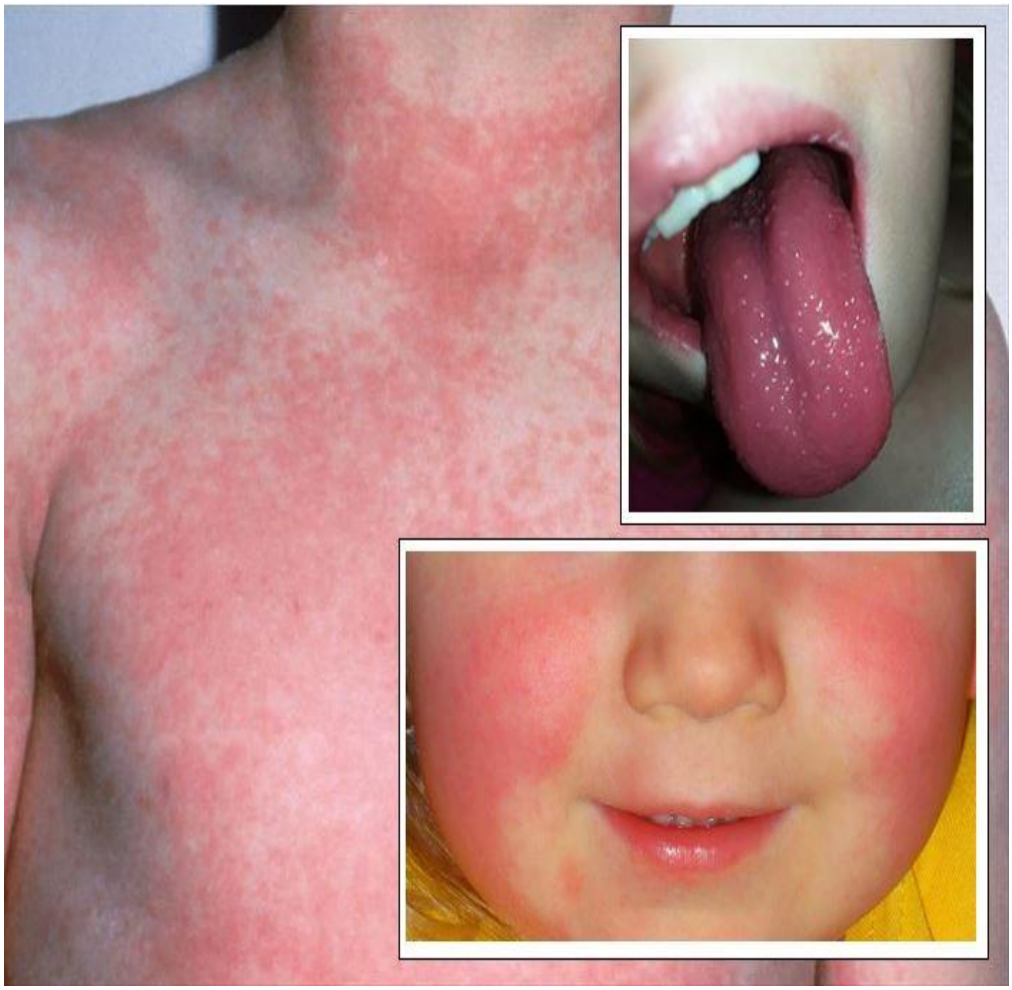
- After 1-2 days, a rash appears on the neck, armpits, and groin that subsequently generalizes to the rest of the body (palms and soles are usually spared).

- The rash begins as scarlet spots, as the rash progresses and becomes more widespread, it begins to resemble sunburn with goose pimples ("sandpaper-like" rash).

- The cheeks commonly appear flushed, giving the area around the mouth a pale appearance in comparison (circumoral pallor).

- Toward the end of the first week, desquamation begins and is most pronounced in the armpits, groin, and tips of the fingers and toes.

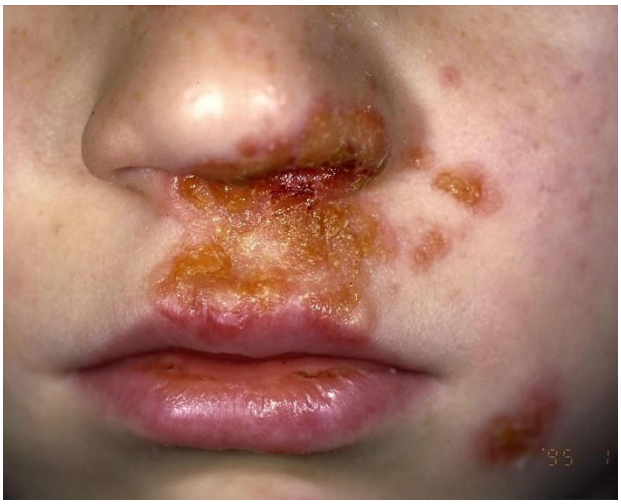
- As with any streptococcal upper respiratory infection, scarlet fever can predispose to acute rheumatic fever and glomerulonephritis.



3. Skin and soft tissue infections:

a. Impetigo (pyoderma):

- A vesicular, blistering eruption eventually leading to formation of a golden yellow crust (honey-crusted lesion), is usually seen in children and newborns, frequently occurs periorally, and can be caused by either Staphylococcuseureus and/or Streptococcus pyogenes.

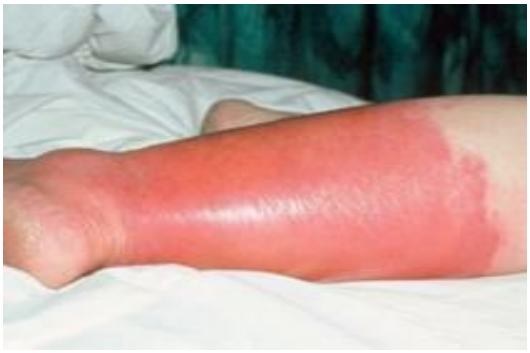


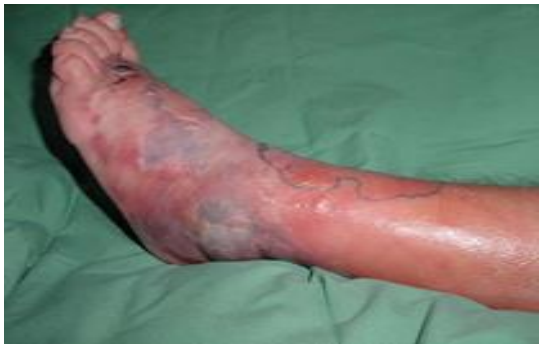
b. Cellulitis and erysipelas:

- Cellulitis and erysipelas are skin infections that develop as a result of bacterial entry via breaches in the skin barrier.

- Cellulitis and erysipelas manifest as areas of skin erythema, edema, and warmth.

- They differ in that erysipelas involves the upper dermis and superficial lymphatics, whereas cellulitis involves the deeper dermis and subcutaneous fat.





4. Invasive streptococcal infections:

a. Puerperal sepsis: this is a life-threatening infection of the endometrium and surrounding structures complicating delivery or abortion. Septicemia and toxic shock may occur.

b. Acute endocarditis: the condition can occur in individuals with normal or damaged heart valves. It is of rapid onset and is highly fatal.

c. Necrotizing fasciitis:

- Necrotizing fasciitis is a severe infection of the subcutaneous tissue and deep fascia that is a surgical emergency.

- The infection is often polymicrobial, but monomicrobial cases due to Streptococcus pyogenes (group A strep) can also occur.

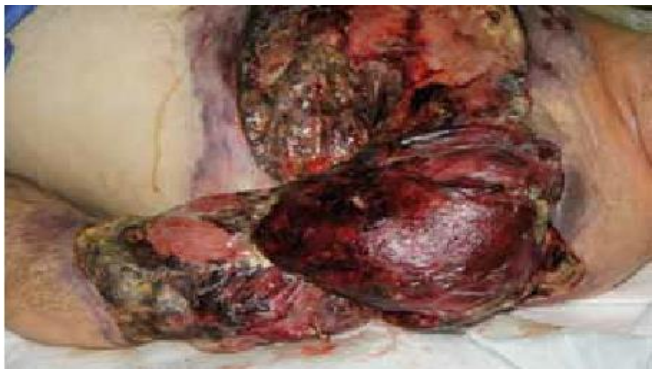
- The destructive nature of this condition let people to refer to S. pyogenes as "flesh-eating bacteria".

- S pyogenes can be transmitted through wounds and spreads rapidly to the deep layers of the skin and fascia due to production of hyaluronidase and other hydrolytic enzymes.

- Typically, there is a sudden onset of severe pain and swelling at a site of trauma or recent surgery.

- Patients quickly become hypotensive and develop septic shock.

- Necrotizing fasciitis is initially treated with aggressive surgical debridement of all necrotic tissue along with empiric broad-spectrum antibiotics due to the high incidence of polymicrobial infection.



d. Toxic shock syndrome.

- Post-Streptococcal sequelae:

- Acute rheumatic fever (ARF) and acute glomerulonephritis (AGN) are non-suppurative conditions.

- Their pathogenesis is based on the hypothesis of an autoimmune mechanism.

- ARF follows pharyngitis but not skin infection, whereas AGN is preceded by either skin or throat infection.

- These pathological events begin 1-4 weeks after an acute streptococcal illness.

1. In the case of Acute rheumatic fever (ARF):

- Antibodies to M protein cross-react with epitopes on heart myosin and sacrolemmal membrane proteins (bacterial and human epitope homology).

- This might evoke an inflammatory response (complement activation) that damages heart valves. Repeated recurrence of rheumatic fever may cause valvular damage.

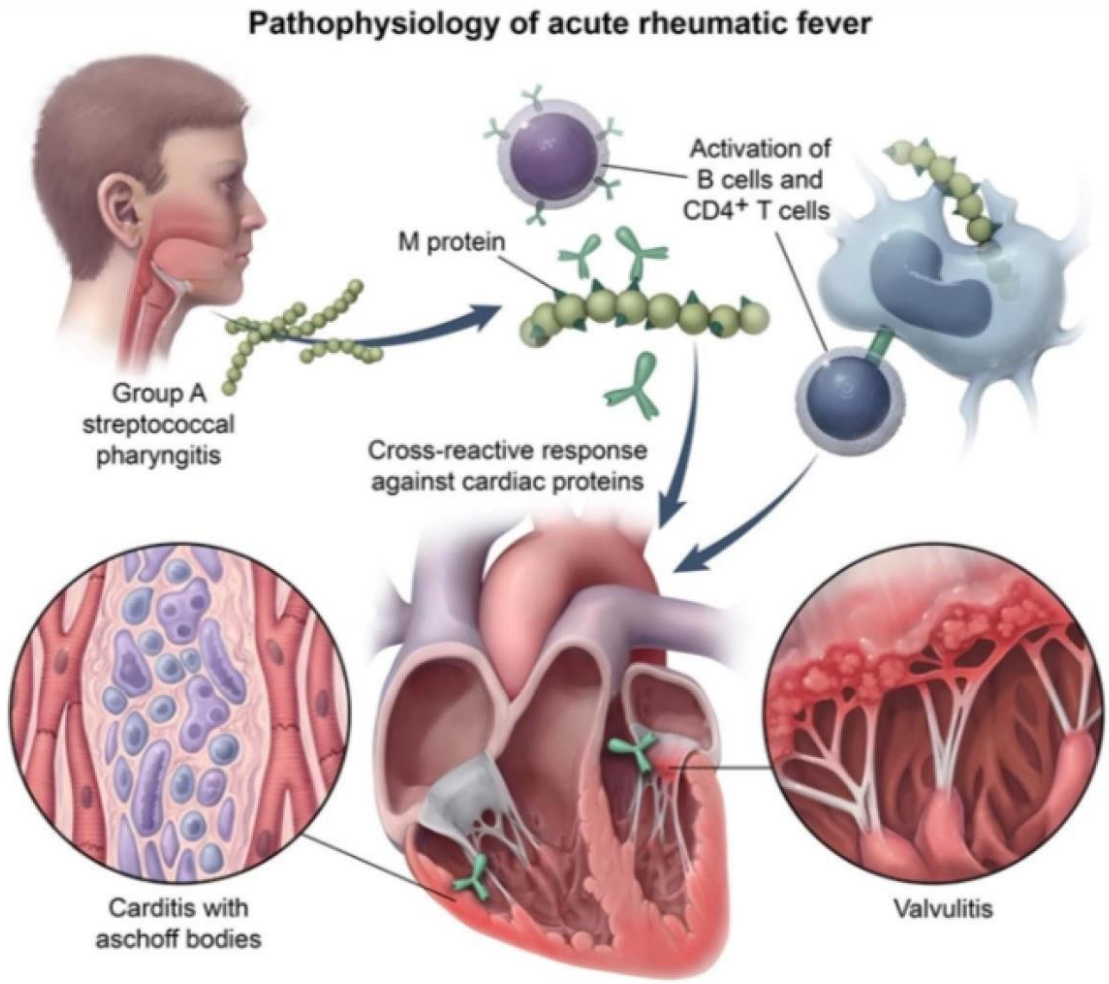
- The mitral valve is most frequently affected, but the aortic valve can also be involved.

- Inflammation of the heart involves all layers, from the pericardium to the endocardium (pancarditis).

- Recurrence of streptococcal pharyngeal infections is common and usually associated with an increased likelihood of rheumatic fever. Therefore, life-long penicillin prophylaxis is recommended following a single attack.

- No penicillin-resistant strains of Streptococcus pyogenes have yet been detected.

- Many patients with rheumatic heart disease eventually require cardiac surgery but early treatment of streptococcal pharyngitis with penicillin will decrease the need for cardiac surgery in these patients.



Features of rheumatic fever	
Epidemiology	• Endemic in developing countries
Pathogenesis	• Occurs following **group A streptococcus** (*Streptococcus pyogenes*) pharyngeal infection • Molecular mimicry between bacterial antigens & self-antigens leads to autoimmune response against cardiac and neuronal tissue

Clinical symptoms	• Early findings
	  • Migratory arthritis
	  • **Pancarditis**
	  • **Sydenham chorea**
	• Late findings (years later)
	  • **Mitral regurgitation/stenosis**
	  • Aortic valve less frequently involved

Prevention & Treatment	• Prompt treatment of streptococcal pharyngitis with **penicillin** • Cardiac valvular surgery often required

2. <u>In case of acute glomerulonephritis (AGN):</u>

- Hypertension, hematuria, nephritic range proteinuria, and RBC casts in the urine following a GAS infection suggest acute post streptococcal glomerulonephritis.

- Patients frequently present with facial edema and dark colored (cola colored) urine.

- The renal damage is due to immune complex deposition on the glomerular basement membrane and activation of complement.

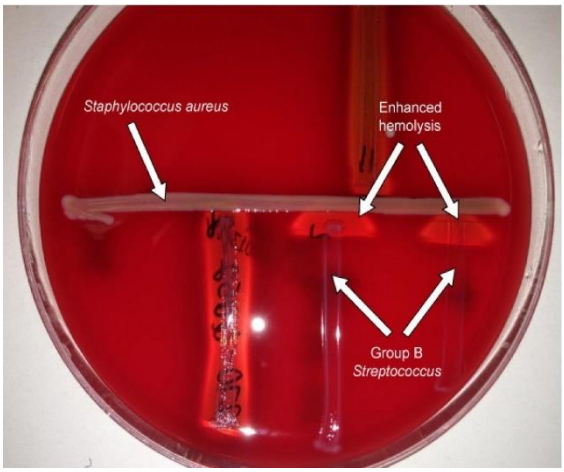
- Complement activation is responsible for the massive inflammatory response and glomerular basement membrane structural damage seen in APSGN

- Recurrence is uncommon, and antibiotic prophylaxis following an initial attack is unnecessary.

Streptococcus agalactiae (Group B Streptococci; GBS)

- Distinguishing Features:

- B-hemolytic.
- Bacitracin resistant.
- Hippurate test positive (Hydrolyze hippurate)
- CAMP test positive (Produces CAMP factor, which enlarges the area of hemolysis formed by S. aureus).
- Note: CAMP stands for the authors of the test, not cyclic AMP.



- Habitats:

- Human vagina (15-20% of women).
- Gastrointestinal tract.

- Mode of transmission: Newborn infected during birth (increased risk with prolonged labor after rupture of membranes).

- Virulence factors and pathogenesis:

- S. agalactiae is group B beta-hemolytic, having a polysaccharide capsule.
- Group B streptococci (GBS) are important etiologic agents of infections particularly during the first two months of life.
- GBS infections are acquired by infants at the time of birth (25 % of pregnant women are vaginal carriers)

- Diseases: Neonatal sepsis which may manifest as pneumonia, septicemia, meningitis and bone and joint infections. The baby who survives is frequently seriously handicapped.

- Prevention:

- The 2002 guidelines for perinatal group B strep prevention recommend universal prenatal screening for group B streptococcal colonization by maternal vaginal and rectal culture at 35-37 weeks gestation.

- In women who culture positive for GBS or in women who have had an infant affected by GBS in the past, intrapartum antibiotic prophylaxis is indicated to prevent neonatal GBS sepsis, pneumonia and meningitis. The incidence of group B streptococcal disease in babies less than a week old is declining due to these recommendations.

- Penicillin remains the first line agent for intrapartum antibiotic prophylaxis, with ampicillin an acceptable alternative.

- Group B for Babies!

Viridians Streptococci (S. Sanguis, S. Mutans)

- Distinguishing Features:

- α hemolytic.

- Optochin resistant (differentiating them from S. pneumoniae, which is α-hemolytic but is optochin sensitive).

- Habitats: human oropharynx (normal flora).

- Mode of Transmission: endogenous.

- Virulence factors and Pathogenesis:

- Viridians streptococci are normal inhabitants of the oral cavity and cause transient bacteremia after dental procedures.

- These organisms are capable of producing extracellular polysaccharides (dextrans) using sucrose as a substrate.

- Dextrans facilitate streptococcal adherence to fibrin. Fibrin and platelets are deposited at sites of endothelial trauma, providing a site for bacterial adherence and colonization during bacteremia leading to the formation of a valvular vegetation.

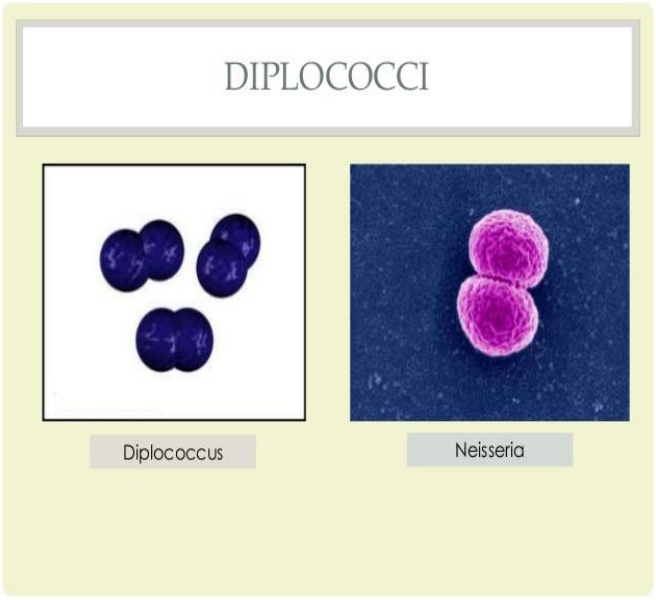
- Without preexisting endothelial damage leading to fibrin and platelet deposition at the cusps of valve leaflets, the viridans streptococci are unable to adhere to the valve and establish an infection leading to endocarditis.

- Diseases:

- Dental caries: S. mutans dextran-mediated adherence glues oral flora onto teeth, forming plaque and causing dental caries.

- Subacute bacterial endocarditis (S. sanguinis) at damaged heart valves. SBE may occur when dental manipulations or trauma to mucosa of upper respiratory tract (tonsillectomy leads to bacteremia).

Streptococcus pneumoniae



- Distinguishing Features:

- On sheep blood agar, colonies show α hemolysis.

- Optochin sensitive.

- Lancet-shaped diplococcic.

- In the laboratory, the optochin and bile solubility tests are used to differentiate pneumococci from S. viridans and Group D Streptococci:
  - Alpha-hemolytic organisms sensitive to optochin are identified as S. pneumoniae while alpha-hemolytic organisms that are optochin-resistant are identified as S. viridans.

  - S. pneumoniae cannot grow in the presence of bile (Lysed by bile) and is considered bile soluble, in contrast to the Group D Streptococci (Enterococci, S. Bovis) which can grow in the presence of bile.

- Habitats: human upper respiratory tract.

- Mode of transmission: Respiratory droplets.

- Virulence factors and pathogenesis:

- The most important virulence factor is the polysaccharide capsule which is antiphagocytic.

- No virulence without capsule.

- IgA protease.

- Diseases:

1. Typical pneumonia:

- Most common cause (especially in sixth decade of life).

- Shaking chills, high fever, lobar consolidation, blood-tinged, "rusty" sputum.

2. Adult meningitis:

- Most common cause.

- Peptidoglycan and teichoic acids are highly inflammatory in the CNS.

- CSF reveals high WBCs (neutrophils) and low glucose, high protein.

3. Otitis media and sinusitis in children: most common cause.

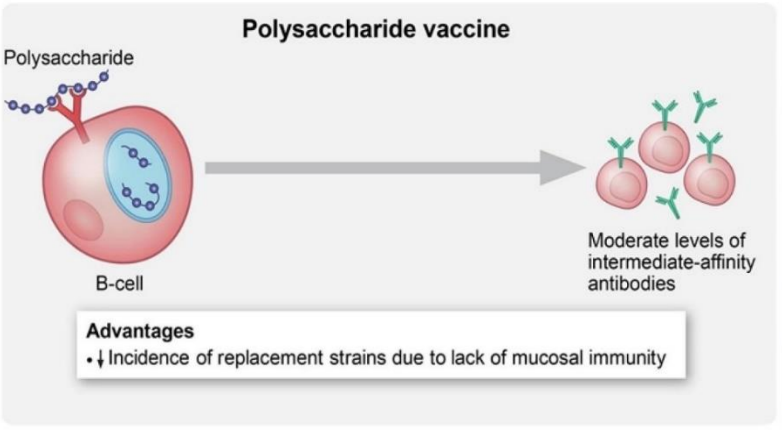
❖ Mnemonic:

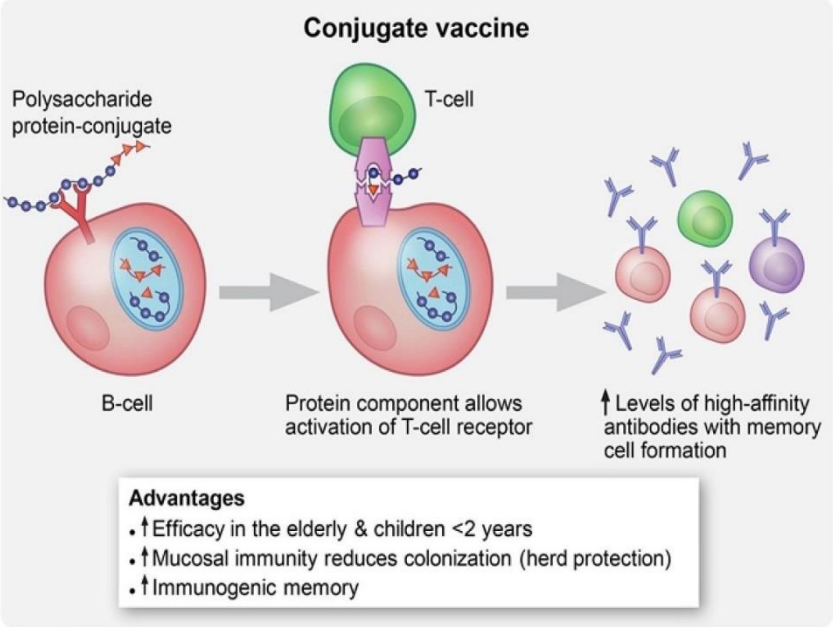
- S. pneumoniae MOPS (Most common cause of: Meningitis, Otitis media (in children), Pneumonia and Sinusitis) are Most OPtochin Sensitive.

❖ N.B:

1. There are more than 80 serotypes of S. pneumoniae based on variants of the capsular polysaccharide.

- The diversity of serotypes makes vaccine development a complex task.
- The 23-valent pneumococcal polysaccharide single-dose vaccine (unconjugated pneumococcal polysaccharide capsule) is recommended for all adults over age 65 and for other patients at high risk for pneumococcal sepsis (HIV patients, asplenic patients, chronic obstructive pulmonary disease patients, and immunosuppressed patients). It is 50-90% efficacious.
- Vaccination does not completely prevent pneumonia, as this vaccine only contains antigen from 23 of the more than 80 different capsular serotypes known.
- There is also a 7-valent conjugated vaccine available for use in children less than 2-years-old, which is about 90% efficacious in these patients.
- The conjugated vaccine contains polysaccharide antigens that are protein-coupled in order to stimulate the T-cell dependent immune (memory) response.
- The adult pneumococcal vaccine is an unconjugated polysaccharide vaccine that, unlike the infant vaccine, does not stimulate a T-helper response.





2. A patient presenting with confusion, headache, fever, and nuchal rigidity should immediately raise suspicion for meningitis.

- In bacterial meningitis, cerebrospinal fluid (CSF) analysis typically shows elevated opening pressure, increased neutrophils, decreased glucose, and elevated protein.
- The morphology of any observed bacteria on CSF Gram stain provides an excellent preliminary identification of the pathogen (whereas cultures take time to grow).
- Streptococcus pneumoniae is the most common cause of bacterial meningitis in adults of all ages. On CSF Gram stain, lancet-shaped Gram-positive cocci are found in pairs.

Genus: Enterococcus

- Genus Features:

- Catalase negative.
- PYR+.

- Species of Medical Importance:

- Enterococcus faecalis.
- Enterococcus faecium.

Enterococcus faecalis/faecium

- Distinguishing Features:

- Catalase-negative.

- Group D gram-positive cocci in chains.

- Lancefield group D includes the enterococci and the nonenterococcal group D streptococci.

- Lancefield grouping is based on differences in the C carbohydrate on the bacterial cell wall.

- Enterococci are gamma-hemolytic (no hemolysis on blood agar).

- Enterococci, hardier than nonenterococcal group D, can grow in hypertonic 6.5% NaCl and bile (lab test).

- PYR test positive.

▪ Habitats: human colon, urethra ± and female genital tract.

▪ Mode of transmission: endogenous.

▪ Virulence factors and Pathogenesis:

- Bile salt tolerance allows survival in bowel and gall bladder.

- During medical procedures on GI or GU tract, E. faecalis → bloodstream → previously damaged heart valves → endocarditis.

- The enterococci are resistant to many antibiotics and can be very difficult to treat:

  - Penicillin is often combined with an aminoglycoside for a synergistic effect, but increasingly, bacteria resistant to both aminoglycosides and B-lactams (including penicillinase-resistant types) are emerging.

  - Additionally, vancomycin-resistant and linezolid-resistant enterococci have emerged.

▪ Diseases:

- Enterococci (E. faecalis and E. faecium) are normal colonic flora that are penicillin G resistant and cause UTI, biliary tract infections, and subacute endocarditis (following GI/GU procedures).

- VRE (vancomycin-resistant enterococci) are an important cause of nosocomial infection.

- Enterococcal endocarditis usually occurs in elderly men who have recently undergone manipulation of areas colonized by this organism, such as the GI or GU tracts (cystoscopy).

- In women, enterococcal endocarditis can occur following obstetrical procedures.

❖ N.B:

▪ Lancefield grouping is based on differences in the C carbohydrate on the bacterial cell wall.
▪ Lancefield group D includes the enterococci (Enterococcus faecalis/faecium) and the nonenterococcal (S. bovis) group D streptococci.
▪ Among nonenterococcal group D streptococci, Streptococcus bovis is the one main human pathogen.
▪ S. bovis can grow in bile but not on 6.5% NaCl.
▪ Streptococcus gallolyticus (formerly S bovis) is a part of the normal flora of the colon, and bacterermia or endocarditis caused by S. bovis is associated with colonic cancer in approximately 25% of cases.

- S. bovis causes a subacute bacterial endocarditis with symptoms similar those of S. viridans SBE.
- The role of S. bovis as causative agent or marker of the disease in colon cancers is unclear, but every patient with S bovis bacteremia with or without endocarditis should be examined for a GI tract malignancy (colon cancer).

❖ Mnemonic:

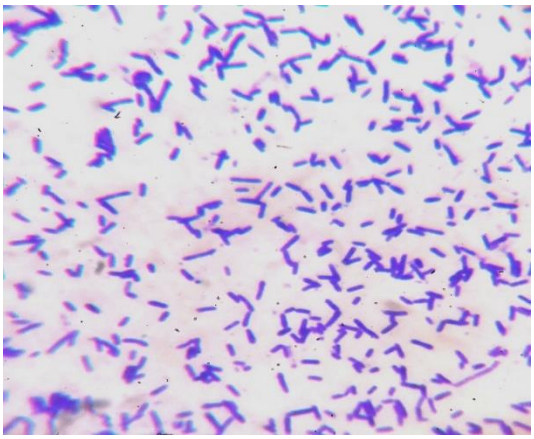
- Bovis in the blood = cancer in the colon.

2. Gram-Positive Rods:

Genus: Bacillus

- Genus Features:

- Gram-positive rods.



- Members of the Bacillus and Clostridium genuses are commonly found in soil and are able to survive high temperatures, desiccation, and chemical agents by forming spores.

- Spore- forming bacteria can survive boiling.

- Spore-forming bacteria can be killed by autoclaving (steaming at 121C for 15 mins).

- The Gram-positive rod-shaped bacteria that form endospores, have two principal subdivisions:
  - The aerobic genus (Bacillus).
  - The anaerobic genus (clostridium).

- Species of Medical Importance:

- Bacillus anthracis.
- Bacillus cereus.

Bacillus anthrax

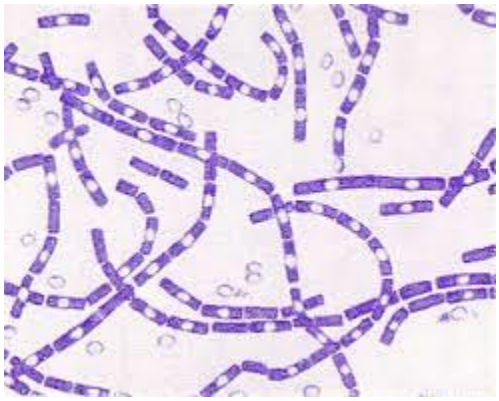
- Distinguishing Features:

- Gram-positive, spore-forming rods.

- Capsule is polypeptide (poly-D-glutamate).

- Potential biowarfare agent.

- On microscopy it forms long chains that are described as being "serpentine" or "medusa head" on appearance.



- Habitats: animals, skins, soils.

- Mode of transmission:

- Anthrax is most commonly acquired occupationally by those who handle livestock that have not been immunized for the disease as well as those who handle the hides of such animals.

- Anthrax is also used as a biological weapon due to the near 100% mortality of the pulmonary form.

- For this reason, an occupational history of exposure to animals or animal products is extremely important; if cutaneous anthrax is suspected in a patient without the risk of occupational exposure, then the potential for bioterrorism should be suspected and public health authorities contacted.

- Virulence factors and pathogenesis:

1. The capsule:

- Bacillus anthracis produces an antiphagocytic capsule that is required for pathogenicity.

- The capsule is unique in that it contains D-glutamate instead of polysaccharide.

- The capsule of B. anthracis has a single antigenic type. It plays its most important role during the establishment of the infection, and a less significant role in the terminal phases of the diseases, which are mediated by the anthrax toxin.

2. Anthrax toxin:

- It is analogous to the A-B model of cholera toxin.

- The toxin consists of three distinct protein factors:
  - The edema factor (EF).
  - The lethal factor (LF).
  - The protective antigen (PA).

- The edema factor (EF) and the lethal factor (LF) from the active (A) domain while the protective antigen (PA) forms the binding (B) domain.

- Protective antigen functions to translocate both edema and lethal factor into the cytosol.

- Neither toxin can take effect without protective antigen.

- Once inside the cell, edema factor acts as a calmodulin-dependent adenylate cyclase that increases cAMP concentration. It causes accumulation of fluid within and between cells (tissue edema) and also results in suppression of neutrophil and macrophage function. This mechanism of action is similar to that of adenylate cyclase toxin, produced by Bordetella pertussis.

▪ Diseases:

- Anthrax is primarily a disease of farm animals. Humans require anthrax as a result of contact with infected animals or animal products. There is no person-to-person transmission of anthrax.

- The disease takes one of three forms, depending on the route of infection. Cutaneous anthrax is the most common form of this disease; pulmonary anthrax accounts for approximately 5% of cases, and gastrointestinal anthrax is a rare occurrence.

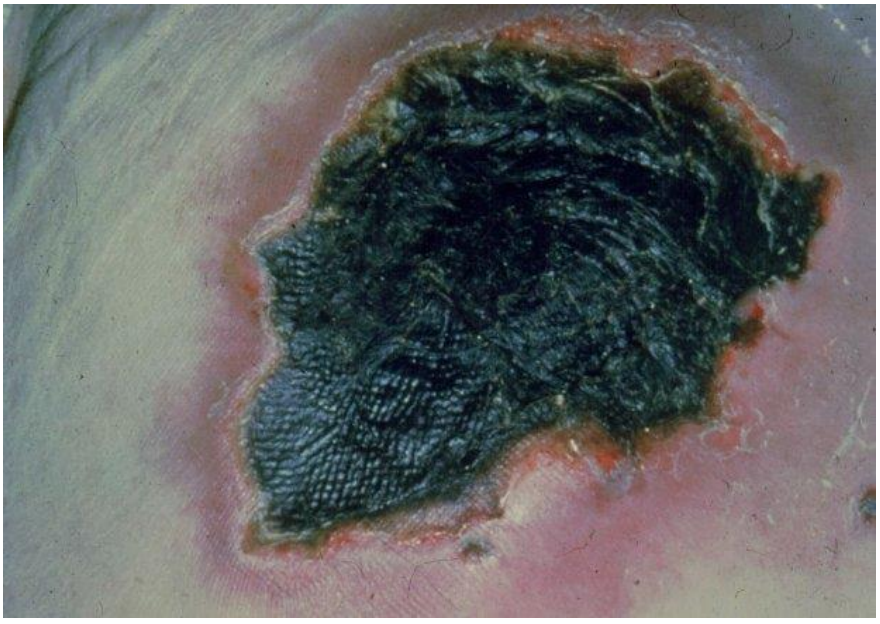
1. Cutaneous anthrax (malignant pustule):

   - This occurs from handling infected material.

   - Spores from the soil or an infected or dead animal enter through a cut or abrasion, usually on an exposed area.

   - The spores germinate and vegetative cells multiply locally forming a small papule which changes rapidly to a vesicle, then a pustule, and finally into a necrotic ulcer which blackens to form a characteristic eschar. The lesion is painless and is surrounded by marked edema.

   - Untreated cases may develop fatal fulminating septicemia.



2. Pulmonary anthrax:

- Pulmonary anthrax is also known as "woolsorters disease" because exposure from handling animal products such as animal hair, wool processing has been associated with infection by Bacillus anthracis.

- The spores of B. anthracis are very small, once they are inhaled, they enter the alveoli and are ingested by macrophages.

- From the lung the organisms rapidly move to mediastinal lymph nodes and cause hemorrhagic mediastinitis.

- Once the spores germinate into vegetative cells, they will begin to produce the three-part anthrax toxin and clinical symptoms will rapidly follow.

- Symptoms initially only consist of myalgia, fever and malaise but rapidly progress to hemorrhagic mediastinitis (widened mediastinum on chest x-ray), bloody pleural effusions, septic shock, and death.

3. Intestinal anthrax: from eating infected meat.

Bacillus cereus

- Distinguishing feature: Gram-positive, spore-forming rods.

- Habitats: found in nature.

- Mode of transmission: Foodborne. Major association with fried rice from Chinese restaurants (reheated rice syndrome).

- Virulence factors and pathogenesis:

- Two possible toxins:

- Emetic toxin: preformed, heat-stable enterotoxin, fast (1-6 hours), similar to S. aureus enterotoxin with vomiting and abdominal cramps; associated with fried rice.

- Diarrheal toxin: produced in vivo (meats, sauces); 18 hours, similar to E. coli labile toxin (LT) increasing cAMP → watery diarrhea.

- Diseases:

- Bacillus cereus causes two types of food poisoning:

1. The "short incubation" or emetic form:

- It has an incubation period of 1 to 6 hours and is characterized by nausea, vomiting and abdominal cramps.

- It resembles staphylococcus aureus food poisoning in its symptoms and incubation period.

- This form is caused by a preformed heat-stable enterotoxin (emetic toxin).
- It is usually associated with improperly refrigerated cooked rice.

2. The "long incubation" or diarrheal form:
- It has an incubation period of 8 to 16 hours and manifests primarily by abdominal cramps and diarrhea.
- This type resembles food poisoning caused by clostridium perfringes. This form is mediated by a heat-labile enterotoxin which activates intestinal adenylate cyclase and causes intestinal fluid secretion.
- It is usually associated with meat dishes.

- Management: supportive care (antibiotics are ineffective against toxins).

Genus: Clostridium

▪ Genus Features:
- Gram-positive Spore forming rods.
- Obligate anaerobic bacilli.

▪ Species of Medical Importance:
- Clostridium tetani.
- Clostridium botulinum.
- Clostridium perfringens.
- Clostridium difficile.

Clostridium tetani

▪ Distinguishing Features:
- Large gram-positive, spore-forming rods.
- Anaerobes.

▪ Habitats: soil.

▪ Mode of transmission:
- Most cases of tetanus result from lacerations or small puncture wounds contaminated with C. tetani spores.
- Ingestion of tetanus toxin doesn't produce the disease.
- In developing countries, most cases of tetanus occur in mothers with incompletely removed placentas and in newborns with unclean and infected umbilical cord stumps.

- Virulence factors and pathogenesis:

- C. Tetani produces the tetanospasmin toxin, a neurotoxin responsible for the symptoms of tetanus.

- It acts centrally at the level of the brain stem and anterior horn cells of the spinal cord.

- Tetanus toxin and botulinum toxin are proteases that cleave SNARE proteins involved in neurotransmission.

- Diseases:

- Clostridium tetani produces disease by the production of potent protein exotoxin, not by bacterial invasion of tissue. Even small amounts of tetanus toxin can be deadly.

- The spores germinate in the traumatized tissue where the blood supply is cut off producing an anaerobic environment with low redox potential. A puncture wound can also cause spores to be injected deeply into the tissue, offering anaerobic medium.

- The vegetative cells of C. tetani grow locally in the necrotic tissue and elaborate tetanospasmin toxin.

- At first, the toxin binds to receptors on the presynaptic membranes of the motor neurons.

- From there, the toxin migrates by the retrograde axonal transport system to the cell bodies of these neurons and next to the spinal cord and brain stem.

- Binding of the toxin in an irreversible event, where it is no longer accessible to neutralization by antitoxin.

- Release of the inhibitory neurotransmitters glycine and gamma-aminobutyric acid (GABA) from these inhibitory neurons (Renshaw cells in the spinal cord) is blocked. The suppression of inhibitory nerve function results in an increased activation of nerves innervating muscles, causing muscle spasms, spastic paralysis, and hyperreflexia.

- The muscle spasms involve both flexor and extensor muscles. Patients with tetanus have spastic muscle contractions:

  - Difficulty opening the jaw (known as lockjaw or "trisrms").

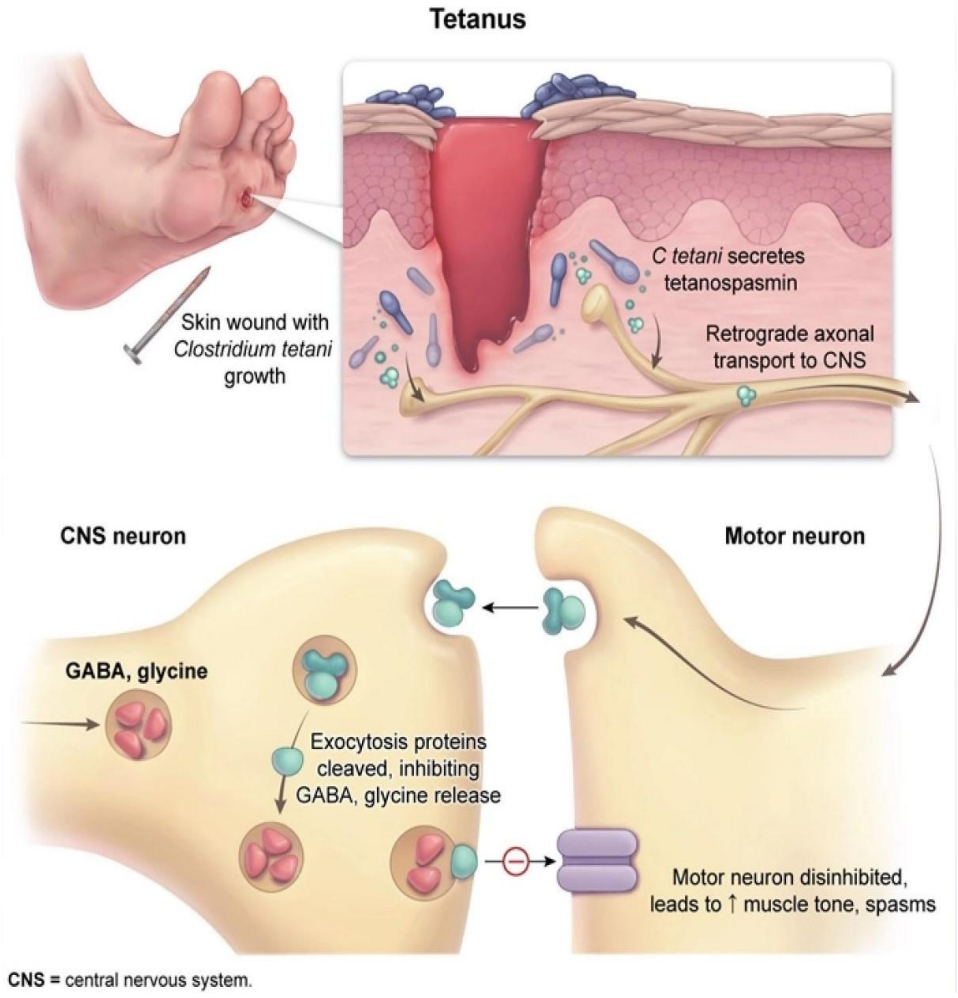
  - A characteristic smile called "risus sardonicus".

  - Contractions of back muscles, resulting in backward arching known as opisthotonos.

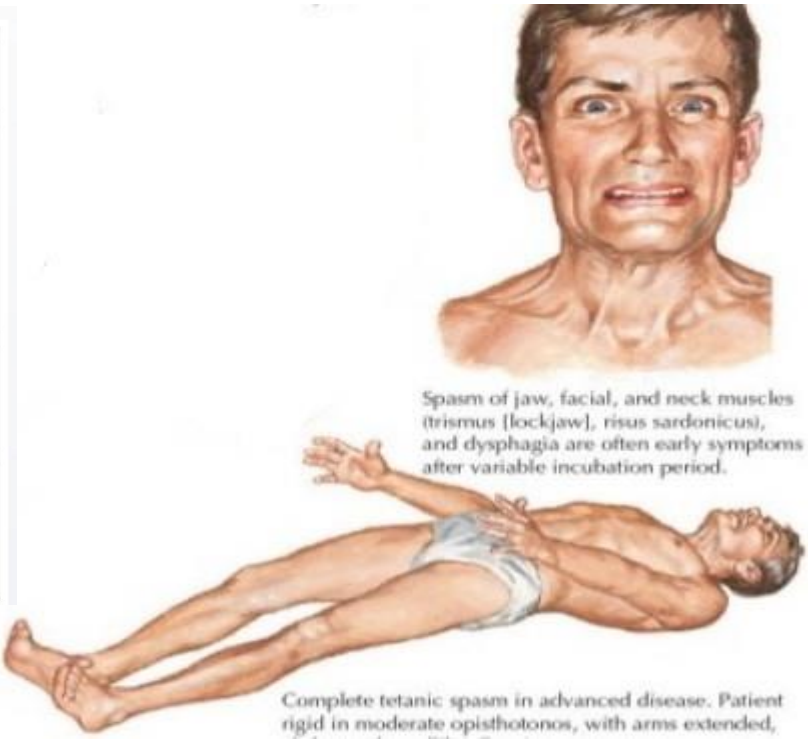
  - Spasmodic contraction may further extend to respiratory muscle → respiratory failure.

  - Patients are extremely irritable, and develop tetanic seizures, brought about by violent, painful muscle contractions following minor stimuli, such as a noise.

- Prevent with tetanus vaccine. Treat with antitoxin +/− vaccine booster, antibiotics, diazepam (for muscle spasms), and wound debridement.



Neonatal tetanus	
Clinical features	• Difficulty feeding, **trismus** • Spasms & hypertonicity ◦ Clenched hands, dorsiflexed feet, **opisthotonus** • Eventual respiratory failure
Treatment	• Supportive care • Antibiotics & tetanus immune globulin
Prevention	• Immunization of pregnant women & those of childbearing age • Hygienic delivery & cord care



❖ Tetanus vaccine:

- Tetanus vaccine has been available since 1925.
- Immunity to Clostridium tetani is produced by vaccination with a formalin-inactivated toxin, also known as toxoid.
- Illness caused by Clostridium tetani (tetanus) can be prevented by proper immunization with a childhood series and a booster immunization every ten years thereafter in adulthood.
- The first vaccine dose is started approximately 2 months after birth.
- An immunized mother will be able to pass IgG through the placenta to the fetus and provide passive immunity against neonatal tetanus until the child receives its first tetanus vaccination at two months of age.
- After the initial childhood vaccine series at 2 months, 4 months, 6 months, 15 to 18 months and 10 to 12 years, booster immunizations are required every ten years to maintain a protective level of antibody.

❖ Mnemonic:

- Tetanus is tetanic paralysis.

Clostridium botulinum

- Distinguishing Features:

- Gram-positive spore-forming rods.
- Anaerobic.

- Habitats: soil/ dust.

- Mode of transmission:

- Whereas Infant botulism is frequently due to consuming C. botulinum spores (honey), adult botulism results from consuming preformed toxin, typically in canned food.
- More than 12% of honey samples contain low numbers of C. botulinum spores.

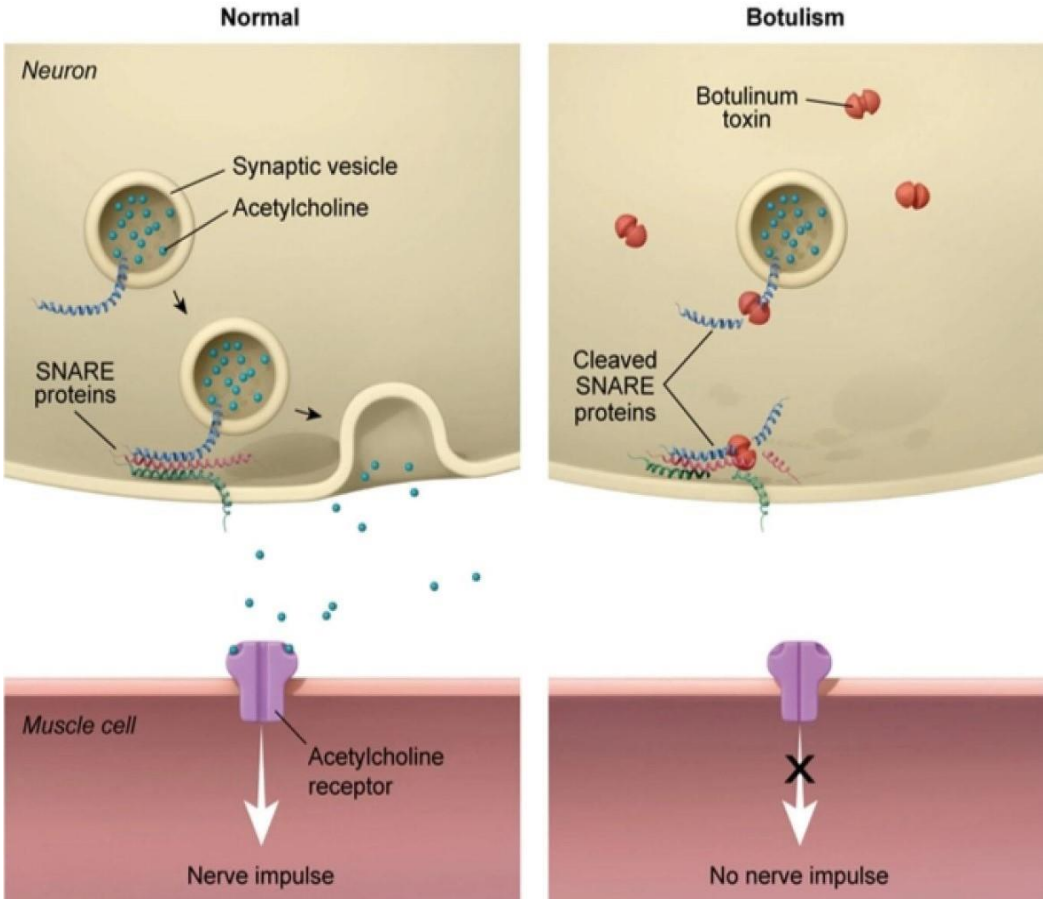
- Virulence factors and pathogenesis:

❖ Botulinum toxins:

- Several toxigenic types of the organism exist, each producing an immunologically distinct form of botulinum toxin.
- Types A, B and E cause human botulism. Type A toxin is the most potent poison known.

- The botulinum toxins are very similar in structure to the tetanus toxin but differ dramatically in their clinical effects because they target different cells in the nervous system.

- The botulinum toxin is specific for peripheral nerve endings at the neuromuscular junction where it inhibits the release of acetylcholine.



- Diseases:

1. Food-borne botulism (Adult botulism):

- Spores, widespread in soil, contaminate vegetables and meat.

- When these foods are canned without adequate sterilization, spores survive and germinate in the anaerobic environment.

- Toxin is produced within the canned food and ingested preformed.

- The highest-risk foods are vegetables such as green beans and mushrooms, smoked and salted fish, and commercially canned salmon.

- The toxin is activated by proteases in the gastric fluid and by gastric acidity.

- The toxin is absorbed in the intestine and is transported systemically via the bloodstream to reach the peripheral neuromuscular synapses.

- The toxin binds to the neuron and prevents the release of acetylcholine across the synaptic cleft. Thus, it produces paralysis of the motor system.

- Botulinum toxin is quick acting so the incubation period may be as short as 12 hours.

- Once the toxin is fixed to the tissue, its actions are very difficult to reverse with antitoxin treatment.

- Antitoxin is only effective if it binds to the toxin before the toxin binds the neuromuscular junction (within 12 hours after ingestion).

- The cranial nerves are affected first [diplopia, dysphagia and dysphonia, dyspnea. (4 'Ds')], followed by a descending, symmetric flaccid paralysis of motor nerves.

- Nausea and vomiting are not usually prominent. No fever is apparent. Death is usually caused by respiratory failure.

2. Infant botulism (floppy baby syndrome):

- In contrast to food poisoning caused by ingestion of preformed toxin, infant botulism results from germination of spores in the gastrointestinal tract where vegetative cells replicate and release the botulinum toxin.

- The disease occurs in infants between 2 weeks and 6 months of age.

- The bacterium can establish itself in the bowel of infants before the establishment of competing intestinal flora.

- The disease is characterized by constipation and weak sucking ability and generalized weakness.

- Almost all cases of the disease recover (In contrast, adult botulism, which results from ingestion of preformed toxin, is almost always very severe).

- In severe cases, the generalized muscle weakness and loss of head control can cause the infant to appear "floppy".

- Infant botulism can usually be diagnosed based on the patient's clinical presentation and food consumption history.

- Culture and isolation of the organism and bioassay of toxins are time-consuming procedures, but rapid in vitro procedures have been developed for the detection of types A, B, E, and F botulinum toxin-producing organisms and their toxins.

- The tests are based on ELISA methodology and polymerase chain reaction techniques.

❖ N.B:

- Focal dystonia is a localized uncontrollable muscle contraction causing pain or discomfort as well as physical deformity in some cases.
- A classic example is cervical dystonia of the sternocleidomastoid muscle, or torticollis.
- Local injection of botulinum toxin (Botox) into the dystonic sternocleidomastoid muscle results in muscular relaxation because the toxin prevents presynaptic release of acetylcholine, the neurotransmitter responsible for muscle contraction, from the nerve terminal at the neuromuscular junction.
- This effect is temporary, however, because regeneration of the nerve terminal eventually occurs (This process takes approximately three months). For this reason, therapeutic botulinum toxin injections must be repeated when the effects begin to diminish.
- Botulinum toxin can also be used cosmetically to reduce the appearance of glabellar and other facial wrinkles.
- It is also used to relax the lower esophageal sphincter in esophageal achalasia, to treat the muscle spasms of multiple sclerosis and Parkinson's disease, and for other conditions resulting from involuntary muscle contraction.

❖ Mnemonic:

- Botulinum is from bad bottles of food and honey (causes a flaccid paralysis).

Clostridium perfringens

- Distinguishing Features:

- Large gram-positive, spore-forming rods.

- Anaerobic.

- Habitats: soil and human colon.

- Mode of transmission: foodborne and traumatic implantation.

- Virulence factors and pathogenesis:

- Spores germinate under anaerobic conditions in tissue.

- Vegetative cells produce:

  - Alpha toxin (phospholipase C) is a lecithinase. It disrupts membranes, damaging RBCs, platelets, WBCs, endothelial cells → massive hemolysis, tissue destruction, hepatic toxicity.

  - Enterotoxin produced in intestines in food poisoning → disrupts ion transport → watery diarrhea, cramps (similar to E. coli); resolution <24 hours.

- Diseases:

1. Gas gangrene (myonecrosis):

- About 95% of cases of gas gangrene are due to Clostridium perfringens.

- The spores of this Gram-positive bacillus are abundant in soil, and patients suffering penetrating injuries can be inoculated with spores.

- Upon entering the host, the C. perfringens spores germinate in the anaerobic environment into vegetative toxin-producing cells.

- Lecithinase is the main toxin of C. perfringens; its concentration correlates with its lethal and necrotic effects.

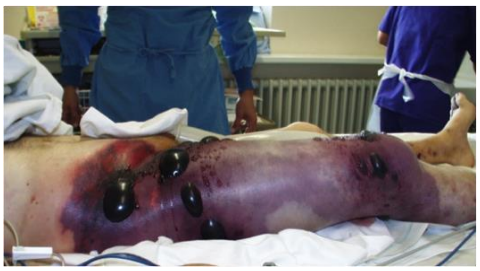
- Lecithinase, also known as phospholipase C or alpha toxin, is an enzyme that catalyzes the splitting of phospholipid molecules in cell membranes causing cell lysis (including RBC hemolysis), tissue necrosis and edema.

- Extensive tissue damage, necrosis, and reduction of blood supply to the affected area result, and the disease continues to spread in the enlarging anaerobic environment.

- The organism rapidly metabolizes carbohydrate, producing a significant amount of gas that can be visualized on plain film radiographs.

- Manifestations include rapid-onset muscle pain, fever, hemorrhagic bullae with dusky surrounding skin, and tissue edema/crepitus.

- Treatment involves emergent debridement and intravenous antibiotics, but even with prompt therapy the prognosis of clostridial myonecrosis, or gas gangrene, is poor and tissue loss is often considerable.



2. Food poisoning:

- Perfringens can also cause a late-onset food poisoning characterized by a transient watery diarrhea.

- This gastroenteritis is caused by toxin formed when large quantities of clostridial spores are ingested.

- The spores germinate in the digestive tract and then begin to elaborate toxin, hence the disease's delayed onset. (This is in contrast to the early-onset food poisoning caused by the preformed toxins of S. aureus and B. cereus).

❖ Mnemonic:

- Perfringens perforates a gangrenous leg.

❖ N.B:

- Clostridium septicum is a spore-forming, exotoxin-producing, gram-positive bacterium that is a normal commensal of the gastrointestinal tract in humans.
- Although the organism is largely nonpathogenic, breakdowns in the gastrointestinal mucosa can lead to invasion with subsequent hematogenous dissemination to healthy muscle tissue, resulting in spontaneous gas gangrene (nontraumatic).
- Unlike C septicum gas gangrene, C perfringens gas gangrene is usually associated with preceding trauma.
- Most cases of spontaneous gas gangrene are triggered by an underlying colonic malignancy, which creates a portal of entry for the bacteria. Inflammatory bowel disease and immunosuppression are also risk factors.
- Manifestations include rapid-onset muscle pain, fever, hemorrhagic bullae with dusky surrounding skin, and tissue edema/crepitus.
- Urgent antibiotics and surgery are required, as the infection is often fatal even with early treatment.

Clostridium difficile

- Distinguishing Features:

- Gram-positive Spore-forming bacillus.

- Anaerobic.

- Habitats: human colon/gastrointestinal tract.

- Mode of transmission: endogenous.

- Virulence factors and pathogenesis:

- When the normal flora is suppressed, due to overuse of antibiotics, C. difficile grows and produces two toxins.

- Toxin A is an enterotoxin that causes fluid accumulation in the bowel, toxin B is a potent cytotoxin.

- Both toxins result in alterations in the actin microfilaments responsible for cytoskeleton of the cells.

- This can cause loosening of the tight junctions between the colonic epithelial cells and hence, diarrhea.

- Both toxins have effects on leukocytes that include induction of tumor necrosis factor, IL-1 and IL-6 that contribute to the inflammatory response (diarrhea that may progress to pseudomembranous colitis).

▪ Diseases:

❖ Antibiotic-associated diarrhea, or pseudomembranous colitis:

- Over 400 types of bacteria inhabit the healthy human gastrointestinal tract as part of the normal gut flora.

- These intestinal bacteria (intestinal biomass) effectively suppress overgrowth of Clostridium difficile and many other potentially pathogenic bacteria by competing for nutrients and adhesion sites within the gut.

- Treatment with antibiotics can alter the intestinal balance of bacteria leading to a potential overgrowth of pathogenic strains and clinical disease.

- Common antibiotics implicated in C difficile colitis include clindamycin, fluoroquinolones, penicillin, and broad-spectrum cephalosporins.

- It causes disease by releasing two toxins that damage the mucosal lining of the large intestine leading to diarrhea (Toxin A) and necrosis (Toxin B) with pseudomembrane formation.

- These toxins disrupt cellular cytoskeletons and intercellular tight junctions, leading to colonocyte apoptosis.

- The colonic mucosa responds to toxin exposure by forming white, patchy pseudomembranes, which consist of a neutrophil- predominant inflammatory infiltrate, fibrin, bacteria, and necrotic epithelium.

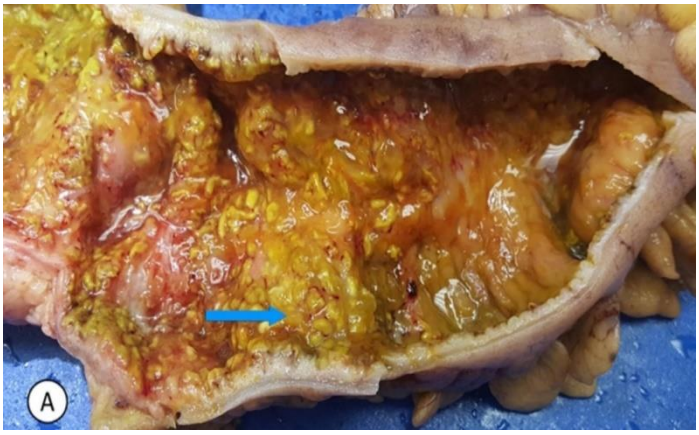
- White/yellow membrane-like plaques seen on colonoscopy are virtually pathognomonic.

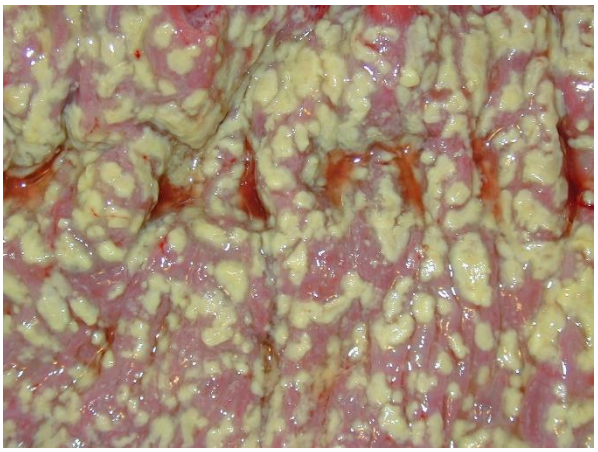
- PCR detection of toxin A and B genes in the stool is the best method for diagnosing C difficile colitis.

- Complication: Patients with severe disease may develop a nonobstructive colonic dilation known as toxic megacolon, which leads to increased risk of colonic perforation.

- Treatment: metronidazole or oral vancomycin, or fidaxomicin. For recurrent cases, consider repeating prior regimen or fecal microbiota transplant.

❖ Mnemonic: Difficile causes diarrhea.





❖ N.B:

- Cases of suspected or proven C difficile infection require additional contact precautions, including handwashing with soap and water (alcohol-based hand sanitizers do not kill the spores), gown for any patient contact, and nonsterile gloves that should be changed after contact with contaminated secretions.
- In addition, a dedicated stethoscope and blood pressure cuff should be left in the patient's room.

Contact precautions	
Organisms	• MDR organisms (eg, MRSA, VRE) • Enteric organisms (eg, *Clostridium difficile*) • Scabies
Infection-control measures	• Hand hygiene **(soap & water for *C difficile*)** • Nonsterile gloves • **Gown** • Private room preferred

MDR = multidrug-resistant; **MRSA** = methicillin-resistant *Staphylococcus aureus*; **VRE** = vancomycin-resistant

Genus: Listeria

- Genus Features:

- Gram-positive, non-spore forming rods.
- Facultative intracellular.
- Tumbling motility.

- Species of Medical Importance: Listeria monocytogenes.

Listeria monocytogenes

- Distinguishing Features:

- Gram-positive rods.

- Beta hemolytic, nonspore-forming rod on blood agar.

- Listeria is immotile at 37 C but demonstrates tumbling motility at 22 C, a fact that can be used to identify potential Listeria isolates.

- It is able to multiply at 4°C, a unique feature that laboratories exploit when culturing the organism, a process called cold enrichment.

- Habitats:

- Widespread: animals (gastrointestinal and genital tracts), unpasteurized milk products, plants, and soil.

- Cold growth: soft cheeses, deli meats, cabbages (coleslaw), hotdogs.

- Mode of transmission:

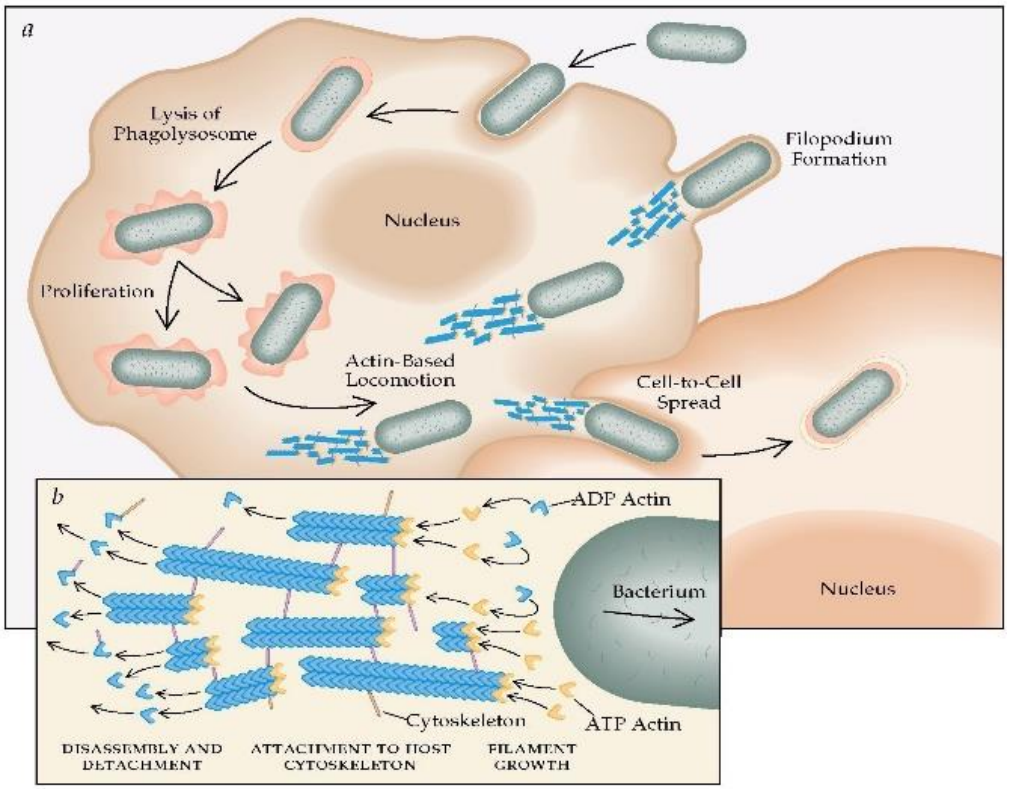
- Foodborne: It can multiply at temperatures as low as 4 C, which allows the bacteria to contaminate refrigerated food (meat, unpasteurized milk, soft cheese, raw vegetables).

- Neonates can acquire Listeria transplacentally or during delivery.

- Virulence factors and pathogenesis:

- Listeriolysin O:

  - a β-hemolysin, facilitates rapid escape of the bacterium from phagosome into cytoplasm where it can grow intracellularly (they are protected from extracellular immune system factors such as the complement system and antibodies), thus evading killing when lysosomal contents are dumped into phagosome; then "jets" directly (by actin filament formation) from cytoplasm to another cell (Forms rocket tails via actin polymerization that allow intracellular movement and cell-to-cell spread across cell membranes, thereby avoiding antibody).



❖ N.B:

- Intracellular bacteria are protected against circulating immune factors such as antibodies and complement but must develop strategies to survive the hostile environment inside macrophages.
- This can be accomplished by blocking the fusion of phagosomes with lysosomes (Salmonella & Mycobacterium tuberculosis), inhibiting phagolysosome acidification (M. tuberculosis), or escaping from the phagosome into the cytosol (Listeria& Shigella).

- Diseases:

1. Listeriosis:

- In adults, listeriosis occurs almost exclusively in the immunocompromised.

- Healthy adults and children (intact cell mediated immunity): generally asymptomatic or presents with mild gastroenteritis.

- In healthy individuals, Listeria infection stimulates the production of cytokines (interferon gamma, tumor necrosis factor-beta, and interleukin-12) that induce a cell-mediated immune response leading to macrophage activation and killing of intracellular Listeria.

- Pregnant women:
  - Symptomatic carriage.
  - Can cause amnionitis, septicemia with fever and chills, and spontaneous abortion.
  - Can cross the placenta in septicemia.

2. Neonatal disease:

- Neonates can acquire Listeria transplacentally or during delivery; they are especially vulnerable to developing meningitis and septicemia because their cell-mediated immunity is not yet fully developed.

- Intact cell-mediated immunity is essential for the elimination of the bacterium from the body.

- Common cause of neonatal meningitis.

- Granulomatosis infantisepticum: in utero transmission; sepsis with high mortality; disseminated granulomas with central necrosis.

3. In immunocompromised patients:

- In patients with compromised cell-mediated immunity (pregnant women, elderly and immunocompromised patients), the organism survives and can cause serious infections.

- Septicemia and meningitis (most common clinical presentation).

- Listeria meningitis-most common cause of meningitis in renal transplant patients and adults with cancer.

▪ Treatment:

- Gastroenteritis is usually self-limited; ampicillin in infants, immunocompromised, and the elderly as empirical treatment of meningitis.

- Ampicillin is the treatment of choice for listeria, which is not sensitive to cephalosporins.

Genus: Corynebacterium

- Genus Features:
- Gram-positive non-spore forming rods.
- Aerobic.

- Species of Medical Importance:
- Corynebacterium diphtheriae.
- Diphtheroids (normal flora).

Corynebacterium diphtheriae

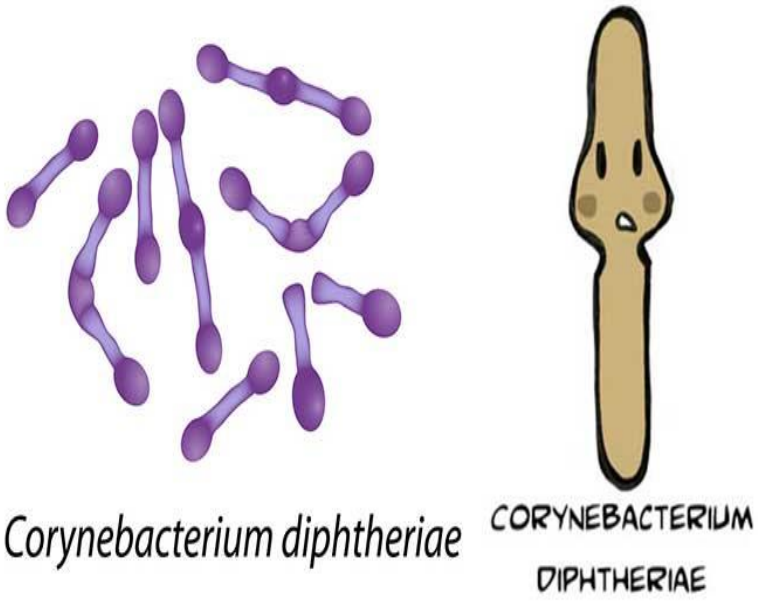
- Distinguishing Features:
- Aerobic, non-spore forming.

- Gray-to-black colonies of club-shaped gram-positive rods arranged in V or L shapes on cysteine-tellurite agar (coryne = club).

- It can also be cultured in Loffler's medium where it will develop cytoplasmic metachromatic granules (visualizable after staining with an aniline dye such as methylene blue).

- Toxin-producing strains have B-prophage carrying genes for the toxin (lysogeny, B-corynephage). The phage from one patient with diphtheria can infect the normal nontoxigenic diphtheroid of another person and cause diphtheria.

- + Elek's test: it is used to test for toxigenicity of C. diphtheriae.



- Habitats: throat and nasopharynx.

- Mode of transmission: bacterium or phage via respiratory droplets.

- Virulence factors and pathogenesis:

- The virulence factor of C. diphtheria is exotoxin. The ability of C. diphtheriae to produce the diphtheria exotoxin is dependent on two elements:

1. The presence of a lysogenic prophage in the bacterial chromosome.

2. Low extracellular concentration of iron: The gene for toxin production occurs on the prophage, but a bacterial repressor protein controls the expression of this gene. The repressor is activated by iron.

- Diphtheria produces a classic two subunit AB exotoxin.

- The B (binding) subunit binds specifically to the heparin-binding epidermal growth factor receptor found on cardiac and neural cells, and is responsible for the toxin's affinity for heart and nervous tissue.

- The B subunit induces endocytosis of the toxin, and the subsequently released A (active) subunit inhibits host cell protein synthesis by catalyzing the ADP-ribosylation of protein elongation factor 2 (EF-2).

- EF-2 is necessary for tRNA to insert new amino acids into the growing protein chain during translation.

- By inhibiting cell protein synthesis, the toxin causes cell death.

- Disease:

- Diphtheria involves both local and systemic pathology:

1. Local pseudomembrane (pseudomembranous pharyngitis):

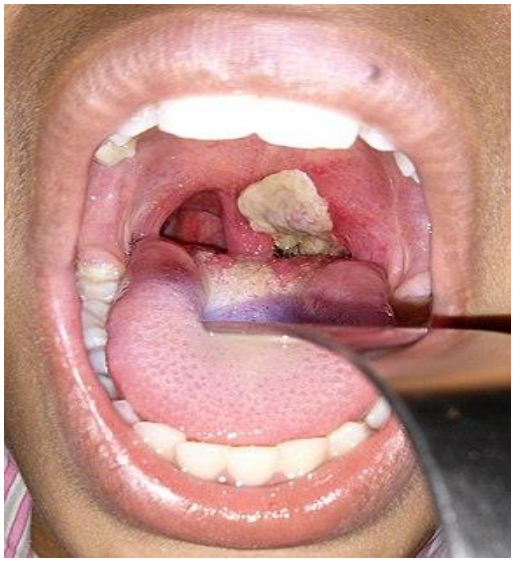
- It develops in the upper respiratory tract (tonsil, pharynx, larynx, and nose).

- It forms due to necrosis of epithelial cells followed by blood leak resulting in a network of fibrin, WBCs and RBCs interlaced with C. diphtheria cells.

- It adheres tightly to the underlying mucosa and will cause bleeding if avulsed.

- Thus, an adherent membrane forms and may spread locally and can cause suffocation (pseudomembranous pharyngitis).

- Diphtheria should be suspected in unvaccinated patients with membranous pharyngitis or obstructive laryngotracheitis with cervical lymphadenopathy (Bull neck) who emigrated or returned recently from an endemic area.



2. Toxemia:

- The diphtheria bacilli do not invade tissues.

- They produce the toxin that is absorbed and disseminated through the blood to the susceptible tissues (mainly heart muscle and peripheral nerves).

- Absorption of the toxin into the bloodstream also results in systemic effects, including significant cardiac and neural toxicity.

- Clinical manifestations include myocarditis, arrhythmia, heart failure, neuropathy, paralysis, and coma.

❖ N.B:

1. Diphtheria is an acute toxin-mediated disease, but not all strains of C. diphtheria express the disease-causing exotoxin.

- Diphtheria acquires virulence via bacteriophage-mediated "infection" with the Tox gene, which codes for the diphtheria AB exotoxin.
- The bacteriophage responsible is called Corynephage beta. The phage Tox gene incorporates into the bacterial chromosome as a prophage and codes for toxin production by C. diphtheriae.
- This process, whereby a bacteriophage infects a host bacterium and integrates its genome into the host bacterium's genome, is termed lysogenization.
- Non-pathogenic Corynebacterium can cause severe pseudomembranous pharyngitis after acquiring the Tox gene via lysogenization by a temperate bacteriophage.

2. Diphtheria is rare in the United States due to widespread administration of the diphtheria-pertussis-tetanus (DPT) childhood vaccine.

- The DPT vaccine contains diphtheria toxoid, which stimulates production of neutralizing antibodies against the binding component (B subunit) of the diphtheria exotoxin.
- Antibody binding prevents the exotoxin from attaching to host cell membrane receptors, thereby preventing disease.

3. Treatment of an acute C. diphtheriae infection requires administration of (in order of importance):

a. Diphtheria antitoxin.

b. Penicillin or erythromycin.

c. DPT vaccine.

- The diphtheria antitoxin inactivates all circulating toxin, but is ineffective against toxin that has already gained access to cardiac or neural cells. Thus, rapid administration of antitoxin is essential.
- The process of administrating antitoxin is often referred to as 'passive immunization,' as it represents the transfer of preexisting, neutralizing antibodies.
- Antibiotic therapy kills the bacteria, thereby halting the release of new exotoxin into the bloodstream and preventing disease transmission.
- Active immunization with the DPT vaccine provides lasting immunity against future diphtheria infection.

Diphtheria	
Epidemiology	• Rare in developed countries due to widespread vaccination • Risk: inadequate vaccination/10-year booster shot
Pathogenesis	• **Toxigenic *Corynebacterium diphtheriae*** colonizes respiratory tract → secretes **diphtheria toxin** → inhibits host protein synthesis (ADP-ribosylation of EF-2) → local and systemic toxin-mediated effects
Manifestations	• Local: **pseudomembranous pharyngitis**, cervical LAD • Systemic: **myocarditis**/heart failure, neurologic toxicity • Pharyngeal edema/pseudomembrane spread can obstruct respiratory tract
Treatment	• **Diphtheria antitoxin** (most important) • Antibiotics

EF-2 = elongation factor-2; **LAD** = lymphadenopathy.

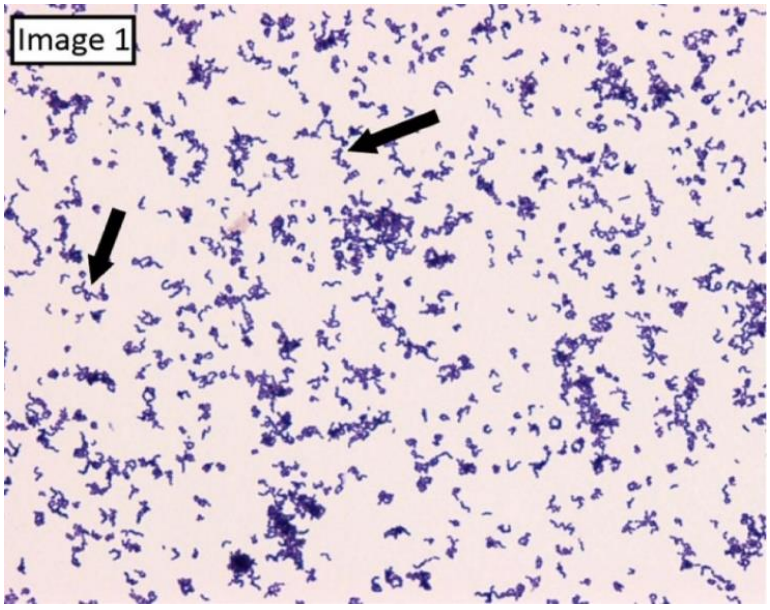
Genus: Actinomyces

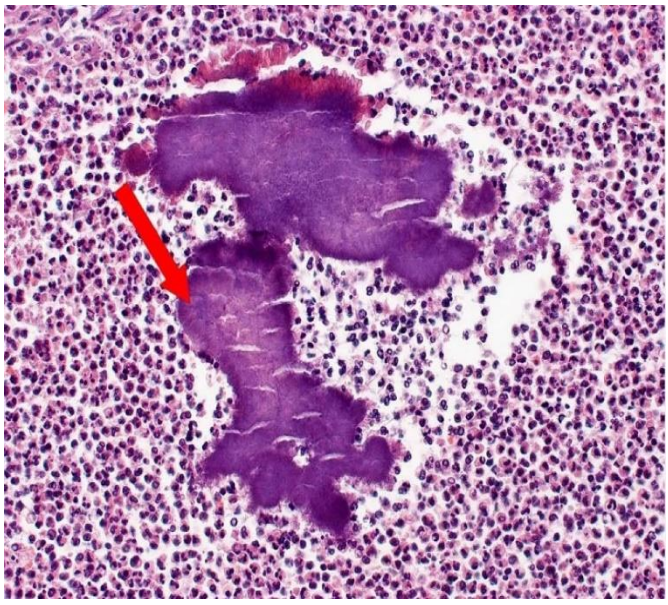
- Genus Characteristics:
- Gram-positive branching rods.
- Anaerobic.
- Non-acid fast.

- Species of Medical Importance: Actinomyces israelii.

Actinomyces israelii

- Distinguishing Features:
- Gram positive anaerobe.
- Branching rods.
- Non-acid fast.
- Actinomyces have a growth pattern similar to that of the mycelial form of fungi, hence their 'fungus-like' name.
- A notable feature of this organism is its ability to form "sulfur granules" which are yellow aggregations of organisms bound together by proteins (Sulfur granules do not actually contain sulfur).
- Sulfur granules grossly appear yellow; however, hematoxylin and eosin staining gives them basophilic appearance under light microscope.

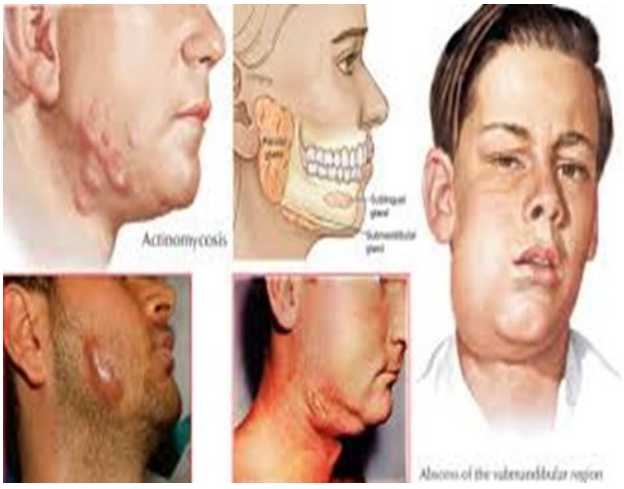




- Habitats: human; normal flora of gingival crevices and female genital tract.
- Mode of transmission: endogenous.
- Virulence factors and pathogenesis: invasive growth in tissues with compromised oxygen supply.
- Diseases:

❖ Cervicofacial actinomycosis:

- Cervicofacial actinomycosis is a condition characterized by the formation of chronic face and neck abscesses complicated by cutaneous sinus tracts.
- History of a slowly growing mass that began in the setting of oral trauma that has recently been draining yellow pus through the skin makes actinomycosis the most likely diagnosis.
- Treatment consists of a prolonged course of parenteral penicillin and surgical debridement.



❖ N.B:

- Pulmonary actinomycosis develops most commonly following aspiration and can be confused with lung abscess, malignancy, or tuberculosis.
- Actinomyces israelii can also cause PID with IUDs.
- Microscopic findings include filamentous, branching, gram-positive bacteria and sulfur granules.

Genus: Nocardia

- Genus Features:

- Gram-positive filaments.
- Obligate aerobes.
- Partially acid fast (some areas of smear will be blue and some red).

- Species of Medical Importance:

- N. Asteroides.
- N. Brasiliensis.

Nocardia Asteroides and Nocardia Brasiliensis

- Distinguishing Features:
- Obligate aerobes.
- Gram-positive branching rods.
- Partially acid fast.
- Habitats: soil and dust.
- Mode of transmission: airborne or traumatic transplantation.
- Virulence factors and pathogenesis:
- No toxins or virulence factors known.
- Immunosuppression and cancer predispose to pulmonary infection.
- Diseases:
- Causes pulmonary infections in immunocompromised and cutaneous infections after trauma in immunocompetent:

1. Cavitary bronchopulmonary nocardiosis:

- Mostly N. asteroides.
- Can be acute, subacute, chronic.
- Symptoms: cough, fever, dyspnea, localized or diffuse pneumonia with cavitation (can mimic TB but with ⊖ PPD).
- May spread hematogenously to brain (brain abscesses) and cause seizure.

2. Cutaneous/subcutaneous nocardiosis:

- Mostly N. brasiliensis.
- Starts with traumatic implantation.
- Symptoms: cellulitis with swelling draining subcutaneous abscesses with granules (mycetoma).

- Treatment: sulfonamides (high dose) or trimethoprim/sulfamethoxazole (TMP-SMX).

❖ Mnemonic:

- Treatment is a SNAP: Sulfonamides (Nocardia); Actinomyces (Penicillin).

Genus: Mycobacterium

- Genus Features:

- Acid fast rods with a waxy cell wall: acid fastness means that once organisms are stained with certain dyes such as carbol-fuchsin (in Ziehl-Neelsen method) or auramine-rhodamine (in fluorescent acid-fast stain), they retain the dye firmly and resist decolorization even by acid-alcohol or strong mineral acids.

- Acid-fastness is due to the high lipid (mycolic acid) content of the cell wall.

- Obligate aerobe.

- They are facultative intracellular pathogens except M. leprea (obligate intracellular pathogen).

- Cell wall: high concentration of lipids containing long chain fatty acids called mycolic acids. This makes mycobacteria highly resistant to Many chemicals.

- Species of Medical Importance:

- M. tuberculosis.
- M. leprae.
- M. avium-intracellulare.
- M. kansasii.
- M. scrofulaceum.
- M. marinum.

Mycobacterium tuberculosis

- Distinguishing Features:

- Aerobic, slow growing on Lowenstein-Jensen medium.

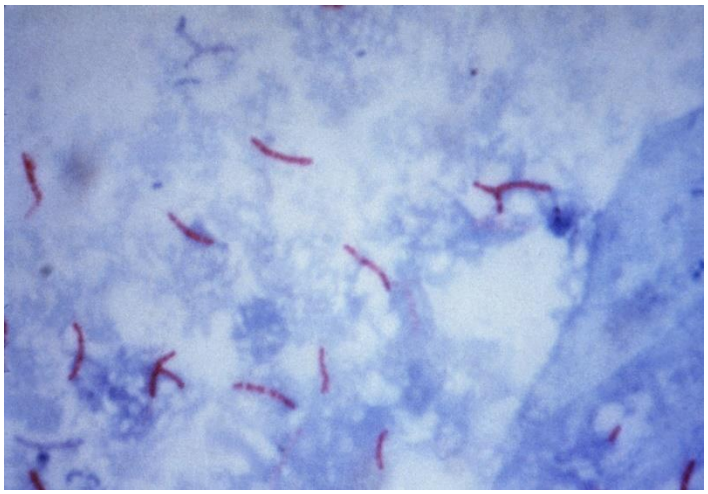
- Catalase +.

- Facultative intracellular pathogen.

- Auramine-rhodamine staining bacilli (fluorescent apple green); no antibody involved (sensitive but not specific).

- Acid fast:
  - Mycobacteria appears weakly positive on Gram stain.
  - The acid-fast stain identifies organisms that have mycolic acid present in their cell walls, including Mycobacterium and some Nocardia species.

- Although it is less sensitive than culture, the acid-fast stained smear allows for immediate microscopic evaluation.
- In the acid-fast stain for mycobacteria, the smear is first treated with an aniline dye (carbolfuchsin).
- The dye (red color) penetrates the bacterial cell wall, where it binds with mycolic acids.
- The slide is then treated with hydrochloric acid and alcohol.
- This acid alcohol dissolves the outer cell membranes of nontuberculous bacteria, but the presence of mycolic acids prevents decolorization of mycobacteria.
- A counterstain (methylene blue) is then applied and taken up by decolorized bacteria.
- As a result, the carbolfuchsin acid-fast stain produces red mycobacteria (initial stain) and blue non-acid fast bacteria.
- The cell membrane and cell wall of mycobacteria are most similar to those in Gram-positive organisms, causing mycobacteria to appear weakly positive on Gram stain.
- Nocardia is a Gram-positive rod that contains mycolic acid in its cell wall.
- Because Nocardia possesses less mycolic acid than do mycobacteria, Nocardia is more weakly acid fast.



- Habitats: human lungs.
- Mode of transmission: The commonest mode of infection is inhalation of droplet nuclei carried by air (aerosol infection) from a patient with open pulmonary tuberculosis.

- Virulence factors and pathogenesis:

1. Cord factor:

- The growth of thick, rope like cords of mycobacterial organisms in a twisted, "serpentine" pattern is consistent with the presence of cord factor.

- The presence of cord factor correlates with virulence; mycobacteria that do not possess cord factor are not able to cause disease.

- More specifically, cord factor is responsible for inactivating neutrophils, damaging mitochondria, and inducing release of tumor necrosis factor.

2. Sulfatides (sulfolipids in cell envelope): Inhibit phagosome-lysosome fusion, allowing intracellular survival.

- Diseases:

1. Primary M. tuberculosis infection:

- It occurs in individuals lacking previous contact with tubercle bacilli.

- TB bacilli are able to survive and multiply intracellularly by inhibiting fusion of phagosome and lysosomes. The accumulating bacilli kill the initial alveolar macrophages.

- Cell mediated immunity response, which is the main immune mechanism against mycobacteria, is intiated about 2-10 weeks after infection.

- Specific Th1 cells recognize infected macrophages and release cytokines (particularly IFN-γ) that activates macrophages and attract more macrophages to the site of infection.

- In addition, IL-12 (produced by activated infected macrophages) promotes the differentiation of Th cells into Th1 cells augmenting the production of IFN-γ.

- When mycobacteria resist the microbicidal effect of the activated macrophages, a characteristic localized inflammatory response called a granuloma (tubercle) develops. This serves to isolate pathogens that resist intracellular destruction.

- The inability of M. tuberculosis to multiply within these tubercles is also due to the low pH and anoxic environment (TB bacilli are strict aerobes), however, it can survive in small numbers in a relatively dormant state (latent tuberculosis infection). This situation is due to a balanced state of host-parasite relationship.

- This occurs in about 90% of the infected people.

- Individuals with this initial tuberculosis infection are tuberculin positive.

- In the remaining 10% of people, TB bacilli overcome the immune system and begin to multiply, resulting in the progression from TB infection to TB disease.

- Symptoms of disease include general malaise, fatigue, night sweat and fever along with persistent cough and bloody sputum.

- The bacilli are transported via lymphatics to the regional lymph glands.

- A few days later, organisms leave the lymph glands to the blood stream.

- This bacillaemic phase of the infection leads to dissemination of organisms throughout the body to more distant tissues (the apices of the lung, the kidney, the brain, and bone).

2. Reactivational (secondary) tuberculosis:

- This form occurs most commonly in the lung apices in previously sensitized individuals with a weakened immune response (malnutrition, immunotherapy for other diseases).

- Failure to maintain the granulomas leads to caseous necrosis, in which the center of the granuloma is liquefied, and the lesions coalesce.

- Erosion exposes the organism to oxygen and spreads them to other parts of the lung with a resulting pneumonia. Hypersensitivity leads to the cavitation.

- Characterized by cough (often with blood-tinged sputum), night sweats, fever, anorexia, and weight loss.

- Tuberculosis may affect other systems. Ex: tuberculosis meningitis, lymphadenitis, renal and intestinal tuberculosis.

❖ Tuberculin skin test:

- It is used widely to screen certain high-risk populations, particularly those who have been exposed to infectious patients.

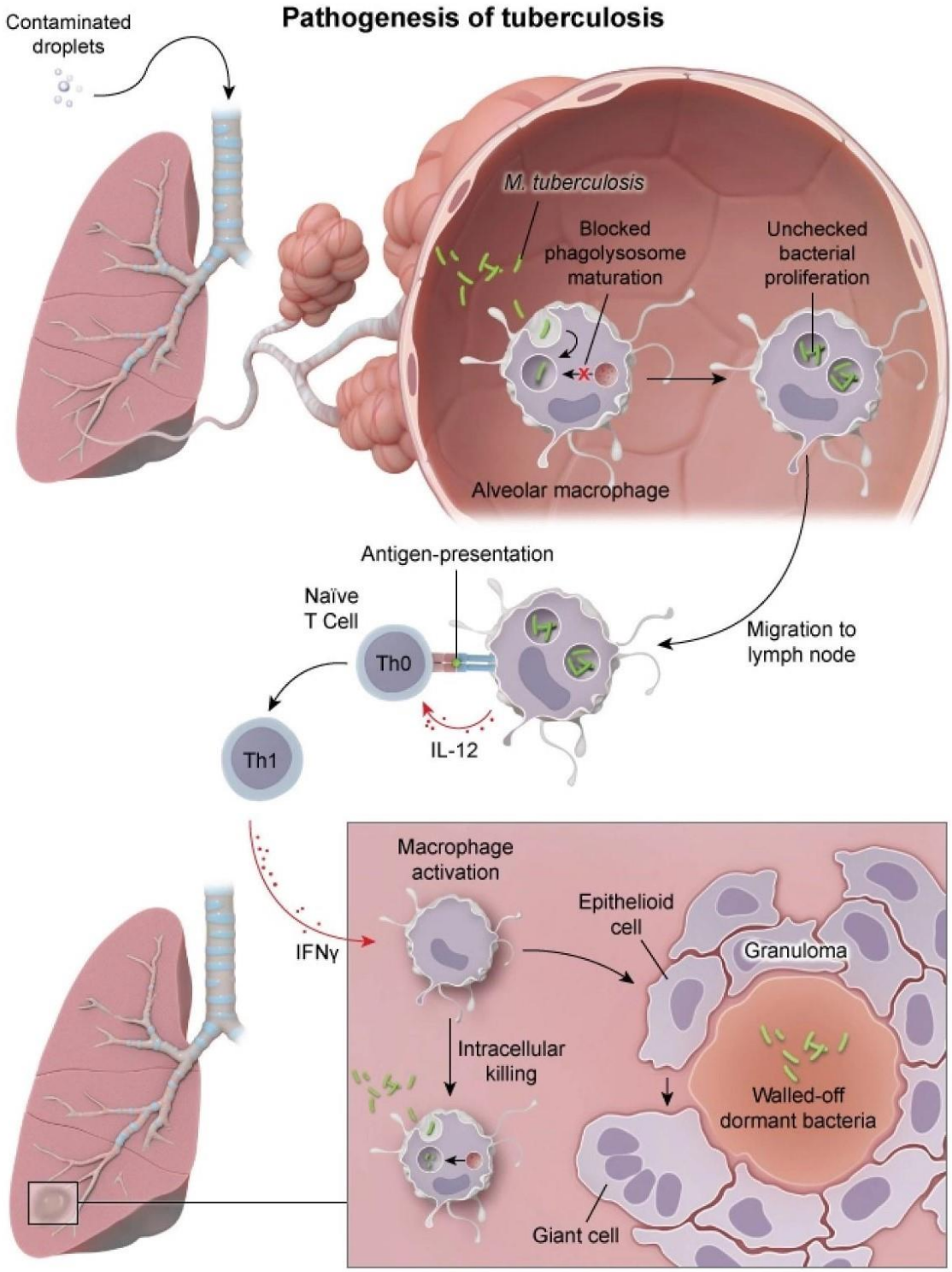
- The test involves an intradermal injection of the purified protein derivative (PDD) of the bacilli.

- After 48-72 hours the injection site is examined for visible and palpable induration.

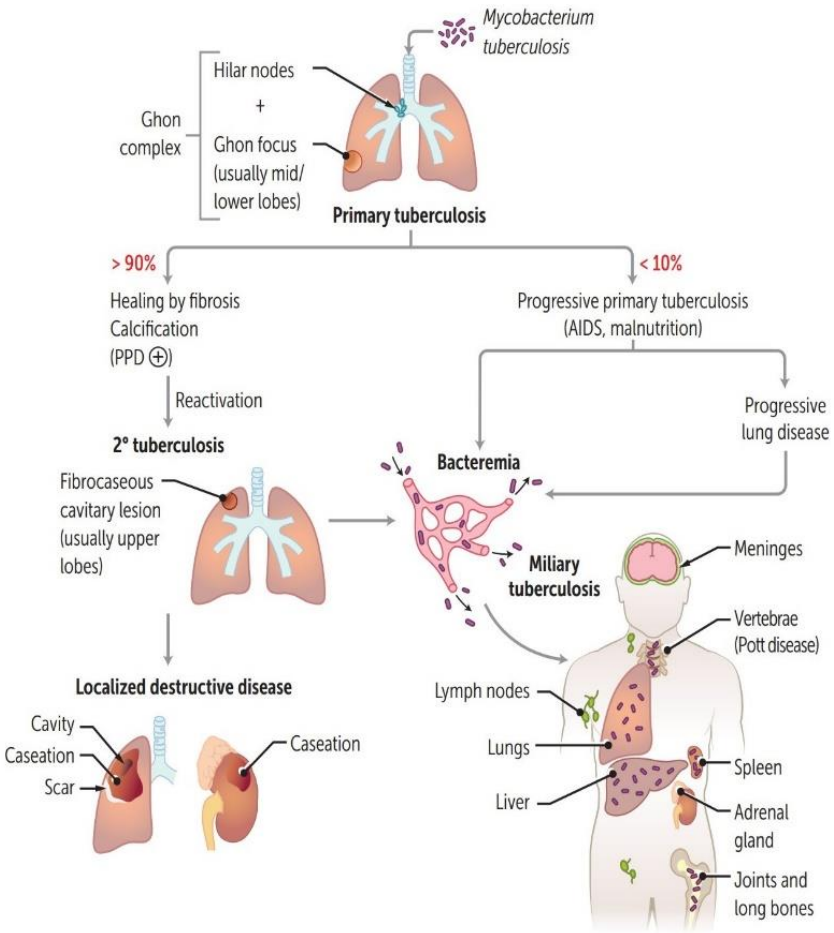
- Because of a possible cross reaction after exposure to other mycobacteria, a single tuberculin test to determine sensitization to mycobacterium tuberculosis is considered positive only if the diameter of the induration at the skin test site measures:

- ≥ 5 mm in immunocompromised patients:
  - Ex: HIV+ or anyone with recent TB exposure; AIDS patients have reduced ability to mount skin test.

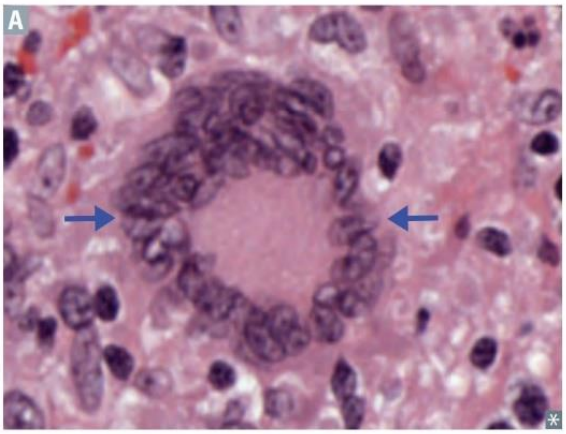
- ≥ 10 mm in sick persons without depression of their immune system:
  - Ex: IV drug abusers, people living in poverty, or immigrants from high TB area.
- ≥ 15 mm in immunocompetent individuals.



Tuberculosis



PPD ⊕ if current infection or past exposure.
PPD ⊖ if no infection and in sarcoidosis or HIV infection (especially with low CD4+ cell count).
Interferon-γ release assay (IGRA) has fewer false positives from BCG vaccination.
Caseating granulomas with central necrosis and Langhans giant cell (single example in A) are characteristic of 2° tuberculosis.



- IGRAs have comparable sensitivity and specificity to tuberculin skin tests, but advantages include their lack of cross-reactivity to the Bacille Calmette-Guerin (BCG) vaccine and that a follow-up visit is not required. Neither skin tests nor IGRAs can be used to distinguish active tuberculosis from LTBI.

Mycobacteria Other than Tuberculosis

- Distinguishing Features:

- Atypical mycobacteria.
- Noncontagious.
- All mycobacteria are acid-fast organisms.
- Found in surface waters, soil, cigarettes.

- Species of Medical Importance:

1. M. avium Intracellulare:

- Causes disseminated non-TB disease in AIDS.

- Often resistant to multiple drugs.

- Prophylaxis with azithromycin when CD4+ count < 50 cells/mm.

2. M. Scrofulaceum: Causes cervical lymphadenitis in children.

3. M. Marinum: Causes hand infection in aquarium handlers.

Mycobacterium leprae

- Distinguishing Features:

- Acid fast rods (seen in punch biopsy).
- Obligate intracellular parasite.
- Cannot be cultured in vitro.
- The nine-banded armadillo can be infected with M. leprae and this animal has become the main source of M. leprae for biochemical and immunological research including development of a vaccine.

- Habitats:

- Human mucosa, skin, and nerves are only significant reservoirs.
- Some infected armadillos in Texas and Louisiana.

- Mode of transmission: Transmission is believed to occur through the respiratory route, although direct cutaneous contact has not been excluded as a mode of transmission.

- Virulence factors and pathogenesis: Obligate intracellular parasite.

- Diseases:

❖ Leprosy:

- Leprosy, or Hansen disease, is a systemic illness caused by Mycobacterium leprae.
- Infection requires prolonged and close contact with patients.
- Leprosy is a chronic infectious disease that primarily affects cooler surfaces areas of the body, such as the skin, ear lobes, mucosa of the nose, mouth, and upper respiratory tract and also the eyes.
- The severity of disease depends on the strength of the cell-mediated immune response, with tuberculoid leprosy the milder form and lepromatous leprosy the more severe form.
- There is usually some degree of irreversible nerve damage. The pathogenesis of leprosy appears to derive from:

1. The ability of M. leprae to survive and replicate within the phagosome of macrophages, in nerve cells and other host cells.

2. The consequent immune response to the organism:

- The disease is not a single clinical entity but presents in two basic forms:

a. Tuberculoid leprosy (TL):

- Cell mediated immune response predominate and form granulomas, resulting in the destruction of most of the mycobacteria. So only few AFB remain in the tissues (paucibacillary or PB leprosy).

- Lesions are few and mainly in the form of hypopigmented maculo-anaesthetic skin lesions (glove and stocking loss of sensation).

- Although skin and peripheral nerves are damaged, TL progresses slowly, sensory loss is mild, and patients usually survive.

- Spontaneous regression of tuberculoid leprosy occurs in over 90% of cases.

b. Lepromatous leprosy (LL):

- Cell mediated immune response is depressed. Although humoral response is predominating, it is not protective as the organism is intracellular.

- The acid-fast bacilli (AFB) are widely disseminated in macrophages and lesions usually contain large numbers of AFB (multibacillary or MB leprosy).

- The AFB form clumps and occur as intra- and extracellular masses known as globi.

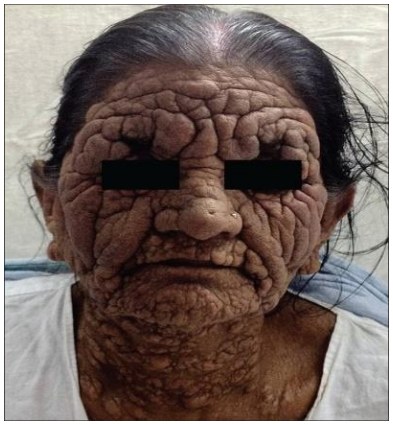
- Lesions are mainly nodular and may form on the face. As the disease progresses, the nose may collapse giving the characteristic leonine facies.

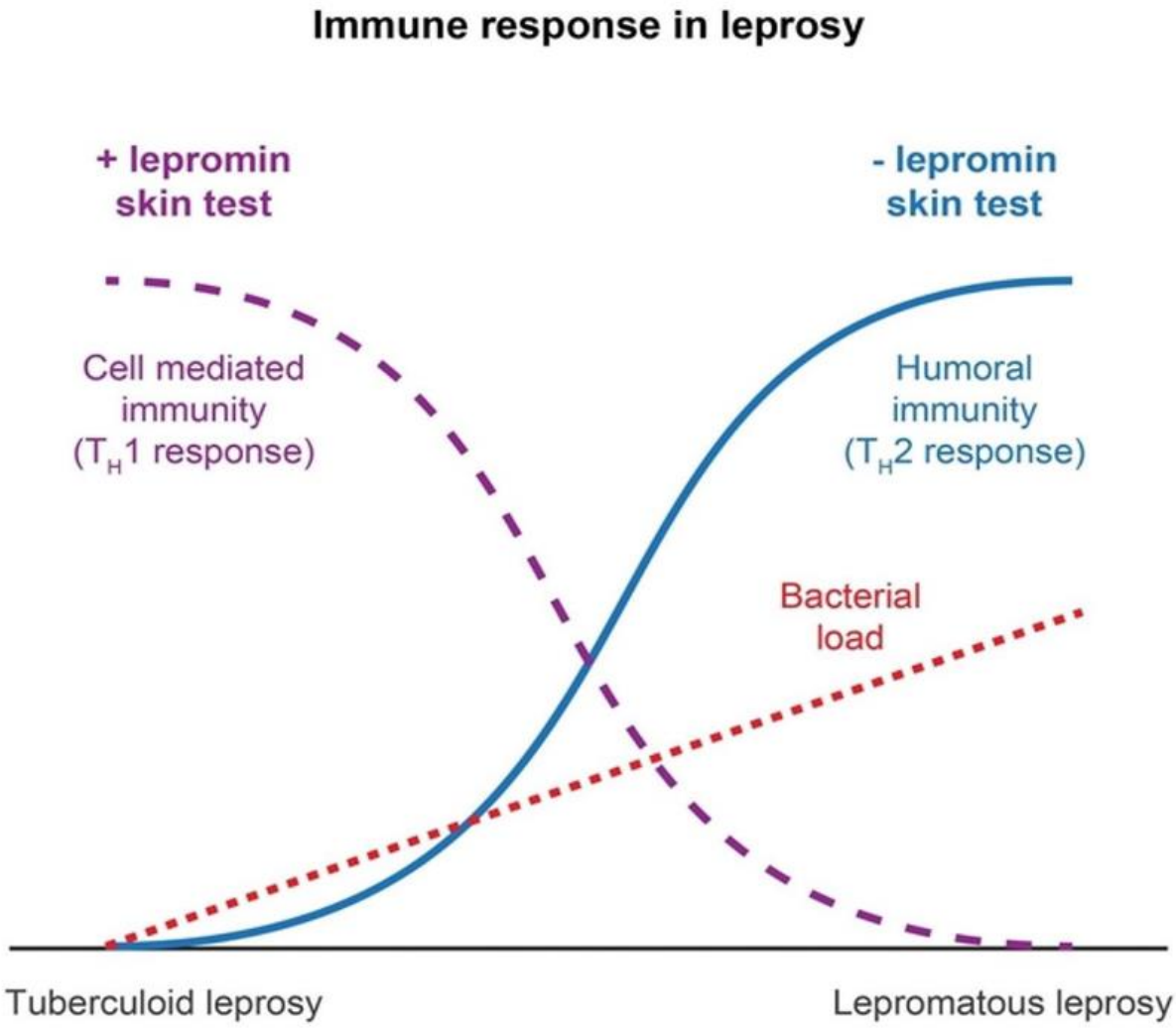
- LL is the more severe form and progresses rapidly. There is a marked sensory loss due to extensive nerve damage.

- Lepromatous form can be Lethal and present with Lion-like facies.

- Diagnosis: Diagnosed via skin biopsy or tissue PCR.

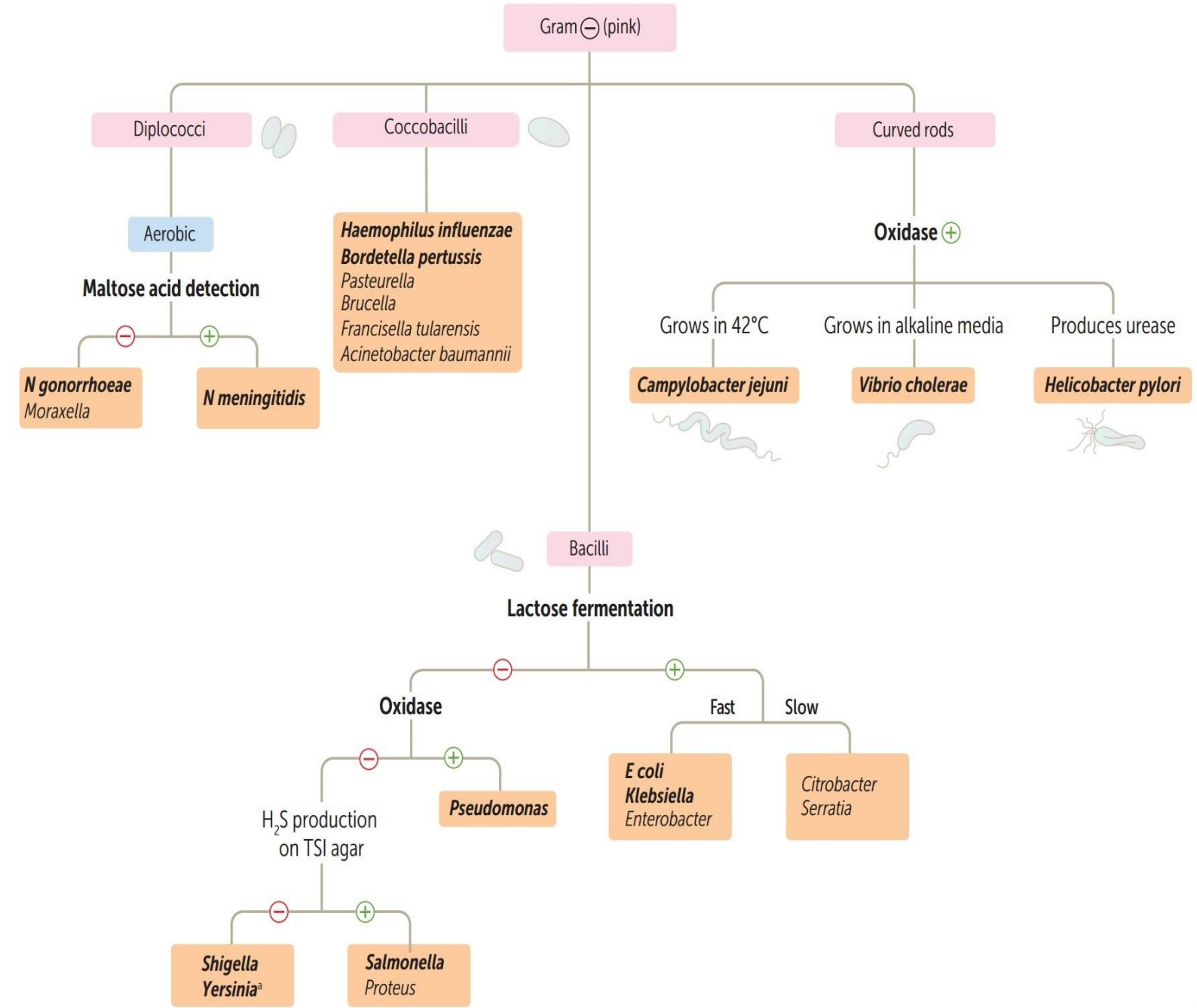
- Treatment: dapsone and rifampin for tuberculoid form; clofazimine is added for lepromatous form.




Immune response in leprosy
+ lepromin skin test
- lepromin skin test
Cell mediated immunity (T_H1 response)
Humoral immunity (T_H2 response)
Bacterial load
Tuberculoid leprosy
Lepromatous leprosy

Gram negative bacteria

Gram-negative lab algorithm



Important **tests** are in **bold.** Important ***pathogens*** are in ***bold italics.***
[a]Pleomorphic rod/coccobacillus

1. Gram- Negative Cocci:

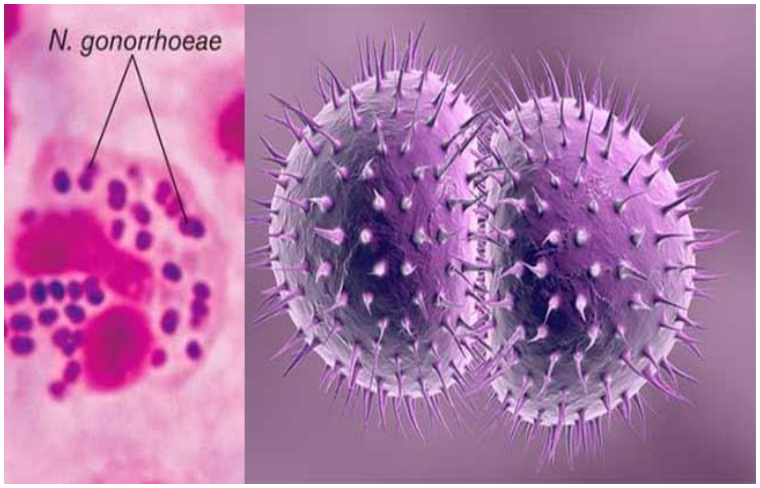
Genus: Neisseria

- Genus Features:

- Gram negative diplococci.
- Oxidase positive.

- Species of Medical Importance:

- Neisseria meningitides.
- Neisseria gonorrhoeae.



Neisseria meningitidis

- Distinguishing Features:

- Gram-negative, kidney bean-shaped diplococcic.

- Ferments maltose in contrast to gonococci. Acid production: MeninGococci (Maltose and Glucose); Gonococci (Glucose).

- Neisseria can be cultured on a chocolate agar-based medium containing various antibiotics, Thayer-Martin VCN (vancomycin/colistin/nystatin) selective medium.

- The antibiotics in this medium kill potential contaminants such as Gram-positive organisms (vancomycin), Gram negative organisms other than Neisseria (colistin) and fungi (nystatin).

- Habitats: human nasopharynx (5-30% carriers).

- Mode of transmission: Respiratory droplets.

- Virulence factors and pathogenesis:

1. Polysaccharide capsule: The polysaccharide capsule, with its antiphagocytic action represents the most important virulence factor.

2. Endotoxin (lipooligosaccharide): LOS is responsible for the endotoxin effect of meningococcal infections.

3. Pili: they are responsible for attachment and adherence.

4. IgA_1 protease: it inactivates secretory IgA1 which allows oropharynx colonization.

- Diseases:

❖ Meningitis and meningococcemia:

- Neisseria meningitidis is the second most common cause of acute bacterial meningitis in adults after S. pneumoniae in the U.S.

- More common in closed community like military recruits and college student.

- Neisseria meningitides is transmitted from person to person by respiratory droplets usually from asymptomatic carriers with nasopharyngeal colonization.

- N. meningitides is normally carried in the nasopharynx in 5-30% of healthy population.

- These carriers represent the source of infection from whom the infection is transmitted by droplets.

- Pili allow the attachment of the organism to the mucosal epithelium of the nasopharynx and together with IgA protease establish bacterial adherence.

- Endocytosis takes place and a slight local inflammation (sore throat) occurs.

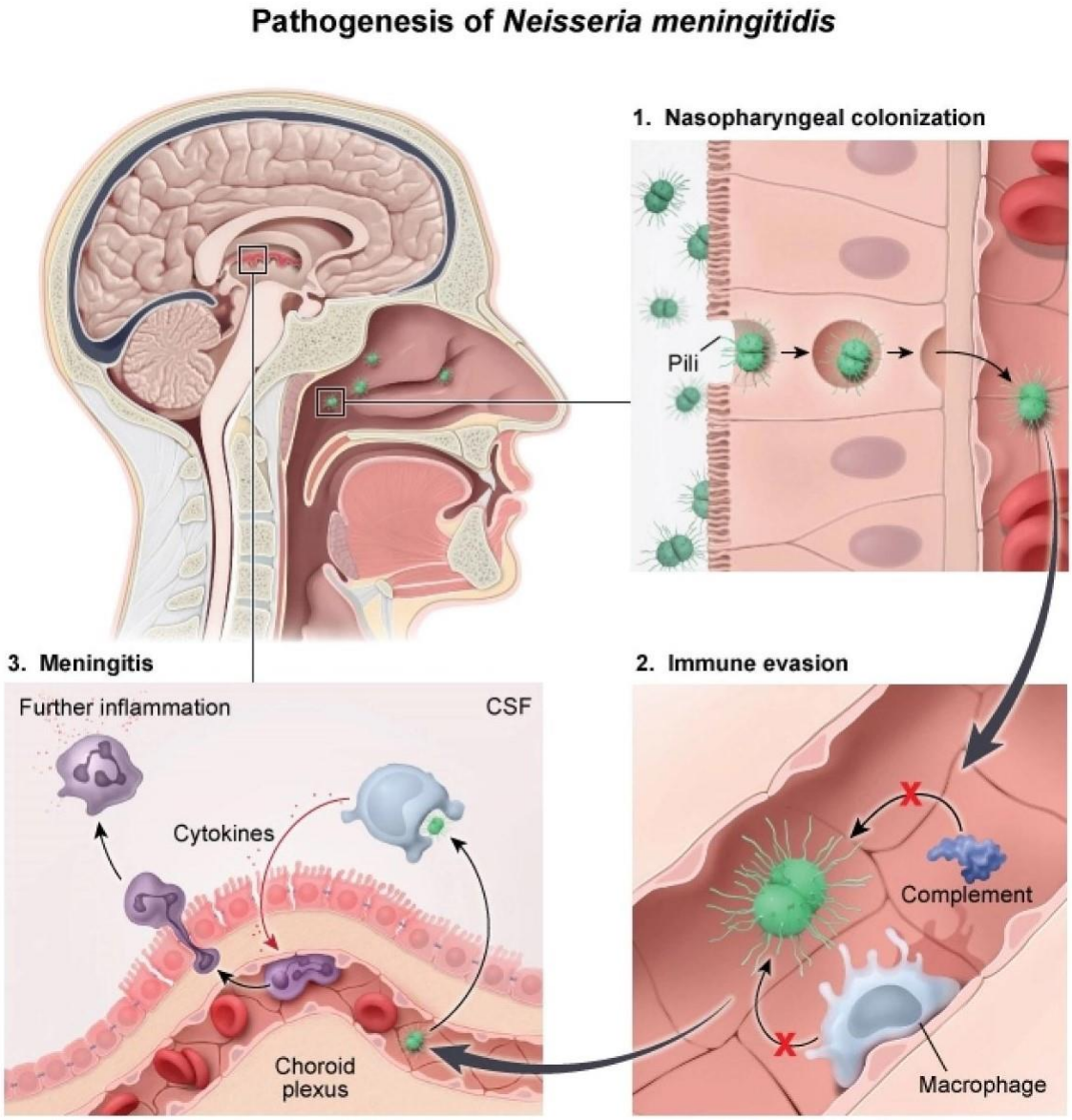
- Neisseria meningitides gains access to the CNS by first colonizing the nasopharynx and subsequently invading the mucosal epithelium and gaining access to the bloodstream.

- Through the blood, it spreads to the choroid plexus, gains access to the CNS through the blood-brain barrier and initiates an inflammatory process.

- The virulence of meningococci is primarily due to invasive capacity of the capsulated organism.

Pharynx → blood → choroid plexus → meninges

- The disease manifests as a sudden severe headache, projectile vomiting, and stiff neck, may progress rapidly to coma and death or resolve with permanent neurological complications (deafness, speech disability, paralysis).



- As in other Gram-negative infections, during the infectious process, the growth and lysis of meningococci causes the release of outer membrane vesicles (OMV) with membrane-attached LOS into the bloodstream.

- This outer membrane LOS acts as an endotoxin and is associated with many of the toxic effects of meningococcal disease.

- Plasma levels of LOS are closely associated with disease manifestations and outcomes in meningococcal infections.

- High levels have been associated with increased rates of severe septic shock, acute respiratory distress syndrome, and death secondary to multi-organ failure.

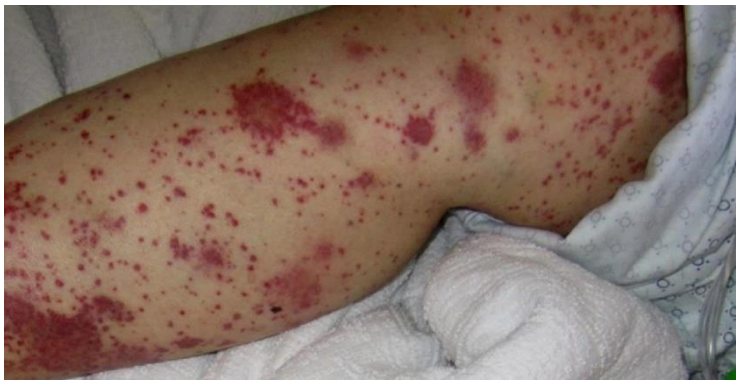
- As with LPS from other gram-negative bacteria, LOS causes sepsis by the induction of a systemic inflammatory response characterized by the production of tumor necrosis factor alpha (TNF-α), interleukin-1β (IL-1β), IL-6, and IL-8.

- Particularly susceptible hosts include:
  - Children under the age of 3 years because they fail to make antibody against the antiphagocytic capsule.
  - Individuals having defect in the terminal complement components.

- N. meningitidis bacteremia may be accompanied by signs and symptoms of sepsis including spiking fevers, chills, arthralgias, and myalgias, as well as purpuric cutaneous lesions and hypotension that may progress shock and death.

- Although N. meningitidis often causes meningitis, N. meningitidis bacteremia frequently occurs without meningeal involvement.

- N. meningitidis may localize also in the joints or endocardium leading to arthritis or endocarditis. Additionally, LOS has been implicated as the cause of cutaneous petechiae and hemorrhagic bullae found in meningococcemia.



- Waterhouse-Friderichsen syndrome is a complication of meningococcemia that may involve bilateral adrenal gland hemorrhagic destruction, disseminated intravascular coagulation (DIC), and shock.

❖ Meningococcal vaccine:

- Antibodies against the polysaccharide capsule confer immunity to N. meningitidis.

- The meningococcal vaccine contains many of the N. meningitidis capsular polysaccharides and stimulates the production of anticapsular antibodies.

- Because the protection provided by the vaccine is incomplete and transient, it is only used in high-risk groups like military recruits and college students.

Neisseria gonorrhoeae

- Distinguishing Features:

- Gram-negative, kidney bean-shaped diplococcic.

- Maltose nonfermenter.

- Isolation of the fastidious N. gonorrhoeae requires a selective medium that will inhibit the growth of the other bacteria normally present in the oral cavity.

- Neisseria gonorrhoeae can be cultured on a chocolate agar-based medium containing various antibiotics, Thayer-Martin VCN (vancomycin/colistin/nystatin) selective medium.

- Habitats: human genital tract.

- Mode of transmission: Sexual contact, or perinatally transmitted.

- Virulence factors and pathogenesis:

1. Pili: mediate attachment to epithelial cells.

2. IgA protease inactivates secretory IgA leading to more adherence to and colonization of mucosa.

3. Lipooligosaccharide (LOS): a modified endotoxin that elicits an inflammatory response.

- Diseases:

1. Gonorrhea:

- Gonococci are introduced onto the mucosal surface by sexual contact.

- Pili and IgA protease allow adherence and colonization to mucosa.

a. Men:

- Gonococci infect the urethra leading to acute urethritis with dysuria and purulent discharge.

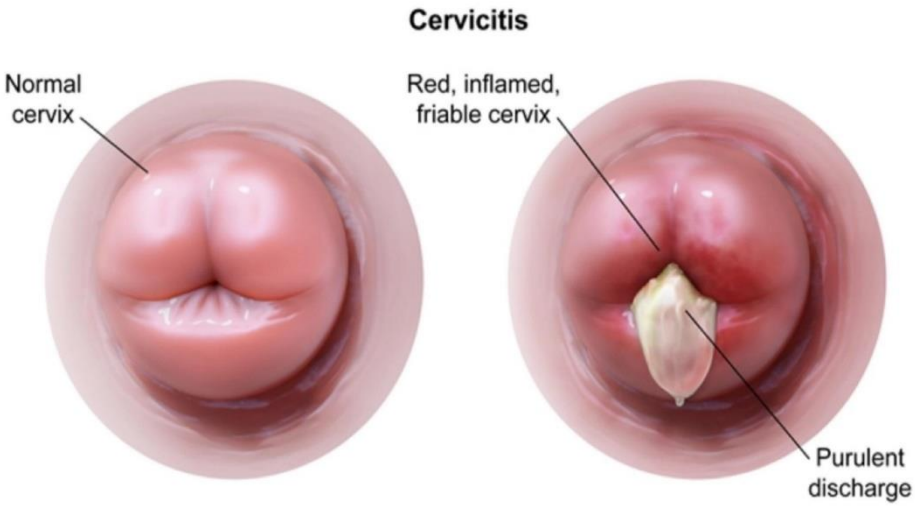
- Classically, Gram stain of urethral discharge from affected patients shows Gram negative diplococci within leukocytes.

- The organism may spread to prostate, bladder and epididymis causing inflammation and swelling.

b. Women:

- Gonococci infect the cervix (not vagina), urethra, vulva and rectum leading to dysuria and cervicitis with a purulent cervical discharge.

- About half of infections in women, however, are asymptomatic and may contribute to persistence and spread of gonorrhea.



2. Vulvovaginitis: infection of the vagina and vulva in young girls due to sexual abuse.

3. Pelvic inflammatory disease (PID):

- It occurs in females due to ascending infection and manifests as endometritis and salpingitis which may lead to ectopic pregnancy or sterility.

- PID may also lead to pelvic peritonitis, tuboovarian abscess or gonococcaemia.

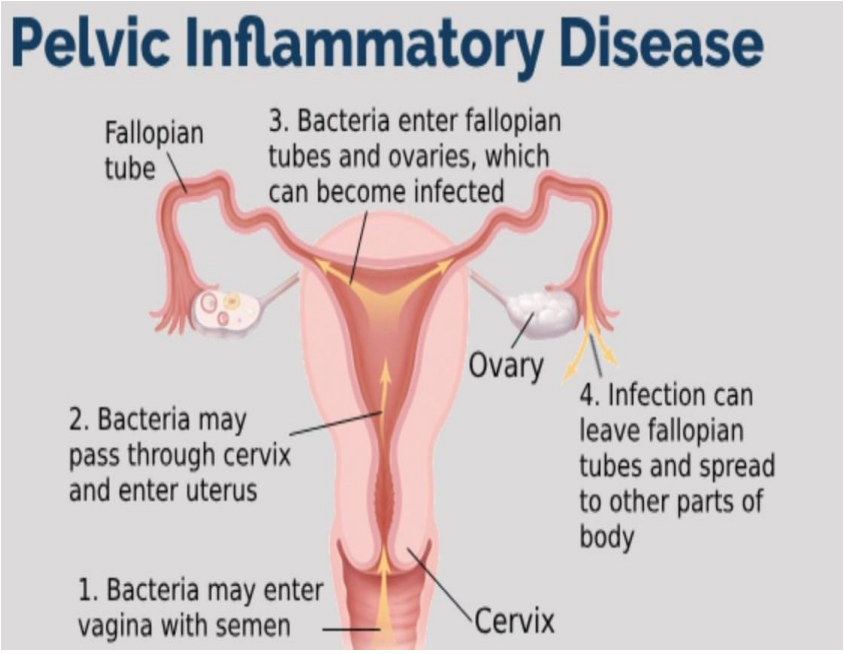
- The most serious complication of PID is permanent damage to the reproductive system resulting in infertility.

- PID is also associated with other infections such as Chlamydia.

- As a result of the inflammatory response to the infection, scarring and blockage of the fallopian tubes can lead to infertility if sperm are unable to bypass the blockage, and ectopic pregnancies if sperm can pass but a fertilized ovum can not.

- The occurrence of infertility as a sequela of PID is estimated to be 15-20% for women who have a single episode of PID and 50-80% for women with multiple episodes of PID.

- Mucopurulent cervicitis with cervical motion tenderness is a frequent indicator of PID caused by N. gonorrhoeae or Chlamydia trachomatis.



4. Neonatal conjunctivitis: it is an acute conjunctivitis in infants born to mothers with gonorrhea. The eyes become infected at the time of delivery and if untreated can lead to blindness.

5. Disseminated gonococcal infection (DGI) or gonococcaemia:

- It occurs more in females (especially the pregnant) and individuals with defects of the terminal complement components.

- Patients typically present with either purulent arthritis or the triad of polyarthralgia, dermatitis, and tenosynovitis

- DGI may result in disseminated intravascular coagulation (DIC) and shock due to the LOS endotoxin.

6. Septic arthritis:

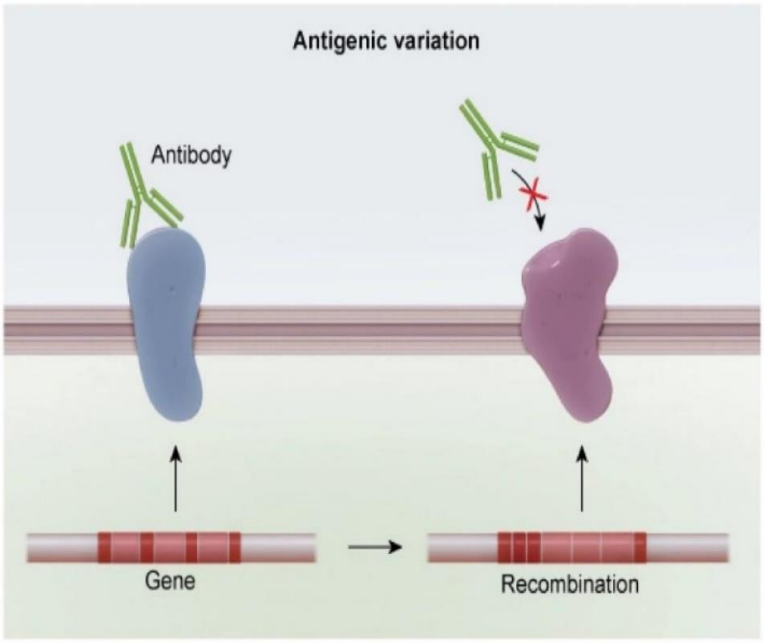
- Arthritis is a complication of disseminated N. gonorrhoeae infection and is the most common cause of septic arthritis in sexually active young adults; therefore, barrier contraception would help prevent this disease (condoms).

- Asymmetric polyarthritis of large joints with fever in a sexually active young adult is characteristic of this disease.

❖ N. gonorrhoeae antigenic variation:

- Those gonococci that have pili are able to adhere to susceptible cells and thereby begin the infectious process.
- When the host produces antibodies directed against gonococcal pili, adherence to the mucosa is inhibited.
- In a given strain at a given time, only a single pilus gene is functional, so only one pilus type is expressed, but the pilus genes are known to undergo antigenic variation at a high frequency.

- Antigenic variation is a process by which the structural genes for pilus proteins undergo recombination with each other to produce new antigenic types of pili, and the array of different antigenic pilus types produced by this mechanism theoretically may be quite large.
- This diversity of pilus protein expression is one reason why development of an effective vaccine directed against the gonococcal pilus is so challenging.



❖ N.B:

- Tubal disease causes approximately 20% of infertility due to female causes.
- The most common cause of tubal-factor infertility is pelvic inflammatory disease (PID).
- PID is most frequently caused by Neisseria gonorrhoeae and Chlamydia trachomatis.
- Infection by either or both of these organisms can often be asymptomatic, but if symptomatic they will initially cause a purulent urethritis followed by ascension to the cervix where infection can further spread to cause purulent infection and inflammation in the endometrium, fallopian tubes and peritoneal cavity.
- Conditions associated with this ascension of infection into the female genital tract and peritoneum include:

A. PID, which is manifest clinically as purulent cervical discharge and cervical motion tenderness.
B. Salpingitis and tubo-ovarian abscess.
C. Peritoneal inflammation including the Fitz-Hugh-Curtis syndrome from inflammation of the hepatic capsule.

- Treatment of gonococcal PID must also always include treatment for C. trachomatis.
- Chlamydia will often co-infect with Neisseria gonorrhoeae.
- A third-generation cephalosporin will treat the gonococcal infection, and further treatment with azithromycin or doxycycline is required to treat the Chlamydia, which is not sensitive to the beta-lactams.
- If the patient was only treated with a cephalosporin, Chlamydia may be allowed to persist and cause an asymptomatic infection leading to fallopian tube scarring and subsequent infertility.

Gonococci	Meningococci
No polysaccharide capsule	Polysaccharide capsule
No maltose acid detection	Maltose acid detection
No vaccine due to antigenic variation of pilus proteins	Vaccine (type B vaccine available for at-risk individuals)
Sexually or perinatally transmitted	Transmitted via respiratory and oral secretions
Causes gonorrhea, septic arthritis, neonatal conjunctivitis (2–5 days after birth), pelvic inflammatory disease (PID), and Fitz-Hugh–Curtis syndrome	Causes meningococcemia with petechial hemorrhages and gangrene of toes B, meningitis, Waterhouse-Friderichsen syndrome (adrenal insufficiency, fever, DIC, shock)
Diagnosed with NAT	Diagnosed via culture-based tests or PCR
Condoms ↓ sexual transmission, erythromycin eye ointment prevents neonatal blindness	Rifampin, ciprofloxacin, or ceftriaxone prophylaxis in close contacts
Treatment: ceftriaxone (+ azithromycin or doxycycline, for possible chlamydial coinfection)	Treatment: ceftriaxone or penicillin G

Moraxella catarrhalis

- Distinguishing Features:
  - Gram-negative diplococcus.
  - Close relative of Neisseria.
- Habitats: normal upper respiratory tract flora.
- Mode of transmission: respiratory droplets.
- Virulence factors and pathogenesis: endotoxin may play role in disease.
- Diseases:
  - Otitis media.
  - Sinusitis.
  - Bronchitis and bronchopneumonia in elderly patients with COPD.

2. Gram-Negative Bacilli:

Genus: Haemophilus

- Genus Features:

- Gram-negative coccobacillary rod.
- Requires growth factors: factors X (hemin) and V (NAD) for growth on chocolate agar (containing red blood cells that have been lysed).
- Chocolate agar provides both X and V factors.
- Grows near S. aureus on blood agar = "satellite phenomenon".

- Species of medical Importance:

- Haemophilus influenza.
- Haemophilus ducreyi.

Haemophilus influenzae

- Distinguishing Features:

- Gram-negative coccobacillary rod.

- Haemophilus influenza is a blood-loving organism that requires both X factor (hematin) and V factor (NAD) to grow.

- Habitats: human nasopharynx.

- Mode of transmission: Infection is transmitted from person-to-person by inhalation of respiratory droplets.

- Virulence factors and pathogenesis:

1. The major virulence factor is the polysaccharide capsule:

- H. influenza can be either encapsulated or unencapsulated (nontypable), with encapsulated strains divided into 6 serotypes (a-f) based on the polysaccharide structure of the capsule.

- Type B capsular material consists of a ribosyl and ribitol phosphate polymer called polyribitol phosphate (PRP).

- The PRP capsule prevents phagocytosis and intracellular killing by neutrophils, allowing the organism to invade the vasculature, persist in the bloodstream, and spread hematogenously to distant sites.

- Antibodies against the type B capsule provide immunity by promoting opsonization and complement fixation.

- H influenza type B used to be a major cause of severe, invasive infections including epiglottitis, meningitis, and bacteremia. However, since the advantage of the conjugate Hib vaccine, most H influenza infections are due to non-type B strains that cause noninvasive disease such as sinusitis, bronchitis, otitis media, and conjunctivitis.

2. IgA protease helps adherence to the mucosa.

- Diseases:

A. Encapsulated types of H. influenza particularly type b (Hib) cause:

1. Bacterial meningitis:

- In children less than 5 years of age (it was the most common cause of meningitis prior to the development of vaccination).

- The introduction of the H. influenza type b (Hib) vaccine has led to a dramatic decrease in the incidence of invasive disease caused by Haemophilus influenza type b including epiglottitis, meningitis, sepsis, and other diseases commonly caused by this bacterium.

2. Epiglottis:

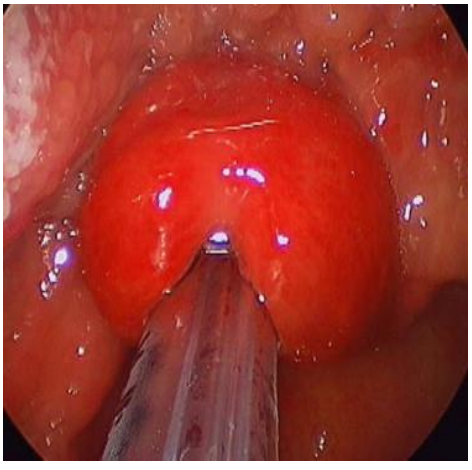
- Acute epiglottitis is a rapidly progressive infection of the epiglottis leading to severe inflammation and edema of the epiglottis and larynx and potentially acute obstruction of the airway especially during laryngoscopy.

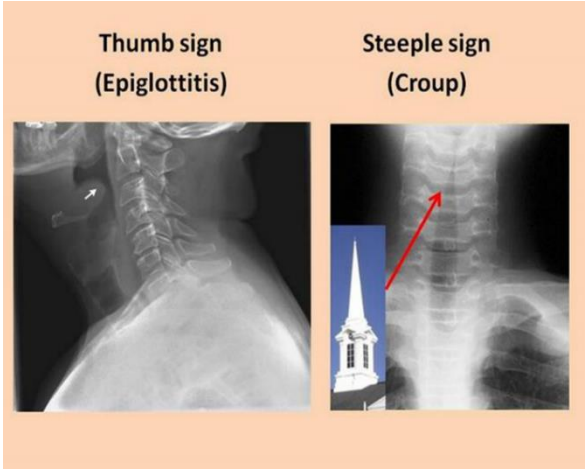
- Small children typically present with fever and dysphagia, while older children and adults complain of sore throat.

- Inspiratory stridor and anxiousness due to compromised diameter of the larynx occur frequently, and patients may present with drooling.

- Diagnosis is confirmed by the presence of an edematous epiglottis that classically appears cherry red though inspection of the epiglottis.

3. Pericarditis, pneumonia, septic arthritis, osteomyelitis, and facial cellulitis in the same age group. These infections are usually accompanied by bacteremia.





B. The nontypable (noncapsulated) strains:

- Strains of H. influenza that do not produce a capsule are referred to as nontypable.

- Nontypable H. influenza strains are part of the upper respiratory tract normal flora, but they can also cause otitis media, sinusitis and bronchitis in adolescents and adults as well as children and vaccination with the H. influenza type b (Hib) vaccine does not confer immunity to any strain except type b.

- The nontypable (noncapsulated) strains cause:

1. Pneumonia and bacteremia in adults and older children in presence of predisposing factors (viral infections, malignancy, and cystic fibrosis).

2. Otitis media:

- H. influenzae along with Streptococcus pneumoniae are the most frequent causes of acute otitis media.

- This infection is primarily seen in children between 6 months and 12 years of age.

- More than 90% of H. influenza strains isolated from middle ear aspirates of infected children are nontypable; the remaining 10% are H. influenza type b.

3. Sinusitis.

- HaEMOPhilus causes Epiglottitis, Meningitis, Otitis media, and Pneumonia.

❖ H. influenzae type b (Hib) conjugate vaccine:

- There are six serotypes of Haemophilus influenzae (a-f); capsular type b is the most invasive strain of H. influenzae and can cause sepsis, meningitis, pneumonia, and other diseases.

- Additionally, there are unencapsulated strains referred to as nontypable Haemophilus influenzae because serotyping is based on antigens in the polysaccharide capsule.

- From the time that the H. influenzae type b (Hib) protein-polysaccharide conjugate vaccine became available in 1987 for childhood immunization beginning at 2 months of age, there has been a dramatic decrease in the incidence of invasive disease caused by Haemophilus influenzae type b.

- Immunity to this and other infectious disease is accomplished during the first months of life by lgG antibodies acquired transplacentally from the mother, but this protection is only transient.

- H. influenzae type b conjugate vaccines prevent disease by the induction of active B-lymphocyte mediated humoral immunity and may decrease oropharyngeal carriage of H. influenzae type b.

- Before the availability of the vaccine, slightly less than 50% of all cases of acute bacterial meningitis in the U.S. were caused by Haemophilus influenzae type b.

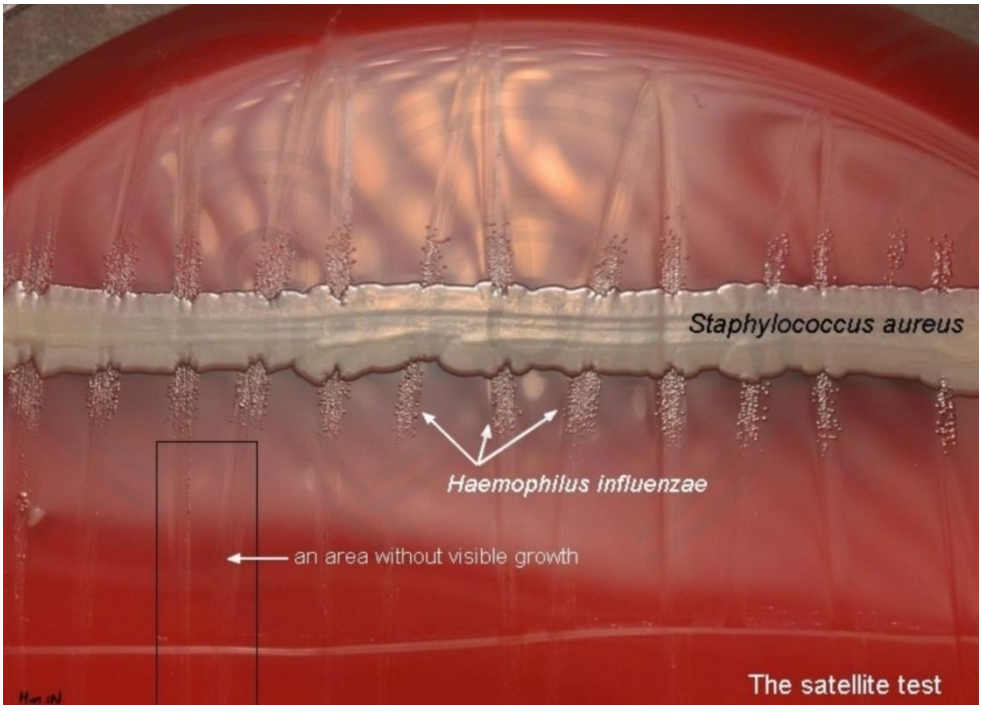
- The change in the vaccination schedule of Hib conjugate vaccine would most likely affect the epidemiology of meningitis.

- The Haemophilus influenza type b (Hib) vaccine consists of PRP (polyribose-ribitol-phosphate) derived from the capsule of H. influenzae type b coupled with either diphtheria or tetanus toxoid.

- When the polysaccharide antigen is conjugated with diphtheria or tetanus protein toxoid the conjugate becomes a T-cell-dependent antigen, and the immunogenicity of the vaccine is thereby increased.

❖ N.B:

- Haemophilus influenza is a blood loving organism and requires both X factor (exogenous hematin) and V factor (NAD+) to support growth.
- Because these factors are found within erythrocytes, optimal concentrations are present only in lysed blood agar (chocolate agar).
- Growth on regular blood agar requires exogenous supplementation of X and V factor.
- Furthermore, 5% sheep blood agar plates not only lack sufficient nutrients to support the growth of Haemophilus species, but they also do not allow the growth of Haemophilus species because of the presence of V factor inactivating enzymes found in the media.
- Growth of Haemophilus species can be achieved on 5% sheep blood agar by cross streaking the medium with Staphylococcus aureus.
- Colonies of H. influenza will grow around the hemolytic S. aureus colonies resulting in the characteristic "satellite" phenomenon.
- When the enzymes of beta hemolytic S. aureus lyse the red blood cells in the medium X factor (hematin) is released, and V factor (NAD+) is actively secreted by staphylococci into the growth medium.
- S. aureus thereby provides the X and V factors necessary to support the growth of Haemophilus species in sheep blood agar.



Haemophilus ducreyi

- Habitats: human genitals.
- Mode of transmission: sexual transmission and direct contact.
- Diseases:

- Chancroid: It presents as one or more deep, painful ulcers (you "do cry" with. H ducreyi) with ragged borders that are associated with a grey exudate and inguinal lymphadenopathy.

Genus: Pasteurella

- Genus Features:

- Gram-negative coccobacillary rods.
- Facultative anaerobic rods.

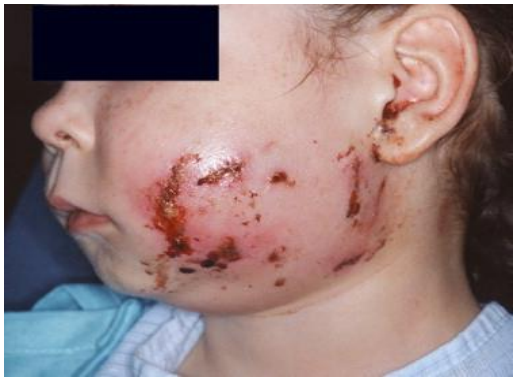
- Species of Medical Importance: Pasteurella multocida.

Pasteurella multocida

- Habitats: mouths of many animals, especially cats and dogs.
- Mode of Transmission: animal bites; particularly from cat bites.
- Virulence factors and Pathogenesis: endotoxin, capsule; spreads rapidly within skin, no exotoxins known.
- Diseases:

- Cellulitis with lymphadenitis: Wound infections with characteristic mouse-like odor, rapidly spreading.

- Frequently polymicrobial infections.



Genus: Francisella

- Genus Features:
- Gram-negative coccobacillary rods.
- Facultative intracellular pathogen.

- Species of Medical Importance: Francisella tularensis.

Francisella tularensis

- Distinguishing Features:
- Gram-negative coccobacillary rods.

- Potential biowarfare agent (the pneumonic form of which is often lethal without treatment).

- Zoonosis: Zoonosis is a disease that can be transmitted to humans from animals. Transmission occurs when an animal infected with bacteria, viruses, parasites, and fungi comes into contact with humans).

- Habitats: many species of wild animals, especially rabbits, deer, and rodents.

- Mode of transmission:
- Tick bite (Dermacentor) → ulceroglandular disease, characterized by fever, ulcer at bite site, and regional lymph node enlargement and necrosis.

- Traumatic implantation while skinning rabbits → ulceroglandular disease.

- Aerosols (skinning rabbits) → pneumonia.

- Ingestion of undercooked, infected meat or contaminated water → typhoidal tularemia.

Genus: Bordetella

- Genus Features:
- Gram-negative coccobacillus.
- Strict aerobes.

- Species of medical Importance: Bordetella pertussis.

Bordetella pertussis

- Distinguishing Features:

- Gram-negative, aerobic coccobacillus.
- Encapsulated organism.

- Habitats: human.

- Mode of transmission: respiratory droplets.

- Virulence factors and pathogenesis:

- Virulence factors include pertussis toxin (disables G_i), adenylate cyclase toxin (↑ cAMP), and tracheal cytotoxin.

1. Pertussis toxin (PTx):

- It is similar to the A-B model of cholera toxin.

- PTx has a potent adenylate cyclase activity by ADP ribosylation of Gi (inhibiting negative regulator of adenylate cyclase, disinhibition) → ↑ cAMP activity that reduces phagocytic activity locally and helps the organism to initiate infection.

- Systemic effects of the toxin include lymphocytosis and increased sensitivity to histamine (resulting in increased capillary permeability, hypotension, and shock).

- This toxin can be inactivated and converted to toxoid for use in vaccines.

2. Adenylate cyclase toxin:

- Bordetella pertussis produces, in addition to pertussis toxin, an exotoxin called adenylate cyclase toxin.

- Like edema factor of bacillus anthracis, adenylate cyclase toxin functions as a calmodulin-dependent adenylate cyclase that causes phagocyte dysfunction and edema.

- The immunosuppression induced by pertussis toxin and adenylate cyclase toxin are important for successful respiratory tract colonization by B. pertussis.

3. Tracheal cytotoxin (TCT):

- TCT is not a classic bacterial exotoxin, since it is not composed of protein, but is a peptidoglycan fragment.

- The toxin kills ciliated respiratory epithelial cells.

- It also stimulates release of IL-1 and so causes fever.

- Diseases:

❖ Whooping cough:

- It is an acute respiratory disease transmitted by droplet.

- Classical pertussis has 3 stages, the catarrhal, paroxysmal and convalescent stages:
  - Catarrhal: low-grade fevers, Coryza.
  - Paroxysmal: paroxysms of intense cough followed by inspiratory gasp "whooP" ("whooping cough"), posttussive vomiting.
  - Convalescent: gradual recovery of chronic cough.

- The most prominent and serious symptom is the severe paroxysmal cough.

- Complications occur such as cyanosis, secondary infections as pneumonia and otitis media.

- The typical disease usually occurs in unimmunized infants.

- Adult pertussis also occurs because vaccine-induced immunity and naturally acquired immunity are not long lived.

Genus: Brucella

- Genus Features:

- Gram-negative coccobacillus.
- Aerobic.
- Facultative intracellular pathogen.
- Zoonosis.

- Species of Medical Importance:

- Brucella abortus: cattle.
- Brucella melitensis: goats.
- Brucella suis: pigs.

Brucella Species

- Distinguishing Features:

- Gram-negative coccobacillus.
- Aerobic.

- Habitats: domestic livestock.

- **Mode of transmission:**

- Unpasteurized dairy products (California and Texas highest number of cases; most associated with travel to Mexico).

- Direct contact with the animal, work in slaughterhouse.

- **Virulence factors and pathogenesis:**

- Endotoxin.

- Facultative intracellular parasite (localizes in cells of the reticuloendothelial system) → septicemia.

- Can form noncaseating granulomas.

- **Diseases:**

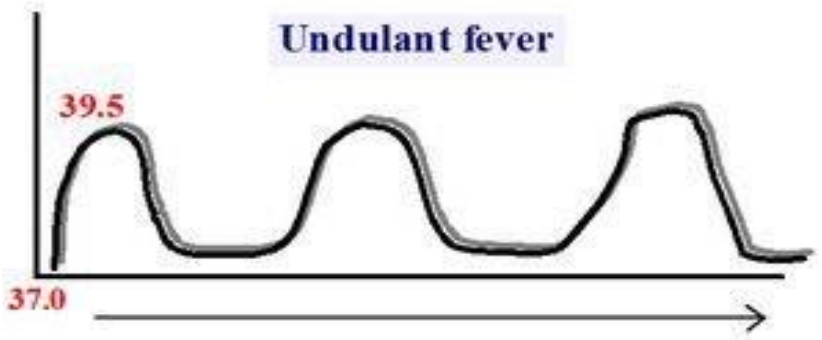
❖ Brucellosis (undulant fever or malta fever):

- May be presented acutely or subacutely or as local disease.

- Systemic symptoms include fever, which is usually prolonged and intermittent (undulant), chills, weakness, malaise, body aches, sweating and headache.

- Undulant means in waves rising and falling pattern.

- Brucellosis may also involve the liver, heart (endocarditis) and central nervous system (meningitis).



Genus: Legionella

- Genus Features:

- Gram-negative rods.
- Stains faintly with Gram stain; silver stains improve visualization.
- Water organisms.

- Species of Medical Importance: Legionella pneumophila.

Legionella pneumophila

- Distinguishing Features:

- Gram-negative rods.
- An intracellular motile rod that stains faintly with Gram stain; silver stains improve visualization.
- Culture must be performed on buffered charcoal yeast extract (BCYE) agar supplemented with L-cysteine and iron.

- Habitats: water source habitat (air conditioning systems, hot water tanks).

- Mode of transmission: Aerosols from contaminated air-conditioning.

- Virulence factors and pathogenesis:

- Aside from endotoxin, no other virulence factors are known.
- The organism replicates intracellularly, therefore cell-mediated immunity is an important host defense.

- Diseases:

1. Legionnaires' disease:

- Legionella should be suspected in patients with recent exposure to contaminated water (cruise trip or hotel stay), radiographic evidence of pneumonia, high fever (>39 C), cough, neurological symptoms (confusion), and gastrointestinal symptoms (diarrhea) such as diarrhea.
- Legionella pneumophila is one of the most common causes of community-acquired pneumonia.
- Predisposing factors include smoking and in chronic lung disease.
- Legionnaires' disease can cause a life-threatening pneumonia if not recognized and treated properly.
- Diagnosis can be difficult because the signs and symptoms are not specific.

- Unlike other atypical pneumonias, the most common x-ray finding in Legionella is a unilobar infiltrate that progresses to consolidation.

- The most common laboratory abnormality seen is hyponatremia, which is frequently associated with Legionella, but not with other causes of pneumonia. The etiology of the hyponatremia is thought to be related to the inappropriate secretion of ADH and/or renal tubulointerstitial disease that impairs the ability to reabsorb sodium.

- The diagnosis is most commonly made by testing for Legionella antigen in the urine.

- Sputum Gram stain often shows many neutrophils, but few or no organisms.

- Treatment is with respiratory fluoroquinolones (levofloxacin) or newer macrolides (azithromycin).

2. Pontiac fever: mild flu-like syndrome.

❖ Mnemonic:

▪ Think of a French legionnaire (soldier) with his silver helmet, sitting around a campfire (charcoal) with his iron dagger—he is no sissy (cysteine).

Overview of *Legionella* pneumonia

Clinical features
- High fever with relative bradycardia
- Headache and confusion
- Watery diarrhea

Laboratory findings
- Hyponatremia
- Sputum Gram stain showing many neutrophils, but few or no organisms

Diagnosis
- *Legionella* urine antigen test

Treatment
- Respiratory fluoroquinolones or newer macrolides

Genus: Campylobacter

- Genus Features:
- Gram-negative comma shaped rods with polar flagella.
- Oxidase-positive.

- Species of Medical Importance: Campylobacter jejuni.

Campylobacter jejuni

- Distinguishing Features:
- Campylobacter is a comma or S shaped Gram-negative rod with a polar flagella that allows it to move in a characteristic "corkscrew" fashion.

- Oxidase +ve.

- Grows well at 42.0°C on selective media (Campylobacter likes the hot campfire).

- Habitats: intestinal tracts of humans, cattle, sheep, dogs, cats, poultry.

- Mode of transmission: fecal-oral route, primarily from poultry.

- Virulence factors and pathogenesis:
- Low infectious dose (as few as 500).

- Invades mucosa of the colon, destroying mucosal surfaces; blood and pus in stools (inflammatory diarrhea).

- Rarely penetrates to cause septicemia.

- Diseases:
- Campylobacter jejuni is the most common cause of acute gastroenteritis in children and adults in industrialized countries.

- In U.S., Campylobacter enteritis > (Salmonella plus Shigella).

- The organism can be acquired from:

1. Domestic animals, such as cattle, sheep, dogs, and chickens. This route of transmission is common in farm and laboratory workers.

2. Contaminated food, such as undercooked poultry and unpasteurized milk.

- Campylobacter species cause inflammatory diarrhea (initially watery, later bloody), accompanied by abdominal cramping, tenesmus, and leukocytes in stool. The abdominal pain may mimic appendicitis.

- Complications:

a. Guillain-Barre syndrome (GBS):

- GBS is a demyelinating syndrome of the peripheral nerves characterized by ascending muscle weakness and paralysis.
- Campylobacter is the most common infectious agent associated with Guillain-Barre syndrome.
- GBS is attributable in 10-30% of instances to C. jejuni infection.

b. Reactive arthritis.

Genus: Helicobacter

- Genus Features:

- Gram-negative comma shaped gastric bacilli with flagella.
- Oxidase positive.

- Species of Medical Importance: Helicobacter pylori.

Helicobacter pylori

- Distinguishing Feature:

- Triple ⊕: catalase ⊕, oxidase ⊕, and urease ⊕.

- Urease positive (Urease hydrolyzes urea into carbon dioxide and ammonia, ammonia permits H. pylori to survive in an acidic environment).

- Habitats: humans.

- Mode of transmission: Fecal-oral.

- Virulence factors and pathogenesis:

1. Urease production and motility are essential for colonization.

- Urease hydrolyzes urea into carbon dioxide and ammonia, the latter permits H. pylori to survive in an acidic environment.

2. H. pylori strains may produce the vacuolating cytotoxin VacA. The toxin inserts itself into the epithelial cell membrane and forms a channel through which bicarbonate and organic anions can be released, providing the bacterium with nutrients.

3. Pathogenic H. pylori strains contain the cag pathogenicity island (cag-PAI). It is a chromosomal region encoding proteins involved in the induction of interlukin-8 production.

- Interlukin-8 is a chemokine which serves as a potent inflammatory mediator recruiting and activating neutrophils in the process of gastritis.

- Diseases:

- Chronic gastritis and duodenal ulcers.

- Associated with several forms of stomach cancer (gastric adenocarcinoma, gastric mucosa-associated lymphoid tissue lymphoma (MALT lymphoma, B-cell lymphomas).

- Can use urea breath test or fecal antigen test for diagnosis.

- Most common initial treatment is triple therapy: Amoxicillin or metronidazole if penicillin allergy + Clarithromycin + Proton pump inhibitor (Antibiotics Cure Pylori). Bismuth-based quadruple therapy if concerned about macrolide resistance.

❖ Urease breath test:

- This test is a screening assay for the presence of urease activity, an indirect means of detecting the presence of Helicobacter pylori, a major cause of duodenal ulcer.
- Culturing the organism from gastric biopsy specimens is considered the definitive confirmatory test but it's invasive.
- The noninvasive urease breath test involves consuming a solution containing isotopically labeled urea.
- When present, urease (a product of H. pylori) degrades the urea into carbon dioxide and ammonia.
- The isotopically labeled carbon dioxide is absorbed into the bloodstream and exhaled in the patient's breath.
- Typically, breath samples are collected 30 minutes after the labeled urea is ingested. This test has excellent sensitivity and specificity for both the initial diagnosis of H. pylori infection and for monitoring treatment success.
- Antibiotic or proton pump inhibitor use during the 2-4 weeks prior to the test may cause false negative results.

Genus: Vibrio

- Genus Features:

- Gram-negative comma shaped rod with polar flagella.
- Oxidase positive.
- Growth on alkaline, but not acidic, media.

- Species of Medical Importance:

- Vibrio cholerae.
- Vibrio parahaemolyticus.
- Vibrio vulnificus.

Vibrio cholerae

- Distinguishing Features:

- Gram-negative, Comma-shaped rods.
- Oxidase-positive.
- They are able to grow on alkaline enrichment medium that kills most organisms of the normal flora of the gut.
- "Shooting star" motility.

- Habitats: Human colon.

- Mode of transmission:

- Fecal-oral route.
- Sensitive to stomach acid and most die in the stomach → Requires high dose (> 10^7 organisms) if stomach acid is normal.
- If V. cholerae is contained in food, then as few as 10^5 V. cholerae are needed because of the buffering capacity of food.
- Achlorhydria is a condition where there is inadequate gastric acid production to maintain the normal gastric pH of less than 4 even with maximal hormonal stimulation. In patients with achlorhydria there is insufficient acid to kill the organism, so very few V. cholera organisms are needed to cause disease.
- It can be pharmacologically induced with long-term proton pump inhibitor therapy (patients on omeprazole treatment) or can be a result of gastritis.

- Virulence factors and pathogenesis:

❖ Cholera enterotoxin (choleragen):

- The toxin binds through its B region to specific receptors on the intestinal epithelial cells and releases the enzymatically active (A) subunit that enters the cells.

- Cholera toxin increases levels of cAMP by increasing the activity of adenylate cyclase in intestinal mucosal cells by a mechanism identical to that of the heat labile toxin produced by Enterotoxigenic E. coli (ETEC).

- Similar to E. coli labile toxin (LT); ADP ribosylates (Gs alpha) activating adenylate cyclase → increased cAMP → efflux of Cl and H_2O.

- Diseases:

❖ Cholera:

- Infection is transmitted by the feco-oral route, through contaminated water or food.

- Direct person-to-person spread is not common because the infectivity dose is high.

- If the bacteria pass the stomach, virulent organisms will penetrate the mucous layer of the small intestine and specifically adhere to its mucosa by fimbriae and other colonization factors.

- V. cholera will then multiply and secrete the potent cholera enterotoxin (CT).

- This causes massive secretion of electrolytes (Na, K, Cl, HCO_3) and water into the lumen of the small intestine.

- Following an incubation period of 6 to 48 hours, cholera begins with an abrupt onset of massive watery diarrhea.

- Several liters of fluid may be secreted within hours, leading to hypovolemic shock.

- The watery diarrhea is speckled with flakes of mucus and epithelial cells "rice-water stool" and contains enormous numbers of vibrio. Vomiting usually occurs.

- The disease runs its course in 2 to 7 days, the outcome depends upon the extent of water and electrolyte loss and the adequacy of treatment.

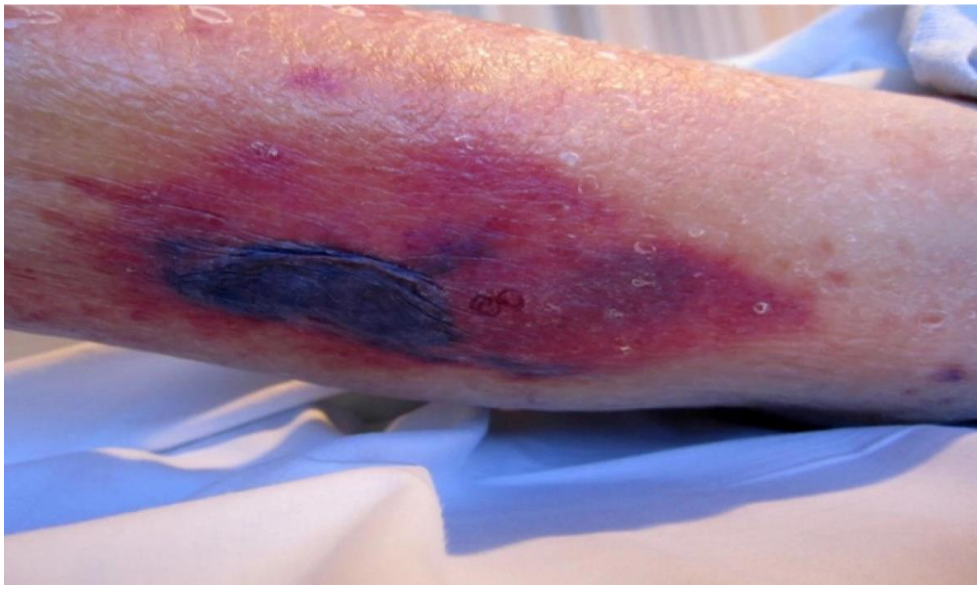
- Because V. cholera does not invade the mucosa or cause enterocyte cell death, no leukocytes or erythrocytes will be visualized on stool microscopy and only epithelial cells and mucous will be seen.

- Death may occur from hypovolemic shock, metabolic acidosis and uremia resulting from acute tubulr necrosis.

- Prompt oral rehydration is necessary.

❖ N.B:

- Vibrio vulnificus is a curved, gram-negative, free-living bacterium that grows in brackish coastal water and marine environments. This bacterium is found in greatest concentrations in the summer months, comprising as much as 8% of the total bacteria in some areas.
- V. vulnificus infections are primarily acquired through the consumption of raw oysters (which concentrate the bacterium) or wound contamination during recreational water activities or the handling of raw seafood.
- Most patients who become ill have liver disease (alcoholic cirrhosis, viral hepatitis); those with iron overload (hemochromatosis) are at particularly high risk as free iron acts as an exponential growth catalyst for the bacterium.
- Healthy patients with V. vulnificus wound contamination usually develop a mild cellulitis, but those with iron overload or liver disease are at high risk for rapidly progressive necrotizing fasciitis with hemorrhagic, bullous lesions and septic shock (hypotension, elevated lactic acid level).
- In these patients, urgent antibiotics, surgery, and blood pressure support are usually required to prevent death.



Genus: Pseudomonas

- Genus Features:

- Non-lactose fermenter, Oxidase-positive Gram-negative rods.
- Aerobic.
- Motile.

- Species of Medical Importance: Pseudomonas aeruginosa.

- Some of the important biochemical properties of the organisms, which can be measured in the lab, are:

1. The ability to ferment lactose and convert it into gas and acid (which can be visualized by including a dye that changes color with changes in pH). Escherichia coli and most of the enterobacteriaceae ferment lactose while Salmonella, Shigella and Pseudomonas aeruginosa do not.

2. The production of H_2S.

3. The production of urease to hydrolyze urea.

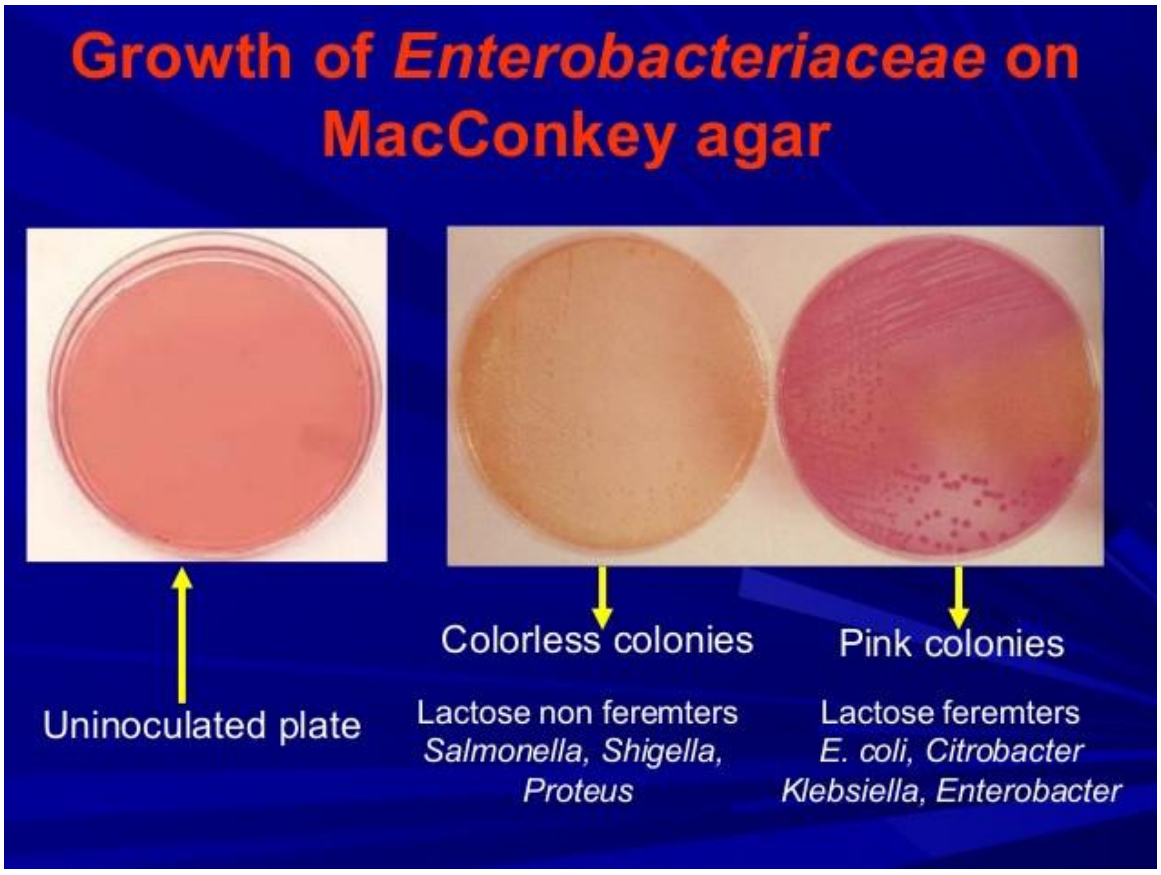
- Some growth media do 2 things at once:

1. They contain chemicals that inhibit the growth of gram-positive bacteria that may be contaminating the sample.

2. They have indicators that change color in the presence of lactose fermentation. The 2 that you should know are:

- EMB agar (Eosine Methylene Blue):
  - Methylene blue inhibits gram-positive bacteria, and colonies of lactose fermenters become deep purple to black in this medium.
  - Escherichia coli colonies gives a metallic green sheen in this medium.

- MacConkey agar:
  - Bile salts in the medium inhibit gram-positive bacteria, and lactose fermenters develop pink colonies.
  - Non-lactose fermenting bacteria leads to formation of white (colorless colonies).



- Oxidase test is used to differentiate non-lactose fermenter bacteria into:

- Oxidase +ve: pseudomonas.
- Oxidase -ve: salmonella, proteus, shigella, yersinia.

- The ability of the bacteria to produce H_2S differentiate oxidase -ve nonlactose fermenter bacteria into:

- Produce H_2S: Salmonella, proteus.
- Does not produce H_2s: shigella, yersinia.

Pseudomonas aeruginosa

- Distinguishing Features:

- Oxidase-positive, motile, Gram-negative Non-lactose fermenting rods.
- Produces pyocyanin and pyoverdine pigment (blue green; also generates reactive oxygen species).
- Emits a grape-like, fruity odor.
- Non-lactose-fermenting colonies on EMB or MacConkey.
- Aerobic (Aeruginosa-aerobic).

- Habitats: ubiquitous in water.

- Mode of transmission: water aerosols, raw vegetables.

- Virulence factors and pathogenesis:

1. Endotoxin: causes inflammation in tissues and gram-negative shock in septicemia.

2. Pseudomonas exotoxin A:

- P. aeruginosa produces several extracellular products, including exotoxin A, collagenase, elastase, fibrinolysin, phospholipase C, and DNAse.

- These substances assist in its invasion and dissemination in human tissues.

- Although they are structurally different, both diphtheria toxin and exotoxin A ribosylate and inactivate elongation factor-2 (EF-2), halting human cell protein synthesis and causing cell death.

- Exotoxin A is a major virulence factor and is responsible for the high mortality associated with P. aeruginosa septicemia.

3. Mucoid polysaccharide Capsule/slime layer:

- Allows formation of pulmonary microcolonies; difficult to remove by phagocytosis.

- Contribute to chronic pneumonia in cystic fibrosis patients due to biofilm formation.

- Diseases:

- Pseudomonas infections are common in neutropenic patients, hospitalized patients, patients with burns and chronic indwelling catheters.

1. Patients with indwelling bladder catheters:

- Pseudomonas aeruginosa is a common cause of urinary tract infections in patients with indwelling bladder catheters.

- Patients with indwelling bladder catheters are at increased risk for urinary tract infections (UTIs) caused by both typical (Escherichia coli, Klebsiella pneumoniae, Staphylococcus saprophyticus, Proteus mirabilis) and opportunistic (Pseudomonas, Enterococcus, other Staphylococci, fungal) organisms.

2. Burn patients:

- Pseudomonas aeruginosa is a major pathogen in burn patients.

- Burn patients are at increased risk of bacterial infection due to loss of the barrier function of the skin, post-burn immune dysfunction, and lack of blood flow to necrotic areas.

3. Diabetic patients:

- It is the most common cause of malignant otitis externa (swimmer's ear), a serious infection of the ear seen in elderly diabetic patients.

- It is most frequently caused by Pseudomonas aeruginosa.

- Patients typically present with exquisite ear pain and drainage.

- The granulation tissue seen within the ear canal is an important characteristic finding of MOE, and the tympanic membrane is usually intact.

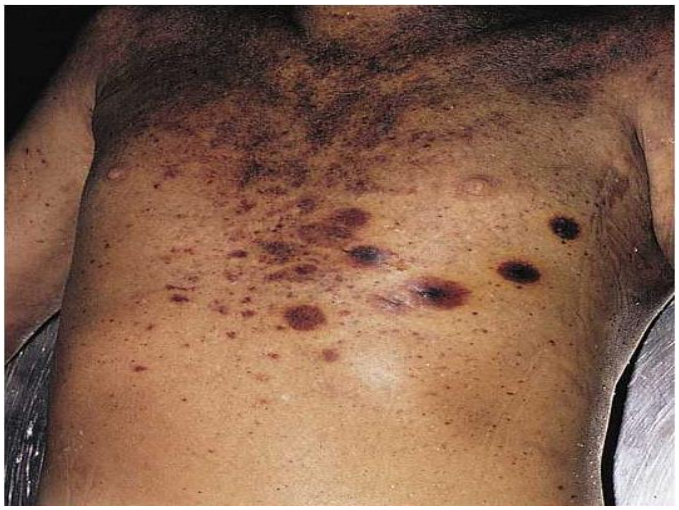
- Progression of this infection can lead to osteomyelitis of the skull base and cranial nerve damage.

4. Immunosuppressed (neutropenic) patients:

- In cases of impaired humoral immunity, there is increased susceptibility to infections with P. aeruginosa as well as other pathogens.

- Ecthyma gangrenosum, a skin finding that is strongly associated with bacteremia by P. aeruginosa. These lesions result from perivascular bacterial invasion of arteries and veins in the dermis and subcutaneous tissue with subsequent release of exotoxins that are destructive to human tissue.

- Enzymes produced by P. aeruginosa such as Exotoxin A (protein synthesis inhibition), Elastase (degrades elastin -important for blood vessel destruction), Phospholipase C (degrades cellular membranes) and pyocyanin (generates reactive oxygen species) are recognized as important virulence factors and play a role in causing the vascular destruction and cutaneous necrosis known as ecthyma gangrenosum.



5. Cystic fibrosis and ventilated patients:

- Recurrent and chronic pneumonias.

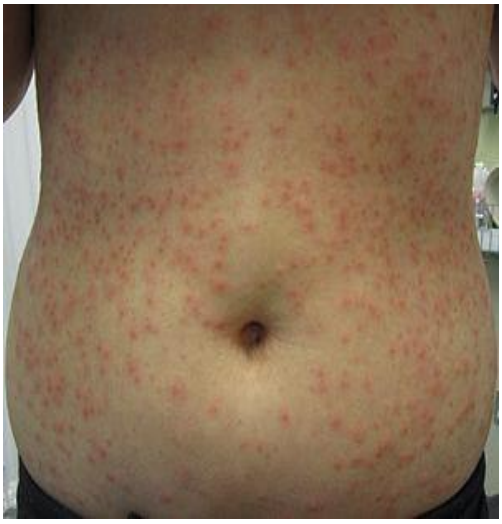
- Mucoid polysaccharide capsule may contribute to chronic pneumonia in cystic fibrosis patients due to biofilm formation.

6. In healthy individuals:

- Hot tub folliculitis is a superficial and self-limited P. aeruginosa infection of the hair follicles that tends to occur in minor outbreaks following exposure to a pool or spa where the chemicals have not been maintained at appropriate levels.

- The culture of a pustule will reveal Gram-negative, oxidase positive, non-lactose fermenting, motile rods that produce pigment (pyocyanin, pyoverdine).

- Many infections by P. aeruginosa often begin with exposure to a water source or creation of a moist environment (swimmer's ear, hot tub folliculitis, burn wound).



7. Osteomyelitis and endocarditis in intravenous drug users (S. Aureus is more common).

❖ Mnemonic:

▪ PSEUDOMONAS is associated with:

- Pneumonia.
- Sepsis.
- Ecthyma gangrenosum.
- UTIs.
- Diabetes.
- Osteomyelitis.
- Mucoid polysaccharide capsule.
- Otitis Externa (swimmer's ear).
- Nosocomial infections (catheters, equipment).
- Addicts (drug abusers).
- Skin infections (hot tub folliculitis, wound infection in burn victims).

❖ Antipseudomnal drugs:

▪ P. aeruginosa is resistant to many antibiotics.

▪ Antibiotics that are effective against P. aeruginosa:

- Cephalosporins such as cefepime and ceftazidime have good anti-pseudomonal coverage.

- Only a few specific penicillins (ticarcillin, piperacillin) and cephalosporins (ceftazidime, cefepime) have activity against it.

- Certain aminoglycosides, fluoroquinolones (ciprofloxacin, levofloxacin), and carbapenems (imipenem, meropenem) are also effective.

- For multidrug resistant strains: colistin, polymyxin B.

❖ CAMPFIRE drugs:

- Carbapenems.
- Aminoglycosides.
- Monobactams.
- Polymyxins (polymyxin B, colistin).
- Fluoroquinolones (ciprofloxacin, levofloxacin).
- ThIRd- and fourth generation cephalosporins (ceftazidime, cefepime).
- Extended spectrum penicillins (piperacillin, ticarcillin).

Distinguishing characteristics of *Pseudomonas aeruginosa* infections

Types of infections
- Pneumonia (especially in cystic fibrosis and ventilated patients)
- Life-threatening infections in neutropenic and burn patients
- Otitis externa (particularly malignant)
- Hot tub folliculitis
- Ecthyma gangrenosum

Microbiology
- Motile aerobic Gram-negative rod
- Nonlactose-fermenting, oxidase-positive
- Produces pyocyanin (blue-green) pigment
- Emits a grapelike, fruity odor
- Produces endotoxin (fever, shock) and exotoxin A (inactivates EF-2)

Effective antibiotics
- Aminoglycosides (gentamicin, tobramycin, amikacin)
- Antipseudomonal penicillins (ticarcillin, piperacillin)
- 3rd and 4th generation cephalosporins (ceftazidime, cefepime)
- Quinolones (ciprofloxacin)
- Monobactams (aztreonam)
- Carbapenems (imipenem, meropenem)

Family: Enterobacteriaceae

- Family Features:

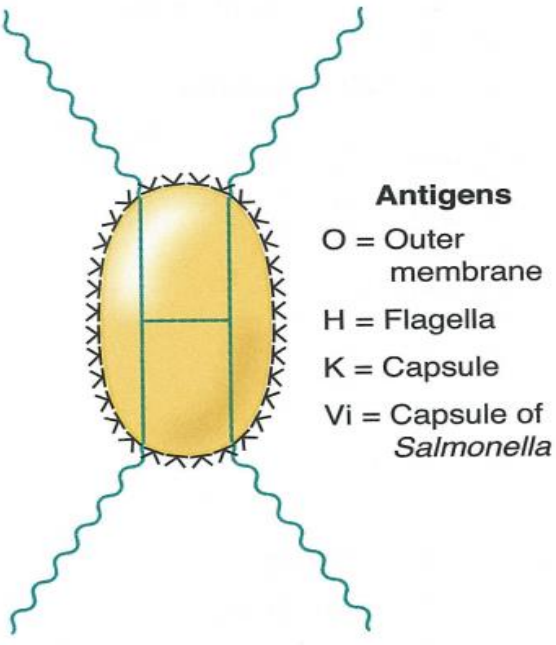
- Gram-negative rods.
- Facultative anaerobes.
- Ferment glucose.
- Oxidase negative.
- Motile except shigella and klebsiella.
- Reduce nitrate to nitrite.

- Family Pathogenesis:

- Endotoxin.
- Some also produce exotoxins.
- Antigens:
  - O= cell envelope or O antigen.
  - H= flagellar (motile cells only) antigen.
  - K= capsular polysaccharide antigen.
  - Vi (virulence)= Salmonella capsular antigen.

- Lab Diagnosis:

- Blood agar.
- Eosin methylene blue or MacConkey agar (differentiate lactose fermentation):
  - Lactose fermenters (colored colonies).
  - Non-lactose fermenters (colorless colonies).



- Lactose Fermenters:

- Mnemonic: Test with MacConKEE'S agar.
  - Citrobacter (Slow).
  - Klebsiella (fast).
  - E. coli (fast).
  - Enterobacter (fast).
  - Serratia (Slow).

- Non-lactose fermenter:

- H_2S producers: Salmonella, proteus.
- Non-H_2S producers: shigella, Yersinia.

Genus: Shigella

- Genus Features:

- Enterobacteriaceae.
- Gram-negative rod.
- Non-lactose fermenters (colorless colonies on EMB or MacConkey).
- Nonmotile.

- Species of medical Importance:

- Shigella sonnei (most common in U.S.).
- Shigella flexneri.
- Shigella dysenteriae (most severe disease).
- Shigella boydii.

Shigella Species

- Distinguishing Features:

- Gram-negative rods.
- Nonmotile (in contrast to Salmonella).
- Non-lactose fermenter (in contrast to E. coli).
- Non H_2S producers (in contrast to Salmonella).

- Habitats: human colon only (no animal carriers).

- Mode of transmission: fecal-oral spread (food washed by contaminated water or by drinking water contaminated with human feces), person to person.

- Virulence factors and pathogenesis:

1. Invasiveness:

- The essential pathogenic process of bacillary dysentery is invasion of mucosal epithelium of the terminal ileum and large intestine where the organism is able to grow.

- The organism does not invade the blood (cell to cell spread only; no hematogenous spread).

- Shigella gains access to the gut mucosal epithelium, specifically by entering M cells in Peyer's patches.

- It then escapes the phagosome, spreads laterally to other epithelial cells, and releases shiga toxin.

- The process of cellular invasion induces a robust inflammatory response in the host; it is this response that is primarily responsible for the diarrhea seen in shigellosis.

2. Shiga toxin:

- Shiga toxin is an exotoxin produced by S. dysentriae.

- The shiga toxin is a classic bacterial AB exotoxin.

- The A subunit inactivates the 60S ribosome of the host, thereby halting protein synthesis and causing cell death.

- The production of toxins is considered less important in the pathogenesis of shigellosis than is cellular invasion because nontoxigenic strains have been found to cause significant disease.

- Mucosal invasion is an essential pathogenic mechanism for Shigella infection and is the most significant factor in causing disease.

- Diseases:

❖ Shigellosis:

- It is characterized by a type of diarrhea in which the stool contains blood and mucus.

- The most common species causing shigellosis in industrialized countries is Shigella sonnei, while the most common strain in developing nations is Shigella flexneri.

- Shigella is highly adapted to surviving the acidity in the stomach, as well as the bacteriostatic action of bile.

- In fact, depending on the age and condition of host, as few as 10 cells of Shigella can cause disease. Even healthy adults will contract shigellosis with an inoculum as small as 200 organisms via the oral route.

- A much larger inoculum of Salmonella (approximately 10^7) is required for successful infection in a susceptible host because salmonella is acid sensitive.

- Other organisms that can cause diarrhea with only a small inoculum include Campylobacter jejuni (500), Entamoeba histolytica (as few as one organism), and Giardia lamblia (as few as one organism).

- Mucosal invasion is the essential pathogenic mechanism for Shigella infection and is the most significant factor in causing disease.

- Shigella invades the gastrointestinal mucosa, specifically via M cells located in Peyer's patches. After entering the M cells, Shigella is able to lyse its containment vacuole and enter the cytosolic compartment.

- Invasion by Shigella triggers a robust host inflammatory response largely mediated by neutrophils.

- Shigella additionally releases shiga toxin, which causes further cell destruction by inhibiting cellular protein synthesis.

- Disease begins with watery diarrhea which progresses to abdominal pain, cramps, diarrhea with blood, mucous and pus, fever, vomiting, and tenesmus (bacillary dysentery). Tenesmus is a painful spasm of the rectum that is associated with an urge to defecate, yet little passage of stool occurs.

Genus: Yersinia

- Genus Features:
- Enterobacteriaceae.
- Gram-negative rods.

- Species of Medical Importance:
- Yersinia pestis.
- Yersinia enterocolitica.

Yersinia pestis

- Distinguishing Features: Gram-negative rods/ coccobacillus that exhibits bipolar staining (resembling a safety pin) on Giemsa or Wright stain.

- Virulence factors and pathogenesis:
- Fraction 1 capsule: the F1 protein form a large gel-like capsule.
- Endotoxin.

- Diseases:

1. Bubonic plague:
- Flea bites infected rodents (rats) and then later uninfected human (Zoonosis).
- The bacteria enter through the skin through a flea bite and travels via the lymphatics to a lymph node, causing it to swell.

- Symptoms:
  - Rapidly increasing fever.
  - Regional buboes (buboes in Greek means groin).
  - Buboes associated with the bubonic plague are commonly found in the armpits, upper femoral, groin and neck region.
  - Leads to septicemia and death if untreated.

2. Pneumonic plague
- Arises from septic pulmonary emboli in bubonic plague or inhalation of organisms from infected individual.
- Highly contagious.

Yersinia Entercolitica

- Distinguishing Features:
- Motile at 25.0°C, nonmotile at 37.0°C.
- Cold growth.

- Habitats: zoonotic.

- Mode of transmission:
- Usually transmitted from pet feces (puppies).
- Unpasteurized milk, pork.

- Virulence factors and pathogenesis:
- Enterotoxin, endotoxin.
- Multiplies in the cold.

- Diseases:

❖ Enterocolitis:
- Presentations may vary with age:
  - Very young: febrile bloody diarrhea (blood and pus).
  - Older kids/young adults: pseudoappendicitis (right lower abdominal pain due to mesenteric adenitis and/or terminal ileitis).
  - Adults: enterocolitis with postinfective sequelae like reactive arthritis.

Genus: Salmonella

- Genus Features:

- Gram-negative rods (Enterobacteriaceae).
- Non-lactose fermenters.
- Motile (salmon swim).
- Salmonellae are named by genus (Salmonella), species (enterica), and subspecies (typhi or enteritidis).

- Species of Medical Importance:

- S. enterica subsp. Typhi.
- S. enterica subsp. Enteritidis.
- S. enterica subsp. Typhimurium.
- S. enterica subsp. Choleraesuis.
- S. enterica subsp. Paratyphi.
- S. enterica subsp. Dublin.

Salmonella enterica Subsp.

- Distinguishing Features:

- Gram-negative rods, highly motile (Salmon Swim) with the Vi capsule.

- Facultative anaerobe, non-lactose fermenting.

- Produces H_2S.

- Sensitive to acid → Large infectious dose of Salmonella (approximately 10^7) is required for successful infection in a susceptible host because salmonella is acid sensitive.

- Habitats: Humans and animals (except Salmonella typhi →no animal reservoirs).

- Mode of transmission:

- Fecal-oral route from human carriers (gall bladder).

- Decreased stomach acid or impairment of mononuclear cells such as in sickle cell disease predisposes to Salmonella infections.

- Virulence factors and pathogenesis:

- The antigens used to define groups and types of salmonella include:

1. The O (somatic) antigens: heat-stable polysaccharides that form part of the cell wall lipopolysaccharide (LPS).

2. The H antigens: flagellin of the flagella.

3. The Vi antigen: capsular polysaccharide antigen.

- Diseases:

1. Food poisoning (gastroenteritis or enterocolitis):

- It is worldwide infection caused by nontyphoidal salmonella strains, commonly S. Enteritidis and S. typhimurium.

- It is the most common salmonella infection.

- Disease transmission is usually linked to food of animal and poultry origins.

- All ages are affected, the incidence is highest in infants.

- The disease is due to an intestinal infection accompanied by severe diarrhea, fever, and abdominal cramps.

- The organism characteristically invades and replicates in the epithelial cell of small and large intestines (not in macrophages) leading to intestinal lesions and bloody diarrhea.

- Treatment depends on correction of dehydration and electrolyte imbalance.

2. Typhoid fever (enteric fever):

- Typhoid fever (also referred to as "enteric fever") is a life-threatening illness caused by the bacterium Salmonella typhi or Salmonella paratyphi. Other species of Salmonella are not associated with typhoid fever.

- The term enteric fever, which may include both typhoid and paratyphoid, has been defined as a generalized infection of the reticuloendothelial system and intestinal lymphoid tissue accompanied by sustained fever and bacteremia.

- Typhoid fever is common in most parts of the world except in industrialized regions such as the United States, Canada, Western Europe, Australia, and Japan.

- In developed nations, typhoid fever is generally not suspected unless there is a history of recent travel to areas where the disease is endemic. Over the past several years, travelers to Asia, Africa, and Latin America have been especially at risk.

- Typhoid fever is a fecal-oral disease that begins after ingestion of S. typhi or paratyphi.

- These organisms penetrate the gut mucosa both via transporters on enterocytes and via phagocytosis by M cells in Peyer's patches.

- The organisms are then phagocytosed by macrophages, within which Salmonella (para) typhi are specially adapted to survive.

- Macrophages carry the infective organisms to the liver, spleen, and bone marrow.

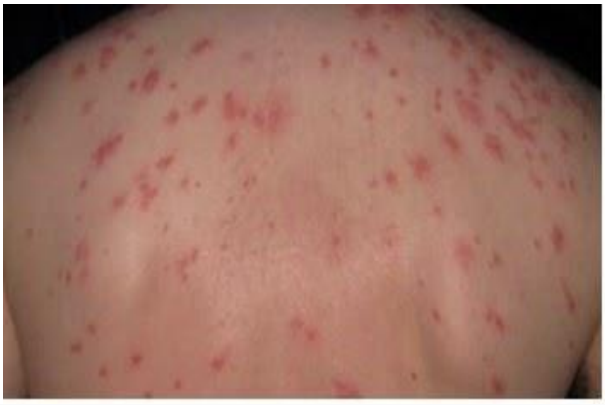
- Hepatosplenomegaly from organism growth ensues. From here, these species are able to cause bacteremia and sepsis.

- The common clinical picture of this disease is mild abdominal cramping with fever and diarrhea OR constipation initially. Subsequently, the patient can develop salmon-colored "rose spots" rash, develop hepatosplenomegaly and recolonization of the gut, leading to hemorrhagic diarrhea.

- Within the gut lumen, S. typhi and paratyphi do more than disseminate, they can cause drastic inflammation within Peyer's patches, causing intestinal hemorrhage as well as potential gut perforation which can cause polymicrobial peritonitis and sepsis, the mechanisms by which typhoid fever can cause death.

- Salmonella typhi and paratyphi also colonize the gallbladder, which allows access to the gut lumen on a virtually limitless basis.

- Patients who do not experience fulminant disease are at risk for becoming chronic carriers of the bacterium and can unknowingly affect dozens of other people.



	Clinical manifestations of typhoid fever
Week 1	• Rising fever • Bacteremia • Relative bradycardia (pulse-temperature dissociation)
Week 2	• Abdominal pain • Rose spots on trunk & abdomen
Week 3	• Hepatosplenomegaly • Intestinal bleeding & perforation

3. Septicemia (or bacteremia):

- It represents about 5-10% of salmonella infections.

- The condition usually occurs in immunocompromised individuals.

- After ingestion of salmonella, they invade the intestinal mucosa and invade the bloodstream early without intestinal lesions.

- Bacteremia results in metastatic abscesses and commonly manifest as osteomyelitis, arthritis, pneumonia, and meningitis.

❖ Salmonella typhi vaccines:

- Oral vaccine contains live attenuated S. typhi.

- IM vaccine contains Vi capsular polysaccharide.

❖ N.B:

1. Many patients with sickle cell disease come to the ER frequently with painful, vaso-occlusive crises; these crises usually respond well to oxygen, IV fluids, and high-dose narcotics.

- The vasoocclusion that is associated with sickle cell anemia not only causes the painful "sickle cell crises," it also causes a relative immune deficiency because the spleen suffers widespread infarction.
- This functional asplenia puts sickle cell patients at an increased risk of infection by encapsulated organisms such as Neisseria, Haemophilus, Streptococcus pneumoniae, and Salmonella species.
- Certain vaccinations are routinely given to patients with sickle cell disease and patients who are asplenic for other reasons (trauma).
- These vaccinations are → the pneumovax for S. pneumoniae, Hib for H. influenza type b, and the Meningitis polysaccharide capsular vaccine for N. meningitidis.
- Salmonella possesses a special capsule called the "Vi antigen" (Vi stands for virulence) which protects the bacterium from opsonization and phagocytosis.
- Salmonella is the most common cause of osteomyelitis in patients with sickle cell anemia followed by E. coli (K antigen capsule), then S. aureus.
- An increased risk of osteomyelitis is present because vaso-occlusive crises cause focal areas of bone necrosis, within which bacteria can easily establish infection.

2. Typhoid strains of Salmonella contain a capsular antigen (Vi) that inhibits neutrophil phagocytosis, neutrophil recruitment, and macrophage-mediated destruction. Therefore, typhoid strains are able to undergo extensive replication within the intracellular space of macrophages with subsequent spread through the lymphatic and reticuloendothelial system, leading to a widespread systemic disease (typhoid fever).

Genus: Proteus

- Genus Features:

- Gram-negative rod (Enterobacteriaceae).
- Peritrichous flagella/highly motile/"swarming motility".
- Non-lactose-fermenting.
- Urease positive.

- Species of medical Importance:

- Proteus mirabilis (90% of infections).
- Proteus vulgaris.

Proteus mirabilis/Proteus vulgaris

- Distinguishing Features:

- Gram-negative rods.
- Highly motile; "swarming" motility on surface of blood agar.
- Oxidase negative.
- Produces H_2S.
- Urease positive.

- Habitats: human colon and environment (water and soil).

- Mode of transmission: endogenous.

- Diseases:

- Proteus infections are usually nosocomial, including:

1. Urinary tract infection especially caused by Proteus Mirabilis.
2. Wound infections and abscess formation.
3. Respiratory infections: otitis media and pneumonia.
4. Septicemia and meningitis.

Genus: Escherichia

- Genus Features:
- Gram-negative rods (Enterobacteriaceae).
- Ferments lactose.

- Species of Medical Importance: Escherichia coli.

Escherichia coli

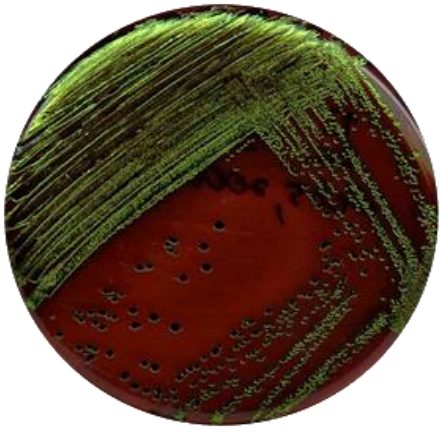
- Distinguishing Features:
- Gram-negative motile enteric rods.

- Able to ferment both lactose and glucose.

- E coli grows well on blood, MacConkey, and eosin methylene blue (EMB) agar plates.

- MacConkey agar:
  - It is a selective and differential medium used to isolate gram negative organisms from contaminants in clinical specimens.
  - The bile salts and crystal violet present in MacConkey agar prevent the growth of gram-positive organisms.
  - Organisms that ferment lactose (E coli, Klebsiella, Enterobacter) cause a local drop in pH, resulting in colonies with a pink-red appearance.
  - Non-lactose fermenting organisms remain colorless.

- EMB agar:
  - It is a selective and differential medium used to isolate and identify enteric pathogens from contaminated clinical specimens.
  - On EMB agar, organisms that ferment lactose, such as E coli, bind to dye in the agar and produce colonies that have a distinct green metallic sheen.



- **Habitats:**

- Human colon; may colonize vagina or urethra.
- Contaminated crops where human fecal fertilizer is used.
- Enterohemorrhagic strains: bovine feces.

- **Mode of transmission:**

- Endogenous.
- Feco-oral.
- Maternal fecal flora.
- Enterohemorrhagic strains: bovine fecal contamination (raw or undercooked beef, milk).

- **Virulence factors and pathogenesis:**

A. Diarrhoeagenic E. coli have:

1. Pili (fimbriae): permits the adhesion of E. coli to the cell surface.

2. Enterotoxins:

Enterotoxigenic *E coli*	Heat-labile toxin (LT)[a]	Overactivates adenylate cyclase (↑ cAMP) → ↑ Cl^- secretion in gut and H_2O efflux	Watery diarrhea: "labile in the Air (Adenylate cyclase), stable on the Ground (Guanylate cyclase)"
	Heat-stable toxin (ST)	Overactivates guanylate cyclase (↑ cGMP) → ↓ resorption of NaCl and H_2O in gut	

3. The shiga like toxin:

- Shiga-like toxins (Stx) or Vero cytotoxins (VT) are produced by Enterohemorrhagic E. coli (EHEC).

- The plasmid coding for this toxin is transmitted to E. coli by a temperate bacteriophage (lysogeny).

- The B subunits recognize the specific receptors on the target cells and induce receptor-mediated endocytosis and internalization of the toxin. Subsequently, the enzymatically active A subunit is released.

- The A subunit then catalyzes the removal of a single specific adenine residue, thereby preventing binding of tRNA to the 60S ribosomal subunit and inhibiting protein synthesis (similar to shiga toxin).

- This leads to intestinal mucosal cell death as well as direct toxicity to renal endothelial cells.

- This mechanism differs from that of diphtheria toxin and exotoxin A of Pseudomonas in that the latter toxins act on EF-2, not the 60S ribosomal subunit.

4. Uropathogenic strains of E. coli have fimbrial adhesins (permits adhesion of the E. coli to the uroepithelium), exotoxins (hemolysins) and K antigen.

5. The LPS: causes endotoxic shock when released into the circulation.

Escherichia coli virulence factors		
Virulence factor	**Mechanism**	**Presentation**
Lipopolysaccharide	Macrophage activation causes widespread release of IL-1, IL-6 & TNF-α	Bacteriemia & septic shock
K1 capsular polysaccharide	Prevents phagocytosis & complement-mediated lysis	Neonatal meningitis
Verotoxin (shiga-like toxin)	Inactivates the 60S ribosomal component, halting protein synthesis & causing cell death	Gastroenteritis (bloody)
Heat-stable/heat-labile enterotoxins	Promotes fluid & electrolyte secretion from intestinal epithelium	Gastroenteritis (watery)
P fimbriae	Allows adhesion to uroepithelium	Urinary tract infections

- Diseases:

1. Urinary tract infection (UTI):

a. Community-acquired UTI:

- E. coli is the commonest cause and accounts for > 80% of infections.

- The uropathogenic strains of E. coli are present in the feces and subsequently colonize the vagina and periurethral region.

- During sexual intercourse or bladder catheterization, E coli can be propelled into the urethra and bladder from the colonized periurethral region.

- These organisms ascend into urethra (urethritis), bladder (cystitis), ureters, renal pelvis (pyelitis) and renal parenchyma (pyelonephritis).

b. Hospital-acquired UTI: it is usually associated with urinary catheters and caused by multi-resistant strains.

❖ N.B:

1. The most common source of E. coli bacteremia is the urinary tract.

- E. coli is the most common cause of urinary tract infection in both healthy adults and elderly patients (E. coli causes approximately 80% of all UTIs).
- Some common predisposing factors to urosepsis are urinary obstruction (BPH), fecal incontinence, a neurogenic bladder secondary to diabetes, and frequent or indwelling catheterization.
- Gram-negative sepsis or septic shock results from the body's systemic reaction to lipopolysaccharide endotoxin (a component in the membranes of some bacteria).

2. The virulence factors expressed by a particular strain of E coli will determine disease characteristics.

- Fimbriae, or pili are one of the most important virulence factors expressed by E coli.
- Without fimbriae, E coli would not be able to bind to uroepithelial cells and infect the bladder, ureters, and kidneys. Instead, the bacteria would simply be washed away during urination.
- UTIs are more common in women than men due to the female urethra being significantly shorter.

2. Neonatal meningitis:

- Group B Streptococcus is the most common cause of neonatal meningitis (0-3 months) in the United States, followed by Escherichia coli and listeria monocytogenes.

- In older infants (>3 months) and adults, the most common pathogens are Streptococcus pneumoniae and Neisseria meningitidis.

- E coli can invade the blood stream of infants from the nasopharynx or gastrointestinal tract and can then travel hematogenously to the meninges.

- The K1 capsular antigen is present in 20%-40% of intestinal E coli isolates and is considered the major virulence factor among E coli strains that cause neonatal meningitis.

- Bacterial capsules are important for most meningeal pathogens.

- They help facilitate survival in the blood by preventing recognition of bacterial antigens, complement deposition, and subsequent phagocytosis.

- The K1 capsule is immunogenic and anti-capsular antibodies are protective against repeat infection.

3. Pneumonia, sepsis, septicemia, and endotoxic shock may follow any of the E. coli infections particularly in neonates.

4. Diarrhea:

- Diarrhoeagenic E. coli include the following types:

a. Enterotoxigenic E. coli (ETEC):

- The LT enterotoxin is very similar to cholera toxin in both structure and mode of action. It attaches via its B subunit to the gut mucosa, then the A subunit enters the cell and activates adenylate cyclase by activating the stimulatory Gs membrane G protein resulting in conversion of ATP to cAMP. Increased level of cAMP induces the active secretion of Cl and inhibits the absorption of Na, creating an electrolyte imbalance and loss of copious amounts of fluids from the intestine.

- The ST stimulates the activity of guanylate cyclase in intestinal epithelial cells leading to formation of cGMP resulting also in loss of fluids from the intestine.

- Enterotoxigenic E. coli (ETEC) strains cause severe diarrhea in infants and children and traveler's diarrhea in adults.

- The usual presentation of ETEC gastroenteritis is watery diarrhea with abdominal cramping, nausea and vomiting, and possibly a low fever.

- The self-limited diarrhea "Traveler's diarrhea" is characteristic of Enterotoxigenic E. coli (ETEC).

- ETEC T = traveler's diarrhea.

b. Enterohemorrhagic E. coli (EHEC):

- Also known as verotoxigenic E. coli or shiga toxin-producing E. coli owing to production of shiga like toxin.

- The Shiga and the Shiga-like toxins inactivate the 60S ribosomal subunit in human cells leading to an inhibition of protein synthesis and eventual cell death.

- EHEC strains cause bloody diarrhea or hemorrhagic colitis (a disease similar to shigella dysentery) and also hemolytic uremic syndrome (HUS) as a potentially fatal complication.

- EHEC do not invade the intestinal mucosa; this is a characteristic of Enteroinvasive E. coli as well as other causes of hemorrhagic diarrhea.

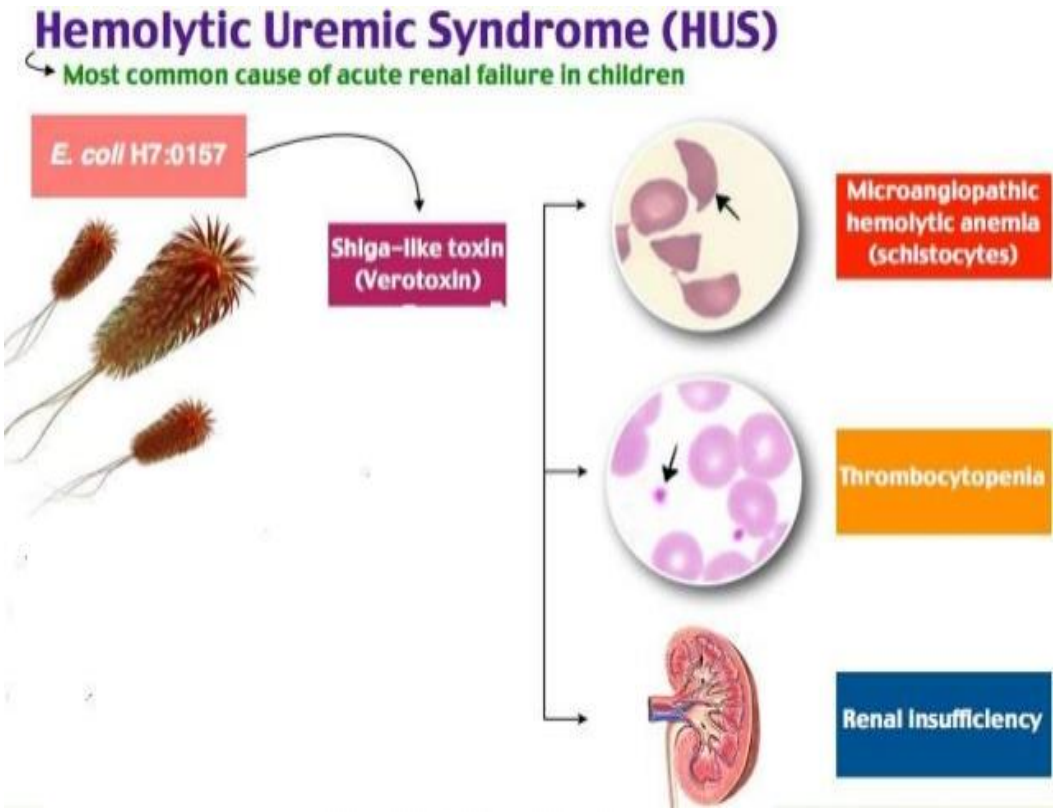
- Hemolytic-uremic syndrome (HUS) triad is characterized by microangiopathic hemolytic anemia (mechanical hemolysis with schistocytes on peripheral blood smear), thrombocytopenia (due to platelet consumption), and renal insufficiency (due to ↓ renal blood flow).

- E. coli serotype O157: H7 is the most common strain associated with the disease.

- Undercooked beef (hamburger) has caused many outbreaks.

- This particular strain of E. coli is unable to ferment sorbitol and does not produce a glucuronidase.

- EHEC H = Hamburger, Hemorrhagic Diarrhea.



c. Enteropathogenic E. coli (EPEC):

- EPEC strains adhere tightly to the intestinal mucosa and interfere with water absorption by mucosal cells.
- They are a common cause of infantile diarrhea.
- EPEC P = pediatric (diarrhea in children).

d. Enteroinvasive E. coli (EIEC):

- EIEC strains cause a disease identical to that caused by shigella spp. but do not produce shiga toxin.
- It invades intestinal mucosa and causes necrosis and inflammation.
- EIEC I = Invasive (dysentery).

Genus: Klebsiella

- Genus Features:
- Gram-negative rods (Enterobacteriaceae).
- Major capsule.

- Species of Medical Importance: Klebsiella pneumoniae.

Klebsiella pneumoniae

- Distinguishing Features:
- Gram-negative rods with large mucopolysaccharide capsule.

- Lactose-fermenting mucoid colonies on MacConkey agar.

- Oxidase negative.

- Habitats: human colon and upper respiratory tract.

- Mode of transmission: endogenous.

- Virulence factors and pathogenesis: The capsule is the most important virulence factor.

- Diseases:

1. Pneumonia:

- An intestinal flora that causes lobar pneumonia in alcoholics and diabetics when aspirated (but this is not the most common cause of pneumonia in alcoholics; S. pneumoniae is).

- Frequent abscesses make it hard to treat; fatality rate high.

- Sputum is generally thick and bloody (currant jelly sputum) but not foul smelling as in anaerobic aspiration pneumonia.

2. Urinary tract infections: catheter-related from fecal contamination of catheters.

❖ Mnemonic:

- 4 A's of KlebsiellA: Aspiration pneumonia, Abscess in lungs and liver, Alcoholics, di-A-betics.

❖ N.B:

- The sputum with S. pneumoniae is described as rusty. The “rust” is simply hemoptysis. As the blood oxidizes, it becomes brownish-red color.
- The sputum with Klebsiella pneumoniae is described as currant jelly. This is simply hemoptysis with mucoid characteristics from a combination of the necrotizing nature of Klebsiella with the organism’s thick mucopolysaccharide coating.
- Interstitial infections such as those caused by Pneumocystis pneumonia (PCP), viruses, Mycoplasma, and sometimes Legionella often give a nonproductive or “dry” cough.

Genus: Gardnerella

- Genus Features:
- Anaerobic Gram-variable rod (pleomorphic).
- Catalase and oxidase negative.

- Species of Medical Importance: Gardnerella vaginalis.

Gardnerella vaginalis

- Distinguishing Features: anaerobic Gram-variable rod (meaning they may stain either negative or positive).

- Habitats: human vagina.

- Mode of transmission: endogenous (normal flora gets disturbed, increased pH).

- Virulence factors and pathogenesis:
- Flourish when the vaginal pH increases, reduction of vaginal Lactobacillus.
- Follows menses or antibiotic therapy.

- Diseases:

❖ Bacterial vaginosis:

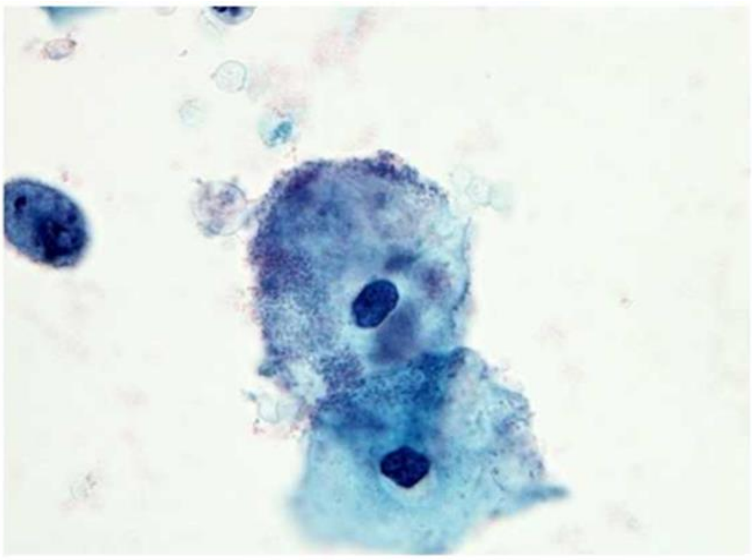
- In bacterial vaginosis, alterations in the normal vaginal flora (specifically loss of lactobacilli and overgrowth of mixed anaerobic organisms) produce a grayish-white discharge with a "fishy" odor that becomes more prominent with addition of potassium hydroxide (Amine whiff test).

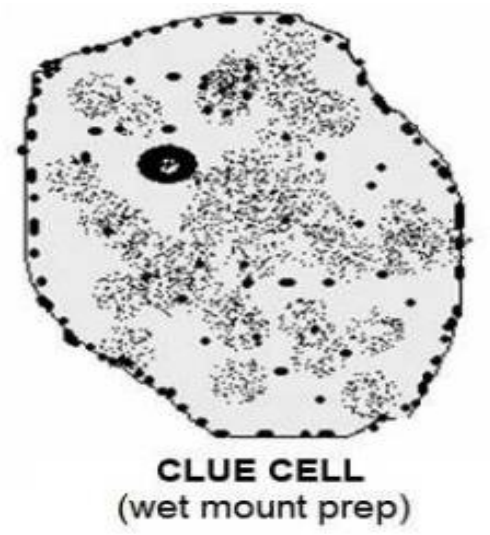
- Wet mount microscopy of the discharge (preferred for diagnosis) and cytologic smears characteristically show clue cells, which are vaginal squamous epithelial cells covered with multiple, small adherent bacteria (G. vaginalis organisms).

- Unlike trichomoniasis or Candida vulvovaginitis, there is no evidence of vaginal inflammation (nonpainful vs vaginitis).

- Associated with sexual activity, but not sexually transmitted.

- Bacterial vaginosis is usually treated with metronidazole or clindamycin, but topical regimens may also be used.





CLUE CELL
(wet mount prep)

Genus: Bacteroides

- Genus Features:

- Gram-negative rod.
- Anaerobic.

- Species of medical Importance: Bacteroides fragilis.

Bacteroides fragilis

- Habitats: human colon; the genus Bacteroides is the predominant anaerobe.

- Mode of transmission: endogenous from bowel defects (from cytotoxic drug use, cancer), surgery, or trauma.

- Virulence factors and pathogenesis:

- Modified LPS has reduced endotoxin activity.
- Capsule is antiphagocytic.

- Diseasess:

- Septicemia, peritonitis (often mixed infections), and abdominal abscess.

- It may cause appendicitis that may perforate to evolve into an intraabdominal abscess.

- Although most infections within the abdominal cavity are polymicrobial, B. fragilis is a common anaerobic gram-negative bacillus that is frequently isolated.

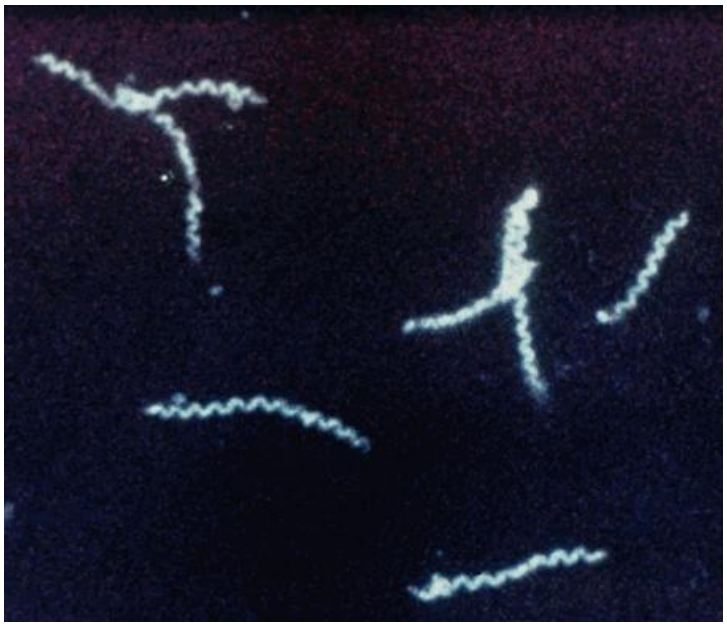
- In addition to B. fragilis, common bacterial isolates from intraabdominal infections include other members of the normal colonic flora such as Escherichia coli, enterococci, and streptococci.

Spirochetes

- Spirochetes are thin-walled, flexible, spiral rods (corkscrew-shaped organism).
- They are motile through the undulation of axial filaments (endoflagella) that lie under the outer sheath.
- Three genera of spirochetes cause human infection:

1. Treponema: which causes syphilis.
2. Leptospira: which causes leptospirosis.
3. Borrelia: which causes Lyme disease (Burgdorferi) and relapsing fever (Recurrentis).

- Treponemes and Leptospira are so thin that they are seen only by dark-field microscopy or direct fluorescent antibody (DFA) microscopy.
- Borrelia are larger (Borrelia is Big). Only Borrelia can be visualized using aniline dyes (Wright or Giemsa stain) in light microscopy due to size.



Genus: Treponema

- Genus Features:

- Spirochetes: spiral with axial filament (endoflagellum).
- Poorly visible on Gram stain but gram-negative envelope.

- Species of Medical Importance: Treponema pallidum.

Treponema pallidum

- Distinguishing Features:

- Thin spirochete, not reliably seen on Gram stain.
- Basically, a gram-negative cell envelope. Outer membrane has endotoxin-like lipids.
- Axial filaments = endoflagella = periplasmic flagella.
- Cannot culture in clinical lab; serodiagnosis.

- Habitats: human genital tract.

- Mode of transmission:

- Sexual exposure leads to venereal syphilis. The bacteria usually enter the body during sexual intercourse.
- Transplacental leading to congenital syphilis.
- Fresh blood transfusion: T. pallidum is not transmitted by stored blood because it dies when stored at 4 C within 3-5 days.

- Virulence factors and pathogenesis:

- Disease characterized by endarteritis resulting in lesions.
- Strong tendency to chronicity.

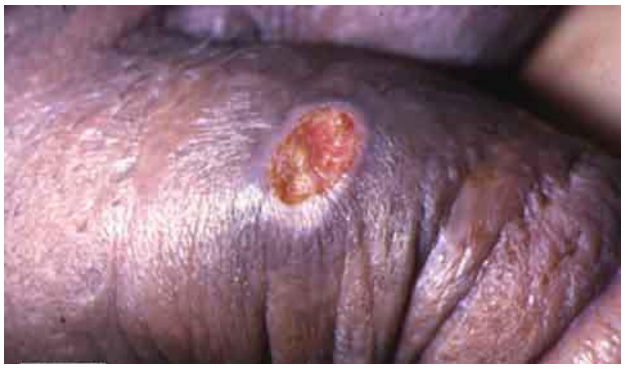
- Diseases:

- Classically, untreated syphilis has three distinct stages and a latent stage between the second and third stages:

1. Primary syphilis:

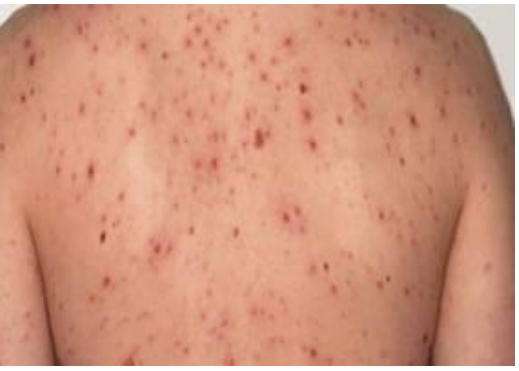
- This is usually manifested as a single hard, painless ulcer called chancre.

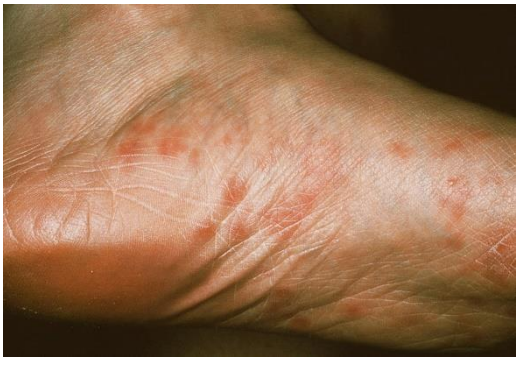
- The regional lymph nodes also become enlarged.
- The chancre represents the primary site of initial multiplication.
- It usually appears on the penis, labia, cervix, anorectal region, or around the mouth.
- The chancre heals within 4-6 weeks, even without treatment.
- Use fluorescent or dark-field microscopy to visualize treponemes in fluid from chancre.

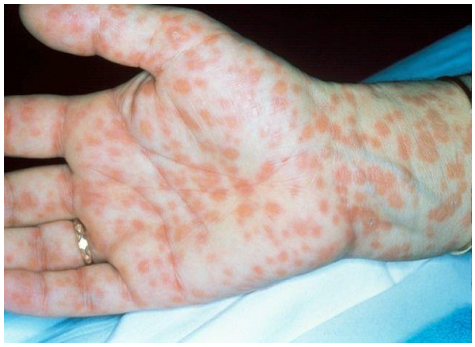


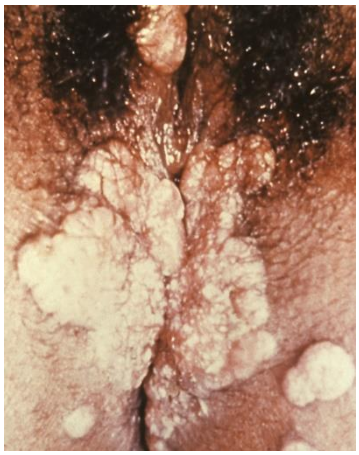
2. Secondary syphilis:

- Secondary syphilis = Systemic.
- Disseminated disease with constitutional symptoms (Fever, fatigue, myalgia, headache, and Generalized nontender lymphadenopathy).
- Maculopapular rash (including palms and soles).
- Condylomata lata (smooth, moist, painless, wart-like white lesions on genitals).
- The symptoms usually last 4-6 months and disappear spontaneously.
- Also confirmable with dark-field microscopy.
- Serologic testing: VDRL/RPR (nonspecific), confirm diagnosis with specific test (FTA-ABS).









3. The latent (hidden) stage:

- There are neither symptoms nor lesions during this stage, and the serology is positive.

- This stage can range from a few months to a lifetime.

4. Tertiary syphilis:

- This may occur in about 40% of untreated cases.

- This stage is characterized by gumma formation (chronic granulomas) in the internal organs and bones and syphilitic lesions that may lead to cardiovascular syphilis and neurosyphilis (tabes dorsalis).

- Aortitis (vasa vasorum destruction)

- Neurosyphilis (tabes dorsalis, general paresis), Argyll Robertson pupil (constricts with accommodation but is not reactive to light; also called "prostitute's pupil" since it accommodates but does not react).

- Signs: broad-based ataxia, ⊕ Romberg, Charcot joint, stroke without hypertension.

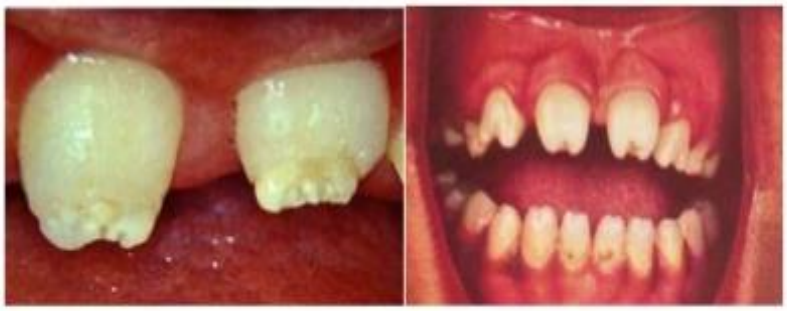
- Serology is positive. For neurosyphilis: test spinal fluid with VDRL, FTA-ABS, and PCR.

❖ Congenital syphilis:

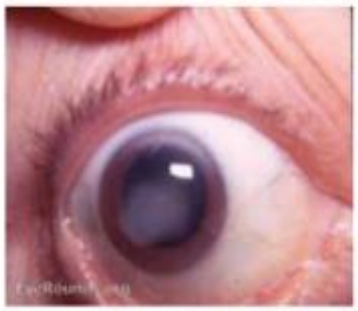
- In utero infection can lead to a stillbirth (a baby born dead) or giving birth to a baby who dies shortly after birth, or latent infections.

- Presents with facial abnormalities such as rhagades (linear scars at angle of mouth), snuffles (nasal discharge), saddle nose, notched (Hutchinson) teeth, mulberry molars, and short maxilla; saber shins; CN VIII deafness.

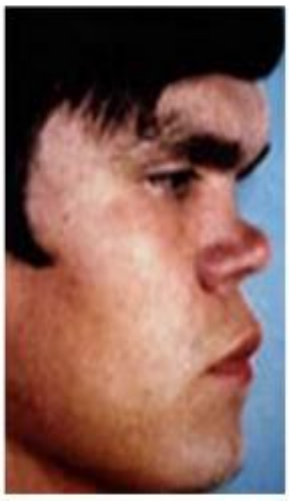
- To prevent, treat mother early in pregnancy, as placental transmission typically occurs after first trimester.



Hutchinson's teeth:
widely spaced, pegged teeth

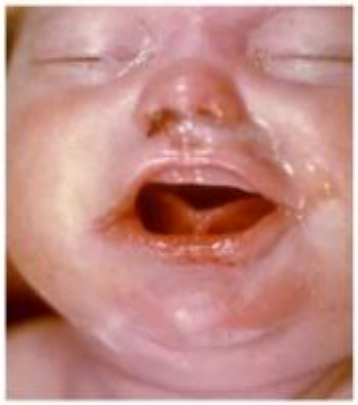


Keratitis



Frontal bossing
Saddle nose

Snuffles



- Diagnosis:

- Because T. pallidum is so thin, it cannot be visualized with standard Gram stain and microscopy. Classically, darkfield microscopy of material scraped from the surface of the cutaneous syphilitic lesion must be employed to visualize T. pallidum. In positive cases, T. pallidum appears as a motile helical organism on darkfield examination.

- The serologic tests can be divided into two groups: nontreponemal tests and treponemal tests:

A. Nontreponemal tests:

- Nontreponemal tests evaluate for the presence of cardiolipin, a byproduct of treponemal infection.

- Examples of nontreonemal tests: rapid plasma reagin (RPR) test and venereal disease research lab (VDRL).

- In the rapid plasma reagin (RPR) test, the patient's serum is mixed with a solution of cardiolipin, cholesterol and lecithin → Aggregation, or "floccutation," of the sample demonstrates the presence of cardiolipin antibodies in the patient's serum.

- This test is considered a nontreponemal serologic test because it does not detect treponemal organisms or antibodies directed against treponemal organisms. Instead, it detects antibodies to human cellular lipids released into the bloodstream after cell destruction by T. pallidum.

- The nontreponemal tests are best used for screening and have a sensitivity of 70-99%.

- Because nontreponemal tests are affected by antitreponemal therapy, they can be used to follow disease progression and therapeutic response.

❖ Mnemonic:

▪ False-Positive results on VDRL with:

- Pregnancy
- Viral infection (Infectious Mononucleosis, Hepatitis).
- Drugs.
- Rheumatic fever.
- Lupus and Leprosy.

B. The treponemal tests:

- The treponemal tests detect specific treponemal antigens and are typically used for confirmation of a positive nontreponemal test or when clinical suspicion remains high despite a negative nontreponemal test.

- Examples of treponemal tests: Fluorescent treponemal antibody absorption (FTA-ABS; most widely used test) and treponema pallidum microhemagglutination (MHA-TP).

- The treponemal tests are not affected by antitreponemal therapy and will remain positive for life.

- A positive FTA-ABS confirms infection with T. pallidum. This specific confirmatory test detects antibodies directed against Treponema (spirochetal antibodies) and is carried out through indirect immunofluorescence of patient serum mixed with whole killed T. pallidum.

- Humoral antibody response often takes 4 weeks to develop, false-negative serologic testing is common early in the course of disease. Nontreponemal tests are particularly susceptible to initial false-negative results (as in this patient); treponemal tests are more sensitive in early infection and are often used preferentially in this setting.

▪ Treatment:

- Benzathine penicillin (long-acting form) for primary and secondary syphilis (no resistance to penicillin); penicillin G for congenital and late syphilis.

- Jarisch-Herxheimer reaction:

- Starts generally during the first 24 hours of antibiotic treatment.
- Flu-like syndrome (fever, chills, headache, myalgia) after antibiotics are started; due to killed bacteria (usually spirochetes) releasing endotoxins. Most cases are self-limited and do not require intervention.

Genus: Leptospira

- Genus Features:
- Spirochetes: thin, with terminal hooks.
- Too thin to visualize, but gram-negative cell envelope.

- Species of medical Importance: Leptospira interrogans.

Leptospira interrogans

- Distinguishing Features:
- Spirochetes with tight terminal hooks.

- Seen on dark-field microscopy but not light microscopy.

- Can be cultured in vitro; aerobic.

- Generally diagnosed by serology.

- Habitats: wild and domestic animals (zoonosis).

- Mode of transmission:
- Contact with animal urine in water. Organism penetrates mucous membranes or enters small breaks in epidermis.

- In U.S., via dog, livestock, and rat urine through contaminated recreational waters (jet skiers) or occupational exposure (sewer workers).

- Virulence factors and pathogenesis: no toxins or virulence factors known.

- Diseases:

❖ Leptospirosis:

- Leptospirosis exhibits a great variety of clinical manifestations, ranging from a mild self-limiting febrile illness (most patients) to a fulminating fatal illness associated with hepatorenal failure (weil's disease).

- Conjunctival suffusion: redness of the conjunctiva that resembles conjunctivitis but is noninflammatory, is an important diagnostic clue.

- Weil disease (icterohemorrhagic leptospirosis): severe form with jaundice and azotemia from liver and kidney dysfunction, fever, hemorrhage, and anemia.

Genus: Borrelia

- Genus Features:
- Larger spirochetes (Borrelia is Big).
- Difficult to culture.

- Species of medical Importance: Borrelia burgdorferi.

Borrelia burgdorferi

- Distinguishing Features:
- Large gram-negative spirochete.

- Best visualized with Giemsa's stain.

- Habitats: white-footed mice (nymphs) and white-tailed deer (adult ticks).

- Mode of transmission:
- By Ixodes deer ticks (also vector for Anaplasma spp. and protozoa Babesia).

- Infected Ixodes ticks are commonly encountered in outdoor recreational areas (camping/hiking in New England).

- Virulence factors and pathogenesis:
- B. burgdorferi invades skin and spreads via the bloodstream to involve primarily the heart, joints, and central nervous system.

- Diseases:

❖ Lyme disease (#1 tick-borne disease in the U.S.):

A. Stage 1 (early localized): erythema migrans (typical "bulls-eye" configuration is pathognomonic but not always present), flu-like symptoms.

- Erythema migrans (EM) is the classic initial skin lesion of Lyme disease.

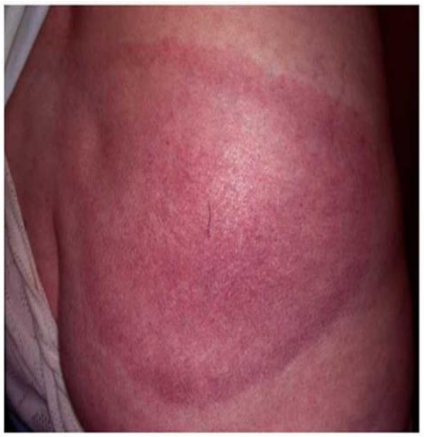
- EM occurs at the site of Borrelia burgdorferi inoculation following a bite by an infected Ixodes tick bite.

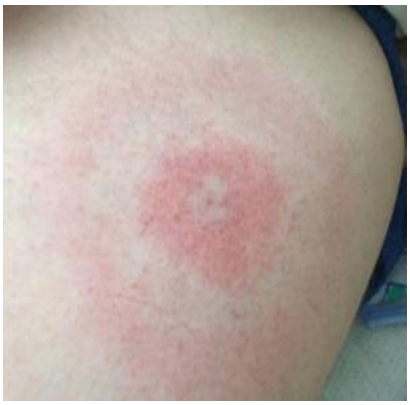
- It begins as an erythematous macule that enlarges with an advancing erythematous border as the bacteria migrate slowly through the skin outward from the inoculation site.

- The classic lesion is erythematous (red) and ring-shaped (annular) due to development of a central clearing.

B. Stage 2 (early disseminated): secondary lesions, carditis, AV block, facial nerve palsy (bilateral), migratory myalgias/transient arthritis.

C. Stage 3 (late disseminated): encephalopathy, chronic arthritis.





- Treatment: doxycycline (1st line as it has the advantage of simultaneously preventing or treating coexisting human granulocytic anaplasmosis, an infection also carried by I. scapularis); amoxicillin and, if severe illness, CNS signs, or heart block, ceftriaxone

❖ Mnemonic:

- A Key Lyme pie to the FACE:
- Facial nerve palsy (typically bilateral).
- Arthritis.
- Cardiac block.
- Erythema chronicum migrans.

Borrelia Recurrentis

- Mode of transmission: human body louse (ticks transmit all known species of borrelia except B. Recurrentis).

- Diseases:

❖ Relapsing fever:

- It is a febrile septicemic disease with sudden onset.

- Fever persists for 3 to 7 days and is followed by an afebrile interval of several days to several weeks.

- Relapses occur as a result of antigenic variations in the causative Borrelia species. Such variations are due to programmed rearrangement of bacterial DNA encoding surface proteins.

- The new antigenic variants will not be destroyed by antibodies directed against the original infecting one. Thus, the patient clinically improves until the new clone multiplies sufficiently to cause another relapse (Borrelia Recurrentis cause Relapsing Fever).

Unusual Bacteria

- Comparison of the Chlamydiaceae, Rickettsiaceae, and Mycoplasmataceae with Typical Bacteria:

	Typical Bacteria (S. aureus)	Chlamydiaceae	Rickettsiaceae	Mycoplasmataceae
Obligate intracellular parasite?	Mostly no	Yes	Yes	No
Make ATP?	Normal ATP	No ATP	Limited ATP	Normal ATP
Peptidoglycan layer in cell envelope?	Normal peptidoglycan	Modified Peptidoglycan (lacks muramic acid)	Normal peptidoglycan	No peptidoglycan

Family: Chlamydiaceae

- Family Features:

- Can not make ATP.
- Obligate intracellular bacteria.
- Cell wall lacks muramic acid.
- Not seen on Gram stain.

- Genera of Medical Importance:

- Chlamydia trachomatis.
- Chlamydophila pneumoniae (formerly Chlamydia pneumoniae).
- Chlamydophila psittaci (formerly Chlamydia psittaci).

Chlamydia trachomatis

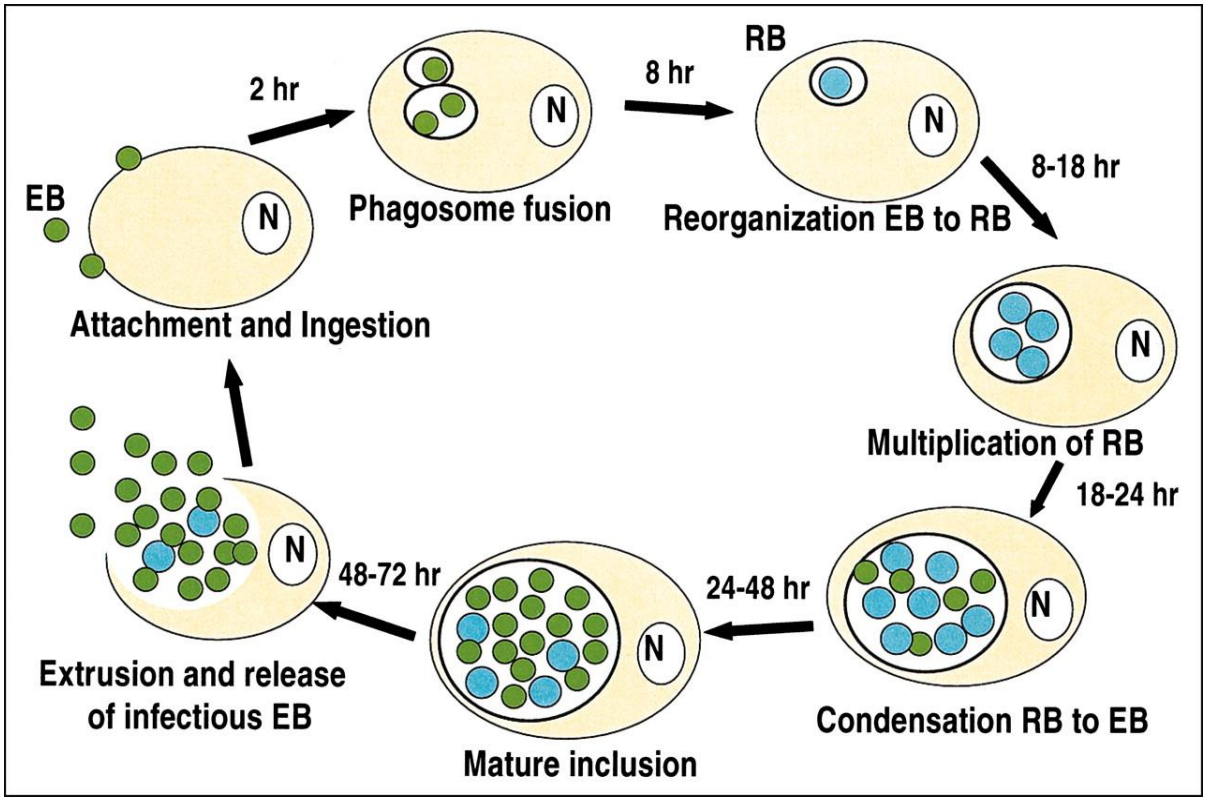
- Distinguishing Features:

- Obligate intracellular bacterium; cannot make ATP.

- Not seen on Gram stain. The chlamydial cell wall lacks classic peptidoglycan (due to reduced muramic acid), rendering β-lactam antibiotics less effective.

- They multiply by binary fission with a biphasic developmental cycle:
  - This cycle explains pathogenesis of diseases caused by chlamydia.
  - There are two morphological forms, elementary bodies (EBs) and reticulate bodies (RBs).

- Elementary bodies are small infectious "Enfectious" particles, metabolically inert, can survive extracellularly but do not replicate.
- The EBs attaches to the host cell and enters by phagocytosis.
- Within the cell it increases in size and becomes reticulate body which is metabolically active.
- The Reticulate body Replicate by binary fission, yielding pleomorphic organisms which mature to new EBs.
- Release of EBs follows rupture of the cell to infect new cells.
- Within the infected cells, the site of replication appears as an inclusion body which can be stained and visualized microscopically. These inclusions are useful in diagnosis.



- Habitats: human genital tract and eyes.
- Mode of transmission: sexual contact and at birth; trachoma is transmitted by hand-to-eye contact and flies.
- Virulence factors and pathogenesis: infection of nonciliated columnar or cuboidal epithelial cells of mucosal surfaces leads to granulomatous response and damage.

- Diseases:

I. Non-sexually transmitted diseases:

A. Ocular infections:

1. Trachoma:

- Serotypes A, B, and C.

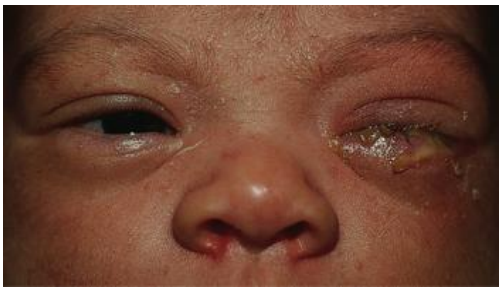
- Leading cause of preventable infectious blindness in Africa.

- Chronic infection cause blindness: Follicular conjunctivitis leading to conjunctival scarring, and inturned eyelashes leading to corneal scarring and blindness.

2. Neonatal conjunctivitis (inclusion conjunctivitis):

- Serotypes D through K.

- It is the most common cause of neonatal conjunctivitis.



B. Neonatal pneumonia: in neonates born vaginally to infected mothers.

II. Genital tract infections (sexually transmitted diseases):

1. Nongonococcal (NGG) urethritis:

- Serotypes D-K (This is the most common bacterial STD in the U.S., although overall, herpes and HPV are more common).

- Cervicitis, salpingitis, procitis and epididymitis.

- Chronic or repeated infections can cause pelvic inflammatory disease (PID), infertility and ectopic pregnancy.

2. Lymphogranuloma venereum (LVG):

- Serotypes L1, 2, 3

- LGV is a chronic disease characterized by an initial painless small ulcer on the genital mucosa that contains cells infected with C. trachomatis.

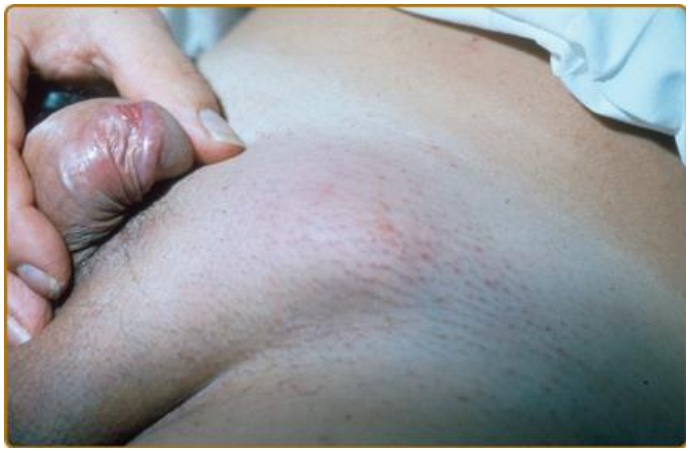
- The painless nature of this ulcer helps distinguish LGV from other entities.

- This ulcer is followed weeks later by swollen, painful inguinal nodes that coalesce, ulcerate, and rupture; these are referred to as buboes.

- Histologically, LGV is characterized by chlamydial inclusion bodies in the cellular cytoplasm.

- The recommended treatment for LGV is doxycycline.

- If left untreated, this condition can cause fibrosis, lymphatic obstruction, and anogenital strictures and fistulas.



Chlamydia trachomatis serotypes	Diseases	Mnemonics
Types A, B, and C	Trachoma: Chronic infection, cause blindness due to follicular conjunctivitis in Africa.	ABC = Africa, Blindness, Chronic infection.
Types D–K	Urethritis/PID, ectopic pregnancy, neonatal pneumonia (staccato cough) with eosinophilia, neonatal conjunctivitis.	D-K = everything else. Neonatal disease can be acquired during passage through infected birth canal.
Types L1, L2, and L3	Lymphogranuloma venereum: small, painless ulcers on genitals with swollen, painful inguinal lymph nodes that ulcerate (buboes).	

- Lab diagnosis: cytoplasmic inclusions seen on Giemsa or fluorescent antibody-stained smear, PCR, and nucleic acid amplification test.

- Treatment: doxycycline or azithromycin (favored because onetime treatment). Add ceftriaxone for possible concomitant gonorrhea.

- Prevention:

- Erythromycin is effective in infected mothers to prevent neonatal disease.

- Treat neonatal conjunctivitis with systemic erythromycin to prevent pneumonia.

Genus: Chlamydophila

- Diseases Caused by Chlamydophila Species:

- C. pneumoniae and C. psittaci cause atypical pneumonia; transmitted by aerosol.

Genus: Rickettesia

- Genus Features:

- Gram-negative bacilli (too small to stain well with Gram stain).
- Obligate intracellular bacteria (do not make sufficient ATP for independent life).

- Species of Medical Importance:

- Rickettsia rickettsii.
- Rickettsia prowazekii.
- Rickettsia typhi.
- Orientia tsutsugamushi (formerly R. tsutsugamushi).
- Ehrlichia spp.

❖ Rickettsial diseases and vector-borne illness:

A. Rash common:

1. Rocky Mountain spotted fever:

- Rickettsia rickettsii, vector is tick (Dermacentor).

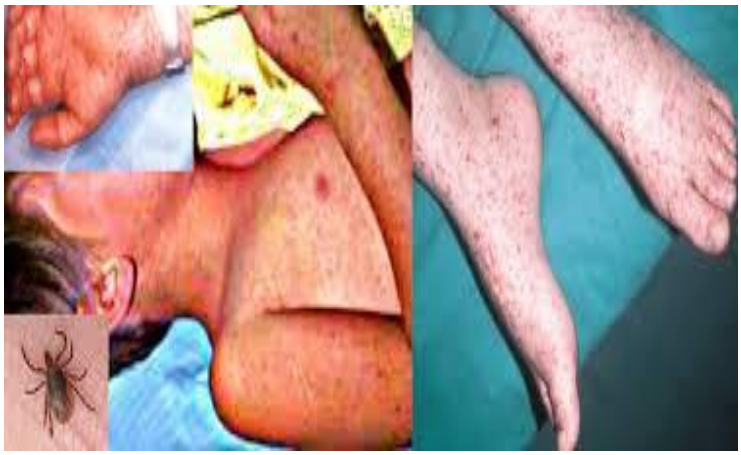
- Despite its name, disease occurs primarily in the South Atlantic states, especially North Carolina.

- Invade endothelial cells lining capillaries, causing vasculitis in many organs including brain, liver, skin, lungs, kidney, and gastrointestinal tract.

- Classic triad: headache, fever, rash (vasculitis).

- Rash (maculopapular → petechial) starts on ankles and wrists and then spreads to the trunk, palms, soles, and face (centripetal rash, from extremities to trunk).

- Palms and soles rash is seen in Coxsackievirus A infection (hand, foot, and mouth disease), Rocky Mountain spotted fever, and 2° Syphilis (you drive CARS using your palms and soles).



2. Typhus:

- Endemic (fleas): R. typhi.

- Epidemic (human body louse): R. prowazekii.

- Rash starts centrally and spreads out, sparing palms and soles.

- Rickettsii on the wRists, Typhus on the Trunk.

B. Rash rare:

1. Ehrlichiosis:

- Ehrlichia chaffeensis, vector is tick.

- Monocytes with morulae (B) in cytoplasm (mulberry-like inclusions).

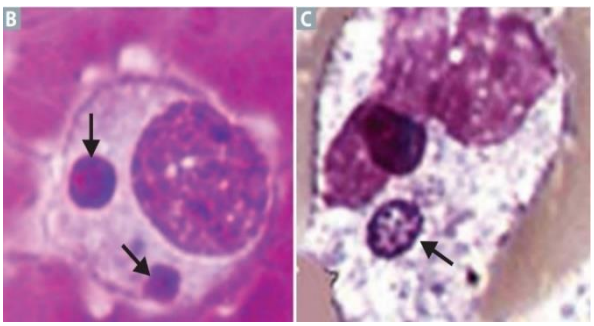
- Present with non-specific symptoms (fever, chills, myalgia, headache) and lymphopenia.

2. Anaplasmosis:

- Anaplasma, vector is Ixodes tick.

- Granulocytes with morulae (C) in cytoplasm.

- MEGA berry: Monocytes = Ehrlichiosis. Granulocytes = Anaplasmosis.



3. Q fever:

- Coxiella burnetii, no arthropod vector.

- Spores inhaled as aerosols from cattle/sheep amniotic fluid.

- A patient with exposure to waste from farm animals who develops a nonspecific illness (myalgia, fatigue, fever [more than 10 days], retroorbital headache with photophobia) with a normal leukocyte count and increased liver enzymes should be evaluated for acute Q fever infection.

- Pneumonia is one of the primary signs of acute Q fever.

- Most common cause of culture ⊖ endocarditis.

- Q fever is Queer because it has no rash or vector and its causative organism can survive outside in its endospore form. Not in the Rickettsia genus, but closely related.

▪ Treatment: doxycycline (inhibitor of bacterial protein synthesis).

Family: Mycoplamataceae

- Family Features:

- Smallest free-living (extracellular) bacteria.

- Missing peptidoglycan (no cell wall). The lack of a cell wall renders these organisms:

1. Resistant to the beta-lactam antibiotics (penicillin and cephalosporins).
2. Unstainable by the Gram stain (but readily stained by Giemsa stain).
3. Variable in shape (pleomorphic).

- They are the only bacteria that contain sterol in the cell membrane.

- Require serum enriched medium containing cholesterol for in vitro culture (Eaton's agar), because their cell membrane is composed of a single cholesterol-rich phospholipid bilayer.

- Genera of medical Importance:

- Mycoplasma pneumoniae.
- Ureaplasma urealyticum.

Mycoplasma pneumoniae

- Distinguishing Features:

- Extracellular, tiny, flexible.

- These organisms completely lack a peptidoglycan cell wall, cell envelope or capsule; not seen on Gram-stained smear.

- Membrane with cholesterol but does not synthesize cholesterol.

- Sputum cultures require a complex acellular medium enriched with cholesterol in order to grow.

- Habitats: human respiratory tract.

- Mode of transmission: respiratory droplets; close contact: families, military recruits, medical school classes.

- Virulence factors and pathogenesis:

- Attaches to respiratory epithelium via P1 protein.

- Inhibits ciliary action.

- Produces hydrogen peroxide, superoxide radicals, and cytolytic enzymes, which damage the respiratory epithelium, leading to necrosis and a bad, hacking cough (walking pneumonia).

▪ Diseases:

❖ Walking pneumonia:

- Pharyngitis which may develop into an atypical pneumonia with persistent hack (little sputum produced).

- Most common atypical pneumonia (along with viruses) in young adults.

- Patients experience a chronic dry nagging cough, low-grade fever and malaise.

- The tell-tale sign is a chest X-ray that looks much worse than the patient appears clinically.

- Clinical sequelae of M. pneumonia infection can go beyond the airways.

- M. Pneumoniae infection can cause hemolysis due to antigenic similarity between antigens in the cell membrane of M. pneumoniae and in the cell membrane of erythrocytes.

- When the immune system mounts a response against these M. pneumonia antigens it also destroys some RBCs resulting in a mild anemia.

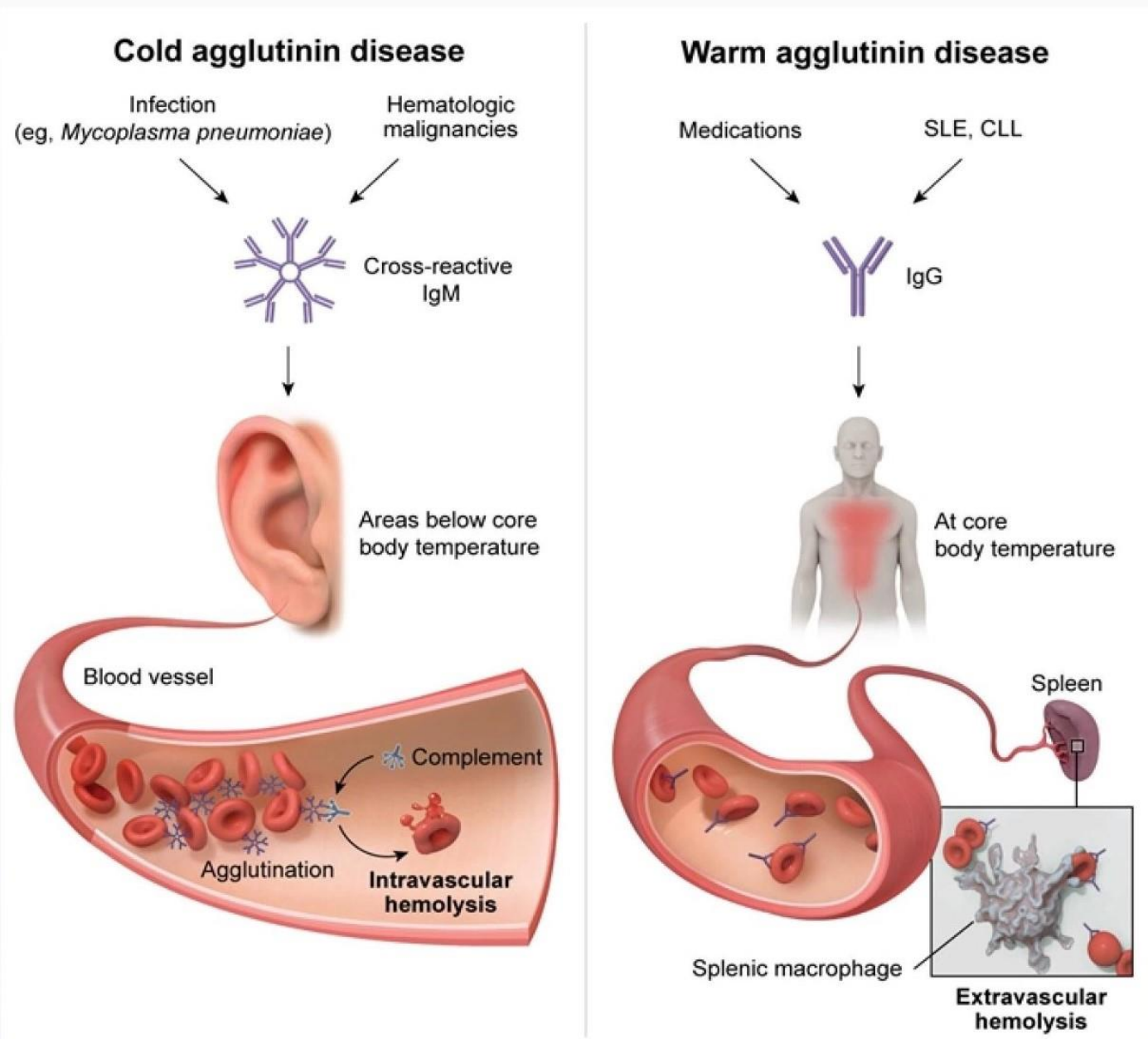
- These antibodies that cross-react between M. pneumoniae and RBCs are called cold agglutinins (IgM) because they are able to agglutinate RBCs in vitro at low temperatures.

- This can be easily done at bedside with blood drawn into an EDTA-containing tube and a cup of ice.

- After the infection has been eliminated and the immune system is no longer activated against M. pneumoniae, the concentration of these antibodies decreases and the anemia spontaneously resolves.

- Cold agglutinins are also associated with Epstein-Barr virus infection and hematologic malignancies in addition to infection with Mycoplasma pneumoniae.

- M. pneumoniae can also cause the Stevens-Johnson syndrome and joint pains among other rare sequelae.



CLL = chronic lymphocytic leukemia; **SLE** = systemic lupus erythematosus.

❖ N.B:

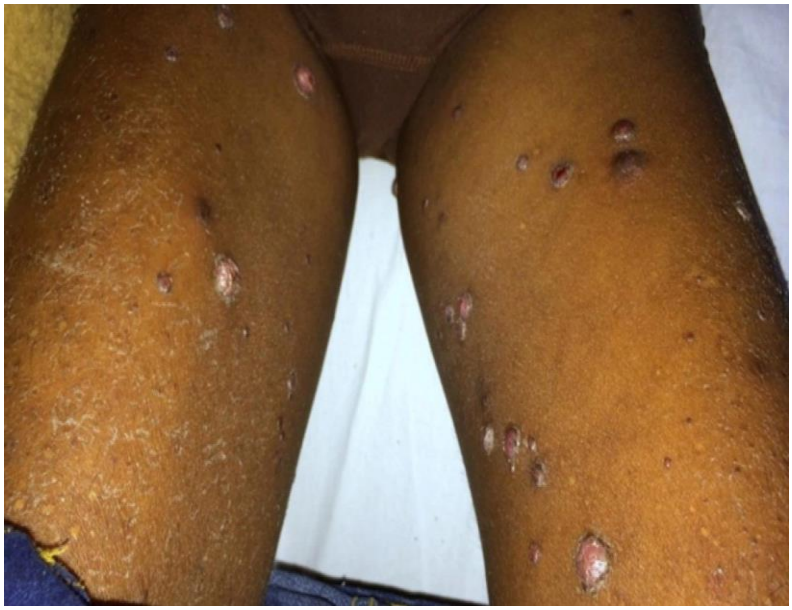
- Organisms in the Mycoplasma genus, including Ureaplasma urealyticum, lack peptidoglycan cell walls.
- Thus, cell wall synthesis inhibitors such as penicillins, cephalosporins, carbapenems, and vancomycin would be ineffective against these organisms.
- Mycoplasma are very small organisms that have only a single phospholipid bilayer membrane separating them from the environment.
- Drugs that are effective against the Mycoplasma genus include anti-ribosomal agents like the macrolides, doxycycline, or fluoroquinolone.

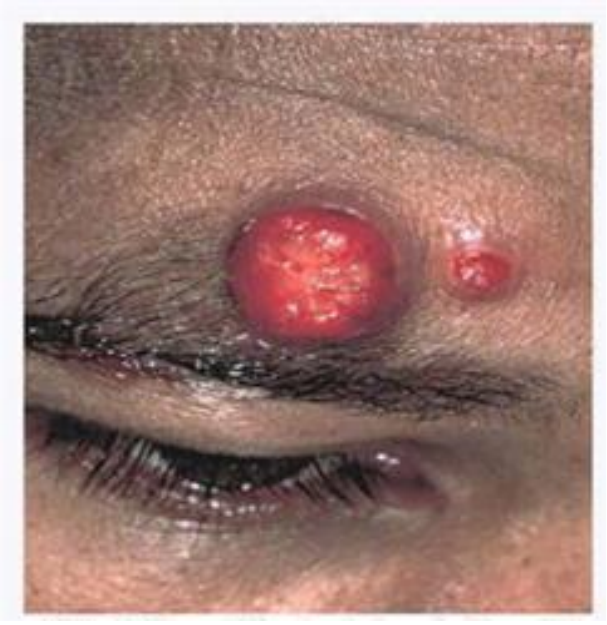
Ureaplasma urealyticum

- Distinguishing Features: Member of family Mycoplasmataceae.
- Virulence factors and Pathogenesis: Urease positive.
- Diseases: Urethritis, prostatitis, renal calculi.

Bartonella hensele

- This organism resides in the oral cavity of cats and is transmitted to humans by cat scratches and bites.
- Bartonella henselae causes cat-scratch disease, bacillary angiomatosis and culture-negative endocarditis.
- Cat-scratch disease is characterized by low fever, lymphadenopathy and a self-limited course.
- Bartonella henselae can also cause bacillary angiomatosis (BA) in immunocompromised patients. BA presents with red-purple papular skin lesions.
- These vascular proliferations may also be found within the viscera. BA can be fatal if left untreated.





Bugs with exotoxins

Bacteria with exotoxins

BACTERIA	TOXIN	MECHANISM	MANIFESTATION
Inhibit protein synthesis			
Corynebacterium diphtheriae	Diphtheria toxin[a]	Inactivate elongation factor (EF-2)	Pharyngitis with pseudomembranes in throat and severe lymphadenopathy (bull neck), myocarditis
Pseudomonas aeruginosa	Exotoxin A[a]		Host cell death
Shigella spp	Shiga toxin (ST)[a]	Inactivate 60S ribosome by removing adenine from rRNA	GI mucosal damage → dysentery; ST also enhances cytokine release, causing hemolytic-uremic syndrome (HUS)
Enterohemorrhagic *E coli*	Shiga-like toxin (SLT)[a]		SLT enhances cytokine release, causing HUS (prototypically in EHEC serotype O157:H7) Unlike *Shigella*, EHEC does not invade host cells
Increase fluid secretion			
Enterotoxigenic *E coli*	Heat-**labile** toxin (LT)[a]	Overactivates adenylate cyclase (↑ cAMP) → ↑ Cl^- secretion in gut and H_2O efflux	Watery diarrhea: "**labile** in the **A**ir (**A**denylate cyclase), **stable** on the **G**round (**G**uanylate cyclase)"
	Heat-**stable** toxin (ST)	Overactivates guanylate cyclase (↑ cGMP) → ↓ resorption of NaCl and H_2O in gut	
Bacillus anthracis	Anthrax toxin[a]	Mimics adenylate cyclase (↑ cAMP)	Likely responsible for characteristic edematous borders of black eschar in cutaneous anthrax
Vibrio cholerae	Cholera toxin[a]	Overactivates adenylate cyclase (↑ cAMP) by permanently activating G_s → ↑ Cl^- secretion in gut and H_2O efflux	Voluminous "rice-water" diarrhea
Inhibit phagocytic ability			
Bordetella pertussis	Pertussis toxin[a]	Inactivates inhibitory G subunit (G_i) → activation of adenylate cyclase → ↑ cAMP	Whooping cough—child coughs on expiration and "whoops" on inspiration (toxin may not actually be a cause of cough; can cause "100-day cough" in adults)

Inhibit release of neurotransmitter			
Clostridium tetani	Tetanospasmin[a]	Both are proteases that cleave SNARE (soluble NSF attachment protein receptor), a set of proteins required for neurotransmitter release via vesicular fusion	Toxin prevents release of **inhibitory** (GABA and glycine) neurotransmitters from Renshaw cells in spinal cord → spastic paralysis, risus sardonicus, trismus (lockjaw)
Clostridium botulinum	Botulinum toxin[a]		Toxin prevents release of **stimulatory** (ACh) signals at neuromuscular junction → flaccid paralysis (floppy baby)

[a]An AB toxin (aka, two-component toxin [or three for anthrax]) with B enabling binding and triggering uptake (endocytosis) of the active A component. The A components are usually ADP ribosyltransferases; others have enzymatic activities as listed in chart.

Bacteria with exotoxins *(continued)*

BACTERIA	TOXIN	MECHANISM	MANIFESTATION
Lyse cell membranes			
Clostridium perfringens	Alpha toxin	Phospholipase (lecithinase) that degrades tissue and cell membranes	Degradation of phospholipids → myonecrosis ("gas gangrene") and hemolysis ("double zone" of hemolysis on blood agar)
Streptococcus pyogenes	Streptolysin O	Protein that degrades cell membrane	Lyses RBCs; contributes to β-hemolysis; host antibodies against toxin (ASO) used to diagnose rheumatic fever (do not confuse with immune complexes of poststreptococcal glomerulonephritis)
Superantigens causing shock			
Staphylococcus aureus	Toxic shock syndrome toxin (TSST-1)	Cross-links β region of TCR to MHC class II on APCs outside of the antigen binding site → overwhelming release of IL-1, IL-2, IFN-γ, and TNF-α → shock	Toxic shock syndrome: fever, rash, shock; other toxins cause scalded skin syndrome (exfoliative toxin) and food poisoning (heat-stable enterotoxin)
Streptococcus pyogenes	Erythrogenic exotoxin A		Toxic shock–like syndrome: fever, rash, shock; scarlet fever

Zoonotic bacteria

Zoonotic bacteria Zoonosis—infectious disease transmitted between animals and humans.

SPECIES	DISEASE	TRANSMISSION AND SOURCE
***Anaplasma* spp**	Anaplasmosis	*Ixodes* ticks (live on deer and mice)
***Bartonella* spp**	Cat scratch disease, bacillary angiomatosis	Cat scratch
Borrelia burgdorferi	Lyme disease	*Ixodes* ticks (live on deer and mice)
Borrelia recurrentis	Relapsing fever	Louse (recurrent due to variable surface antigens)
***Brucella* spp**	Brucellosis/undulant fever	Unpasteurized dairy
Campylobacter	Bloody diarrhea	Feces from infected pets/animals; contaminated meats/foods/hands
Chlamydophila psittaci	Psittacosis	Parrots, other birds
Coxiella burnetii	Q fever	Aerosols of cattle/sheep amniotic fluid
Ehrlichia chaffeensis	Ehrlichiosis	*Amblyomma* (Lone Star tick)
Francisella tularensis	Tularemia	Ticks, rabbits, deer flies
***Leptospira* spp**	Leptospirosis	Animal urine in water; recreational water use
Mycobacterium leprae	Leprosy	Humans with lepromatous leprosy; armadillo (rare)
Pasteurella multocida	Cellulitis, osteomyelitis	Animal bite, cats, dogs
Rickettsia prowazekii	Epidemic typhus	Human to human via human body louse
Rickettsia rickettsii	Rocky Mountain spotted fever	*Dermacentor* (dog tick)
Rickettsia typhi	Endemic typhus	Fleas
***Salmonella* spp (except *S typhi*)**	Diarrhea (which may be bloody), vomiting, fever, abdominal cramps	Reptiles and poultry
Yersinia pestis	Plague	Fleas (rats and prairie dogs are reservoirs)

CHAPTER 2

MYCOLOGY

- Mycology is the study of fungi (molds, yeasts, and mushrooms)
- All fungi are Eukaryotic (true nucleus, 80S ribosomes, mitochondria).
- They are heterotrophic (can't make their own food) and require organic carbon.
- They are saprophytic (lives on dead organic material).
- Have a rigid cell wall containing glucan and chitin.
- Ergosterol is the major membrane sterol.

Fungal morphology

1. Molds are multicellular fungi organized into hyphae:

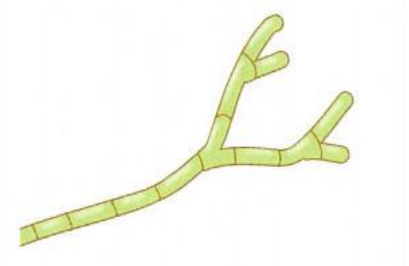
- Hyphae = filamentous cellular units of molds.

a. Nonseptate Hyphae:

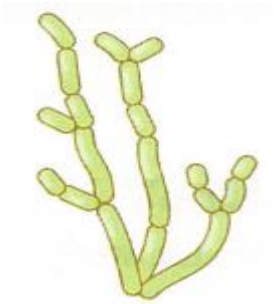
- No cross walls.
- Broad hyphae with irregular width.
- Broad angle of branching.

b. Septate Hyphae:

- With cross walls.
- Width is fairly regular (tube-like).



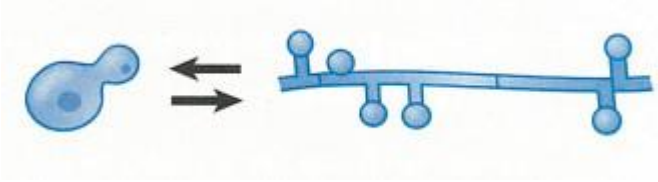
2. Pseudohyphae (candida albicans): hyphae with constrictions at each septum.



3. Yeasts are unicellular (round to oval) fungi.

4. Dimorphic Fungi:

- Fungi able to convert from hyphal to yeast or yeast-like forms.
- At room temperature (cold temperature), it grows as a mold.
- At body temperature, it grows as a yeast.



❖ Mnemonic:

▪ Body Heat Changes Shape for the dimorphic fungi:

- Blastomyces.
- Histoplasma.
- Coccidioides.
- Sporothrix.

❖ Fungi reproduction:

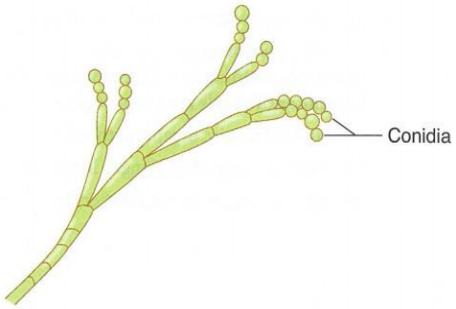
▪ Yeast reproduction is typically by an asexual process called budding.

▪ Molds reproduction is typically by spore formation, which can be sexual or asexual.

▪ Spore types:

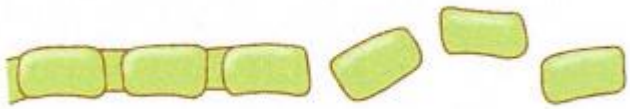
1. Conidia:

- Asexual spores.
- Formed off of hyphae.
- Common.

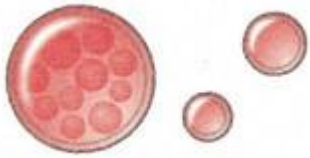


2. Blastoconidia: "Buds" on yeasts (asexual budding daughter yeast cells).

3. Arthroconidia: Asexual spores formed by a "joint".



4. Spherules and Endospores (Coccidioides): Spores inside the spherules in tissues.



- All medically important fungi may be divided into a few groups according to the area of involvement:

1. Cutaneous mycoses include dermatophytosis and pityriasis versicolor.

2. Subcutaneous mycoses include Sporotrichosis.

3. Mycoses with systemic involvement (most often affecting the lungs) are histoplasmosis, coccidioidoses and blastomycosis.

4. Opportunistic mycoses mainly affect immunosuppressed patients. These include Candida, Aspergillus, Mucor and Rhizopus species.

Non-Systemic fungal infection

Cutaneous Fungal Infections (Without Systemic Disease)

1. Malassezia furfur:

- Normal skin flora (lipophilic yeast).

- Pityriasis or tinea versicolor:

- Superficial infection of keratinized cells. The infection is localized to the stratum corneum of the skin.

- Caused by Malassezia furfur, a yeast-like fungus (not a dermatophyte despite being called tinea).

- Can occur any time of year but common in summer (hot, humid weather).

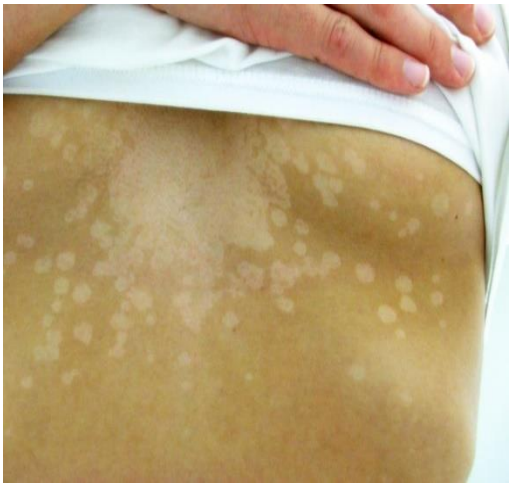
- Degradation of lipids produces acids that damage melanocytes and cause hypopigmented and/or pink patches.

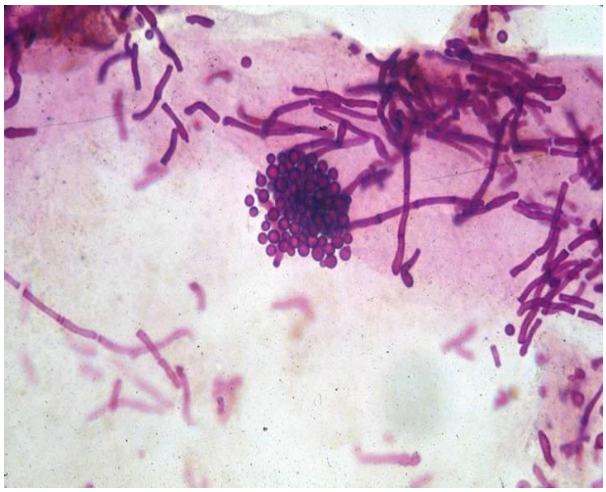
- Hypopigmented areas are commonly located on the back and chest and are more visible on sun tanned skin because affected areas do not tan.

- Pityriasis versicolor is usually asymptomatic and is only problematic in terms of its cosmetic appearance.

- KOH mount of skin scales: spaghetti and meatballs appearance on microscopy. KOH degrades human tissues leaving hyphae and yeast visible.

- Treatment: topical and/or oral antifungal medications, selenium sulfide.

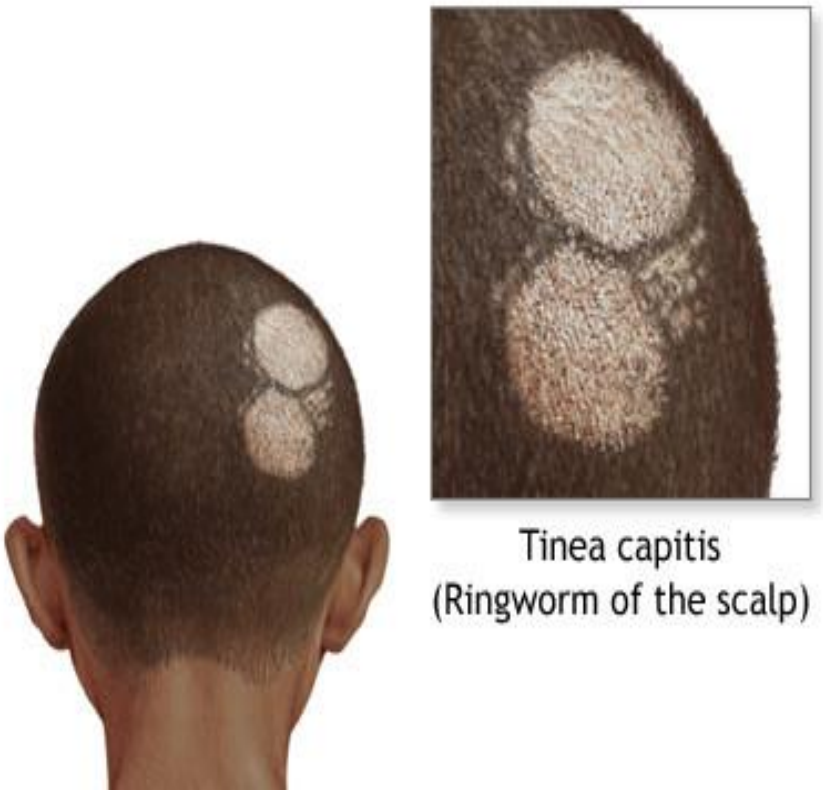




2. Dermatophytes:

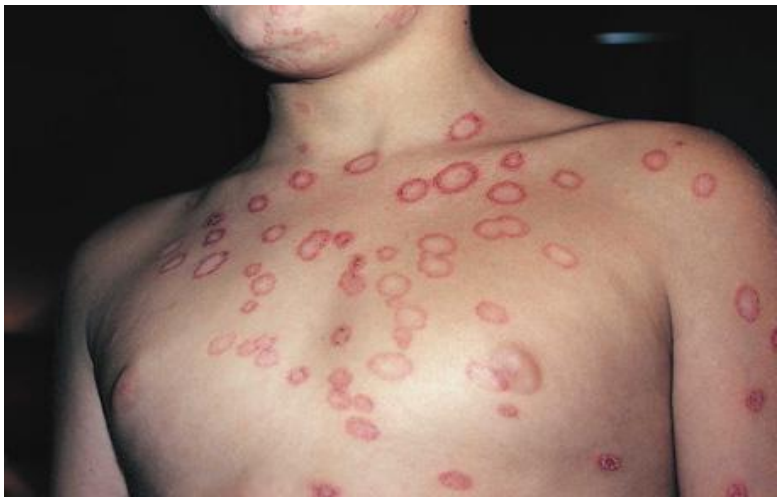
- Filamentous fungi (monomorphic).
- Infect only skin and hair and/or nails (do not disseminate).
- Three genera: Trichophyton, Microsporum, Epidermophyton.
- Dermatophytic Infections = Tineas (Ringworms)

- Tinea is the clinical name given to dermatophyte (cutaneous fungal) infections.
- Itching is the most common symptom of all tineas.
- Tinea capitis occurs on head, scalp.
- Associated with lymphadenopathy, alopecia, scaling.
- The most serious of the tinea capitis is favus (tinea favosa), which causes permanent hair loss and is very contagious.

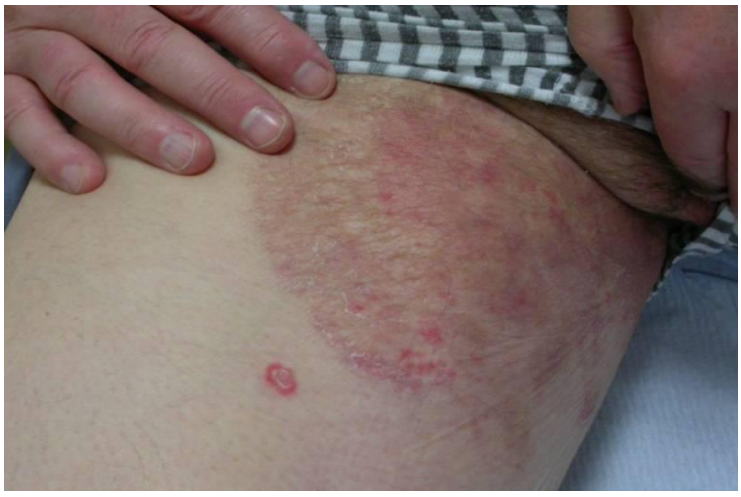


Tinea capitis
(Ringworm of the scalp)

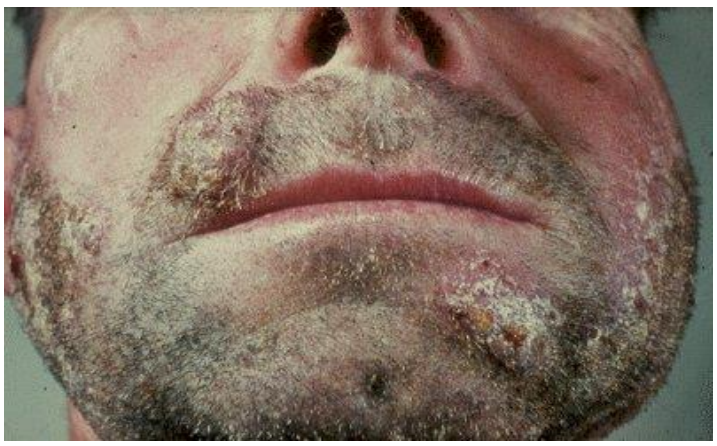
- Tinea corporis occurs on body. Characterized by erythematous scaling rings ("ringworm") and central clearing. Can be acquired from contact with an infected cat or dog.



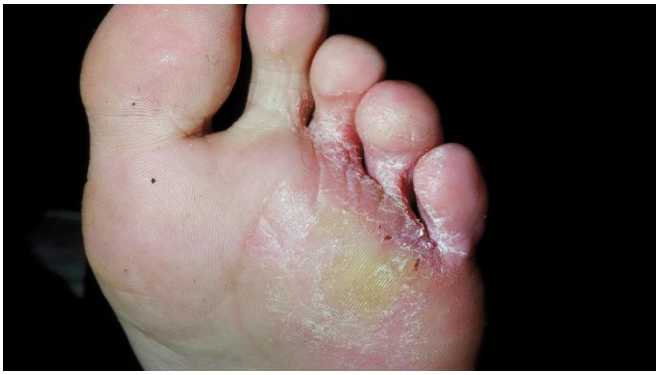
- Tinea cruris occurs in inguinal area. Often does not show the central clearing seen in tinea corporis.



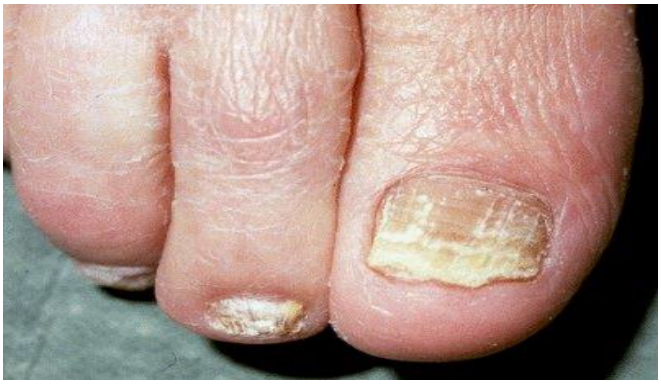
- Tinea barbae = ringworm of the bearded region.



- Tinea pedis (athlete's foot).



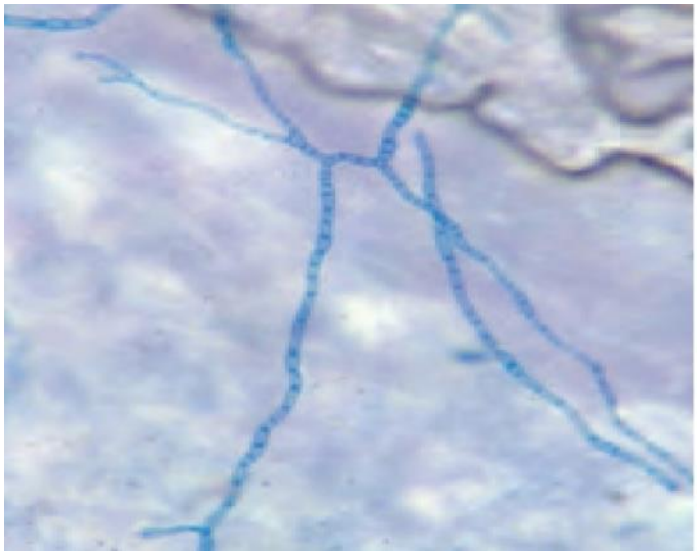
- Tinea unguium occurs on nails (Onychomycosis).

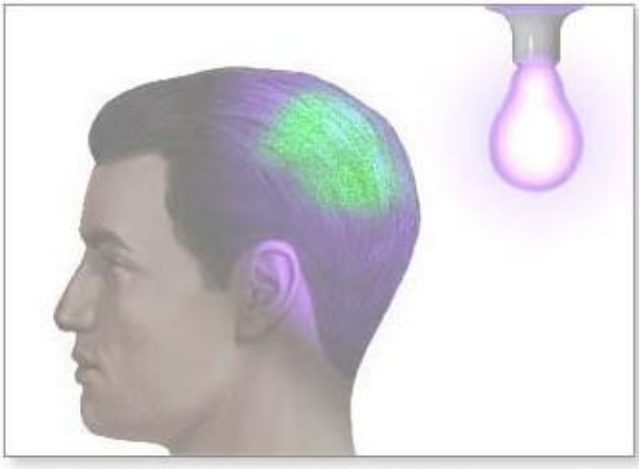


- Diagnosis:

- Microsporum fluoresces a bright yellow green with Wood lamp.

- Branching septate hyphae visible on KOH preparation with blue fungal stain.



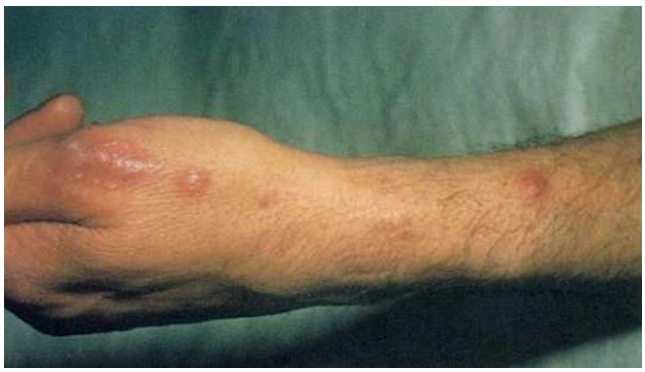


Infectious organisms glowing under Wood's lamp illumination

Subcutaneous Mycoses

❖ Sporothrix schenckii:

- Sporothrix is a dimorphic fungus found in the natural environment in the form of mold (hyphae).
- It forms cigar shaped yeast in tissues.
- It resides on the bark of trees, shrubs and garden plants, and on plant debris in soil.
- It enters the body through breaks in the skin (often via thorn prick) and spreads along the lymphatics.
- Sporotrichosis (rose gardner disease) is common in gardeners.
- The initial lesion (a reddish nodule that later ulcerates along draining lymphatics) appears at the site of the thorn prick or other skin injury.
- Biopsy of the lesion would reveal a granuloma consisting of histiocytes, multinucleated giant cells, and neutrophils, surrounded by plasma cells.



- The subcutaneous nodules pictured above are consistent with sporotrichosis, a subcutaneous mycosis caused by Sporothrix schenckii.
- The diagnosis of sporotrichosis is made by culturing the affected area and isolating Sporothrix schenckii.
- Treatment: Itraconazole or amphotericin B.

Systemic Fungal Infections

- Important systemic fungi in the U.S:

- Histoplasma.
- Coccidioides.
- Paracoccidioides.
- Blastomyces.

- All of them can cause pneumonia and can disseminate.

- All are caused by dimorphic fungi: cold (20°C) = mold; heat (37°C) = yeast. The only exception is coccidioides, which is a spherule (not yeast) in tissue.

- Treatment: fluconazole or itraconazole for local infection; amphotericin B for systemic infection.

- Systemic mycoses can mimic TB (granuloma formation), except, unlike TB, have no person-person transmission.

Histoplasma capsulatum

- Histoplasma capsulatum is a dimorphic fungus that is found as a mold in soil.

- It is also present in bird and bat droppings and is endemic to the Mississippi and Ohio River basins.

- Patients may report a history of exploring caves (exposure to bats) or cleaning bird cages or coops.

- H. capsulatum is transmitted by the respiratory route when bird or bat droppings containing fungal spores are inhaled.

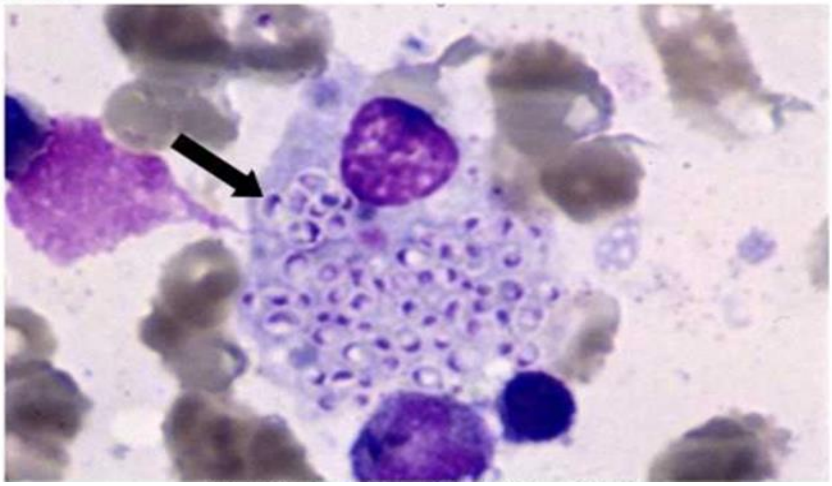
- In the lungs, the fungus is ingested by macrophages, and is seen on light microscopy as small intracellular oval bodies.

- While the majority of immunocompetent hosts remain asymptomatic, some may develop acute pulmonary disease (cough, fever, pleuritic chest pain and pulmonary infiltrates).

- Furthermore, individuals with underlying lung disease may develop chronic pulmonary histoplasmosis, a condition that clinically resembles tuberculosis (patients present with cough, malaise, weight loss and cavitations in the upper lung lobes).

- The immune reaction to Histoplasma closely resembles that induced by M. tuberculosis: a cellular response with formation of granuloma.

- The radiographic changes of chronic lung disease resemble those of pulmonary tuberculosis; cavitary lesions form in the upper lung lobes, and calcified nodes and fibrotic scarring may also be present.
- Disseminated disease occurs in immunocompromised individuals.
- Chest x-ray of a patient with disseminated histoplasmosis may show diffuse pulmonary infiltrates with hilar adenopathy.
- Because the fungus targets histiocytes and the reticuloendothelial system → lymphadenopathy and hepatosplenomegaly.

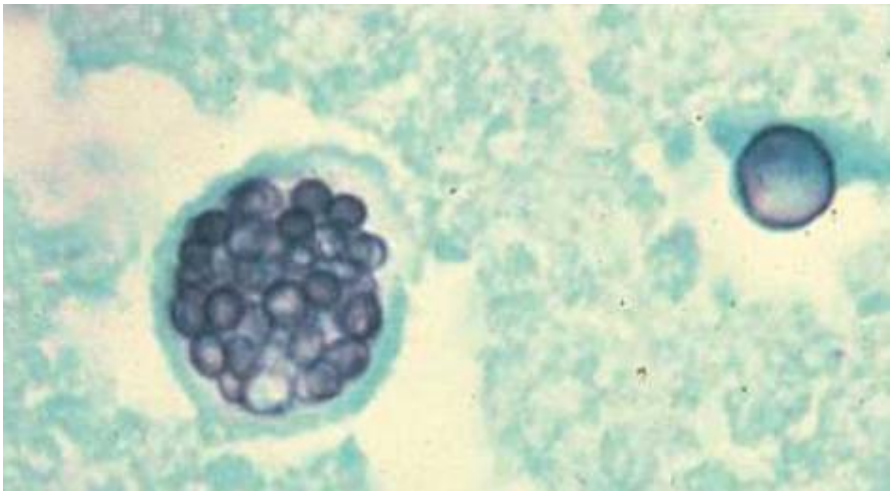


- Examination of lung biopsy specimens and bone marrow aspirates shows oval or round yeasts within macrophages.
- This image shows small ovoid bodies within a macrophage.
- Histo hides (within macrophages).
- Culture on Sabouraud agar will grow hyphae (as Histoplasma is a dimorphic fungus).
- Histoplasma antigen in blood and urine can be detected by immunoassay.
- Serologic tests (complement fixation, immunodiffusion) can be used to measure the level of anti-Histoplasma antibodies.

Coccidioides Immitis

- Coccidioides immitis is a dimorphic fungus that has a mold form (hyphae) at 25°C-30° C and an endospore form (spherules containing endospores, a unique characteristic of Coccidioides) at body temperature (37° C-400 C).

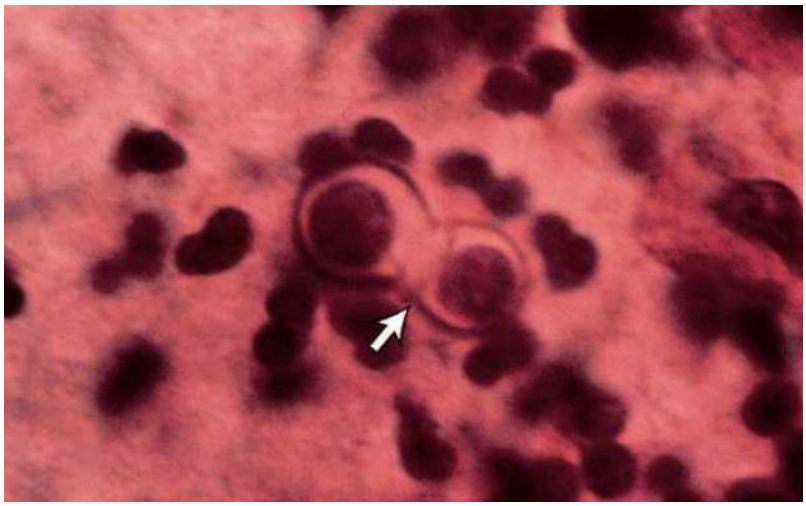
- C. immitis is endemic to the southwestern United States (southern and central California, Arizona, New Mexico, and western Texas), northern Mexico, and some regions of Central and South America.
- Patients with coccidioidomycosis are likely to live in or have recently traveled to an endemic area (recent travel to Arizona).
- C. immitis is transmitted by spore inhalation. Spores are formed by fragmentation of hyphae.
- Associated with dust exposure in endemic areas (archeological excavations, earthquakes).
- Once inside the lungs, the spores turn into spherules that contain endospores. The spherules subsequently rupture and release endospores that disseminate to other organs and tissues. Each endospore is capable of forming a new spherule.
- In immunocompetent hosts, C. immitis causes lung disease, which can be asymptomatic, or cause flu-like symptoms (cough, fever, and myalgia) accompanied by erythema nodosum.
- In total, C. immitis can present in five ways: acute pneumonia, chronic progressive pneumonia, pulmonary nodules and cavities, extrapulmonary nonmeningeal disease, and meningitis.
- Can disseminate to bone and skin → Arthralgias (desert rheumatism), Erythema nodosum (desert bumps) or multiforme.
- The more severe manifestations are largely reserved for immunocompromised hosts.



- Microscopic examination of body fluids, sputum, and tissue samples in 10% KOH or silver stain shows thick-walled spherules packed with endospores.
- The image above shows a large spherule filled with small round endospores.
- Culture on Sabouraud's agar and serology are also important in making the diagnosis.

Blastomyces dermatitidis

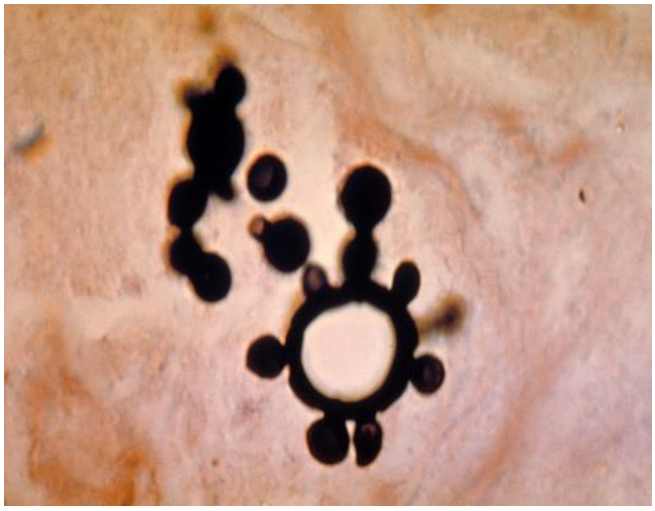
- Blastomyces is a dimorphic fungus. The mold form (branching hyphae) predominates in the environment, with average temperatures of 25 -30° C. In the human body (37-40° C), it assumes the yeast form (single cells).
- Fungus endemic to the Great Lakes, and Ohio and Mississippi River regions.
- Infection occurs by inhalation of aerosolized fungus from the environment.
- In the lungs, Blastomyces assumes yeast form, multiplies and induces a granulomatous response.
- In about half of immunocompetent individuals, blastomycosis remains asymptomatic.
- In others it may present as a lung infection or cause a flu-like illness (fever, chills, myalgia, headache, nonproductive cough) or pneumonia (fever, cough, pleuritic chest pain). The infection may become chronic. Pulmonary blastomycosis is characterized by granuloma formation.
- In immunocompromised patients, blastomycosis can cause disseminated disease.
- Patients may experience skin lesions (verrucous lesions can simulate SCC), and bone pain (caused by lytic lesions).



- Sputum stain with KOH is diagnostic. It reveals round yeast with thick, doubly refractive walls "broad based budding".
- Blasto buds broadly

Paracoccidioides brasiliensis

- Paracoccidioides brasiliensis is a dimorphic fungus and the causative agent of the disease paracoccidioidomycosis.
- Paracoccidioides brasiliensis is endemic in Latin America.
- It also causes pneumonia and can disseminate.
- Diagnosis: it forms budding yeast with "captain's wheel" formation.

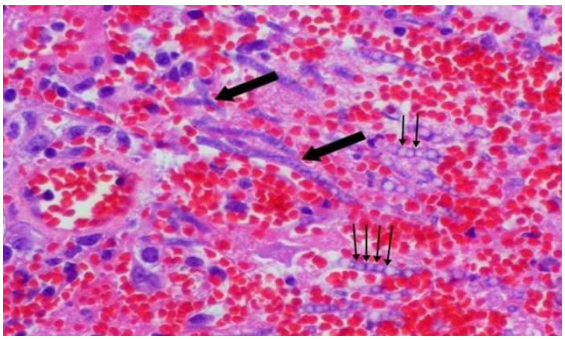


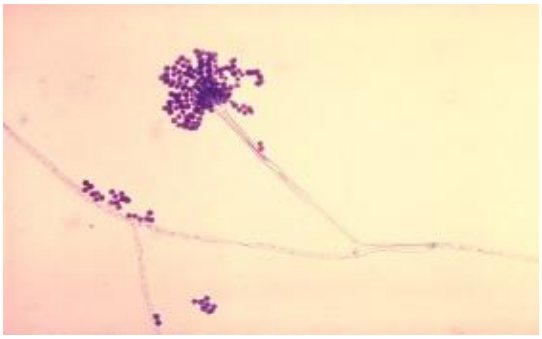
- Paracoccidio parasails with the captain's wheel all the way to Latin America.

Opportunistic Fungi

Aspergillus Fumigates

- Aspergillus fumigatus is a mold that is widely present in organic matter.
- It forms septate hyphae that branch at 45° angles (V-shaped branching).
- Think "A" for Acute Angles in Aspergillus.





- The spores of Aspergillus are inhaled with the air and are cleared by the mucus and ciliated epithelium of the respiratory tract.
- This fungus is widely distributed in the environment and commonly grows on decaying vegetables. It is monomorphic, existing only in mold form (multicellular hyphae).
- Some species of Aspergillus produce Aflatoxins (associated with hepatocellular carcinoma).
- Aspergillus causes the following conditions:

1. Invasive aspergillosis develops in immunosuppressed patients:

- The prolonged neutropenia associated with leukemia and lymphoma treatment is a strong risk factor for invasive aspergillosis.
- It most commonly affects the lung, causing the formation of lung granulomas with development of fever, pleuritic chest pain, and hemoptysis.
- Aspergillus has a predilection for blood vessels and can spread hematogenously, causing infection and infarcts involving the skin, paranasal sinuses, kidneys, endocardium, and brain.
- Diagnosis is made by light microscopy of tissue specimens, which shows V-shaped, narrow, septate hyphae invading the tissue.
- Amphotericin B is used to treat invasive aspergillosis.

2. Aspergillomas are fungus balls caused by Aspergillus that grow in old lung cavities:

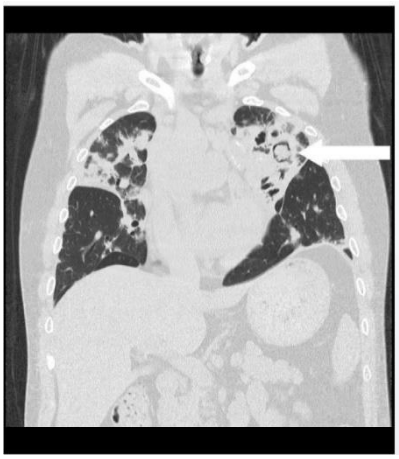
- Colonizing aspergillosis occurs in old lung cavities (from tuberculosis, emphysema, or sarcoidosis).

- Aspergillus does not invade the lung tissue, but grows inside the cavity, forming a "fungus ball" or aspergilloma.

- This condition may be asymptomatic, or it may cause cough and hemoptysis.

- On chest x-ray, an aspergilloma will appear as a radiopaque structure that shifts when the patient changes position.

- They are often surgically removed.



3. Allergic bronchopulmonaray spergillosis (ABPA) occurs in patients with asthma and presents with wheezing and have migratory pulmonary infiltrates:

- Aspergillus fumigatus is a low virulence fungus that generally does not cause significant infections except in immunocompromised or debilitated patients.

- It may, however, colonize the bronchial mucosa and complicate asthma or cystic fibrosis via a hypersensitivity reaction.

- The result is allergic bronchopulmonary aspergillosis (ABPA).

- ABPA occurs in 5% to 10% of steroid-dependent asthmatics.

- Patients with this condition have very high serum IgE levels, eosinophilia, and IgE plus IgG serum antibodies to Aspergillus.

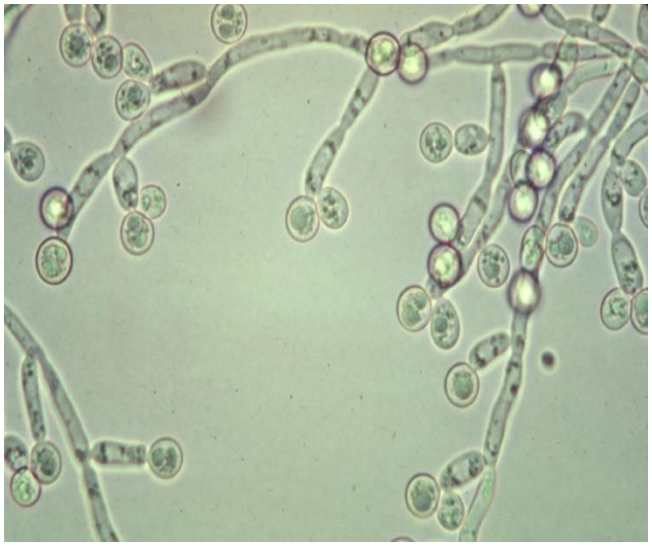
- There is intense airway inflammation and mucus plugging with exacerbations and remissions.

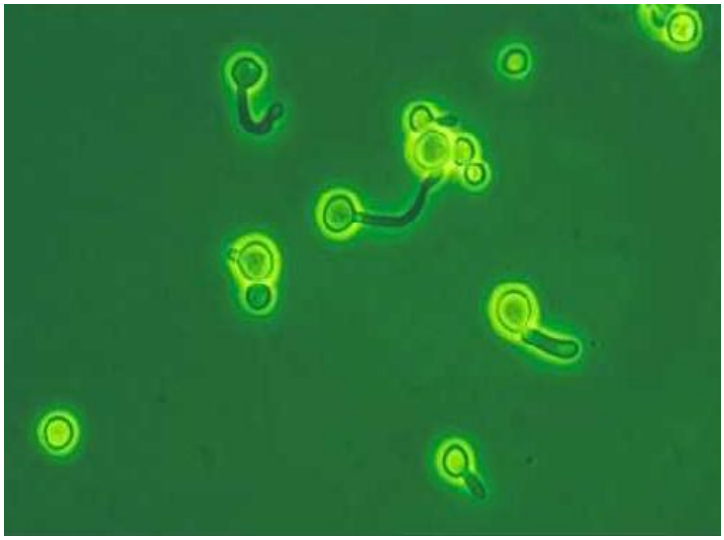
- Repeated exacerbations may produce transient pulmonary infiltrates and proximal bronchiectasis.

- Treatment is with corticosteroids.

Candida albicans

- Alba = white.
- Candida albicans is the most common cause of opportunistic mycosis.
- It can affect any organ and cause generalized candidemia.
- Dimorphic; forms pseudohyphae and budding yeasts at 20°C, germ tubes at 37°C.
- Candida albicans can gives rise to true hyphae, termed "germ tubes," when incubated at 37°C for 3 hours.
- Germ tubes are specific for C. albicans; they are not seen with any other Candida species.





- Candida albicans is a normal inhabitant of the GI tract (including the oral cavity) in up to 40% of the population. Thus, it is a common contaminant of sputum cultures. The presence of Candida in sputum does not indicate disease.
- Candida can cause localized disease in immunocompetent people with decreased local immune defenses.
- Superficial Candida infections are associated with antibiotic use, corticosteroid use, diabetes mellitus, HIV and other immunosuppressing illnesses.

- Potential manifestations include:

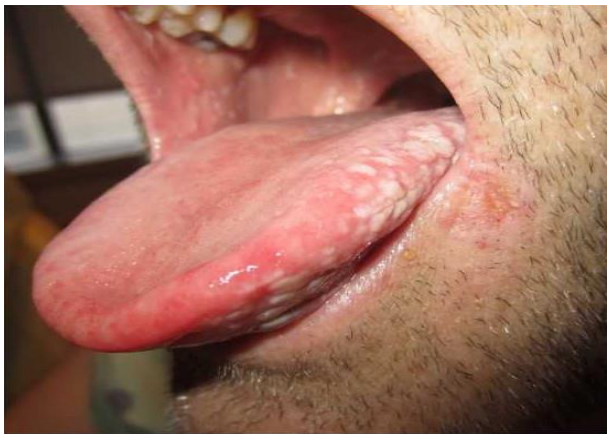
1. Oral thrush:

- Oral thrush most commonly presents with white patches on the oral mucosa (pseudomembranous candidiasis) that can be easily scraped off, revealing an erythematous mucosal surface underneath.

- Oral thrush occurs in denture wearers, diabetics, immunosuppressed patients, and patients receiving steroids, antibiotics, or chemotherapy.

- They may be asymptomatic or experience burning pain.

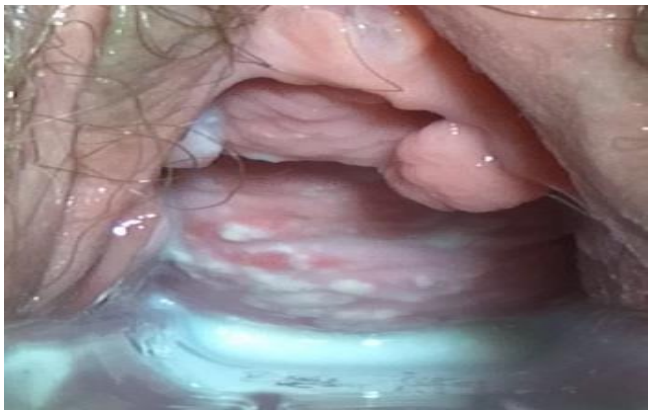
- Unexplained oral thrush in an otherwise healthy person suggests the possibility of HIV infection.



2. Vulvovaginal candidiasis:

- Patients complain of vulvar erythema and a thick white vaginal “cottage cheese” discharge.

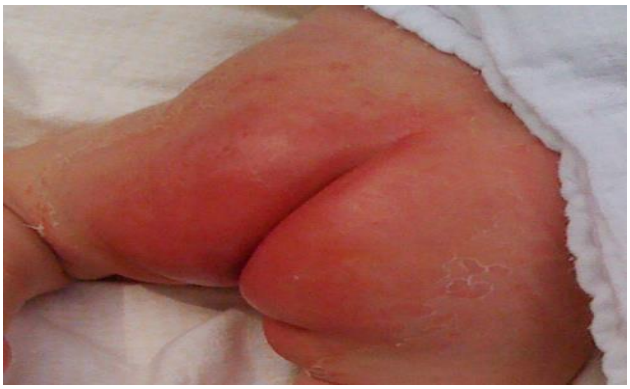
- This candidiasis is associated with antibiotic and contraceptive use, pregnancy, diabetes mellitus, and HIV.



3. Cutaneous candidiasis:

- This can occur in areas exposed to heat or high humidity (dishwashers' hands, infants' groins "diaper rash").

- Signs include erythema, a vesiculopapular rash, maceration, and fissuring.



- In immunocompromised patients, Candida can also cause disseminated disease that may affect any organ system. Potential manifestations include pneumonia, esophagitis, right-sided endocarditis (IV drug users), abscesses, and candidemia (sepsis).

❖ N.B:

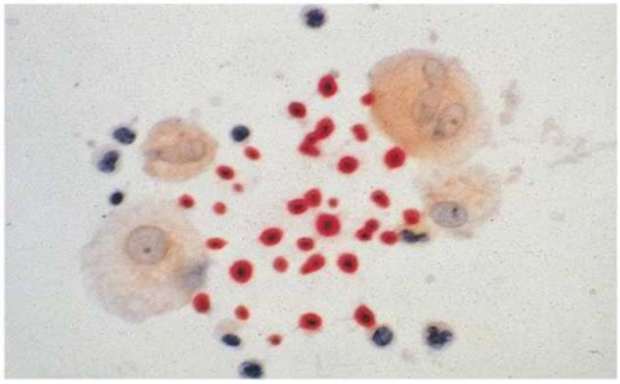
- Nosocomial blood stream infections (BSIs) is most common in those who have long-term (>12 days) central venous access as this provide sufficient time for skin flora to colonize the internal/external lumen of the catheter and subsequently spread to the bloodstream. Patient receiving parental nutrition (through a central venous catheter) are at high risk for candidemia (candida can colonize the catheter).

Most common pathogens causing nosocomial bloodstream infections
• Coagulase-negative staphylococci • *Staphylococcus aureus* • Enterococci • *Candida* species

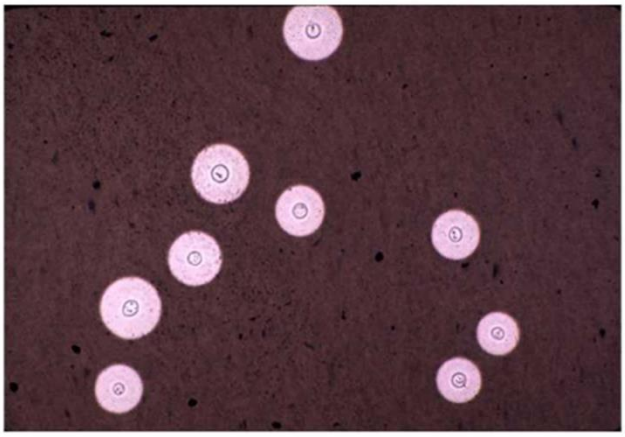
Cryptococcus neoformans

- C. neoformans exists in the yeast form (single cells) only.
- It has a thick polysaccharide capsule with antiphagocytic properties.
- This fungus is present in soil and bird (especially pigeon droppings).
- It infects humans via the respiratory tract and enters the lungs (lung is the most likely primary focus of C. neoformans infection).
- Inhaled yeast enters the lungs and is cleared in immunocompetent persons by macrophages and T-cells.

- In people with an impaired cellular immune response, C. neoformans causes symptomatic disease.
- Lung infection by Cryptococcus is usually asymptomatic; from the lungs the infection may disseminate to other organs.
- C. neoformans has a predilection for the CNS.
- The diagnosis of pulmonary cryptococcosis is made by microscopic examination of bronchopulmonary washings and lung tissue.
- Cryptococcal yeast appears red on mucicarmine stain. Mucicarmine stain is used to detect the polysaccharide capsule of Cryptococcus neoformans.



- The image above shows a red-stained capsule, which is typical for this fungus.
- In patients with HIV, sarcoidosis, or leukemia, and in those on high-dose steroid therapy, Cryptococcus commonly causes meningitis.
- Meningitis is the most common presentation of Cryptococcus neoformans infection.
- Headache, nausea, vomiting, and confusion are the common symptoms.
- CSF findings include low glucose, increased protein, and low leukocyte count (particularly in HIV-positive patients).
- Lymphocytes predominate, although some neutrophils may also be seen.
- Diagnosis is made by examining cerebrospinal fluid stained with India ink.
- The round budding yeast have peripheral clearings or "halos," due to their thick polysaccharide capsules.



- The image shows Cryptococcus neoformans with India ink staining.
- The India ink preparation stains the background while the organism remains transparent (negative stain technique).
- The thick polysaccharide capsule surrounding the yeast is seen as a peripheral clearing between the background and the dark central part of the organism "Soap bubble" lesions in brain.
- Latex agglutination test detects polysaccharide capsular antigen and is more sensitive and specific.
- Treatment: amphotericin B + flucytosine followed by fluconazole for cryptococcal meningitis.

Mucor, Rhizopus, Absidia (Zygomycophyta)

- Mucor, Rhizopus, and Absidia fungi exist in mold form only.
- They are transmitted by spore inhalation and cause mucormycosis.
- Mucormycosis is very strongly associated with diabetic ketoacidosis.
- Patients with underlying immunosuppression (due to solid organ transplantation, hematologic malignancies, or glucocorticoid therapy) are also at high risk.
- Mucormycosis tends to affect the paranasal sinuses. Patients complain of facial and periorbital pain, headache, and purulent nasal discharge, and may have cranial nerve involvement.
- Rhizopus has an affinity for ketones and high blood glucose because of its enzyme, ketone reductase. These fungi proliferate in blood vessel walls, causing necrosis of the downstream tissue. Black eschar (necrotic tissue) may be seen on the palate or nasal turbinates is a characteristic finding.

- The findings of facial pain, headache, and black necrotic eschar in the nasal cavity in a patient with diabetic ketoacidosis are highly suggestive of mucormycosis.

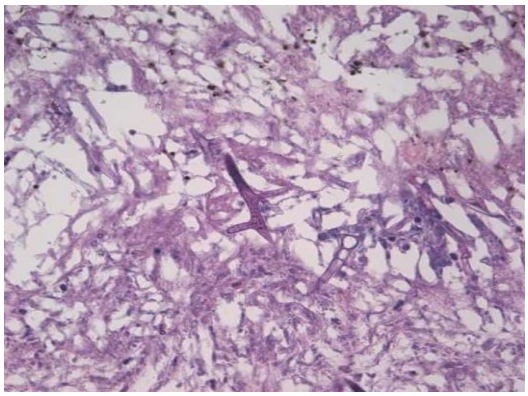
- Fungi proliferate in blood vessel walls, penetrate cribriform plate, and enter brain.

- Mucormycosis can rapidly spread to the CNS, causing confusion, neurological deficits and death. This is a condition that requires prompt diagnosis and treatment.

- Rhinocerebral, frontal lobe abscess; cavernous sinus thrombosis can develop.

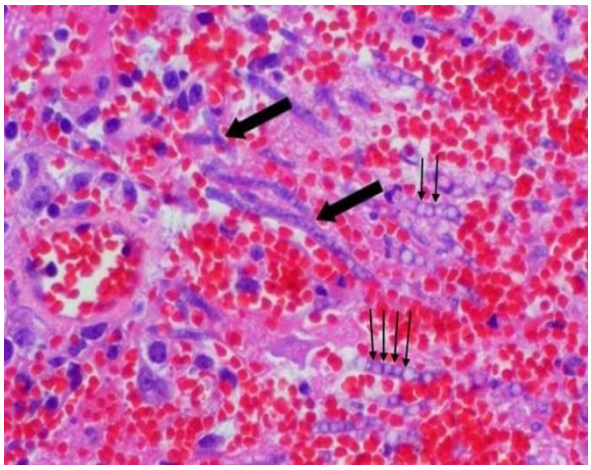
- Treatment consists of surgical debridement and amphotericin B.

- Mucormycosis is diagnosed by light microscopy of a tissue specimen (mucosal biopsy). They form broad, nonseptate hyphae that branch at wide, often 90° angles.



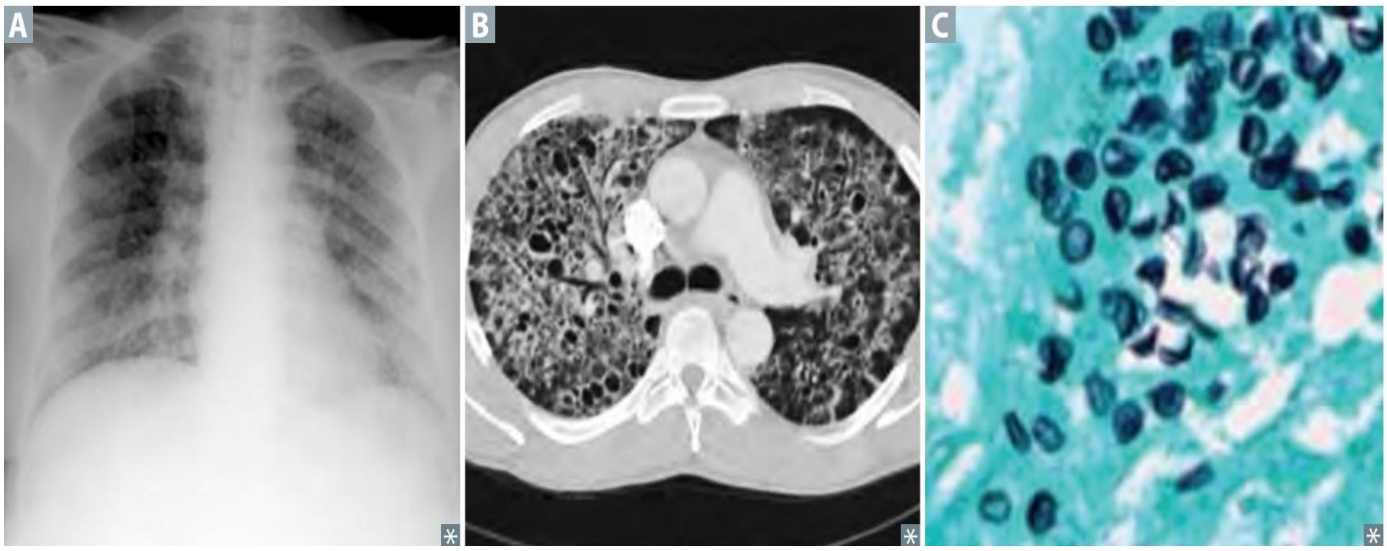
- Mucormycosis must be differentiated from invasive aspergillosis, as Aspergillus fumigatus can also affect the paranasal sinuses of immunosuppressed patients, causing similar symptoms.

- On light microscopic evaluation of the affected tissue, Aspergillus is seen as septate hyphae with V-shaped branching (45° angle).



Pneumocystis jirovecii (formerly P. carinii)

- Yeast-like fungus (originally classified as protozoan).
- The mode of transmission is by inhalation.
- Most infections are asymptomatic.
- Immunosuppression (AIDS) predisposes to disease.
- Causes Pneumocystis pneumonia (PCP), a diffuse interstitial pneumonia.
- Diffuse, bilateral ground-glass opacities on CXR/CT.
- Diagnosed by lung biopsy or lavage.
- Disc-shaped yeast forms on methenamine silver stain of lung tissue.
- Treatment/prophylaxis: TMP-SMX, pentamidine, dapsone (prophylaxis as single agent, or treatment in combination with TMP), atovaquone.
- Start prophylaxis when CD4+ count drops to < 200 cells/mm^3 in HIV patients.



CHAPTER 3

PARASYTOLOGY

- A parasite is an organism that lives at the expense of another (called host) deriving food or food and shelter from it and usually causing a certain amount of harm or injury to its host.

- The infected host is classified as:

- Intermediate host in which larval or asexual stages develop.
- Definitive host in which the adult or sexual stages occur.

- Parasites are classified as protozoans or metazoans.

Important Protozoan parasites

GI infections

1. Giardia lamblia:

- Mode of transmission: Cysts in water.

- Giardiasis:

- Giardia is the most common enteric parasite in the U.S. and Canada, and is a common cause of diarrhea in campers/hikers.

- Their life cycle alternates between an actively swimming trophozoite and an infective, resistant cyst.

- Upon ingestion of the cyst contained in contaminated water or food, excystation (cyst is converted to trophozoites) occurs in the stomach and the duodenum in the presence of acid and pancreatic enzymes.

- The trophozoites pass into the small bowel where they multiply rapidly.

- The trophozoites attach to the enterocytes via its ventral adhesive disk.

- Giardia-induced loss of intestinal brush border surface area, villus flattening, inhibition of disaccharidase activities, and eventual overgrowth of enteric bacterial flora appear to be involved in the pathophysiology of giardiasis (Bloating, flatulence, foul-smelling, fatty diarrhea).

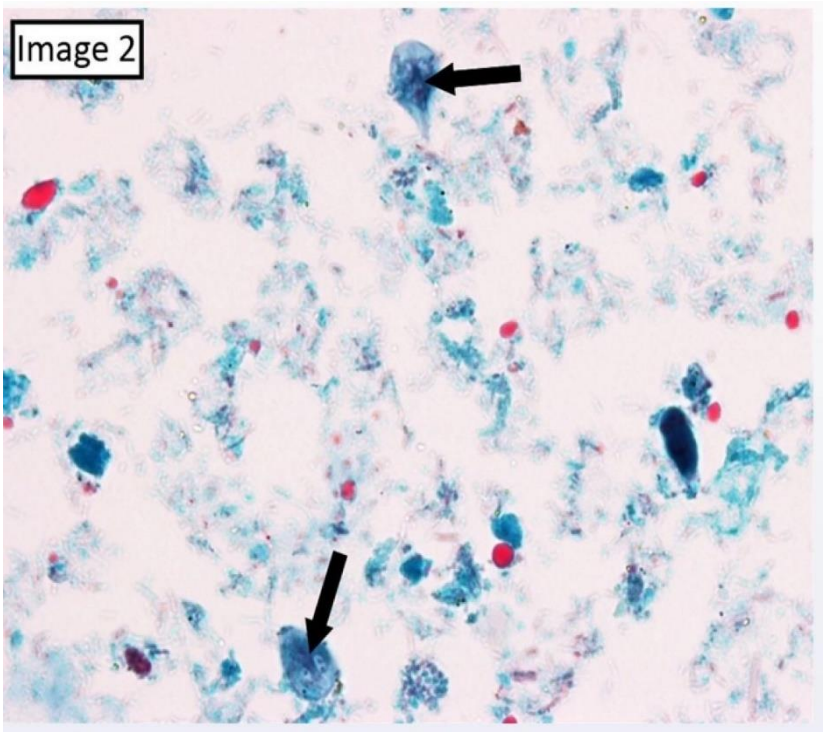
- As trophozoites pass into the large bowel, encystation (trophozoites is converted back to cysts).

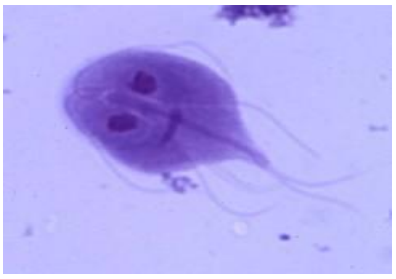
- Cysts are passed into the stool, and the cycle is repeated.

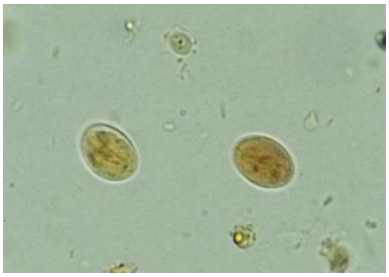
- Secretory IgA which impairs adherence, is the major component of adaptive immunity against G. lamblia infection. Conditions causing IgA deficiency predispose patients to chronic giardiasis.

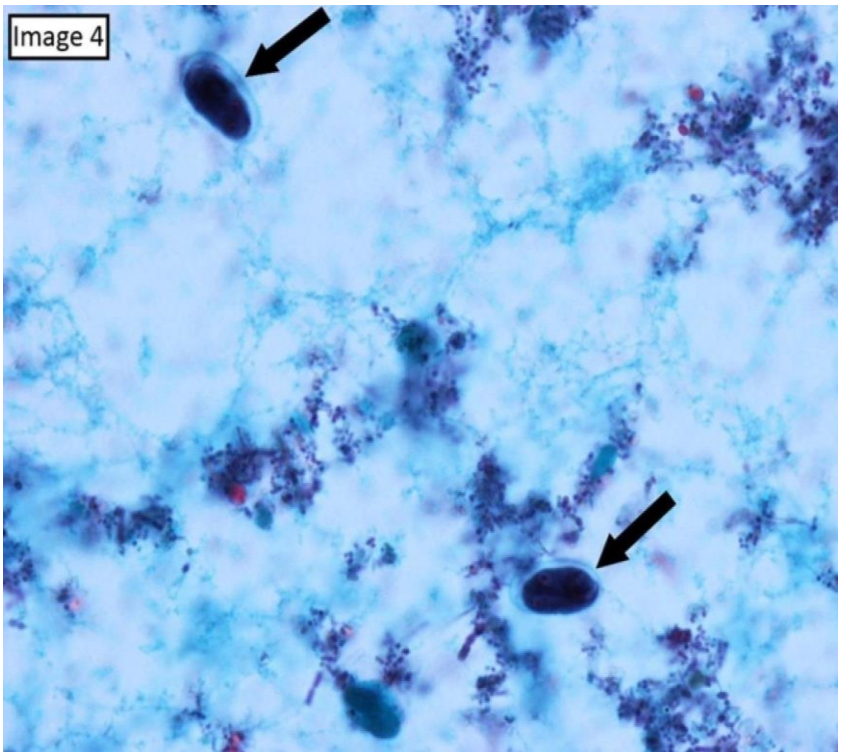
- Diagnosis: This iodine-stained stool smear shows Giardia lamblia cysts.

- Treatment: Metronidazole.









2. Entamoeba histolytica:

- Mode of transmission: Cysts in water.

- Amebiasis:

- E. histolytica is transmitted via ingestion of the cystic form (infective stage) of the protozoa.

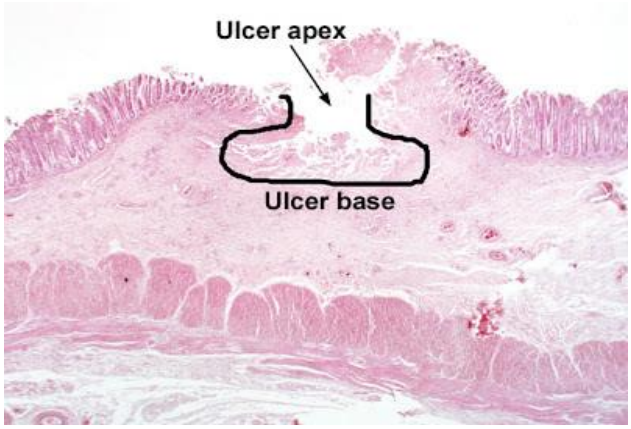
- In developed countries, amebiasis primarily affects immigrants from and travelers to endemic regions.

- In the terminal ileum or colon, cyst is converted to trophozoites (invasive form).

- Although most cases of amebiasis are asymptomatic, dysentery and invasive extraintestinal disease can occur.

- The trophozoites can penetrate and invade the colonic mucosal barrier, leading to tissue destruction (ulcers), inflammatory bloody diarrhea (amebic dysentery), and colitis resembling inflammatory bowel disease.

- Histology may show flask-shaped ulcer.



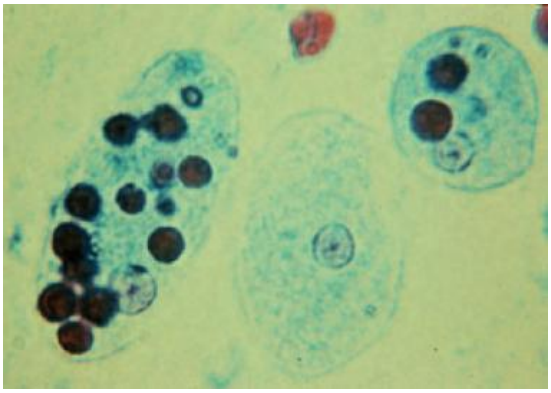
- In addition, the trophozoites can spread hematogenously via the portal circulation to the liver or even to more distant organs.

- Amebic liver abscess is the most common manifestation of invasive amebiasis, but other organs can also be involved, including pleuropulmonary, cardiac, cerebral, renal, genitourinary, peritoneal, and cutaneous sites.

- Diagnosis:

- Serology and/or trophozoites with RBCs in the cytoplasm (the image below) or cysts (with up to 4 nuclei) in stool.

- Entamoeba Eats Erythrocytes



- Treatment: Metronidazole; iodoquinol for asymptomatic cyst passers.

3. Cryptosporidium:

- Mode of transmission: Oocysts in water.

- Diseases:

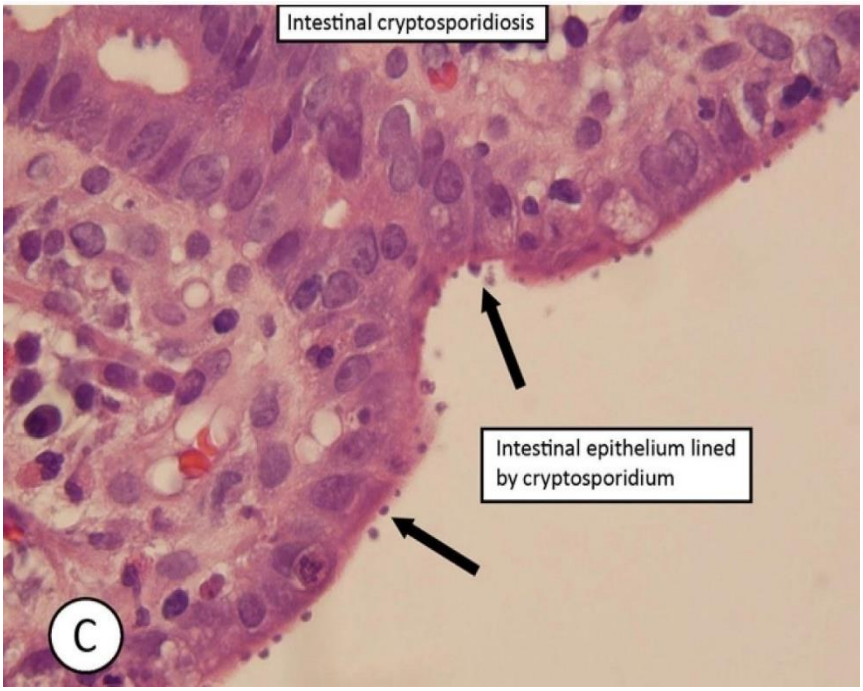
- Severe diarrhea in AIDS.

- Mild disease (watery diarrhea) in immunocompetent hosts.

- Cryptosporidiosis mainly affects children.

- Diagnosis: Oocysts on acid-fast stain.

- Treatment: Prevention by filtering city water supplies; nitazoxanide in immunocompetent hosts.



CNS infections

1. Toxoplasma gondii:

- Mode of transmission:

- Humans typically acquire the infection due to:
  - Incidental ingestion of oocytes from cat feces (the definitive host).
  - Consumption of undercooked meat from farm animals (pigs, chickens, goats) that ingested oocytes and developed tissue infection.
  - Crosses placenta (pregnant women should avoid cats).

- Diseases:

- T. gondii has 2 distinct life cycles:
  - The sexual cycle occurs only in cats, the definitive host.
  - The asexual cycle occurs in other mammals (including humans).

- It consists of 2 forms:
  - Tachyzoites (the rapidly dividing form observed in the acute phase of infection).
  - Bradyzoites (the slowly growing form observed in tissue cysts).

- T. gondii oocysts, tachyzoites, and bradyzoites can cause infection in humans.

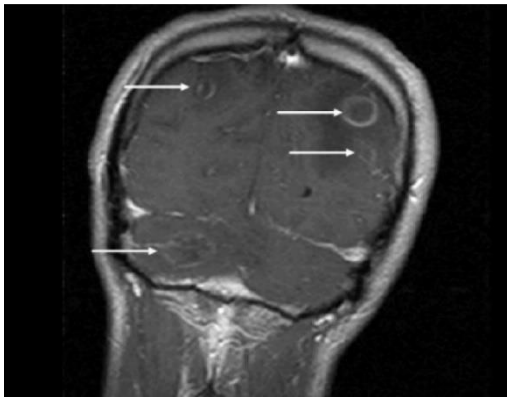
- The cat is the definitive host of this protozoan, with humans infected following ingestion of contaminated food or water.

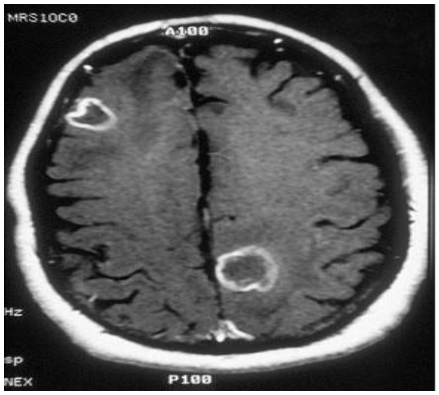
- Toxoplasma gondii is an obligate intracellular protozoan with a worldwide distribution. Cell mediated immunity is very important to eradicate obligate intracellular parasites.

- Immunocompromised individuals exposed to T. gondii can develop encephalitis with multiple necrotizing brain lesions, resulting in fever, headache, altered mental status, focal neurologic findings, and seizures.

- MRI or contrast CT of the brain demonstrates multiple ring-enhancing lesions in both hemispheres, with MRI the more sensitive of the two studies.

- In AIDS patients, the radiographic finding of ring-enhancing lesions in both cerebral hemispheres is most often indicative of toxoplasmosis.





❖ Congenital toxoplasmosis:

- Congenital toxoplasmosis is a transplacental infection (acquired in utero).

- The fetus is affected only if the mother is infected with toxoplasmosis during the first six months of pregnancy.

- Most cases in pregnant women arise due to the consumption of undercooked, contaminated meat. Therefore, pregnant women are advised to avoid raw, cured, and undercooked meat in order to reduce the risk of infection.

- Pregnant women should be warned not to handle cat litter in order to prevent contact with Toxoplasma, which is often found in cat feces.

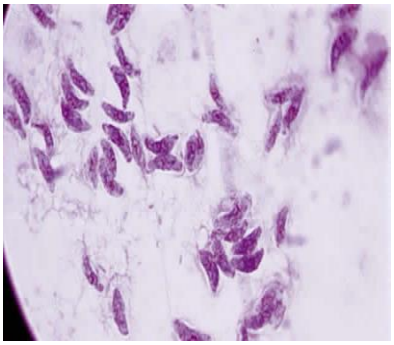
- Hydrocephalus, intracranial calcifications and chorioretinitis form the classic triad of congenital toxoplasmosis.

- Hydrocephalus occurs due to CNS inflammation and is evidenced by macrocephaly and enlargement of the ventricles.

- Chorioretinitis refers to inflammation of the choroids and the retina that can leave cotton-like white/yellow scars on the retina visible on fundoscopy.

- Affected neonates also have hepatosplenomegaly, rash and multiple neurological abnormalities such as seizures, altered muscle tone and ocular movement defects.

▪ Diagnosis: Serology, biopsy (tachyzoite).



- Treatment: First-line treatment of toxoplasmosis includes a combination of pyrimethamine and sulfadiazine.

***Toxoplasma* encephalitis**	
Setting	• Exposure to cat feces with subsequent ingestion of ***Toxoplasma gondii*** • Reactivation in setting of immunosuppression • Primarily **AIDS** with **CD4 count <100/mm^3**
Clinical	• Headache • Confusion • Fever • Focal neurologic deficits/seizures
Diagnostic	• Positive *Toxoplasma gondii* IgG • Multiple **ring-enhancing** brain lesions
Therapeutic	• Sulfadiazine & pyrimethamine (leucovorin) • Antiretroviral initiation • Prophylaxis: **TMP-SMX** (CD4 count <100/mm^3)

TMP-SMX = trimethoprim-sulfamethoxazole.

❖ N.B:

- The two most common causes of focal brain lesions in HIV-positive patients are toxoplasmosis and primary central nervous system (CNS) lymphoma.
- Other less common causes of focal brain lesions in this patient population include primary brain tumors (glioblastoma multiforme), metastatic carcinoma, and abscesses containing less common infectious agents (Cryptococcus neoformans, Mycobacterium tuberculosis).
- Most often, primary CNS lymphoma occurs in immunocompromised patients and is of B-lymphocyte origin.
- Latent EBV infection is strongly associated with AIDS-related primary CNS lymphoma.

2. Naegleria fowleri:

- Mode of transmission: Swimming in freshwater lakes (participation in recreational water activities); enters the brain via cribriform plate.

- Diseases:

- It is the causal agent of primary amebic meningoencephalitis (PAM), which is an acute, fulminant, and rapidly fatal infection of the central nervous system (CNS).

- It is called "Brain eating amoeba". Only over a 10 survivors of PAM due to N. fowleri have been reported in literature.

- Diagnosis: motile trophozoites in CSF.

- Treatment: Amphotericin B has been effective for a few survivors.

Primary amoebic encephalitis	
Pathophysiology	• Infection with ***Naegleria fowleri*** (free-living, motile protozoan) • Exposure during **recreational water activities** (eg, boating, diving) • Mucosal invasion → penetration of cribriform plate → meningoencephalitis
Presentation	• Acute fever, confusion, headache, photophobia, CN palsies • CSF - ↑ protein, neutrophils; **motile trophozoites** on wet mount
Management	• Nearly all cases are **fatal** • Antibiotics (eg, amphotericin B) administered but usually insufficient

CN = cranial nerve; **CSF** = cerebrospinal fluid.

3. Trypanosoma brucei:

- Two subspecies: Trypanosoma brucei rhodesiense, Trypanosoma brucei gambiense.

- Mode of transmission: trypomastigotes in saliva of tsetse fly contaminate bite.

- African sleeping sickness: Indurated chancre at bite site (painful bite), enlarged lymph nodes, recurring fever (due to antigenic variation), somnolence, coma.

- Diagnosis: trypomastigotes in blood films or CSF.

- Treatment: Suramin for acute cases or melarsoprol for chronic.

Hematologic infections

1. Plasmodium (P. vivax/ovale, P. falciparum, P. malariae):

- Mode of transmission: female Anopheles mosquito.

- Diseases (Malaria):

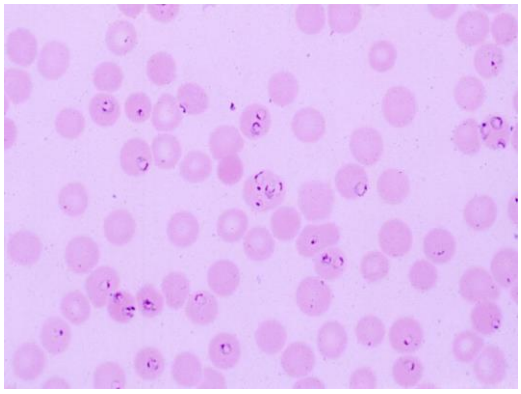
- Malaria is a potentially life-threatening disease caused by infection with Plasmodium protozoa transmitted by an infective female Anopheles mosquito.

- After a mosquito takes a blood meal, it injects the malarial sporozoites into the blood to reach hepatocytes (liver phase) within minutes and reproduce asexually to merozoites.

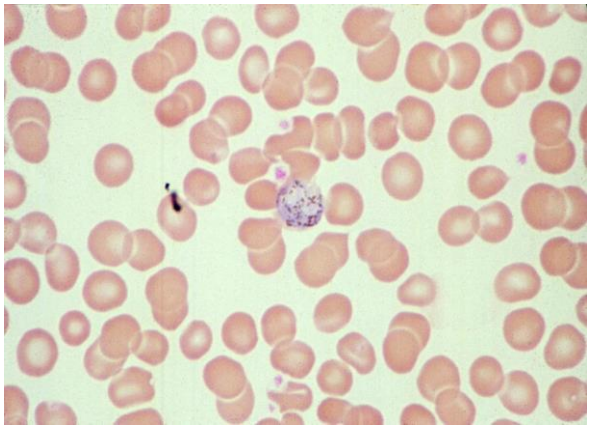
- These merozoites rapidly enter erythrocytes, where they develop into trophozoites and then into schizonts over a period of days (during the erythrocytic phase of the life cycle).

- Rupture of infected erythrocytes containing the schizont results in anemia, fever and merozoite release.

- The merozoites enter new red cells, and the process is repeated, resulting in an increase in parasite burden.

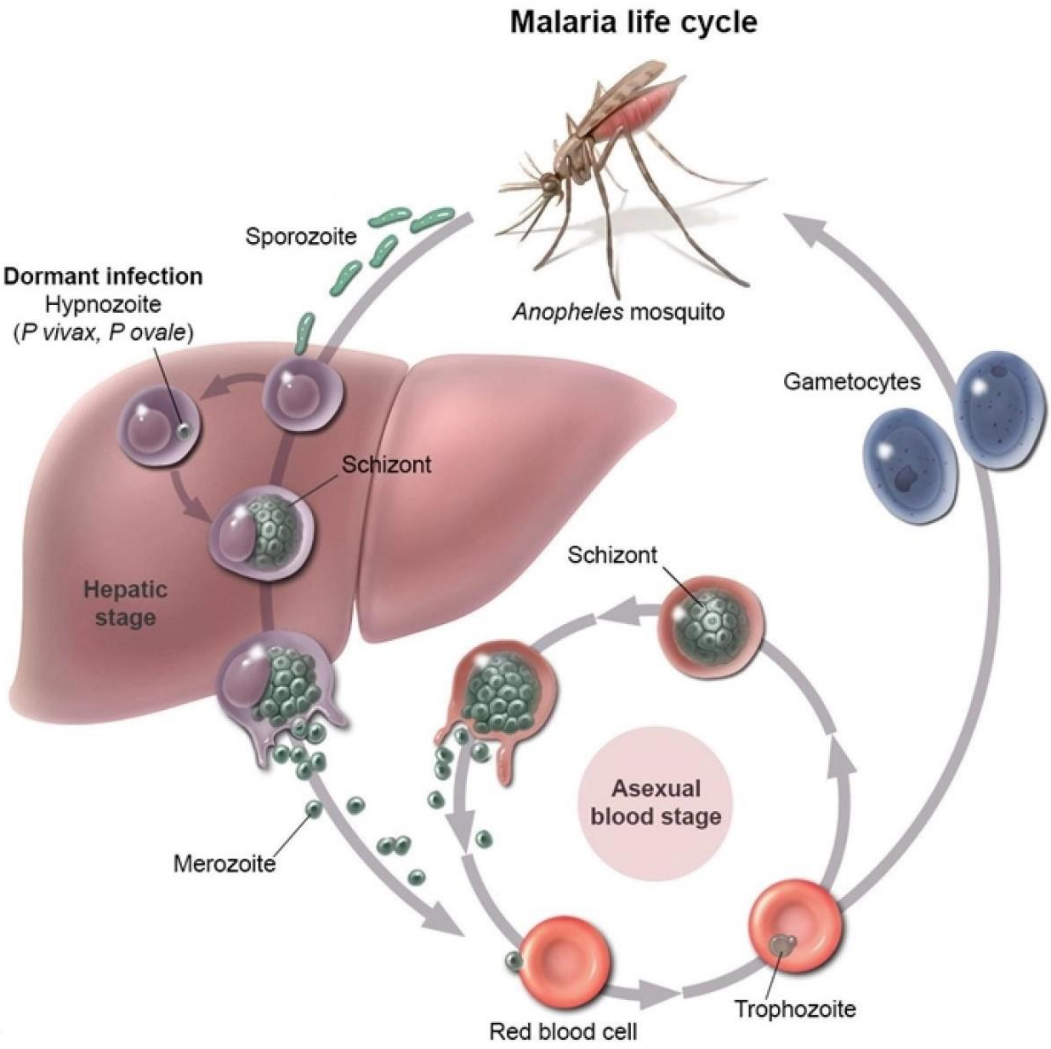


❖ The image above illustrates the trophozoite form, or immature-ring form of the malarial parasite within peripheral erythrocytes. Red blood cells infected with trophozoites do not bind to the endothelial cells of blood vessels.



❖ The image above shows a mature schizont within an erythrocyte. These red blood cells (RBCs) are sequestered in the spleen when malaria proteins, called sequestrins, on the RBC surface bind to endothelial cells within that organ. Sequestrins are only on the surfaces of erythrocytes that contain the schizont form of the parasite.

- Clinical symptoms of malaria include fever, headache, anemia, splenomegaly.



- P. vivax/ovale:
  - 48-hr cycle (tertian, includes fever on first day and third day, thus fevers are actually 48 hr. apart); dormant form (hypnozoite) in liver.

- P. falciparum:
  - Severe; irregular fever patterns; parasitized RBCs occlude capillaries in brain (cerebral malaria), kidneys, lungs.

  - P. falciparum can cause cerebral malaria, pulmonary edema, rapidly developing anemia, and renal problems.

- An important reason that the consequences of P. falciparum infection are so severe is due to its ability to adhere to endothelial cell walls → vascular obstruction.

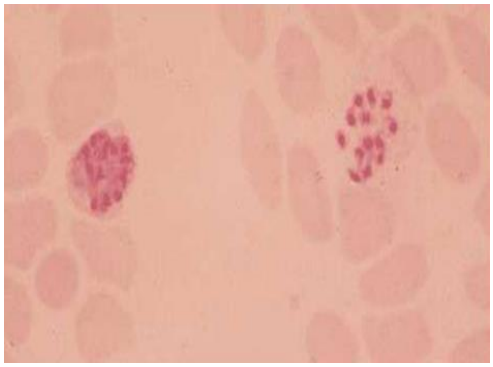
- When a red blood cell (RBC) becomes infected with P falciparum, the organism produces adhesion proteins that bind to endothelial cells.

- The adherence of these infected RBCs causes them to clump together in the blood vessels in many areas of the body, causing microvascular damage and leading to much of the damage incurred by the parasite.

- P. Malariae: 72-hr cycle (quartan).

▪ Diagnosis:

- The diagnosis is frequently made by examination of a peripheral blood thin smear.



- On the slide above, the abnormal RBC on the left contains a schizont, while the abnormal cell on the right is ready to rupture to release the merozoites within it.

▪ Treatment:

- Chloroquine and atovaquone-proguanil are effective in eradicating chloroquine-sensitive Plasmodia from the bloodstream but has no activity against the latent hepatic infections established by P. vivax and P. ovale. Primaquine must be used in addition to completely eradicate infections by these organisms and prevent relapses.

- Some African species are chloroquine-resistant; these can be treated with mefloquine, a quinine analog.

- Mefloquine chemoprophylaxis for malaria must be continued for 4 weeks after return from endemic region to ensure the elimination of hepatic schizonts (which develops in the liver over 8-30 days).

2. Babesia:

- Mode of transmission: Ixodes tick (same as Borrelia burgdorferi of Lyme disease; may often coinfect humans).

- Disease (Babesiosis):

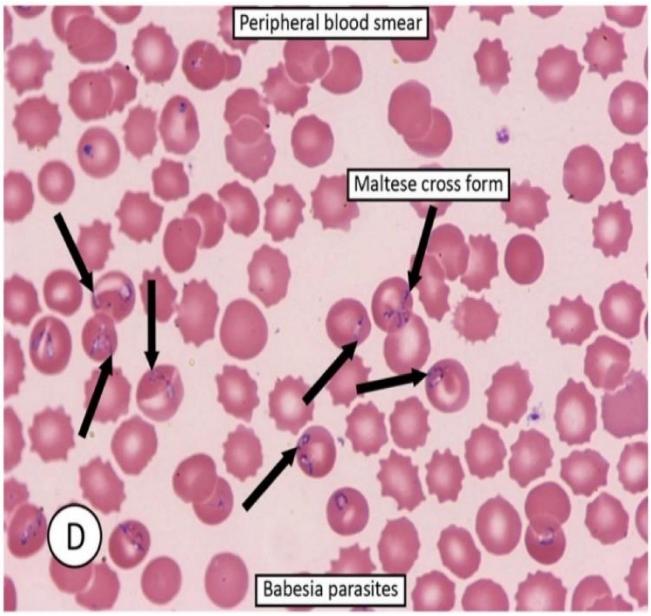
- Babesiosis is a tick-borne malaria-like illness caused by species of the intraerythrocytic protozoan Babesia microti.

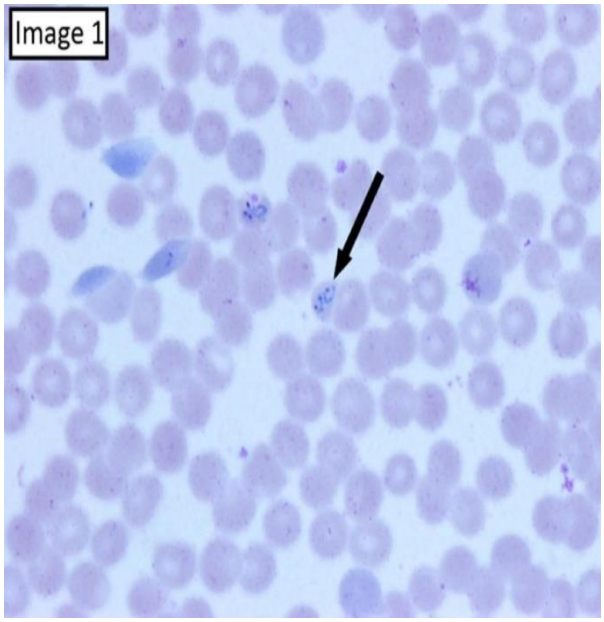
- Babesiosis: fever and hemolytic anemia; predominantly in northeastern United States; asplenia risk of severe disease.

- Diagnosis:

- Blood smear: intraerythrocytic ring inclusions, "Maltese cross" in giemsa stain of blood smear.

- Treatment: Atovaquone + azithromycin.





Babesiosis	
Epidemiology	• Agent: *Babesia microti* • Vector: *Ixodes scapularis* **tick** (same for Lyme disease & HGA) • Geographic distribution: Northeastern United States
Clinical manifestations	• Signs & symptoms: Fevers, fatigue, myalgias, headache, **flulike symptoms** (if **severe**: ARDS, CHF, DIC, splenic rupture) • Laboratory studies: **Anemia, thrombocytopenia**, ↑LFTs
Diagnosis	• Thin blood smear (Wright/Giemsa stains): **Intraerythrocytic** pleomorphic **ring forms** (occasional "Maltese crosses")

ARDS = acute respiratory distress syndrome; **CHF** = congestive heart failure; **DIC** = disseminated intravascular coagulation; **HGA** = human granulocytic anaplasmosis; **LFTs** = liver function tests.

Visceral infections

1. Trypanosoma cruzi:

- Mode of transmission: Reduviid bug (kissing bug) feces, deposited in a painless bite (much like a kiss).

- Chagas disease (American trypanosomiasis):

- Trypanosoma cruzi is a parasite transmitted by an insect that lives in the walls of rural areas "the reduviid bug".

- Trypanosoma cruzi is endemic in rural areas of Central and South America.

- When the histiocytes or other inflammatory cells ingest the parasites, they transform into amastigotes.

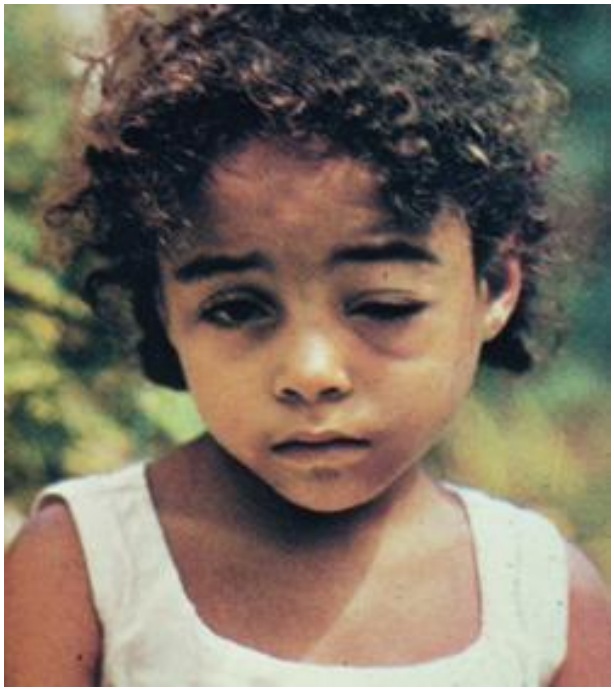
- In the amastigote form, parasites can multiply in the cells of virtually every organ and tissue.

- After local multiplication, the organisms can assume the trypomastigote form and invade the bloodstream, carrying the infection to all parts of the body.

- In the acute phase, the heart is the main target organ. The severity of the acute infection widely varies, ranging from asymptomatic infection to severe tissue destruction.

- The myocardium develops focal myonecrosis, contraction band necrosis, interstitial fibrosis, and lymphocytic infiltration.

- Unilateral periorbital swelling (Romaña sign) characteristic of acute stage.



- In chronic stage of chagas disease, this parasite can also destroy the myenteric plexi in the esophagus, intestines, and ureters, causing secondary achalasia, megacolon, and megaureter, respectively.

- Dysphagia for liquids and difficulty belching in association with a dilated esophagus and absent peristalsis in the smooth muscle portion of the esophagus is diagnostic of achalasia.

- When a patient from Central or South America, presents with achalasia, however, suspect infection by Trypanosoma cruzi (American trypanosomiasis).

- T. cruzi infection can cause similar changes in the sigmoid colon and ureter, respectively causing megacolon and megaureter.

▪ Diagnosis: trypomastigotes in blood smear.

▪ Treatment: Benznidazole or nifurtimox.

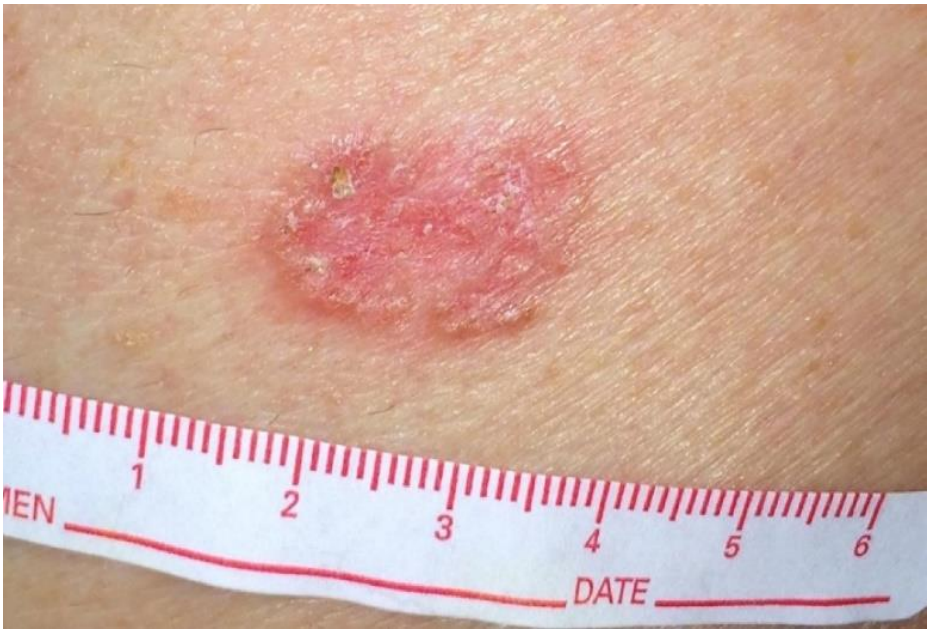
2. Leishmania donovani:

▪ Mode of transmission: Sand-fly.

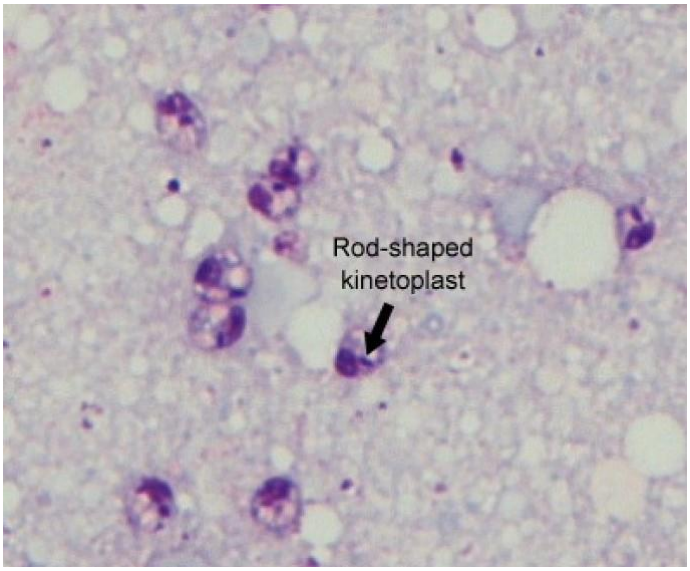
▪ Diseases:

- Visceral leishmaniasis (kala-azar): spiking fevers, hepatosplenomegaly, pancytopenia.

- Cutaneous leishmaniasis: characterized by chronic, pinkish papule that evolve into a nodule or plaque.



- Diagnosis: Macrophages containing amastigotes with rod-shaped kinetoplasts.



- Treatment: Amphotericin B, sodium stibogluconate.

Sexually transmitted infections

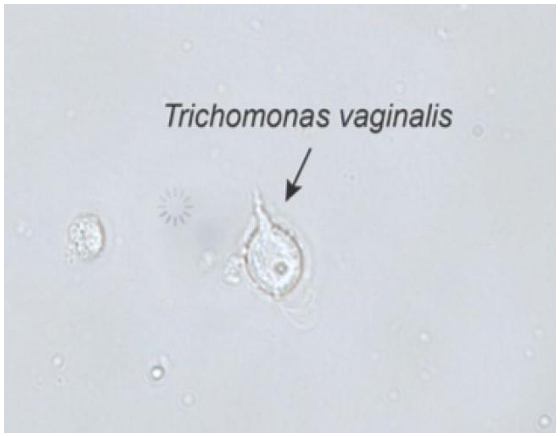
❖ Trichomonas vaginalis:

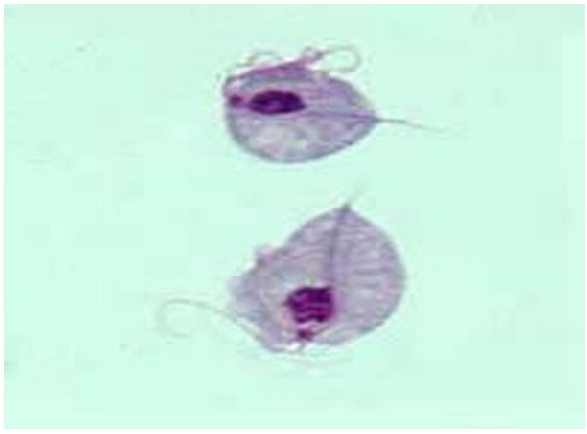
- Mode of transmission: Sexual (cannot exist outside human because it cannot form cysts).

- Disease (Trichomoniasis):

- Vaginitis: foul-smelling, greenish discharge; itching and burning, do not confuse with Gardnerella vaginalis, a gram-variable bacterium associated with bacterial vaginosis.

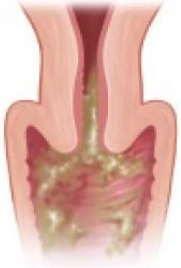
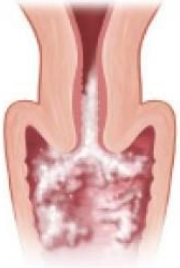
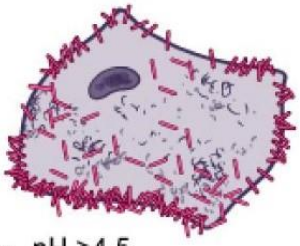
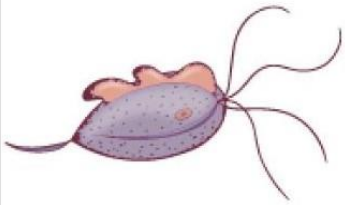
- Diagnosis: Trophozoites (motile) on wet mount; "strawberry cervix".





- Treatment: Metronidazole for patient and partner (prophylaxis).

Differential diagnosis of vaginitis

Diagnosis	Bacterial vaginosis (*Gardnerella vaginalis*)	Trichomoniasis (*Trichomonas vaginalis*)	Candida vaginitis (*Candida albicans*)
Examination	 • Thin, off-white discharge with fishy odor • No inflammation	 • Thin, yellow-green, malodorous, frothy discharge • Vaginal inflammation	 • Thick, "cottage cheese" discharge • Vaginal inflammation
Laboratory findings	 • pH >4.5 • Clue cells • Positive whiff test (amine odor with KOH)	 • pH >4.5 • Motile trichomonads	 • Normal pH (3.8-4.5) • Pseudohyphae
Treatment	Metronidazole or clindamycin	Metronidazole; treat sexual partner	Fluconazole

KOH = potassium hydroxide.

Important metazoan parasites

1. Nematodes

Intestinal infections

1. Enterobius vermicularis (pinworm):

- Mode of transmission: Feco-oral.

- Enterobiasis:

- It is the most common helminthic infection in the United States, occurring most frequently in school children ages 5-10 years.

- Enterobius vermicularis has a simple life cycle, which contributes to its high prevalence.

- The adult worms live in the human intestine, particularly in the cecum and appendix.

- In contrast to other worms that release their eggs into the intestine, the female worm migrates out through the rectum onto the perianal skin to deposit eggs (most commonly at night).

- Larvae inside the eggs mature within 6 hours and can either be ingested by the same individual (autoinfection) or spread to other humans.

- The presence of eggs and worms in the perianal area causes an inflammatory reaction that results in perianal itching, also known as pruritus ani.

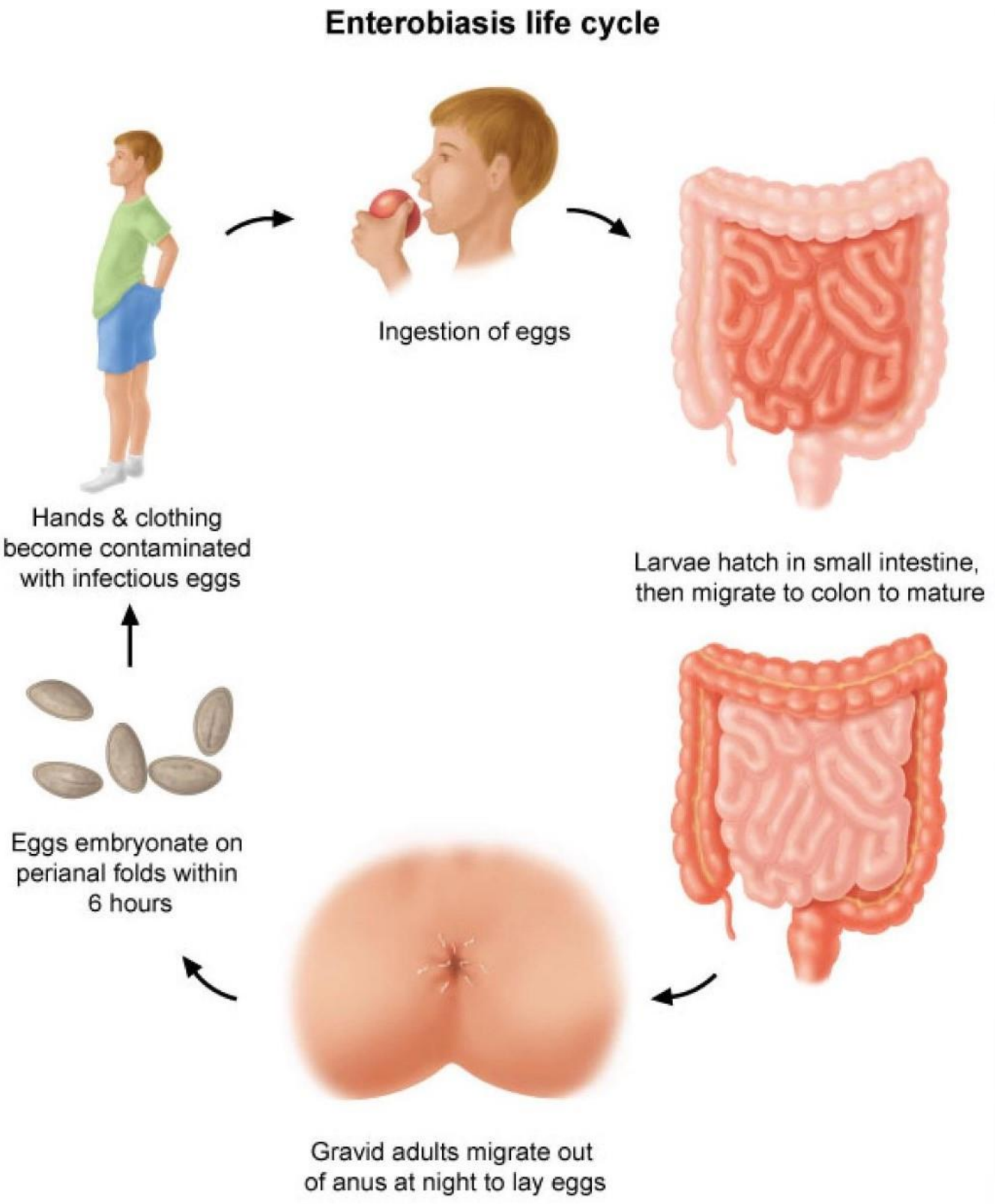
- Abdominal pain, nausea, and vomiting can also manifest in patients with a heavy worm burden.

- Diagnosis: it is made by the "Scotch tape" test, which reveals the presence of oval, asymmetrically flattened eggs with a bean-shaped appearance.

- Treatment:

- Albendazole or mebendazole is the first-line treatment.

- Pyrantel pamoate, an alternate agent, is preferred in pregnant patients.


Enterobiasis life cycle
Ingestion of eggs
Larvae hatch in small intestine, then migrate to colon to mature
Gravid adults migrate out of anus at night to lay eggs
Eggs embryonate on perianal folds within 6 hours
Hands & clothing become contaminated with infectious eggs

2. Ascaris lumbricoides (giant roundworm):

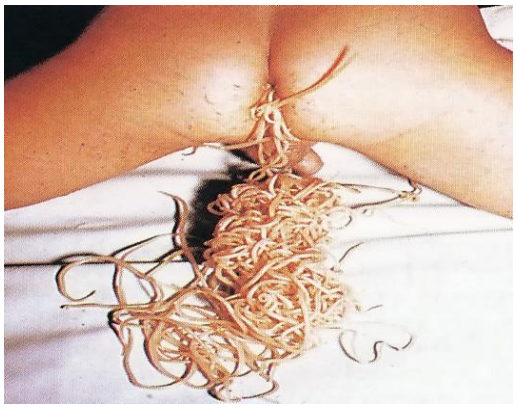
- Mode of transmission: Fecal-oral; eggs visible in feces under microscope.

- Disease (Ascariasis):

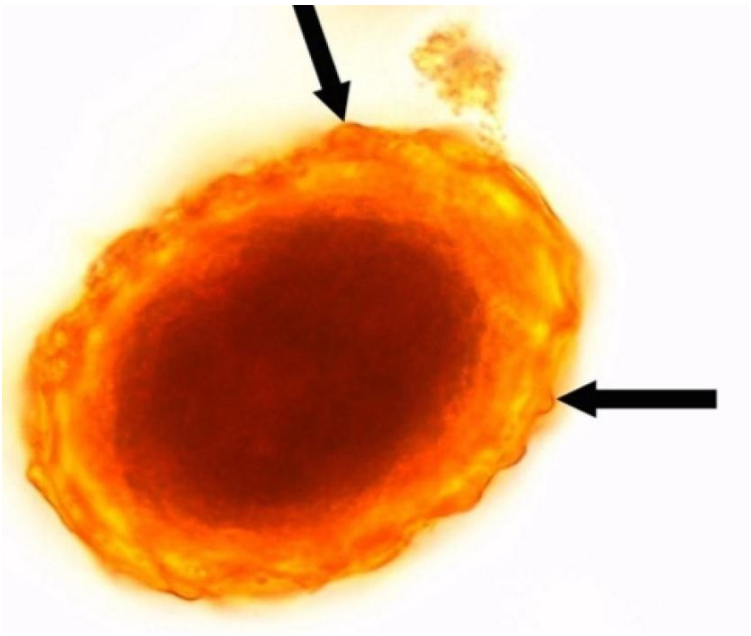
- It is the most common helminth worldwide.

- Ingestion of the egg → larva migrates through lungs (cough) and mature in small intestine.

- It causes intestinal infection with possible obstruction at ileocecal valve or biliary duct.



- Diagnosis: Stool microscopy reveals an oval egg with a thick outer shell.



- Treatment: Bendazoles.

3. Strongyloides stercoralis:

- Mode of transmission: Larvae in soil penetrate the skin.

- Strongyloidiasis:

- It is a disease caused by the roundworm Strongyloides stercoralis.

- The infection is transmitted by filariform (infectious) larvae found in soil contaminated with human feces.

- On contact, the larvae penetrate the skin and migrate hematogenously to the lungs.

- There they enter the alveoli and travel up the bronchial tree to the pharynx, where they are swallowed.

- When the larvae reach the intestine, they develop into adults that lay eggs within the intestinal mucosa.

- These hatch into rhabditiform (noninfectious) larvae that migrate into the intestinal lumen to be excreted in the stool.

- Some rhabditiform larvae can molt directly into filariform larva within the intestine and re-infect the host by penetrating the intestinal wall or perianal skin.

- This cycle of autoinfection can result in a massive increase in worm burden, leading to widespread dissemination of the parasites throughout the body (hyperinfection). The ensuing inflammation can be severe enough to cause multiorgan dysfunction and septic shock.

- Hyperinfection occurs most often in patients taking immunosuppressants (corticosteroids) or with HTLV-1 infection. These patients have impaired Th_2-directed cellular immunity (mediated by the antihelmintic action of eosinophils and basophils).

- Presentation:

- Most patients are asymptomatic, but some present with chronic, intermittent gastrointestinal or pulmonary symptoms.

- Intestinal infection causing vomiting, diarrhea, epigastric pain (may feel like peptic ulcer).

- Pruritic, erythematous, linear streaks (known as larva currens) may occur on the thighs and buttocks as the larva migrate subcutaneously away from the perianal region.

- Diagnosis: The diagnosis is made by finding rhabditiform (noninfectious) larvae in the stool, as the eggs and adult parasites are usually seen only in intestinal biopsies.

- Treatment: Ivermectin or bendazoles.

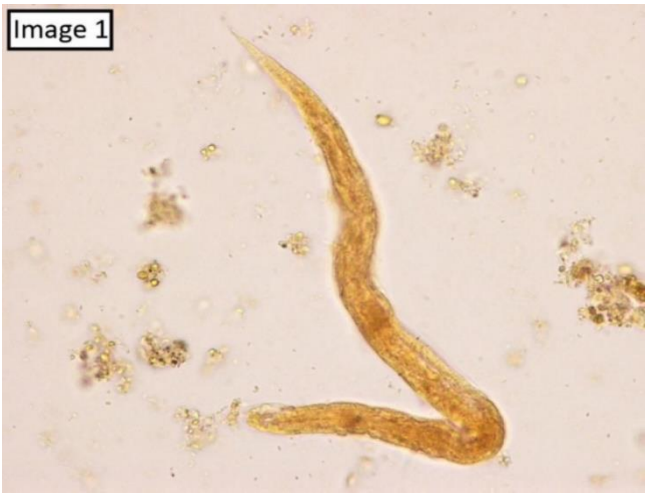
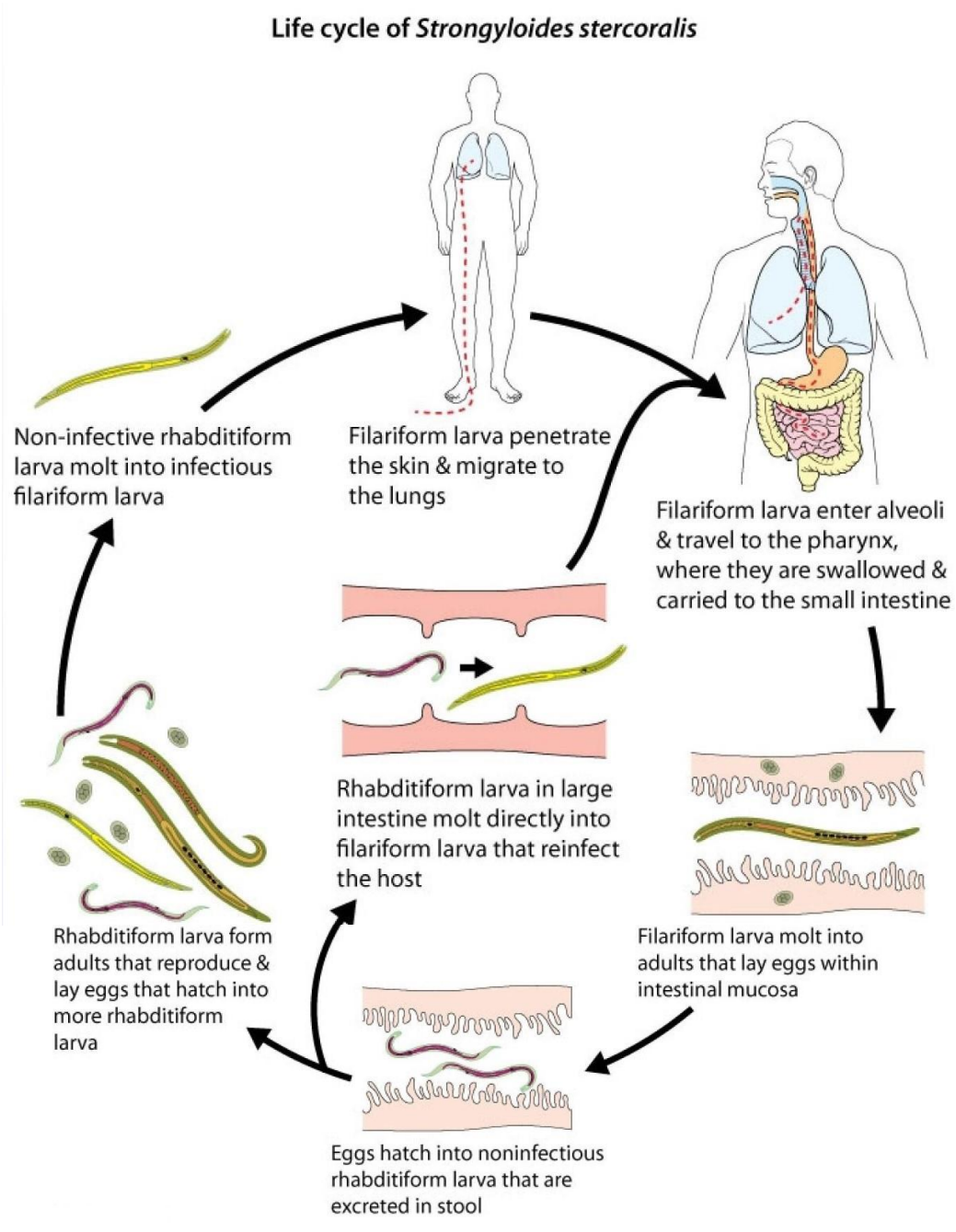


Image 1


Life cycle of Strongyloides stercoralis
Non-infective rhabditiform larva molt into infectious filariform larva
Filariform larva penetrate the skin & migrate to the lungs
Filariform larva enter alveoli & travel to the pharynx, where they are swallowed & carried to the small intestine
Rhabditiform larva in large intestine molt directly into filariform larva that reinfect the host
Filariform larva molt into adults that lay eggs within intestinal mucosa
Rhabditiform larva form adults that reproduce & lay eggs that hatch into more rhabditiform larva
Eggs hatch into noninfectious rhabditiform larva that are excreted in stool

4. Hookworms (Ancylostoma duodenale, Necator americanus):

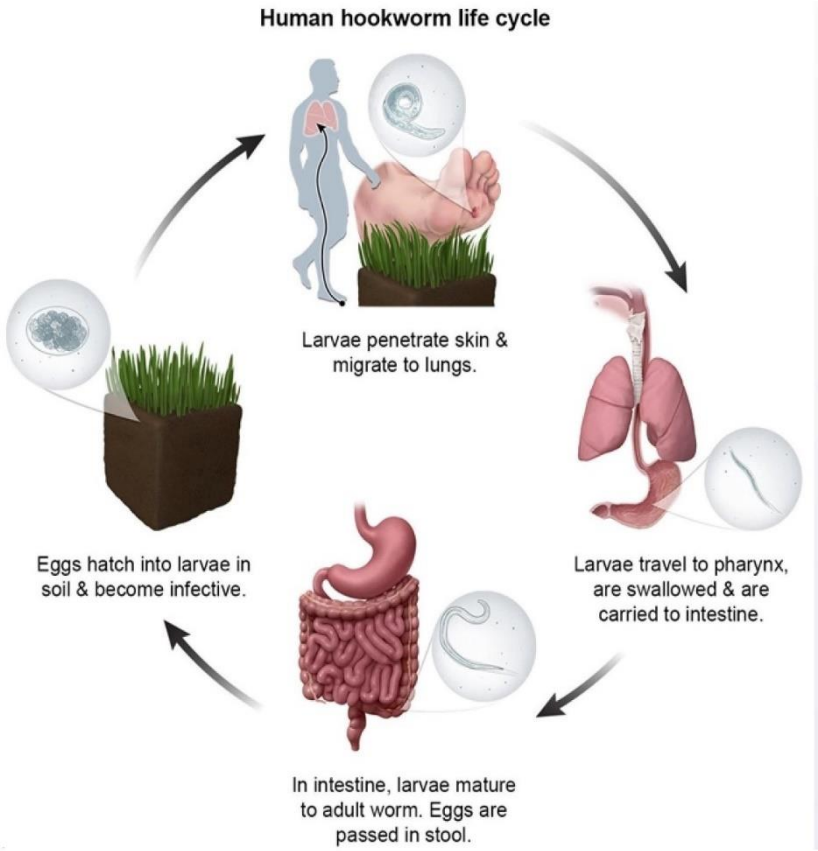
- Mode of transmission: Larvae penetrate skin of bare feet.

- Diseases:

- Intestinal infection causing microcytic anemia by sucking blood from intestinal walls.

- It may also migrate to the lungs → pneumonitis.

- Treatment: Bendazoles or pyrantel pamoate.



5. Trichinella spiralis:

- Mode of transmission: Fecal-oral; undercooked meat (esp. pork).

- Diseases:

- Intestinal infection; larvae enter bloodstream and encyst in striated muscle cells → inflammation of muscle.

- Trichinosis: fever, vomiting, nausea, periorbital edema, myalgia.

- Treatment: Bendazoles.

Tissue infection

1. Onchocerca volvulus:

- Mode of transmission: Female blackfly bite.

- Diseases: Hyperpigmented skin and river blindness (black flies, black skin nodules, "black sight"); allergic reaction to microfilaria possible.

- Treatment: Ivermectin (ivermectin for river blindness).

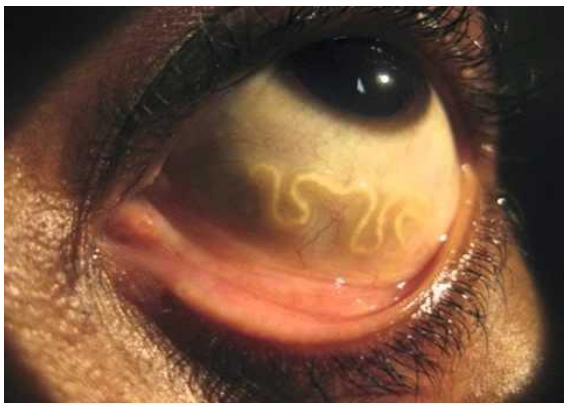
2. Loa loa:

- Mode of transmission: Deer fly, horse fly, mango fly.

- Diseases:

- African eye worm.

- Migration of adult worms is not painful and seldom noticed unless they reach conjunctiva or bridge of nose.



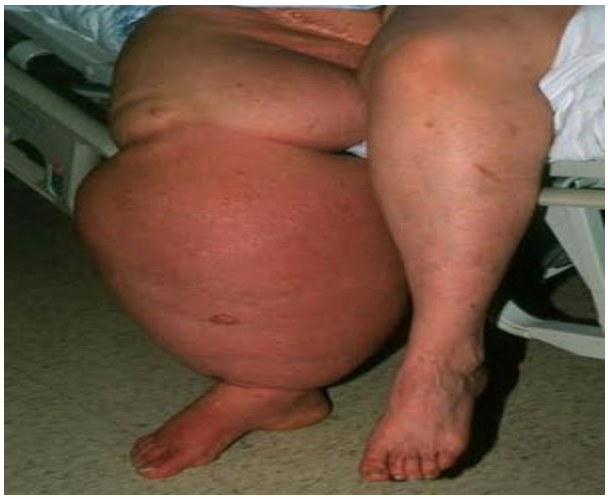
- Treatment: Diethylcarbamazine.

3. Wuchereria bancrofti:

- Mode of transmission: Female mosquito.

- Diseases:

- Elephantiasis: Worms block lymphatic vessels→ lymphedema, lymphangitis and lymphadenitis, takes 9 ms-1 yr after bite to become symptomatic.



- Treatment: Diethylcarbamazine.

4. Toxocara canis:

- Mode of transmission: Fecal-oral, eggs ingested from handling puppies.

- Diseases: Visceral larva migrans (larva wanders aimlessly until they die, cause inflammation).

- Treatment: Bendazoles.

❖ Nematode routes of infection:

- Ingested:

- Enterobius, Ascaris, Toxocara, Trichinella
- You'll get sick if you EATT these!

- Cutaneous:

- Strongyloides, Ancylostoma, Necator.
- These get into your feet from the SANd.

- Bites:

- Loa loa, Onchocerca volvulus, Wuchereria bancrofti.
- Lay LOW to avoid getting bitten.

2. Cestodes (tapeworms)

Taenia solium

- Diseases:

- Humans can be both intermediate and definitive host.

- Ingestion of larvae encysted in undercooked pork (humans are the definitive host) → Intestinal infection.

- Ingestion of eggs excreted in feces of infected human carriers (humans are the intermediate host)→ larva penetrate intestinal wall and migrate via the blood to brain, heart and lungs → Cysticercosis, neurocysticercosis.

- T. solium is endemic in central and south America and neurocysticercosis should be considered in patients from these areas who develop seizures or neurologic symptoms.

- Treatment: Praziquantel; albendazole for neurocysticercosis.

Neurocysticercosis	
Microbiology	• Ingestion of ***Taenia solium*** **(pork tapeworm) eggs** excreted in feces of human carriers
Clinical presentation	• Common in **Central & South America**, sub-Saharan Africa, Asia • Prolonged incubation (months to years) • **Seizures**, focal neurologic symptoms, intracranial hypertension (CSF obstruction)
Diagnosis	• CT/MRI - **cysts**, **scolex** • **Eosinophilia**, ↑ESR
Management	• Antiparasitic therapy (albendazole)

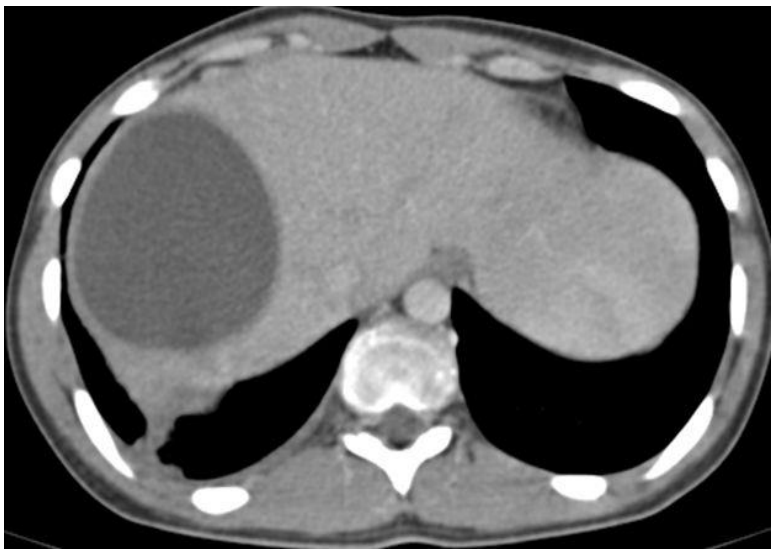
CSF = cerebrospinal fluid; **ESR** = erythrocyte sedimentation rate.

Diphyllobothrium latum

- Mode of transmission: Ingestion of larvae from raw freshwater fish.
- Diseases: Vitamin B_{12} deficiency (tapeworm competes for B_{12} in intestine) → megaloblastic anemia.
- Treatment: Praziquantel.

Echinococcus granulosus

- Mode of transmission:
  - Ingestion of eggs from dog feces.
  - Sheep are an intermediate host.
- Diseases: Hydatid cysts in liver, causing anaphylaxis if antigens released (hydatid cyst injected with ethanol or hypertonic saline to kill daughter cysts before removal).
- Treatment: Albendazole.



3. Trematodes (flukes)

Schistosoma

- Mode of transmission:

- Snails are the host in the water; cercariae penetrate skin of humans.

- Infection is not possible in the United States due to absence of the specific freshwater snails necessary for larval development.

- Diseases:

- Humans acquire schistosomiasis via contact with the freshwater habitat of snails, the intermediate host that incubates the infectious larvae.

- Once released from the snails, larvae penetrate the intact skin of humans and enter the vascular and lymphatic vessels.

- They subsequently travel to the liver and mature into adults over a period of several weeks.

- After maturation, the adult worms migrate to specific destinations:
  - The mesenteric venules of the intestine (S. japonicum and S. mansoni).
  - The vesical venous plexus (S. haematobium).

- The adult worms remain in these blood vessels for life (5-30 years), adhering to the vessel wall with suckers and releasing eggs into circulation.

- Eggs released by S. japonicum and S. mansoni have a tendency to penetrate the bowel wall and be excreted in the feces.

- They also frequently traverse the portal venous system and lodge in the liver.

- S. haematobium eggs tend to pierce the vesical and ureteral walls and be expelled in the urine. On exposure to fresh water, these eggs release larvae that can infect snails and perpetuate the life cycle.

- The clinical manifestations of schistosomiasis result from a TH_2 mediated immune response directed against the eggs.

- This results in granulomatous inflammation and fibrosis, which ultimately causes ulceration and scarring of the bowel or bladder/ureters, depending on the infectious species.

- Eggs that settle into the presinusoidal radicals of the portal vein cause periportal "pipestem" fibrosis (pathognomonic for hepatic schistosomiasis), which eventually results in restriction of portal venous flow and portal hypertension.

- S. haematobium chronic infection has high association with bladder carcinoma in Egypt and Africa.

- Diagnosis: eggs in stool. S. mansoni and japonicum have subterminal spine in their eggs but S. haematobium has a terminal spine.

- Treatment: Praziquantel.

Schistosomiasis		
***Schistosoma* species**	**Location**	**Symptoms**
S haematobium	North Africa, sub-Saharan Africa, the Middle East	**Urinary schistosomiasis:** Terminal hematuria, dysuria & frequent urination; hydronephrosis, pyelonephritis & squamous cell carcinoma of the bladder
S mansoni	Sub-Saharan Africa, the Middle East, South America & the Caribbean	**Intestinal schistosomiasis:** Diarrhea & abdominal pain; intestinal ulceration → iron deficiency anemia
S japonicum	Asia, particularly in China, the Philippines & Japan	**Hepatic schistosomiasis:** Hepatomegaly, splenomegaly; periportal fibrosis & subsequent portal hypertension

Clonorchis sinensis

- Mode of transmission:

- Undercooked fish.

- Diseases:

- Biliary tract inflammation → pigmented gallstones.

- Associated with cholangiocarcinoma.

- Treatment: Praziquantel

❖ Parasite hints:

Association	Organism
Biliary tract disease, cholangiocarcinoma	Clonorchis sinensis
Brain cysts, seizures	Taenia solium (cysticercosis)
Hematuria, squamous cell bladder cancer	Schistosoma haematobium
Liver (hydatid) cysts	Echinococcus granulosus
Microcytic anemia	Ancylostoma, Necator
Myalgias, periorbital edema	Trichinella spiralis
Perianal pruritus	Enterobius
Portal hypertension	Schistosoma mansoni, Schistosoma japonicum
Vitamin B12 deficiency	Diphyllobothrium latum

Ectoparasites

- A parasite that lives on the outside of its host.

Sarcoptes scabiei

- Mode of transmission: Scabies is due to infestation by the Sarcoptes scabiei mite, which burrows into the skin and spreads through direct person-to-person contact but may occasionally occur due to contact with contaminated fomites (bedsheets, clothing).

- Presentation:

- It usually presents with an intensely pruritic rash in the flexor surfaces of the wrist, lateral surfaces of the fingers, and the finger webs.

- The rash is often worse at night and is due to a delayed type IV hypersensitivity reaction to the mite, mite feces, and mite eggs.

- Scabies can also involve other parts of the body (elbows, axillary folds, nipples, and areola in women; scrotum and penis in men), but less commonly affects the back and head (except in children).

- Skin examination usually shows excoriations with small, crusted, red papules scattered around the region. Patients can also develop small vesicles, pustules, or wheals. Linear burrows are the most specific finding in scabies, although they are often obscured by excoriations.

- Diagnosis: Diagnosis is confirmed by skin scrapings from excoriated lesions that show mites, ova, and feces under light microscopy.

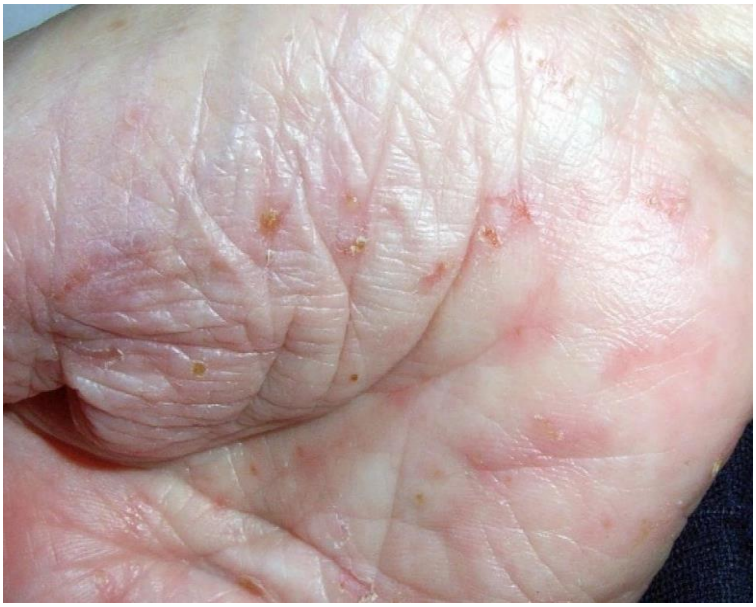
- Treatment:

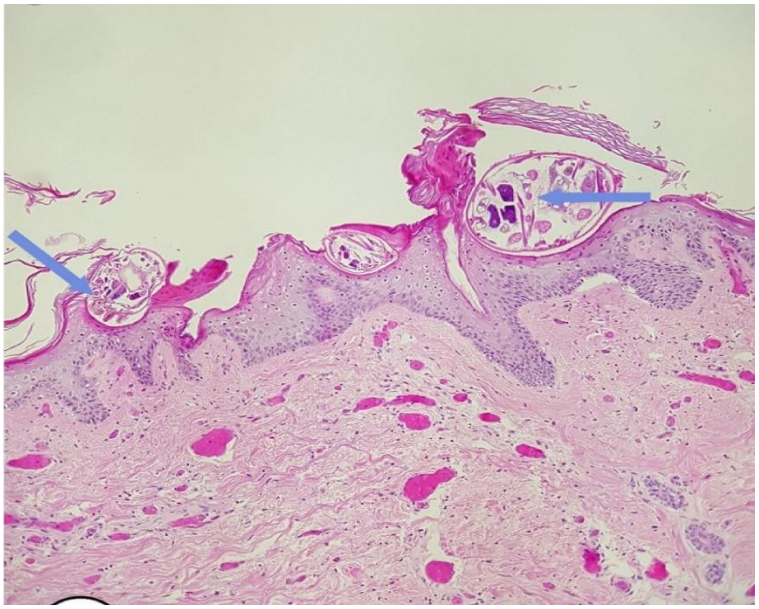
- Treatment is required to prevent discomfort, transmission, and potential complications (secondary bacterial infection).

- First-line therapy includes topical permethrin, which blocks mite neurotransmission by impairing voltage-gated sodium channels. Permethrin cream is applied from the neck to the soles of the feet and left on for 8-14 hours.

- Oral ivermectin, an antiparasitic agent that binds chloride ion channels in invertebrate nerve and muscle cells, is an alternate medication for classic scabies and is used with permethrin for crusted scabies.

- Washing/drying all clothing/bedding, treat close contacts.





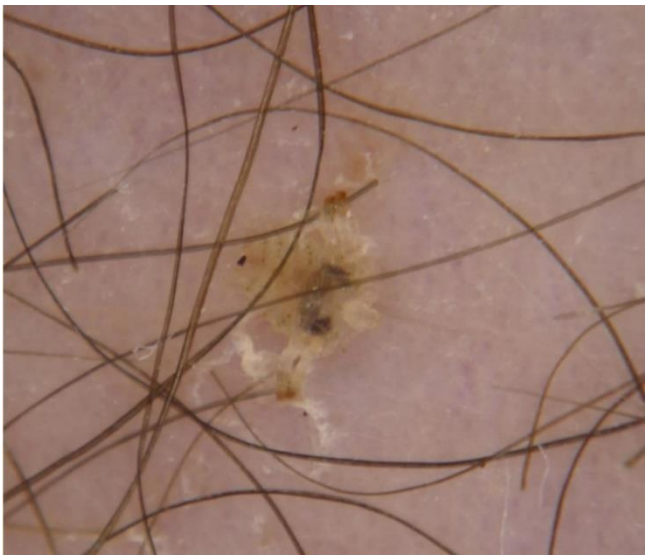
Pediculus humanus/ Phthirus pubis

- Mode of transmission:

- Body lice can transmit Rickettsia prowazekii (epidemic typhus) and Borrelia recurrentis (relapsing fever).

- Phthirus pubis, the human pubic louse, is a translucent parasite approximately 1 mm long with crab-like claws that allow it to grip pubic hair. Transmission usually occurs during sexual contact whereby an infected individual transmits the louse to an uninfected individual by skin-to-skin contact. Therefore, condoms are unable to prevent transmission (it is not transmitted by fluids).

- Teenagers and young adults are affected most commonly due to higher numbers of sexual partners.



- Presentation:

- Blood-sucking lice that cause intense pruritus with associated excoriations due to scratching, commonly on scalp and neck (head lice), waistband and axilla (body lice), or pubic and perianal regions (pubic lice).

- Visualization of the louse or nits (oval, white, louse eggs on the hair shaft) confirms the diagnosis.

- Treatment:

- First-line treatment includes a topical permethrin cream to the affected area. Permethrin blocks parasite sodium ion conduction in nerve cell membrane channels and results in louse paralysis and death.

CHAPTER 4

VIROLOGY

Nature of human viruses

A. **Viruses differ from bacteria in the following:**

- Viruses are very small in size, so they can only be seen under the electron microscope.
- Viruses contain only one type of nucleic acid (DNA or RNA), never both.
- They are obligatory intracellular parasites (can only replicate inside living cells).
- They cannot be cultivated in the laboratory on artificial culture media.
- They are not susceptible to antibacterial antibiotics.

B. **Virus particles are called virions:**

- Virions are composed of either RNA or DNA that is encased in a protein coat called a capsid.
- They are either naked or enveloped, depending on whether the capsid is surrounded by a lipoprotein envelope.

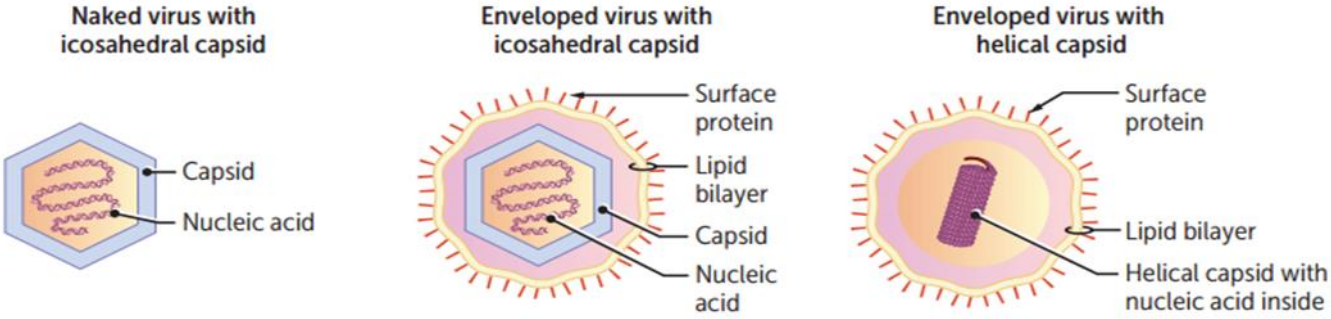
C. **Viral genome:**

- Viral genome may be single stranded or double stranded, linear or circular, and segmented or nonsegmented.
- Its characteristics are used as one criterion for viral classification.

D. **Viral capsid:**

- Capsid is composed of structural units called capsomers, which are aggregates of viral-specific polypeptides.
- They are classified as helical, icosahedral (a 20-sided polygon), or complex; used as a criterion for viral classification.
- Viral nucleocapsid refers to the capsid and enclosed viral genome.
- <u>Serves four functions:</u>

- Protects the viral genome.
- The site of receptors necessary for naked viruses to initiate infection.
- The site of antigenic determinants important in some serologic tests.
- Stimulates antibody production.



D. Viral envelope:

- The viral envelope surrounds the nucleocapsid of enveloped viruses and is composed of an outer lipid bilayer coat acquired from the host cell plasma or nuclear membranes.

- Most enveloped nucleocapsid viruses acquire lipid bilayer envelopes by budding through the plasma membrane of the host cell.

- However, the herpesviruses (which include cytomegalovirus) bud through and acquire the lipid bilayer envelope from the host cell nuclear membrane.

- It contains molecules that are necessary for enveloped viruses to initiate infection, act as a stimulus for antibody production, and serve as antigens in serologic tests; it also forms the basis of ether sensitivity of a virus.

❖ N.B:

1. For synthesis of viral components, viruses must first synthesize messenger RNA (mRNA). Specific messenger RNAs are transcribed from the viral nucleic acid and are translated in the cell ribosomes to form viral components.

- Transcription of mRNA varies according to the type of viral nucleic acid whether DNA or RNA, ds or ss, positive or negative sense strand, as follows:

A. DNA viruses: in which mRNA can be formed using the host's own RNA polymerase to transcribe from negative sense strand → positive sense strand.

B. RNA viruses: these are 4 groups of RNA viruses in which RNA cannot be transcribed like DNA viruses, as host polymerases do not work from viral RNA. The virus must provide its own polymerases. RNA viruses produce mRNA as follows:

a. In dsRNA viruses, one strand is first transcribed by viral RNA-dependent RNA polymerase into mRNA.

b. In ssRNA viruses there are 3 distinct routes to the formation of mRNA:

- The strand with positive sense acts directly as mRNA.
- The strand with negative sense must first be transcribed using viral RNA-dependent RNA polymerase into positive sense strand, which can then act as mRNA.
- Retroviruses which contain positive ssRNA, by the action of reverse transcriptase will produce complementary ssDNA. Thus, it is converted to dsDNA which enters the nucleus and is either integrated in host cell genome using transformation or is transported by host polymerase into mRNA.

2. For a naked (nonenveloped) RNA molecule to induce viral protein synthesis in the host cell, it must act as mRNA capable of using the host's intracellular machinery for translation.
- In other words, the RNA molecule must be single-stranded and positive sense (SS+).
- Generally speaking, only naked SS+ RNA molecules are infectious (rhinovirus), whereas the naked single-stranded negative sense (SS-) RNA molecules and the naked double-stranded RNA molecules are not.
- Separated RNA of a positive sense virus can directly cause infection though it may be less infectious than the whole virus particle.
- RNA of a negative sense virus is not infectious by itself because it cannot be replicated or translated in absence of the viral RNA polymerase.
- Thus, naked viruses containing single-stranded positive-sense RNA can be infectious, whereas naked viruses containing single- or double-stranded negative sense RNA are not infectious.

3. Successful viral replication requires the synthesis of viral proteins through the translation of viral-specific mRNA by the host cell ribosomal machinery.
- Eukaryotic translation is a monocistronic process, meaning that a finalized mRNA sequence codes only for a single protein product.
- In contrast, many prokaryotic mRNAs and viral genomes are polycistronic, meaning they contain multiple cistrons, or protein-coding sequences, within the same transcript.
- Because viruses must use eukaryotic ribosomes for protein synthesis, they must convert their polycistronic genome into monocistronic mRNA through 1 or more of the following processes:

A. The viral genome may already be segmented into multiple pieces, with each piece functioning as an individual mRNA strand.
B. A transcription promoter can precede each gene within the viral genome to form individual mRNA strands (similar to eukaryotic DNA transcription).
C. Precursor RNA strands can undergo alternative splicing to produce different finalized mRNA sequences.
D. The viral genome can serve as (or be transcribed into) a single mRNA that is translated into 1 long polyprotein and subsequently cleaved into separate proteins.

- For functional individual viral proteins to be generated, the polyprotein product must be cleaved by a specific viral protease (often part of the polyprotein).
- Viruses that demonstrate this particular method of viral replication include single-stranded, positive-sense, linear, nonsegmented RNA viruses such as echovirus (Picornaviridae family).

4. Ether and other organic solvents can inactivate the "enveloped" viruses, which by definition have an outer lipid bilayer coat acquired from the host cell plasma or nuclear membranes.
- The solvent-induced disruption or dissolution of the envelope lipid results in a loss of viral infectivity.
- Non-enveloped viruses are generally resistant to the action of ether.

Viral classification

- Classification is based on chemical and physical properties of virions.
- Viruses are classified into major families (DNA and RNA viruses), which are further subdivided by physiochemical and serologic characteristics into genera.
- DNA viruses:

- Contain double-stranded DNA (except for parvovirus).
- Are naked viruses (except for herpesviruses, hepadnaviruses, and poxviruses).
- All are linear except papilloma-, polyoma-, and hepadnaviruses (circular).
- Have icosahedral capsids (except for poxviruses).
- All replicate in the nucleus (except for poxviruses).

❖ N.B:

- DNA viruses All replicate in the nucleus (except poxvirus).
- RNA viruses All replicate in the cytoplasm (except influenza virus and retroviruses).

General rule	Comments
DNA Viruses mnemonic: Pardon Papa As He has Pox	Parvovirus, Papillomavirus, Polyomavirus, Adenovirus, Hepadanavirus, Herpes virus, Poxvirus
Are double stranded	Except parvo (single stranded).
Are linear	Except papilloma and polyoma (circular, supercoiled) and hepadna (circular, incomplete).
Are icosahedral	Except poxvirus (complex).
Are Naked	Except poxviruses, herpesviruses, and hepadnaviruses
Replicate in the nucleus	Except poxvirus (carries own DNA-dependent RNA polymerase, so it can transcribe its own DNA in the cytoplasm).

Parvoviridae

- Virus Characteristics:
- ssDNA virus, linear (smallest DNA virus).
- Naked, icosahedral.

- Viruses of Medical Importance: B_{19}.

Parvovirus (B_{19})

- Habitats: human respiratory tract.

- Mode of transmission: respiratory route, saliva and vertical transmission.

- Pathogenesis:
- Parvovirus B_{19} is attach to human erythroid cells via the blood group P antigen (globoside).

- The P antigen is expressed by mature erythrocytes, erythroid progenitors, megakaryocytes, placenta, and the fetal liver and heart.

- Immature cells of the erythroid family are most vulnerable to parvovirus B_{19} infection, which is why adult bone marrow and fetal liver are principal targets. Viral replication causes cell death.

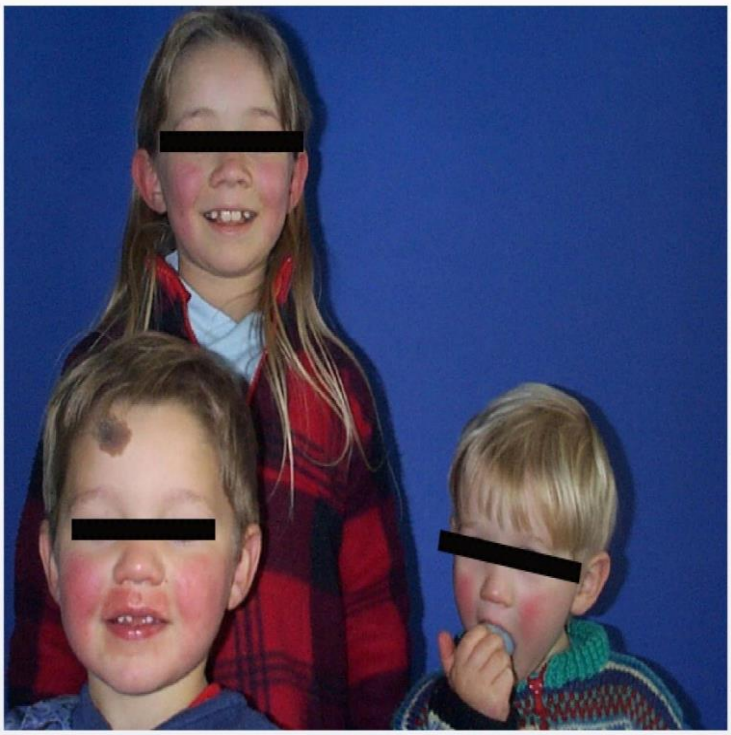
- Diseases: B_{19} is associated with erythema infectiosum ("fifth disease"), aplastic crises in those with sickle cell anemia, and hydrops fetalis (particularly when infection occurs before the 20th week of gestation).

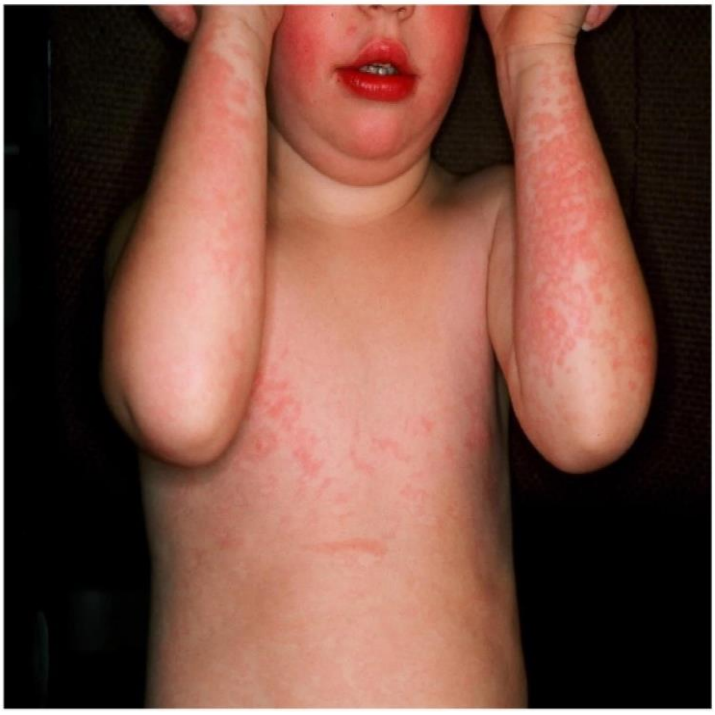
1. Erythema infectiosum ("fifth disease"):
- The prodrome of low-grade fever, headache, malaise, and upper respiratory symptoms followed by the sudden appearance of an erythematous malar rash with circumoral pallor 2-5 days later is characteristic of erythema infectiosum (''fifth disease").

- This rash has a "slapped-cheek" appearance and usually spares the nasolabial folds.

- As the facial rash fades, an erythematous rash in a reticular, lacelike pattern often appears on the trunk and extremities.

- The rash of erythema infectiosum due to infection of the endothelial cells as well as deposition of immune complexes.





2. Aplastic crisis in sickle cell patients:

- This virus is highly tropic for erythroid precursor cells and replicates predominantly in the bone marrow.

- Aplastic crisis in sickle cell patients is usually secondary to parvovirus B 19 infection of erythroid precursor cells in the bone marrow. Destruction of the erythroid precursor cells by this virus diminishes the number of reticulocytes available to replace the deformed and/or removed erythrocytes.

- Normally, If the bone marrow were able to respond appropriately to the degree of anemia, the reticulocyte count would be elevated (normal reticulocyte count is 0.5-1.5% of red cells). But in aplastic anemia, the patient's reticulocyte count persists at the low end of normal.

3. Hydrops fetalis:

- It also causes hydrops fetalis if transmitted from mother to fetus (pregnant woman with with arthralgia with or without rash → hydrops fetalis or spontaneous abortion).

Papillomaviridae

- Virus Characteristics:
- dsDNA virus, circular.
- Naked, icosahedral.

- Viruses of Medical Importance: Human papilloma virus (HPV).

Human Papilloma Virus (HPV)

- Habitats: human skin and genitals.

- Mode of transmission: direct or sexual contact.

- Pathogenesis:
- Virus infects basal cell layer of the stratified squamous epithelium and mucous membranes.

- Hyperkeratosis leads to the formation of the "wart".

- HPV replicate in the nucleus of squamous epithelial cells. The genome is contained within a spherical protein capsid with 7 Early proteins (E1 to E7), and 2 Late structural proteins (L1 and L2). Based on L1 gene sequence difference there are more than 100 HPVs genotypes.

- Important types include:
  - The high-risk types HPV 16 & 18 associated with cervical cancer.
  - The low-risk types HPV 6 & 11 cause most genital warts (benign).

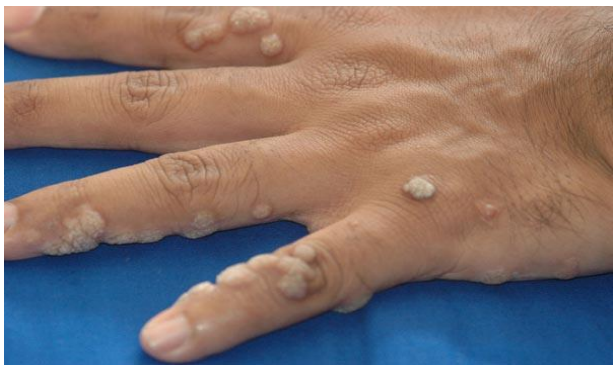
- Diseases:

A. Cutaneous warts:

- Serotypes (1, 2, 6, 11).

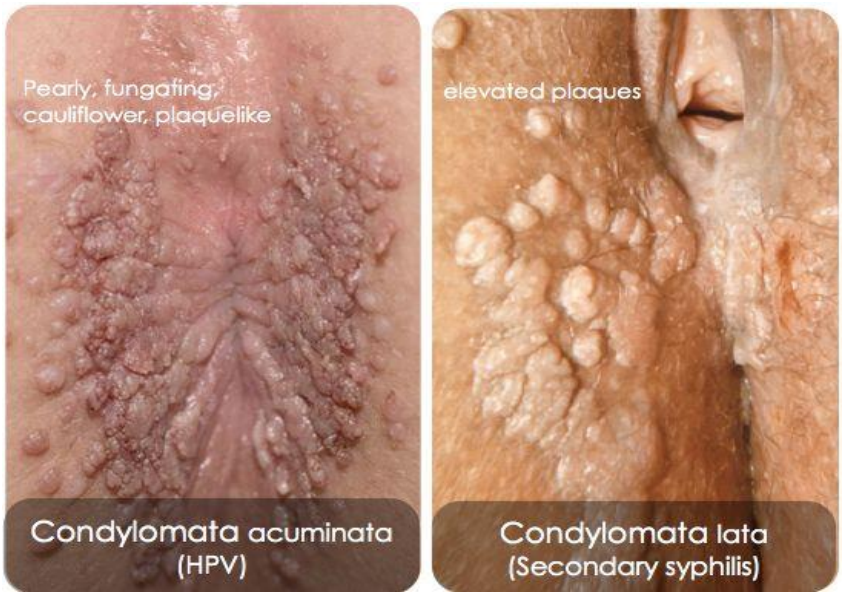
- HPV produces a chronic infection of basal cell layer of the stratified squamous epithelium.

- Hyperkeratosis leads to the formation of warts.



B. Anogenital warts (Condylomata acuminate):

- Over 90% of genital warts are serotypes 6 and 11 (benign).



C. Cervical and anal carcinoma:

- Serotypes 16 and 18 are preneoplastic (cervical intraepithelial neoplasia; CIN).

- 95% of cases of CINs contain HPV DNA.

- Integration of HPV DNA leads to high expression of E6 and E7 genes leading to overproduction of E6 and E7 proteins leading to inactivation of the two suppressor genes p53 and retinoblastoma → induction of abnormal mitosis.

- Human papillomavirus (HPV) types 16 and 18 are strongly associated with anal and cervical squamous cell carcinoma.

- Immunodeficiency states (AIDS) increase the host's susceptibility to HPV infection and more severe infection. Consequently, HIV infection is associated with a higher incidence of anogenital carcinomas.

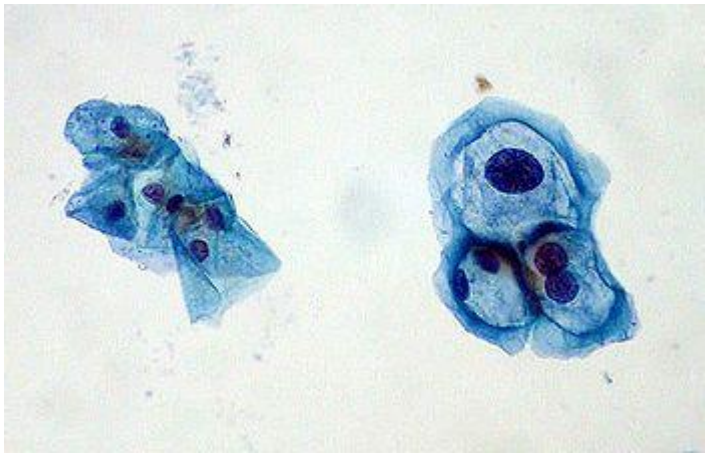
- HIV-positive homosexual males (men who have sex with men) are more prone to developing anal squamous cell carcinoma (anal intercourse is hypothesized to be related), and HIV-positive females are more prone to developing cervical squamous cell carcinoma.

▪ Diagnosis:

- Cutaneous: clinical grounds.

- Genital: finding of koilocytic cells (cells with perinuclear cytoplasmic vacuolization and nuclear enlargement) in Pap smears.

- In situ DNA probes and PCR can be used to confirm any diagnosis and type the HPV strain involved.



- Prevention:

- A vaccine composed of HPV capsid proteins produced by recombinant DNA technology.

- Safe sex practices.

Polyomaviridae

- Virus Characteristics:
- dsDNA virus, circular.
- Naked, icosahedral.

- Viruses of Medical Importance:
- BK Virus.
- JC Virus.

BK Virus

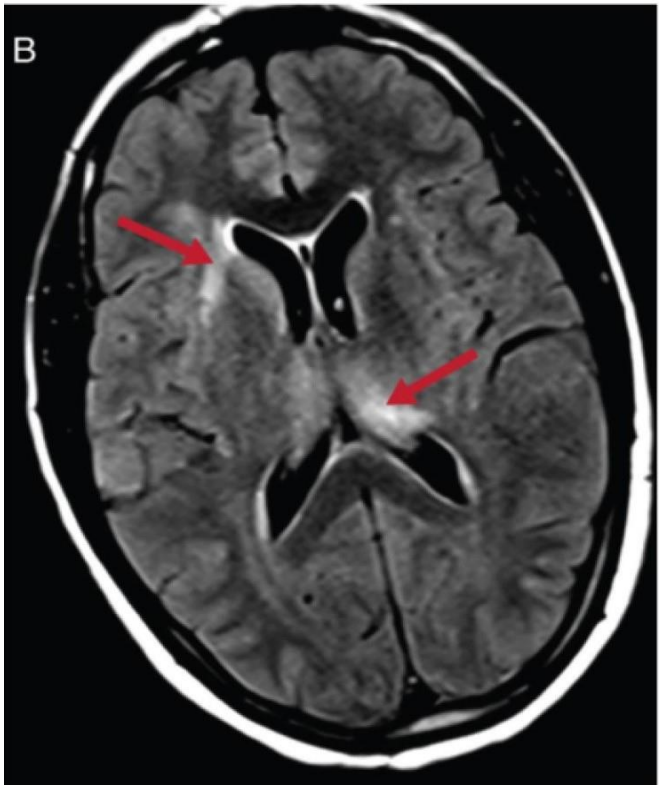
- Habitats: human respiratory tract.
- Mode of transmission: respiratory route.
- Pathogenesis: Latent infection in kidney (Rental tubular epithelial cells).
- Diseases: Renal disease in AIDS patients.

❖ Mnemonic: BK: Bad Kidney.

JC Virus

- Habitats: human respiratory tract.
- Mode of transmission: respiratory route.
- Pathogenesis: Latent infection in oligodendrocytes → demyelination.
- Diseases:
- Progressive multifocal leukoencephalopathy (PML) in AIDS and transplant patients.
- This condition usually presents with slowly progressive confusion, ataxia, and motor deficits.
- Brain MRI reveals multifocal areas of white matter demyelination with no mass effect or enhancement.

❖ Mnemonic: JC: Junky Cerebrum.



Adenoviridae

- Virus Characteristics:

- dsDNA, nonenveloped, icosahedral.
- They were isolated from adenoidal tissue of children, hence the name adenovirus.

- Viruses of medical Importance: Adenovirus.

Adenovirus

- Habitats: ubiquitous in humans and animals.

- Mode of transmission:

- Adenovirus is transmitted via direct contact, fecal-oral route, or respiratory droplets.

- Adenovirus infection occurs in outbreaks in crowded quarters (day care centers, camp dormitories, military barracks).

- Pathogenesis:

- Adenoviruses are the only viruses with fibers protruding from the capsid.

- The fiber is the organ of attachment, a strong hemagglutinin and toxic to human cells.

- Diseases:

A. Acute respiratory disease (ARD) and pneumonia:

- Spring and winter peak incidence.

- Children, young military recruits and college students.

B. Pharyngoconjunctivitis fever:

- Specifically, this condition is characterized by acute, self-limited, febrile pharyngitis, cough, nasal congestion, conjunctivitis, and enlarged cervical nodes.

- This condition accounts for only 2-4% of the acute viral diseases of the upper respiratory tract in civilian populations. However, pharyngoconjunctival fever is epidemiologically much more common in small groups of individuals who are living in crowded quarters.

- Thus, adenovirus outbreaks are seen more often among military recruits or campers.

C. Epidemic keratoconjunctivitis:

- Highly contagious.

- Associated with foreign particles in eye.

D. Acute hemorrhagic cystitis:

- A urinary tract infection characterized by dysuria and hematuria is most likely hemorrhagic cystitis.

- Acute hemorrhagic cystitis in children may be caused by bacteria or viruses, though in a majority of cases no infectious agent can be cultured from the urine.

- The most common known viral cause of acute hemorrhagic cystitis in children (and males in particular) is adenovirus.

E. Gastroenteritis: Daycare, not as common as rotavirus.

Hepadnaviridae

- Virus Characteristics:

- dsDNA, circular.
- Enveloped, icosahedral.

- Viruses of medical Importance: Hepatitis B virus (we will talk about it in details in hepatitis).

Herpesviridae

- Virus Characteristics:

- Large dsDNA.
- Enveloped, icosahedral.
- Derives envelope from nuclear membrane.
- Intranuclear inclusion bodies.
- Establishes latency.

- Viruses of medical Importance:

- Herpes simplex virus 1 and 2 (HSV).
- Varicella-zoster virus (VZV).
- Epstein-Barr virus (EBV).
- Cytomegalovirus (CMV).
- Human herpesvirus 6 (HHV-6).
- Human herpesvirus 8 (HHV-8).

Viruses that cause latent infection	
Site of latency	**Virus**
Sensory neurons	Herpes simplex & varicella zoster viruses
B lymphocytes	Epstein-Barr virus
Myeloid cells	Cytomegalovirus, human herpesvirus 6 & 7
CD4 T lymphocytes	Human immunodeficiency virus
Stratified squamous epithelial cells	Human papilloma virus
Renal tubular epithelial cells	BK virus
Neuroglial cells	JC virus

HSV-1 and HSV-2

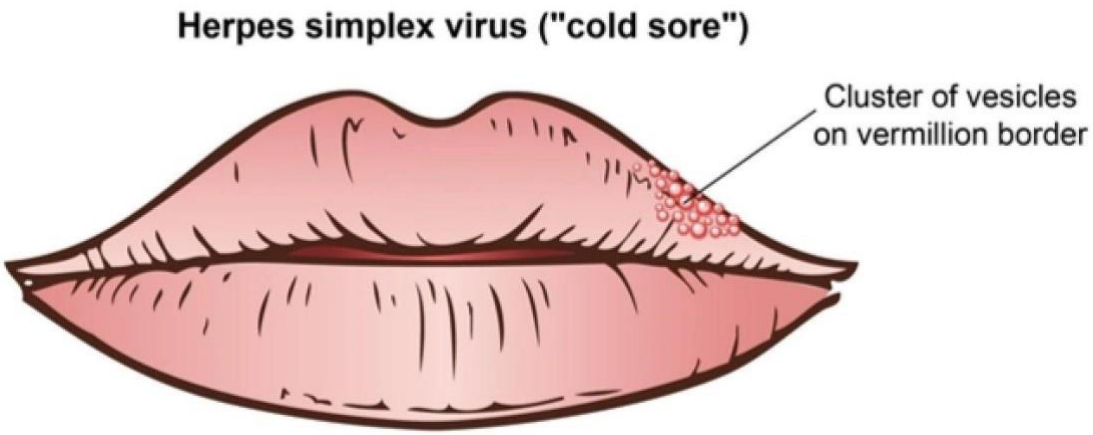
- Habitats: human mucosa and ganglia.
- Mode of transmission: close personal contact (kissing, sexual contact).
- Pathogenesis:

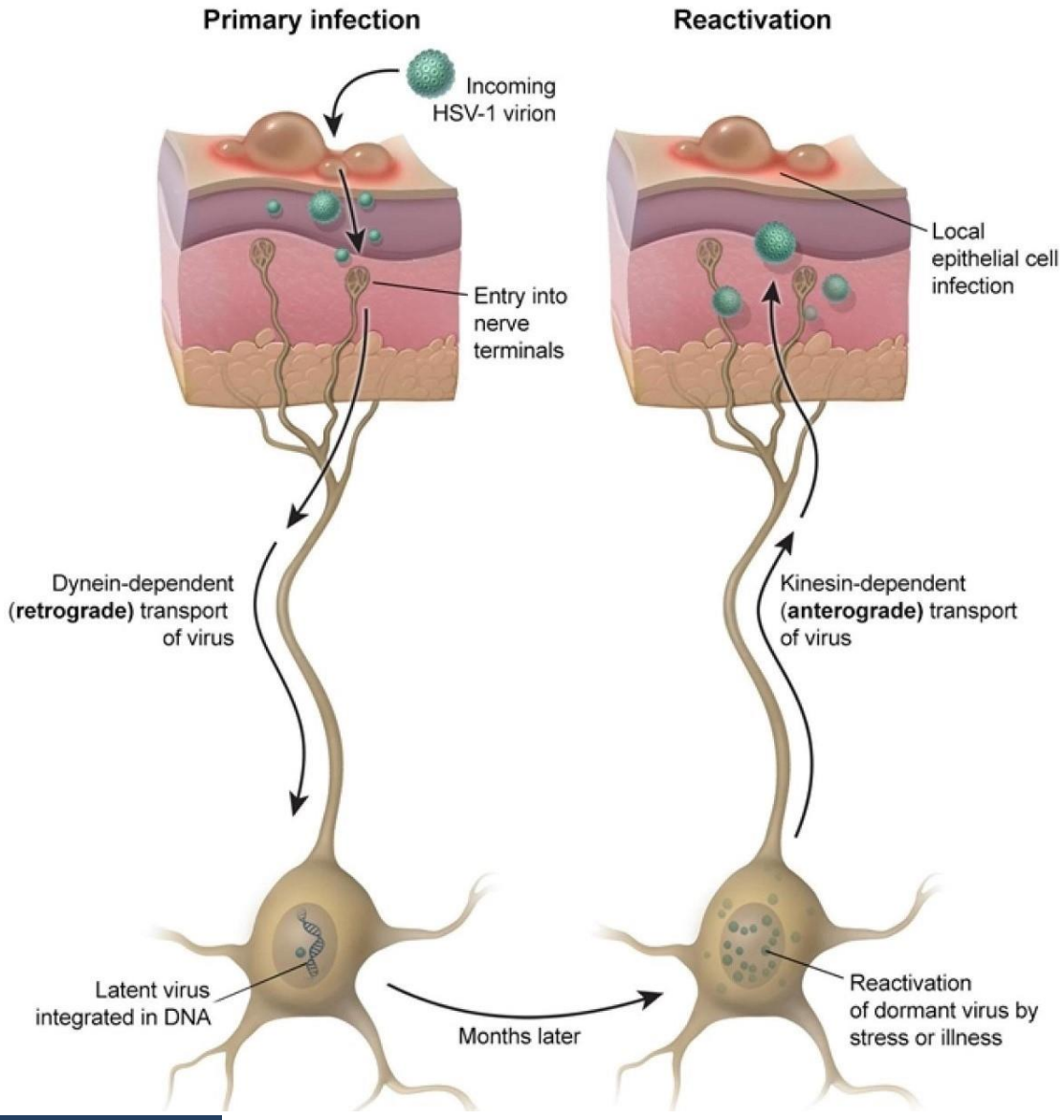
- HSV establishes infection in the mucosal epithelial cells and leads to the formation of vesicles.
- The virus travels up the ganglion to establish lifelong latent infection.
- Stress triggers reactivation of virus in nerve and recurrence of vesicles.

- Diseases: The rule of thumb is that HSV-1 infections generally occur above the waist and HSV-2 infections generally occur below the waist.

A. Gingivostomatitis and cold sores:

- Herpetic gingivostomatitis is the most common clinical manifestation of primary herpes simplex (HSV-1) infection; it occurs most often in children aged 1-3 years and results in fever, vesiculoulcerative lesions of the oral mucous membranes, and localized lymphadenopathy.
- Latent in trigeminal ganglion.
- Reactivation of a latent HSV infection in the trigeminal ganglia generally results in more limited perioral blisters or cold sores (recurrent herpes labialis).
- Intranuclear inclusions are characteristic of herpesviruses, which replicate predominantly within the host cell nucleus.





B. Keratoconjunctivitis:

- Generally, with lid swelling and vesicles.
- Dendritic ulcers may be seen.
- Untreated and repeat attacks may result in blindness.

C. Temporal lobe encephalitis:

- Herpes simplex virus type 1 (HSV-1) encephalitis is the most common cause of fatal sporadic encephalitis, or inflammation of the brain parenchyma.
- Viral meningitis is more common with HSV-2 than with HSV-1
- HSV-1 encephalitis results from primary oropharyngeal infection that travels via the olfactory tract or from the reactivation of latent virus in the trigeminal ganglion with subsequent spread into the cerebral vault.

- Symptoms of acute encephalitis include acute onset of headache, fever, mental status changes (lethargy, disorientation), cranial nerve deficits (Bell's palsy), and seizures.

- Temporal lobe damage can result in receptive aphasia and personality changes (hypersexuality, aggression).

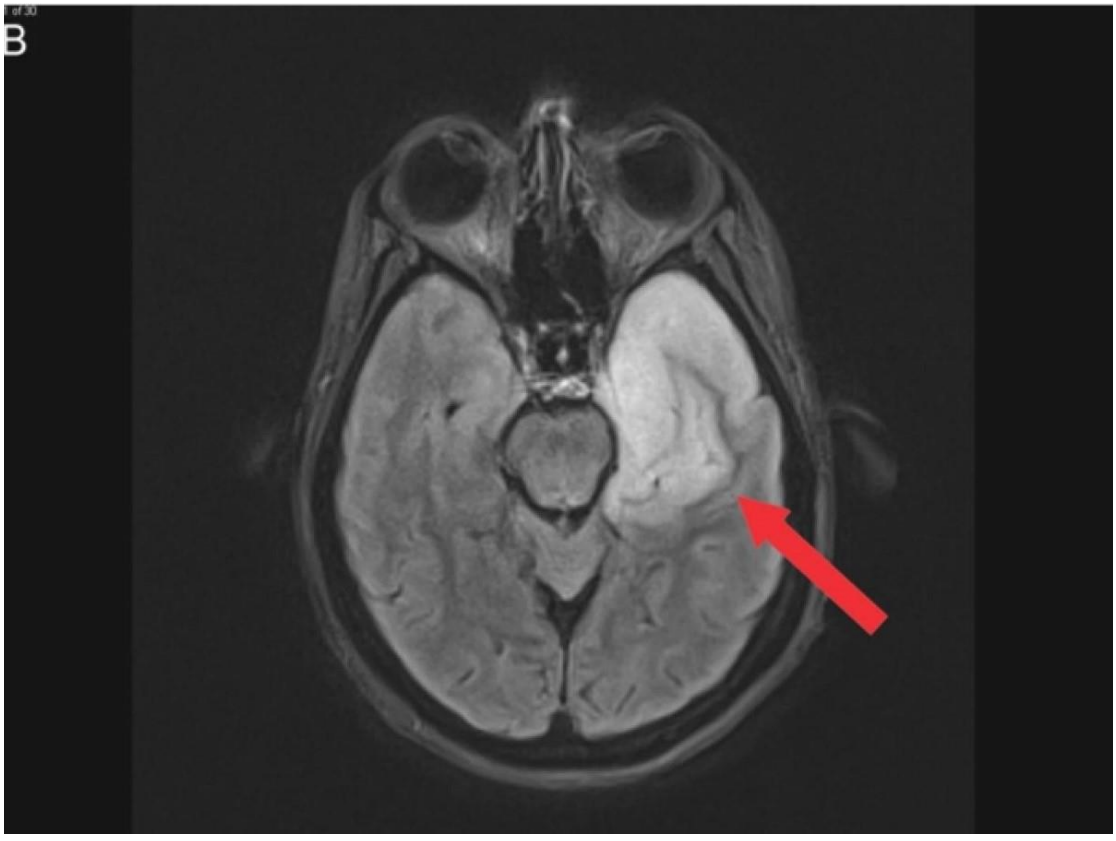
- CT, MRI (red arrow), and post-mortem macroscopic brain examination reveal edema and hemorrhagic necrosis of the temporal lobe.

- Unilateral involvement is most common, but bilateral necrosis can also occur. Definitive diagnosis is made by polymerase chain reaction testing of cerebrospinal fluid.

- If untreated, 70% mortality rate.

HSV encephalitis	
Pathogenesis	HSV-1 → olfactory tract → olfactory cortex (temporal lobe)
Presentation	Fever, headache, seizures, aphasia, mental status/behavior changes
Diagnosis	Temporal lobe hemorrhage/edema on brain imaging Cerebrospinal fluid PCR for HSV
Treatment	Intravenous acyclovir

HSV-1 = herpes simplex virus type 1.



D. Genital infections (herpes genitalis):

- It is the classic presentation of HSV-2 infection.

- It manifests as extensive bilateral painful vesicular lesions in the genital area, accompanied by fever, dysuria and inguinal lymphadenopathy.

- Latent infection within the S_2, S_3, and S_4 dorsal root (sensory) ganglia.

- HSV-2 produces a recurrent, painful genital rash ("herpes genitalis") when the latent virus is reactivated in the sacral sensory ganglia.

E. Neonatal herpes:

- Infection with HS-2 during passage through infected birth canal.

- It is the most serious complication of genital herpes.

- Infections are usually severe:
  - Disseminated with liver involvement and high mortality.
  - Encephalitis (high mortality).
  - Skin, eyes, or mouth.

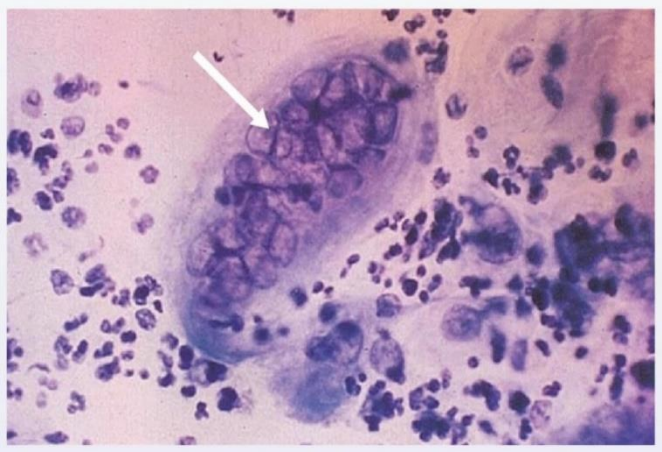
- Diagnosis:

- Diagnosis can be established by polymerase chain reaction testing for viral DNA, direct fluorescence antibody testing, viral culture, or Tzanck smear.

- Tzanck smear:
  - It requires the application of a stain solution (most commonly the Wright-Giemsa stain) to epithelial cells that are scraped from an ulcer base.

  - The presence of multinucleated giant cells with cowdry type A intranuclear inclusions suggests a herpes simplex virus (HSV) or varicella zoster virus (VZV) infection.

  - Tzanck heavens I do not have herpes.



Varicella Zoster Virus (HHV-3)

- Habitats: human mucosa and nerves.

- Mode of transmission: respiratory droplets.

- Pathogenesis:

- VZV enters the respiratory tract → replicates in the local lymph nodes → primary viremia → spleen and liver → secondary viremia → skin (rash) → latent in the dorsal root ganglia.

- Reactivation of virus due to stress or immunocompromise causes vesicular lesions and severe nerve pain.

- Diseases:

A. Chickenpox:

- Primary infection with VZV typically occurs in children and is a highly contagious condition described as chickenpox.

- Clinically, children with chickenpox present with fever, malaise, pharyngitis, and a generalized vesicular and pruritic rash.

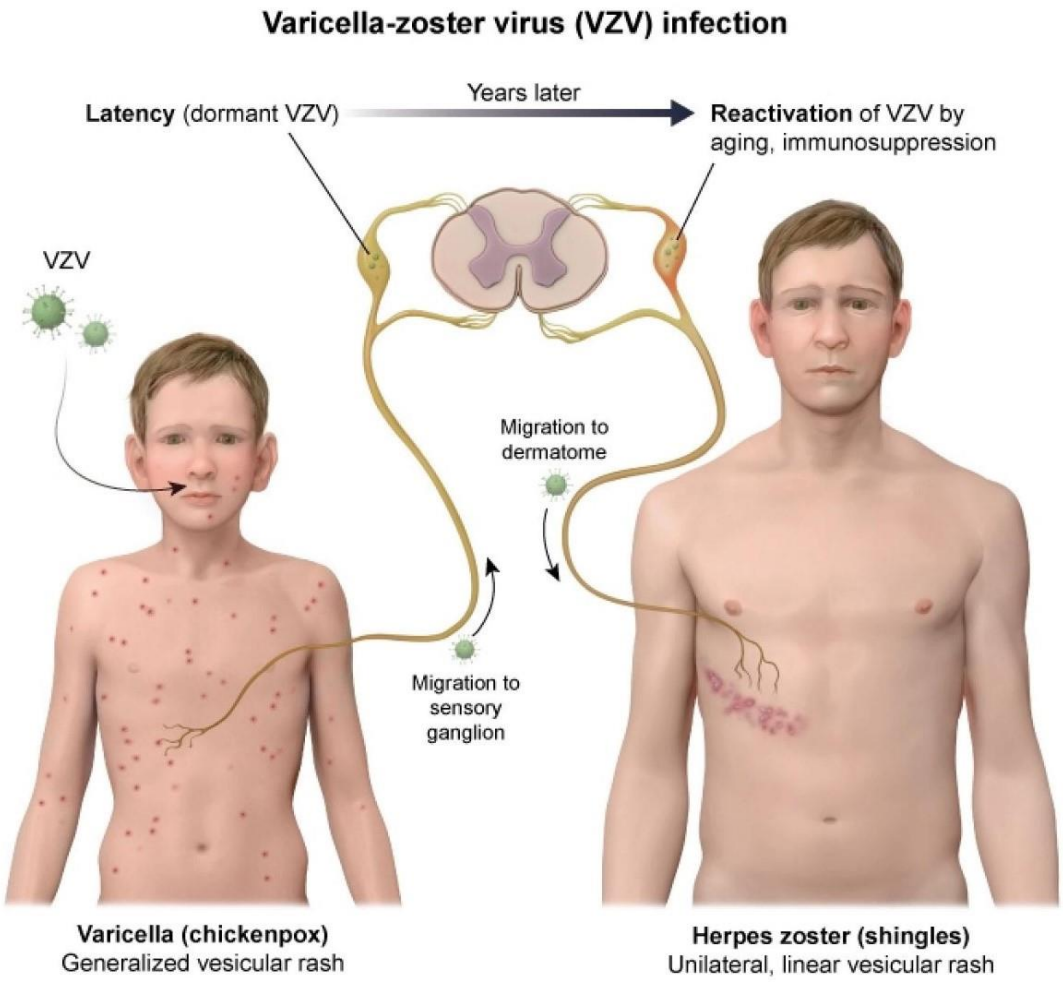
- The skin lesions appear as successive crops on the face, trunk, and limbs, and most have crusted within 6 days of onset.

- The vesicles changes to pustules which dry to form scabies which heal without scar formation.

- Children who have experienced chickenpox infection are typically resistant to future episodes of chickenpox but can develop herpes zoster later in life.

- Varicella lgG antibodies suggest an antecedent primary varicella-zoster virus (VZV) infection. These antibodies generally confer immunity against chickenpox reinfection but not against herpes zoster, which is reactivation of VZV.

- The varicella vaccine is recommended for all children aged 12-18 months, women of childbearing age, adults with sustained risk of exposure, and household contacts of immunocompromised hosts.



B. Zoster (shingles):

- The natural history of VZV infection of the dorsal root ganglia involves reactivation that results in a painful, vesicular skin eruption along the sensory dermatomes (a phenomenon called herpes zoster, or "shingles").

- A unilateral vesicular rash localized on a single dermatome in an elderly patient is most likely herpes zoster.

- Herpes zoster arises when latent varicella zoster virus (VZV) infection is reactivated within a single dorsal root sensory ganglion.

- Shingles arises most commonly in the thoracic or trigeminal dermatomes.

- The clinical combination of varicella IgG antibodies and a dermatome-centered, vesicular, painful rash is strongly suggestive of herpes zoster (shingles).

- Localized dermatomal pain that persists for more than one month after a zoster eruption is termed post herpetic neuralgia and is the most common neurological complication of VZV infection.

- Post-herpetic neuralgia is typically described as "stabbing" may be constant or intermittent, and often lasts for several months.

▪ Diagnosis:

- Tzanck smear-Cowdry type A, intranuclear inclusions.

- Antigen detection by PCR.

Epstein-Barr Virus (HHV-4)

▪ Habitats: humans.

▪ Mode of transmission: Saliva (kissing disease).

▪ Pathogenesis:

- Virus infects nasopharyngeal epithelial cells, salivary and lymphoid tissues → latent infection of B cells (EBV binds to CD21 and acts as a B-cell mitogen) and leads to polyclonal activation that result in the appearance of antibodies directed against a wide range of self and heterophile antigens.

- Infection of B lymphocytes is followed by a marked T cell response which is detected as large number of atypical lymphocytes in the peripheral blood.

▪ Diseases:

1. Heterophile-positive infectious mononucleosis (glandular fever):

- EBV is generally transmitted from an asymptomatic virus shedder to a susceptible individual through saliva transfer (kissing).

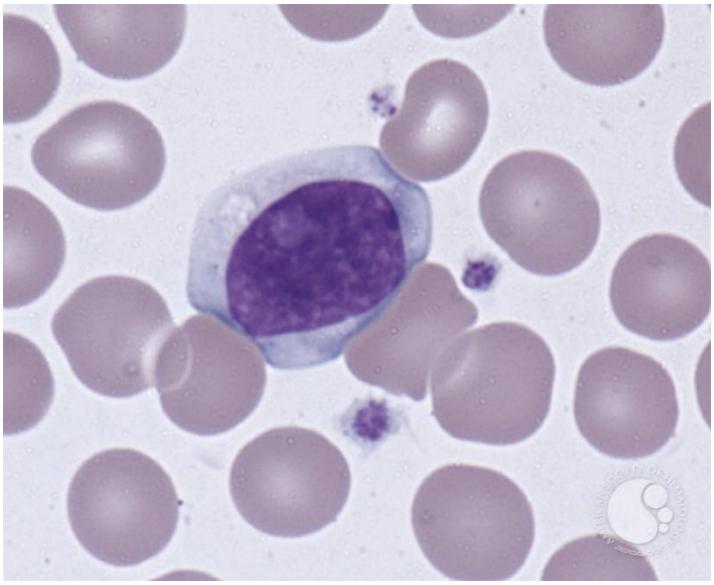
- Typical clinical and laboratory features of Epstein-Barr mononucleosis include fever, pharyngitis, lymphadenopathy, hepatosplenomegaly, atypical lymphocytosis, and a positive Monospot test (positive heterophile antibodies). Avoid contact sports until resolution due to risk of splenic rupture.

- One serologic means of diagnosing EBV infection is the Monospot test, which detects a heterogeneous group of IgM antibodies that react with the heterophile antigens present on horse red blood cells.

- Agglutination of these horse RBCs by human serum is a sensitive and highly specific test for EBV infection in the B-cell compartment of the human host (positive Monospot test).

- Cytomegalovirus (CMV), human herpesvirus 6 (HHV-6), and toxoplasmosis are all known to cause mononucleosis-like syndromes characterized by negative Monospot tests. CMV is the most common cause of heterophile antibody-negative (Monospot-negative) mononucleosis.

- Use of amoxicillin in mononucleosis can cause characteristic maculopapular rash.



2. Lymphoproliferative disease:

- Essential to viral invasion of cells and the viral tropism for specific tissues is the initial attachment of the virion envelope or capsid surface proteins to the complementary host cell surface receptors.

- Many viruses bind to normal host cell plasma membrane receptors in order to enter host cells.

- Known host cell receptor include:
  - CD4 and HIV gp120.
  - CD21 and EBV.
  - Erythrocyte P antigen and parvovirus B19 .
  - The EBV envelope glycoprotein gp350 binds to the cellular receptor of B lymphocytes (CD21).

- CD21 is normally present on the surface of B cells and nasopharyngeal epithelial cells.

- Thus, a monoclonal anti-CD21 antibody could interfere with the attachment of EBV to cells.

- Epstein-Barr virus (EBV) commonly infects B cells, stimulating them to enter the cell cycle and proliferate continuously (a process termed "transformation" or "immortalization") mediating their transformation into long-term proliferating cell lines.

- This process is accomplished when EBV binds to the cell surface and the EBV-encoded oncogenes activate proliferative and anti-apoptotic signaling pathways within the B cell.

- In an immunocompetent host, EBV-induced B cell proliferation is held in check by a vigorous cell-mediated and humoral immune response.

- Latent Epstein-Barr virus (EBV) infection is present in up to 90% of normal individuals, with reactivation common in the immunosuppressed (those with AIDS).

- As a result, AIDS patients have an increased incidence of EBV-associated non-Hodgkin's lymphomas, including the aggressive diffuse large B-cell lymphomas and Burkitt's lymphoma.

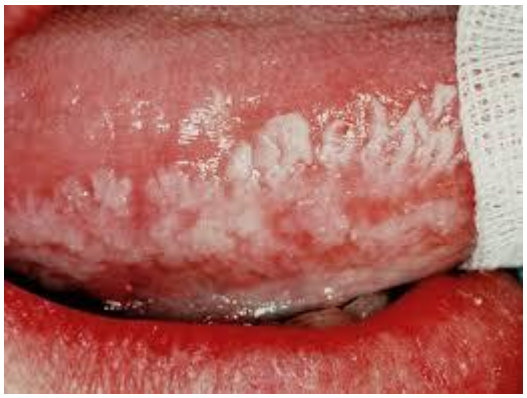
- The use of highly active antiretroviral therapy to replenish CD4+ T-cell counts in HIV-positive patients can lower the increased risk of developing HIV-induced non-Hodgkin's lymphoma.

3. Hairy oral leukoplakia:

- Leukoplakia is a benign lesion of the tongue caused by hyperplasia of the squamous mucosa that can evolve into dysplasia → carcinoma in situ → invasive carcinoma.

- Leukoplakia appears as white patches or plaques on the oral mucosa. However, these lesions cannot be easily removed with scraping (unlike candida oral thrush).

- AIDS patients.



4. Malignancies associated with EBV infection:

- Burkitt's lymphoma.

- Nasopharyngeal carcinoma.

- Hodgkin lymphoma.

▪ Diagnosis:

- Heterophile-antibody positive (IgM antibodies that recognize the Paul Bunnell antigen on sheep and bovine RBCs).

- Serology based on EBV viral antigens.

Cytomegalovirus (HHV-5)

- Habitats: humans.

- Mode of transmission: saliva, sexual, parenteral (Blood transfusion), in utero.

- Pathogenesis:

- Primary infection occurs in 40-60% of individuals and the virus persist in the host for live (latent infection).

- Latent in mononuclear cells (CMV).

- Reactivation is common.

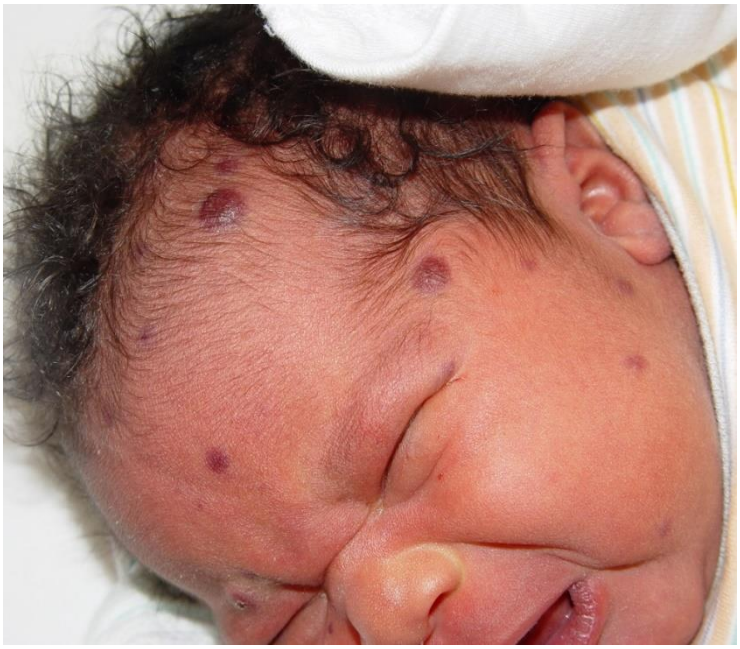
- Diseases:

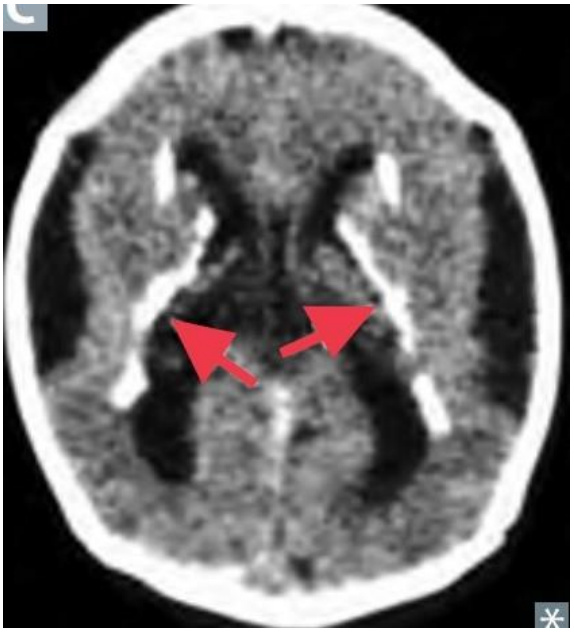
A. Congenital infection (Cytomegalic inclusion disease):

- Most common in utero infection in U.S.

- Disease ranges from infected with no obvious defects to severe cytomegalic inclusion disease characterized by jaundice, microcephaly, hepatosplenomegaly, thrombocytic purpura ("blueberry muffin baby"), pneumonitis, and CNS damage to death.

- CMV- related complications observed in infants exposed to the virus in utero include chorioretinitis (most common eye-related problem), sensorineural deafness, periventricular calcifications, seizures, jaundice, hepatomegaly, splenomegaly, and microcephaly.





B. Heterophile-negative mononucleosis:

- Cytomegalovirus (CMV) is a rare cause of disease in the immunocompetent, with the virus more typically responsible for subclinical infection.

- A heterophile antibody-negative mononucleosis syndrome is the most frequent clinical manifestation of CMV infection in the immunocompetent.

- When primary CMV infection does result in clinically evident illness, afflicted individuals appear to have a systemic mononucleosis-like syndrome characterized by fever, malaise, myalgia, atypical lymphocytosis, and elevated liver transaminases.

- In contrast to EBV mononucleosis, heterophil antibodies are not usually present in patients with CMV mononucleosis.

C. Interstitial pneumonitis to severe systemic infection:

- Due to reactivation in a transplanted organ or in an AIDS patient.

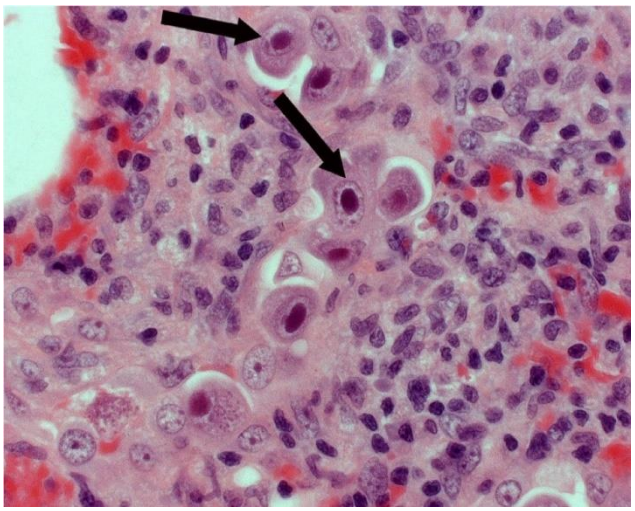
- In the immunocompromised, primary or reactivated CMV infection can result in severe retinitis, pneumonia, esophagitis, colitis, and/or hepatitis.

- This lung biopsy shows an enlarged, centrally located epithelial cell with intranuclear inclusions, findings characteristic of cytomegalovirus (CMV).

- The finding of interstitial pneumonia in a transplant patient with intranuclear inclusion bodies histologically points to opportunistic infection with CMV.

D. CMV retinitis: common in AIDS patients ("sightomegalovirus").

- Diagnosis: Infected cells have characteristic "Owl-eye" inclusion. Basophilic intranuclear inclusions.

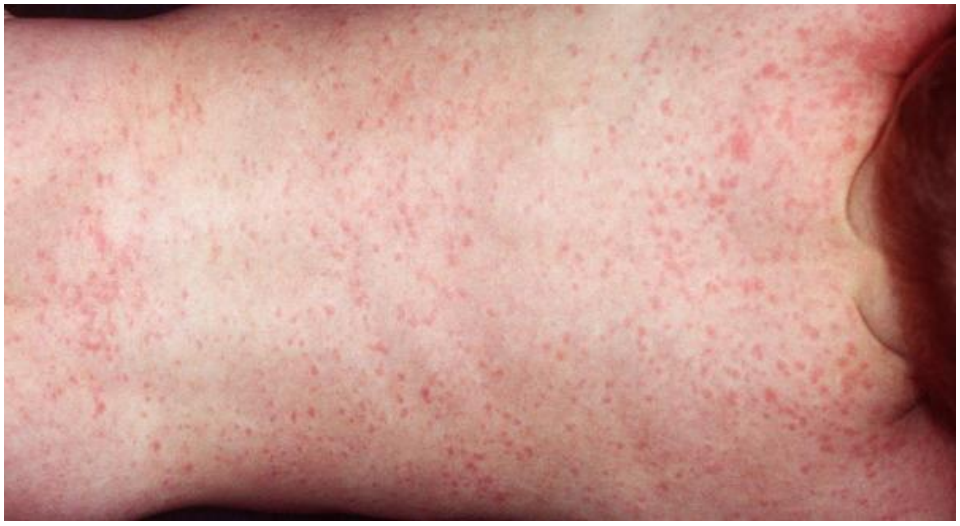


Human herpesviruses 6 and 7

- Reservoir: humans.
- Mode of transmission: respiratory droplets and saliva.
- Pathogenesis: replicates in peripheral blood mononuclear cells.
- Diseases:

❖ Roseola infantum (exanthem subitum):

- Primary symptoms include high fever for 3-5 days followed by an erythematous maculopapular rash.
- The rash usually starts on the trunk and spreads to the face and extremities.
- Febrile seizures can be caused by any febrile illness, and HHV-6 is one of the most common causes.
- The diagnosis is based on clinical presentation, and the infection is typically benign and self-limited.
- HHV-7 → less common cause of roseola.



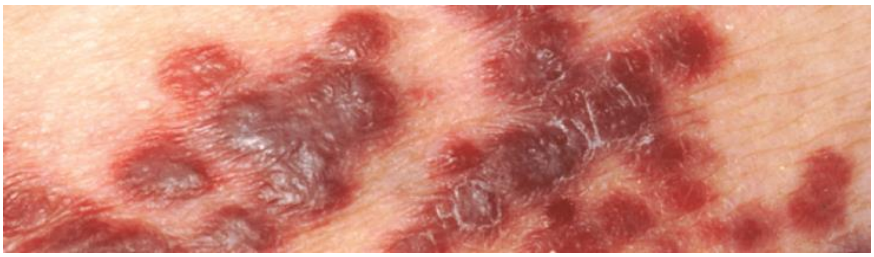
HHV-8

- Habitat: humans.
- Transmission: sexual contact, saliva, vertical.

- Pathogenesis: HHV-8 has a gene that turns on vascular endothelial growth factor (VEGF), which plays a direct role in the development of Kaposi sarcoma

- Diseases:

- Kaposi sarcoma:
  - It is a systemic disease (tumor of endothelial cells) that can present with cutaneous lesions with or without internal involvement.
  - Biopsy with lymphocytic inflammation
  - Seen in HIV/AIDS and transplant patients.



Poxviridae

- Virus Characteristics:
  - Large dsDNA, enveloped.
  - Replicates in the cytoplasm.
  - Potential biowarfare agent.

- Viruses of Medical Importance:
  - Variola/Smallpox.
  - Molluscum contagiosum.
  - Cowpox (milkmaid blisters).

Variola/Smallpox

- Habitat:
  - Humans.
  - Variola has 1 serotype, which made eradication (1977) possible.

- Mode of transmission: respiratory route.

- Pathogenesis:
  - Via inhalation, the virus enters the upper respiratory tract and disseminates via Lymphatics → viremia.
  - After a secondary viremia, the virus infects all dermal tissues and internal organs.

- Diseases:

- Prodrome of flu-like illness for 2-4 days.

- Prodrome followed by rash, which begins in the mouth and spreads to the face, arms and legs, hands, and feet and can cover the entire body within 24 hours.

- All vesicles are in the same stage of development (synchronous rash).

- Eradication was achieved by world-wide use of the live attenuated vaccine.

Molluscum contagiosum

- Habitat: humans.

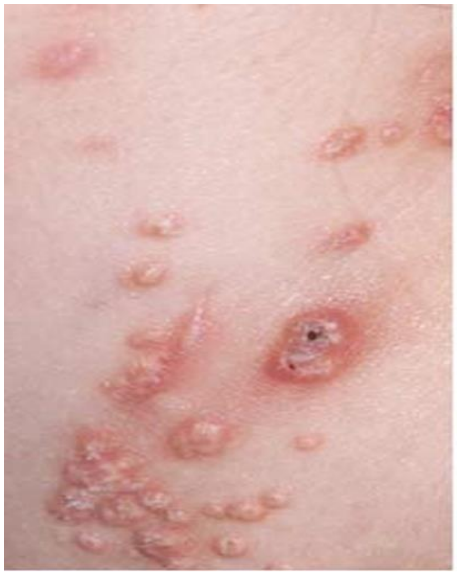
- Mode of transmission: direct contact (sexual) and fomites.

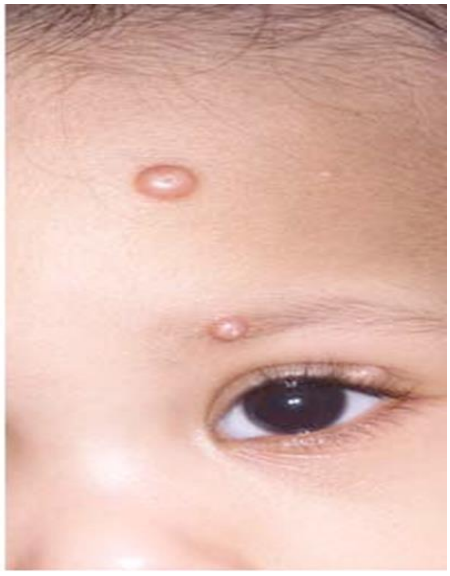
- Pathogenesis: replication in dermis.

- Diseases:

- Single or multiple (<20) benign, wart-like tumors.

- Flesh-colored papule with central umbilication.





❖ Summary of herpesvirus infections:

Virus	Site of primary infection	Clinical presentation of primary infection	Site of latency	Clinical presentation of recurrent infection
HSV-1	Mucosa	Gingivostomatitis, keratoconjunctivitis, temporal lobe encephalitis.	Trigeminal ganglia.	Cold sores.
HSV-2	Mucosa	Genital herpes, neonatal herpes.	Sacral ganglia	Genital herpes
VZV	Mucosa	Chickenpox	Dorsal root ganglia	Shingles (zoster)
EBV	Mucosal epithelial cells, B cells	Mononucleosis (heterophil +)	B cells	Asymptomatic shedding of virus.
CMV	Mononuclear cells, epithelial cells	Mononucleosis (heterophil -), cytoplasmic inclusion disease.	Mononuclear cells	Asymptomatic shedding of virus.
HHV-6	Mononuclear cells	Roseola infantum	Mononuclear cells	Asymptomatic shedding of virus.
HHV-8	Dermis	Kaposi sarcoma		

RNA Viruses

- RNA viruses:

- All are ssRNA (like our mRNA), except "repeato-virus" (reovirus) is dsRNA.

- Are enveloped except for caliciviruses, picornaviruses, reoviruses, and hepeviruses (CPR and hepevirus).

- Have helical capsids except for caliciviruses, picornaviruses, reoviruses, flaviviruses, togaviruses, retroviruses and hepeviruses (CPR, FTR and hepevirus).

- Are classified positive, negative, or ambisense (part of the nucleotide sequence is of positive-sense, part is of negative-sense) depending on the ability of virion RNA to act as messenger RNA (mRNA).

- Replicate in the cytoplasm except for orthomyxoviruses and retroviruses (OR have both a cytoplasmic and a nuclear phase).

- N.B:

- Some RNA viruses are segmented (different genes on different pieces of RNA):
  - Reovirus.
  - Orthomyxovirus.
  - Bunyavirus.
  - Arenavirus.
- ROBA sounds like robot, pieces.

❖ Positive-Stranded RNA Viruses:

- (+) RNA Viruses: Calicivirus, Hepevirus, Picornavirus, Flavivirus, Togavirus, Coronavirus, Retrovirus (Call Henry Pico and Flo To Come Rightaway).

OR

- Positive-stranded RNA viruses: I went to a retro (retrovirus) toga (togavirus) party, where I drank flavored (flavivirus) Corona (coronavirus) and ate hippy (hepevirus) California (calicivirus) pickles (picornavirus).

Caliciviridae

- Family Characteristics:
- Naked, icosahedral.
- Positive sense ssRNA.

- Viruses of medical Importance:
- Norovirus: most common cause of viral gastroenteritis in developed countries.

Norovirus

- Habitats: human gastrointestinal tract.

- Mode of transmission: fecal-oral route, contaminated food and water.

- Diseases:

❖ Acute gastroenteritis:

- 60% of all nonbacterial gastroenteritis in U.S in older children and adults.

- Nausea, vomiting, diarrhea.

- Watery diarrhea; no blood or pus in stools.

- It is resistant to inactivation by acid, bile, and pancreatic enzymes. As a result, norovirus is easily transmitted via fecal-oral spread (foodborne, person-to-person via contaminated bodily fluids) and results in outbreaks in crowded settings (schools, hospitals, cruise ships, nursing homes).

- The incubation period is 1-2 days; symptoms develop acutely and self-resolve within days.

- The diagnosis is typically based solely on clinical presentation, but polymerase chain reaction testing may be performed to confirm an outbreak.

Norovirus	
Epidemiology	• Single-stranded RNA genome • Most common cause of viral gastroenteritis in developed countries
Clinical features	• Acute onset; resolves in 2-3 days • Vomiting &/or diarrhea (no blood or mucus)

Hepeviridae

- Family Characteristics:
- Naked, icosahedral.
- Positive sense ssRNA.

- Viruses of medical Importance: Hepatitis E Virus (we will talk about it in details in the hepatitis viruses).

Picornaviridae

- Family Characteristics:
- Naked, icosahedral.
- Positive sense ssRNA.

- Viruses of medical Importance:

1. Enteroviruses (acid stable):

- The enteroviruses are a family of single-stranded RNA viruses that include the coxsackie viruses, echoviruses and polioviruses.

- They do not typically cause gastroenteritis. Enteroviruses are so-named because of their fecal-oral transmission and ability to replicate in the GI tract without being denatured or degraded by the acid environment.

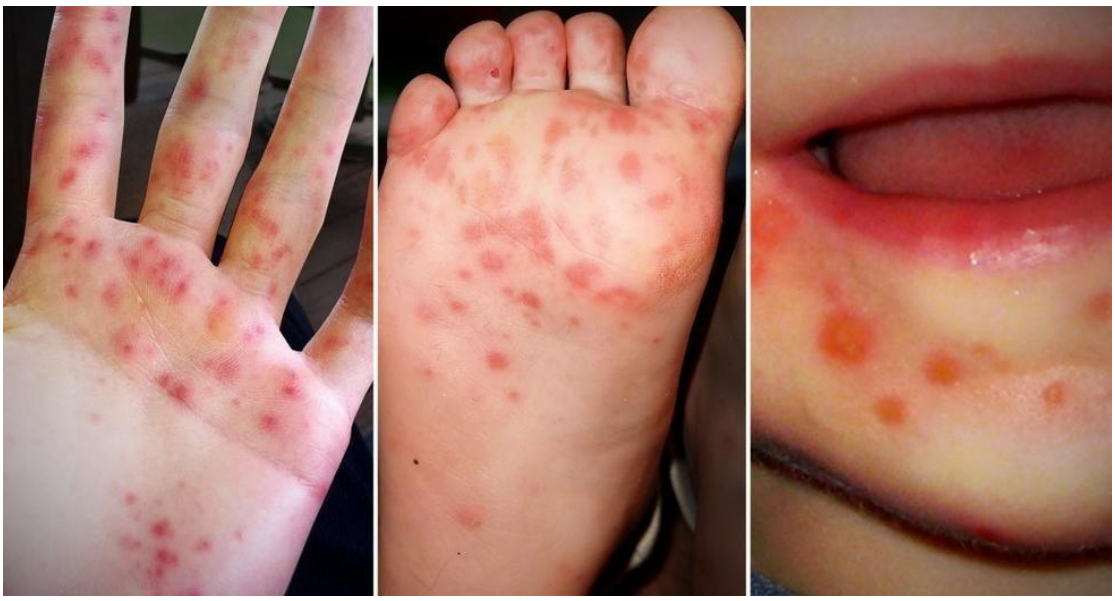
- This explains why enteroviruses can colonize or infect the GI tract, whereas rhinoviruses are limited to colonization or infection of the upper respiratory tract.

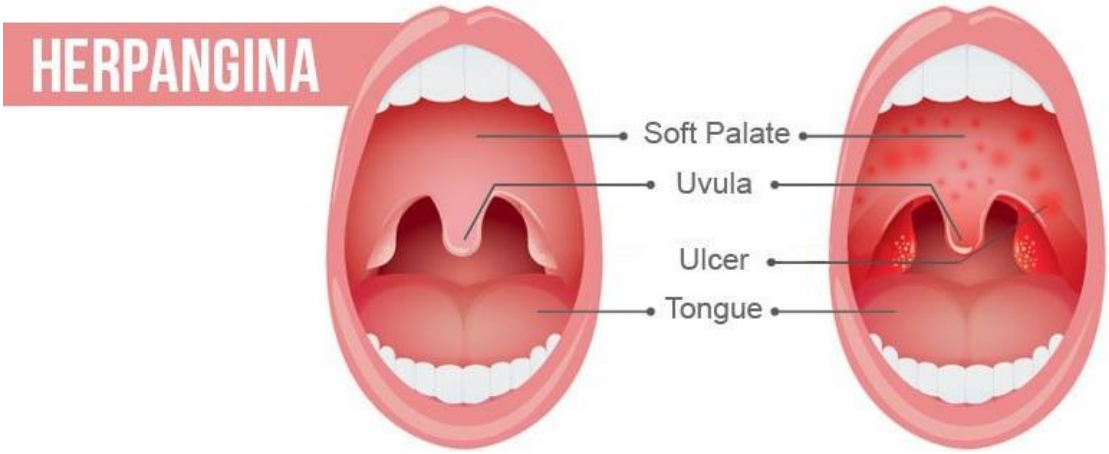
- Enterovirus infection is the most common cause of aseptic meningitis, accounting for up to 90% of cases.

A. Polio virus: poliomyelitis.

B. Coxsackievirus:
   - Aseptic meningitis.
   - Herpangina (acute painful febrile pharyngitis, characterized by vesicles on the fauces and tongue which rapidly ulcerate without skin findings, coxsaki A).
   - Hand, foot, and mouth disease (erythematous papules on the palms and soles with vesicles and ulcers in oral mucosa and around the mouth, coxsaki A).
   - Myocarditis; pericarditis (coxsaki B).

C. Echoviruses: aseptic meningitis.





Enterovirus	
Epidemiology	• Infants & young children • Summer season • Fecal-oral transmission
Clinical features	• Herpangina (oral ulcerations) • Hand-foot-and-mouth disease (oral & extremity ulcerations) • Aseptic meningitis • Myocarditis

2. Rhinoviruses (acid labile):
   - Once the pH drops below 5.0 (as in the stomach, which can have a pH of 1.0), the acid-labile rhinoviruses are inactivated.
   - Cause of common cold; > 100 serologic types.
   - Rhino has a runny nose.

3. Heparnaviruses: HAV.

❖ Mnemonic:

- Picornaviruses: Polio virus, Enterovirus, Echovirus, Coxsackie virus, Rhinovirus, Hepatits A (PEE Co Rn A Viruses).

❖ N.B:

1. Enteroviruses, including coxsackievirus and echovirus, are the most common causes of aseptic meningitis.

- Patients with viral meningitis classically present with headache, fever, and neck stiffness.
- Focal neurologic signs, stupor, coma, and signs of severe meningeal irritation are usually absent; their presence should prompt an investigation into other possible diagnoses, especially bacterial meningitis, encephalitis, and intracranial hemorrhage.
- Cerebrospinal fluid analysis in patients with viral meningitis typically shows a lymphocytic pleocytosis, modestly elevated protein level, and normal glucose level.
- A negative Gram stain and culture further supports this diagnosis.
- While the majority of cases of viral meningitis will have normal glucose levels, remember that it is possible to have mildly decreased or mildly increased levels of glucose in the CSF, especially when serum blood glucose levels are elevated (diabetics).

Differences between bacterial & viral meningitis

	Viral	Bacterial
Common microbes	• **Enteroviruses** (most common) • Arboviruses • Herpes simplex virus type 2	• **Adults**: *Streptococcus pneumoniae* & *Neisseria meningitidis* • **Neonates**: Group B *Streptococcus* & gram-negative bacilli
CSF cell differential	• WBC count often **<500** cells/mm^3 • Lymphocytic predominance	• WBC count often **>1000** cells/mm^3 • Neutrophilic predominance
CSF glucose & protein	• Glucose levels are **normal** or slightly reduced • Protein generally <150 mg/dL	• Glucose levels **<45 mg/dL** • Protein is often >250 mg/dL
CSF Gram stain & culture	• No organisms identified	• Often positive for a specific organism

CSF = cerebrospinal fluid; WBC = white blood cell.

2. Polio classically occurs in unvaccinated immigrant patients from endemic geographic regions.
- Symptoms of fever, malaise and aseptic meningitis occur first, and can be followed by severe myalgias and asymmetric paralysis (classically affecting the legs).
- Damage to the anterior horn lower motor neuron cell bodies produces this hyporeflexic paralysis.

3. There are three antigenic types of polio virus (1, 2, 3).
- There are two effective vaccines containing the three antigenic types. The vaccines used for vaccine immunization are Sabin which is a live oral poliovirus vaccine (OPV) and Salk which is inactivated poliovirus vaccine (IPV).
- Differences between Sabin vaccine and Salk vaccine:

	Sabin vaccine oral polio vaccine (OPV)	**Salk vaccine inactivated polio vaccine (IPV)**
Type	Live attenuated virus	Formalin inactivated (killed) whole poliovirus.
Route of administration	Oral	Intramuscular
Protective immunity	- Local intestinal immunity by secretory IgA against reinfection. It breaks transmission in the population. - Systemic immunity by humoral IgG and IgM antibodies → prevent invasion of of CNS.	- No local immunity (so virus is still able to multiply in the gut) - Systemic immunity only.
Dissemination of vaccine virus in the community	Vaccine virus passes in the stools and can be transmitted to non-immunized children.	No
Use in pregnancy	Contraindicated	Safe
Use in immunosuppressed individuals and their household contacts	Contraindicated	Safe
Potential limiting factor	- Rare occurrence of vaccine associated poliomyelitis due to mutation of the virus to the wild type. - Loss of potency due to improper storage (properly stored at 4° C)	None of these
Allergy (minor local reaction)	No	Yes

- Allergy because IPV contains trace amounts of streptomycin, polymyxin B and neomycin, hypersensitivity reactions can occur among people sensitive to them.

- Breast- feeding is not a contraindication to immunization against poliovirus.

Flaviviridae

- The family (Flaviviridae) gets its name from Yellow Fever virus. Flavus means yellow in Latin (Yellow fever in turn was named because of its propensity to cause jaundice).

- Family Characteristics:

- Enveloped, icosahedral.
- Positive sense ssRNA.
- Arthropod-borne (arboviruses).

- Viruses of medical Importance:

- St. Louis encephalitis virus (SLE): Encephalitis (vector is Culex species).
- West Nile encephalitis virus (WNV): Encephalitis (vector is Culex species).
- Dengue virus (vector is Aedes mosquitoes).
- Yellow fever virus (vector is Aedes mosquitoes).
- Zika virus (vector is Aedes mosquitoes).
- Hepatitis C virus (HCV; we will talk about it in details in the hepatitis viruses).

Yellow fever

- Vector: arbovirus transmitted by Aedes mosquitoes.

- Host: Virus has a monkey or human reservoir.

- Diseases:

❖ Yellow fever disease:

- Yellow fever is endemic in equatorial countries of Africa and south America.

- The virus enters through the bite of an infected mosquito, multiplies in local lymph nodes, and is then released into the blood to be localized in the liver, spleen, kidneys, and bone marrow.

- After an incubation period of 3-6 days, there is high fever, black vomitus, jaundice, albuminuria, bleeding from nose, gum, hematemesis, and melena due to liver damage.

- The high mortality rate in severe cases is due to liver and kidney failure.

- May see Councilman bodies (eosinophilic apoptotic globules) on liver biopsy.

Dengue virus

- There are 4 different serotypes (DENV1-4).
- Vector: Aedes egypti mosquito.
- Host: monkeys (main reservoir) and humans.
- Diseases:

1. Dengue fever:

- The disease is a febrile illness (high-grade fever) with severe pain in the bones, muscles, retroorbital, and joints (breakbone fever), headache and skin rash.
- Complete recovery is the role.
- Primary infection leads to lifelong immunity against the same serotype, but individuals can be infected with a different serotype.

2. Dengue hemorrhagic fever:

- Secondary infection with a different viral serotype can cause a more severe illness, possibly due to antibody-dependent enhancement of infection, enhanced immune complex formation, and/or accelerated (not blunted) T-lymphocyte responses.
- DHF, which can be a serious manifestation of secondary infection, is due to increased capillary permeability and can be manifested by marked thrombocytopenia, prolonged fever, respiratory/circulatory failure, and shock.
- Patients also develop more significant hemorrhagic tendencies (petechiae with tourniquet application) and spontaneous bleeding.

Zika virus

- Vector: Aedes mosquito bites, Sexual and vertical transmission are also possible.
- Diseases:

- Causes conjunctivitis, low-grade pyrexia, and itchy rash in 20% of adult cases.

❖ Congenital Zika syndrome:

- The neurotropic virus can cross the placenta and infect and destroy fetal neural progenitor cells, causing congenital Zika syndrome and possible fetal demise.

- Fetal brain development is impaired due to disruption of normal proliferation, migration, and differentiation of neurons.

- Classic findings in affected newborns include microcephaly with facial features out of proportion to head size, seizures, hypertonia, and ocular abnormalities.

- Loss of brain mass (cortical thinning, ventriculomegaly) as well as subcortical calcifications are typically present.

- Pregnant women should be counseled to avoid traveling to areas with ongoing Zika transmission (South and Central America, Asia, Africa, Mexico, the Caribbean).

- Diagnosis: Diagnosis is confirmed by detection of Zika RNA (real-time reverse transcriptase PCR) in serum, urine, or cerebrospinal fluid.

- Treatment: The mainstay of treatment for surviving infants is supportive care with management of feeding difficulties, hydrocephalus, and seizures.

Togaviridae

- Family Characteristics:
- Enveloped, icosahedral.
- Positive sense ssRNA.

- Viruses of medical Importance:
- Toga CREW:

1. Chikungunya virus (co-infection with dengue virus can occur; vector is Aedes mosquitoes).
2. Rubella.
3. Eastern equine encephalitis virus (EEE): Encephalitis.
4. Western equine encephalitis virus (WEE): Encephalitis.

Chikungunya virus

- The Aedes mosquito also transmits the virus causing chikungunya, a febrile illness with flulike symptoms, prominent polyarthralgia/arthritis (hands, wrists, ankles), and diffuse macular rash.

- As a result, many areas have had simultaneous outbreaks of both dengue and chikungunya.

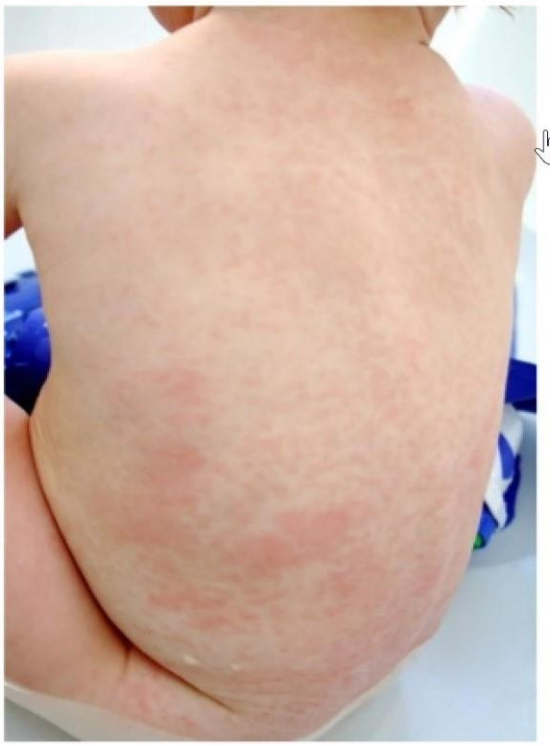
- Preventive measures against both infections include protective barriers (bed nets, window screens) and insect spraying.

Rubella virus

- Host: humans.
- Vector: none.
- Mode of transmission: respiratory droplets.
- Diseases:

1. German (3-day) measles:

- Among the acute viral exanthems, measles (rubeola) and German measles (rubella) are characterized by a maculopapular rash that begins on the face and spreads centrifugally (from the center to extremities) to the trunk and extremities.
- Compared to rubeola, the rash of rubella typically spreads faster and does not darken or coalesce.
- In a susceptible child, a febrile maculopapular rash that begins on the face and spreads to the trunk and extremities is suggestive of rubeola (measles) or rubella (German measles).
- The additional finding of postauricular and occipital lymphadenopathy indicates that rubella is the most likely etiology which is caused by a togavirus.
- Many immigrants to the United States will not have completed the vaccination regimen recommended by the Centers for Disease Control and Prevention including the MMR vaccine.



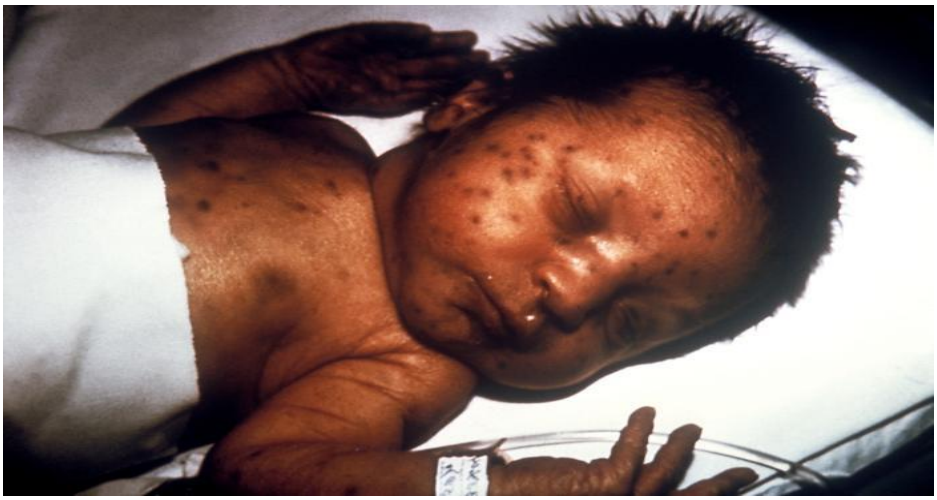
2. Congenital rubella syndrome:

- Congenital rubella findings include "blueberry muffin" appearance, indicative of extramedullary hematopoiesis (can also be seen in congenital CMV infection).

- The congenital rubella syndrome is predominantly characterized by neonatal defects of the head (microcephaly, mental retardation), eyes (cataracts), ears (deafness), and heart/cardiovascular system (patent ductus arteriosus, pulmonary artery stenosis).

- The most classic clinical triad of congenital rubella includes congenital cataracts (white pupils), sensory-neural deafness, and patent ductus arteriosus.

- Maternal rubella infection produces a low-grade fever, a maculopapular rash with cephalocaudal progression, and posterior auricular and suboccipital lymphadenopathy. Most adult women patients develop polyarthritis and polyarthralgia as sequelae.



▪ Prevention:

- Live attenuated rubella virus vaccine (given in combination with that of measles and mumps; MMR) is currently recommended not only for children at 12-15 months and again at 4-6 years of age, but also in non-pregnant women of childbearing age who lack serum antibody against rubella.

- At the time of vaccination, women are strongly advised to avoid pregnancy for the next four weeks.

- This vaccination policy has markedly decreased the incidence of congenital rubella.

❖ N.B:

1. Arbovirus meningoencephalitis is caused by members in the togaviridae and flaviviridae families.

- Infection is acquired from the bite of infected mosquito.
- The virus multiplies in the reticuloendothelial cells, released into the blood (viraemia) which coinceded with the onset of fever, the virus then reaches the CNS causing meningitis or meningoencephalitis.

2. Febrile illness with signs of meningitis (neck stiffness, headache) and encephalitis (confusion, seizure, tremor, focal deficits), raising strong suspicion for an outbreak of arbovirus meningoencephalitis (West Nile virus, La Crosse virus).

- Arboviruses are small RNA viruses that are harbored by birds and small mammals and are transmitted to humans via biting arthropods (mosquitos, ticks, fleas), primarily during the summer months when arthropod populations are at their peak.
- Infections in otherwise healthy individuals usually result in an asymptomatic low-level viremia that is cleared within a week by the humoral antibody response.
- In contrast, patients who cannot mount a rapid, effective antibody response (elderly, immunocompromised) may have persistent and more severe viremia; these individuals often develop a severe flu-like illness (fever, headache, fatigue, arthralgias) that may progress to meningitis and/or encephalitis.
- Most arbovirus infections are self-limited and resolve with supportive care, but some cases of meningoencephalitis are fatal.
- Because no vaccines are currently available for North American arboviruses, prevention primarily relies on the elimination of vector arthropod breeding grounds (stagnant water) and the early identification of infected arthropods (and birds) so that aerosolized insecticide can be sprayed.

3. West Nile virus is an enveloped RNA virus that is found in warm climates worldwide.

- The virus replicates extensively within birds and is passed to mosquitos (Culex species) during blood feeding. Viral accumulation in mosquito salivary glands can lead to human transmission.
- Most infected patients develop low-level viremia that is neutralized within a week by the humoral immune response; these individuals typically remain asymptomatic.
- However, a minority of patients (particularly the young, elderly, or immunosuppressed) are unable to mount an effective immune response and develop significant clinical manifestations, including:

- West Nile fever: a self-limited, flu-like illness that is often associated with a maculopapular rash.
- Neuroinvasive disease: the virus is neurotropic and often causes meningitis (fever, headache, neck stiffness), encephalitis (confusion, tremors, seizures), and/or flaccid paralysis (asymmetric weakness of limbs, fasciculations).

- Cerebrospinal fluid usually shows a lymphocytic pleocytosis.
- Most patients recover completely with supportive care, but some with neuroinvasive disease have long-term neurologic sequelae or die.

Common arboviral infections	
Viral pathogens	• West Nile virus • St. Louis encephalitis virus • La Crosse virus • Eastern & Western equine encephalitis viruses
Clinical syndromes	• Most common: asymptomatic to flu-like illness (eg, fever, headache, myalgias) • Meningitis (eg, neck stiffness, headache) • Encephalitis (eg, confusion, tremors, focal neurological deficits) • Acute asymmetric flaccid paralysis: West Nile virus
Transmission	• Arthropod (eg, mosquito, tick) bites • Host: birds and small mammals

Coronaviridae

- Family Characteristics:

- Enveloped, helical.
- Positive sense ssRNA.

- Viruses of Medical Importance:

- Coronavirus.
- Severe acute respiratory syndrome coronavirus (SARS-CoV).

Coronavirus

- Second most common cause of the common cold.

- Winter/spring peak incidence.

SARS-CoV

- Habitat: birds and small mammals.

- Mode of transmission:

- Respiratory droplets.

- Virus is also found in urine, sweat, and feces.

- Disease:

❖ Severe acute respiratory syndrome (SARS):

- Atypical pneumonia.

- Clinical case definition includes fever > 100.4°F, flu-like illness, dry cough, dyspnea, and progressive hypoxia.

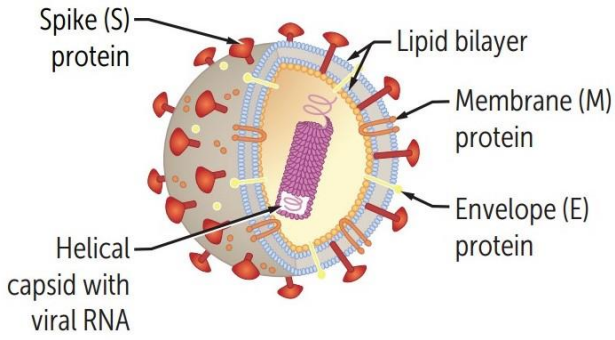
- Chest x-ray may show patchy distribution of focal interstitial infiltrates.

Severe acute respiratory syndrome coronavirus 2

- SARS-CoV-2 is a novel ⊕ ssRNA coronavirus and the cause of the ongoing COVID-19 pandemic.

- Mode of transmission:

- Spreads primarily through respiratory droplets and aerosols.
- Host cell entry occurs by attachment of viral spike protein to angiotensin-converting enzyme 2 receptor on cell membranes.



- Presentation:

- Clinical course varies; often asymptomatic.

- Symptoms include fever, dry cough, shortness of breath, fatigue. More specific: anosmia (loss of smell), dysgeusia (altered taste).

- Potential complications include respiratory failure, hypercoagulability, shock, organ failure, death.

- Risk factors for severe illness or death include increasing age, obesity, diabetes, hypertension, chronic kidney disease, and severe cardiopulmonary illness.

- Diagnosis: Diagnosed by RT-PCR (most common); antigen and antibody tests are available.

- Treatment: Treatment options for hospitalized adults include remdesivir (nucleoside analog), convalescent plasma, and dexamethasone (to treat cytokine release syndrome).

Retroviridae

- Family Characteristics:

- Enveloped Positive sense ssRNA.

- It contains the enzyme reverse transcriptase (responsible for a unique feature of replication not found in other viruses).

- Viruses of medical Importance:

A. Oncovirus group:

- Human T-cell lymphotropic virus (HTLV): Adult T-cell leukemia.

B. Lentivirus group:

- Human immunodeficiency viruses 1 and 2 (HIV-1 and HIV-2); acquired immunodeficiency syndrome.

Human Immunodeficiency Virus (HIV)

- Distinguishing Characteristics: HIV-1 is the major cause of AIDS worldwide, while AIDS caused by HIV-2 is much less severe, slower in progression, and limited mostly to west Africa.

Comparison of HIV-1 & HIV-2		
	HIV-1	**HIV-2**
Geographic location	Worldwide	West Africa
Viral load	High	Low
Progression to AIDS	7-10 years	10-25 years
Intrinsic resistance	Limited	NNRTIs, fusion inhibitors

NNRTIs = non-nucleoside reverse transcriptase inhibitors.

- The HIV virion contains:
  - Enveloped, truncated, conical capsid.
  - Two copies of the ss (+) RNA.
  - RNA-dependent DNA polymerase (reverse transcriptase).
  - Integrase.
  - Protease.

- The viral genome consists of 2 identical copies of positive sense ssRNA, each of which has a copy of the virus genes:

1. env gene: encodes gp160, a precursor glycoprotein that is cleaved to form the 2 envelope glycoproteins gp120 and gp41. Rapid mutation in this gene results in many gp120 antigenic variants.

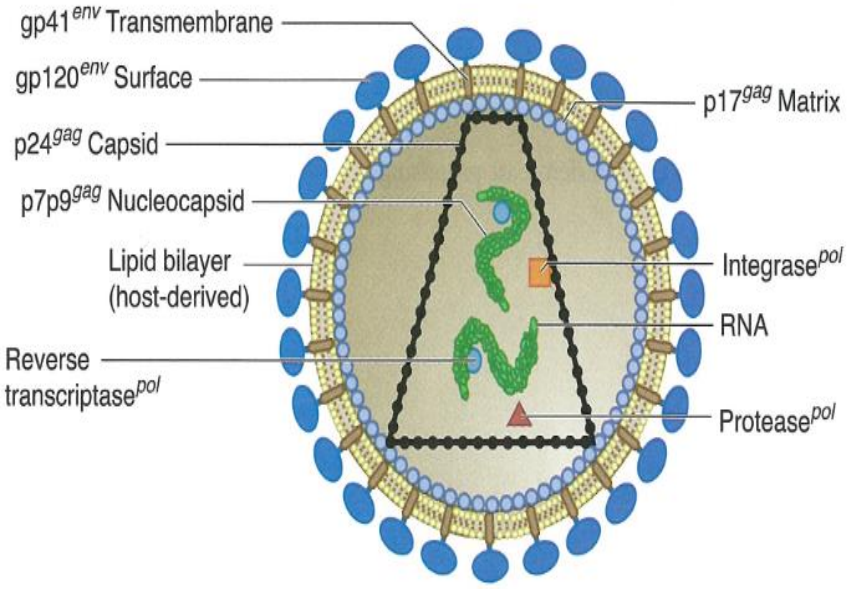
2. pol gene: encodes the enzymes reverse transcriptase, integrase and protease which participate in viral replication.

3. gag gene: encodes the core proteins, the most important of which is p24.

- The viral envelope is composed of a lipid membrane and contains 2 virus-specific glycoproteins:

A. gp 120: protrudes from the surface and is responsible for viral binding to host cell receptors.

B. gp 41: is embedded in the envelope and mediates the fusion of the viral envelope with cell membrane at the time of infection.



- Habitat: human TH cells and macrophages.

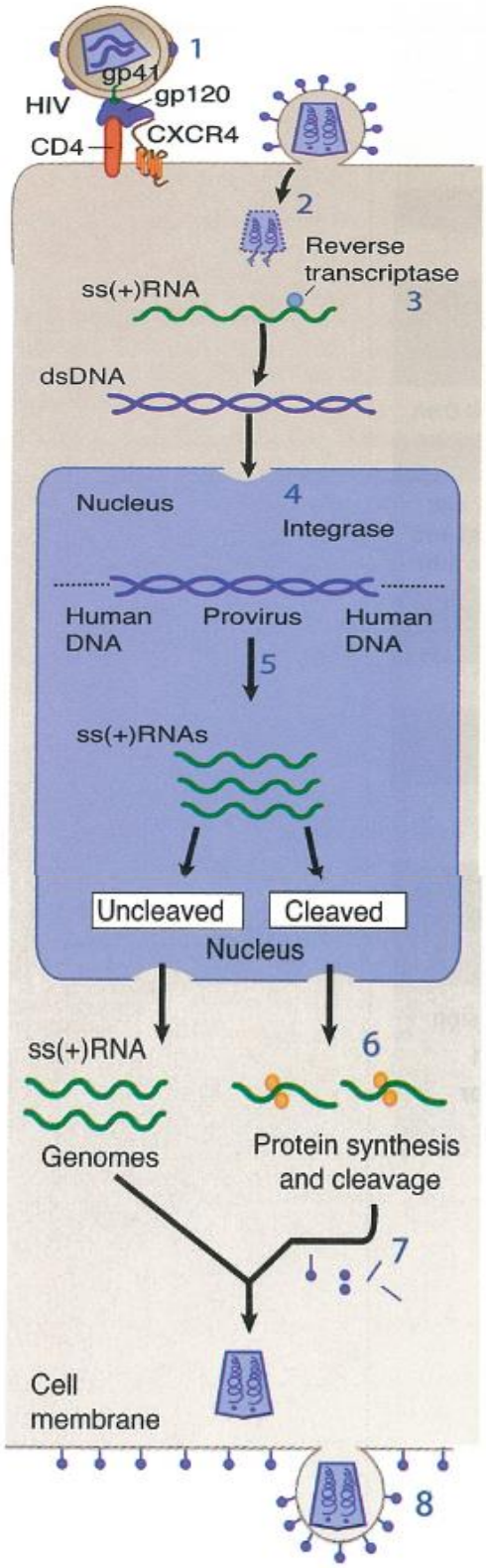
- Mode of transmission:

- Sexual contact.

- Bloodborne (transfusions, dirty needles).

- Vertical transmission.

3. HIV life cycle:



1. Surface gp120 of HIV binds to CD4 of T-helper cells, macrophages, microglia, and coreceptors (*CCR5* and *CXCR4*) found on macrophages and TH cells, respectively.
2. HIV is taken into the cell, losing the envelope; the RNA is uncoated.
3. The RNA is copied using the virion-associated reverse transcriptase; ultimately dsDNA with long terminal repeats is made.
4. The DNA and integrase migrate to nucleus and the DNA is integrated into host DNA forming the **provirus**.

   The provirus remains in the host DNA.

   The rate of viral replication is regulated by the activity of the regulatory proteins (*tat/rev*, *nef*, etc).

   Tat upregulates transcription.

   Rev regulates transport of RNA to cytoplasm.

   Co-infections (e.g., mycobacterial) stimulate the HIV-infected cells to produce more virus.
5. Transcription produces ss(+)RNA, some cleaved and some remain intact.
   - Cleaved RNA will be used as mRNA.
   - Uncleaved RNA is used as genomic RNA.
6. Translation produces the proteins some of which are polyproteins that are cleaved by the HIV protease.
7. Assembly
8. Maturation/release of virus

Gene	Product(s)	Function
Structural Genes		
Gag	Group-specific antigens	Structural proteins
	p24	Capsid protein
	p7p9	Core nueleocapsid proteins
	p17	Matrix protein
Pol	Reverse transcriptase	Produces dsDNA provirus
	Integrase	Viral DNA integration into host cell DNA
	Protease	Cleaves viral polyprotein
Env	gp120	Surface protein that binds to CD4 and coreceptors CCR5 (macrophages) and CXCR4 (T cells); tropism
	gp41	Transmembrane protein for viral fusion to host cell
Regulatory Genes		
Tat	Transactivator	Transactivator of transcription (upregulation)
Rev	Regulatory protein	Upregulates transport of unspliced and spliced transcripts to the cell cytoplasm
Nef	Regulatory protein	Decreases CD4 and MHC I expression on host cells; manipulates T-cell activation pathways; required for progression to AIDS

- Pathogenesis:

- Cells infected by HIV:

a) CD4 T helper cells.
b) Macrophages and monocytes.
c) Dendritic cells.
d) Oligodendrocytes, astrocytes, neurons and glial cells.
e) Follicular dendritic cells (in lymph nodes).

- Except for the CD4 T lymphocytes, these cells are not necessarily destroyed by the virus; therefore, they act as reservoir for further T cell infection.

- Following sexual transmission, the dendritic cells in the genital mucosa bind to the virus and carry it from the site of infection to the lymph nodes where other cells (especially T helper cells) become infected.

- Infection of CD4 T helper cells by HIV leads to their depletion by 2 mechanisms:

  - Direct killing of infected cells by the virus as a result of viral replication.
  - Killing of infected CD4 cells by CD8 cytotoxic lymphocytes which constitutes the main immune response to HIV infection.

- Diseases:

❖ Acquired immunodeficiency syndrome (AIDS):

- Asymptomatic infection → persistent, generalized lymphadenopathy → symptomatic → AIDS-defining conditions.

- The course of the illness follows the decline in CD4 T cells.

- The clinical picture of HIV infection can be divided into 3 stages:

A. Acute (early) stage:

- After an incubation period of 2-4 weeks, HIV-infected individuals suffer from an acute flu-like illness (acute retroviral syndrome).

- The most common symptoms are fever, maculopapular rash, oral ulcers, lymphadenopathy, sore throat and malaise.

- The number of CD4 cells is usually normal.

- This symptomatic phase lasts for 7-10 days, after which the cytotoxic T cells (the main immune response to HIV) and antibodies dramatically reduce HIV levels. This induced immune response succeeds in controlling, but not eliminating the virus.

B. Latent stage (clinical latency):

- Acute infection is followed by an extended period of clinical latency.

- During this phase, a large amount of HIV particles is being produced by lymph node cells but remains sequestered within the lymph nodes (during clinical latency the virus itself does not enter a latent stage).

- The patient is usually asymptomatic, and viremia is low or absent.

- The duration of this period (which may extend up to 10 years) depends on a number of factors including the virus type, immune response and use of antiretroviral therapy.

- Clinical latency is actually attributed to the ability of HIV to evade the aggressive immune response through:
  - High rate of viral mutation (esp. env gene).
  - Integration of the virus in the chromosome of infected cells shielded from recognition by the immune system.
  - Downregulation of MHC 1 expression on infected cells by the virus preventing recognition of these cells by cytotoxic T cells.
  - Loss of CD4 T cell responses which leads to impaired functions of other cells of the immune system.

C. Immunodeficiency (late) stage:

- Viral replication and gradual depletion of CD4 T cells continue until finally, some years after initial infection, full-blown AIDS develops.

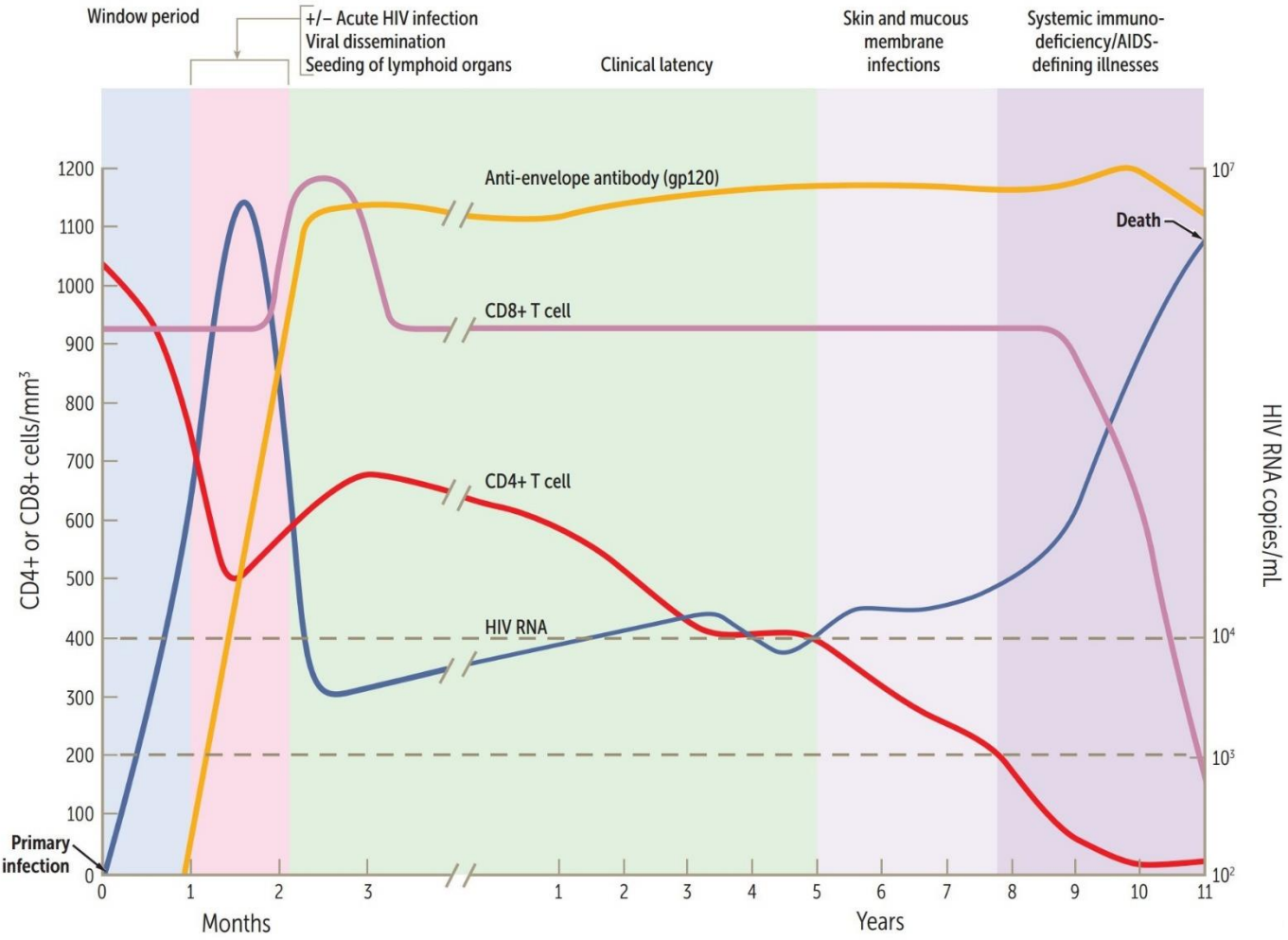
- This occurs when CD4 T cell count falls below 200/mm (normal count: 500-1500 mm).

- The infected person becomes particularly susceptible to opportunistic infections and cancers characteristic of AIDS such as Kaposi sarcoma and certain lymphoma.

- Patients suffer debilitating weight loss, diarrhea, and neurogenic manifestations.

- Infections are the major cause of death in AIDS patients.

Time course of untreated HIV infection



- Common diseases of HIV-positive adults:

- As CD4+ count ↓, risks of reactivation of past infections (TB, HSV, shingles), dissemination of bacterial infections and fungal infections (coccidioidomycosis), and non-Hodgkin lymphomas:

Pathogen	Presentation	Findings
< 500 cells/mm3		
Candida albicans	Oral thrush	Scrapable white plaque, pseudohyphae on microscopy
EBV	Oral hairy leukoplakia	Unscrapable white plaque on lateral tongue
HPV	Squamous cell carcinoma, commonly of anus (men who have sex with men) or cervix (women)	
HHV-8	Kaposi sarcoma	Biopsy with lymphocytic inflammation
< 200 cells/mm3		
Histoplasma capsulatum	Fever, weight loss, fatigue, cough, dyspnea, nausea, vomiting, diarrhea	Oval yeast cells within macrophages
HIV	Dementia	
JC virus (reactivation)	Progressive multifocal leukoencephalopathy	Nonenhancing areas of demyelination on MRI
Pneumocystis jirovecii	Pneumocystis pneumonia	"Ground-glass" opacities on CXR
< 100 cells/mm3		
Aspergillus fumigatus	Hemoptysis, pleuritic pain	Cavitation or infiltrates on chest imaging
Bartonella henselae	Bacillary angiomatosis	Biopsy with neutrophilic inflammation
Cryptococcus neoformans	Meningitis	Thickly encapsulated yeast on India ink stain
Candida albicans	Esophagitis	White plaques on endoscopy; yeast and pseudohyphae on biopsy
Cryptosporidium spp.	Chronic, watery diarrhea	Acid-fast oocysts in stool
CMV	Retinitis, esophagitis, colitis, pneumonitis, encephalitis	Linear ulcers on endoscopy, cotton-wool spots on fundoscopy. Biopsy reveals cells with intranuclear (owl eye) inclusion bodies.
EBV	B-cell lymphoma (non-Hodgkin lymphoma, CNS lymphoma)	CNS lymphoma: ring enhancing, may be solitary (vs. Toxoplasma)
Mycobacterium avium-intracellulare, Mycobacterium avium Complex	Nonspecific systemic symptoms (fever, night sweats, weight loss) or focal lymphadenitis	
Toxoplasma gondii	Brain abscesses	Multiple ring-enhancing lesions on MRI

HIV-associated esophagitis		
Pathogen	**Endoscopic findings**	**Microscopic findings**
Candida albicans	Patches of adherent, grey/ white **pseudomembranes** on erythematous mucosa	**Yeast** cells & pseudohyphae invading mucosal cells
HSV-1	Small **vesicles** → **"punched-out" ulcers**	Eosinophilic intranuclear inclusions (**Cowdry type A**) in multinuclear squamous cells at ulcer margins
CMV	**Linear** ulcerations	Intranuclear & cytoplasmic **inclusions**

CMV = cytomegalovirus; **HSV** = herpes simplex virus.

- <u>HIV diagnosis:</u>

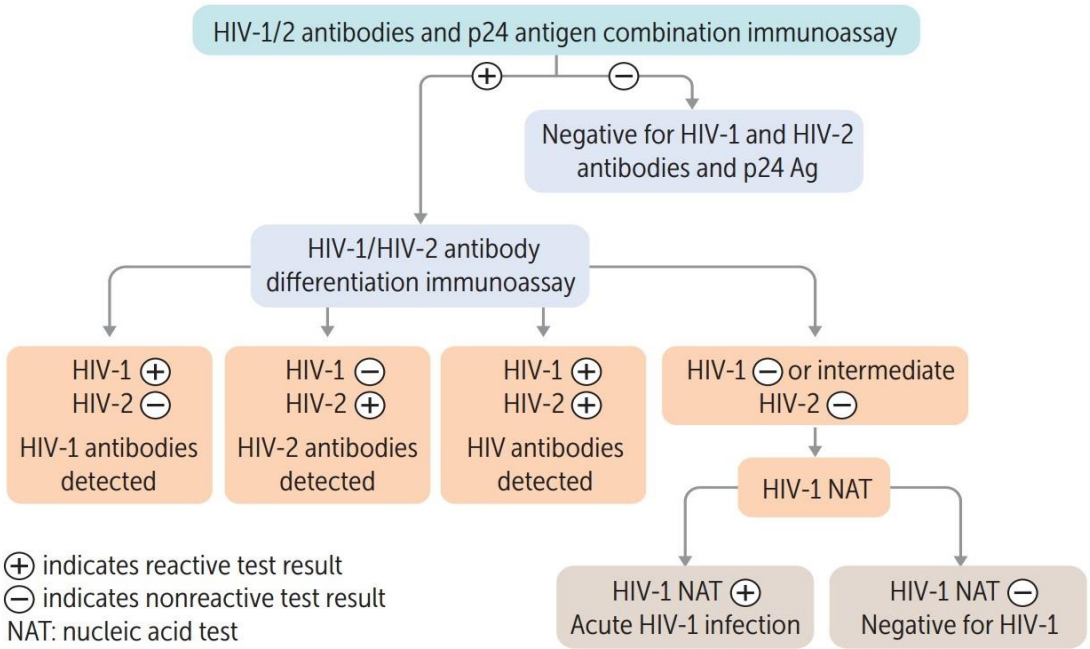
- Presumptive diagnosis made with HIV-1/2 Ag/Ab immunoassays.

- These immunoassays detect viral p24 Ag capsid protein and IgG Abs to HIV-1/2.

- Very high sensitivity/specificity.

- Viral load tests determine the amount of viral RNA in the plasma. High viral load associated with poor prognosis. Also use viral load to monitor effect of drug therapy.

- Use HIV genotyping to determine appropriate therapy.



- AIDS diagnosis:
  - ≤ 200 CD4+ cells/mm^3 (normal: 500–1500 cells/mm^3).
  - CD4 percentage < 14%.
  - HIV ⊕ with AIDS-defining condition (Pneumocystis pneumonia).

- Western blot tests are no longer recommended by the CDC for confirmatory testing.

- HIV-1/2 Ag/Ab testing is not recommended in babies with suspected HIV due to maternally transferred antibody. Use HIV viral load instead.

❖ N.B:

1. HIV infection progresses at varying rates among individuals, and it has been observed that some individuals remain uninfected after persistent exposure to HIV virus.
   - These differences in disease progression and viral infectivity are explained by the role of chemokine receptors in viral entry into the cell.
   - Surface gp120 of HIV binds to CD4 of T-helper cells, macrophages, microglia, and coreceptors (CCR5 and CXCR4) found on macrophages and TH cells, respectively.
   - The HIV virus uses the CD4 protein as a primary receptor, and the chemokine receptor CCR5 serves as a coreceptor.
   - Both CD4 and CCR5 are bound by the HIV viral outer envelope protein gp120.
   - After binding to CD4 and the CCR5 chemokine receptor, the HIV virus enters the cell by fusion with the cell membrane.
   - If cells do not express the CCR5 protein on their membrane, HIV virus binds CD4 but is unable to enter the cell.
   - Deletion of both alleles of the gene that codes for the CCR5 receptor (homozygous CCR5 32 deletion) renders the individual resistant to HIV infection.
   - Individuals with deletion of one copy of this gene (heterozygous) can be infected with HIV, but develop clinical symptoms later than patients with two copies of the CCR5 gene.
   - Homozygous CCRS mutation → immune.
   - Heterozygous CCRS mutation → slow course.

2. The risk of HIV infection occurring in an infant born to an HIV-positive mother who received no prenatal antiretroviral therapy is estimated to be 13-39%.
   - Studies have shown that maternal prophylaxis during pregnancy with the nucleoside analog zidovudine (ZDV, AZT), a retroviral reverse transcriptase inhibitor, reduces the risk of perinatal transmission by about two-thirds in HIV positive women who have not previously received antiretroviral therapy.

- Negative-Stranded RNA Viruses:

- Paramyxovirus, Rhabdovirus, Filovirus, Orthomyxovirus, Bunyavirus, Arenavirus

- Must transcribe ⊖ strand to ⊕. Virion brings its own RNA-dependent RNA polymerase.

- Mnemonic for the ss (-) RNA viruses:

  - Pain Results From Our Bunions Always. Gives them in order of size. You can remember these are the negative ones because pain is a negative thing.

OR

  - Always Bring Polymerase Or Fail Replication.

Paramyxovirida

- Family Characteristics:

- Enveloped, helical nucleocapsid.
- Negative sense ssRNA.
- All contain surface F (fusion) protein, which causes respiratory epithelial cells to fuse and form multinucleated cells.

- Viruses of Medical Importance:

- Measles.
- Mumps.
- Parainfluenza.
- Respiratory syncytial virus (RSV).
- Human metapneumovirus (human MNV).

Measles Virus

- Habitat: human respiratory tract.

- Mode of transmission: respiratory route.

- Pathogenesis: The virus replicates locally in the mucosa and regional lymph nodes of the upper respiratory tract followed by viremia and localization of virus in skin and mucous membranes.

- Diseases:

1. Measles:

- Rubeola is caused by infection with the measles virus, a member of the paramyxovirus family.

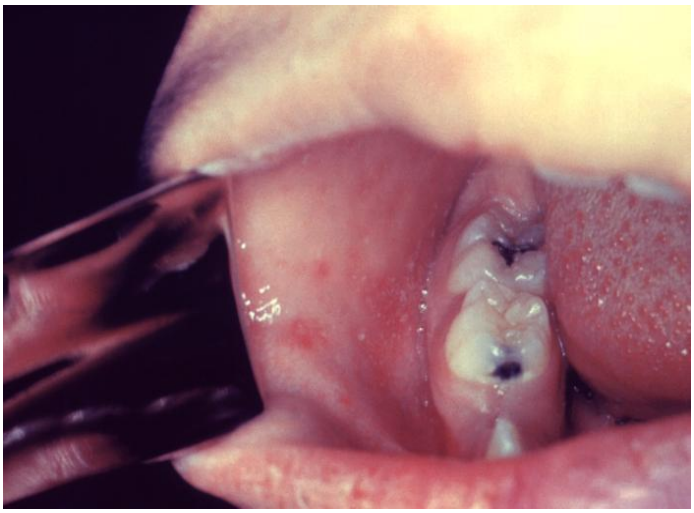
- A classic clinical manifestation of measles is the presence of Koplik spots, small whitish, bluish, or grayish specks on the buccal mucosa adjacent to the second molars.

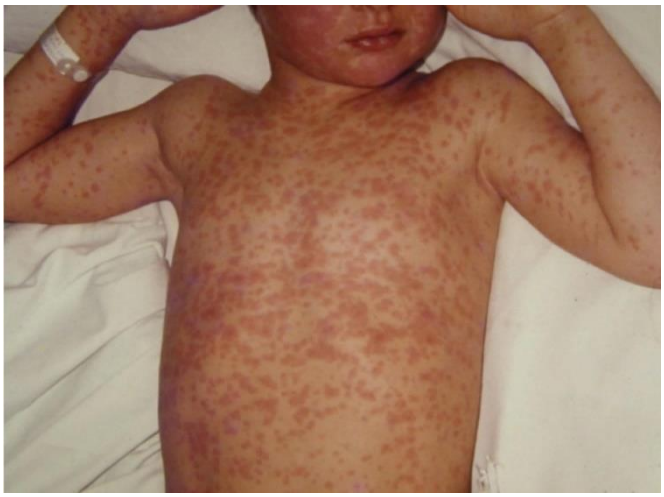
- Koplik spots are sometimes likened to "grains of sand" on an erythematous base.

- When the spots are accompanied by Cough, Coryza, and Conjunctivitis, measles infection is likely (mnemonic: CCCK: Cough, Coryza, Conjunctivitis, and Koplik spots).

- In typical measles, the appearance of these symptoms heralds the development of a maculopapular rash that starts at the head/neck and spreads downward within 1-2 days.

- Vitamin A supplementation can reduce measles mortality in malnourished or vitamin-deficient children. Vitamin A helps prevent and treat these ocular complications (keratitis and corneal ulceration). In addition, it reduces risk of other comorbidities (pneumonia, encephalitis), recovery time, and length of hospital stay.





2. Possible sequelae after measles infection:

A. Subacute sclerosing panencephalitis (SSPE): Rare late complication (occurs years later). Defective measles virus persists in brain, acts as slow virus → Chronic CNS degeneration (personality changes, dementia, autonomic dysfunction, death.

B. Encephalitis (1:1000): symptoms appear within few days of rash.

C. Giant cell pneumonia: rare except in immunosuppressed.

- Prevention: live attenuated vaccine (MMR).

Mumps Virus

- Distinguishing Characteristics:

- Negative sense ssRNA.
- Helical.
- Enveloped.

- Habitat: human respiratory tract.

- Mode of transmission: person to person via respiratory droplets.

- Disease:

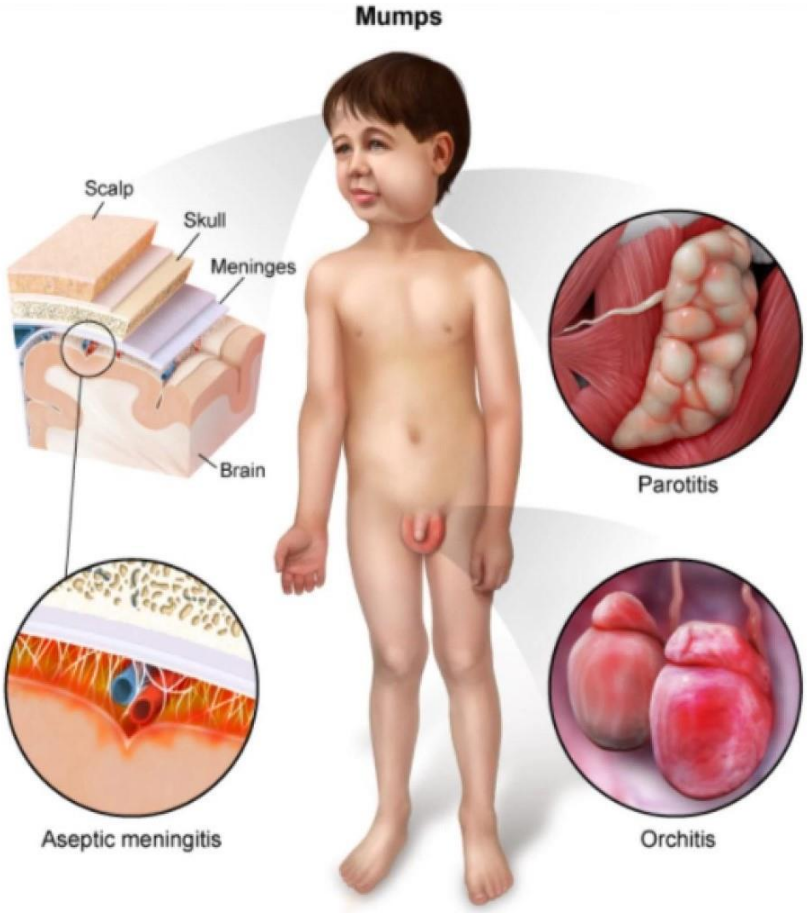
❖ Mumps:

- Asymptomatic to bilateral parotitis with fever, headache, and malaise.

- Complications include pancreatitis, orchitis (leads to sterility especially after puberty), and aseptic Meningitis.

- Mumps makes your parotid glands and testes as big as POM-poms.

- Prevention: live attenuated vaccine (MMR).



Parainfluenza

- Habitat: human respiratory tract.

- Mode of transmission: respiratory route.

- Diseases:

❖ Croup (Acute laryngotracheobronchitis):

- When a child with a history of recent URI develops a brassy, barking cough (seal-like) and breathing difficulties (inspiratory stridor), it is likely that acute laryngotracheitis (croup) has developed.

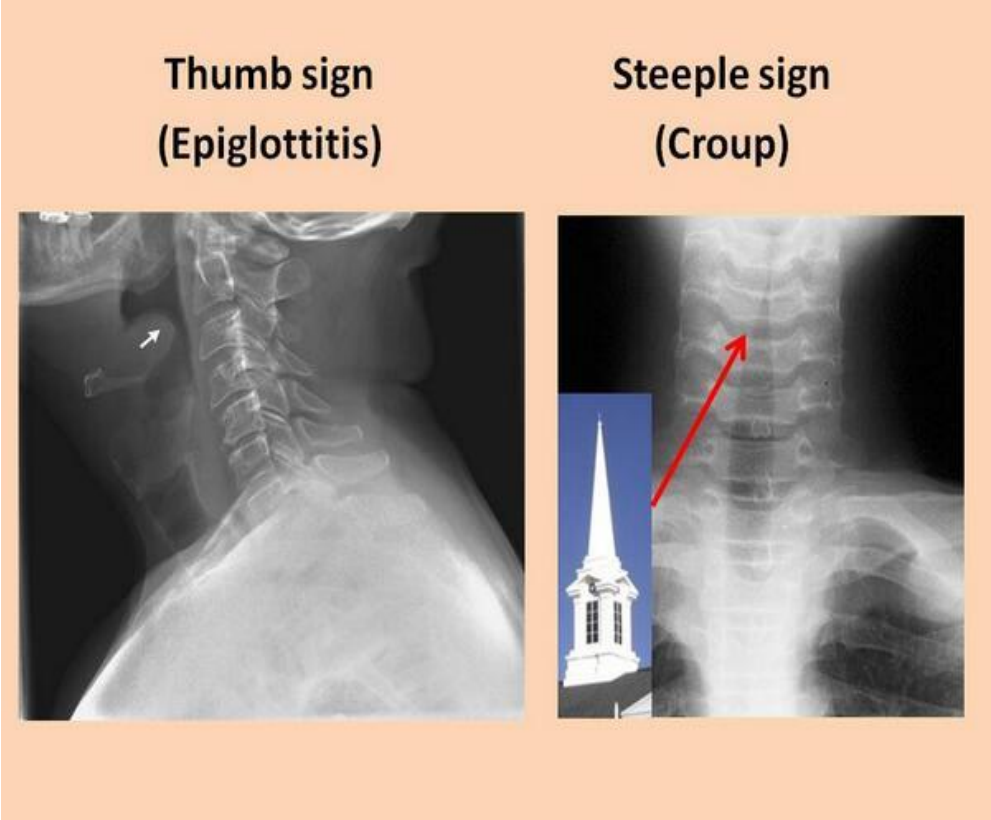
- The dyspnea associated with croup occurs when inflamed subglottic tissue obstructs the upper airway.

- Narrowing of upper trachea and subglottis leads to characteristic steeple sign on X-ray.

- Croup is typically caused by the standard URI viruses, with the parainfluenza viruses (members of Paramyxoviridae) most commonly responsible.

- Racemic epinephrine is used in emergent cases.

	Epiglottitis	Croup
Organism	H. influenza	Parainfluenza virus
X-ray	Thumb sign	Steeple sign



Respiratory syncytial virus (RSV)

- Habitat: human respiratory tract.
- Mode of transmission: respiratory route.
- Diseases:

❖ Bronchiolitis in babies:

- RSV is an important cause of lower respiratory tract disease in infants and young children, under one year of age.
- Viral bronchiolitis is a lower respiratory tract infection that usually occurs before age 2 and is most commonly caused by respiratory syncytial virus (RSV).
- Bronchiolitis typically starts with rhinorrhea and congestion followed by cough, low-grade fever, and increased work of breathing.
- Classic findings include hypoxemia, tachypnea, and retractions with diffuse wheezes and crackles.
- In older infants and children, bronchiolitis is typically a mild, self-limited illness; however, in young infants (age <2 months) or those born prematurely, symptoms may be severe and lead to apnea and/or respiratory failure.
- Palivizumab (monoclonal antibody against F protein) prevents pneumonia caused by RSV infection in premature infants.
- Palivizumab for Paramyxovirus (RSV) Prophylaxis in Preemies.

Bronchiolitis	
Epidemiology	• Age <2 • RSV most common cause
Clinical presentation	• Antecedent nasal congestion/discharge & cough • Wheezing/crackles & respiratory distress (eg, tachypnea, retractions, nasal flaring) • Apnea in high-risk patients

Rhabdoviridae

- Family Characteristics:

- Negative sense ssRNA.
- Bullet shaped.
- Enveloped, helical.

- Viruses of Medical Importance:

- Rabies.
- Vesicular stomatitis virus (foot and mouth disease).

Rabies Virus

- Reservoir:

- Worldwide: dogs are primary reservoir.

- In U.S: More commonly from bat, raccoon, and skunk bites than from dog bites.

- Mode of transmission: contaminated saliva of infected rabid animal through biting.

- Pathogenesis: Once deposited in a wound, the virus stays local for a period of days or weeks before binding to nicotinic acetylcholine receptors on peripheral nerve axons and traveling retrograde to the central nervous system (dorsal root ganglia and spinal cord), where replication occurs. The virus then spreads to other organs through neural pathways.

- Disease:

❖ Rabies:

- Common manifestations of rabies include a nonspecific, flu-like prodrome (malaise, anorexia, mild fever, headache, nausea, vomiting) and a subsequent acute neurologic syndrome that includes agitation, persistent fever, variable consciousness, and painful spasms with swallowing or inspiration.

- Pharyngeal muscle spasms cause dysphagia, which can lead to the avoidance of food and water (hydrophobia).

- Dysphagia along with hypersalivation due to autonomic dysfunction results in the "mouth foaming" seen in rabies encephalitis.

- Generalized flaccid paralysis and coma follow the acute neurologic phase, with most patients dying within two weeks of becoming comatose.

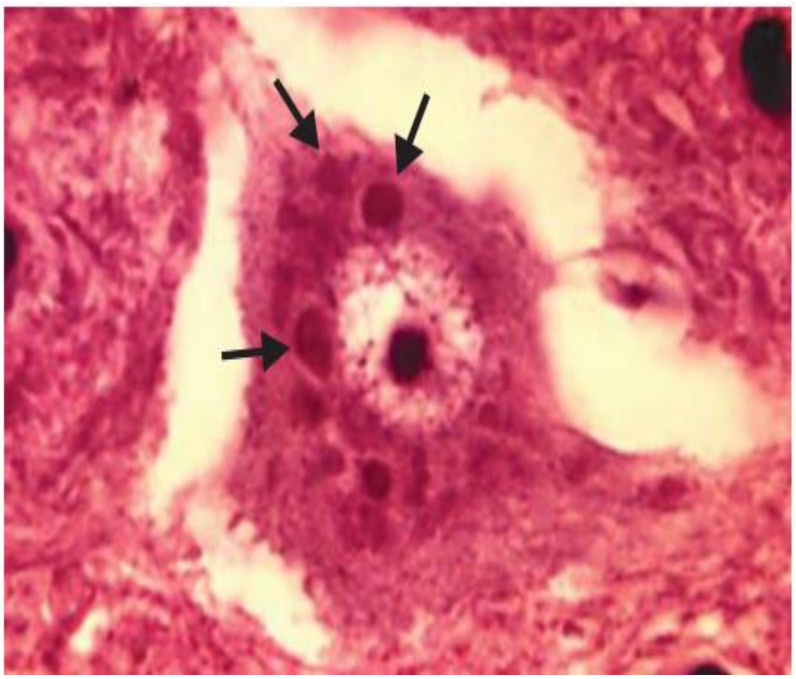
- The clinical presentation of restlessness, agitation, and dysphagia progressing to coma 30 to 50 days following an exposure to cave bats is strongly suggestive of rabies encephalitis.

- Massive replication occurs within the central nervous system and the rabies virus spreads to other organs through neural pathways; it is thought that at this point, postexposure prophylaxis is no longer effective.

▪ Diagnosis:

- Clinical diagnosis.

- Demonstration of Negri bodies (intracytoplasmic inclusion bodies commonly found in Purkinje cells of cerebellum and in hippocampal neurons) in infected animals by direct immunofluorescence.



▪ Treatment:

- If symptoms are evident: none (postexposure prophylaxis is no longer effective).

- If suspect → Postexposure prophylaxis:
  - Immediate and thorough washing of all bite wounds and scratches with soap or a detergent and water.
  - One dose of human rabies immunoglobulin (hRIG).
  - Five doses of rabies vaccine (day of 0,3, 7, 14, 28).

- Prevention:

- The FDA approved rabies vaccine consists of various rhabdovirus strains grown in tissue cell culture and then inactivated to produce killed virus vaccine.

- The CDC currently recommends prophylactic vaccination for individuals at high risk of exposure to the rabies virus (veterinarians, animal handlers, cave explorers, laboratory workers handling infected tissues, and individuals who stay for more than 30 days in developing countries where rabid dogs are prevalent).

- Vaccination program for domestic animals (U.S.).

Human rabies		
Pathogenesis	Transmission of rabies virus by bite from infected mammal	
Reservoir	• United States: Bats (most common), raccoons, skunks, foxes • Developing world: Dogs	
Clinical features	**Encephalitic**	• Hydrophobia • Aerophobia • Pharyngeal spasm, spastic paralysis • Agitation
	Paralytic	• Ascending flaccid paralysis
Postexposure prophylaxis	Rabies immune globulin & rabies vaccine immediately after exposure to high-risk wild animal	
Prognosis	Coma, respiratory failure & death within weeks	

Filoviridae

- Family Characteristics:
- Negative sense ssRNA.
- Enveloped, helical.

- Viruses of Medical Importance:
- Ebola virus.
- Marburg virus.

Ebola virus

- Mode of transmission:
- Transmission requires direct contact with bodily fluids or fomites (including dead bodies); high incidence of nosocomial infection.

- Diseases:

❖ Ebola:

- It targets endothelial cells, phagocytes, hepatocytes.

- Presents with abrupt onset of flu-like symptoms, diarrhea/vomiting, high fever, myalgia.

- Can progress to DIC, diffuse hemorrhage, shock.

- High mortality rate, no definitive treatment. Supportive care.

- Strict isolation of infected individuals and barrier practices for health care workers are key to preventing transmission.

Orthomyxoviridae

- Family Characteristics:
- Negative sense ssRNA.
- Enveloped.
- Helical.
- Segmented (8 segments).

- Viruses of Medical Importance: Influenza viruses.

Influenza virus

- Distinguishing Features:

- The nucleocapsid is enclosed in an envelope consisting of a lipid bilayer and two surface glycoproteins, a hemagglutinin (H) and a neuraminidase (N) which are the major antigenic determinants.

- H (Hemagglutinin): surface glycoproteins that bind to sialic acid (N-acetylneuraminic acid) receptors and promotes viral entry.

- N (Neuraminidase): clips off sialic acids, thus aiding in spreading of the virus (promotes progeny virion release).

- Habitat:

- Influenza A (birds, pigs, humans).
- Influenza B (humans only).

- Mode of transmission:

- Direct contact.
- Respiratory droplets.

- Pathogenesis:

- Influenza viruses are divided into types A, B, and C on the basis of nucleoprotein antigen.

- In types A and B, the H and N antigens undergo genetic variation, which is the basis for the emergence of new strains, type C is antigenically stable.

A. Influenza A virus only is further classified into subtypes based upon H and N antigens.

- 16 H subtypes and 9 N subtypes are now recognized circulating in birds, humans, swine and horses.
- A new subtype usually initiate panzootic in birds or pandemic in humans due to lack of pre-existing protective immunity to the new virus.

- Currently the most famous subtypes are:

  - H_1N_1: circulating in humans and is causing the 2009 influenza pandemic (swine flu).
  - H_5N_5: circulating in birds causing avian flu and affecting humans who closely handle infected birds.

B. Influenza B virus infects mammals only, but generally not as severe as type A.

- Disease:

❖ Influenza:

- Infection of mucosal cells results in cellular destruction and desquamation of superficial mucosa.

- The patient suffers from chills, malaise, fever, muscle pain (myalgia) and respiratory symptoms as rhinitis and pharyngitis.

- Pure viral pneumonia may occur but more commonly bacterial pneumonia associate with influenza (most commonly by S. aureus, S. pneumonia, and H. influenza).

- Patients recently infected with influenza are vulnerable to secondary bacterial infection because of virally induced damage to the mucociliary clearance mechanisms of the respiratory epithelium.

- The elderly and heavy smokers are particularly at risk for this complication.

- Gene reassortment:

- Because the influenza virus genome is segmented, genetic reassortment can occur when a host cell is infected simultaneously with viruses of two different parent strains.

- If a cell is infected with two strains of type A virus, for example, some of the progeny virions will contain mixture of genome segments from the two strains.

- This process of genetic reassortment probably accounts for the periodic appearance of the novel type A strains that cause influenza pandemics.

- Influenza epidemics are two types:

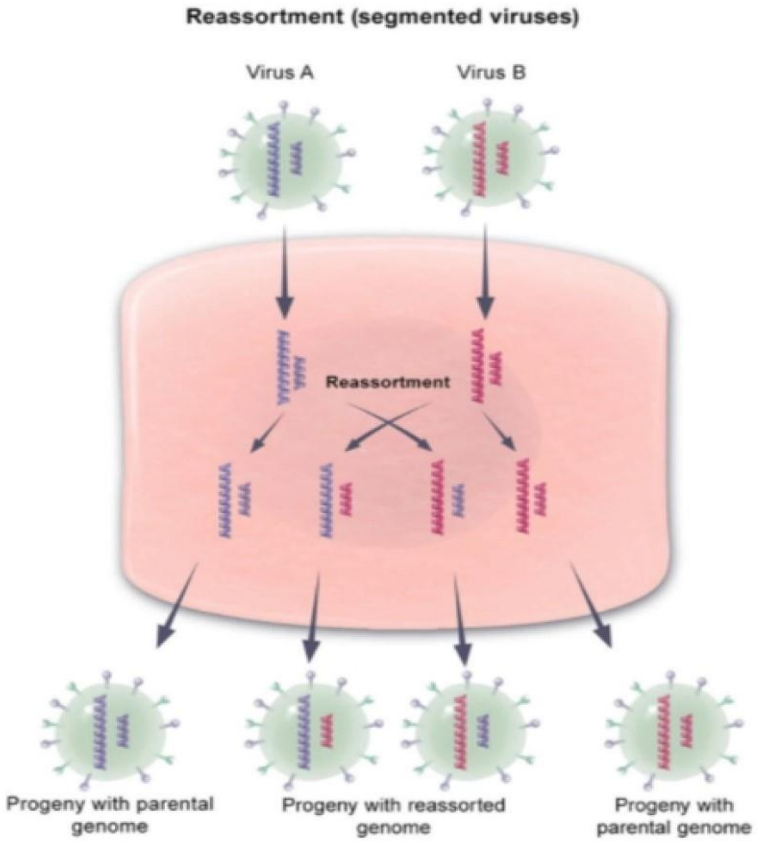
1. Yearly epidemics are caused by both type A and type B viruses.

2. The rare severe influenza pandemics are always caused by type A virus.

- Two different mechanisms of antigenic changes are responsible for producing the strains that cause these two types of epidemics:

A. Antigenic shift:

- Influenza A only.
- Coinfection of cells with two different strains of influenza A (such as when segments of human flu A virus reassort with swine flu A virus).
- Production of a new agent to which population has no immunity.
- Responsible for strains that cause influenza pandemics.
- Viruses with segmented genomes (orthomyxoviruses and rotaviruses) are capable of genetic shifts through reassortment.



B. Antigenic drift:

- Influenza A and B.
- Minor (antigenic drift) changes based on random mutation in hemagglutinin or neuraminidase genes.
- Responsible for strains that cause yearly influenza epidemics.

- Sudden shift is more deadly than gradual drift.

- Prevention:

- Reformulated vaccine ("the flu shot") contains viral strains most likely to appear during the flu season:

A. Killed vaccine:

- Two strains of influenza A (H_3N_2, H_1N_1, for example) and one strain of influenza B are incorporated into the vaccine.

- Killed viral vaccine is most frequently used.

B. Live, attenuated vaccine:

- Intranasal administration.

- Similar composition.

- Currently recommended for children >5 years.

❖ N.B:

- A major determinant of viral tropism for the specific tissues of specific hosts is the extent to which the viral surface proteins can bind to complementary host cell plasmalemma receptors.
- A mutation in a viral encoded envelope glycoprotein can therefore dramatically affect the range of host cells that the virus can attach to or infect.
- One such example would be a mutation in the hemagglutinin of an influenza A strain that was previously confined to domestic livestock.
- If the mutation conferred a new binding affinity for a neuraminic acid containing glycoprotein on the surface of human nasopharyngeal epithelial cells, then the virus would no longer be a threat only to domestic livestock and humans would be vulnerable to infection.
- Changes in host range are most commonly caused by a mutation in the viral encoded surface glycoprotein that mediates virion attachment to target host cell plasmalemma receptors.

Bunyavirida

- Family Characteristics:

- Negative sense ssRNA.
- Enveloped viruses.
- Mostly arboviruses, except Hantavirus.

- Viruses of Medical Importance:

- California encephalitis.
- LaCrosse encephalitis.
- Hantavirus: hemorrhagic fever, pneumonia.
- Sandfly/Rift Valley fevers.

Arenaviridae

- Family Characteristics:

- Negative sense ssRNA.
- Pleomorphic, enveloped.

- Viruses of Medical Importance:

- Lymphocytic choriomeningitis virus (LCMV).
- Lassa fever virus.

- Double-Stranded RNA Viruses:

Reoviridae

- Family Characteristics:

- Linear dsRNA.
- Naked virus.
- Segmented.

- Viruses of Medical Importance:

- Rotavirus.
- Colorado tick fever virus.

Rotavirus

- Mode of transmission: feco-oral.

- Diseases:

- It is a major cause of infectious diarrhea in children worldwide and typically presents in those age <5 with acute, self-limited fever and watery diarrhea that may lead to dehydration and electrolyte abnormalities.

- Major cause of acute diarrhea in the United States during winter, especially in day care centers, kindergartens.

- Rotavirus invades the villous epithelium of the duodenum and proximal jejunum. Infection causes diarrhea via multiple mechanisms including villous blunting (loss of absorptive capacity), proliferation of secretory crypt cells (secretory diarrhea), and reduced brush border enzymes (accumulation of unmetabolized disaccharides in the small intestine lumen).

- Rotavirus vaccination is protective and has resulted in a dramatic reduction in disease incidence.

- CDC recommends routine vaccination of all infants.

- ROTAvirus = Right Out The Anus.

Hepatitis viruses

	Hepatitis A	Hepatitis B	Hepatitis C	Hepatitis D	Hepatitis E
	"Infectious" (HAV)	"Serum" (HBV)	"Post-transfusion Non A, Non B" (HCV)	"Delta" (HDV)	"Enteric" (HEV)
Family	Picornavirus	Hepadnavirus	Flavivirus	Deltavirus (Defective)	Hepevirus
Features	RNA (Naked Capsid)	DNA (Enveloped)	RNA (Enveloped)	Circular (RNA Enveloped)	RNA (Naked capsid)
Transmission	Feco-oral	Parenteral, sexual	Primarily blood (IVDU, posttransfusion)	Parenteral, sexual	Feco-oral
Disease Presentation	- Asymptomatic (usually) - Mild acute - No chronicity - No sequalae	- Acute → complete self-resolve; occasionally severe. - Chronic: 5-10% adults, 90% infants. - Primary hepatocellular carcinoma, cirrhosis.	- Acute is usually subclinical. - 80% become chronic. - Primary hepatocellular carcinoma, cirrhosis	- Co-infection with HBV: occasionally severe - Superinfection with HBV: often severe - Cirrhosis, fulminant hepatitis	- Normal patients: mild. - Pregnant patients: Fulminant hepatitis. - No chronic
Liver biopsy	- Hepatocyte swelling, monocyte infiltration, Councilman bodies.	- Granular eosinophilic "ground glass" appearance due to accumulation of surface antigen within infected hepatocytes; cytotoxic T cells mediate damage.	- Lymphoid aggregates with focal areas of macrovesicular steatosis.	- Similar to HBV.	- Patchy necrosis.
Mortality	< 0,5%	1-2%	0,5-1%	High to very high	- Normal patients: 1-2% - Pregnant patients: 25%

❖ N.B:

- Signs and symptoms of all hepatitis viruses: episodes of fever, jaundice, ALT and AST. May see Councilman bodies (eosinophilic apoptotic globules) on liver biopsy.
- HAV and HEV are fecal-oral: The vowels hit your bowels. Naked viruses do not rely on an envelope, so they are not destroyed by the gut.

Hepatitis A virus

- The pathogen responsible for hepatitis A is an RNA picornavirus with an average incubation period of 30 days.

- Transmission occurs through the fecal-oral route and is common in areas with overcrowding and poor sanitation.

- Outbreaks frequently result from contaminated water or food, with raw or steamed shellfish (oysters) being the typical, and often tested, culprit in the United States.

- Because hepatitis A virus (HAV) is transmitted through the fecal-oral route, improved sanitary conditions (frequent handwashing, appropriate food heating, and the avoidance of food and water in endemic areas) serve to limit outbreaks of infection.

- When HAV contamination is a concern, the virus can be inactivated with water chlorination, bleach (1:100 dilution), formalin, or boiling to 85° C for one minute.

- Clinical presentation:

- Hepatitis A virus infection is most often silent or subclinical ("anicteric," with no jaundice observed) in children but can also present as an acute, self-limited illness characterized by jaundice, malaise, fatigue, anorexia, nausea, vomiting and right upper quadrant pain.

- It's currently estimated that 40-70% of adults within the United States have anti-HAV IgG antibodies, with most positive individuals having never experienced an icteric illness.

- Hepatomegaly is commonly seen. AST and ALT spike early in the illness, followed by increases in bilirubin and alkaline phosphatase.

- Development of antibodies to HAV confers lifelong immunity.

- Fortunately, hepatitis A infection is a self-limiting disease and does not progress to chronic hepatitis, cirrhosis, or hepatocellular carcinoma.

- Treatment of hepatitis A infection is largely supportive, with complete recovery expected in 3-6 weeks.

- Laboratory diagnosis:

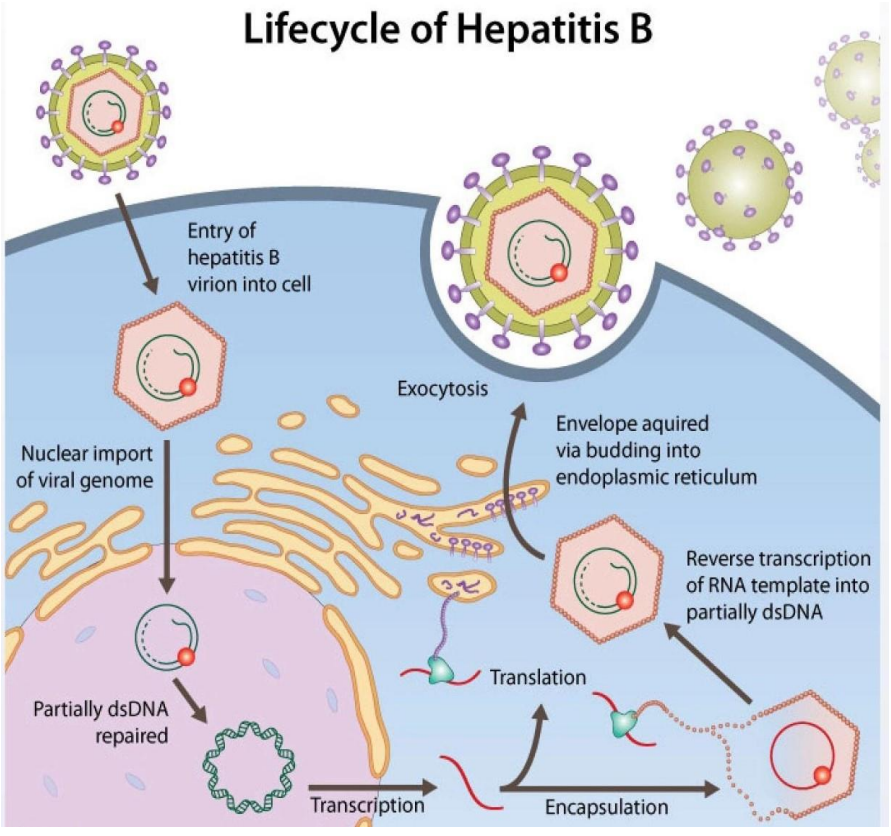
- Marked elevation of liver transaminases and bilirubin.

- Detection of anti-HAV IgM is diagnostic of acute phase. Detection of ant-HAV IgG indicates immunity and may persist for decades.

- Detection of HAV particles in stool or blood by RIA, PCR or electron microscope.

Hepatitis B virus

- Hepatitis B virus (HBV) is a member of the DNA-containing Hepadnaviridae family.
- The HBV genome is a partially double stranded circular DNA molecule, and replication of this genome is accomplished through a reverse transcriptase DNA polymerase that creates an intermediate + single-stranded RNA template.
- After the virion enters the cell, the capsid is released into the cytoplasm and the viral genome is transferred into the nucleus.
- The viral DNA is then repaired to form a fully double-stranded circular minichromosome that is capable of being transcribed into viral mRNAs.
- Reverse transcriptase (which has both RNA- and DNA-dependent DNA-polymerase activity) acts on this RNA template to create a single-stranded DNA intermediate that is then converted back into circular, partially double-stranded DNA.
- The mature capsid is then enveloped by a portion of the endoplasmic reticulum containing virally coded proteins to form the completed virion.
- The hepatitis B virus replicates via the following sequence: double-stranded DNA → template +RNA → progeny double-stranded DNA.



- Hepatitis B virus can be detected in all bodily fluids with the exception of stool.

- Blood is the primary means of HBV transmission, but the virus can also be spread by exposure to semen, saliva, sweat, tears, breast milk, and pathologic effusions.

- Individuals at highest risk for contracting hepatitis B virus include intravenous drug abusers, men who have sex with men, health-care workers subject to needle-stick accidents, patients on dialysis, and blood product recipients.

- Hepatitis B virus infection can produce one of three syndromes:

1. Acute hepatitis with complete resolution.
2. Chronic hepatitis (with or without cirrhosis and the attendant increased risk of hepatocellular carcinoma).
3. Fulminant hepatitis with massive liver necrosis.

- By far the most common outcome in HBV-infected adults (> 95%) is acute hepatitis with mild or subclinical symptoms that eventually completely resolve.

- The HBV genome encodes numerous protein sequences, including three antigens, a DNA polymerase, and a transcriptional transactivator from the X region.

1. HBsAg: is a noninfective envelope glycoprotein that forms spheres and tubules 22 nm in diameter.

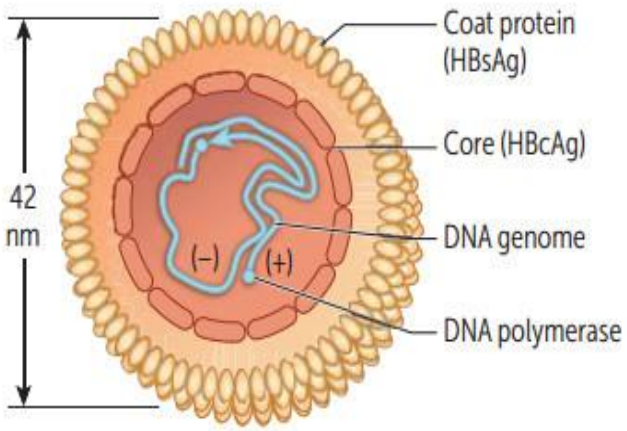
2. HBcAg: is a nucleocapsid core protein that resides within hepatocytes and assembles virion.

3. HBeAg: is a nucleocapsid core and precore protein that is a marker of high infectivity.

4. The DNA polymerase: uses reverse transcriptase to replicate the genome.

5. The transcriptional transactivator of viral genes from the X region:

- It is necessary for viral replication.
- It is also thought to function in the deregulation of hepatocyte replication and the subsequent development of hepatocellular carcinoma in those individuals infected with HBV.



Virus particle

- Laboratory diagnosis:

- The serologic markers for hepatitis B infection appear on a predictable timeline with a long asymptomatic incubation period (lasting an average of 6-8 weeks) followed by acute disease lasting several weeks to months.

- Serological markers for the hepatitis B virus include the following:

A. HBsAg:

- The first virological marker detected in the serum after inoculation, it precedes both the elevation of serum aminotransferases and the onset of clinical symptoms.

- It remains detectable during the entire symptomatic phase of acute hepatitis B and suggests infectivity.

B. Anti-HBs:

- Appearing in the serum after either successful HBV vaccination or the clearance of HBsAg, this marker remains detectable for life.

- It serves as an indicator of noninfectivity and immunity.

- However, there is a time lag between the disappearance of HBsAg and the appearance of anti-HBs in the serum, which is termed the "window period".

C. HBcAg: This marker is not detectable in serum as it is normally sequestrated within the HBsAg coat.

D. Anti-HBc:

- Appearing in the serum shortly after the emergence of HBsAg, this marker remains detectable long after the patient recovers.

- The IgM fraction signals the acute/Recent phase infection, whereas the IgG fraction signal prior exposure or chronic infection.

- Because IgM anti-HBc is present in the "window period," it is an important tool for diagnosis when HBsAg has been cleared and anti-HBs is not yet detectable.

- Thus, IgM anti-HBc is the most specific marker for diagnosis of acute hepatitis B.

E. HBeAg:

- This antigen is detectable shortly after the appearance of HBsAg and indicates active viral replication and high infectivity.

- It is associated with the presence of HBV DNA. HBeAg tends to disappear shortly after aminotransferase levels peak and before HBsAg is eliminated and is followed by the appearance of anti-HBe.

F. Anti-HBe: This marker suggests the cessation of active viral replication and low infectivity.

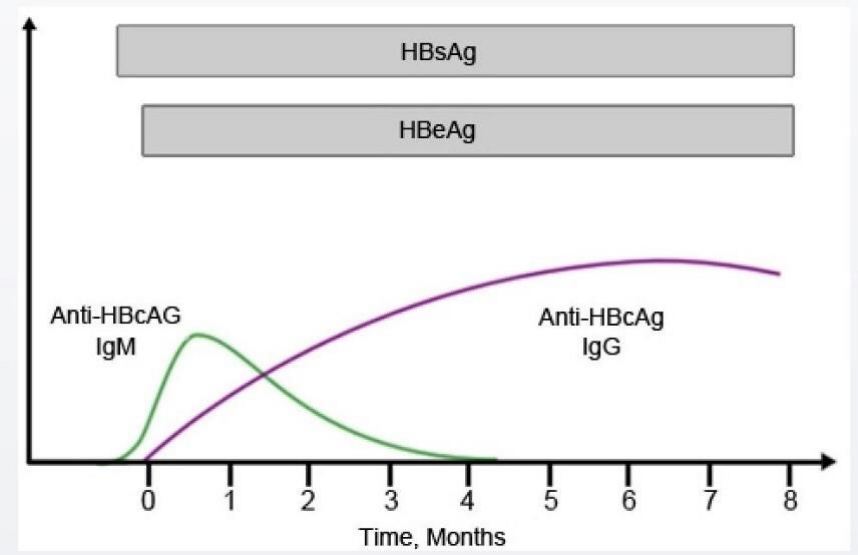
- Interpretation of hepatitis B serology:

Tests	Results	Typical interpretation
HBsAg **Anti-HBc** **Anti-HBs**	Negative Negative Negative	Susceptible individual
HBsAg **HBeAg** **DNA Polymerase** **HBV DNA**	Positive Positive Positive Positive	Viremia stage
HBsAg **IgM anti-HBc** **Anti-HBs**	Positive Positive Negative	Acute HBV infection
HBsAg **IgM anti-HBc** **Anti-HBs**	Negative Positive Negative	Window phase
HBsAg **IgM anti-HBc** **IgG anti-HBc** **HBeAg** **Anti-HBe** **Anti-HBs**	Positive Negative Positive Positive Negative Negative	Chronic HBV infection (High infectivity)
HBsAg **IgM anti-HBc** **IgG anti-HBc** **HBeAg** **Anti-HBe** **Anti-HBs**	Positive Negative Positive Negative Positive Negative	Chronic HBV infection (Low infectivity)
HBsAg **IgG anti-HBc** **Anti-HBs**	Negative Positive Positive	Immune individual following natural infection
HBsAg **Anti-HBc** **Anti-HBs**	Negative Negative Positive	Immune individual following HBV Vaccination

❖ N.B:

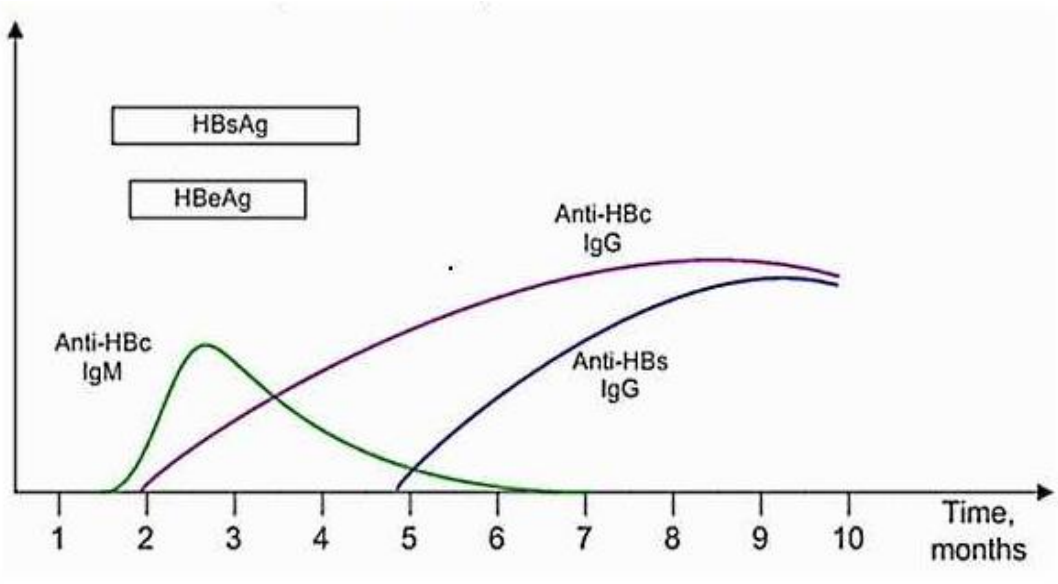
1. In this serologic marker graph, it appears that this patient has a persistence of HBsAg and HBeAg over a long period with low to moderate levels of anti-HBcAg IgG and no detectable Anti-HBsAg.

- These findings are suggestive of an acute hepatitis B infection that has not resolved, but rather has progressed to a highly infectious chronic hepatitis B (note the persistence of HBeAg and lack of anti-HBeAg).



2. In the next serologic marker graph, this patient now has undetectable levels of HBsAg and HBeAg but has moderate to high levels of anti-HBc igG and anti-HBs.

- These findings are suggestive of an acute hepatitis B infection that has completely resolved.



3. Vertical transmission of hepatitis B from pregnant females to the unborn child can occur with active hepatitis B infection.

- Typically, such transmission takes place during the passage of the fetus through the birth canal, but transplacental infection can also occur.
- This is especially common in those women who developed acute hepatitis B infection in the third trimester.
- The presence of HBeAg (a soluble protein that is a marker of viral replication and increased infectivity) in the mother significantly increases the risk of vertical transmission of the virus.
- Were this woman HBeAg negative, her neonate's risk of infection would be 20%.
- If she were HBeAg positive, however, her neonate's risk of infection would be 95%.
- Moreover, should the infant become infected, his chance of progression to chronic hepatitis is 90%.

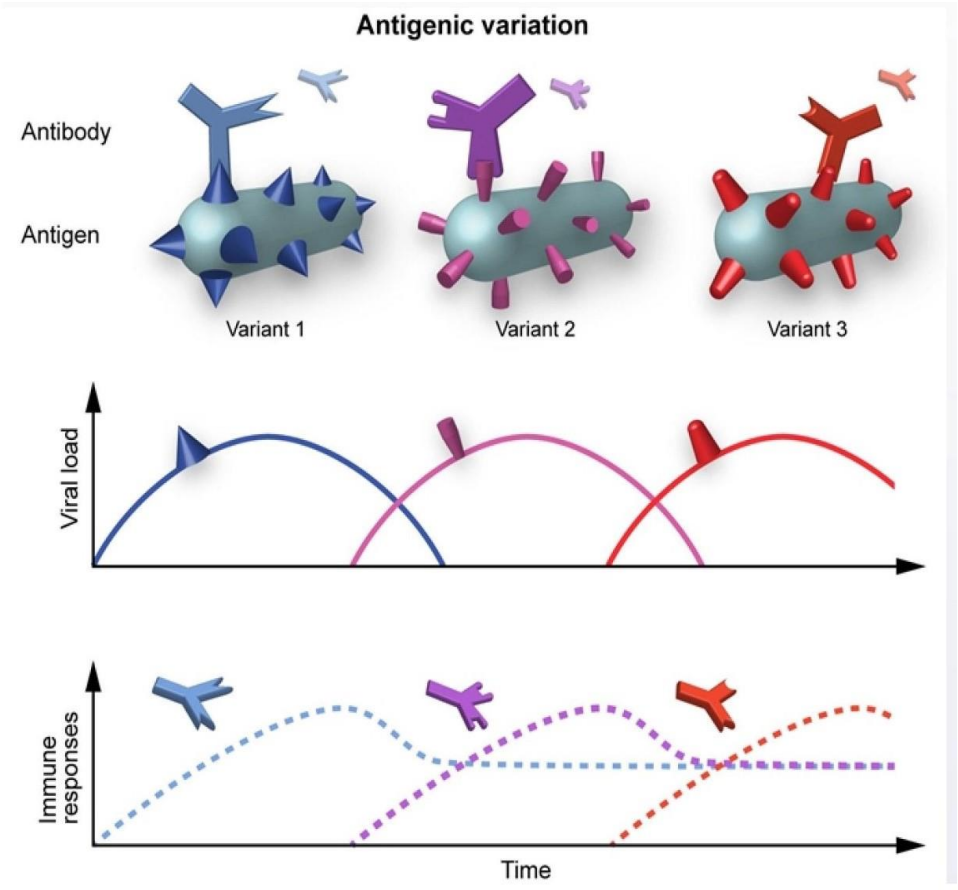
- Viral replication occurs rapidly in infected infants due to immune system immaturity in newborns. The chance of progression to chronic hepatitis is 90% without treatment, which is higher than the chance of progression in adults (<5%) and children (20%-30%).
- Over time, chronically infected newborns are at significant risk of disease progression to cirrhosis and/or hepatocellular carcinoma.
- Because of this concern, the newborns of all mothers with active hepatitis B are passively immunized at birth with hepatitis B immune globulin (HBIG), followed by active immunization with recombinant HBV vaccine.

4. The hepatitis B vaccine is a safe and highly effective recombinant vaccine that contains HBsAg, a surface antigen that stimulates the production of anti-HBsAg in the host.

- Anti-HBsAg is a protective antibody and confers immunity to vaccinated individuals.
- Therefore, seronegative individuals who receive the vaccination will develop immunity and be positive for anti-HBsAg but negative for HBsAg.

Hepatitis C virus

- Hepatitis C virus has six or more genotypes and multiple subgenotypes, as demonstrated by the genetic differences in the encoding of its two envelope glycoproteins.
- This genetic variation has led to the development of a hypervariable region of the envelope glycoprotein that is especially prone to frequent mutation.
- Moreover, there is no proofreading 3'-5' exonuclease activity built into the virion-encoded RNA polymerase.
- As a result, the RNA polymerase makes many errors during replication, and several dozen subspecies of hepatitis C virus are typically present in the blood of an infected individual at any one time.
- Because of this remarkable variety in the antigenic structure of the HCV envelope proteins, production of host antibodies lags behind the production of new mutant strains of HCV and effective immunity against infection is not conferred.
- The tremendous antigenic variety of HCV has significantly slowed efforts to develop a vaccine against the virus.



Hepatitis D virus

- Often referred to as the delta agent or the hepatitis delta virus, hepatitis D virus is a replication-defective RNA virus that is only capable of causing infection when encapsulated with HBsAg of the hepatitis B virus.
- The HBsAg of hepatitis B virus must coat the HDAg of hepatitis D virus before it can infect hepatocytes and multiply.
- Therefore, HDV infection can arise either as an acute coinfection with hepatitis B virus (with the HBV established first to provide the HBsAg for the HDV) or as a superinfection of a chronic HBV carrier.
- In a population that is universally vaccinated with recombinant HBsAg, then, the hepatitis D virus would not be able to replicate and would cease to be a significant threat.

Hepatitis E virus

- Hepatitis E virus is an unenveloped, single-stranded RNA virus spread through the fecal-oral route.
- Infection with HEV occurs primarily in young and middle-aged adults living in Asia, sub-Saharan Africa, and Mexico, with an average incubation period of six weeks.
- While the virus is shed in the stool during the acute illness, the disease is typically self-limited and not associated with either chronic liver disease or a carrier state.
- The most concerning feature of hepatitis E is the high mortality rate observed in infected pregnant women.

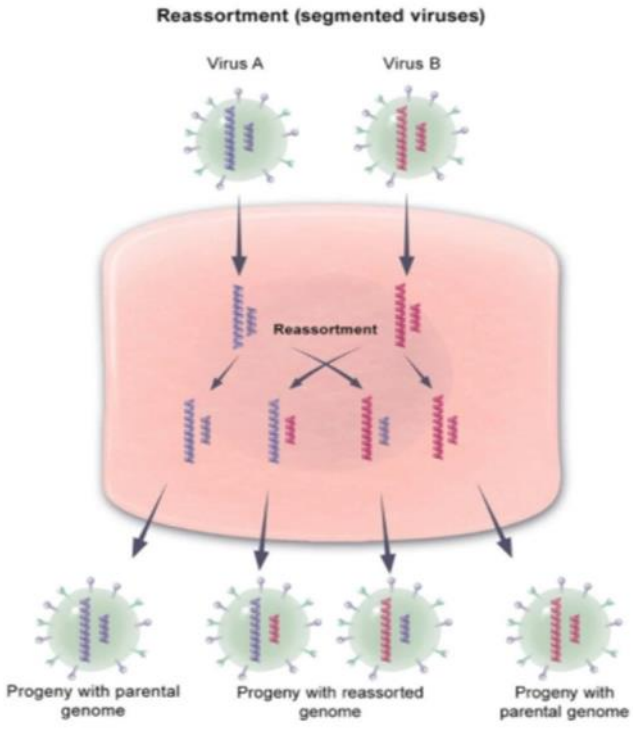
Viral genetics

Recombination

- Recombination refers to the exchange of genes between 2 chromosomes via crossing over within homologous regions.
- The resulting progeny can have recombined genomes with traits from both parent viruses.

Reassortment

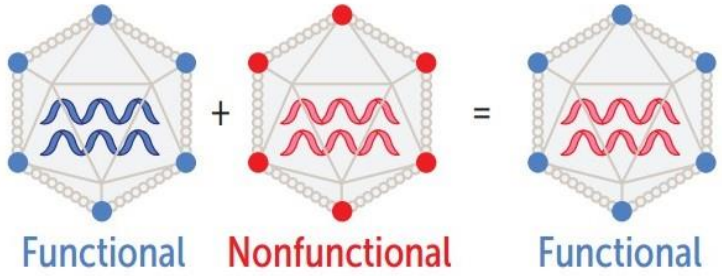
- When viruses with segmented genomes (influenza virus) exchange genetic material.
- For example, the 2009 novel H1N1 influenza A pandemic emerged via complex viral reassortment of genes from human, swine, and avian viruses.
- Has potential to cause antigenic shift.



Complementation

- When 1 of 2 viruses that infect the cell has a mutation that results in a nonfunctional protein, the nonmutated virus "complements" the mutated one by making a functional protein that serves both viruses.

- For example, hepatitis D virus requires the presence of replicating hepatitis B virus to supply HBsAg, the envelope protein for HDV.

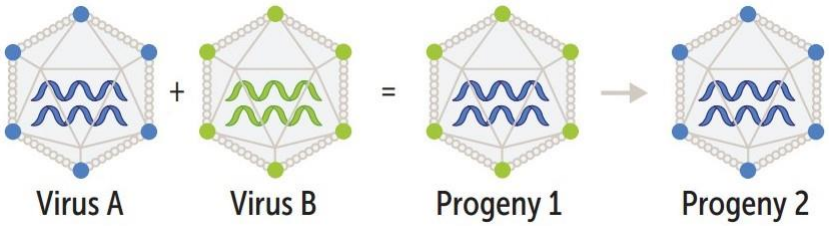


Phenotypic mixing

- Occurs with simultaneous infection of a cell with 2 viruses.

- For progeny 1, genome of virus A can be partially or completely coated (forming pseudovirion) with the surface proteins of virus B (phenotypic masking). Type B protein coat determines the tropism (infectivity) of the hybrid virus.

- Progeny from subsequent infection of a cell by progeny 1 will have a type A coat that is encoded by its type A genetic material.



CHAPTER 5

Systems

❖ Bugs causing food poisoning:

- S. aureus and B. cereus food poisoning starts quickly and ends quickly.

Microorganism	Source of infection
B. cereus	Reheated rice.
C. botulinum	Improperly canned foods, honey.
C. perfringens	Reheated meat.
E. coli O157:H7	Undercooked beef.
Salmonella	Poultry, meat, and eggs.
S. aureus	Meats, mayonnaise, custard; preformed toxin
V. parahaemolyticus and V. vulnificus	Contaminated seafood

- V. vulnificus can also cause wound infections from contact with contaminated water or shellfish.

❖ Bugs causing diarrhea:

Bloody diarrhea	
Campylobacter	Comma- or S-shaped organisms; growth at 42°C.
E. histolytica	Protozoan; amebic dysentery; liver abscess.
Enterohemorrhagic E. coli	O157:H7; can cause HUS; makes Shiga-like toxin.
Enteroinvasive E. coli	Invades colonic mucosa.
Salmonella	Lactose ⊖; flagellar motility; has animal reservoir, especially poultry and eggs.
Shigella	Lactose ⊖; very low ID50; produces Shiga toxin (human reservoir only); bacillary dysentery.
Y. enterocolitica	Day care outbreaks, pseudoappendicitis.

Watery diarrhea	
C. difficile	Pseudomembranous colitis; caused by antibiotics; occasionally bloody diarrhea.
C. perfringens	Also causes gas gangrene.
Enterotoxigenic E. coli	Travelers' diarrhea; produces heat-labile (LT) and heat-stable (ST) toxins.
Protozoa	Giardia, Cryptosporidium.
V. cholera	Comma-shaped organisms; rice-water diarrhea; often from infected seafood.
Viruses	Rotavirus, norovirus, enteric adenovirus.

❖ Common causes of pneumonia:

Neonates (< 4 wk)	Children (4 wk–18 yr)	Adults (18–40 yr)	Adults (40–65 yr)	Elderly
- **Group B streptococci** - **E. coli**	- Viruses (RSV) - Mycoplasma - C. trachomatis (infants-3 yr) - C. pneumonia (school-aged Children) - S. pneumonia. - Runts May Cough Chunky Sputum	- Mycoplasma - C. pneumoniae - S. pneumoniae - Viruses (influenza)	- S. pneumoniae - H. influenzae - Anaerobes - Viruses - Mycoplasma	- S. pneumoniae - Influenza virus - Anaerobes - H. influenzae - Gram-negative rods

Special groups	
Alcoholic/IV drug user	S. pneumoniae, Klebsiella, S. aureus.
Aspiration	Anaerobes (Peptostreptococcus, Fusobacterium, Prevotella, Bacteroides).
Atypical	Mycoplasma, Legionella, Chlamydia
Cystic fibrosis	Pseudomonas, S. aureus, S. pneumonia.
Immunocompromised	S. aureus, enteric gram-negative rods, fungi, viruses, P. jirovecii (with HIV).
Nosocomial (hospital acquired)	S. aureus, Pseudomonas, other enteric gram-negative rods.
Postviral	S. aureus, H. influenzae, S. pneumoniae

❖ Common causes of meningitis:

Newborn (0–6 mo)	children (6 mo–6 yr)	6–60 yr	60 yr +
- **Group B streptococci** - **E. coli** - **Listeria**	- S. pneumoniae - N. meningitidis - H. influenzae type B - Enteroviruses	- S. pneumonia. - N. meningitidis (#1 in teens) - Enteroviruses - HSV	- S. pneumonia. - Gram-negative rods. - Listeria.

- Give ceftriaxone and vancomycin empirically (add ampicillin if Listeria is suspected).
- Viral causes of meningitis: enteroviruses (especially coxsackievirus), HSV-2 (HSV-1 = encephalitis), HIV, West Nile virus (also causes encephalitis), VZV.
- In HIV: Cryptococcus spp.
- Note: Incidence of H. influenzae meningitis has ↓ greatly with introduction of the conjugate H. influenzae vaccine in last 10–15 years. Today, cases are usually seen in unimmunized children.

❖ CSF findings in meningitis:

	Opening pressure	Cell type	Protein	Sugar
Bacterial	↑	↑ PMNs	↑	↓
Fungal/TB	↑	↑ lymphocytes	↑	↓
Viral	Normal/↑	↑ lymphocytes	Normal/↑	Normal

❖ Infections causing brain abscess:

- Most commonly viridans streptococci and Staphylococcus aureus.
- If dental infection or extraction precedes abscess, oral anaerobes commonly involved.
- Multiple abscesses are usually from bacteremia; single lesions from contiguous sites: otitis media and mastoiditis → temporal lobe and cerebellum; sinusitis or dental infection → frontal lobe.
- Toxoplasma reactivation in AIDS.

❖ Osteomyelitis:

- Hematogenous osteomyelitis is predominantly a disease of children that most frequently affects the long bones.
- Staphylococcus aureus is implicated in most cases secondary to a bacteremic event.
- Streptococcus pyogenes (group A streptococcus) is the second most common cause of hematogenous osteomyelitis.

Risk Factor	Associated infection
Assume if no other information is available	S. aureus (most common overall)
Sexually active	Neisseria gonorrhoeae (rare), septic arthritis more common
Sickle cell disease	Salmonella and S. aureus
Prosthetic joint replacement	S. aureus and S. epidermidis
Vertebral involvement	S. aureus, Mycobacterium tuberculosis (Pott disease)
Cat and dog bites	Pasteurella multocida
IV drug abuse	Pseudomonas, Candida, S. aureus are most Common

❖ Urinary tract infections:

- Cystitis presents with dysuria, frequency, urgency, suprapubic pain, and WBCs (but not WBC casts) in urine.

- Primarily caused by ascension of microbes from urethra to bladder.

- Predisposing factors:

- Male infants with congenital defects, vesicoureteral reflux.
- Elderly: enlarged prostate.
- Ten times more common in women (shorter urethras colonized by fecal flora).

- Ascension to kidney results in pyelonephritis, which presents with fever, chills, flank pain, costovertebral angle tenderness, hematuria, and WBC casts.

- Other predisposing factors: obstruction, kidney surgery, catheterization, GU malformation, diabetes, pregnancy.

❖ UTI bugs:

Species	Features	Comments
Escherichia coli	Leading cause of UTI. Colonies show green metallic sheen on EMB agar.	Diagnostic markers: - ⊕Leukocyte esterase = evidence of WBC activity. - ⊕Nitrite test = reduction of urinary nitrates by bacterial species (E. coli). - ⊕Urease test = urease-producing bugs (Proteus, Klebsiella).
Staphylococcus Saprophyticus	2nd leading cause of UTI in sexually active women.	
Klebsiella pneumoniae	3rd leading cause of UTI. Large mucoid capsule and viscous colonies.	
Serratia marcescens	Some strains produce a red pigment; often nosocomial and drug resistant.	
Enterococcus	Often nosocomial and drug resistant.	
Proteus mirabilis	Motility causes "swarming" on agar; produces urease; associated with struvite stones.	
Pseudomonas aeruginosa	Blue-green pigment and fruity odor; usually nosocomial and drug resistant.	

❖ ToRCHeS infections:

- Microbes that may pass from mother to fetus.
- Transmission is transplacental in most cases, or via delivery (especially HSV-2).
- Nonspecific signs common to many ToRCHeS infections include hepatosplenomegaly, jaundice, thrombocytopenia, and growth retardation.
- Other important infectious agents include Streptococcus agalactiae (group B streptococci), E. coli, and Listeria monocytogenes, all causes of meningitis in neonates. Parvovirus B19 causes hydrops fetalis.

Agent	Mode of transmission	Maternal manifestations	Neonatal manifestions
Toxoplasma gondii	Cat feces or ingestion of undercooked meat	Usually asymptomatic; lymphadenopathy (rarely)	Classic triad: chorioretinitis, hydrocephalus, and intracranial calcifications −/+ "blueberry muffin" rash
Rubella	Respiratory droplets	Rash, lymphadenopathy, Arthritis	Classic triad: PDA (or pulmonary artery hypoplasia), (cataracts, and deafness −/+ "blueberry muffin" rash
CMV	Sexual contact, organ Transplants	Usually asymptomatic; mononucleosis-like illness	Hearing loss, seizures, petechial rash, "blueberry muffin" rash, periventricular calcifications
HIV	Sexual contact, needle-stick	Variable presentation depending on CD4+ count	Recurrent infections, chronic diarrhea.
Herpes simplex virus-2	Skin or mucous membrane Contact	Usually asymptomatic; herpetic (vesicular) lesions	Encephalitis, herpetic (vesicular lesions)
Syphilis	Sexual contact	Chancre (1°) and disseminated rash (2°) are the two stages likely to result in fetal infection	Often results in stillbirth, hydrops fetalis; if child survives, presents with facial abnormalities (notched teeth, saddle nose, short maxilla), saber shins, CN VIII deafness

❖ Red rashes of childhood:

Agent	Associated syndrome/Disease	Clinical Presentation
Coxsackievirus type A	Hand-foot-mouth disease	Oval-shaped vesicles on palms and soles, vesicles and ulcers in oral mucosa
HHV-6	Roseola (exanthem subitum)	Asymptomatic rose-colored macules appear on body after several days of high fever; can present with febrile seizures; usually affects infants
Measles virus	Measles (rubeola)	Beginning at head and moving down; rash is preceded by cough, coryza, conjunctivitis, and blue-white (Koplik) spots on buccal mucosa
Parvovirus B19	Erythema infectiosum (fifth disease)	"Slapped cheek" rash on face (can cause hydrops fetalis in pregnant women)
Rubella virus	Rubella (German measles)	Pink coalescing macules begin at head and move down → fine desquamating truncal rash, postauricular lymphadenopathy
Streptococcus pyogenes	Scarlet fever	Erythematous, sandpaper-like rash with fever and sore throat
VZV	Chickenpox	Vesicular rash begins on trunk; spreads to face and extremities with lesions of different ages

❖ Sexually transmitted infections:

Disease	Clinical Features	Organism
AIDS	Opportunistic infections, Kaposi sarcoma, lymphoma	HIV
Chancroid	Painful genital ulcer with exudate, inguinal adenopathy	Haemophilus ducreyi (it's so painful, you "do cry")
Chlamydia	Urethritis, cervicitis, conjunctivitis, reactive arthritis, PID	Chlamydia trachomatis (D–K)
Condylomata acuminate	Genital warts, koilocytes	HPV-6 and -11
Genital herpes	Painful penile, vulvar, or cervical vesicles and ulcers; can cause systemic symptoms such as fever, headache, myalgia	HSV-2, less commonly HSV-1
Gonorrhea	Urethritis, cervicitis, PID, prostatitis, epididymitis, arthritis, creamy purulent Discharge	Neisseria gonorrhoeae
Hepatitis B	Jaundice	HBV

Lymphogranuloma Venereum		Infection of lymphatics; painless genital ulcers, painful lymphadenopathy (buboes)	C. trachomatis (L1–L3)
1° syphilis		Painless chancre	Treponema pallidum
2° syphilis		Fever, lymphadenopathy, skin rashes, condylomata lata	
3° syphilis		Gummas, tabes dorsalis, general paresis, aortitis, Argyll Robertson pupil	
Trichomoniasis	Vaginitis, strawberry cervix, motile in wet prep	Trichomonas vaginalis	

Characteristics of ulcerative sexually transmitted diseases			
Disease	**Causative agent**	**Features of primary lesion**	**Initial lesion painful?**
Chancroid	*Haemophilus ducreyi*	• Multiple & deep ulcers • Base may have gray to yellow exudate • Organisms often clump in long parallel strands ("school of fish")	**Yes**
Genital herpes	Herpes simplex virus 1 & 2	• **Multiple**, small, grouped ulcers • Shallow with erythematous base • Multinucleated giant cells & intranuclear inclusions (Cowdry type A)	**Yes**
Granuloma inguinale (donovanosis)	*Klebsiella granulomatis*	• Extensive & progressive ulcerative lesions without lymphadenopathy • Base may have granulation-like tissue • Deeply staining gram-negative intracytoplasmic cysts (Donovan bodies)	No
Syphilis	*Treponema pallidum*	• Single, indurated, well-circumscribed ulcer • Clean base • Thin, delicate, corkscrew-shaped organisms on dark-field microscopy	No
Lymphogranuloma venereum	*Chlamydia trachomatis*	• Small & shallow ulcers • Large, painful, coalesced inguinal lymph nodes ("buboes") • Intracytoplasmic chlamydial inclusion bodies in epithelial cells & leukocytes	No

❖ Pelvic inflammatory disease:

- Chlamydia trachomatis (subacute, often undiagnosed), Neisseria gonorrhoeae (acute).
- C. trachomatis: most common bacterial STI in the United States.
- Cervical motion tenderness (chandelier sign), purulent cervical discharge.
- PID may include salpingitis, endometritis, hydrosalpinx, and tubo-ovarian abscess.
- Salpingitis is a risk factor for ectopic pregnancy, infertility, chronic pelvic pain, and adhesions.

❖ Nosocomial infections:

- E. coli (UTI) and S. aureus (wound infection) are the two most common causes.

RisK Factor	Pathogen	Unique signs/symptoms
Altered mental status, old age, aspiration	Polymicrobial, gram-negative bacteria, often anaerobes	Right lower lobe infiltrate or right upper/middle lobe (patient recumbent); purulent malodorous sputum
Antibiotic use	Clostridium difficile	Watery diarrhea, leukocytosis
Decubitus ulcers, surgical wounds, drains	S. aureus (including MRSA), gram-negative anaerobes	Erythema, tenderness, induration, drainage from surgical wound sites
Intravascular catheters	S. aureus (including MRSA), S. epidermidis (long term), Enterobacter	Erythema, induration, tenderness, drainage from access sites
Mechanical ventilation, Endotracheal intubation	Late onset: P. aeruginosa, Klebsiella, Acinetobacter, S. aureus.	New infiltrate on CXR, sputum production, sweet odor (Pseudomonas)
Renal dialysis unit, Needlestick	HBV	
Urinary catheterization	E. coli, Klebsiella, Proteus spp.	Dysuria, leukocytosis, flank pain or costovertebral angle tenderness
Water aerosols	Legionella	Signs of pneumonia, GI symptoms (nausea, vomiting)

❖ Bugs affecting unimmunized children:

Clinical Presentation	Findings/labs	Pathogen
Dermatologic		
Rash	Beginning at head and moving down with postauricular lymphadenopathy	Rubella virus
	Beginning at head and moving down; rash preceded by cough, coryza, conjunctivitis, and blue white (Koplik) spots on buccal mucosa	Measles virus

Neurologic		
Meningitis	Microbe colonizes nasopharynx	H. influenzae type B
	Can also lead to myalgia and paralysis	Poliovirus
Respiratory		
Epiglottitis	Fever with dysphagia, drooling, and difficulty breathing due to edematous "cherry red" epiglottis; "thumbprint sign" on X-ray	H. influenzae type B (also capable of causing epiglottitis in fully immunized children)
Pharyngitis	Grayish oropharyngeal exudate (pseudomembranes may obstruct airway), painful throat	Corynebacterium diphtheriae (elaborates toxin that causes necrosis in pharynx, cardiac, and CNS tissue)

❖ Bug hints (if all else fails):

Characteristic	Organism
Asplenic patient (due to surgical splenectomy or autosplenectomy (chronic sickle cell disease)	Encapsulated microbes, especially SHiN (S. pneumonia, H. influenzae type B, N. meningitidis)
Branching rods in oral infection, sulfur granules	Actinomyces israelii
Chronic granulomatous disease	Catalase ⊕ microbes, especially S. aureus
"Currant jelly" sputum	Klebsiella
Dog or cat bite	Pasteurella multocida
Facial nerve palsy	Borrelia burgdorferi (Lyme disease)
Fungal infection in diabetic or immunocompromised patient	Mucor or Rhizopus spp.
Health care provider	HBV (from needlestick)
Neutropenic patients	Candida albicans (systemic), Aspergillus
Organ transplant recipient	CMV
PAS ⊕	Tropheryma whipplei (Whipple disease)
Pediatric infection	Haemophilus influenzae (including epiglottitis)
Pneumonia in cystic fibrosis, burn infection	Pseudomonas aeruginosa
Pus, empyema, abscess	S. aureus
Rash on hands and feet	Coxsackie A virus, Rickettsia rickettsia, 2RY Syphilis (you drive CARS using your palms and soles)
Sepsis/meningitis in newborn	Group B strep
Surgical wound	S. aureus
Traumatic open wound	Clostridium perfringens

❖ N.B:

1. Central venous catheters (CVCs) are commonly used in critically ill patients for hemodynamic monitoring and administration of fluids and medications, especially medications that cannot be given peripherally (vasopressors, TPN, chemotherapy).

- Infection, phlebitis, and bacteremia are the major complications of intravascular catheters, especially CVCs.
- Infection involving CVCs often originates from the patient's skin flora or bacteria on the hands of health care workers.
- Gram-positive cocci account for the overwhelming majority of these infections, with the most common pathogens being coagulase-negative staphylococci and Staphylococcus aureus.
- The most important steps for the prevention of central venous catheter infections are as follows:

- Proper hand washing
- Full barrier precautions during insertion of a central line
- Chlorhexidine for skin disinfection
- Avoidance of the femoral insertion site
- Removal of catheter(s) when no longer needed.

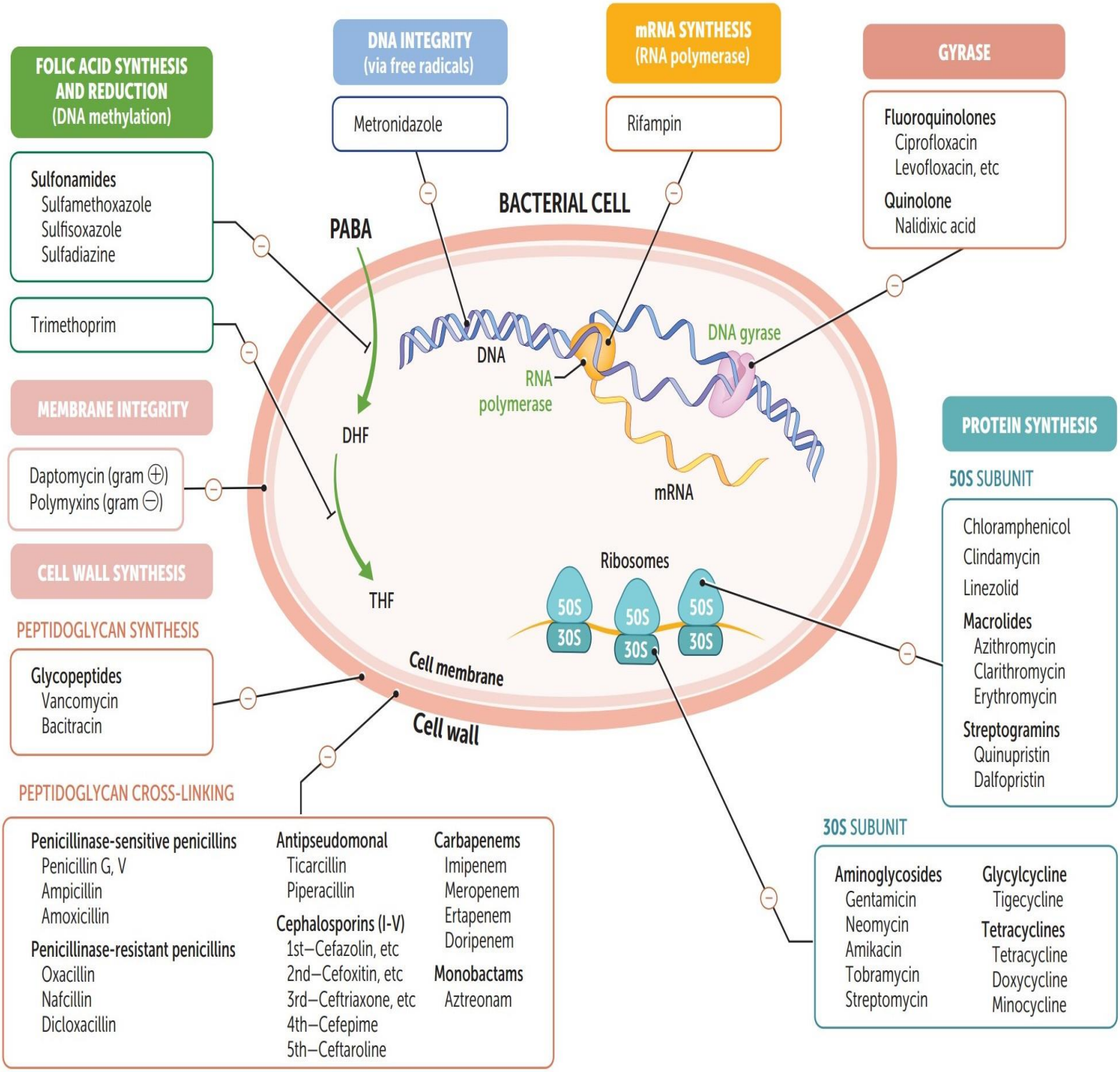
2. Alcohols, including ethanol and isopropanol, are widely used as disinfecting agents in the health care setting.

- They are commonly used to clean the skin before immunization or venipuncture and to disinfect external surfaces of equipment.
- They function by disorganizing the lipid structure in membranes, causing them to be leaky, and by denaturing cellular proteins.
- They are rapidly bactericidal and also tuberculocidal, fungicidal, and virucidal, but do not destroy bacterial spores.

CHAPTER 6

Antimicrobials

- Bacteriostatic antibiotics: drugs that stop the growth of bacteria but don't decrease the number of the bacteria (inhibit the growth of the organisms).
- Bactericidal antibiotics: drugs that decrease the growth of bacteria and its number (direct killing of the organisms).
- Most of the antibiotics are bactericidal.



Inhibitors of cell wall synthesis

- All cell-wall synthesis inhibitors are bactericidal.

Penicillins

- Mechanisms of action:

- D-Ala-D-Ala is the amino acid sequence on peptidoglycan precursor molecules that is recognized by the enzyme transpeptidase, the enzyme that catalyzes the final crosslinking step in peptidoglycan cell wall.

- Penicillins are structural analogs of D-Ala-D-Ala that bind penicillin-binding proteins (transpeptidases) and inhibit it by binding covalently to its active site.

- The result is failed synthesis of the bacterial peptidoglycan cell wall.

- The weakened cell wall integrity causes osmotic lysis of the bacterium.

- Subgroups and antimicrobial activity:

1. Very narrow spectrum:

- Drugs: methicillin, nafcillin, oxacillin, cloxacillin, dicloxacillin.

- Beta-lactamase resistant because bulky R group blocks access of β-lactamase to β-lactam ring.

- Spectrum: known or suspected S. aureus (except MRSA; resistant because of altered penicillin-binding protein target site).

- Nafcillin is commonly used empirically to treat skin and soft tissue infections (folliculitis, abscesses) for which S. aureus is the usual cause.

- "Use naf (nafcillin) for staph".

2. Narrow spectrum:

- Drugs: Penicillin G (IV and IM form), penicillin V (oral).

- Beta-lactamase sensitive.

- Spectrum: Mostly used for gram-positive organisms (S. pneumoniae, S. pyogenes, Actinomyces). Also used for gram-negative cocci (mainly N. meningitidis) and spirochetes (namely T. pallidum).

- Bactericidal for gram-positive cocci, gram-positive rods, gram-negative cocci, and spirochetes.

3. Broad spectrum:

- Drugs: Aminopenicillins (ampicillin and amoxicillin).

- Beta-lactamase sensitive, also combine with clavulanic acid to protect against destruction by β-lactamase.

- Spectrum: Wider spectrum, gram-positive cocci (not staph), E. coli, H. influenzae, Listeria monocytogenes (ampicillin), Borrelia burgdorferi (amoxicillin), H. pylori (amoxicillin).

- AmOxicillin has greater Oral bioavailability than ampicillin.

4. Extended spectrum:

- Drugs: Piperacillin, ticarcillin.

- Antipseudomonal, beta-lactamase sensitive.

- Spectrum: increased activity against gram-negative rods, including Pseudomonas aeruginosa.

▪ General considerations:

- Activity enhanced if used in combination with beta-lactamase inhibitors (clavulanic acid, sulbactam) to protect them from destruction by β-lactamase.

- Synergy with aminoglycosides against pseudomonal and enterococcal species.

▪ Pharmacokinetics:

- Most are eliminated via active tubular secretion; dose reduction needed only in major renal dysfunction.

- Nafcillin and oxacillin eliminated largely in bile; ampicillin undergoes enterohepatic cycling but excreted by the kidney.

▪ Side effects:

- Hypersensitivity:
  - Incidence 5 to 7% with wide range of reactions (types I-IV).
  - Urticarial skin rash common, but severe reactions, including anaphylaxis are possible.
  - Interstitial nephritis with methicillin.
  - Assume complete cross-allergenicity between individual penicillins.

- Others:
  - GI distress (NVD), especially ampicillin.
  - Jarisch-Herxheimer reaction in treatment of syphilis.

- Mechanism of resistance:

- Many Gram positive and Gram-negative bacteria have acquired resistance to the penicillin family of antibiotics.

- One mechanism of resistance is production of beta-lactamase, an enzyme that disrupts the beta-lactam ring of penicillins and cephalosporins, effectively inactivating these medications.

- Certain antibiotics have combinations of chemical groups around the beta-lactam ring that prevent beta-lactamase access.

- These antibiotics are considered beta-lactamase resistant. It is through this mechanism that third generation cephalosporins have better activity against Gram negative bacteria than first or second generation cephalosporins.

- Clavulanic acid, Sulbactam and Tazobactam (CAST) are beta-lactamase inhibitors that extend the spectrum of penicillin-family antibiotics to include beta-lactamase producing organisms such as S. aureus, H. influenzae, Bacteroides, and other gram-negative bacteria.

β-lactamase inhibitors

- β-lactamase inhibitors Include Clavulanic Acid, Sulbactam, and Tazobactam (CAST).

- Examples: amoxicillin-clavulanate, ceftazidime-avibactam, ampicillin-sulbactam, piperacillin-tazobactam).

- Often added to penicillin antibiotics to protect the antibiotic from destruction by β-lactamase.

Cephalosporins

- Mechanisms of action:

- Identical to penicillins.

- Penicillins and cephalosporins function by irreversibly binding to penicillin-binding proteins. Transpeptidases are one form of penicillin-binding protein that function to cross-link peptidoglycan in the bacterial cell wall.

- Inhibition of transpeptidase leads to cell wall instability and bacteriolysis.

- Subgroups and antimicrobial activity:

1. First generation:

- Drugs: Cefazolin, cephalexin.

- Spectrum: gram-positive cocci (not MRSA), Proteus mirabilis, E. coli, Klebsiella pneumoniae.

- 1st generation: PEcK.

- Cefazolin used prior to surgery to prevent S. aureus wound infections.

- Pharmacokinetics: none enter CNS.

2. Second generation:

- Drugs: cefaclor, cefoxitin, cefuroxime, cefotetan. 2nd graders wear fake fox fur to tea parties.

- Spectrum: gram-positive cocci, Haemophilus influenzae, Enterobacter aerogenes, Neisseria spp, Serratia marcescens, Proteus mirabilis, E. coli, Klebsiella pneumoniae.

- 2nd generation: HENS PEcK.

- Pharmacokinetics: no drugs enter the CNS, except cefuroxime.

3. Third generation:

- Drugs: Ceftriaxone (IM) and cefotaxime (parenteral), ceftazidime and cefixime (oral).

- Spectrum: gram-positive and gram-negative cocci, serious gram-negative infections resistant to other β-lactams.

- Ceftriaxone: meningitis, gonorrhea, disseminated Lyme disease.

- Ceftazidime: Pseudomonas.

- Pharmacokinetics: most enter CNS; important in empiric management of meningitis and sepsis.

4. Fourth generation:

- Drugs: Cefepime (IV).

- Spectrum: gram-negative organisms, with activity against Pseudomonas and gram-positive organisms.

- Resistant to most beta-lactamases.

- Enters CNS.

5. Fifth generation:

- Ceftaroline.

- Spectrum: broad gram-positive and gram-negative organism coverage.

- Unlike 1st-4th generation cephalosporins, ceftaroline covers MRSA, and Enterococcus faecalis.

- Does not cover Pseudomonas.

▪ Organisms not covered by cephalosporins are "LAME":

- Listeria monocytogenes (ampicillin +/- gentamycin).

- Atypicals as Chlamydia, Mycoplasma (tetracyclin or macrolides).

- MRSA (vancomycin).

- Enterococci (ampicillin +/- gentamycin).

- Exception: ceftaroline covers MRSA.

▪ Pharmacokinetics:

- Renal clearance similar to penicillins, with active tubular secretion blocked by probenecid.

- Dose modification in renal dysfunction.

- Do not adjust ceftriaxone in renal failure because it's eliminated by the liver.

- Avoid ceftriaxone in neonates because it decreases biliary metabolism → biliary sludge, we give them cefotaxime.

- Ceftriaxone is largely eliminated in the bile.

▪ Side effects:

- Hypersensitivity:

  - Incidence: 2%.

  - Wide range, but rashes and drug fever most common.

  - Positive Coombs test, but rarely autoimmune hemolytic anemia.

  - Assume complete cross-allergenicity between individual cephalosporins and partial cross-allergenicity with penicillins (about 5%).

- If there is rash to penicillin → use cephalosporin, if there is anaphylaxis to penicillin → use non-B lactam (for gram-positive organisms, consider macrolides; for gram-negative rods, consider aminoglycosides or aztreonam in case of renal impairment).

- Others:
- Disulfiram-like effect: cefotetan.

- ↑ nephrotoxicity of aminoglycosides.

▪ Mechanism of resistance:
- Inactivated by cephalosporinases (a type of β-lactamase).

- A change in the structure of penicillin-binding proteins that prevents cephalosporin binding is one mechanism of bacterial resistance to cephalosporins.

Imipenem and Meropenem (penems)

▪ Drugs: Doripenem, Imipenem, Meropenem, Ertapenem. (DIME antibiotics are given when there is a 10/10 [life-threatening] infection).

▪ Mechanism of action: Same as penicillins and cephalosporins.

▪ Spectrum:
- Broad spectrum antibiotics that cover Gram-positive cocci, gram-negative rods (Enterobacter, Pseudomonas spp.), and anaerobes.

- Resistant to beta-lactamases.

- Important in-hospital agents for empiric use in severe life-threatening infections.

▪ Pharmacokinetics:
- Imipenem is given with cilastatin, a renal dehydropeptidase inhibitor, which inhibits imipenems metabolism to a nephrotoxic metabolite. Meropenem is stable to dehydropeptidase I.

- Undergo renal elimination, So adjust the dose in renal dysfunction.

▪ Side effects:
- GI distress.

- Drug fever (partial cross-allergenicity with penicillins).

- CNS effects, including seizures with imipenem in overdose or renal dysfunction.

- Mechanism of resistance: Inactivated by carbapenemases.

Monobactam

- Drugs: Aztreonam.

- Mechanism of action:

- Same as for penicillins and cephalosporins.

- Resistant to beta-lactamases.

- Clinical Use:

- Gram-negative rods only, no activity against gram positives or anaerobes.

- For penicillin-allergic patients and those with renal insufficiency who cannot tolerate aminoglycosides.

- No cross-allergenicity with penicillins or cephalosporins.

- Toxicity: Usually nontoxic; occasional GI upset.

Vancomycin

- Mechanism of action: Inhibits cell wall peptidoglycan formation by binding D-ala D-ala portion of cell wall precursors.

- Spectrum:

- Gram-positive bugs only, serious, multidrug-resistant organisms, including:
  - MRSA.
  - Enterococci.
  - Clostridium difficile (oral dose for pseudomembranous colitis).

- Pharmacokinetics:

- Used orally (not absorbed) in colitis.

- Enters most tissues (bone), but not CNS.

- Eliminated by renal filtration (important to decrease dose in renal dysfunction).

- Side effects:

- Well tolerated in general, but NOT trouble free.

- Nephrotoxicity, Ototoxicity, Thrombophlebitis, diffuse flushing "red man syndrome" due to histamine release with rapid IV injection (can largely prevent by pretreatment with antihistamines and slow infusion rate).

▪ Resistance:

- Vancomycin-resistant staphylococcal (VRSA) and enterococcal (VRE) strains emerging.

- The mechanism of vancomycin resistance in organisms such as VRE is a substitution of D-lactate in the place of D-alanine during the process of peptidoglycan cell wall synthesis. This prevents the binding of vancomycin to its usual D-alanine-D-alanine binding site in the cell wall.

❖ N.B:

1. Impinems are used to treat unknown life-threatening infections, but vancomycin is used to treat gram-positive life-threatening infections.

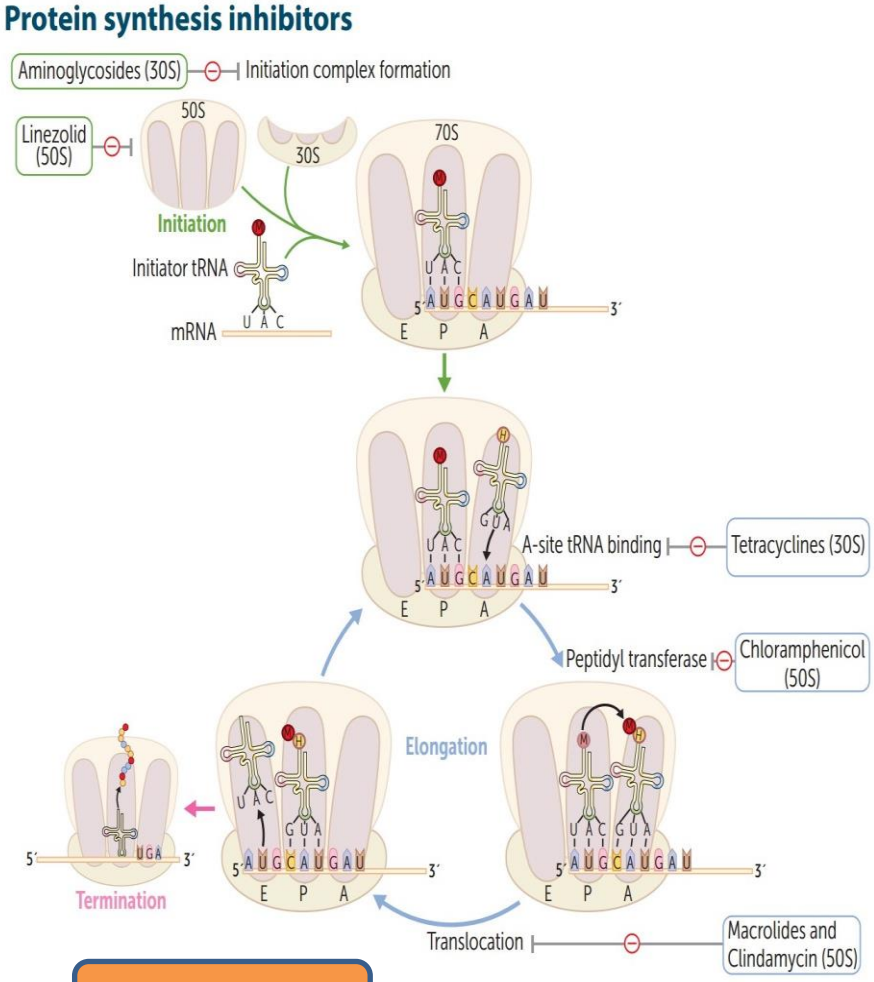
2. Inhibitors of cell wall synthesis:

	B-lactams	**Vancomycin**
Target	PBPs	D-ala terminus
Side effects	Hypersensitivity	Red-man syndrome

3. Daptomycin is a lipopeptide antibiotic with activity toward Gram-positive organisms.

- It is used for treating skin and skin structure infections and bacteremia (with or without endocarditis) due to Staphylococcus aureus, including methicillin-resistant S. aureus (MRSA).
- Daptomycin disrupts the bacterial membrane through the creation of transmembrane channels.
- These channels cause leakage of intracellular ions leading to depolarization of the cellular membrane and inhibition of macromolecular (DNA, RNA, and protein) synthesis, which ultimately leads to cell death.
- Daptomycin cannot permeate the outer membrane of Gram-negative bacteria, which is why it is ineffective in the treatment of Gram-negative infections.
- Daptomycin also binds to and is inactivated by pulmonary surfactant, thus it is not effective in treating pneumonias.
- Daptomycin is associated with increased CPK levels and an increased incidence of myopathy, particularly in patients using other drugs associated with myopathy (statins).
- Monitoring of CPK levels with assessment for muscle pain and weakness should be performed regularly in patients taking daptomycin.

Inhibitors of bacterial protein synthesis



Specifically target smaller bacterial ribosome (70S, made of 30S and 50S subunits), leaving human ribosome (80S) unaffected.

All are bacteriostatic, except aminoglycosides (bactericidal) and linezolid (variable).

30S inhibitors

Aminoglycosides
Tetracyclines

50S inhibitors

Chloramphenicol, Clindamycin
Erythromycin (macrolides)
Linezolid

"Buy AT 30, CCEL (sell) at 50."

Aminoglycoside

- Drugs: Gentamicin, Neomycin, Amikacin, Tobramycin, Streptomycin.

- Activity and clinical uses:

- Bactericidal, accumulated intracellularly in microorganisms via an O_2-dependent uptake → ineffective against anaerobes.

- Irreversible inhibition of initiation Complex through binding of the 30S subunit. Can cause genetic code misreading of mRNA. Also block translocation.

- Useful spectrum includes gram-negative rods; gentamicin, tobramycin, and amikacin often used in combinations.

- Streptomycin used in tuberculosis; is the DOC for bubonic plague and tularemia.

- Neomycin for bowel surgery.

- Pharmacokinetics: Renal elimination proportional to GFR, and major dose reduction needed in renal dysfunction.

- Side effects:

- Nephrotoxicity (6 to 7% incidence) includes proteinuria, hypokalemia, acidosis, and acute tubular necrosis: usually reversible, but enhanced by vancomycin, amphotericin B, cisplatin, and cyclosporine.

- Neuromuscular blockade with ↓ release of Ach → may enhance effects of skeletal muscle relaxants (absolute contraindication with myasthenia gravis).

- Ototoxicity (2% incidence) from hair cell damage; includes deafness (irreversible) and vestibular dysfunction (reversible); toxicity may be enhanced by loop diuretics.

- Teratogenic.

- Mnemonic:

- AmiNOglycosides: NO anaerobes, cause Nephrotoxicity, Neuromuscular blockade and Ototoxicity.

- Mechanism of resistance:

- Mutations of the genes that encode ribosomal proteins are responsible for aminoglycoside resistance because they modify the ribosomal binding sites for these drugs.

- The enterococci have achieved aminoglycoside resistance by producing aminoglycoside-modifying enzymes (bacterial transferase enzymes) that transfer different chemical groups (acetyl groups, methyl group, adenyl groups, and phosphate groups) to the aminoglycoside antibiotic molecule outside of the bacterium, thereby decreasing the ability of these drugs to bind to ribosomes and exert their antimicrobial effects.

Tetracyclines

- Drugs: Tetracycline, doxycycline, minocycline.

- Mechanism of action: Bacteriostatic; bind to 30S and prevent attachment of aminoacyl-tRNA.

- Activity and clinical uses:

- Drugs' ability to accumulate intracellularly makes them very effective against Rickettsia and Chlamydia.

- "Broad-spectrum" antibiotics, with good activity versus chlamydial and mycoplasma species, H. pylori (GI ulcers), Rickettsia, Borrelia burgdorferi, Brucella, Vibrio, and Treponema (backup drug).

- Also used to treat acne.

- Specific drugs:
- Doxycycline: more activity overall than tetracycline and has particular usefulness in prostatitis because it reaches high levels in prostatic fluid.

- Demeclocycline: used in syndrome of inappropriate secretion of ADH (SIADH; blocks ADH receptor function in collecting ducts).

- Pharmacokinetics:
- Kidney for most (adjust the dose in renal dysfunction).

- Liver for doxycycline.

- Doxycycline is fecally eliminated and can be used in patients with renal failure.

- Limited CNS penetration.

- Do not take tetracyclines with milk (Ca2+), antacids (Ca2+ or Mg2+), or iron-containing preparations because divalent cations inhibit drugs' absorption in the gut.

- Side effects:
- Tooth enamel dysplasia and possible ↓ bone growth in children (avoid).

- Tetracycline use during pregnancy can cause fetal bone growth retardation and discoloration of the deciduous teeth.

- Phototoxicity (demeclocycline, doxycycline).

- Mechanism of resistance: ↓uptake or ↑efflux out of bacterial cells by plasmid-encoded transport pumps.

Glycylcycline

- Drugs: Tigecycline.

- Mechanism of action: Tetracycline derivative. Binds to 30S, inhibiting protein synthesis. Generally bacteriostatic.

- Clinical Use:
- Broad-spectrum anaerobic, gram ⊖, and gram ⊕ coverage.

- Multidrug-resistant organisms (MRSA, VRE) or infections requiring deep tissue penetration.

- Adverse effects: GI symptoms: nausea, vomiting.

Chloramphenicol

- Mechanism of action:

- Bacteriostatic.

- Chloramphenicol elicits its antibacterial effect by binding to the ribosomal 50S subunit and inhibiting the peptidyl transferase enzyme. Thus, it suppresses bacterial protein synthesis.

- Activity and clinical uses:

- Bacteriostatic with a wide spectrum of activity.

- Meningitis (Haemophilus influenzae, Neisseria meningitidis, Streptococcus pneumoniae) and Rocky Mountain spotted fever (Rickettsia rickettsia).

- Limited use owing to toxicities but often still used in developing countries because of low cost.

- Pharmacokinetics:

- Metabolized by hepatic glucuronidation, and dose reductions are needed in liver dysfunction and in neonates.

- Inhibition of cytochrome P450.

- Side effects:

- Chloramphenicol can cause both dose-dependent and dose-independent aplastic anemia (pancytopenia).

- Dose-dependent aplastic anemia associated with chloramphenicol is reversible after the medication is withdrawn.

- Dose-independent anemia is usually severe and may be fatal.

- "Gray baby syndrome" in premature infants because they lack liver UDP-glucuronyl transferase.

- Mechanism of resistance: Plasmid-encoded acetyltransferase inactivates the drug.

Clindamycin

- Mechanism of action:

- Bacteriostatic. Blocks peptide transfer (translocation) at 50S ribosomal subunit.

- Not a macrolide but has the same mechanisms of action and resistance.

- Narrow spectrum:

- Anaerobic infections (Bacteroides spp., Clostridium perfringens) in aspiration pneumonia, lung abscesses, and oral infections. Also effective against invasive group A streptococcal infection.

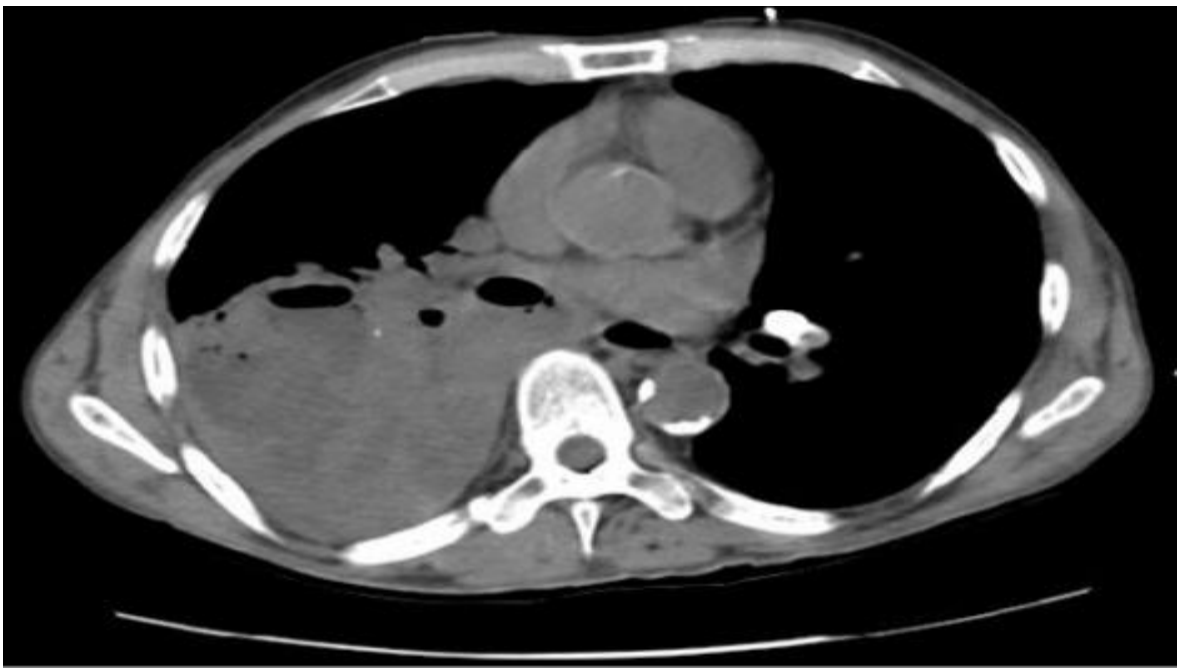
- Clindamycin treats anaerobic infections above the diaphragm vs. metronidazole treats anaerobic infections below the diaphragm.

- Concentration in bone has clinical value in osteomyelitis due to gram-positive cocci.

- Side effect: Pseudomembranous colitis (C. difficile overgrowth), fever, diarrhea.

❖ N.B:

- This CT scan shows a lung abscess that can be identified by the presence of air-fluid levels.
- Clindamycin has the most activity against oral anaerobes and also covers aerobic Gram-positive organisms such as S pneumoniae.
- Alcoholics are more likely than the general population to develop pulmonary infections and abscesses involving combinations of anaerobic oral flora (Bacteroides, Prevotella, Fusobacterium, and Peptostreptococcus) and aerobic bacteria.
- Alcoholics with poor oral hygiene have increased numbers of oral bacteria, further increasing this risk.
- In addition, alcohol may impair the phagocytic and/or bactericidal action of alveolar macrophages, predisposing to infection.
- Clindamycin covers most of these organisms and is thus the antibiotic of choice for treating lung abscesses.



Macrolide

- Drugs: erythromycin, azithromycin, clarithromycin.

- Mechanism of action: Bacteriostatic. Inhibit protein synthesis by blocking translocation; bind to the 23S rRNA of the 50S ribosomal subunit.

- Clinical use: Atypical pneumonias (Mycoplasma, Chlamydia, Legionella), STIs (Chlamydia), gram-positive cocci (streptococcal infections in patients allergic to penicillin), and B. pertussis.

- Pharmacokinetics: Clarithromycin and erythromycin inhibit cytochrome P-450 → increases serum concentration of theophylline, oral anticoagulants.

- Side effects:

- Macrolides stimulate motilin receptors and cause gastrointestinal distress (erythromycin, azithromycin > clarithromycin)

- Used in treatment of diabetic gastroparesis.

- Toxicity:

- MACRO: Gastrointestinal Motility issues, Arrhythmia caused by prolonged QT interval, acute Cholestatic hepatitis, Rash, eOsinophilia.

- Mechanism oF resistance: Methylation of 23S rRNA-binding site prevents binding of drug.

❖ N.B:

- In young males, urethritis is usually a sexually transmitted infection.
- Sexually transmitted urethritis is classified as either gonococcal (due to Neisseria gonorrhoeae infection) or non-gonococcal (most commonly caused by Chlamydia trachomatis).
- A single intramuscular dose of ceftriaxone can treat gonococcal urethritis, but since coinfection with C. trachomatis is common, it is recommended that affected patients be treated for both infections empirically.
- C. trachomatis is a Gram negative obligate intracellular microorganism that lacks a peptidoglycan cell wall.
- Because there is no peptidoglycan cell wall, beta-lactam-based antibiotics like cephalosporins are not effective.
- Doxycycline and macrolide antibiotics (erythromycin, azithromycin) are effective against C. trachomatis infection.

Oxazolidinones

- Drugs: Linezolid.
- Mechanism of action: Inhibit protein synthesis by binding to 50S subunit and preventing formation of the initiation complex.
- Spectrum: Treatment of VRSA, VRE, and drug-resistant pneumococci.
- Side effects: Bone marrow suppression (especially thrombocytopenia), peripheral neuropathy, and serotonin syndrome (due to partial MAO inhibition).
- Mechanism oF resistance: Point mutation of ribosomal RNA.

❖ N.B:

1. Quinupristin and dalfopristin are streptogramins that work via several mechanisms.
   - Binding to sites on 50S ribosomal subunit, they prevent the interaction of amino-acyl-tRNA with acceptor site and stimulate its dissociation from the complex.
   - Used parenterally in severe infections caused by vancomycin-resistant staphylococci (VRSA) and enterococci (VRE), as well as other drug- resistant, gram-positive cocci.

2. Treatment of highly resistant bacteria:
   - MRSA: vancomycin, daptomycin, linezolid, tigecycline, ceftaroline.
   - VRE: linezolid and streptogramins (quinupristin, dalfopristin).
   - Multidrug-resistant P aeruginosa, multidrug-resistant Acinetobacter baumannii: polymyxins B and E (colistin).

Some antibiotics commonly used for invasive methicillin-resistant *Staphylococcus aureus* infections

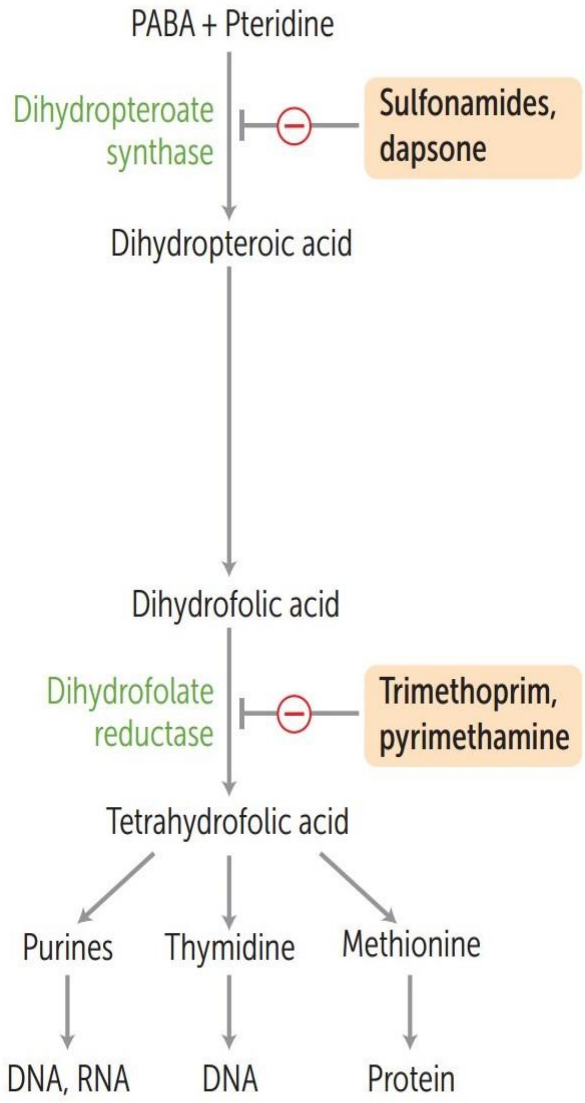
	Mechanism of action	Adverse effects
Vancomycin	Blocks glycopeptide polymerization by binding tightly to D-alanyl-D-alanine	• Red man syndrome • Nephrotoxicity
Daptomycin	Depolarizes cellular membrane by creating transmembrane channels	• Myopathy & CPK elevation • Note: Inactivated by pulmonary surfactant
Linezolid	Inhibits bacterial protein synthesis by binding to 50S subunit	• Thrombocytopenia • Optic neuritis • High risk for serotonin syndrome

CPK = creatine phosphokinase.

Inhibitors of nucleic acid synthesis

Inhibitors of Folic Acid Synthesis

- Drugs: sulfonamides, trimethoprim, and pyrimethamine.



Sulfonamides

- Mechanism of action:

- Para-aminobenzoic acid (PABA) antimetabolites inhibit dihydropteroate synthase → Inhibit folate synthesis.

- Bacteriostatic (bactericidal when combined with trimethoprim).

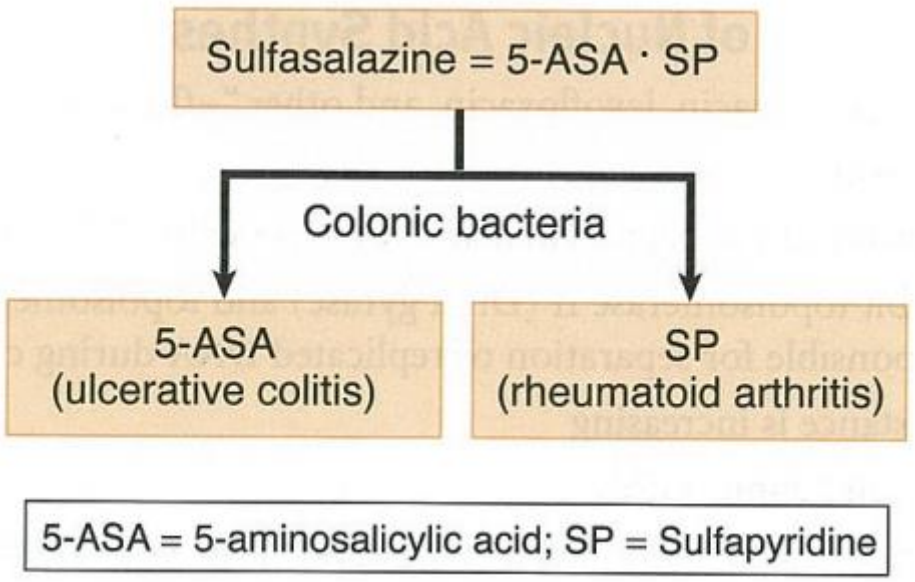
- Activity and clinical uses:

- Sulfonamides alone are limited in use because of multiple resistance.

- DOC in Nocardia (SNAP).

- Sulfasalazine is a prodrug used in ulcerative colitis and rheumatoid arthritis.

- Sulfadiazine is used in burns.



- Combination with dihydrofolate reductase inhibitors:

- Synergy (causing sequential block of folate synthesis).

- ↓ resistance.

- Uses of trimethoprim-sulfamethoxazole [TMP-SMX, cotrimoxazole]:

- Urinary tract infections and most common opportunistic infections in AIDS.

- Fungus: Pneumocystis jiroveci (back-up drugs are pentamidine and atovaquone).

- Protozoa: Toxoplasma gondii (sulfadiazine + pyrimethamine).

- Pharmacokinetics:

- Sulfonamides are hepatically acetylated (conjugation).

- High protein binding → Drug interaction (displace other drugs from albumin as warfarin).

- Side effects:
  - Hypersensitivity (rashes, Stevens-Johnson syndrome).
  - Hemolysis in G6PD deficiency.
  - Phototoxicity.
  - Nephrotoxicity (tubulointerstitial nephritis).
  - Kernicterus in neonates (avoid in third trimester).
- Mechanism of resistance: Altered enzyme (bacterial dihydropteroate synthase), ↓ uptake, or ↑ PABA synthesis.

Dapsone

- Mechanism of action: Similar to sulfonamides, but structurally distinct agent.
- Clinical Use: Leprosy (lepromatous and tuberculoid), Pneumocystis jirovecii prophylaxis, or treatment when used in combination with TMP.
- Adverse effects: Hemolysis if G6PD deficient, methemoglobinemia, agranulocytosis.

Trimethoprim

- Mechanism of action: Bacteriostatic. Inhibits bacterial dihydrofolate reductase.
- Clinical use: Used in combination with sulfonamides [TMPSMX], causing sequential block of folate synthesis.
- Toxicity: Megaloblastic anemia, leukopenia, granulocytopenia (May alleviate with supplemental folinic acid).

❖ N.B:

- Trimethoprim, methotrexate, and pyrimethamine all prevent the reduction of folic acid to tetrahydrofolate by inhibiting dihydrofolate reductase.
- Methotrexate is a folate antimetabolite that targets rapidly proliferating cells by halting DNA synthesis through the irreversible binding of dihydrofolate reductase.

Direct Inhibitors of Nucleic Acid Synthesis: Quinolones

- Drugs: Ciprofloxacin, enoxacin, norfloxacin, ofloxacin; respiratory fluoroquinolones (gemifloxacin, levofloxacin, moxifloxacin).

- Mechanisms of action:

- Quinolones are bactericidal and interfere with DNA synthesis.

- Inhibit topoisomerase II (DNA gyrase) and topoisomerase IV.

- Activity and clinical uses:

- Urinary tract infections (UTIs), particularly when resistant to cotrimoxazole.

- Sexually transmitted diseases (STDs)/pelvic inflammatory diseases (PIDs): chlamydia, gonorrhea.

- Skin, soft tissue, and bone infections by gram-negative organisms.

- Diarrhea to Shigella, Salmonella, E. coli, Campylobacter.

- Drug-resistant pneumococci (levofloxacin).

- Pharmacokinetics:

- Must not be taken with antacids (Iron and calcium limit their absorption).

- Eliminated mainly by kidney by filtration and active secretion (inhibited by probenecid).

- Reduce dose in renal dysfunction.

- Side effects:

- May cause tendonitis or tendon rupture in people > 60 years old and in patients taking prednisone. Fluoroquinolones hurt attachments to your bones.

- Contraindicated in pregnant women, nursing mothers, and children < 18 years old due to possible damage to cartilage (inhibition of chondrogenesis).

- Phototoxicity, rashes.

- CNS effects (insomnia, dizziness, headache).

- Mechanism of resistance: Resistance is increasing. Chromosome-encoded mutation in DNA gyrase, plasmid-mediated resistance, efflux pumps.

❖ Antibiotics that cause photosensitivity:

1. Tetracyclines.
2. Sulfonamides.
3. Quinolones.

Unclassified antibiotic: Metronidazole

▪ Mechanism of action:

- Forms toxic free radical metabolites in the bacterial cell that damage DNA.

- Bactericidal, antiprotozoal.

▪ Clinical uses:

- Antiprotozoal: Giardia, Trichomonas, and Entamoeba.

- Antibacterial: strong activity against most anaerobic gram-negative Bacteroides species, Clostridium species, Gardnerella, and H. pylori (Used with a proton pump inhibitor and clarithromycin for "triple therapy").

▪ Side effects:

- Metallic taste.

- Disulfiram-like effect (severe flushing, tachycardia, hypotension):
  - Oral metronidazole can cause disulfiram-like effects when combined with alcohol.
  - Metronidazole's interaction with alcohol is thought to result from its inhibition of alcohol oxidizing enzymes, which causes acetaldehyde to accumulate and thus the unpleasant effects.
  - Disulfiram is used in recovering alcoholics to prevent them from relapsing to alcohol use.

Polymyxins

▪ Drugs: Colistin (polymyxin E), polymyxin B.

▪ Mechanism of action:

- Cation polypeptides that bind to phospholipids on cell membrane of gram ⊖ bacteria.

- Disrupt cell membrane integrity → leakage of cellular components → cell death.

▪ Clinical use:

- Salvage therapy for multidrug-resistant gram ⊖ bacteria (P aeruginosa, E coli, K pneumoniae).

- Polymyxin B is a component of a triple antibiotic ointment used for superficial skin infections.

- Adverse effects: Nephrotoxicity, neurotoxicity (slurred speech, weakness, paresthesia), respiratory failure.

❖ Antibiotics for STDs:

1. T. pallidum (syphilis): Benzathine penicillin G.
2. Gonorrhea: ceftriaxone.
3. Chlamydia: azithromycin or doxycycline.
4. Trichomonas: metronidazole.
5. Gardenella: metronidazole.

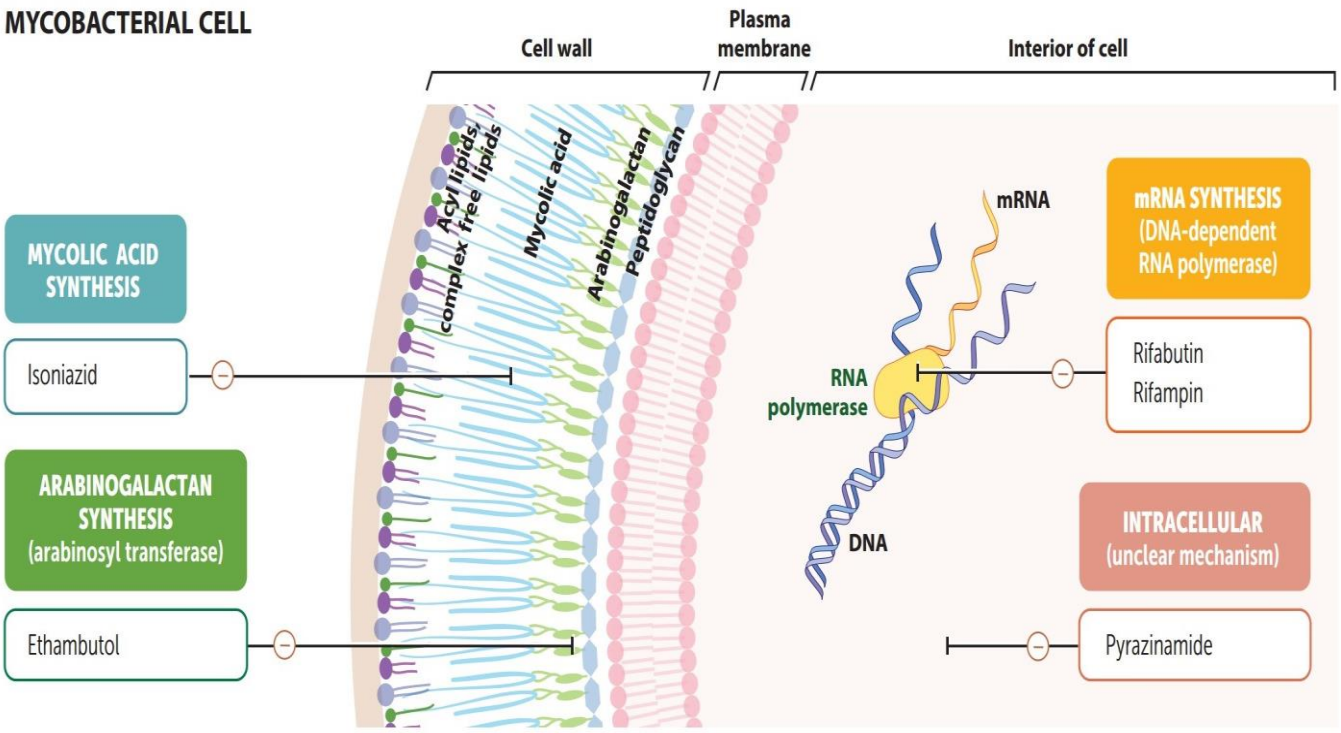
❖ Antibiotics for ticks (lyme or RMSF): Doxycycline.

❖ Antibiotics for UTIs, cystitis, prostatitis: TMP/SMX or cipro, nitrofurantoin in pregnancy.

Common antibiotic resistance mechanisms	
Penicillins	• Beta-lactamase, ESBL • Mutated PBP • Mutated porin protein
Vancomycin	• Mutated peptidoglycan cell wall • Impaired influx/increased efflux
Quinolones	• Mutated DNA gyrase • Impaired influx/increased efflux
Aminoglycosides	• Aminoglycoside-modifying enzymes • Mutated ribosomal subunit protein • Mutated porin protein
Tetracyclines	• Impaired influx/increased efflux • Inactivated enzyme
Rifamycins	• Mutated RNA polymerase

ESBL = extended-spectrum beta-lactamase; **PBP** = penicillin-binding protein.

Antitubercular drugs



- The rise in HIV infections in the United States has been paralleled by a rise in M. tuberculosis infections, typically from reactivation of latent infection after the dramatic onset of HIV-induced immunosuppression.

- Combination drug therapy is the rule to delay or prevent the emergence of resistance and to provide additive (possibly synergistic) effects against Mycobacterium tuberculosis.

- The primary drugs in combination regimens are isoniazid (INH), rifampin, ethambutol, and pyrazinamide.

❖ Antimycobacterial drugs:

Bacterium	Prophylaxis	Treatment
M. tuberculosis	Isoniazid	Rifampin, Isoniazid, Pyrazinamide, Ethambutol (RIPE for treatment)
M. avium–intracellulare	Azithromycin, rifabutin	- More drug resistant than M. tuberculosis. - Azithromycin or clarithromycin + ethambutol. - Can add rifabutin or ciprofloxacin.
M. leprae	N/A	- Long-term treatment with dapsone and rifampin for tuberculoid form. - Add clofazimine for lepromatous form.

Rifamycins

- Drugs: Rifampin, rifabutin.

- Mechanism of action:

- Rifampin inhibits DNA-dependent RNA polymerase, thereby preventing transcription.

- The subsequent lack of mRNA leads to a deficiency of those proteins necessary for mycobacterial survival.

- Clinical use:

- Currently, rifampin is used as a component of multiagent therapy in the treatment of mycobacterial infections or leprosy (delay resistance to dapsone when used for leprosy) and as prophylactic monotherapy in those exposed to H. influenzae or N. meningitides.

- Rifampin is the preferred prophylaxis for persons who have been definitively exposed to N. meningitidis.

- Rifampin has been used successfully for chemoprophylaxis for household members and close contacts of patients with invasive meningococcal disease and is the most likely chemoprophylactic agent to be administered in this subject.

- Rifampin is used for chemoprophylaxis because it penetrates well into the respiratory tract and will eliminate nasopharyngeal colonization.

- Pharmacokinetics:

- Rifampin is an inducer of the CYP450 system in the liver (rifAMPin AMPiifies CYP450), so other drugs processed through this mechanism will be metabolized more rapidly when taken in conjunction with rifampin.

- Toxicity:

- Rifampin's most notable side effect is an orange discoloration of secretions (urine, breast milk and tears), and patients should be alerted to the fact that contact lenses will be permanently stained orange.

- Minor hepatotoxicity and drug interactions (cytochrome P-450).

- Rifabutin favored over rifampin in patients with HIV infection due to less cytochrome P-450 stimulation. rifAMPin AMPiifies cytochrome P-450, but rifabutin does not.

- Mechanism of resistance: Rifampin is well known to induce resistance in many bacterial pathogens when used as monotherapy. Resistance occurs through spontaneous genetic mutations in the bacterial DNA-dependent RNA polymerase.

❖ Mnemonic:

- Rifampin's 4 R's:

- RNA polymerase inhibitor.
- Ramps up microsomal cytochrome P-450.
- Red/orange body fluids.
- Rapid resistance if used alone.

Isoniazid

- Mechanism of action:

- ↓ synthesis of mycolic acids.

- Prodrug requiring conversion by bacterial catalase peroxidase.

- Bacterial catalase peroxidase (encoded by KatG gene) needed to convert INH to active metabolite.

- Clinical use:

- Mycobacterium tuberculosis.

- The only agent used as solo prophylaxis against TB.

- Pharmacokinetics:

- It is metabolized in the liver via acetylation.

- The rate of acetylation is genetically determined (Different INH half-lives in fast vs. slow acetylators).

- Toxicity:

- INH-induced peripheral neuropathy is caused by pyridoxine (vitamin B6) deficiency. INH binds the active form of pyridoxine, resulting in renal excretion. Most patients have large enough stores of pyridoxine to tolerate increased excretion; however, those with malnourishment, pregnancy, or certain comorbid illnesses (diabetes mellitus) may develop a deficiency.

- INH use should generally be combined with vitamin B6 (pyridoxine) to prevent peripheral neuropathy that can be a side effect of INH.

- INH Injures Neurons and Hepatocytes → Neurotoxicity (peripheral neuritis), hepatotoxicity.

- Isoniazid induces pyridoxine deficiency → insufficient heme formation in early red blood cells → Sideroblastic anemia (use vitamin B_6).

- SLE in slow acetylators.

▪ Mechanism of resistance: Mutations leading to underexpression of KatG.

Pyrazinamid

▪ Mechanism of action:

- Uncertain.

- Pyrazinamide is a prodrug that is converted to the active compound Pyrazinoic acid.

- Works best at acidic pH (in host phagolysosomes).

▪ Clinical uses: Mycobacterium tuberculosis.

▪ Toxicity: Hyperuricemia, hepatotoxicity.

❖ N.B:

▪ Pyrazinamide (PZA) works best at relatively acidic pHs, as within phagolysosomes.
▪ Of the first-line agents for Mycobacterium tuberculosis, only pyrazinamide requires an acidic environment (as is present within macrophage phagolysosomes) to exert antimicrobial effects.
▪ It is therefore most bactericidal to M. tuberculosis (MTB) organisms engulfed by macrophages.
▪ The other first-line agents for MTB, namely isoniazid, rifampin, and ethambutol, have better activity against extracellular MTB (including organisms in necrotic foci and/or tissue cavities) than PZA.

Ethambutol

▪ Mechanism of action:

- Ethambutol is an antimycobacterial agent that inhibits carbohydrate polymerization by inhibiting arabinosyl transferase, thereby preventing peptidoglycan cell wall synthesis.

- Ethambutol is not effective against other organisms.

▪ Clinical uses: Ethambutol is an effective component of the multi-agent antibiotic regimen used in the treatment of M. tuberculosis and atypical mycobacterial infections.

▪ Toxicity:

- The most notable side effect of ethambutol is optic neuritis (red-green color blindness).

- This side effect clinically manifests as decreased visual acuity, central scotoma, or color-blindness and may be reversible with discontinuation of the drug.

- As with all other antimycobacterial agents, ethambutol can also cause hepatotoxicity.

❖ Mnemonic: Pronounce ethambutol "eyethambutol" for optic neuritis.

Drugs for treatment of tuberculosis		
Drug	**Mechanism of action**	**Side effects**
(R)ifampin	Inhibition of bacterial DNA-dependent **R**NA polymerase	GI side effects, **r**ash, **r**ed-orange body fluids, cytopenias
(I)soniazid	Inhibition of **mycolic acid** synthesis	Neurotoxicity (give vitamin B_6/pyridoxine), hepatotoxicity
(P)yrazinamide	Unclear	Hepatotoxicity, hyperuricemia
(E)thambutol	Inhibition of arabinosyl transferase (?)	Optic neuropathy

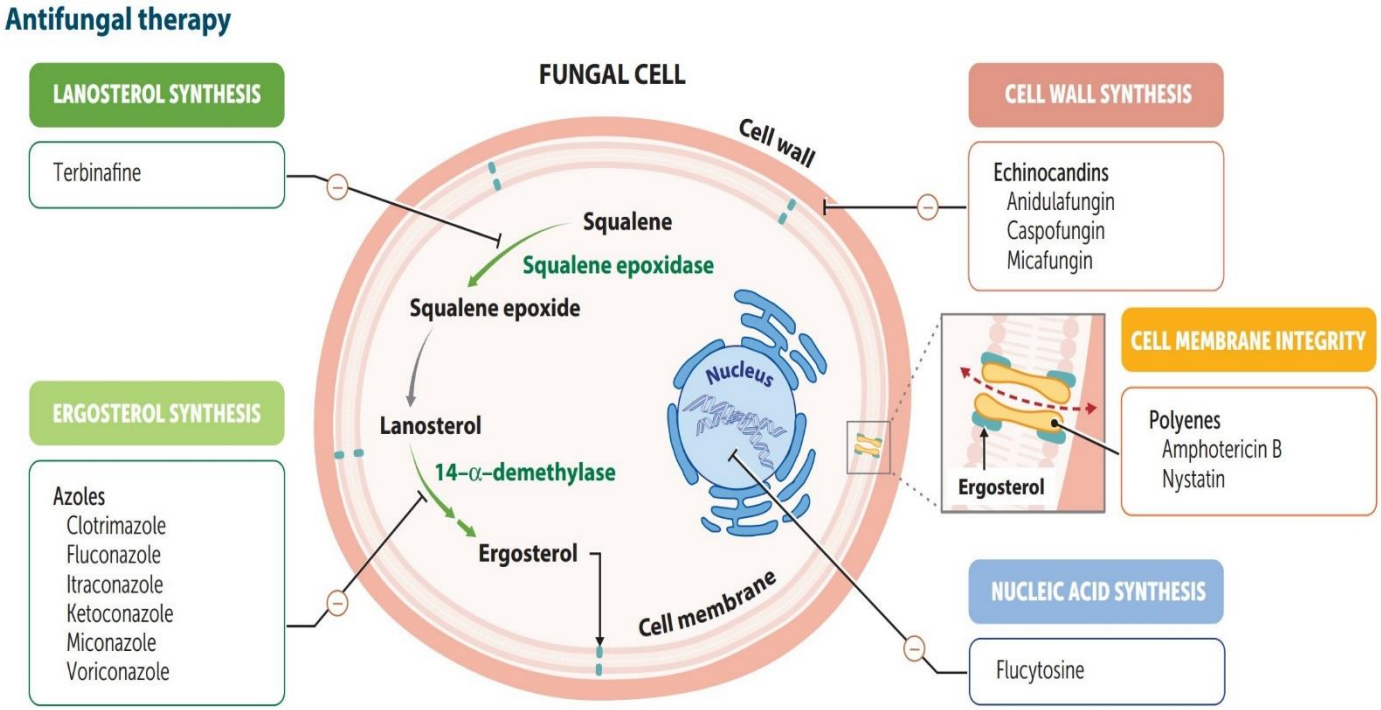
GI = gastrointestinal.

❖ N.B:

1. Mycobacterium avium complex (MAC) is a common opportunistic pathogen that causes disseminated disease in HIV+ patients.
   - MAC is resistant to many of the typical antimycobacterial drugs.
   - As such, disseminated infection is treated with clarithromycin or azithromycin in combination with rifabutin or ethambutol.

2. Common precipitating drugs of glucose-6-phosphate dehydrogenase (G6PD) deficiency anemia include: dapsone, antimalarials, sulfonamide antibiotics (TMP-SMX).

Antifungal therapy



- <u>The main classes of antifungal medications currently in use include:</u>

- Polyenes (Amphotericin B and nystatin): Bind ergosterol molecules in fungal cell membranes, creating pores and causing cell lysis.

- Azoles (ketoconazole, fluconazole, itraconazole and voriconazole): Inhibit ergosterol synthesis.

- Echinocandins (caspofungin and micafungin): Inhibit glucan synthesis (a component of the fungal cell wall).

- Pyrimidines: Flucytosine is the only agent in this class of antifungals. It is converted to 5. fluorouracil within the fungal cell and interferes with fungal RNA and protein synthesis.

Amphotericin B

- <u>Mechanism of action:</u>

- The polyenes (amphotericin B and nystatin) depend on the amount of ergosterol (unique to fungi) incorporated into fungal cell membranes for their efficacy.

- These drugs bind to ergosterol molecules, forming pores in the membrane and allowing leakage of ions (especially K) from the cells.

- This disruption of cell membrane integrity leads to cell lysis.

- Amphotericin "tears" holes in the fungal membrane by forming pores.

▪ Activity and clinical uses:

- Serious, systemic mycoses.

- Cryptococcus (amphotericin B with/without flucytosine for cryptococcal meningitis), Blastomyces, Coccidioides, Histoplasma, Candida, Mucor.

▪ Pharmacokinetics:

- Amphotericin B given by slow IV infusion.

- Poor penetration into the CNS (Intrathecally for fungal meningitis).

▪ Side effects:

- Amphotericin B preferentially binds the ergosterol of fungal cell membranes, leading to fungal cell lysis.

- This drug is relatively selective, because it has a higher affinity for ergosterol (in fungal membranes) than for cholesterol (in human cell membranes).

- This drug does, however, bind cholesterol to a degree, which explains a large number of its adverse effects.

- Its main toxicities include:

1. Acute infusion-related reactions, such as fever, chills, rigors, and hypotension.
   - Acute infusion-related reactions are common, and most frequent during initial infusions (often diminish with subsequent infusions).
   - Premedication with antipyretics and antihistamines can lessen the severity of these effects.

2. Amphotericin B causes a dose-dependent nephrotoxicity because it decreases the glomerular filtration rate.
   - Permanent loss of renal function is thought to be related to the cumulative total dose.
   - Increasing BUN and creatinine levels indicate declining renal function.
   - Renal function should be closely monitored in patients undergoing treatment with Amphotericin.
   - Concomitant administration of other nephrotoxic drugs (aminoglycosides, cyclosporine) should be avoided.

3. Amphotericin B can also cause significant electrolyte abnormalities (hypomagnesemia and hypokalemia).
   - Severe hypokalemia and hypomagnesemia are commonly seen during therapy, and often require daily supplementation.
   - These effects occur in the majority of patients within the first week of therapy.
   - Electrolytes should be monitored daily and replaced as needed.

4. Anemia occurs due to suppression of renal erythropoietin synthesis: This effect may be severe in HIV patients taking zidovudine (a drug that also suppresses bone marrow function).

5. Thrombophlebitis at the site of injection may occur ("amphoterrible").

- The most important adverse effects of amphotericin B are nephrotoxicity, hypokalemia, and hypomagnesemia. Protect by Supplemental K and Mg, use of liposomal amphotericin B, hydration, or by drug combinations (flucytosine), permitting ↓ in amphotericin B dose.

▪ Mechanism of resistance:

- The polyenes (amphotericin B and nystatin) depend on the amount of ergosterol incorporated into fungal cell membranes for their efficacy.

- Decreasing the cell membrane ergosterol content is a major mechanism of polyene resistance.

Nystatin

▪ Mechanism of action: Same as amphotericin B.

▪ Clinical Use: Nystatin (too toxic for systemic use) used topically for localized infections (candidiasis thrush); topical for diaper rash or vaginal candidiasis.

Azoles

▪ Drugs: Clotrimazole, fluconazole, itraconazole, ketoconazole, miconazole, voriconazole.

▪ Mechanism of action: Azoles" are fungicidal and interfere with the synthesis of ergosterol by inhibiting 14-α-demethylase, a fungal P450 enzyme, which converts lanosterol to ergosterol.

▪ Activity and clinical uses:

- Local and less serious systemic mycoses.

- Fluconazole for chronic suppression of cryptococcal meningitis in AIDS patients (Only fluconazole penetrates into the CSF) and candidal infections of all types.

- Itraconazole for Blastomyces, Coccidioides, Histoplasma.

- Clotrimazole and miconazole for topical fungal infections.

- Voriconazole for Aspergillus and some Candida. Isavuconazole for serious Aspergillus and Mucor infections.

- Pharmacokinetics:

- Only fluconazole penetrates into the CSF and can be used in meningeal infection.

- The azoles inhibit the activity of the human P450 cytochrome oxidase system. This property leads to multiple drug interactions. All azoles (ketoconazole, fluconazole, itraconazole and voriconazole) increase the serum concentrations of drugs metabolized by liver P450 enzymes.

- Side effects:

- ↓ Synthesis of steroids (especially with ketoconazole), including cortisol and testosterone →↓ libido, gynecomastia, menstrual irregularities.

- Liver dysfunction.

- Mechanism of resistance: Resistance occurs via decreased intracellular accumulation of azoles.

Flucytosine

- Mechanism of action: Inhibits fungal DNA and RNA biosynthesis by conversion to 5-fluorouracil by fungal cytosine deaminase which after triphosphorylation is incorporated into fungal RNA and inhibits its synthesis.

- Clinical use: Systemic fungal infections (especially meningitis caused by Cryptococcus) in combination with amphotericin B.

- Toxicity: Bone marrow suppression.

Terbinafine

- Mechanism of action: Terbinafine inhibits synthesis of ergosterol of the fungal membrane by inhibiting the enzyme squalene epoxidase.

- Clinical use:

- Dermatophytosis (especially onychomycosis: fungal infection of finger or toenails).

- Possibly superior to griseofulvin in onychomycoses.

- Toxicity: GI upset, headaches, hepatotoxicity, taste disturbance.

Echinocandin

- Drugs: Caspofungin, micafungin, Anidulafungin.
- Mechanism of action: Echinocandins (caspofungin and micafungin) are a newer group of antifungal medications that inhibit synthesis of the polysaccharide glucan, an essential component of the fungal cell wall.
- Clinical use: Invasive aspergillosis, Candida.
- Toxicity: GI upset, flushing (by histamine release).

Griseofulvin

- Mechanism of action:
  - Interferes with microtubule function; disrupts mitosis.
  - Deposits in keratin-containing tissues (nails).
- Clinical use:
  - Oral treatment of superficial infections; inhibits growth of dermatophytes (tinea, ringworm).
  - Active only against dermatophytes (orally, not topically) by depositing in newly formed keratin and disrupting microtubule structure.
- Toxicity: Teratogenic, carcinogenic, confusion, headaches, ↑ cytochrome P-450 and warfarin metabolism.

Antiprotozoal therapy

Infection	Drug of Choice
Amebiasis	Metronidazole
Giardiasis	Metronidazole
Trichomoniasis	Metronidazole
Toxoplasmosis	Pyrimethamine + sulfadiazine
Leishmaniasis	Sodium Stibogluconate
Trypanosomiasis	Nifurtimox (Chagas disease) Arsenicals (African)

Anthelminthic therapy

- Most intestinal nematodes: mebendazole or pyrantel pamoate.
- Most cestodes and trematodes: praziquantel.

Antimalarial drugs

- Treatment of Chloroquine-Sensitive Malaria:
  - P. falciparum: Chloroquine.
  - P. malariae: Chloroquine.
  - P. vivax, P. ovale: Chloroquine + primaquine.
- Treatment of Chloroquine-resistant Malaria:
  - Prophylaxis: mefloquine.
  - Treatment: quinine.
- Side effects:
  - Hemolytic anemia in G6PD deficiency.
  - Cinchonism (quinine).

Chloroquine

- Mechanism of action:
  - Blocks detoxification of heme into hemozoin.
  - Heme accumulates and is toxic to plasmodia.
- Clinical Use:
  - Treatment of plasmodial species other than P. falciparum (frequency of resistance in P. falciparum is too high).
  - Resistance due to membrane pump that ↓ intracellular concentration of drug.
  - Treat P. falciparum with artemether/lumefantrine or atovaquone/proguanil.

- For life-threatening malaria, use quinidine in US (quinine elsewhere) or artesunate.

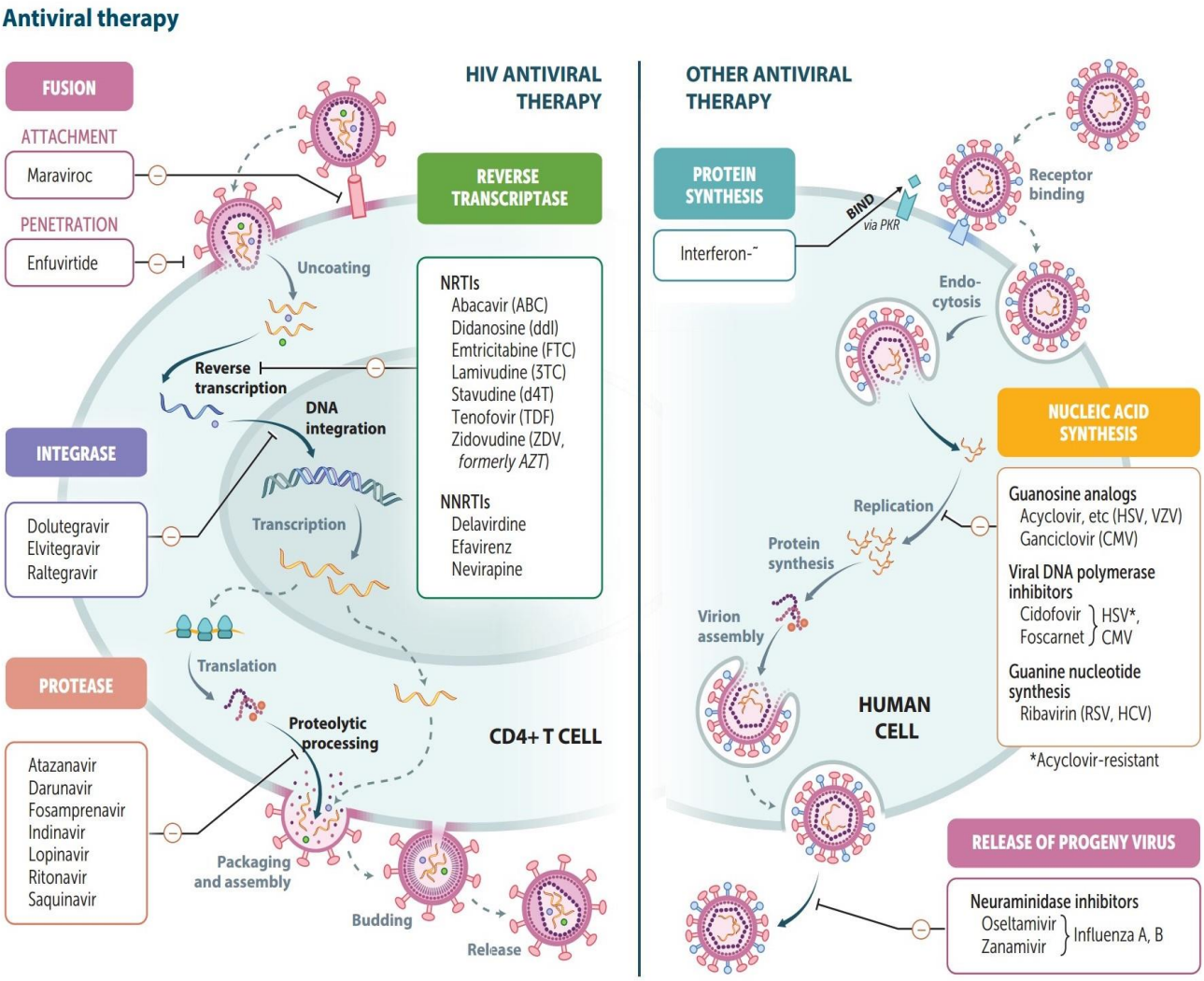
▪ Adverse effects: Retinopathy; pruritus (especially in dark-skinned individuals).

Anti-mite/louse therapy

▪ Used to treat scabies (Sarcoptes scabiei) and lice (Pediculus and Pthirus):

- Permethrin (blocks Na → channels neurotoxicity).
- Ivermectin → Binds to glutamate-gated chloride channels, causing hyperpolarization.
- Malathion (acetylcholinesterase inhibitor).
- Lindane (blocks GABA channels → neurotoxicity).

Antiviral therapy



Acyclovir, famciclovir, valacyclovir

- Mechanism of action:

- Nucleoside analog antiviral drugs must be phosphorylated into nucleotides in order to function.

- Nucleotides are nucleosides with 1 or more attached phosphates.

- Nucleoside analog drugs such as acyclovir, valacyclovir, famciclovir, and ganciclovir require viral phosphorylating enzymes (viral thymidine kinase) for conversion into their monophosphate form.

- Once that has taken place, cellular kinases can further convert the drug nucleoside monophosphate into a nucleoside triphosphate that interferes with herpesvirus replication (inhibit viral DNA polymerase by chain termination).

- Clinical use:

- HSV and VZV.
- Used for HSV induced mucocutaneous and genital lesions as well as for encephalitis. Prophylaxis in immunocompromised patients.
- Weak activity against EBV.
- No activity against CMV.
- No effect on latent forms of HSV and VZV.
- For herpes zoster, use famciclovir.
- Valacyclovir, a prodrug of acyclovir, has better oral bioavailability.

- Side effects:

- Obstructive crystalline nephropathy and acute renal failure if not adequately hydrated.
- Acyclovir is excreted principally in the urine via glomerular filtration and tubular secretion. When the acyclovir concentration in the collecting duct exceeds its solubility, crystallization, crystalluria, and renal tubular damage may result.
- In most cases, this toxic complication is transient and can be prevented (as well as treated) with adequate hydration and dosage adjustment, which includes slowing the rate of intravenous infusion.

- Mechanism of resistance: Resistance possibly due to changes in DNA polymerase or to mutated viral thymidine kinase.

Ganciclovir

- Mechanisms of action: Similar to that of acyclovir

- Activity and clinical uses:

- HSV, VZV, and CMV.
- Mostly used in prophylaxis and treatment of CMV infections, including retinitis, in AIDS and transplant patients.
- Valganciclovir, a prodrug of ganciclovir, has better oral bioavailability.

- Side effects:

- Dose-limiting hematotoxicity (leukopenia, thrombocytopenia), mucositis, fever, rash, and crystalluria (maintain hydration).

- Seizures in overdose.

- Mechanism of resistance: similar to acyclovir.

Foscarnet

- Mechanism of action:

- Not an antimetabolite, but still inhibits viral DNA and RNA polymerases.

- Binds to pyrophosphate-binding site of enzyme. Does not require activation by viral kinase.

- Foscarnet = pyrofosphate analog.

- Clinical use:

- CMV retinitis in immunocompromised patients when ganciclovir fails; acyclovir resistant HSV.

- Identical to ganciclovir, plus > activity versus acyclovir-resistant strains of HSV.

- Side effects:

- Nephrotoxicity, electrolyte abnormalities (hypo- or hypercalcemia, hypo- or hyperphosphatemia, hypokalemia, hypomagnesemia) can lead to seizures.

- Foscarnet can chelate calcium and promote nephrotoxic renal magnesium wasting.

- Foscarnet-induced renal wasting of magnesium may lead to hypomagnesemia and a reduction in the release of parathyroid hormone, which contributes to the hypocalcemic state.

- Both hypocalcemia and hypomagnesemia can promote seizures.

- Avoid pentamidine IV (nephrotoxicity and hypocalcemia).

- Mechanism of resistance: Mutated DNA polymerase.

Cidofovir

- Mechanism of action:
- Nucleotide analogue that directly inhibits viral DNA polymerase.
- Does not require phosphorylation by viral kinase.
- Clinical use:
- CMV retinitis in immunocompromised patients; acyclovir resistant HSV.
- Long half-life.
- Toxicity: Nephrotoxicity (coadminister with probenecid and IV saline to ↓ toxicity).

Amantadine

- Mechanisms of action: Blocks attachment, penetration, and uncoating of influenza A virus.
- Clinical uses: prophylaxis mainly, but may ↓ duration of flu symptoms by 1-2 day.
- Side effects:
- CNS effects: nervousness, insomnia, and seizures.
- Causes atropine-like peripheral effects and livedo reticularis.

Zanamivir and Oseltamivir

- Mechanisms of action: Inhibit neuraminidases of influenza A and B → ↓ release of progeny virus.
- Clinical uses:
- Treatment and prevention of both influenza A and B.
- Beginning therapy within 48 hours of symptom onset may shorten duration of illness.

Remdesivir

- Mechanisms of action: Prodrug of an ATP analog. The active metabolite inhibits viral RNA-dependent RNA polymerase and evades proofreading by viral exoribonuclease (ExoN) → ↓ viral RNA production.
- Clinical uses: Recently approved for treatment of COVID-19 requiring hospitalization.

HIV therapy

- Highly active antiretroviral therapy (HAART): often initiated at the time of HIV diagnosis.

- Strongest indication for:

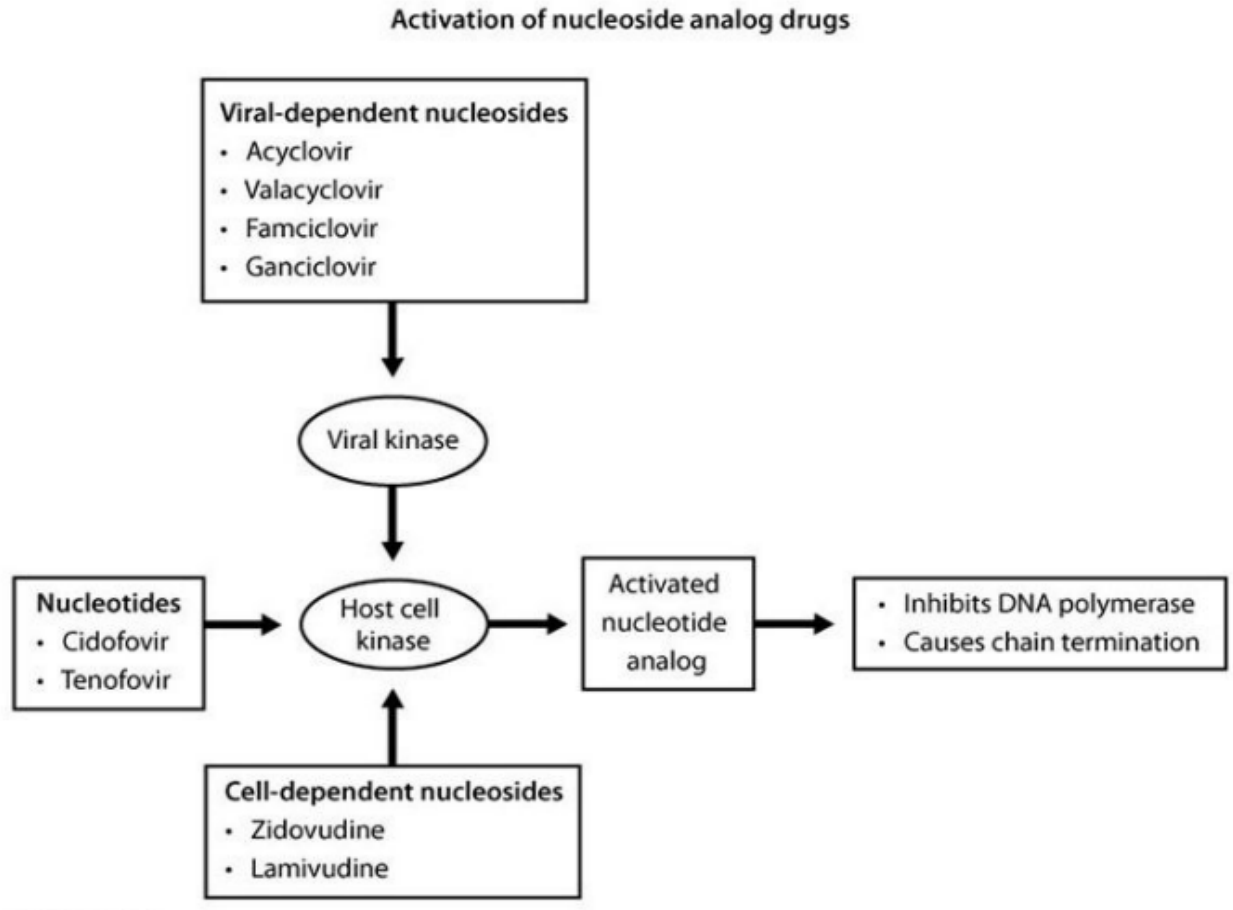
- Patients presenting with AIDS-defining illness.
- Low CD4+ cell counts < 500 cells/mm^3, or high viral load.

- Regimen consists of 3 drugs to prevent resistance: 2 NRTIs and preferably an integrase inhibitor.

- All ARTs are active against HIV-1 and HIV-2 with the exception of NNRTIs.

Drug	Mechanism	Toxicity
Protease inhibitors		
Atazanavir **Darunavir** **Fosamprenavir** **Indinavir** **Lopinavir** **Ritonavir** **Saquinavir**	- Assembly of virions depends on HIV-1 protease (pol gene), which cleaves the polypeptide products of HIV mRNA into their functional parts. Thus, protease inhibitors prevent maturation of new viruses. - Ritonavir can "boost" other drug concentrations by inhibiting cytochrome P-450. - All protease inhibitors end in –navir. - Navir (never) tease a protease.	▪ All protease inhibitors have the following important adverse effects: 1. Lipodystrophy leads to increased deposition of fat on the back and abdomen and decreased adipose tissue on the extremities. This gives patients a "buffalo hump" appearance with central obesity and peripheral wasting. 2. Hyperglycemia is a side effect associated with all protease inhibitors that results from increased insulin resistance and may lead to frank diabetes. 3. Inhibition of P-450 also occurs with some protease inhibitors and may cause interactions with other drugs. ▪ Rifampin should not be administered with protease inhibitors because rifampin increases the activity of P-450 and will therefore decrease the serum levels of protease inhibitor; rifabutin should be used instead for Mycobacterial infections in patients on protease inhibitors. ▪ Indinavir can cause nephrotoxicity and nephrolithiasis.

NRTIs		
Abacavir (ABC) **Didanosine (ddI)** **Emtricitabine (FTC)** **Lamivudine (3TC)** **Stavudine (d4T)** **Tenofovir (TDF)** **Zidovudine (ZDV, formerly AZT)**	- Competitively inhibit nucleotide binding to reverse transcriptase and terminate the DNA chain (lack a 3' OH group). - Tenofovir is a nucleoTide; the others are nucleosides and need to be phosphorylated to be active. - ZDV is used for general prophylaxis and during pregnancy to ↓ risk of fetal transmission.	- Bone marrow suppression (can be reversed with granulocyte colony-stimulating factor [G-CSF] and erythropoietin), peripheral neuropathy, lactic acidosis (nucleosides), Anemia (ZDV), Nephrotoxicity (tenofovir), pancreatitis (didanosine). - Abacavir contraindicated if patient has HLA-B*5701 mutation due to ↑ risk of hypersensitivity.
NNRTIs		
Delavirdine **Efavirenz** **Nevirapine**	- Bind to reverse transcriptase at site different from NRTIs. - Do not require phosphorylation to be active or compete with nucleotides.	- Rash and hepatotoxicity are common to all NNRTIs. - Vivid dreams and CNS symptoms are common with efavirenz. - Delavirdine and efavirenz are contraindicated in pregnancy.
Integrase inhibitors		
Raltegravir **Elvitegravir** **Raltegravir**	Disrupts the ability of HIV to integrate its genome into the host cell's chromosomes, thus preventing host cellular machinery from being used to synthesize HIV mRNA	↑ creatine kinase.
Fusion inhibitors		
Enfuvirtide	Antiretroviral agents that selectively bind to the HIV envelope transmembrane glycoprotein gp41 prevent the conformational changes necessary for the viral membrane to fuse with the target cellular membrane.	- Skin reaction at injection sites. - Enfuvirtide inhibits fusion.
Maraviroc	Binds CCR-5 on surface of T cells/monocytes, inhibiting interaction with gp120.	- Maraviroc inhibits docking.



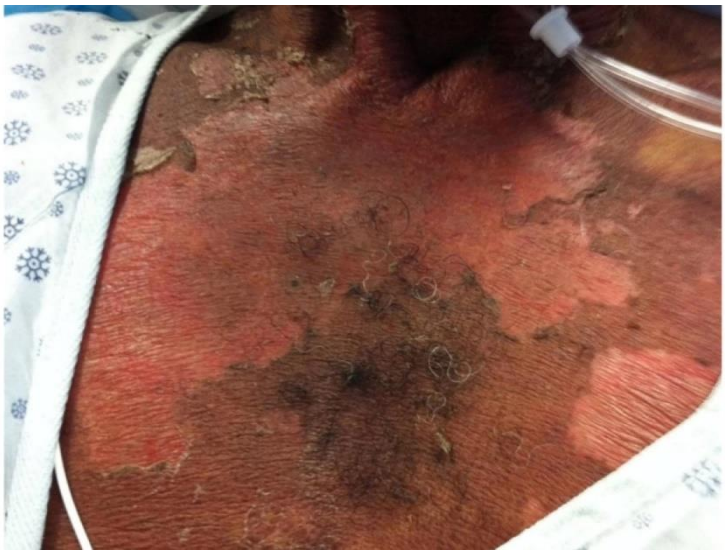
❖ N.B:

1. Zidovudine (AZT) competitively binds to reverse transcriptase and is incorporated into the viral genome as a thymidine analog.

- However, AZT has an azido group in place of the hydroxyl group normally found on the 3' end of thymidine.
- Because a free 3' hydroxyl group is required for new nucleotides to be added to replicating DNA, the azido group on AZT prevents DNA chain elongation.
- AZT does not have a 3'-OH group, making 3'-5' phosphodiester bond formation impossible.

2. Abacavir hypersensitivity reaction (AHR) is an allergic reaction that develops in 2%-8% of patients and is strongly associated with the HLA-B*57:01 allele of the human leukocyte antigen (HLA) system.

- AHR occurs due to direct binding of abacavir to a segment on the HLA-B*57:01 molecule, which alters the presentation of self-peptides to the immune system and results in a delayed hypersensitivity reaction (type IV).
- Manifestations are mediated by a cytotoxic T-cell response and typically include fever, malaise, gastrointestinal symptoms, and a delayed rash.
- Abacavir discontinuation results in rapid improvement.
- A negative test for the HLA-B*57:01 allele has almost a 100% negative predictive value for AHR. Therefore, genetic testing is usually done prior to administering the medication.



3. As ganciclovir is used to treat cytomegalovirus infections, it is commonly administered to patients with advanced HIV.
- Neutropenia is a significant adverse effect of ganciclovir therapy, and its incidence is increased with coadministration of zidovudine.
- Both drugs can affect DNA synthesis in hematopoietic stem cell lines, resulting in bone marrow suppression.

4. Thymidine kinase-deficient (and therefore acyclovir-resistant) varicella zoster virus isolates tend to be obtained almost exclusively from AIDS patients.
- These immunocompromised patients are best treated with either foscarnet (a pyrophosphate analog viral DNA polymerase inhibitor that does not require viral kinase activation) or with cidofovir (a broad-spectrum antiviral nucleotide analogue that can be converted to the active triphosphate solely by cellular kinases, thus its efficacy does not depend on the presence of a virally encoded kinase).

5. Visual impairment in an HIV-infected patient is most commonly secondary to cytomegalovirus-induced retinitis. Treatment options for cytomegalovirus-induced retinitis include ganciclovir, foscarnet, and cidofovir.

6. Prophylaxis in HIV patients:

Cell count	Prophylaxis	Infection
CD4 < 200 cells/mm3	TMP-SMX	Pneumocystis pneumonia
CD4 < 100 cells/mm3	TMP-SMX	Pneumocystis pneumonia and toxoplasmosis

Hepatitis C therapy

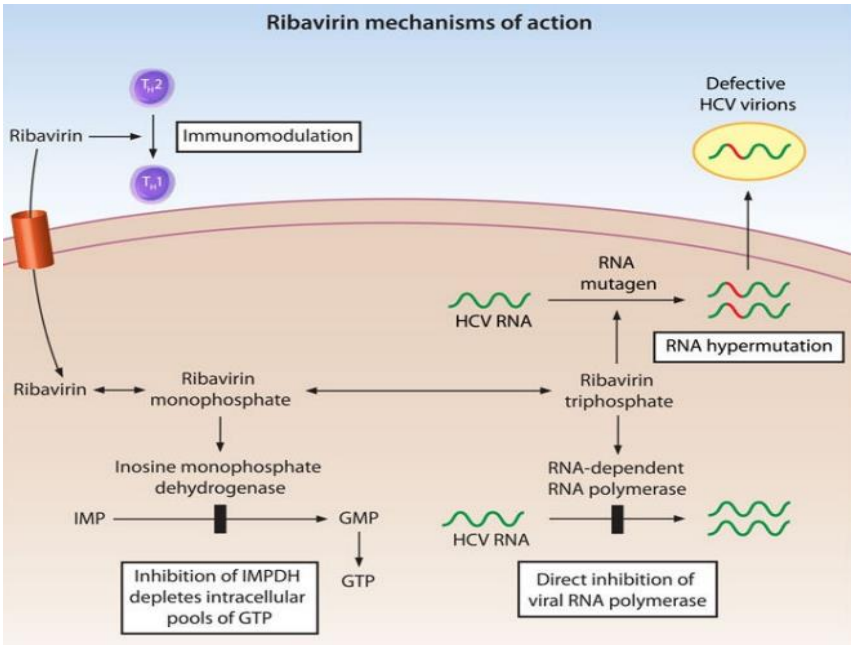
- Chronic HCV infection is treated with different combinations of the following drugs; none is approved as monotherapy.

- Developed based on understanding of HCV replication cycle.

Drug	Mechanism	Clinical use
NS5A inhibitors		
Ledipasvir **Ombitasvir**	Inhibits NS5A, a viral phosphoprotein that plays a key role in RNA replication, unknown exact mechanism.	Headache, diarrhea.
NS3/4A inhibitors		
Simeprevir **Grazoprevir**	Inhibits NS3/4A, a viral protease, preventing viral replication.	Grazoprevir: Photosensitivity reactions, rash. Simeprevir: Headache, fatigue.
NS5B inhibitors		
Sofosbuvir **Dasabuvir**	Inhibits NS5B, an RNA-dependent RNA polymerase acting as a chain terminator.	Toxicity: fatigue, headache, nausea.
Ribavirin	- Monophosphorylated form inhibits synthesis of guanine nucleotides by competitively inhibiting inosine monophosphate dehydrogenase. - Triphosphate inhibits viral RNA polymerase.	- Chronic HCV, also used in RSV (palivizumab preferred in children) - Toxicity: hemolytic anemia; severe teratogen.

❖ N.B:

- Ribavirin is a nucleoside antimetabolite drug that interferes with duplication of viral genetic material. Its mechanism of action is thought to be multifactorial and includes inducing lethal hypermutation, inhibiting RNA polymerase and inosine monophosphate dehydrogenase (depleting GTP).
- Treatment of chronic hepatitis C involves the use of interferon alpha and ribavirin.



Interferons

- Mechanism of action: Glycoproteins normally synthesized by virus-infected cells, exhibiting a wide range of antiviral and antitumoral properties.

- Clinical use:

- IFN-α: chronic hepatitis B and C, Kaposi sarcoma, hairy cell leukemia, condyloma acuminatum, renal cell carcinoma, malignant melanoma.

- IFN-β: multiple sclerosis.

- IFN-γ: chronic granulomatous disease.

- Toxicity: Neutropenia, myopathy.

Infection control techniques

- Goals include the reduction of pathogenic organism counts to safe levels (disinfection) and the Inactivation of self-propagating biological entities (sterilization).

- Autoclave: Pressurized steam at > 120°C. May be sporicidal.
- Alcohols: Denature proteins and disrupt cell membranes. Not sporicidal.
- Chlorhexidine: Denatures proteins and disrupts cell membranes. Not sporicidal.
- Hydrogen peroxide: Free radical oxidation. Sporicidal.
- Iodine and iodophors: Halogenation of DNA, RNA, and proteins. May be sporicidal.
- Quaternary amines: Impair permeability of cell membranes. Not sporicidal.

Antibiotics to avoid in Pregnancy

Antibiotic	Adverse effect
Sulfonamides	Kernicterus
Aminoglycosides	Ototoxicity
Fluoroquinolones	Cartilage damage
Clarithromycin	Embryotoxic
Tetracyclines	Discolored teeth, inhibition of bone growth
Ribavirin (antiviral)	Teratogenic
Griseofulvin (antifungal)	Teratogenic
Chloramphenicol	Gray baby syndrome

❖ Mnemonic: SAFe Children Take Really Good Care.

Antimicrobial prophylaxis

Clinical scenario	Medication
Exposure to meningococcal infection	Ceftriaxone, ciprofloxacin, or rifampin
High risk for endocarditis and undergoing surgical or dental procedures	Amoxicillin
History of recurrent UTIs	TMP-SMX
Malaria prophylaxis for travelers	Atovaquone-proguanil, mefloquine, doxycycline, primaquine, or chloroquine (for areas with sensitive species)
Pregnant patients carrying group B strep	Intrapartum penicillin G or ampicillin
Prevention of gonococcal conjunctivitis in newborn	Erythromycin ointment on eyes
Prevention of postsurgical infection due to S. aureus	Cefazolin; vancomycin if ⊕ for MRSA
Prophylaxis of strep pharyngitis in child with prior rheumatic fever	Benzathine penicillin G or oral penicillin V

CHAPTER 7

Immunology

- The immune system is designed to produce a coordinated response to the introduction of foreign substances or antigens into the body.

- It is organizationally divided into two complementary arms: The innate (or native or natural) immune system and the adaptive (or acquired or specific) immune system.

- Innate immunity provides the body's early line of defense against microbial invaders.

- Once the barriers of the innate immune response have been breached, the adaptive immune response is activated in an antigen-specific fashion to provide for the elimination of antigen and lasting protection from future challenge.

- Comparison of Innate and Adaptive Immunity:

Characteristics	Innate	Adaptive
Specificity	For structures shared by groups of microbes	For specific antigens of microbial and nonmicrobial agents
Diversity	Limited	High
Memory	No	Yes
Self-reactivity	No	No
Components		
Anatomic and chemical barriers	Skin, mucosa, chemicals (lysozyme, interferon α and β), temperature, PH.	Lymph nodes, spleen, mucosal associated lymphoid tissues.
Blood proteins	Complement	Antibodies
Cells	Neutrophils, macrophages, monocytes, dendritic cells, natural killer (NK) cells (lymphoid origin)	Lymphocytes (other than NK cells)

- These features of adaptive immunity are designed to give the individual the best possible defense against disease:

- Specificity is required, along with memory, to protect against persistent or recurrent challenge.

- Diversity is required to protect against the maximum number of potential pathogens.

- Specialization of function is necessary so that the most effective defense can be mounted against diverse challenges.

- The ability to distinguish between invaders and one's own cells and tissues (self-versus non-self) is vital in inhibiting a response to one's own cells (autoimmunity).

❖ N.B:

- <u>The innate and adaptive arms of the immune response do not operate independently of one another:</u>

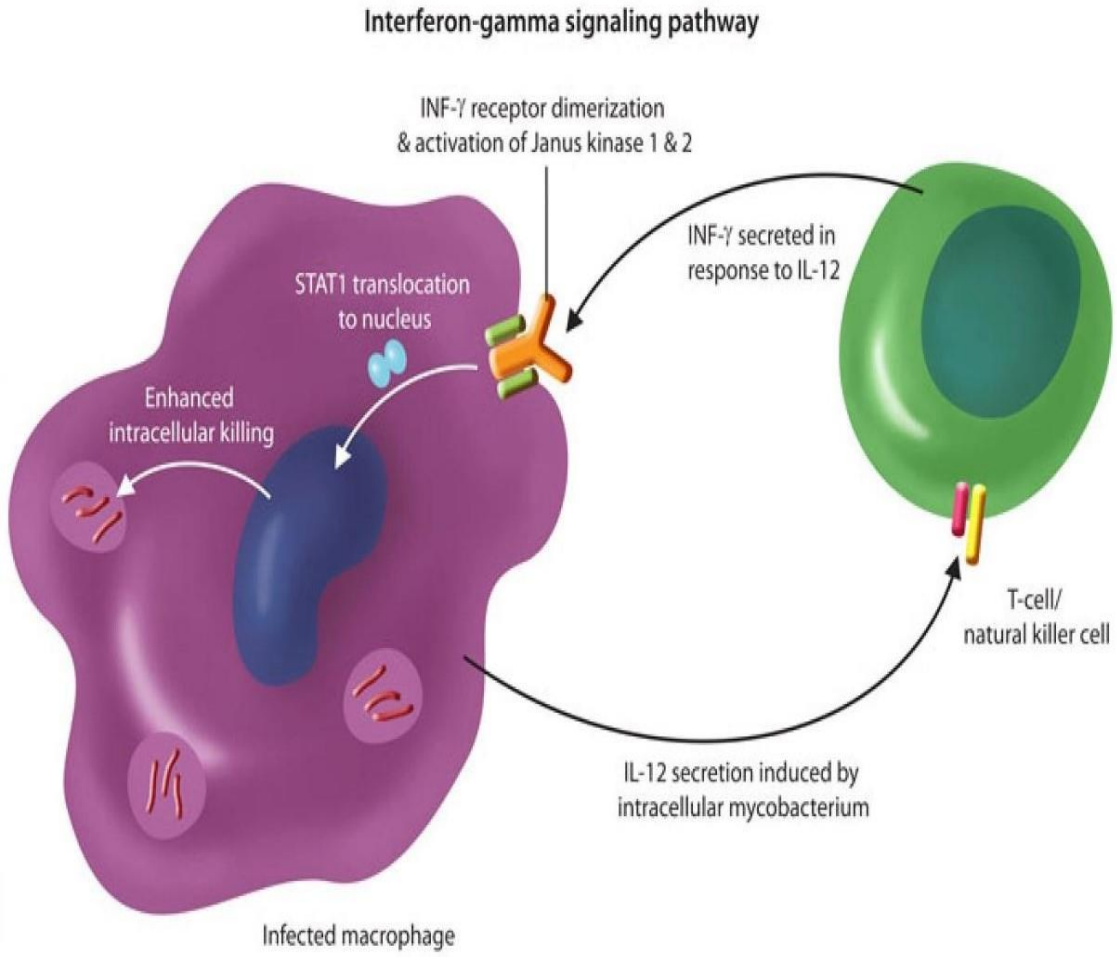
1. Phagocytic cells process and display antigen to facilitate stimulation of specific T lymphocytes.

2. Macrophages secrete immunoregulatory molecules (cytokines), which help trigger the initiation of specific immune responses.

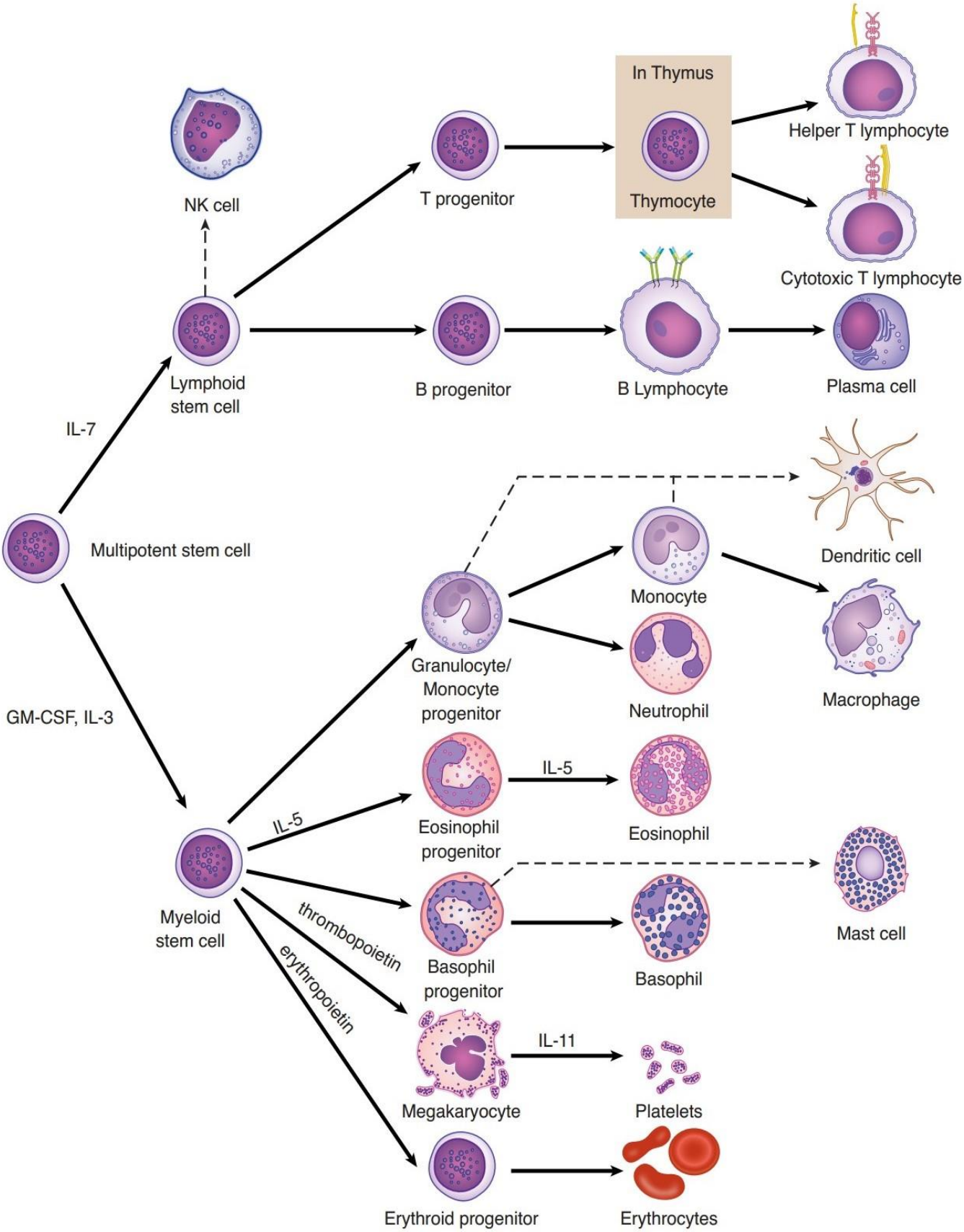
3. T. lymphocytes produce cytokines, which enhance the microbicidal activities of phagocytes.

4. Antibodies produced by plasma cells bind to pathogens and activate the complement system to result in the destruction of the invaders.

5. Antibodies produced by B lymphocytes bind to pathogens and assist with phagocytosis (opsonization).



Cells of the Immune System

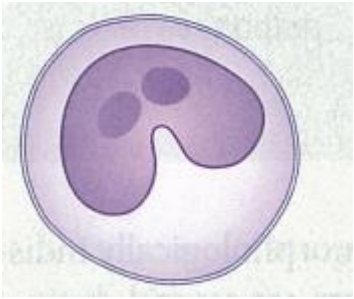


- The cells of the immune system arise from a pluripotent stem cell in the bone marrow.
- Differentiation of this cell will occur along one of two pathways, giving rise to either a common lymphoid progenitor cell or a common myeloid progenitor cell.
- The common lymphoid progenitor cell gives rise to B lymphocytes, T lymphocytes, and natural killer (NK) cells.
- The myeloid progenitor gives rise to erythrocytes, platelets, basophils, mast cells, eosinophils, neutrophils, monocytes, macrophages, and dendritic cells.

Myeloid cells

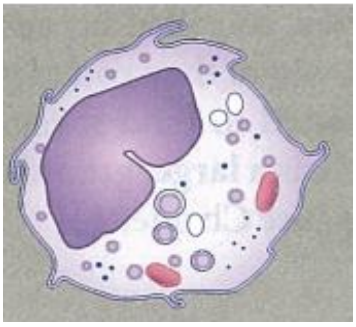
1. Monocytes:

- Location: Bloodstream.
- Identification: Kidney bean shaped nucleus, CD14 positive.
- Function: Phagocytic, differentiate into tissue macrophages.



2. Macrophages:

- Location: Tissues.
- Identification: Ruffled membrane, cytoplasm with vacuoles and vesicles, CD14 positive.
- Function: Phagocytosis, secretion of cytokines.

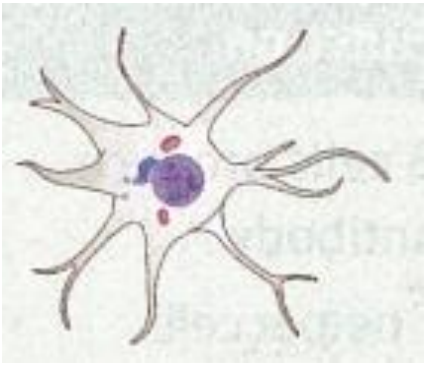


3. Dendritic cells:

- Location: Epithelia, tissues.

- Identification: Long cytoplasmic arms.

- Function: Antigen capture, transport, and presentation.

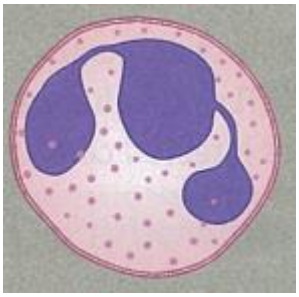


4. Neutrophils:

- Location: Bloodstream.

- Identification: Multilobed nucleus (3-5 lobes); small pink granules.

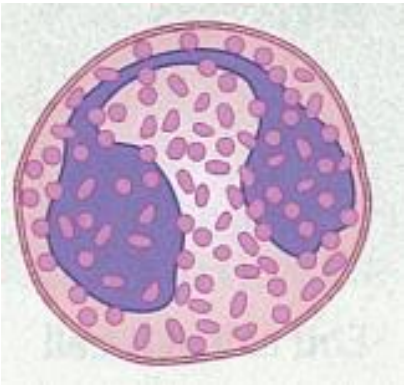
- Function: Phagocytosis and activation of bactericidal mechanisms.



5. Eosinophils:

- Location: Bloodstream.

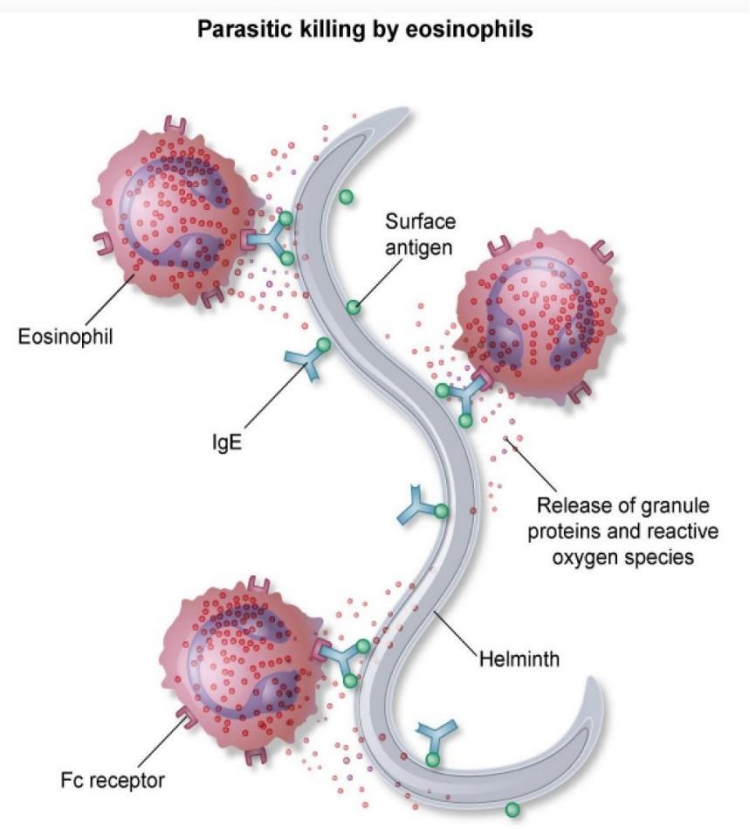
- Identification: Bilobed nucleus, large pink granules.



- Function:

A. Defense against parasitic infections:

- When a parasite enters the bloodstream, it is bound by free IgE.
- The resultant antigen-antibody complex then binds an IgE Fc receptor located on the eosinophil cell surface.
- Upon binding, the eosinophil releases major basic protein and other enzymes from its granules, substances that damage and destroy antibody-bound parasites.
- This mechanism is an example of antibody-dependent cellular cytotoxicity (ADCC), a method of killing that depends on the ability of the immune cell to recognize specific antibody bound to a cell and trigger the death of that cell without the use of complement.
- Macrophages and NK cells also rely on ADCC.



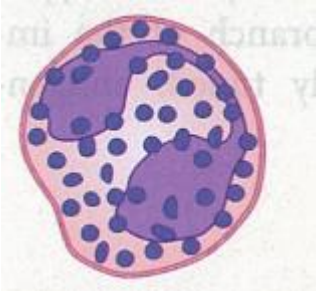
B. Regulation of type I hypersensitivity reactions: Eosinophilic granules contain histaminase, an enzyme that degrades histamine, which helps to reduce the severity of atopic symptoms.

6. Basophils:

- Location: Bloodstream.

- Identification: Bilobed nucleus, large blue granules.

- Function: Nonphagocytic, release pharmacologically active substances (histamine) during allergic responses.

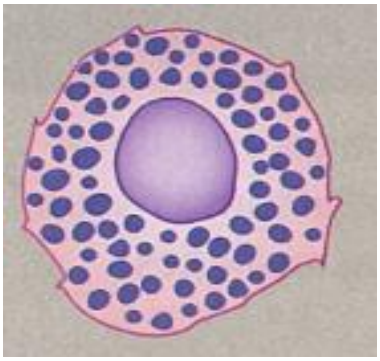


7. Mast cells:

- Location: tissues, mucosa, and epithelia.

- Identification: Small nucleus, cytoplasm packed with large blue granules.

- Function: Release of granules containing histamine during allergic responses.



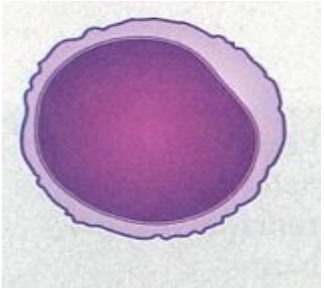
lymphoid cells

1. Lymphocytes:

- Location: Bloodstream, lymph nodes, spleen.

- Identification:
  - Large, dark nucleus, small rim of cytoplasm.
  - B cells: CD19, 20, 21.
  - T cells: CD3.
  - TH cells: CD4.
  - CTLs: CD8.

- Function:
  - B cells produce antibody.
  - T helper cells regulate immune responses.
  - Cytotoxic T cells (CTLs) kill altered or infected cells.

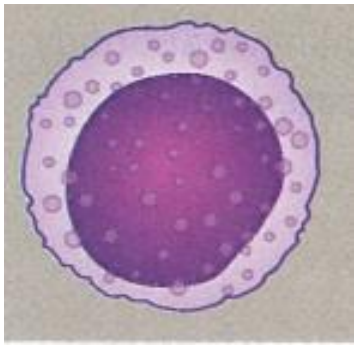


2. Natural killer (NK) lymphocytes:

- Location: Bloodstream.

- Identification: Lymphocytes with large cytoplasmic granules CD16 CD56 positive.

- Function: Kill tumor/virus cell targets or antibody-coated target cells (ADCC).



❖ N.B:

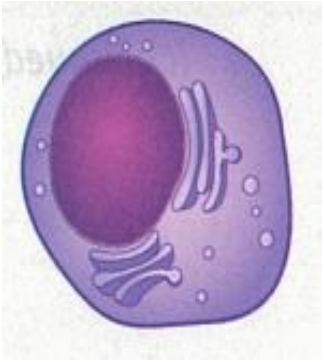
- Natural killer cells are derived from lymphoid stem cells and comprise approximately 10% of all circulating lymphocytes.
- Do not express CD4, CD8 or CD3 molecules on their surface. They do express either CD16 or CD 56.
- Do not require the thymus for maturation and are present in athymic patients.
- Have no antigen-specific activities, do not require exposure to antigen for activation, and do not possess antigen memory ability.
- NK cells recognize and kill cells with decreased or absent MHC class I antigen cell surface expression, such as virus infected cells and tumor cells.
- They are large cells with cytoplasmic granules containing perforins, which produce holes in target cell membranes, and granzymes, chemicals that induce target cell apoptosis.
- The target cell subsequently undergoes apoptosis.

3. Plasma cells:

- Location: Lymph nodes, spleen, mucosal associated lymphoid tissues, and bone marrow.

- Identification: Small dark nucleus, intensely staining Golgi apparatus.

- Function: End cell of B-cell differentiation, produce antibody.



❖ In a Nutshell:

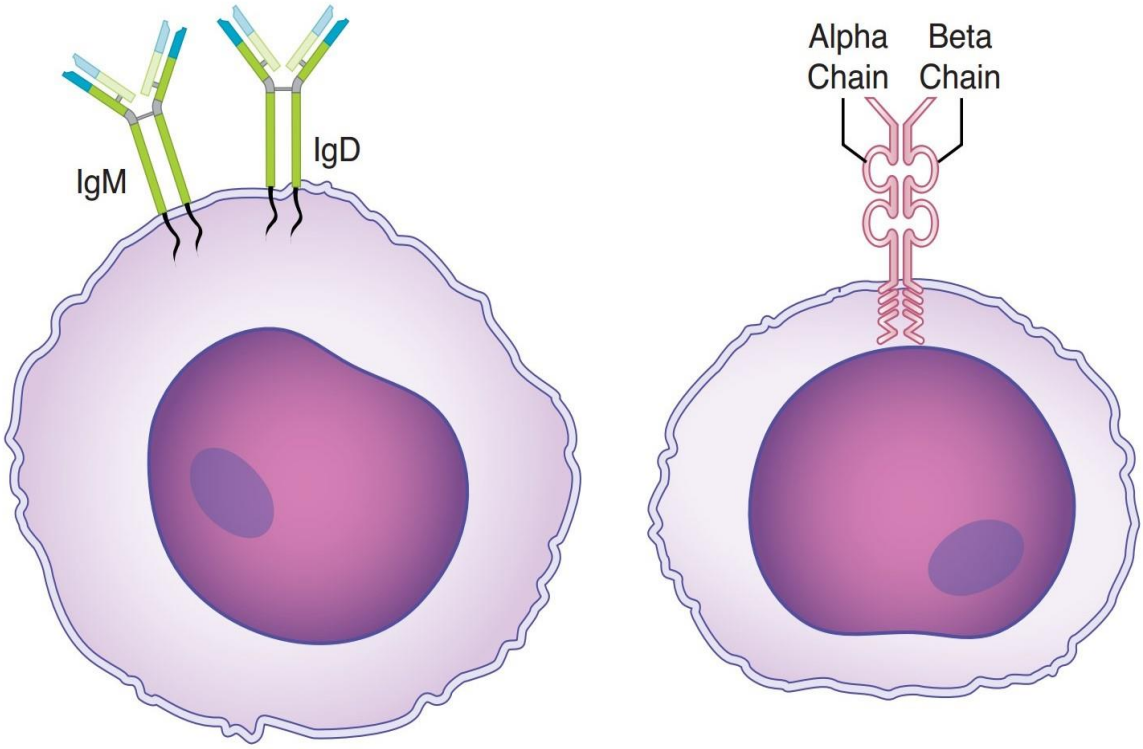
- Myeloid cells are in the innate branch.
- Lymphoid cells (except NK cells) are in the adaptive branch.
- B lymphocytes, so called because they complete their development in the bone marrow, and T lymphocytes, so called because they pass from their origin in the bone marrow into the thymus, where they complete their development.

The antigen recognition molecules of lymphocytes

- Each of the cells of the lymphoid lineage is clinically identified by the characteristic surface molecules that they possess.

- The B lymphocyte, in its mature ready-to-respond form (the naive B lymphocyte), wears molecules of two types of antibody or immunoglobulin called IgM and IgD embedded in its membrane.

- The naive T cell wears a single type of genetically related molecule, called the T-cell receptor (TCR), on its surface.

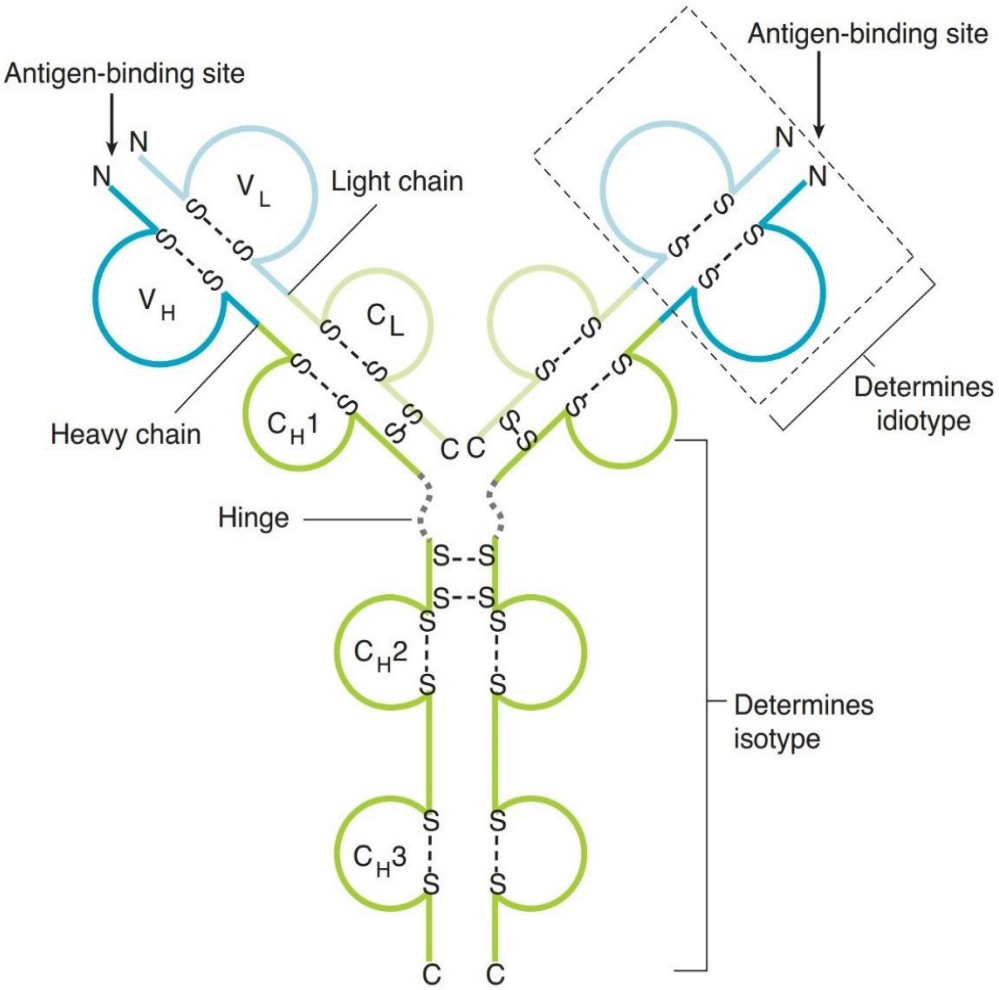


❖ <u>In a Nutshell:</u>

- The naive B-cell antigen receptors are IgM and IgD.
- The T-cell antigen receptor is made of α and β chains.

B lymphocytes antigen receptors

- The antigen receptor of the B lymphocyte, or membrane-bound immunoglobulin, is a four-chain glycoprotein molecule.
- This immunoglobulin has two identical halves, each composed of a long, or heavy chain, and a shorter, light chain.
- The two halves are held together by disulfide bonds into a shape resembling a "Y" and some flexibility of movement is permitted between the halves by disulfide bonds forming a hinge region.
- On the N -terminal end of the molecule where the heavy and light chains lie side by side, a "pocket" is formed whose three-dimensional shape will accommodate the noncovalent binding of one, or a very small number, of related antigens.
- The unique three-dimensional shape of this pocket is called the idiotype.



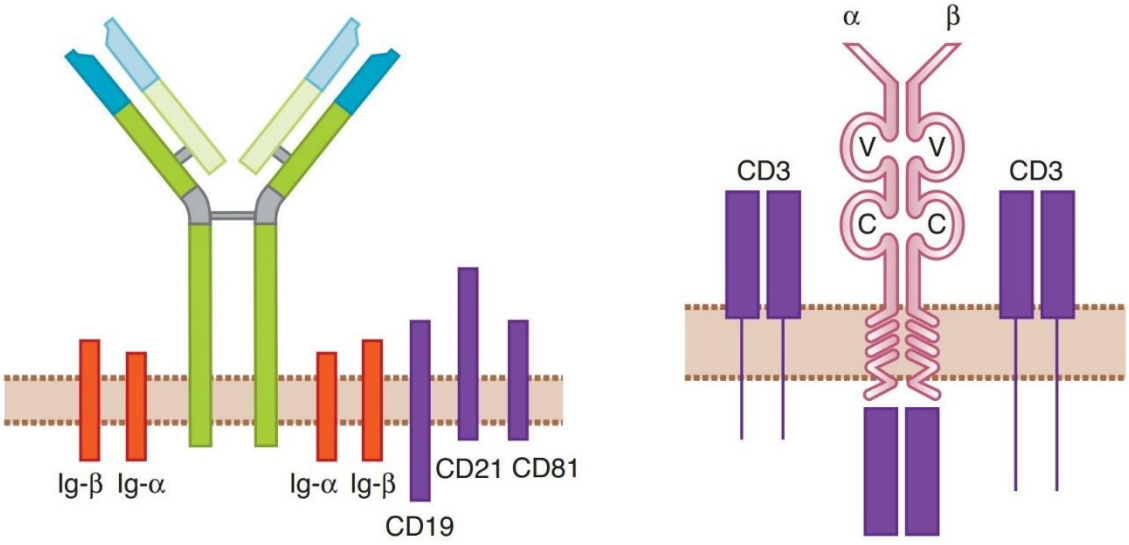
B-Lymphocyte Antigen Recognition Molecule
(Membrane-Bound Immunoglobulin)

❖ In a Nutshell:

- Membrane-bound Ig has two heavy and two light chains.
- A "hinge" region joins the heavy chains.
- The idiotype of the molecule resides in the N-terminal pocket of heavy and light chains.
- The isotype of the molecule is determined by domain toward the C-terminus.

T lymphocytes antigen receptors

- The antigen receptor of the T lymphocyte is composed of two glycoprotein chains that are similar in length and are thus designated α and β chains.
- On the N-terminal end of the molecule, a groove is formed between the two chains, whose three-dimensional shape will accommodate the binding of a small antigenic peptide presented on the surface of an antigen-presenting cell (macrophage, dendritic cell, or B lymphocyte).
- This groove forms the idiotype of the TCR.
- Notice that there is no hinge region present in this molecule, and thus its conformation is quite rigid.
- The membrane receptors of B lymphocytes are designed to bind unprocessed antigens of almost any chemical composition, whereas the TCR is designed to bind only cell-bound peptides.
- Also, although the B-cell receptor is ultimately modified to circulate freely in the plasma as secreted antibody, the TCR is never released from its membrane-bound location.
- In association with these unique antigen-recognition molecules on the surface of B and T cells, accessory molecules are found whose function is in signal transduction.
- Thus, when a lymphocyte binds to an antigen complementary to its idiotype, a cascade of messages transferred through its signal transduction complex will culminate in intracytoplasmic phosphorylation events, which will activate the cell.
- In the B cell, this signal transduction complex is composed of two single-chain immunoglobulin relatives known as Ig-α and Ig-β and two other molecules designated CD (cluster of differentiation) 19 and 21.
- In the T cell, the signal transduction complex is a multichain structure called CD3.



B-Cell Signal Transduction Complex **T-Cell Signal Transduction Complex**

Lymphocyte Signal Transduction

Property	B-Cell Antigen Receptor	T-Cell Antigen Receptor
Idiotypes/Lymphocyte	1	1
Isotypes/Lymphocyte	2 (IgM and IgD)	1 (α/β)
Is secretion possible?	Yes	No
Number of combining sites/molecule	2	1
Mobility	Flexible (hinge region)	Rigid
Signal-transduction molecules	Igα, Ig-β, CD19, CD21	CD3

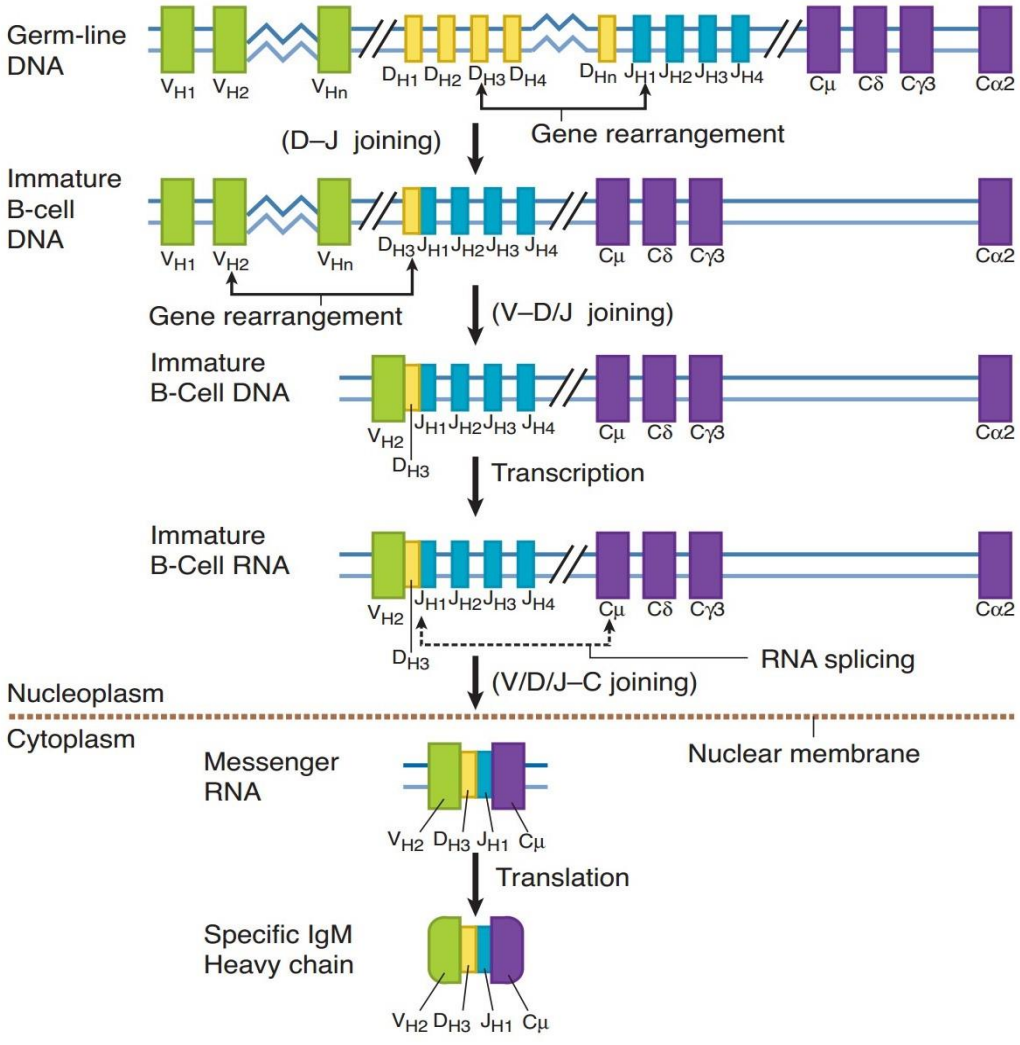
The generation of receptor diversity

- Because the body requires the ability to respond specifically to all of the millions of potentially harmful agents it may encounter in a lifetime, a mechanism must exist to generate the millions of idiotypes of antigen receptors necessary to meet this challenge.

- If each of these idiotypes were encoded separately in the germline DNA of lymphoid cells, it would require more DNA than is present in the entire cell.

- The generation of this necessary diversity is accomplished by a complex and unique set of rearrangements of DNA segments that takes place during the maturation of lymphoid cells.

- In the first place, it was discovered that individuals inherit a large number of different segments of DNA, which may be recombined and alternatively spliced to create unique amino acid sequences in the N -terminal ends (variable domains) of the chains that compose their antigen recognition sites.



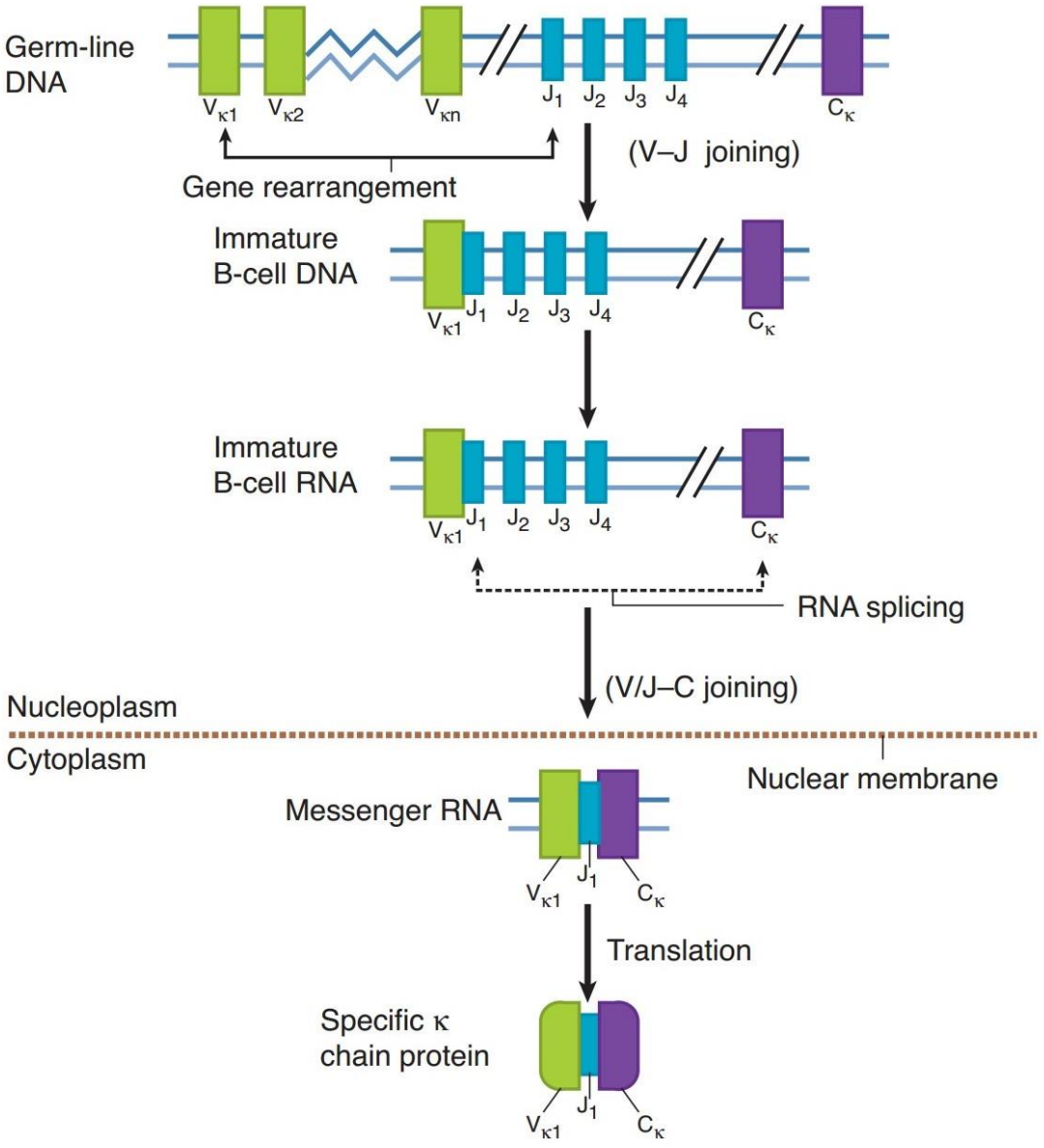
Production of Heavy (B-Cell) or Beta (T-Cell) Chains of Lymphocyte Antigen Receptors

- For example, to produce the heavy chain variable domain of their antigen receptor, B-lymphocyte progenitors select randomly and in the absence of stimulating antigen to recombine three gene segments designated variable (V), diversity (D), and joining (J) out of hundreds of germline-encoded possibilities to produce unique sequences of amino acids in the variable domains (VDJ recombination).

- An analogous random selection is made during the formation of the β chain of the TCR.

- Next, the B-lymphocyte progenitor performs random rearrangements of two types of gene segments (V and J) to encode the variable domain amino acids of the light chain.

- An analogous random selection is made during the formation of the α chain of the TCR.

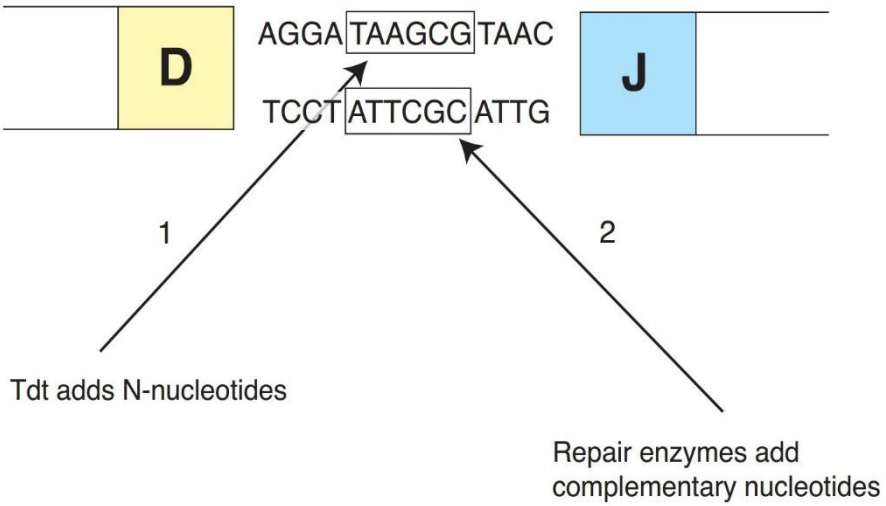


Production of Light (B-Cell) or Alpha (T-Cell) Chain of a Lymphocyte Antigen Receptor

- While heavy chain gene segments are undergoing recombination, the enzyme terminal deoxyribonucleotidyl transferase (Tdt) randomly inserts bases (without a template on the complementary strand) at the junctions of V, D, and J segments (N-nucleotide addition).

- When the light chains are rearranged later, Tdt is not active, but it is active during the rearrangement of all gene segments in the formation of the TCR.

- This generate more diversity than the random combination of V, D, and J segments alone.



- Needless to say, many of these gene segment rearrangements result in the production of truncated or nonfunctional proteins.

- When this occurs, the cell has a second chance to produce a functional strand by rearranging the gene segments of the homologous chromosome.

- If it fails to make a functional protein from rearrangement of segments on either chromosome, the cell is induced to undergo apoptosis or programmed cell death.

- In this way, the cell has two chances to produce a functional heavy (or β) chain. A similar process occurs with the light or α chain. Once a functional product has been achieved by one of these rearrangements, the cell shuts off the rearrangement and expression of the other allele on the homologous chromosome (a process known as allelic exclusion).

- This process ensures that B and T lymphocytes synthesize only one specific antigen-receptor per cell.

- Because any heavy (or β) chain can associate with any randomly generated light (or α) chain, one can multiply the number of different possible heavy chains by the number of different possible light chains to yield the total number of possible idiotypes that can be formed. This generates yet another level of diversity.

Mechanism	Cell in Which It Is Expressed
Existence in genome of multiple V, D, J Segments	B and T cells
VDJ recombination	B and T cells
N-nucleotide addition	B cells (only heavy chain) T cells (all chains)
Combinatorial association of heavy and light chains	B and T cells
Somatic hypermutation	B cells only, after antigen stimulation

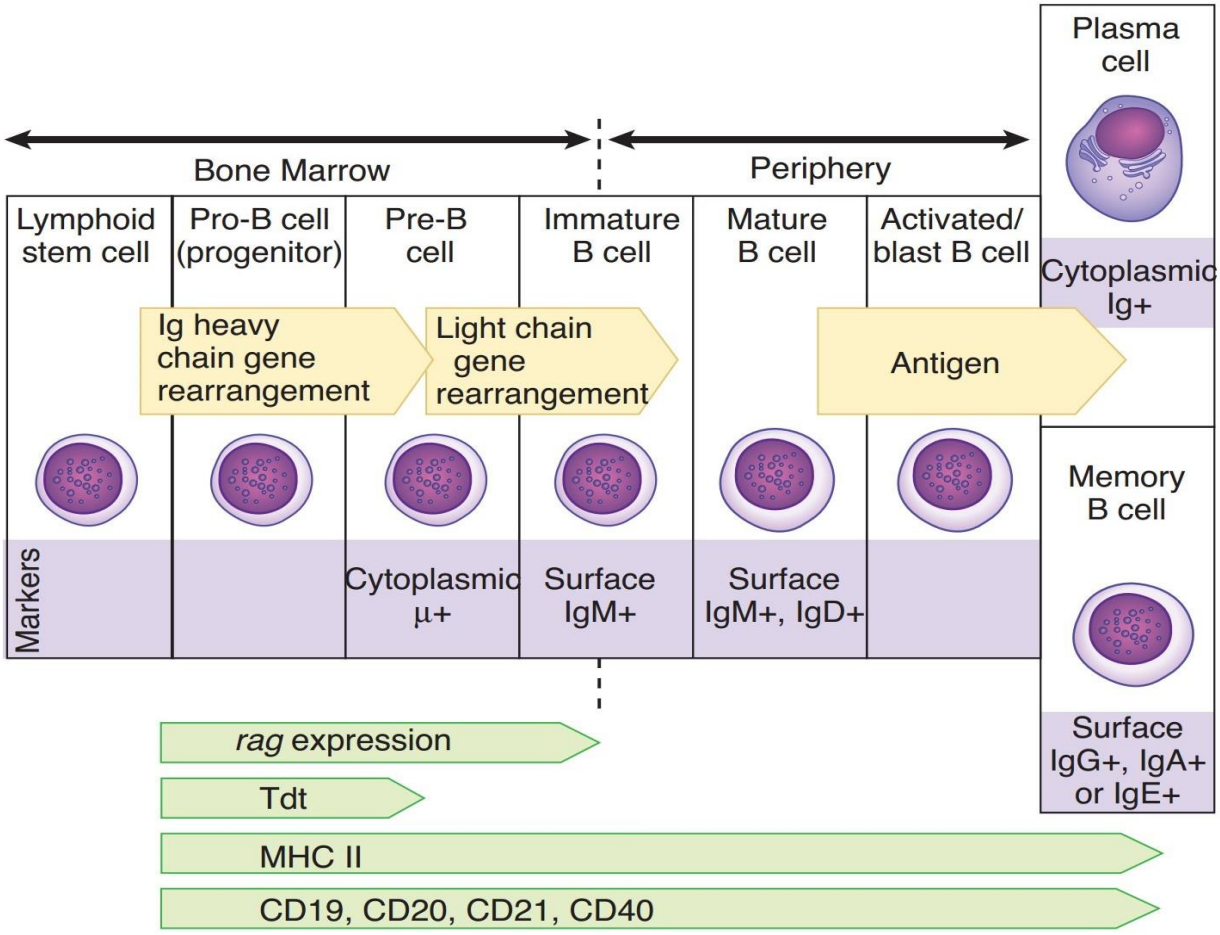
❖ N.B:

- In Severe combined immunodeficiency (SCID), there is an autosomal recessive nonsense mutation in rag 1 or rag2 genes (which encodes for recombinase enzyme needed for gene rearrangement) → Total lack of B and T cells → Total defects in humoral and cell mediated immunity.

The Selection of Lymphocytes

Selection of B lymphocytes

- As lymphoid progenitors develop in the bone marrow, we have seen that they make random rearrangements of their germline DNA to produce the unique idiotypes of antigen-recognition molecules that they will use throughout their lives.
- Primary lymphoid organs are sites of lymphoid-cell development (lymphopoiesis): Bone marrow (B lymphocytes), Thymus (T lymphocytes).
- Secondary lymphoid organs are sites of antigen exposure: Spleen, Lymph nodes, Mucosal-associated lymphoid tissues.
- B lymphocytes complete their development in the Bone marrow.
- Because these gene segment rearrangements occur randomly and in the absence of stimulation with foreign antigen, it stands to reason that many of the idiotypes of receptors produced could have a binding attraction or affinity for normal body constituents. These cells, if allowed to develop further, could develop into self-reactive.
- Lymphocytes that could cause harm to the host. Therefore, one of the key roles of the bone marrow stroma and interdigitating cells is to remove such potentially harmful products.
- Cells whose idiotype has too great affinity for normal cellular molecules are either deleted in the bone marrow (clonal deletion) or inactivated in the periphery (clonal anergy).
- In such a way, only those cells that are selectively unresponsive (tolerant) to self-antigens are allowed to leave the bone marrow.

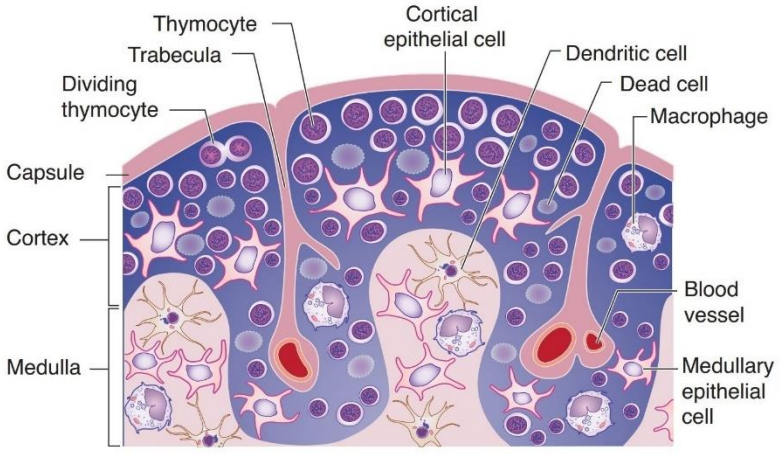


B-Cell Differentiation

Selection of T lymphocytes

- Immature lymphocytes destined to the T-cell lineage leave the bone marrow and proceed to the thymus, the second primary lymphoid organ dedicated to the maturation of T cells.

- The thymus is a bilobed structure located above the heart that consists of an outer cortex packed with immature T cells and an inner medulla into which cells pass as they mature.



- As the developing thymocytes begin to express their TCRs, they are subjected to a rigorous two-step selection process.

- Because the TCR is designed to bind antigenic peptides presented on the surface of antigen-presenting cells (APCs) in the body, a selection process is necessary to remove those cells that would bind to normal self-antigens and cause autoimmunity, as well as those that have no attraction whatsoever for the surfaces of APCs.

- This is accomplished by exposure of developing thymocytes to high levels of a unique group of membrane-bound molecules known as major histocompatibility complex (MHC) antigens.

- The MHC is a collection of highly polymorphic genes on the short arm of chromosome 6 in the human.

Class I gene products	Class II gene products
HLA-A HLA-B HLA-C	HLA-DP HLA-DQ HLA-DR

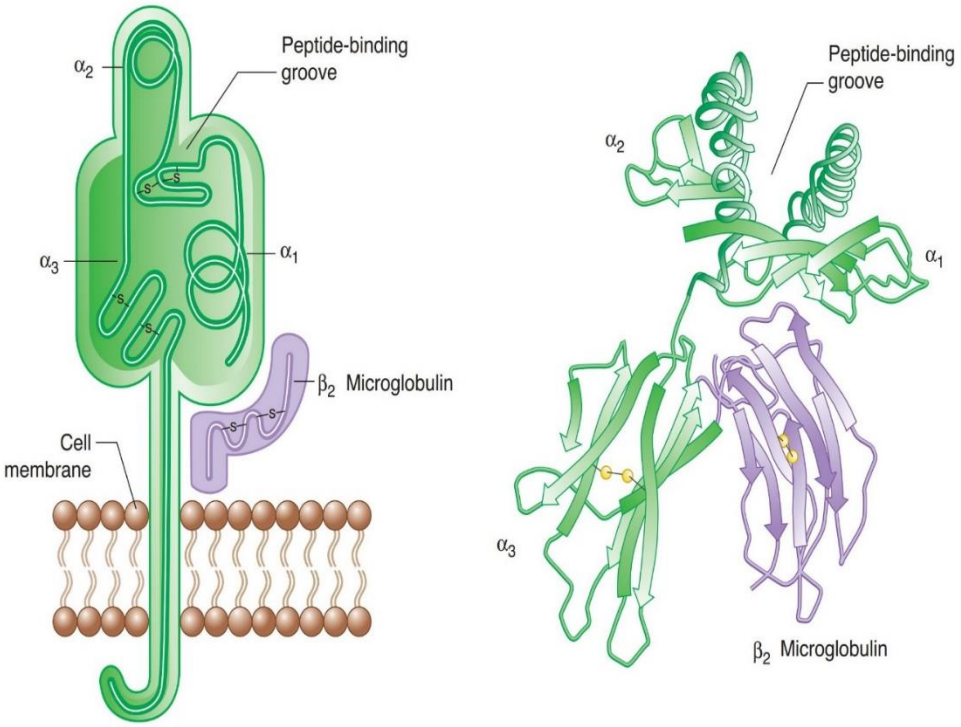
- There are two classes of cell-bound MHC gene products (classes I and II):

A. MHC Class I molecules:

- Expressed on all nucleated cells in the body.

- They are expressed in codominant fashion, meaning that each cell expresses two A, two B, and two C products (one from each parent).

- The molecules (A, B, and C) consist of an α heavy chain with three extracellular domains and an intracytoplasmic carboxy-terminus.



- A second light chain, β2-microglobulin.

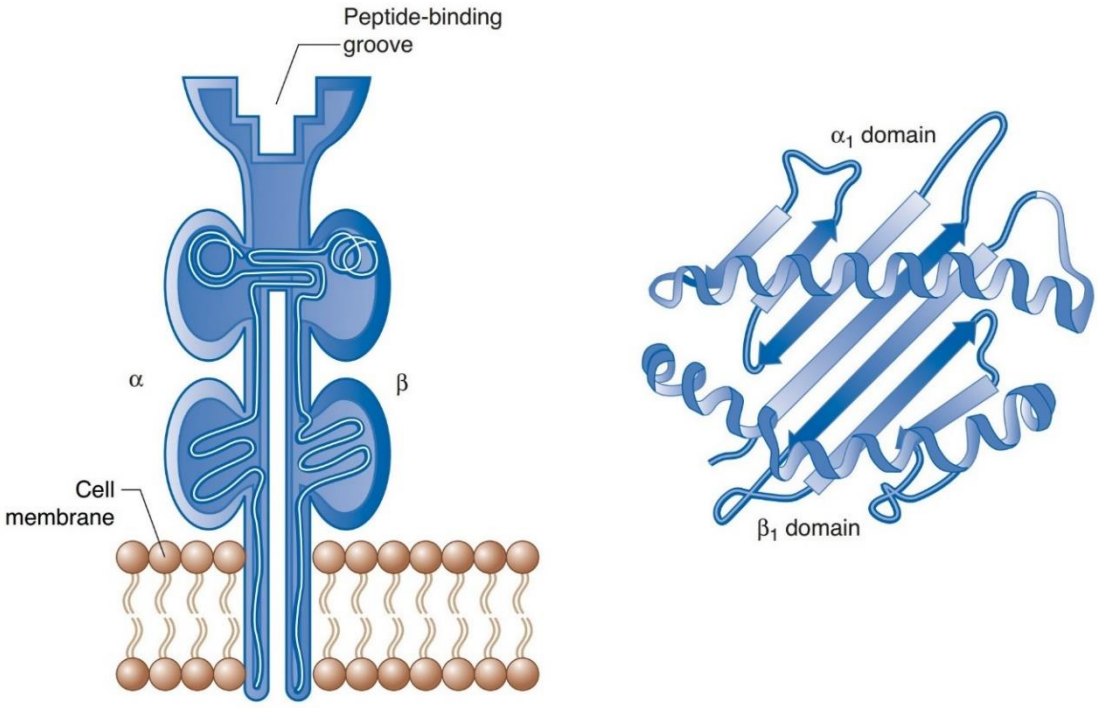
- A groove between the first two extracellular domains of the α chain is designed to accommodate small peptides to be presented to the TCR.

B. MHC Class II molecule:

- Expressed (also codominantly) on the antigen-presenting cells of the body (macrophages, B lymphocytes, dendritic cells, and Langerhans cells).

- The molecules are two chain structures of similar length, called α and β, and each possesses two extracellular domains and one intracytoplasmic domain.

- A groove that will accommodate peptides to be presented to the TCR is formed at the N-terminal end of both chains.



- Within the thymus, each of these MHC products, loaded with normal self-peptides, is presented to the developing thymocytes:

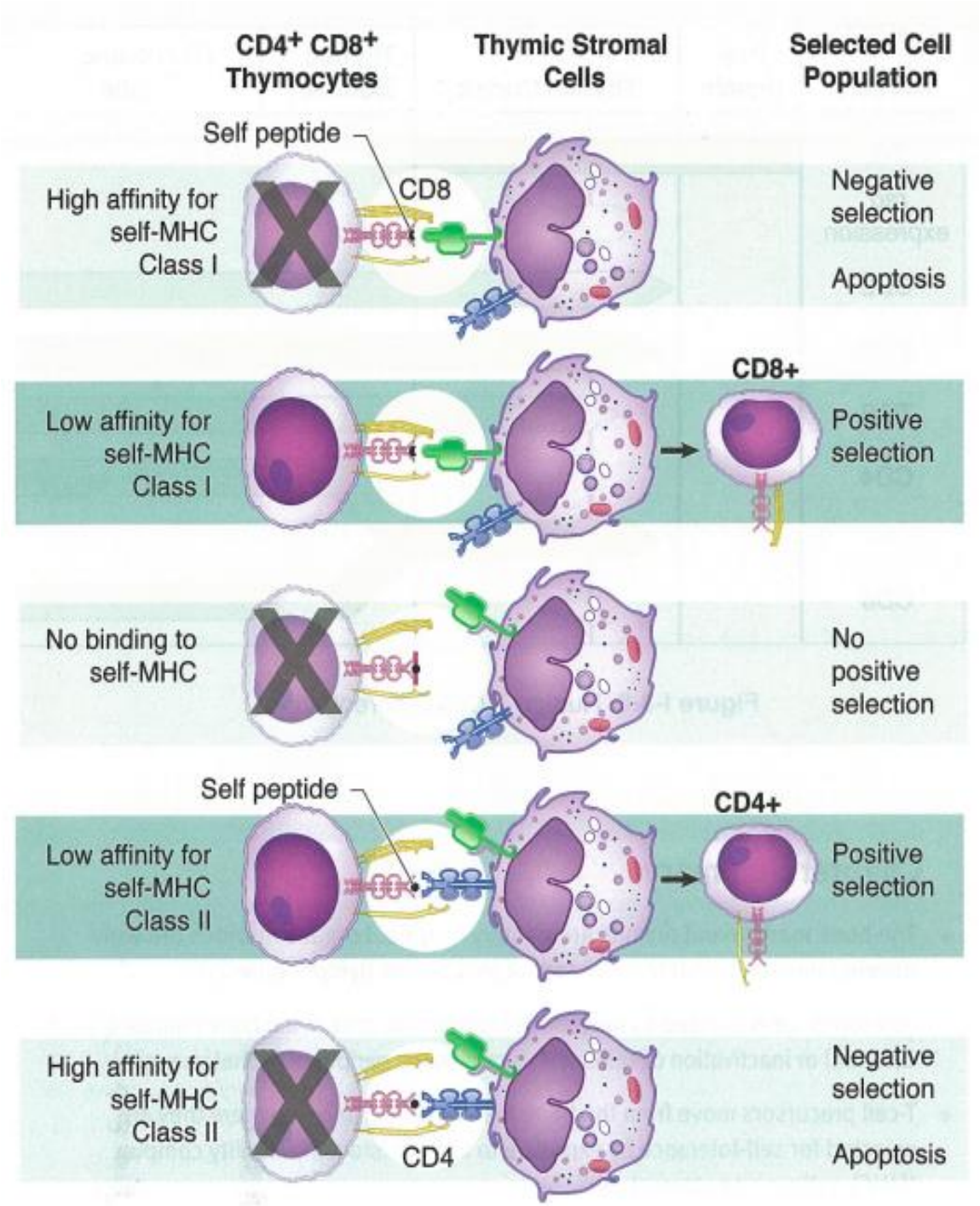
1. Those that have TCRs capable of binding with low affinity will receive a positive selection signal to divide and establish clones that will eventually mature in the medulla.

2. Those that fail to recognize self-MHC at all will not be encouraged to mature (failure of positive selection).

3. Those that bind too strongly to self MHC molecules will be induced to undergo apoptosis (negative selection) because these cells would have the potential to cause autoimmune disease.

- Although immature thymocytes express two accessory molecules on their surfaces designed to stabilize the interaction between MHC and TCR called CD4 and CD8, as the affinity of the TCR for class I or class II MHC is "evaluated;' the cells are directed to express only CD8 if their TCR binds class I molecules and only CD4 if their TCR binds class II molecules.

- This selection process is an extraordinarily rigorous one. A total of 95 to 99% of all T-cell precursors entering the thymus are destined to die there.



- Only those with TCRs appropriate to protect the host from foreign invaders will be permitted to leave to the periphery: CD4+ cells that recognize class II MHC are destined to become "helper" T cells (TH), and CD8+ cells that recognize class I MHC are destined to become cytotoxic T cells (CTLs).

❖ In a nutshell:

- Immature T-lymphocytes express both the CD4 and CD8 cell surface antigens in addition to a complete TCR or a pro-TCR.
- These lymphocytes exist in the thymic cortex where they undergo positive selection and in the thymic medulla where they undergo negative selection.
- Cells with "good" receptors receive positive selection.
- Cells with "useless" receptors receive no positive selection.
- Cells with "bad" receptors receive negative selection.
- CD4+ cells that recognize class II MHC = TH cells.
- CD8+ cells that recognize class I MHC = CTLs.

Lymphocyte Recirculation and Homing

- Lymphocytes of the B- and T-cell lineages that have completed their selection in the bone marrow and thymus respectively are now mature, naive lymphocytes ready to begin their role in the surveillance of the body against invaders.

- These mature, naive lymphocytes will begin the process of recirculation through the body, which is essential for ensuring that the limited number of cells with receptors for a specific antigen is enabled to search for that antigen throughout the body.

- Naive cells preferentially recirculate through the peripheral (secondary) lymphoid organs, the lymph nodes, spleen, and mucosal-associated lymphoid tissue (MALT) to maximize the chances of encounter with foreign antigen and thereby initiate specific immune responses.

Lymph nodes

- Lymph nodes are the small nodular aggregates of secondary lymphoid tissue found along the lymphatic channels of the body and are designed to initiate immune responses to tissue-borne antigens.

- Functions are nonspecific filtration by macrophages, storage of B and T cells, and immune response activation.

- Each lymph node is surrounded by a fibrous capsule that is punctured by afferent lymphatics, which bring lymph into the subcapsular sinus.

- The fluid percolates through an outer cortex area that contains aggregates of cells called follicles.

- The lymph then passes into the inner medulla and the medullary sinus before leaving the node through the hilum in an efferent lymphatic vessel.

- Ultimately, lymph from throughout the body is collected into the thoracic duct, which empties into the vena cava and returns it to the blood.

- <u>Follicle:</u>

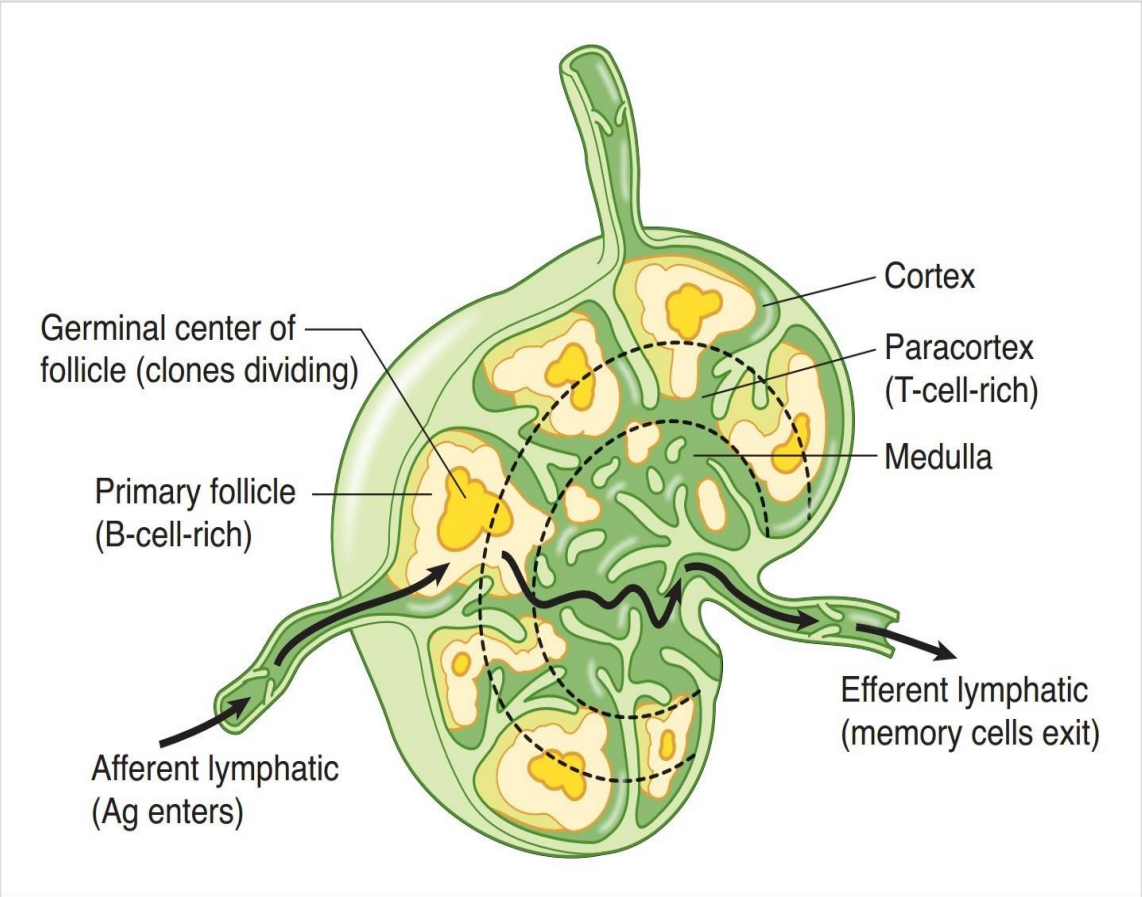
- In outer cortex.
- Site of B-cell localization and proliferation.
- 1° follicles are dense and dormant.
- 2° follicles have pale central germinal centers and are active.

- Paracortex:

- Houses T cells.
- Region of cortex between follicles and medulla.
- Not well developed in patients with DiGeorge syndrome.
- Paracortex enlarges in an extreme cellular immune response (EBV and other viral infections → paracortical hyperplasia → lymphadenopathy).

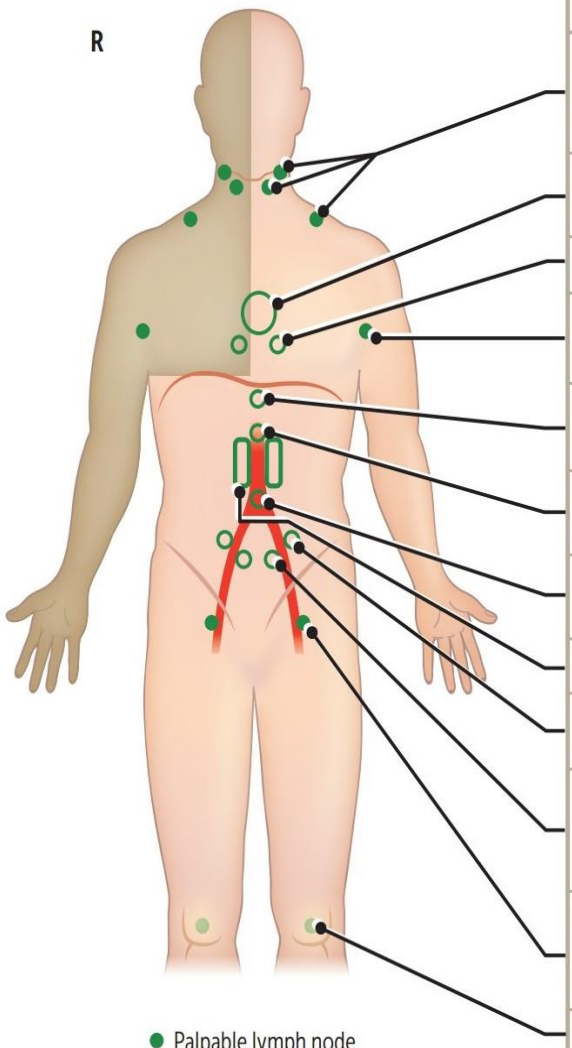
- Medulla:

- Consists of medullary cords (closely packed lymphocytes and plasma cells) and medullary sinuses.
- Medullary sinuses communicate with efferent lymphatics and contain reticular cells and macrophages.



Compartmentalization of a Lymph Node

Lymphatic drainage associations



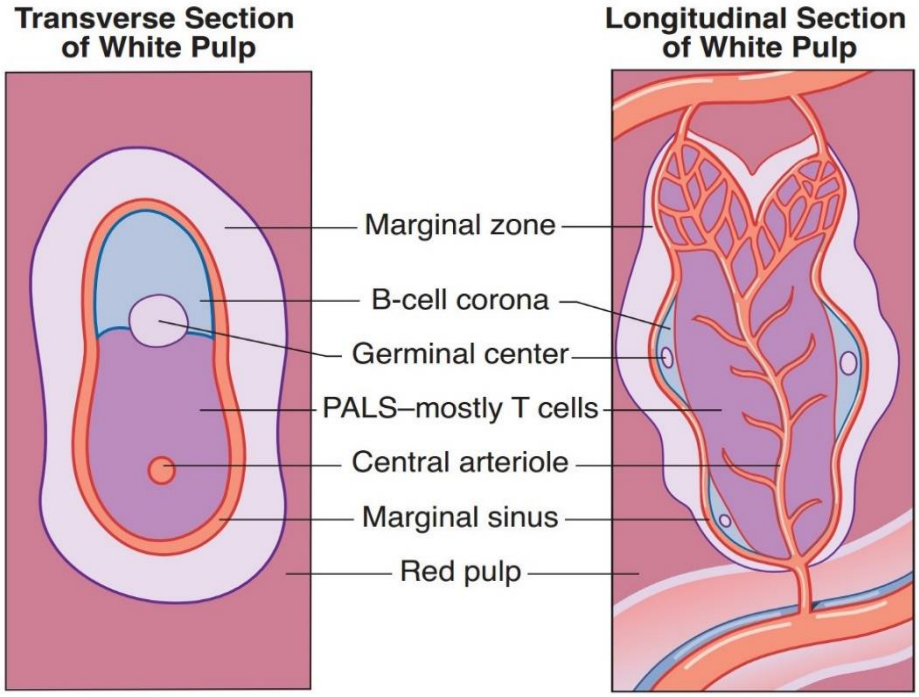
Lymph node cluster	Area of body drained	Associated pathology
Cervical, supraclavicular	Head and neck	Upper respiratory tract infection Infectious mononucleosis Kawasaki disease
Mediastinal	Trachea and esophagus	Primary lung cancer Granulomatous disease
Hilar	Lungs	Granulomatous disease
Axillary	Upper limb, breast, skin above umbilicus	Mastitis Metastasis (especially breast cancer)
Celiac	Liver, stomach, spleen, pancreas, upper duodenum	Mesenteric lymphadenitis Typhoid fever Ulcerative colitis Celiac disease
Superior mesenteric	Lower duodenum, jejunum, ileum, colon to splenic flexure	
Inferior mesenteric	Colon from splenic flexure to upper rectum	
Para-aortic	Testes, ovaries, kidneys, uterus	Metastasis
External iliac	Cervix, superior bladder, and body of uterus	Sexually transmitted infections Medial foot/leg cellulitis (superficial inguinal)
Internal iliac	Lower rectum to anal canal (above pectinate line), bladder, vagina (middle third), cervix, prostate	
Superficial inguinal	Anal canal (below pectinate line), skin below umbilicus (except popliteal area), scrotum, vulva	
Popliteal	Dorsolateral foot, posterior calf	Lateral foot/leg cellulitis

Right lymphatic duct drains right side of body above diaphragm into junction of the right subclavian and internal jugular vein

Thoracic duct drains below the diaphragm and left thorax and upper limb into junction of left subclavian and internal jugular veins (rupture of thoracic duct can cause chylothorax)

Spleen

- The spleen is the secondary lymphoid organ designed to initiate immune responses to blood-borne antigens.
- A single splenic artery enters the capsule at the hilum and branches into arterioles, which become surrounded by lymphocytes, the periarteriolar lymphoid sheaths (PALS).
- This constitutes the white pulp.
- T cells are found in the periarteriolar lymphatic sheath (PALS) within the white pulp.
- B cells are found in follicles within the white pulp.
- The marginal zone, in between the red pulp and white pulp, contains APCs and specialized B cells, and is where APCs capture blood-borne antigens for recognition by lymphocytes.
- Macrophages found nearby in spleen remove encapsulated bacteria.
- The arterioles ultimately end in vascular sinusoids, which make up the red pulp.
- From here, venules collect blood into the splenic vein, which empties into the portal circulation.



Structure of the Spleen

The First Response to Antigen

- An immunogen is a substance that can stimulate the immune system to produce an immune response. The terms immunogen and antigens are used interchangeably.

- For a molecule to be an immunogen, it needs to fit three basic criteria:

- It must be recognized as foreign.
- It must have a certain degree of chemical complexity.
- It must have a large molecular weight.

- B lymphocytes are capable of recognizing molecules of almost any chemical composition.

- T lymphocytes recognize peptides only when presented to them in the groove of an MHC molecule on the surface of an antigen-presenting cell.

Antigenic determinants or epitopes

- The immune system does not recognize the antigen molecule as a whole but reacts to structurally limited parts of the molecule called epitopes.

- They are very small, composed of just four to five amino acids or monosaccharide residues.

- They determine the specificity of the antigen.

- The same antigen may possess different epitopes.

- Antigens that share one or more similar epitopes are known as cross-reactive (heterophile antigens).

- Heterophile antigens are a group of similar antigens found in unrelated animals.

- Heterophile antibodies produced against heterophile antigens of one species will cross react with others.

Hapten

- This is a low molecular weight substance which is incapable of inducing immune response alone but when coupled with a carrier molecule (protein) it can act as an antigen.

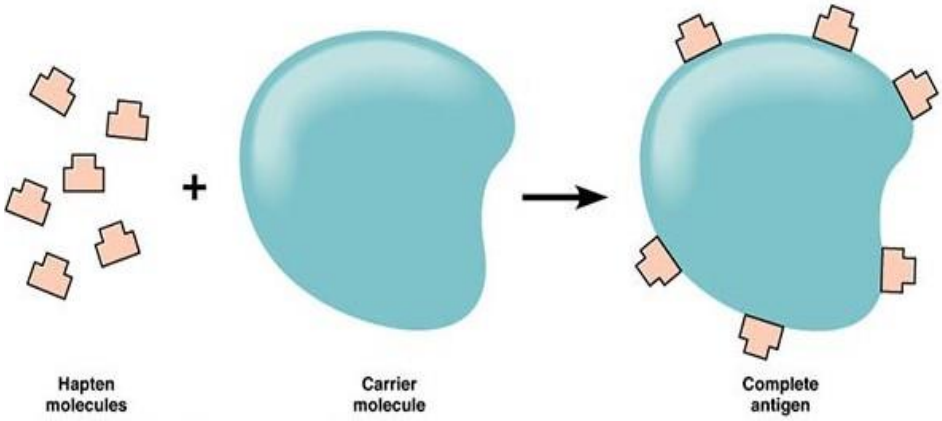
- Examples of haptens are drugs (penicillin).

- Drug allergies to penicillin and other agents such as streptomycin, aspirin, sulfa drugs, succinyl choline, and some opiates can be induced by small doses of the drug and are not consequences of the pharmacologic or physiologic effects of the drugs.

- Most drugs are low molecular weight compounds that are not capable of inducing immune responses by themselves they act as haptens.

- Inside the body, however, these agents can become conjugated to body proteins (the carrier), and the hapten-carrier conjugate serves as the immunogen for the ensuing allergic response.

- Typically, an allergic response occurs 7 to 14 days following exposure, and the first symptoms may be mild. Subsequent drug exposures can result in severe and life-threatening anaphylaxis.



Acute inflammatory response

- Antigens are normally introduced into the body across the mucosa or the epithelia.

- The acute inflammatory response is often the first response to this invasion and represents a response of the innate immune system to block the challenge.

- The first step in the acute inflammatory response is activation of the vascular endothelium in the breached epithelial barrier.

- Cytokines and other inflammatory mediators released in the area as a result of tissue damage induce expression of selectin-type adhesion molecules on the endothelial cells.

- Neutrophils are usually the first cell to bind to the inflamed endothelium and extravasate into the tissues, peaking within 6 hours.

- Monocytes, macrophages, and even eosinophils may arrive 5 to 6 hours later in response to neutrophil-released mediators.

Steps in Extravasation

- The extravasation of phagocytes into the area requires four sequential, overlapping steps:

A. Step 1: Rolling

- Phagocytes attach loosely to the endothelium by low-affinity, selectin-carbohydrate interactions.

- E-selectin molecules on the endothelium bind to mucin-like adhesion molecules on the phagocyte membrane and bind the cell briefly, but the force of blood flow into the area causes the cell to detach and reattach repeatedly, rolling along the endothelial surface until stronger binding forces can be elicited.

B. Step 2: Activation by chemoattractants

- Chemokines released in the area during inflammation, such as interleukin 8 (IL-8), complement split product C5a, and N-formyl peptides produced by bacteria bind to receptors on the phagocyte surface and trigger a G-protein-mediated activating signal.

- This signal induces a conformational change in integrin molecules in the phagocyte membrane that increases their affinity for immunoglobulin-superfamily adhesion molecules on the endothelium.

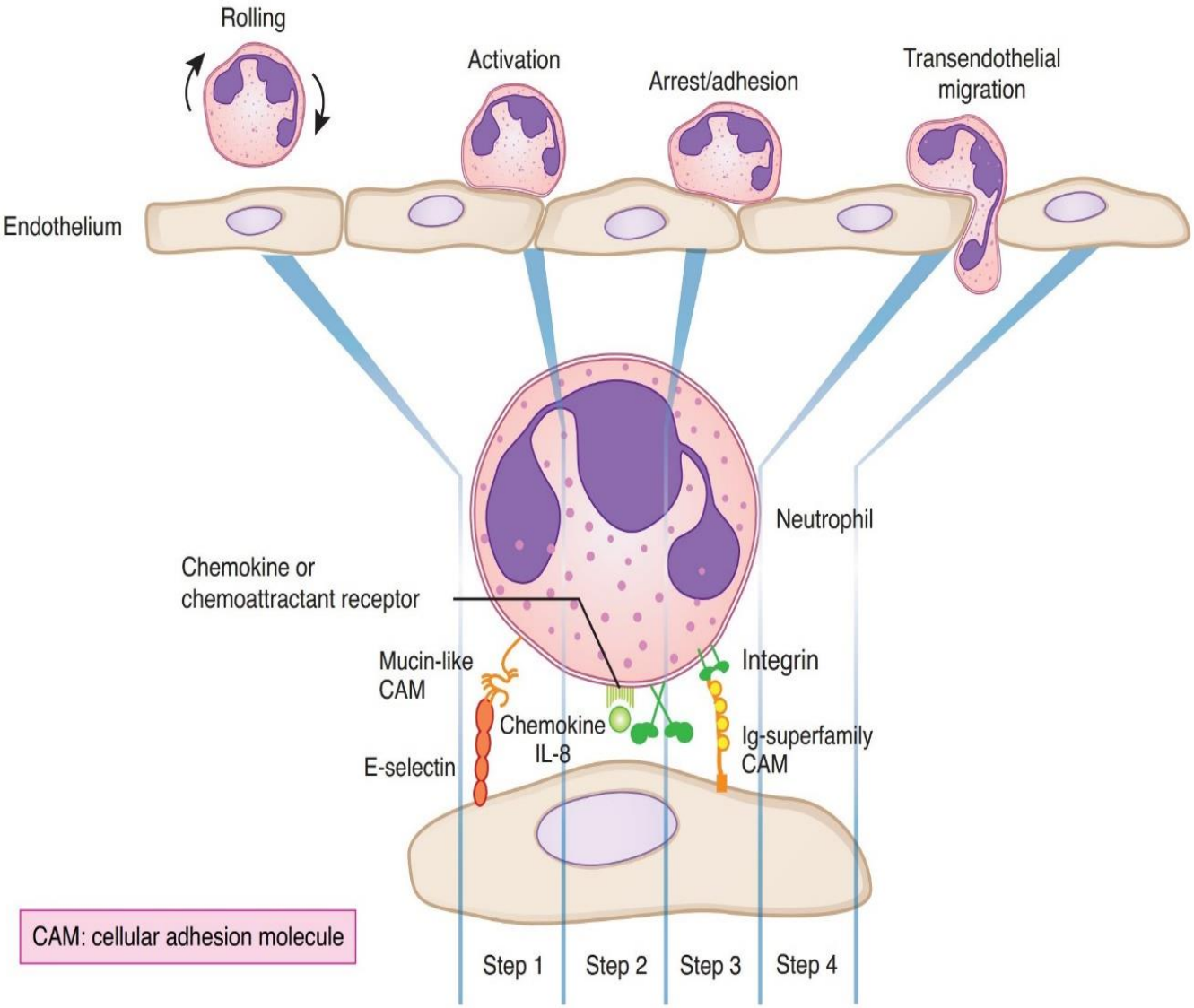
C. Step 3: Arrest and adhesion

- Interaction between integrins and Ig-superfamily cellular adhesion molecules (Ig CAMs) mediates the tight binding of the phagocyte to the endothelial cell.

- These integrin-Ig-CAM interactions also mediate the tight binding of phagocytes and their movement through the extracellular matrix.

D. Step 4: Transendothelial migration

- The phagocyte extends pseudopodia through the vessel wall and extravasates into the tissues.



Steps of Phagocyte Extravasation

❖ N.B:

1. Leukocyte adhesion deficiency is a rare autosomal recessive disease in which there is an absence of CD18, which is the common β2 chain of a number of integrin molecules. A key element in the migration of leukocytes is integrin-mediated cell adhesion, and these patients suffer from an inability of their leukocytes to undergo adhesion-dependent migration into sites of inflammation.

- The first indication of this defect is often omphalitis, a swelling and reddening around the stalk of the umbilical cord.
- These patients susceptible to suffer recurrent, chronic bacterial infections.
- These patients frequently have abnormally high numbers of granulocytes in their circulation, but migration into sites of infection is not possible, so abscess and pus formation do not occur.
- Bacterial infections in these patients can be treated with antibiotics, but they recur.

2. Neutrophils release chemoattractive factors that call in other phagocytes:

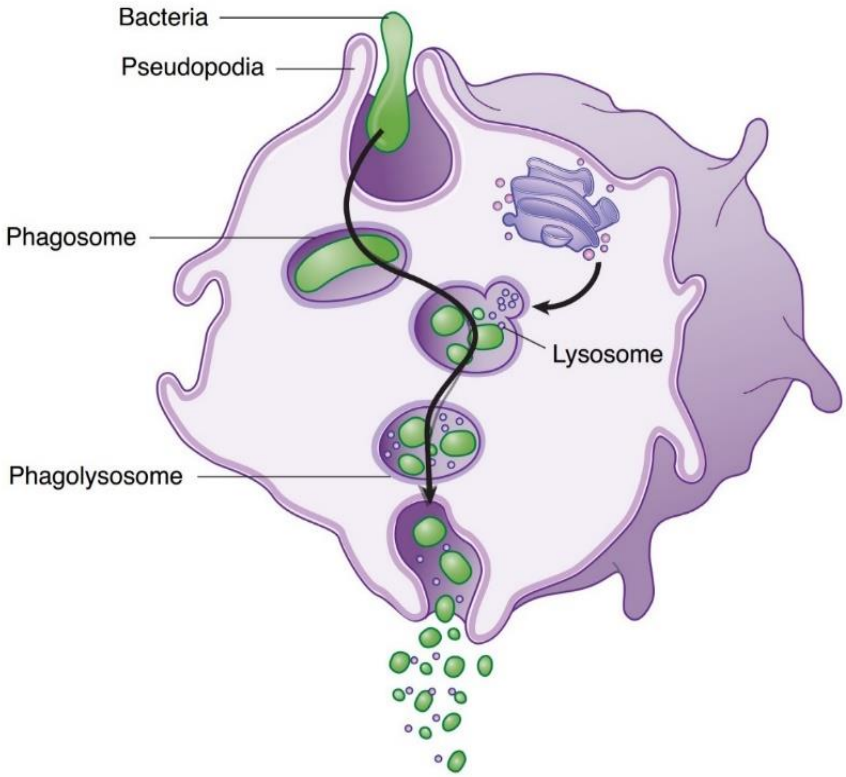
Chemoattractive Molecule	Origin
Chemokines (IL-8)	Tissue mast cells, platelets, neutrophils, monocytes, macrophages, eosinophils, basophils, lymphocytes
Complement split product C5a	Classical or alternative pathways
Leukotriene B4	Membrane phospholipids of macrophages, monocytes, neutrophils, mast cells →arachidonic acid cascade → lipoxygenase pathway.
Formyl methionyl peptides	Released from microorganisms

Phagocytosis

- Once chemotaxis of phagocytic cells into the area of antigen entry is accomplished, these cells ingest and digest particulate debris, such as microorganisms, host cellular debris, and activated clotting factors.

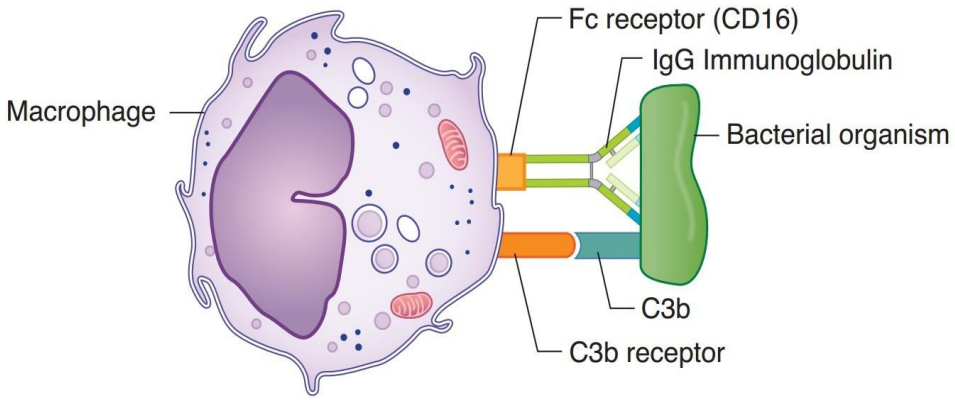
- <u>This process, called phagocytosis, involves:</u>

- Extension of pseudopodia to engulf attached material.
- Fusion of the pseudopodia to trap the material in a phagosome.
- Fusion of the phagosome with a lysosome to create a phagolysosome.
- Digestion.
- Exocytosis of digested contents.

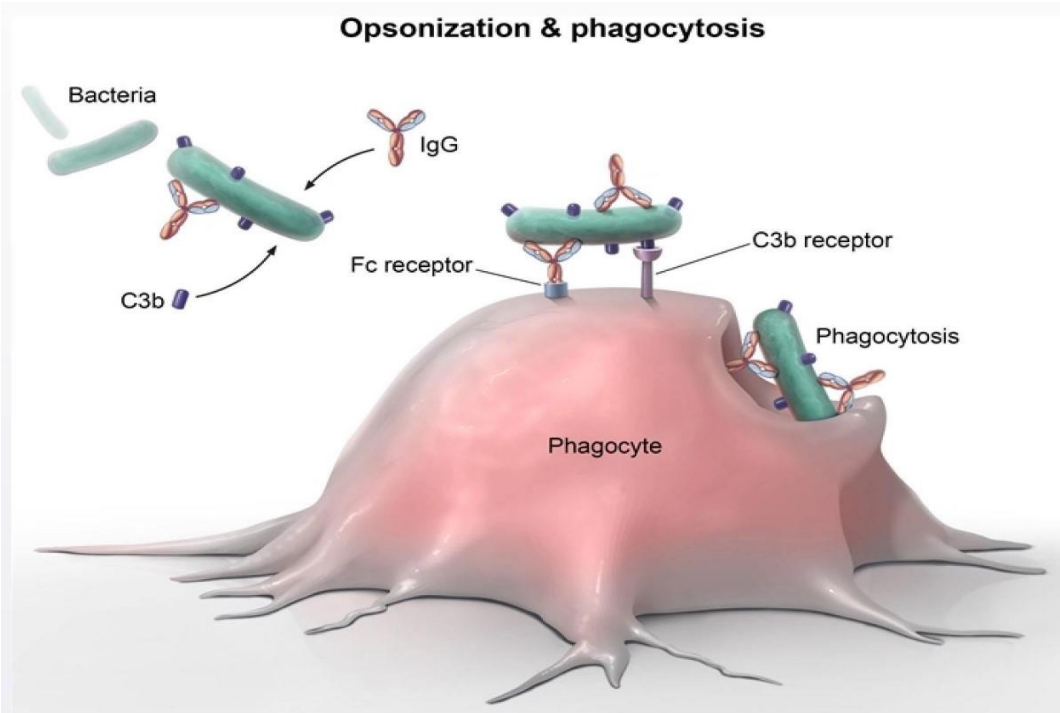


Opsonization

- Both macrophages and neutrophils have membrane receptors for certain types of antibody (IgG) and certain complement components (C3b).
- If an antigen is coated with either of these materials, adherence and phagocytosis is enhanced.
- Thus, antibody and complement are called opsonins, and the means by which they enhance phagocytosis is called opsonization.
- Protein A of Staphylococcus aureus impedes opsonization by binding to the Fc component of lgG.



Opsonization of Bacteria with Antibody and Complement C3b



Intracellular Killing

- During phagocytosis, a metabolic process known as the respiratory burst activates a membrane-bound oxidase that generates oxygen metabolites, which are toxic to ingested microorganisms.

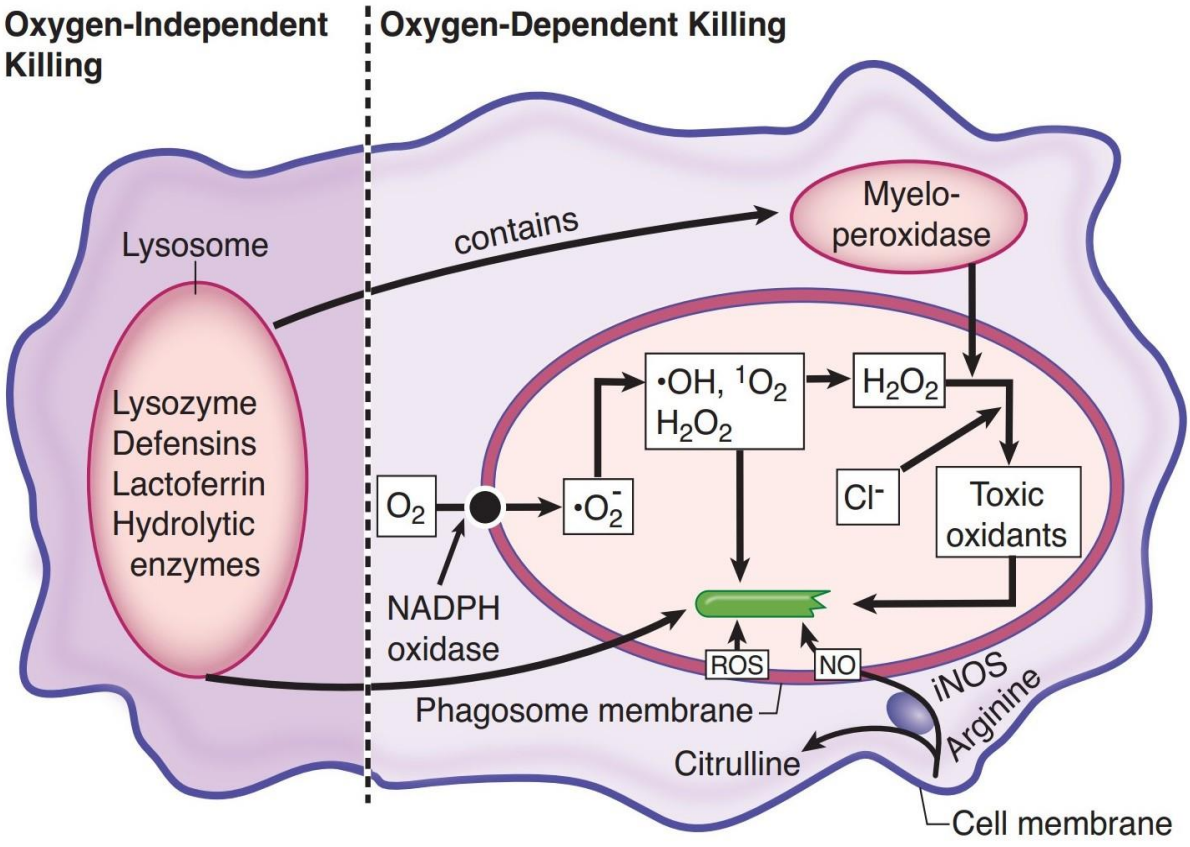
- Two oxygen-dependent mechanisms of intracellular digestion are activated as a result of this process:

1. NADPH oxidase reduces oxygen to superoxide anion, which generates hydroxyl radical and hydrogen peroxide, which are microbicidal.

2. Myeloperoxidase in the lysosomes acts on hydrogen peroxide and chloride ions to produce hypochlorite (the active ingredient in household bleach), which is microbicidal.

- In addition, the lysosomal contents of phagocytes contain oxygen-independent degradative materials:

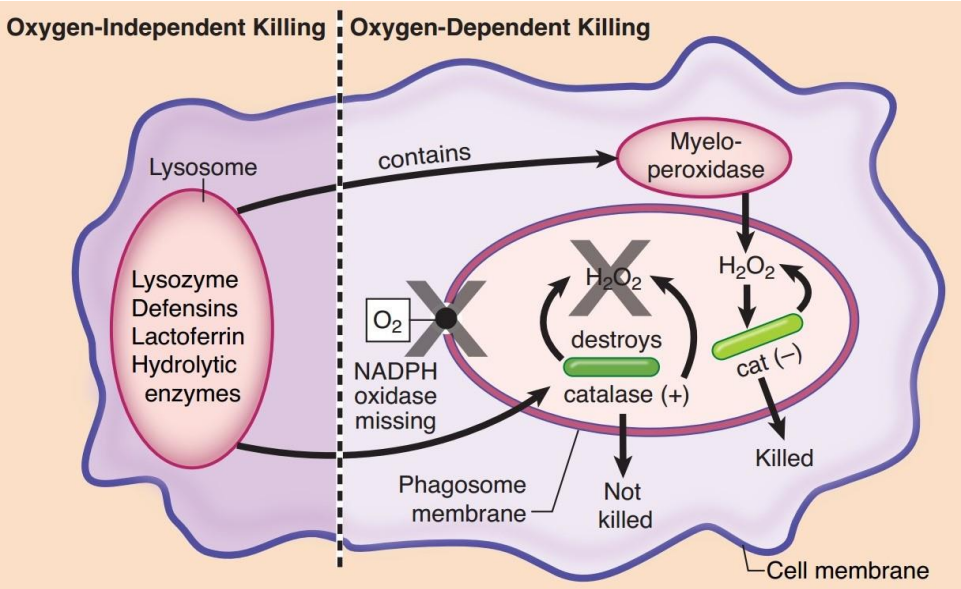
- Lysozyme: digests bacterial cell walls by cleaving peptidoglycan.
- Defensins: circular peptides that form channels in bacterial cell membranes.
- Lactoferrin: chelates iron.
- Hydrolytic enzyme.



❖ N.B:

1. Chronic granulomatous disease (CGD) is an inherited deficiency in the production of neutrophils NADPH oxidase.

- This defect eliminates the phagocyte's ability to produce many critical oxygen-dependent intracellular metabolites (O_2 -, OH, and H_2O_2).
- The two other intracellular killing mechanisms remain intact (myeloperoxidase + H_2O_2 → HOCl and lysosomal contents).
- If the patient is infected with a catalase-negative organism, the H_2O_2 waste product produced by the bacterium can be used as a substrate for myeloperoxidase, and the bacterium is killed.
- If, however, the person is infected with a catalase-positive organism (Staphylococcus aureus, Pseudomonas cepacia, Serratia marcescens, Nocardia species, Aspergillus species) the myeloperoxidase system lacks its substrate (because these organisms destroy H_2O_2), and the patient is left with the oxygen-independent lysosomal mechanisms that prove inadequate to control rampant infections.
- Thus, CGD patients suffer from chronic, recurrent infections with catalase-positive organisms.

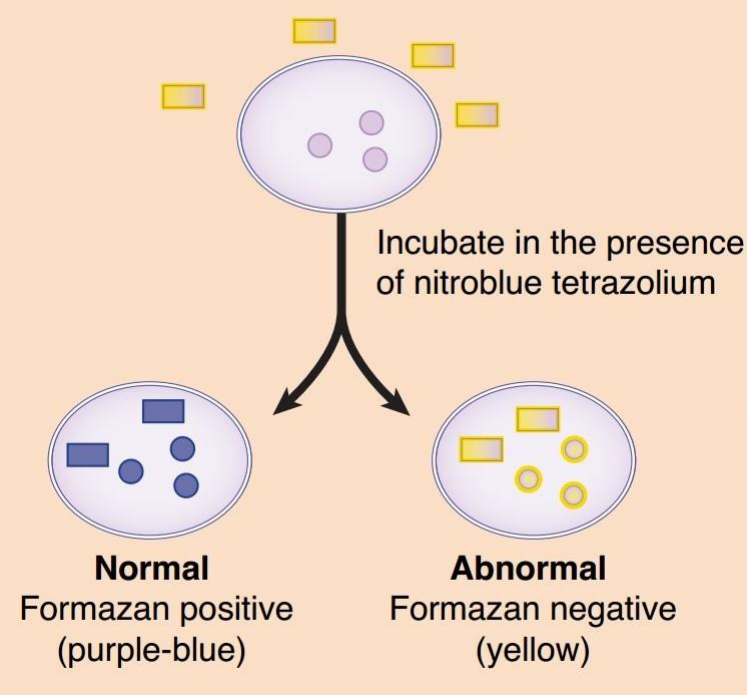


- Failures of phagocytic cells to generate oxygen radicals are easily detected by the nitroblue tetrazolium.

- The nitroblue tetrazolium test is carried out by adding nitroblue tetrazolium to a sample of patient neutrophils:

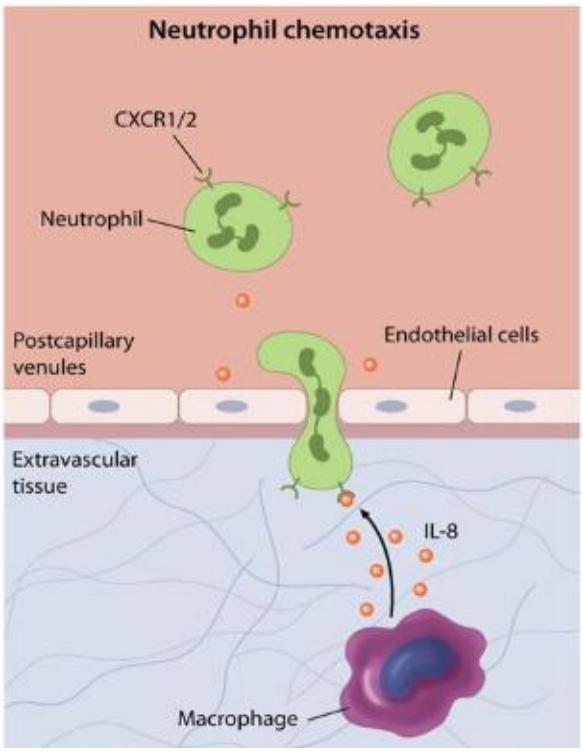
- Properly functioning neutrophils are able to produce reactive oxygen species such as superoxide, and these chemicals are able to reduce nitroblue tetrazolium, leading to formation of a dark blue pigment within the cells.

- Cells from patients with CGD are unable to reduce nitroblue tetrazolium because they cannot produce reactive oxygen species due to a genetic defect resulting in NADPH oxidase deficiency.



2. Pus consists of a thin, protein-rich fluid, known as liquor puris, and dead leukocytes, primarily neutrophils.

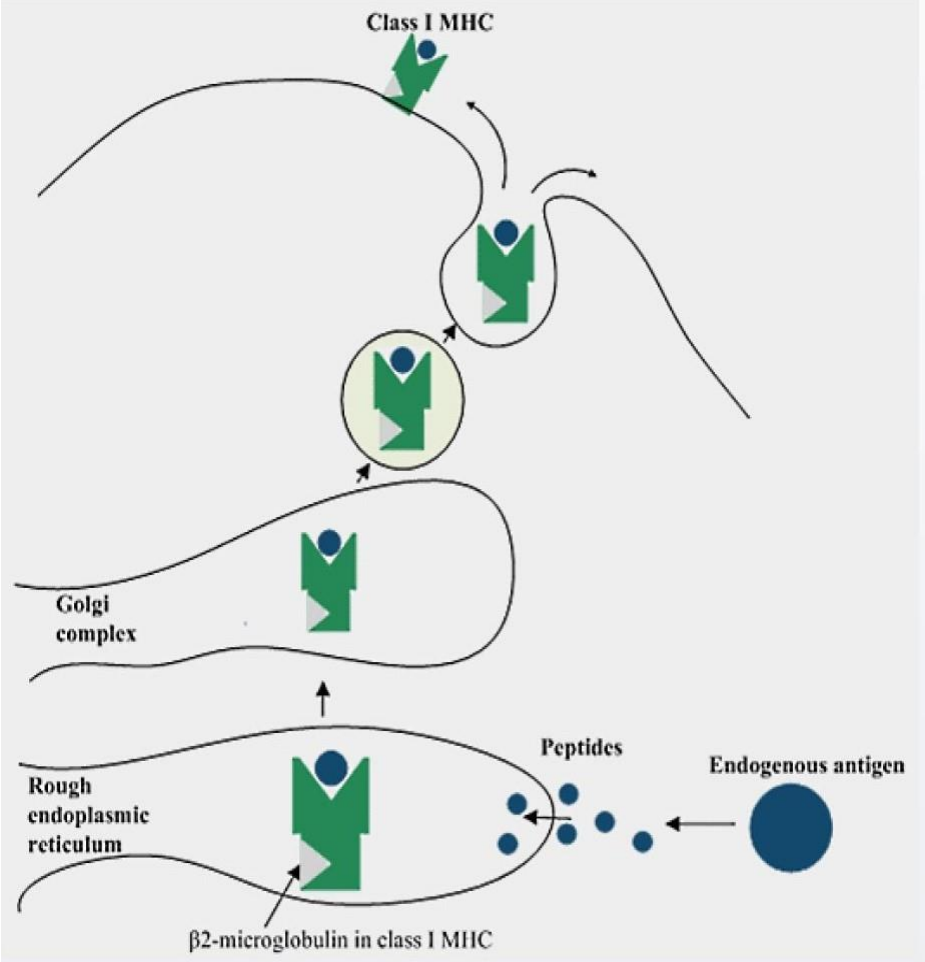
- During infection, macrophages and surrounding endothelial cells release cytokines such as interleukin-8 (IL-8) that trigger neutrophils to enter the site of infection via chemotaxis.
- IL-8 also induces phagocytosis in neutrophils once they have arrived.
- Interleukin-8 is a chemokine produced by macrophages that induces chemotaxis and phagocytosis in neutrophils.
- Other significant chemotactic agents include n-formylated peptides, leukotriene B4, and complement component C5a.



The Processing and Presentation of Antigen

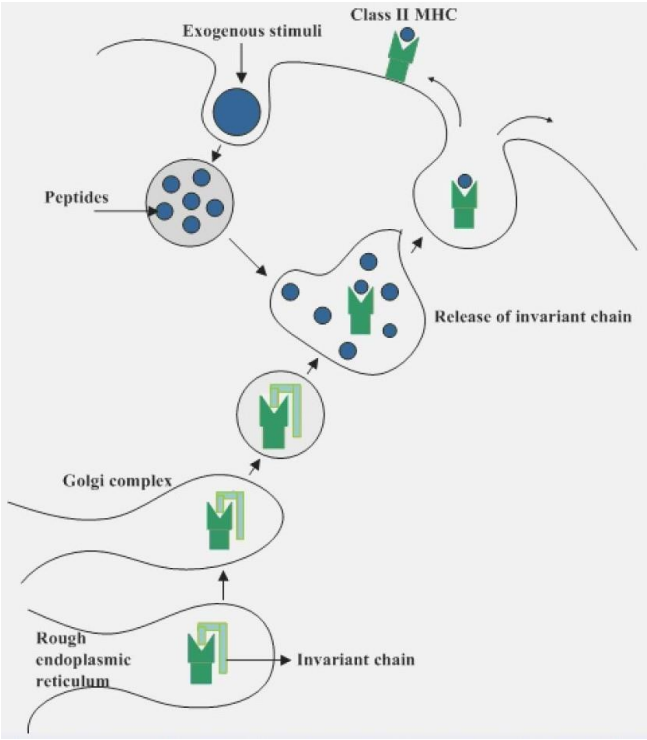
MHC class I

- MHC molecules are designed to bind small peptides and present them to T cells.
- The class I molecule is synthesized in the endoplasmic reticulum of the cell and proteins are loaded there by an endogenous pathway.
- Proteins synthesized in the cell cytosol are routinely degraded in proteasomes, and the peptides from these proteins are transported through a peptide transporter, known as the TAP complex, into the endoplasmic reticulum, where they have the opportunity to bind to freshly synthesized MHC class I proteins.
- These are then transported to the cell membrane where they may be presented to CD8+ T lymphocytes.



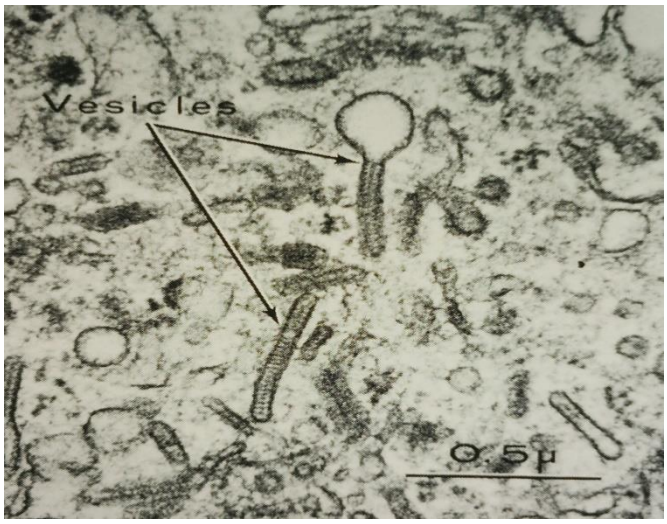
MHC class II

- Class II MHC peptides are displayed only by antigen presenting cells.
- MHC Class II is used to present antigens that antigen presenting cells (dendritic cells, macrophages and B-lymphocytes) have encountered in the body and have taken up by phagocytosis or endocytosis.
- Material in the environment such as bacterial organisms or freely circulating antigenic material is taken up by antigen presenting cells and degraded by acidification after phagosome-lysosome fusion.
- MHC Class II molecules are synthesized in the rough endoplasmic reticulum and routed to the endosomes by the Golgi apparatus.
- Each MHC class II molecule has a peptide fragment called an invariant chain bound to its antigen binding site.
- Fusion of the vesicles containing MHC Class II with the acidified phagolysosomes containing foreign antigen leads to degradation of the invariant chain and loading of antigen onto the MHC Class II molecule.
- The MHC Class II molecule-protein antigen complexes are then displayed on the surface of antigen presenting cells where they are available to bind the T-cell receptors (TCR) on T-lymphocytes and initiate a T-cell response to the antigen they display.
- Without lysosomal acidification, antigen processing in association with MHC class II antigens would not occur, and MHC Class II would be unable to bind antigen and, therefore, unable to bind the TCR.



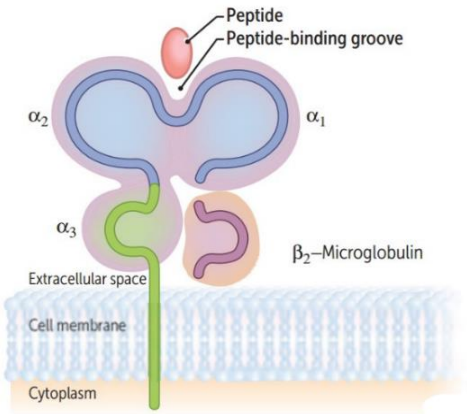
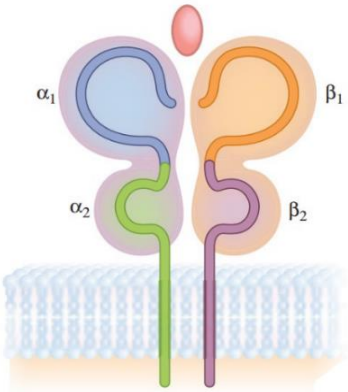
❖ N.B:

- Langerhans cells are dendritic cells found in the skin that act as professional antigen presenting cells.
- Langerhans cells are the form of dendritic cell most commonly found in the skin and mucous membranes.
- These cells are derived from the myeloid cell line and they possess characteristic racquet-shaped intracytoplasmic granules known as Birbeck granules.



Major histocompatibility complex I and II

MHC encoded by HLA genes. Present antigen fragments to T cells and bind T-cell receptors (TCRs).

	MHC I	MHC II
LOCI	HLA-A, HLA-B, HLA-C MHC I loci have 1 letter	HLA-DP, HLA-DQ, HLA-DR MHC II loci have 2 letters
BINDING	TCR and CD8	TCR and CD4
STRUCTURE	1 long chain, 1 short chain	2 equal-length chains (2 α, 2 β)
EXPRESSION	All nucleated cells, APCs, platelets (except RBCs)	APCs
FUNCTION	Present endogenous antigens (eg, viral or cytosolic proteins) to CD8+ cytotoxic T cells	Present exogenous antigens (eg, bacterial proteins) to CD4+ helper T cells
ANTIGEN LOADING	Antigen peptides loaded onto MHC I in RER after delivery via TAP (transporter associated with antigen processing)	Antigen loaded following release of invariant chain in an acidified endosome
ASSOCIATED PROTEINS	β_2-microglobulin	Invariant chain
STRUCTURE		

❖ HLA subtypes associated with diseases:

A3	Hemochromatosis.
B27	Psoriatic arthritis, Ankylosing spondylitis, arthritis of Inflammatory bowel disease, Reactive arthritis (formerly Reiter syndrome). PAIR
DQ2/DQ8	Celiac disease.
DR2	Multiple sclerosis, hay fever, SLE, Goodpasture syndrome.
DR3	Diabetes mellitus type 1, SLE, Graves' disease, Hashimoto thyroiditis.
DR4	Rheumatoid arthritis, diabetes mellitus type 1.
DR5	Pernicious anemia, vitamin B 12 deficiency, Hashimoto thyroiditis.

- Because of the extreme polymorphism of the HLA system in humans, when tissues are transplanted between nonidentical individuals, cells of the transplant are often targeted by CTLs as abnormal.

Activation of T Lymphocyte

- The binding of the TCR of the mature, naive T cell to the MHC peptide complex of the APC provides the first signal to the T cell to begin its activation. This provides the antigenic specificity of the response. The interaction is stabilized by the coreceptors CD4 and CD8 which bind to MHC class II and MHC class I molecules, respectively.

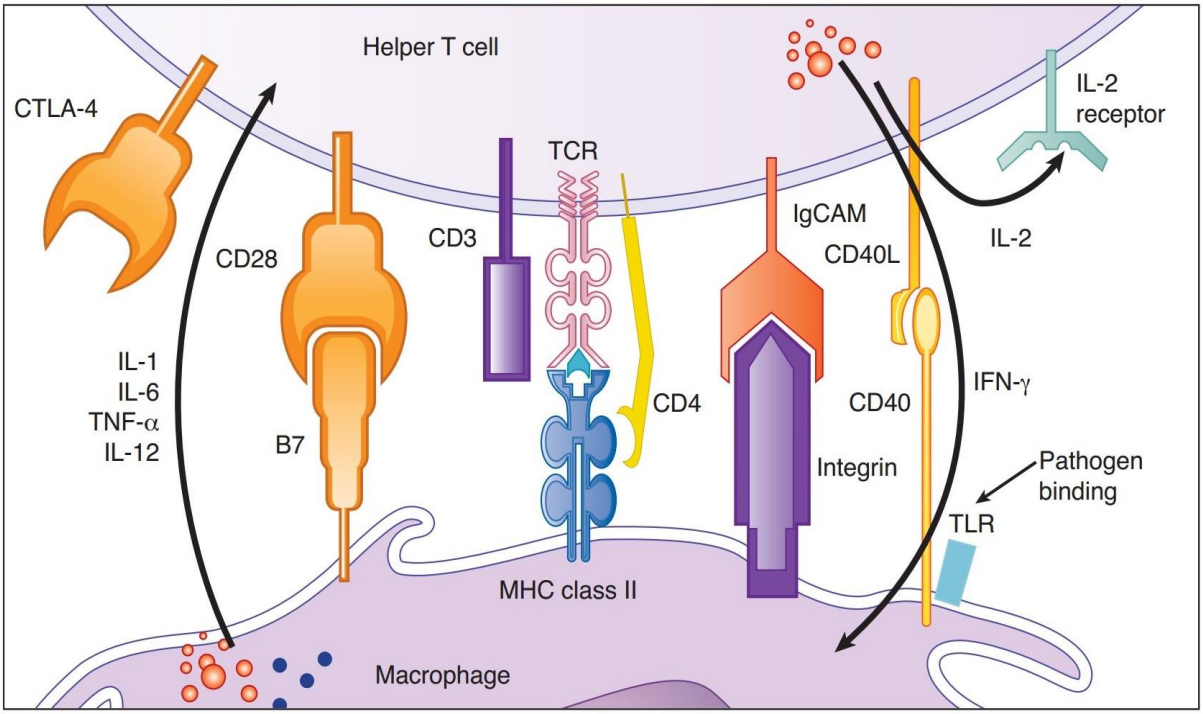
- The costimulatory molecules B7 (CD80/86) on APCs bind to CD28 on the mature, naïve T cells, providing the second signal necessary for successful activation.

- Under normal conditions, B7 is expressed at low levels on APCs. In the presence of infection or inflammation, the expression will increase, enhancing activation of the mature, naïve T cells. Later in the immune response, B7 will preferentially bind to CTLA-4 or PD-1, effectively turning off the T-cell response.

- Intimately associated with the T cell receptor is the CD3 signal transduction complex. Interaction of cell adhesion molecules on the surface of the APCs and T cells allows for the formation of the immune synapse.

- The proliferation of naive T cells in response to antigen recognition is mediated principally by an autocrine growth pathway, in which the responding T cell secretes its own growth-promoting cytokines and also expresses receptor molecules for these factors.

- IL-2 is the most important growth factor for T cells and stimulates the proliferation of clones of T cells specific to that antigen.



❖ N.B:

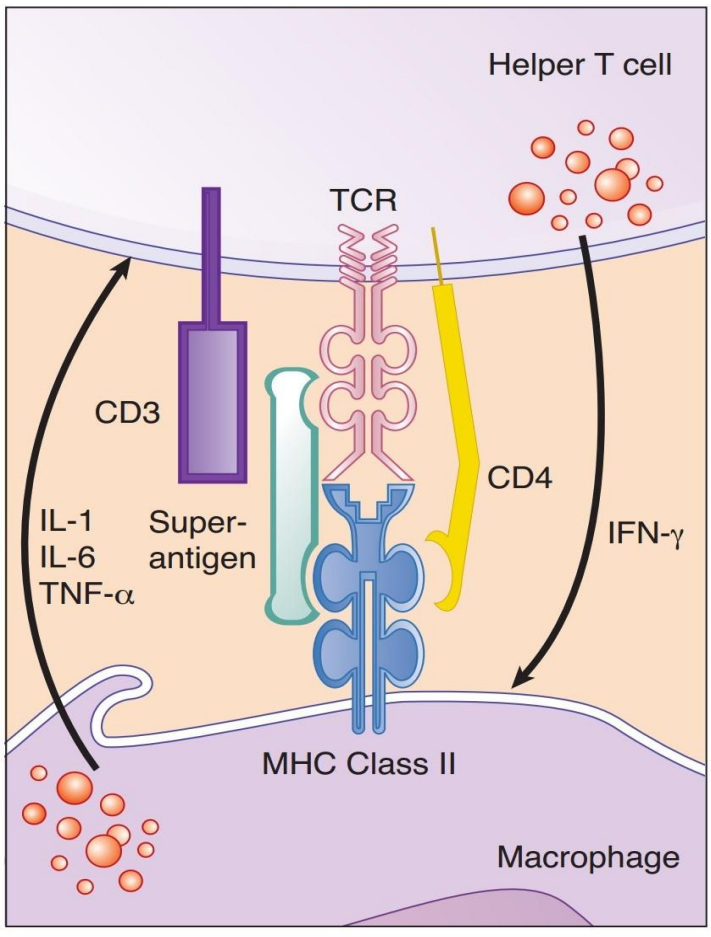
1. Several surface molecules are involved in the activation of mature, naive T lymphocytes:

A. First (primary) signal: recognition of the MHC-peptide complex by the T cell receptor and coreceptors (CD4 and CD8).

B. Second (costimulatory) signal: recognition of B7 by CD28.

2. Superantigens are viral or bacterial proteins that cross-link the variable β domain of a T-cell receptor to an α chain of a class II MHC molecule.

- This cross-linkage provides an activating signal that induces T-cell activation and proliferation, in the absence of antigen-specific recognition of peptides in the MHC class II groove.
- Because superantigens bind outside of the antigen-binding cleft, they activate any clones of T cells expressing a particular variable B sequence and thus cause polyclonal activation of T cells, resulting in the overproduction of IFN-γ.
- This, in turn, activates macrophages, resulting in overexpression of proinflammatory cytokines (IL-1, IL-6 and TNF-α).
- Excess amounts of these cytokines induce systemic toxicity.
- Molecules produced during infectious processes and known to act as superantigens include staphylococcal enterotoxins, toxic-shock syndrome toxin-1 (TSST-1), and streptococcal pyrogenic exotoxins.



Superantigen Activation

Development of the Th1, Th2, and Th17

- The activated CD4+ (helper) T lymphocytes, which have thus been generated in the lymph nodes and spleen following antigen administration, are now ready to serve as the orchestrators of virtually all the possible effector mechanisms that will arise to destroy the antigenic invaders.

- The effector mechanisms that are controlled totally or at least in part by Th cells include antibody synthesis, macrophage activation, cytotoxic T-cell killing, and NK cell killing.

- There are 3 major classes of helper T (Th) cell that arise from the same precursor, the naive Th lymphocyte (or Th_0 cell):

- Th1.
- Th2.
- Th17.

- The pattern of differentiation is determined by the antigen or type of pathogen causing the infection and the cytokines produced in response to the antigen.

A. Differentiation of a Th0 cell into a ThI cell:

- Differentiation of a Th0 cell into a Th1 cell is stimulated by intracellular pathogens (viruses and intracellular bacteria). These pathogens induce a strong innate immune response with the resultant production of IL-12 by macrophages and IFN-γ by NK cells.

- In turn, Th1 cells secrete high levels of the inflammatory cytokine IFN-γ which does the following:

  - Amplifies the Th1 response.
  - Inhibits the Th2 response.
  - Activates macrophages.

B. Differentiation of a Th0 cell into a Th2 cell:

- Differentiation of a Th0 cell into a Th2 cell seems to be encouraged in response to large extracellular parasites such as helminths or allergens.

- Due to the inability to phagocytose these pathogens, there is not significant macrophage or NK-cell stimulation.

- In this way, naive Th0 cells seem to produce IL-4 constitutively, and in the absence of IL-12 stimulation, these cells will upregulate their production of IL-4 to encourage differentiation into Th2 cells.

- Several cytokines are produced by Th2 cells, including IL-4, IL-5, IL-10, and IL-13.

- These cytokines not only determine the stimulatory pathways that the cells will employ, but they also expand and develop the cells of the respective subset.

- For example, IFN-γ produced by Th1 cells promotes further Th1 development and inhibits the proliferation of Th2 cells.

- IL-4 and IL-10 produced by Th2 cells promote Th2 differentiation and inhibit the activation of THI cells.

- Thus, each subset amplifies itself and cross regulates the other set so that immune responses become increasingly polarized over time, reaching extremes in cases where antigen exposure becomes chronic.

C. Differentiation of a Th0 cell into a Th17 cell:

- Differentiation of a Th0 cell into a Th17 cell occurs in the presence of extracellular bacterial and fungal infections.

- Local cells react to the infection by secreting IL-1, IL-6, and TGF-β, inducing the development of Th17 cells.

- The activated Th17 cells will in turn secrete the cytokines IL-17, IL 21 and IL-22:

- IL-17 induces local cells to increase chemokine production recruiting neutrophils.
- IL-22 stabilizes interactions between cells in the endothelium decreasing permeability.
- IL-17 and IL-22 induce secretion of anti-microbials by the endothelium.

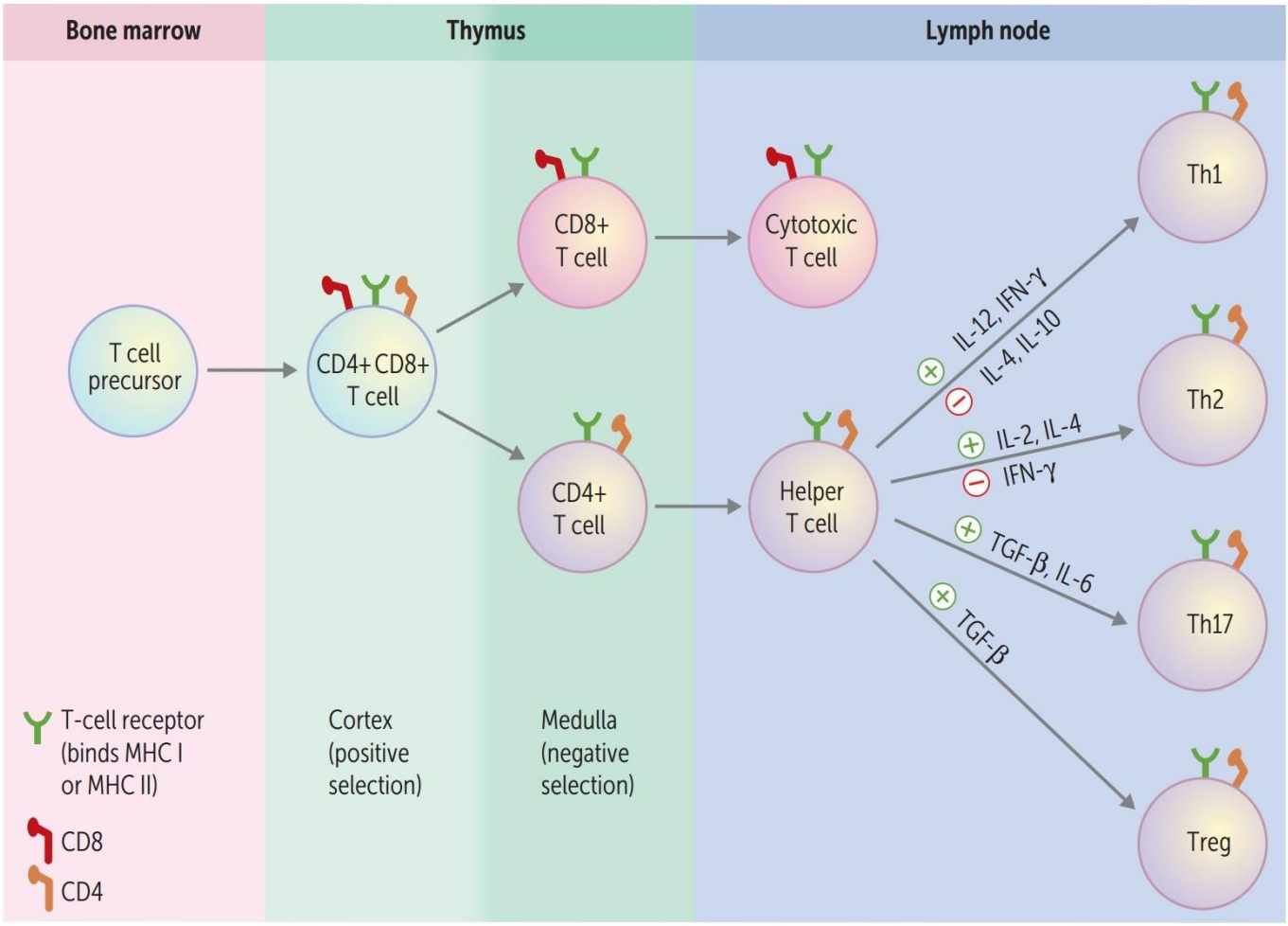
D. Another population of T cells that arises from the Th0 is the T regulatory cell (T Reg cell):

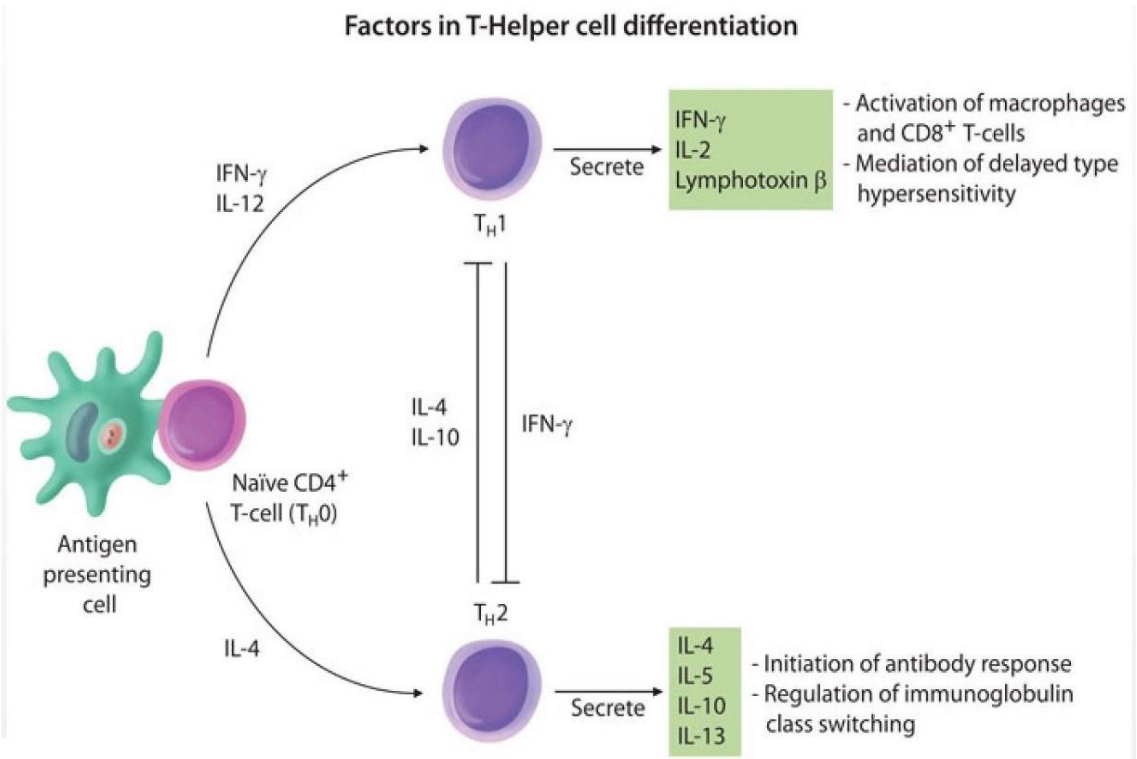
- Help maintain specific immune tolerance by suppressing CD4 and CD8 T-cell effector functions. Have been shown to be critical for the prevention of autoimmunity.

- Identified by expression of CD3, CD4, CD25, and FOXP3.

- Activated regulatory T cells (Tregs) produce anti-inflammatory cytokines (IL-10, TGF-β).

- IPEX (Immune dysregulation, Polyendocrinopathy, Enteropathy, X-linked) syndrome:
  - X linked recessive disorder (male child).
  - Genetic deficiency of FOXP3 → autoimmunity.
  - Characterized by enteropathy, endocrinopathy, nail dystrophy, dermatitis, and/or other autoimmune dermatologic conditions.
  - Associated with diabetes in male infants.


Bone marrow
Thymus
Lymph node
T cell precursor
CD4+ CD8+ T cell
CD8+ T cell
CD4+ T cell
Cytotoxic T cell
Helper T cell
Th1
Th2
Th17
Treg
IL-12, IFN-γ
IL-4, IL-10
IL-2, IL-4
IFN-γ
TGF-β, IL-6
TGF-β
T-cell receptor (binds MHC I or MHC II)
CD8
CD4
Cortex (positive selection)
Medulla (negative selection)


Factors in T-Helper cell differentiation
IFN-γ
IL-12
Secrete
IFN-γ
IL-2
Lymphotoxin β
- Activation of macrophages and CD8+ T-cells
- Mediation of delayed type hypersensitivity
TH1
IL-4
IL-10
IFN-γ
Naïve CD4+ T-cell (TH0)
Antigen presenting cell
TH2
IL-4
Secrete
IL-4
IL-5
IL-10
IL-13
- Initiation of antibody response
- Regulation of immunoglobulin class switching

T cell subsets

	Th1 cell	Th2 cell	Th17 cell	Treg
SECRETES	IFN-γ, IL-2	IL-4, IL-5, IL-6, IL-10, IL-13	IL-17, IL-21, IL-22	TGF-β, IL-10, IL-35
FUNCTION	Activates macrophages and cytotoxic T cells to kill phagocytosed microbes	Activates eosinophils and promotes production of IgE for parasite defense	Immunity against extracellular microbes, through induction of neutrophilic inflammation	Prevents autoimmunity by maintaining tolerance to self-antigens
INDUCED BY	IFN-γ, IL-12	IL-2, IL-4	TGF-β, IL-1, IL-6	TGF-β, IL-2
INHIBITED BY	IL-4, IL-10 (from Th2 cell)	IFN-γ (from Th1 cell)	IFN-γ, IL-4	IL-6
IMMUNODEFICIENCY	Mendelian susceptibility to mycobacterial disease		Hyper-IgE syndrome	IPEX

Development of Cytotoxic T Lymphocytes

- Like CD4+ T cells, CD8+ T cells require both a primary and a costimulatory signal to become activated.
- The main difference between them is that CD8+ T cells recognize their specific antigen presented by MHC class I molecules and rely upon the cytokines produced by T helper cells to proliferate and ultimately differentiate into cytotoxic T lymphocytes (CTLs).

Activation of B lymphocytes

- As mature naive B lymphocytes leave the bone marrow following successful rearrangement of their membrane immunoglobulin receptor genes, they recirculate throughout the body, attracted to follicular areas of the lymph nodes and spleen.

- If antigen entering these secondary lymphoid organs binds to and cross-links the idiotypes of the immunoglobulin, this provides the first signal for the activation of the B lymphocyte.

- The antigens that B lymphocytes encounter are divided into 2 categories: thymus-independent (TI) antigens and thymus-dependent (TD) antigens.

A. Thymus Dependent-Antigen Activated B Lymphocytes:

- Most antigens introduced in the body fall into the category of thymus-dependent (TD) antigens.

- Response to such molecules requires the direct contact of B cells with helper T cells and are influenced by cytokines secreted by these cells.

- After the cross-linking of receptors on the B-cell surface with antigen, the material is endocytosed and processed via the exogenous pathway to generate an MHC class II-peptide complex, which is then inserted into the membrane of the professional APCs.

- Simultaneously, expression of B7 is upregulated on the B lymphocytes, making the cells effective presenters of antigen to CD4+ T cells in the area. Once a CD4+ T cell recognizes its specific peptide displayed on MHC class II molecules, the 2 cells form a conjugate. The CD4+ T cell is activated and differentiates into a helper T cell.

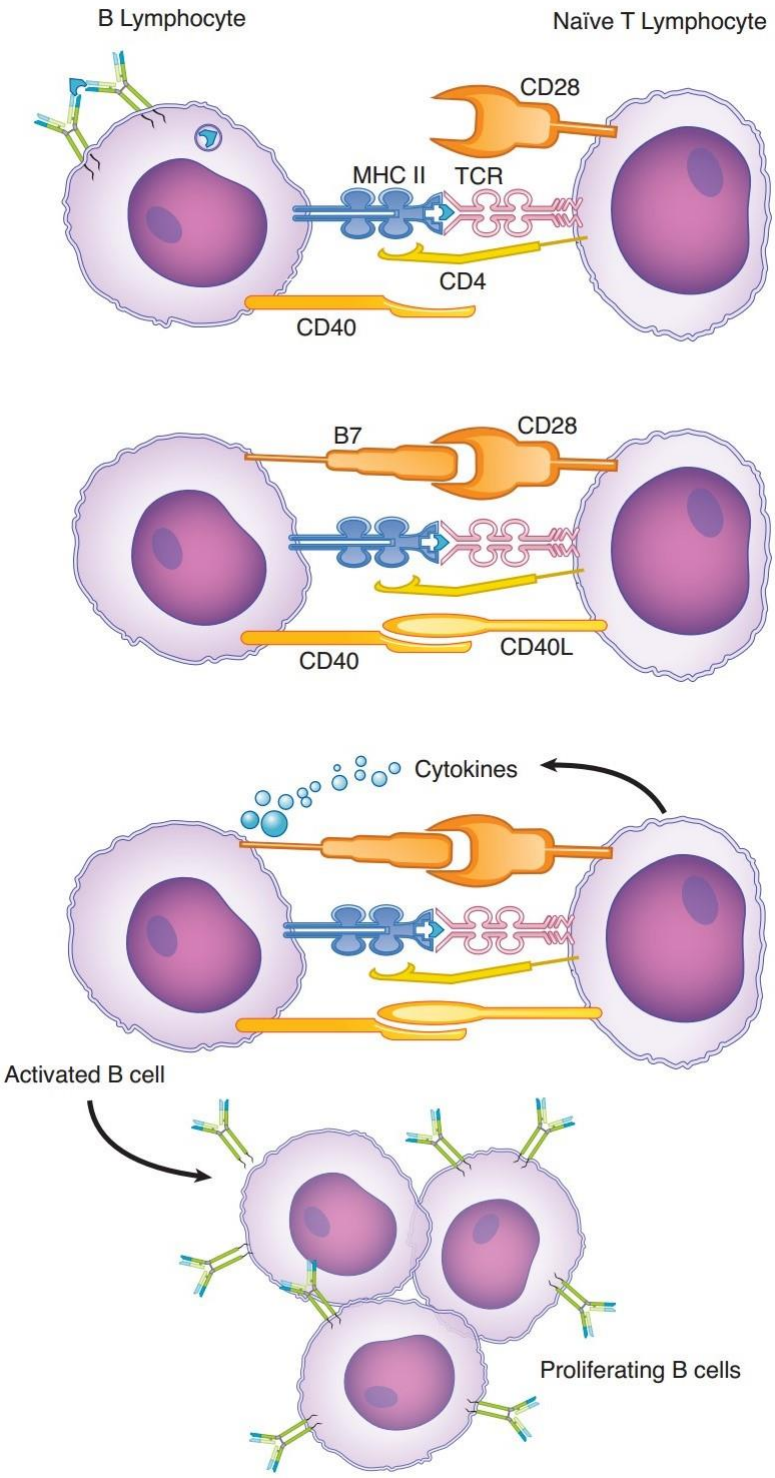
- Expression of CD40L on the surface of the helper T cell is upregulated and interacts with CD40 on the B cell to provide the second signal for B-cell activation.

- The B cells respond by proliferating and differentiating into plasma cells and memory B cells.

B. Thymus Independent-Antigen Activated B Lymphocytes:

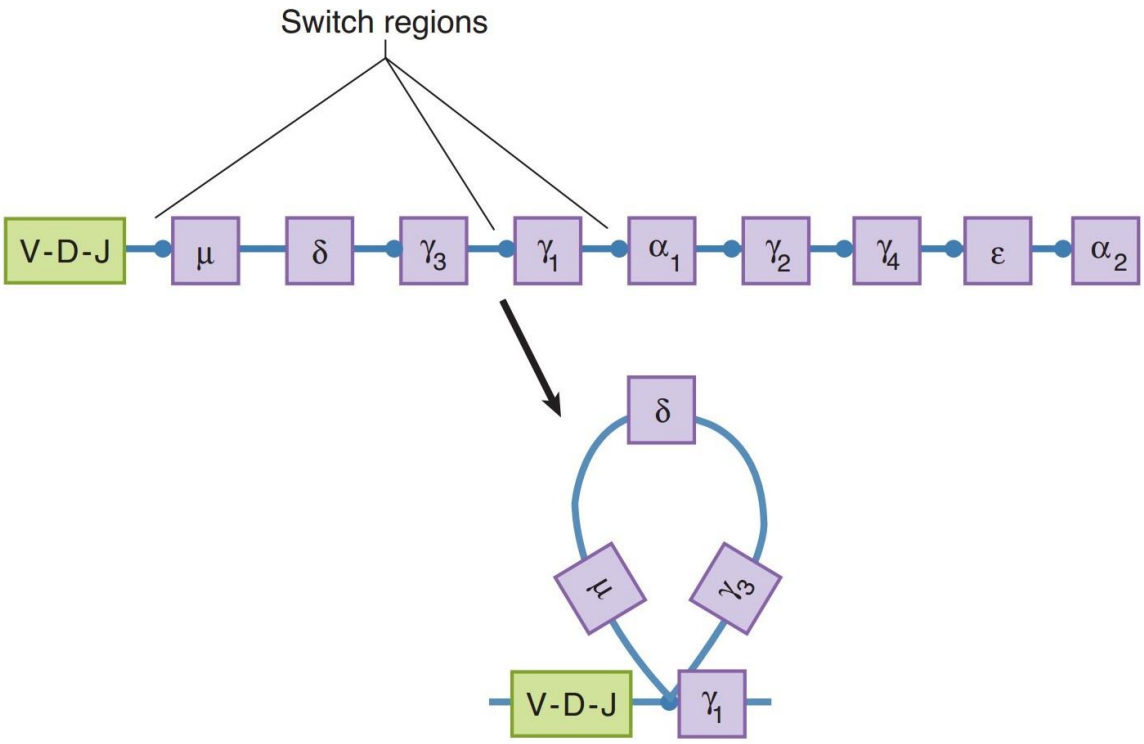
- Certain mature, naive B lymphocytes are capable of being activated by macromolecules such as lipids, polysaccharides, and lipopolysaccharides without having to interact with helper T cells. These antigens are called thymus-independent (TI) antigens, and they directly stimulate B cells to proliferate and differentiate into plasma cells.

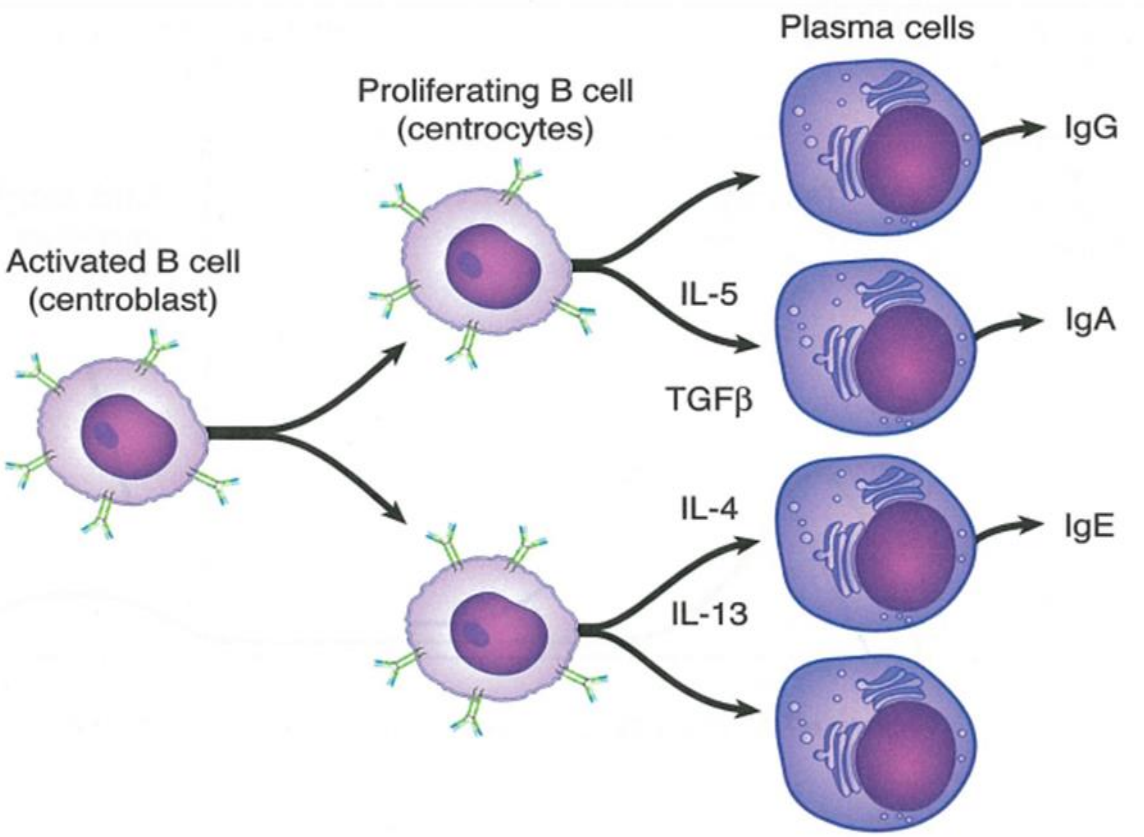
- The response to thymus-independent antigens is generally weaker than the response to other classes of antigens, resulting in the secretion of IgM antibodies only (no class switching) and the absence of immunologic memory.


B Lymphocyte
Naïve T Lymphocyte
CD28
MHC II
TCR
CD4
CD40
B7
CD28
CD40
CD40L
Cytokines
Activated B cell
Proliferating B cells

Isotype switching

- As the B lymphocyte receives cytokine signals from the activated Th2 cells in the secondary lymphoid organs, it is induced to undergo isotype switching, changing the heavy-chain constant domains to classes of antibodies with new and different effector functions.
- It does this by rearranging the DNA encoding the constant region of the heavy chain by activating switch regions that cause the intervening DNA to be looped out, excised, and degraded.
- The idiotype is then joined to a new constant region domain coding, and an antibody molecule with identical antigenic specificity but a new effector function is produced.
- This isotype switch is one-way: Because the excised DNA is degraded, a cell that has begun to produce an isotype downstream from IgM coding can never produce IgM again.
- This is why IgM is the principal immunoglobulin of the primary immune response when antigen is first encountered, and it is replaced in later responses by antibodies of different isotypes.
- Isotype switching (from IgM to other types of immunoglobulins) occurs in the germinal centers late in the primary response.
- Isotype switching first requires interaction of the CD40 receptor on activated B-cells with the CD40 ligand expressed by activated T-cells.
- Afterward, isotype switching can occur through genetic rearrangement of the heavy chain constant regions.
- This process is modulated by T-cell cytokines such as IL-2, IL-4, IL-5, IL-6, and IFN-γ.
- Th2 helper cells release IL-13 which, together with IL-4, preferentially promotes B-cell IgE production.
- Th2 cells also secrete Il-5, which activates eosinophils and promotes IgA synthesis.
- One hypothesis for the pathogenesis of asthma is an excess of Th2 cell activity relative to Th 1 cell activity, causing excessive IgE production, an abnormal propensity to Type I hypersensitivity reactions, and an associated chronic eosinophilic bronchitis.


Switch regions
V-D-J
μ
δ
γ3
γ1
α1
γ2
γ4
ε
α2
δ
μ
γ3
V-D-J
γ1


Plasma cells
Proliferating B cell
(centrocytes)
IgG
Activated B cell
(centroblast)
IL-5
IgA
TGFβ
IL-4
IgE
IL-13

❖ X-Linked Hyper-IgM Syndrome:

- Hyper-IgM syndrome results from an inability of B-lymphocytes to undergo isotype switching from IgM to other immunoglobulin isotypes such as IgD, IgG, IgE and IgA due to genetic deficiencies in the CD-40 T-lymphocyte ligand that is essential in inducing B-cells to switch classes.
- Therefore, Th cells from these patients will fail to express functional CD40L on their membrane and will thereby fail to give the costimulatory signal necessary for the B-cell response to T-dependent antigens, so only IgM antibodies are produced.
- The B-cell response to T-independent antigens is unaffected.
- The disease results in elevated or normal levels of IgM and a failure to synthesize all other isotypes of immunoglobulin heavy chain constant regions.
- Clinical effects of this illness include recurrent sinus and airway infections (deficient IgA).
- Treatment is with intravenous gamma globulin.

Somatic hypermutation

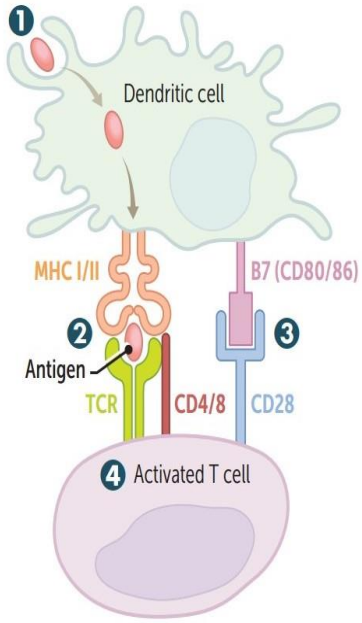
- During the activation of B lymphocytes by Th2 cells, intense proliferation of the B cells results in the formation of germinal centers in the follicles of the lymph nodes and spleen.
- During the intense proliferative response of the B cell, random mutations in the coding of the variable domain region may occur. This is called somatic hypermutation.
- Clones of cells with higher receptor affinity will begin to predominate in the germinal center. This clonal selection results in the predominance of clones capable of producing antibodies with increasing affinity for the antigen, a process known as affinity maturation.

T- and B-cell activation

APCs: B cells, dendritic cells, Langerhans cells, macrophages.
Two signals are required for T-cell activation, B-cell activation, and class switching.

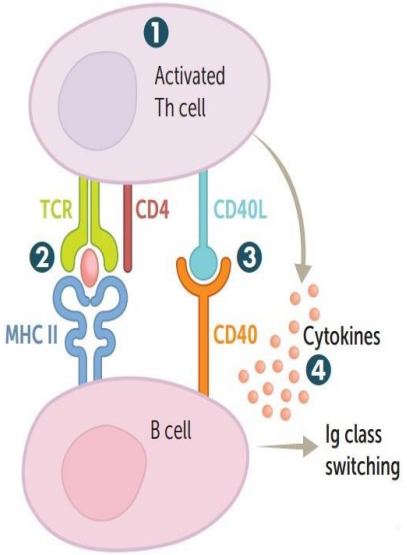
T-cell activation

1. Dendritic cell (specialized APC) samples antigen, processes antigen, and migrates to the draining lymph node.
2. T-cell activation (signal 1): antigen is presented on MHC II and recognized by TCR on Th (CD4+) cell. Endogenous or cross-presented antigen is presented on MHC I to Tc (CD8+) cell.
3. Proliferation and survival (signal 2): costimulatory signal via interaction of B7 protein (CD80/86) on dendritic cell and CD28 on naïve T cell.
4. Th cell activates and produces cytokines. Tc cell activates and is able to recognize and kill virus-infected cell.



B-cell activation and class switching

1. Th-cell activation as above.
2. B-cell receptor–mediated endocytosis; foreign antigen is presented on MHC II and recognized by TCR on Th cell.
3. CD40 receptor on B cell binds CD40 ligand (CD40L) on Th cell.
4. Th cells secrete cytokines that determine Ig class switching of B cells. B cells are activated, undergo class switching and affinity maturation, and begin producing antibodies.

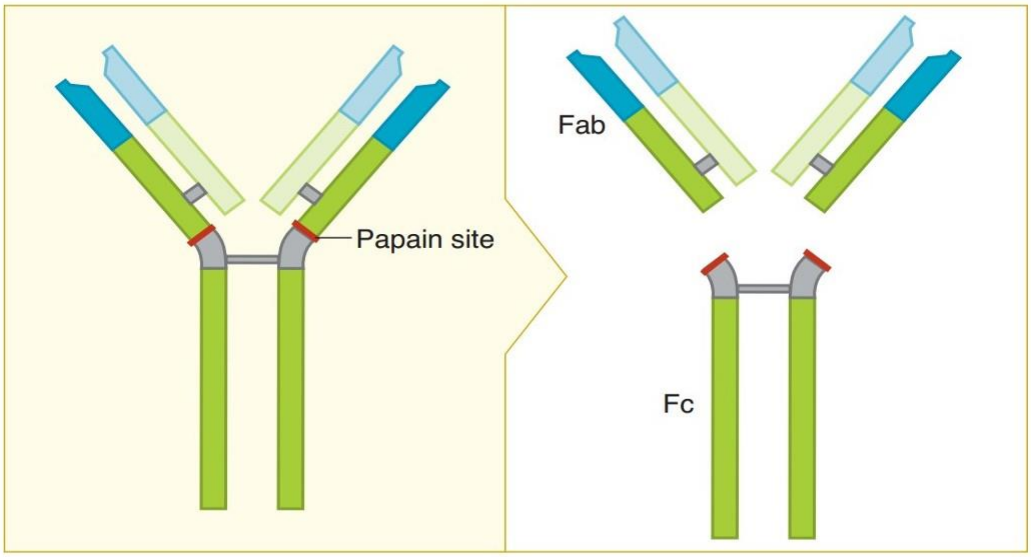


Humoral immunity

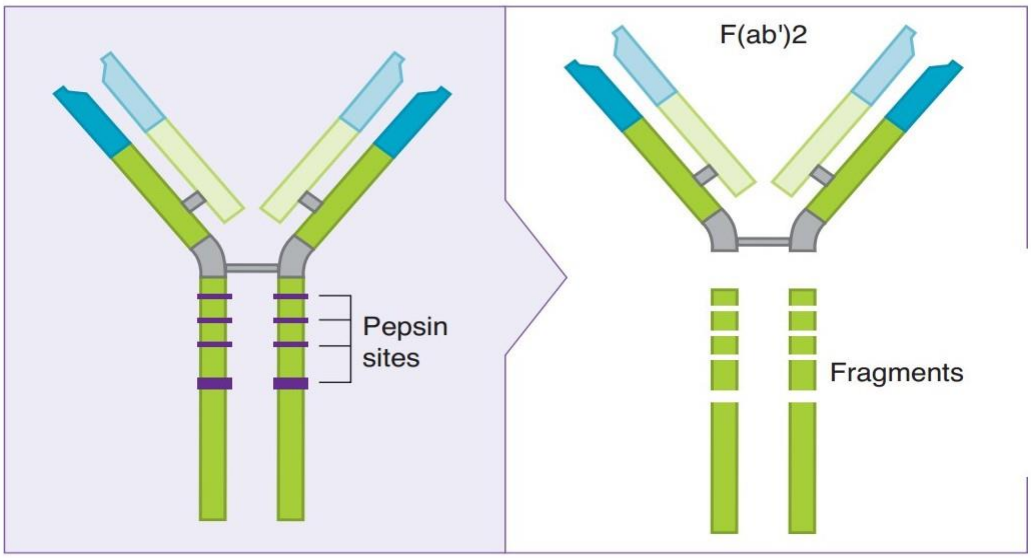
- The biologic function of segments of the antibody molecule was first elucidated by digestion of these molecules with proteolytic enzymes.

- If an antibody molecule is digested with papain, cleavage occurs above the disulfide bonds that hold the heavy chains together. This generates three separate fragments, two of which are called Fab (fragment antigen binding), and one is called Fe (fragment crystallizable).

- Cleavage of the antibody molecule with pepsin generates one large fragment called F(ab')2 and a digested Fc fragment. The bridging of antigens by antibody molecules is required for agglutination of particulate antigens or the precipitation of soluble antigens.



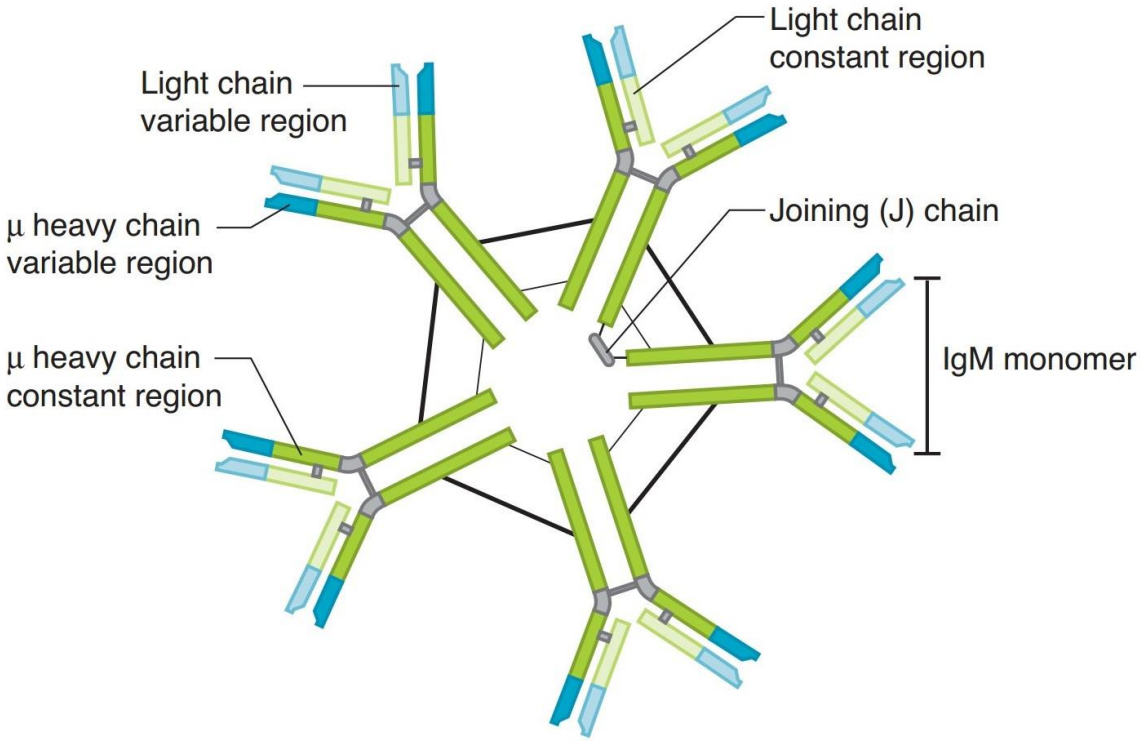
Proteolytic Cleavage with Papain



Proteolytic Cleavage with Pepsin

The primary humoral response

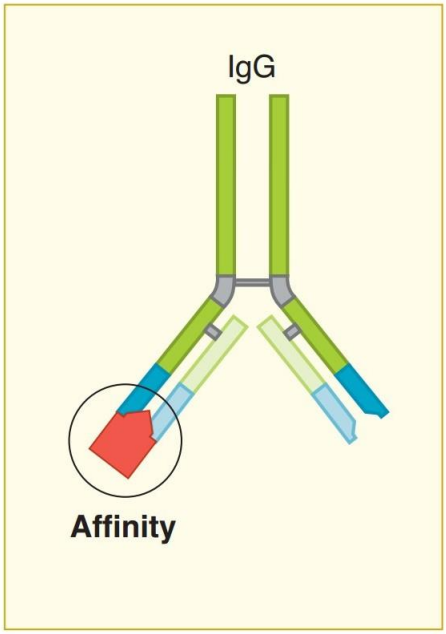
- The first isotype of immunoglobulin that can be produced by a B cell with or without T-cell help is IgM.
- This is because coding for the constant domains of the heavy chain of IgM (μ chains) are the first sequences downstream from the coding for the idiotype of the molecule.
- The IgM molecule on the surface of the B cell is a monomer, but the secreted form of this molecule is a pentamer, held together in an extremely compact form by a J chain synthesized by the cell.

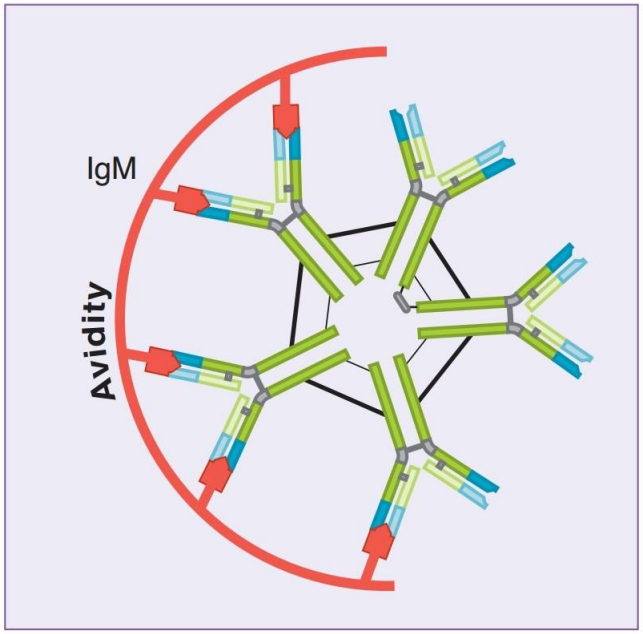


- The design of the IgM pentamer maximizes the effector functions critical to the body early during antigenic challenge.
- Because of its multimeric structure (5 of the Y-shaped monomers joined into one unit), plasma IgM has five times the capacity for binding antigenic epitopes as any monomeric immunoglobulin unit.
- The valence of the molecule is therefore 10. In other words, 10 identical epitopes can be simultaneously bound, as compared with 2 for the monomeric structure.
- This makes IgM the most effective immunoglobulin isotype at "sponging" the free antigen out of the tissues and proves critical, as the humoral response evolves, in trapping antigen so that it can be presented to the lymphocytes that will ultimately refine the choice of effector mechanism.

- The multimeric structure of IgM also makes it the most effective antibody at activating complement, a set of serum proteases important in mediating inflammation and antigen removal.

- Serum IgM is incapable of binding to cellular Fc receptors and thus cannot act as an opsonin or a mediator of antibody-dependent cell mediated cytotoxicity (ADCC).



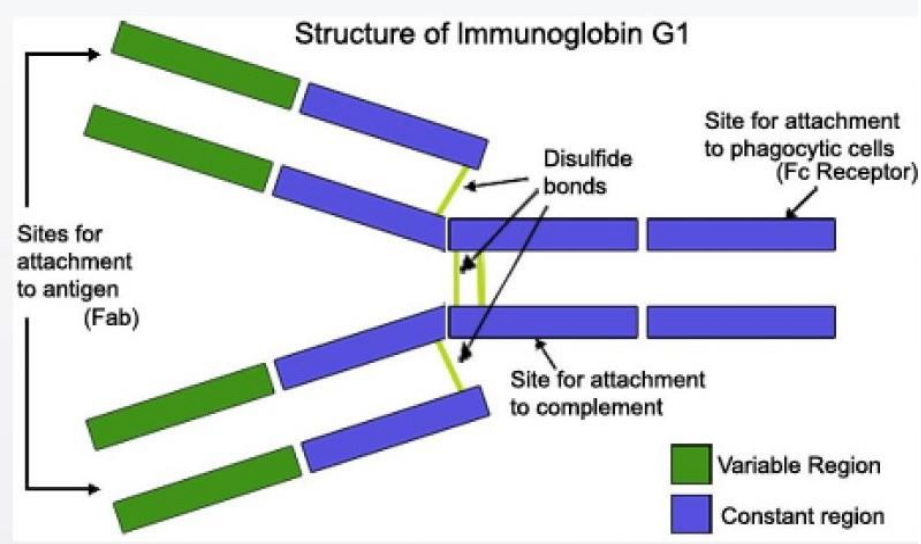


- Although the binding strength (affinity) of the idiotype for the epitope may not be strong early in the immune response, the IgM molecule possesses the highest avidity (number of combining sites available to bind epitopes) of any immunoglobulin molecule produced in the body.

Antibodies of secondary immune responses

1. Ig G:

- The predominant isotype of immunoglobulin that begins to be produced after IgM during the primary immune response is IgG.
- IgG is a monomeric molecule.
- IgG activates complement, acts as an opsonin, and mediates ADCC. It is also actively transported across the placenta by receptor-mediated transport and thus plays a crucial role in protection of the fetus during gestation.
- Macrophages, neutrophils and B-lymphocytes express cell surface proteins known as Fc receptors (FcR) that bind specifically to the Fc portion of IgG molecules. This binding is essential for the process of opsonization.



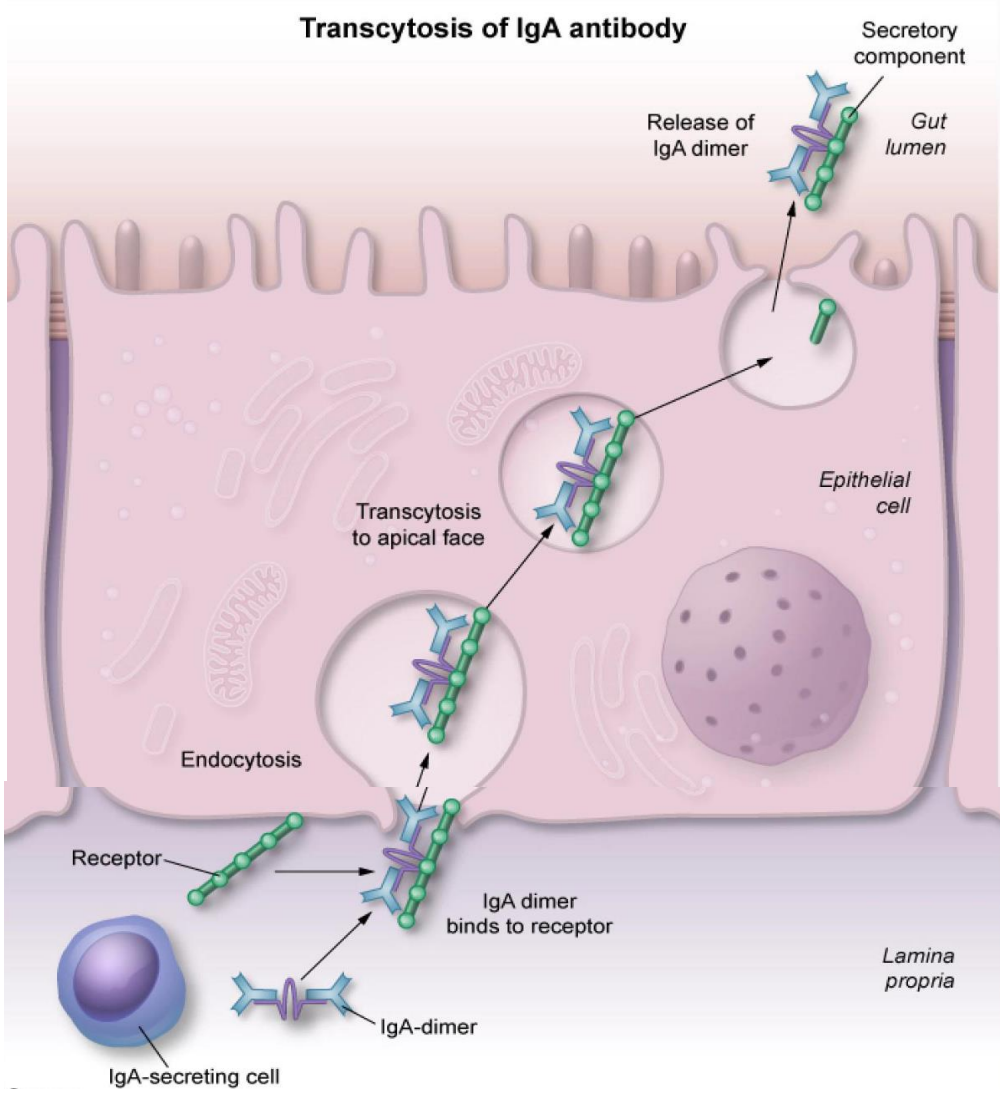
2. Ig A:

- Two forms: It is present in serum as a monomer, while its secretory form is a dimer.
- Secretory IgA generally exists as a dimer and serves as a major protective defense of the mucosal surfaces of the body.
- Its sole function appears to be the inhibition of binding of toxins or adhesive microbial components to the mucosa of the digestive, respiratory, and urogenital systems.
- It does not activate complement or act as an opsonin.
- It is particularly important as a component of the colostrum, or the first breast milk fed to an infant after birth, where it functions to provide the infant with passive mucosal immunity.

- Secretory IgA (that which is released across the mucosa of the respiratory, digestive, and urogenital tracts) differs from serum IgA in an important fashion.

- As the IgA dimer is produced by plasma cells and B lymphocytes, it becomes bound to poly-Ig receptors on the basolateral side of the epithelia, is endocytosed, and is released into the lumen bound to a secretory piece that is the residue of the receptor.

- The secretory component thus serves an important function in transepithelial transport, and once in the lumen of the tract, has a function in protecting the molecule from proteolytic cleavage.



❖ N.B:

1. IgA protease is an enzyme produced by Neisseria gonorrhoeae and Neisseria meningitidis.

- Both of these organisms gain access to the human bloodstream by penetrating mucosal surfaces, N. Gonorrhoeae in the genital region and N. meningitidis in the nasopharynx.
- Secretory IgA exists on mucosal surfaces and in secretions and acts to bind and inhibit the action of pili and fimbriae as well as other cell surface antigens that normally mediate mucosal adherence and penetration.
- IgA protease cleaves secretory IgA at its hinge region rendering it ineffective.

2. Selective IgA deficiency is the most commonly occurring primary immunodeficiency. It is thought to occur due to failure of B-cells to switch from IgM to IgA production.

- Most commonly these patients are asymptomatic, but classically this immunodeficiency predisposes to recurrent sinopulmonary and GI tract infections due to the absence of secretory IgA.
- Recurrent otitis media, sinusitis, bronchitis or pneumonias are caused by encapsulated bacteria, such as H. influenzae or S. pneumoniae.
- Gastrointestinal infections manifest as recurrent acute or chronic diarrhea due to viral, bacterial, and G. lamblia infections.
- Patients with selective IgA deficiency often form IgG antibodies directed against IgA (anti-IgA antibodies). When transfused with blood or blood products containing small amounts of IgA these patients may develop potentially fatal anaphylactic reactions.
- Gamma-globulin preparations should not be used for treatment of these patients as it may increase the synthesis of anti-IgA antibodies because the patient's body recognizes it foreign.

3. Ig E:

- IgE binds allergenic antigen at its Fab sites and binds Fc receptors on mast cells and basophils.

- Once multiple IgE molecules bind antigen and the Fc receptor on the mast cell or basophil and subsequently cross-link with each other, these cells will degranulate thereby releasing multiple vasoactive substances allergen mediating immediate (type I) hypersensitivity.

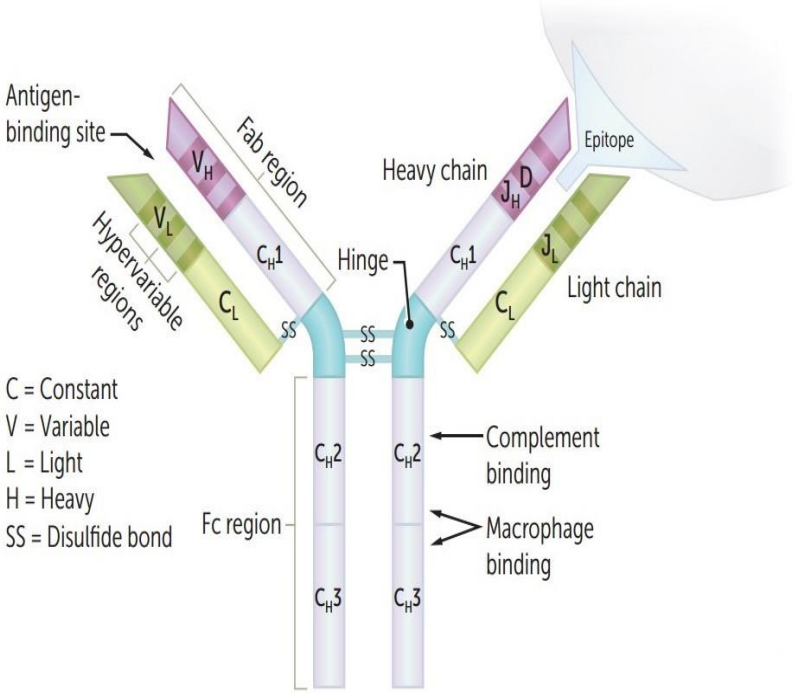
- It does not activate complement or act as an opsonin.

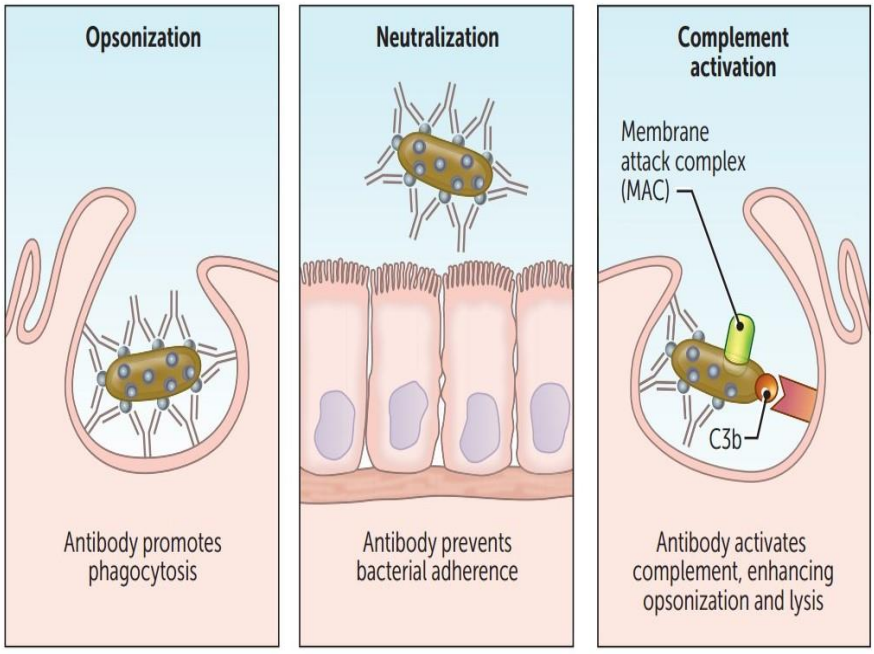
❖ Immunodeficiencies Involving B Lymphocytes:

- Patients with B-cell deficiencies usually present with recurrent pyogenic infections with extracellular pathogens.
- The absence of immunoglobulins for opsonization and complement activation is a major problem.
- The T-cell immune system is intact, and T-cell activities against intracellular pathogens, delayed type hypersensitivity, and tumor rejection are normal.

Antibody structure and function

Fab (containing the variable/hypervariable regions) consisting of light (L) and heavy (H) chains recognizes antigens. Fc region of IgM and IgG fixes complement. Heavy chain contributes to Fc and Fab regions. Light chain contributes only to Fab region.





Fab:

- Fragment, antigen binding
- Determines idiotype: unique antigen-binding pocket; only 1 antigenic specificity expressed per B cell

Fc:

- Constant
- Carboxy terminal
- Complement binding
- Carbohydrate side chains
- Determines isotype (IgM, IgD, etc)

Generation of antibody diversity (antigen independent)

1. Random recombination of VJ (light-chain) or V(D)J (heavy-chain) genes
2. Random addition of nucleotides to DNA during recombination by terminal deoxynucleotidyl transferase (TdT)
3. Random combination of heavy chains with light chains

Generation of antibody specificity (antigen dependent)

4. Somatic hypermutation and affinity maturation (variable region)
5. Isotype switching (constant region)

Complement

- The complement system is a set of interacting proteins released into the blood after production in the liver.

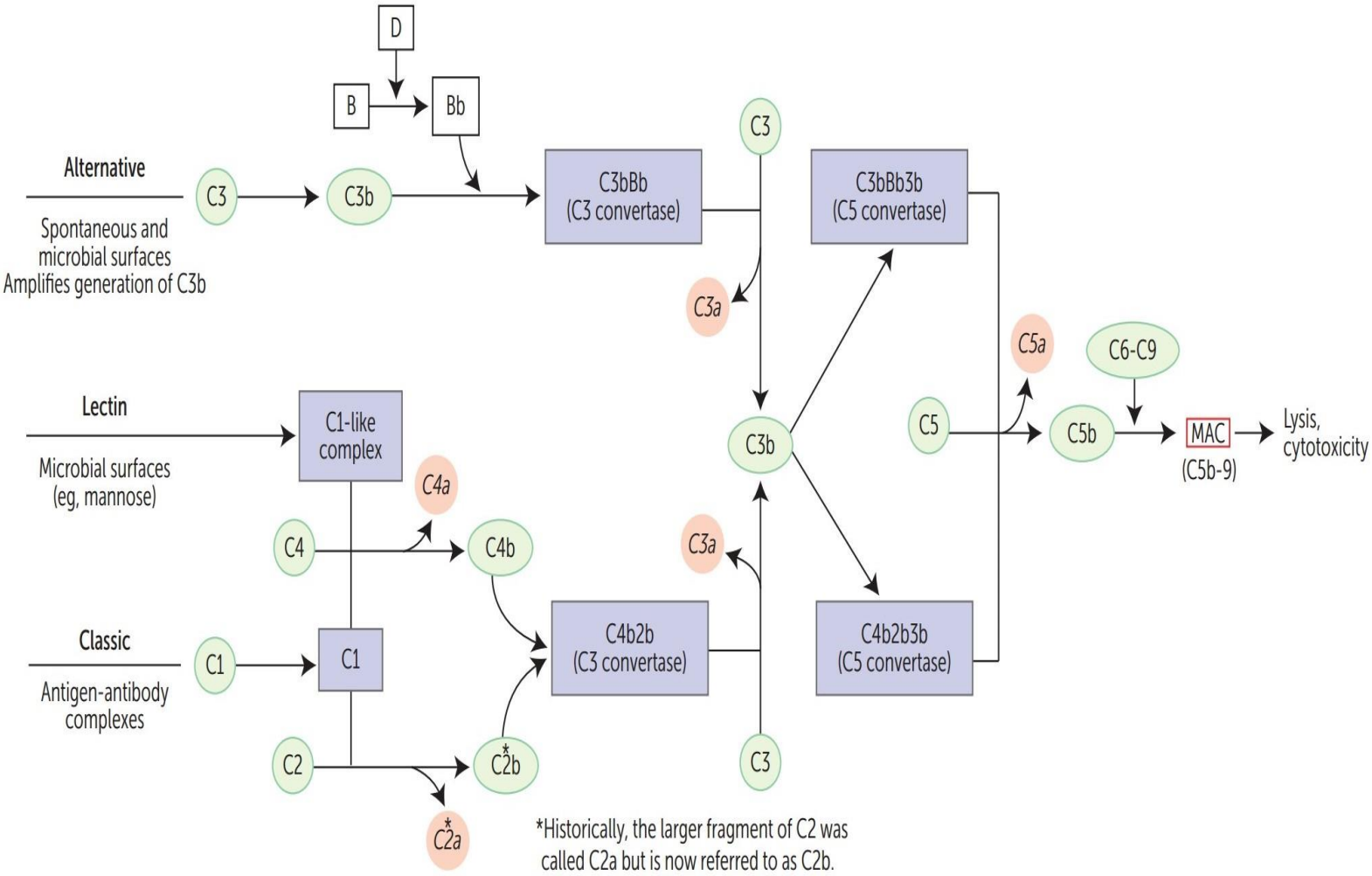
- System of hepatically synthesized plasma proteins that play a role in innate immunity and inflammation. Membrane attack complex (MAC) defends against gram ⊖ bacteria.

- Activation:

- Classic pathway: IgG or IgM mediated. GM makes classic cars.

- Alternative pathway: microbe surface molecules.

- Lectin pathway: mannose or other sugars on microbe surface.



- Function:

- Mediate inflammation, enhance phagocytosis by opsonization, and cause lysis of particles by membrane pore formation.

- C3b: opsonization.

- C3a, C4a, C5a: anaphylaxis.

- C5a: neutrophil chemotaxis.

- C5b-9: cytolysis by MAC.

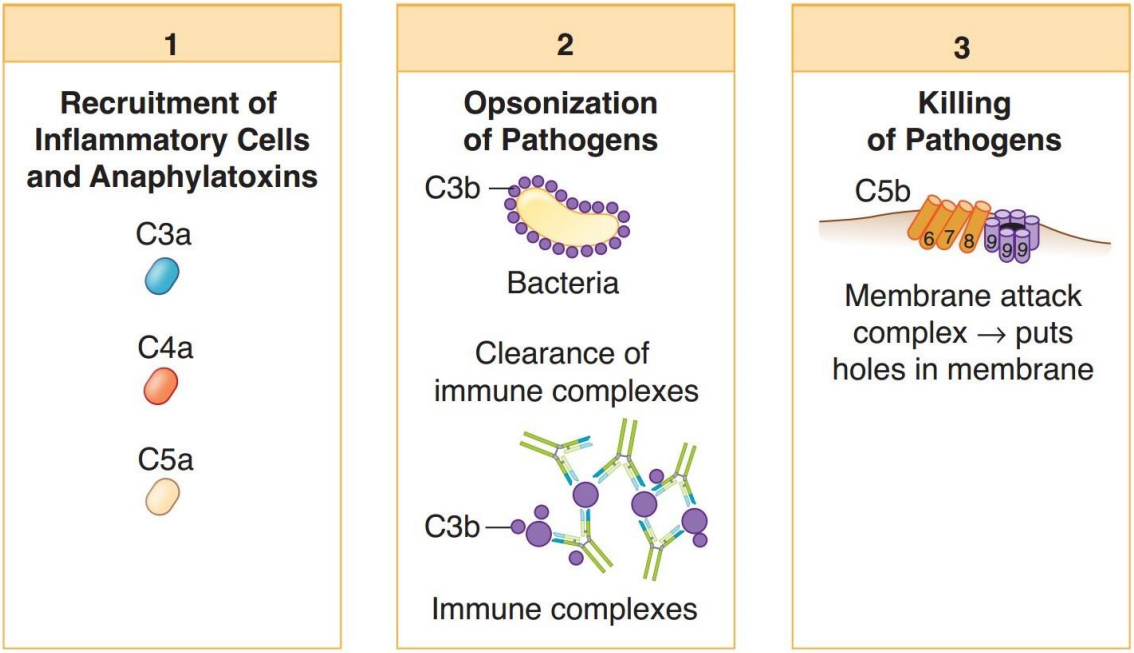
- Opsonins: C3b and IgG are the two 1° opsonins in bacterial defense; enhance phagocytosis.

- C3b also helps clear immune complexes.

- Opsonin (Greek) = to prepare for eating.

❖ N.B:

- Even though gram-positive bacteria may be resistant to the membrane attack complex of complement, the early components of the cascade mediate localized inflammation and opsonize the bacteria.



- Inhibitors:

- Decay-accelerating factor (DAF, aka CD55) and C1 esterase inhibitor help prevent complement activation on self-cells.

- Complement disorders:

1. Complement protein deficiencies:

A. Early complement deficiencies (C1-C4): Increases risk of severe, recurrent pyogenic sinus and respiratory tract infections; susceptibility to type III hypersensitivity reactions (due to C3 deficiency).

B. Terminal complement deficiencies (C5-C9): Terminal complement deficiency increases susceptibility to recurrent Neisseria bacteremia.

2. Complement regulatory protein deficiencies:

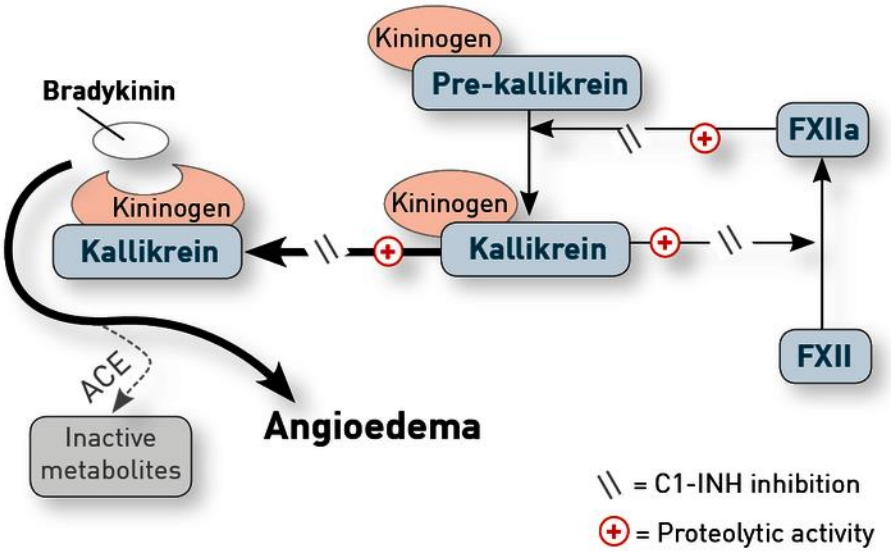
A. C1 esterase inhibitor deficiency:

- Causes hereditary angioedema due to unregulated activation of kallikrein → ↑ bradykinin. Characterized by ↓ C4 levels.

- ACE inhibitors are contraindicated (also ↑ bradykinin).

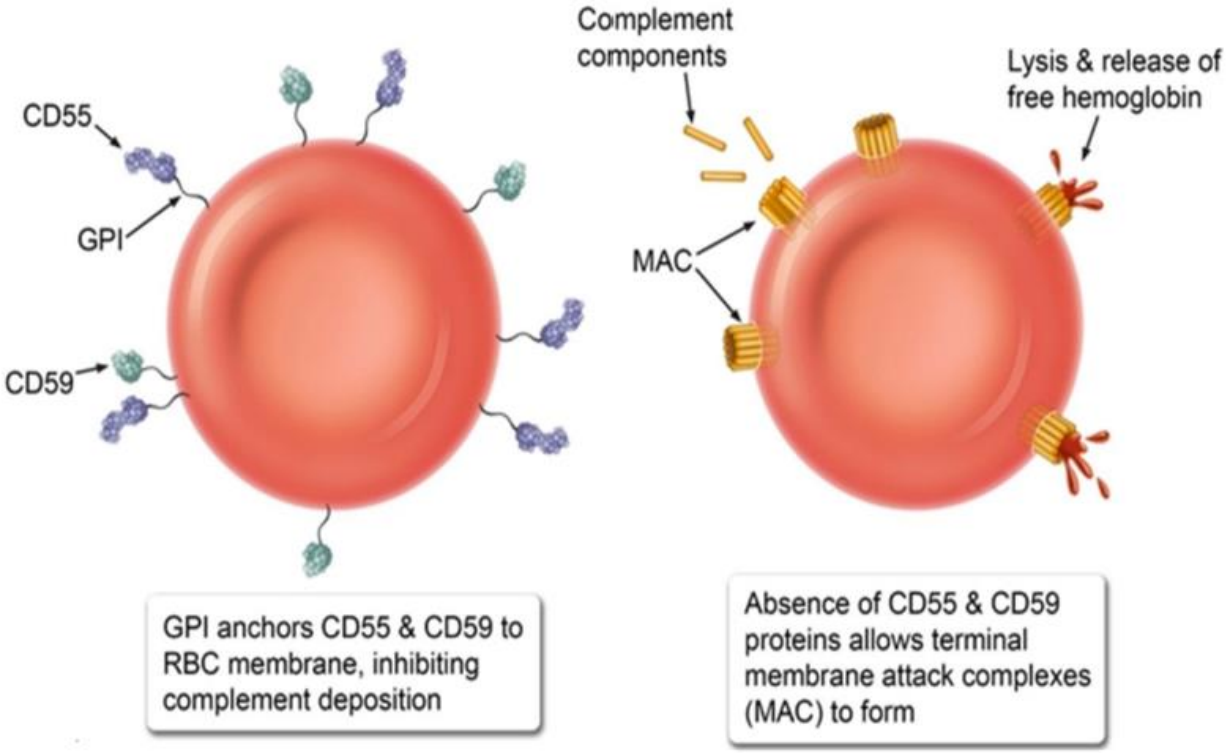
- Symptoms include facial swelling (without urticaria), life-threatening laryngeal edema, and gastrointestinal manifestations (nausea/vomiting, colicky pain, diarrhea).

- Management of acute attacks involves supportive care and the administration of C1 INH concentrate or a kallikrein inhibitor.



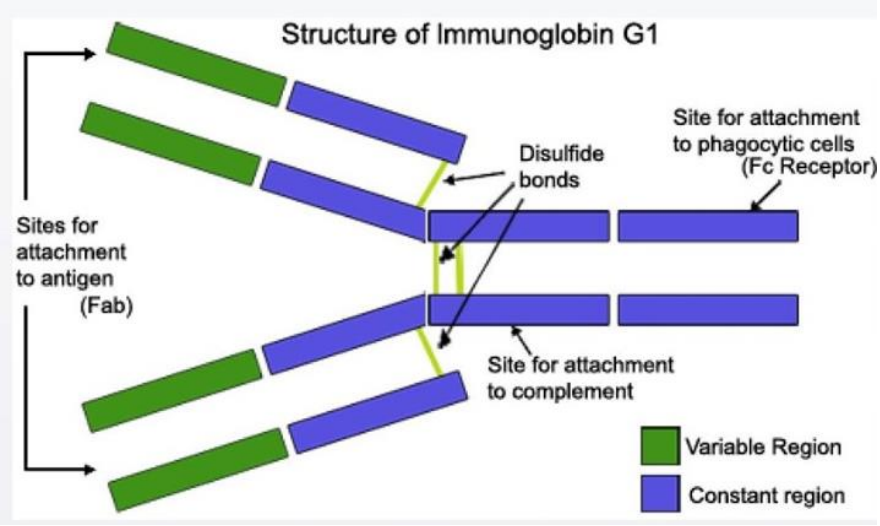
Facial swelling during a hereditary angioedema (HAE) attack

B. DAF (GPI-anchored enzyme) deficiency: Causes complement-mediated lysis of RBCs and paroxysmal nocturnal hemoglobinuria.



❖ N.B:

- Both IgG antibodies and IgM antibodies are capable of, and essential for, triggering the classical complement pathway after binding a C1 molecule.
- The classical pathway would not be able to proceed in the absence of either IgM or IgG.
- C1 is the complement component that when activated is able to release the catalytic factors responsible for the next steps in the classical complement pathway.
- In order to be activated, C1 must bind the Fc portions of two different antibodies at specific C1 binding sites.
- Because IgM circulates in pentameric form (five IgM molecules joined together at their Fc regions by a J chain peptide), it is much more effective in initiating the complement cascade than IgG which circulates in monomeric form (a single circulating immunoglobulin).
- The complement binding site on both IgG and IgM is located in the Fc portion closer to the hinge region.



The Generation of Cell-Mediated Effector Mechanisms

- The cell-mediated arm of the immune response (CMI) is designed to identify and eradicate antigenic stimuli that arise from inside the cells of the body.

- This occurs when cells of the host become infected with intracellular pathogens, such as viruses, some parasites and bacteria, or when malignant transformation causes cells to express aberrant surface molecules.

- In such cases, Th1 cells primed in the lymph nodes and spleen serve to provide the cytokine stimuli to activate the three potential effector cells to destroy the infected or altered cells: macrophages, cytotoxic CD8+ T lymphocytes (CTLs), and NK cells.

Activation of macrophages

- One example of a cell-mediated effector mechanism that is enhanced by the action of Th1 cells is macrophage killing.

- This is a critical protective mechanism in the defense against organisms invading macrophages and attempting to live there (mycobacteria, Leishmania) or in the case where phagocytosed microbes have protective mechanisms that make them resistant to intracellular digestion (Listeria).

- In CMI against phagocytosed microbes, the specificity of the response arises from T cells, but the actual effector function is mediated by the phagocytes.

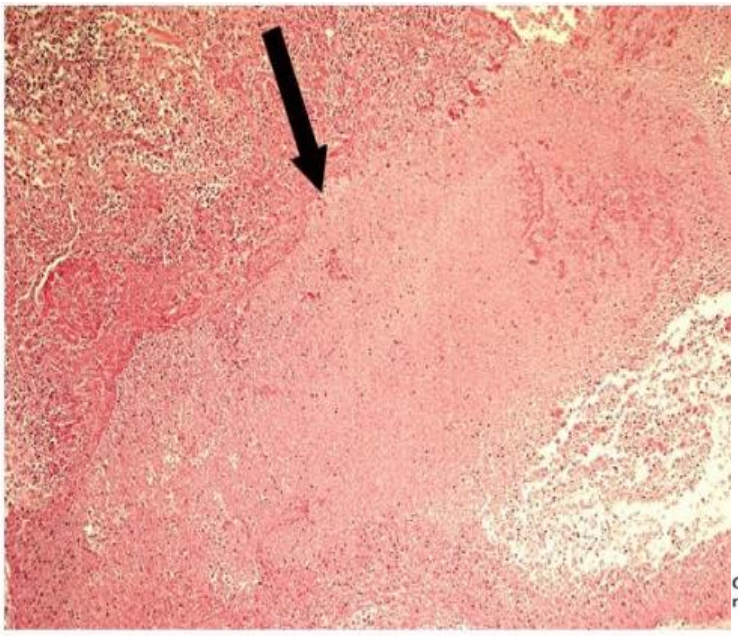
- This provides an important link between the adaptive and innate immune responses, and in essence, converts phagocytes into agents of the adaptive immune response. The most important cytokine elaborated by Th1 cells and CD8+ T lymphocytes to enhance the microbicidal capabilities of phagocytes is IFN-γ.

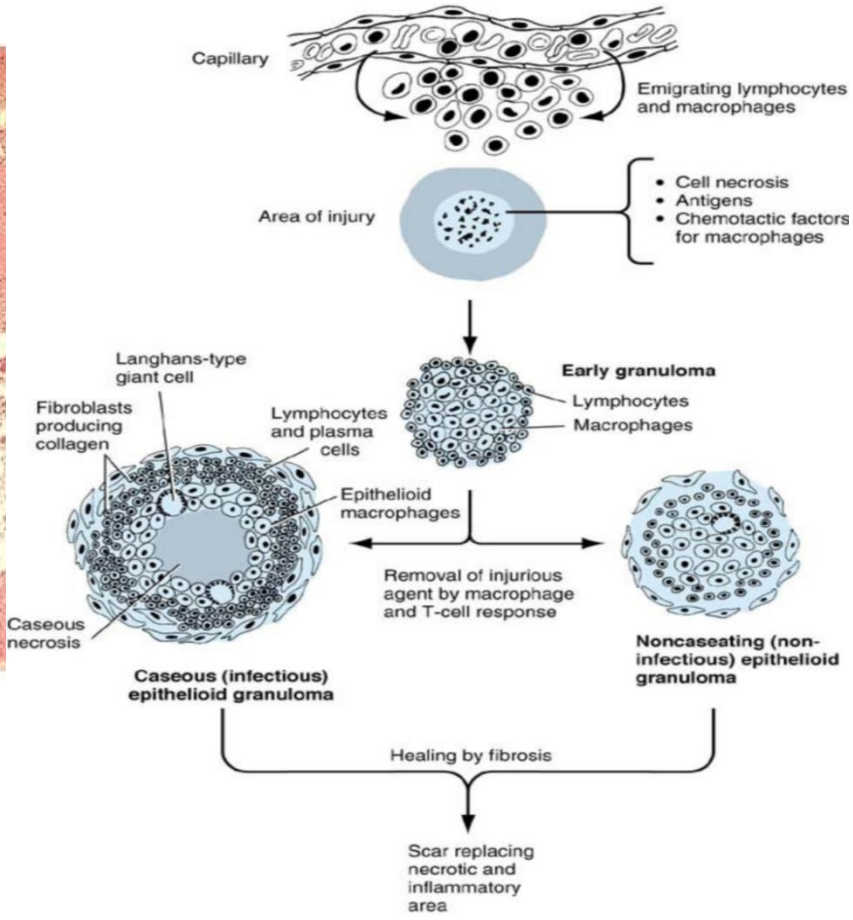
❖ N.B:

1. Within the lymph nodes and lung, mature T helper cells produce interferon-γ (IFN- γ).

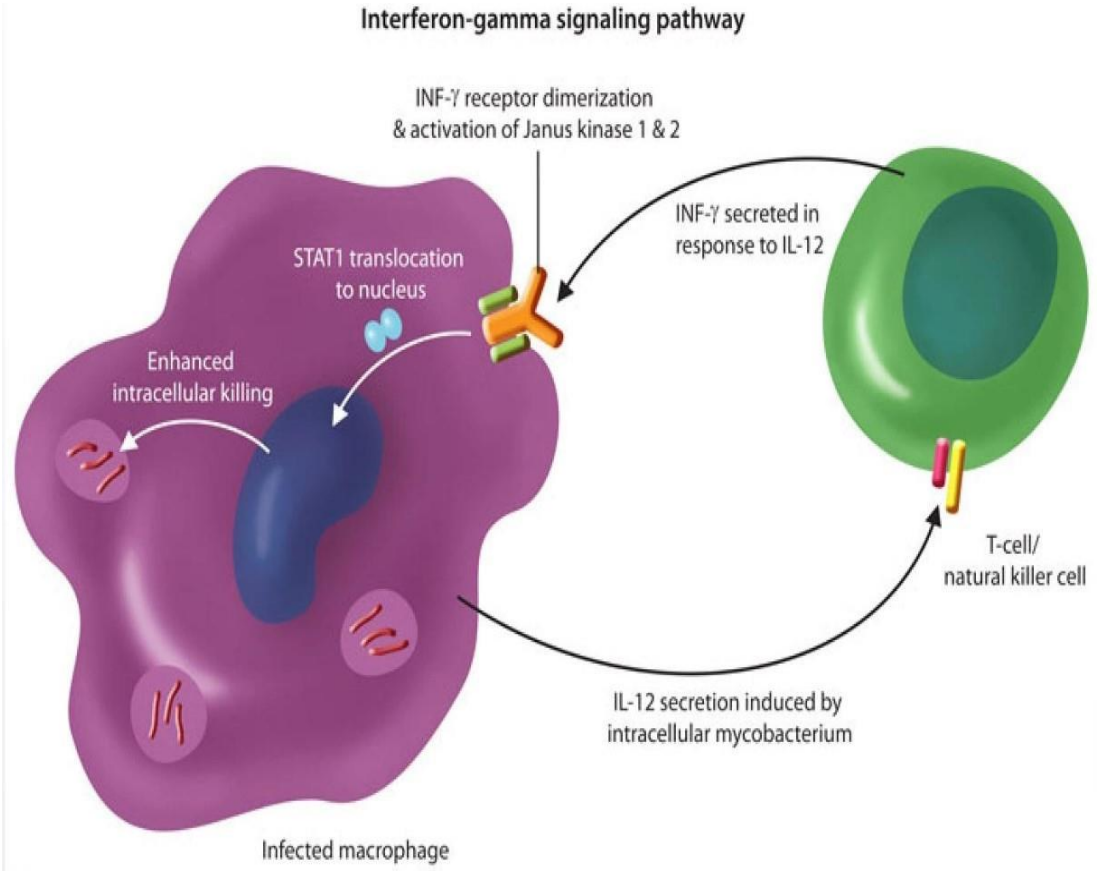
- This interferon is a key mediator in the maturation of macrophages, thereby enabling them to contain an infection with Mycobacterium tuberculosis.
- IFN- γ is responsible for the formation of the phagolysosome (which contains harsh bactericidal acids) in infected macrophages.
- It also stimulates the release of inducible nitric oxide synthase (iNOS), an enzyme that initiates a series of reactions that ultimately produce reactive nitrogen intermediates and free radicals. These products are capable of destroying various components of the mycobacterial cell.
- The role of IFN- γ is important in other regards as well, since it is responsible for both granuloma formation and caseous necrosis.
- The activated macrophages (which were initially stimulated by IFN- γ) produce TNF, which recruits monocytes to the area.

- These monocytes differentiate into epithelioid histiocytes and cluster in a circular fashion around the M. tuberculosis organisms.
- This immune reaction causes "walling off' of the tuberculous foci with the creation of caseating granulomas, which consist of epithelioid cells, Langhans multinucleated giant cells, fibroblasts and collagen.
- For most individuals, the formation of this caseating granuloma (arrow in below image) successfully limits the bacteria from spreading and effectively controls the infection.
- Without the T helper cell and its associated IFN- y, the host would be unable to mount an effective response and the tuberculous infection would progress unchecked.





2. Host defense against mycobacterial infections depends on the interactions between macrophages and T cells.
- Interferon gamma (IFN-gamma), a Th1 cytokine, is a key factor in the elimination of these infections.
- Specifically, macrophages infected with mycobacteria produce interleukin 12, which in turn stimulates T cells and natural killer cells to produce IFN-gamma.
- IFN-gamma then binds to its receptor, leading to receptor dimerization and activation of Janus kinases 1 and 2.
- This results in nuclear signaling via STAT1 and transcription of IFN-gamma-regulated genes, which promote mycobacterial killing by phagocytes.
- Autosomal recessive deficiencies of the IFN-gamma receptor (or other elements of this pathway) result in disseminated mycobacterial disease in infancy or early childhood.
- Once identified, these patients require lifelong treatment with continuous antimycobacterial antibiotics.



3. IL-12 stimulates the differentiation of "naive" T-helper cells into the Th1 subpopulation.
- Without Th1 cells, the synthesis of IFN-γ required for activation of macrophages does not occur.
- Activated macrophages are necessary for delayed hypersensitivity reactions and cytotoxicity against intracellular organisms, such as mycobacteria.
- Patients with IL-12 receptor deficiency suffer from severe mycobacterial infections due to the inability to mount a strong cell mediated granulomatous immune response. They are treated with IFN-γ.

4. The findings of hilar adenopathy, pulmonary infiltrates, and non-caseating lung granulomas in an African American female point to a diagnosis of sarcoidosis.
- Sarcoidosis is thought to result from a dysregulated cell-mediated immune response to an unidentified antigen that results in the formation of granulomas.
- Granuloma formation is a manifestation of cell-mediated immunity driven by products of Th 1 type CD4+ helper T cells, particularly IL-2 and interferon-γ (IFN-γ), which stimulate Th1 type cell proliferation and macrophage activation, respectively.
- Immunologic abnormalities in sarcoidosis include intraalveolar and interstitial accumulation of CD4+ T cells, resulting in high CD4:CD8 T-cell ratios in bronchoalveolar lavage (BAL) fluid.

5. In silicosis, there may be disruption of macrophage phagolysosomes by internalized silica particles.
- Macrophage killing of intracellular mycobacteria may be impaired as a result, causing increased susceptibility of patients with silicosis to pulmonary tuberculosis.

Activation of cytotoxic T lymphocytes

- The CTL recognizes the cell it will ultimately kill by interaction between its TCR and MHC class I antigens on the surface of the target cell.

- CTLs are capable of differentiation and cloning by themselves in the presence of the appropriate peptide/class I MHC antigen stimulus but are much more effective in so doing if they are assisted by signals from Th1 cells.

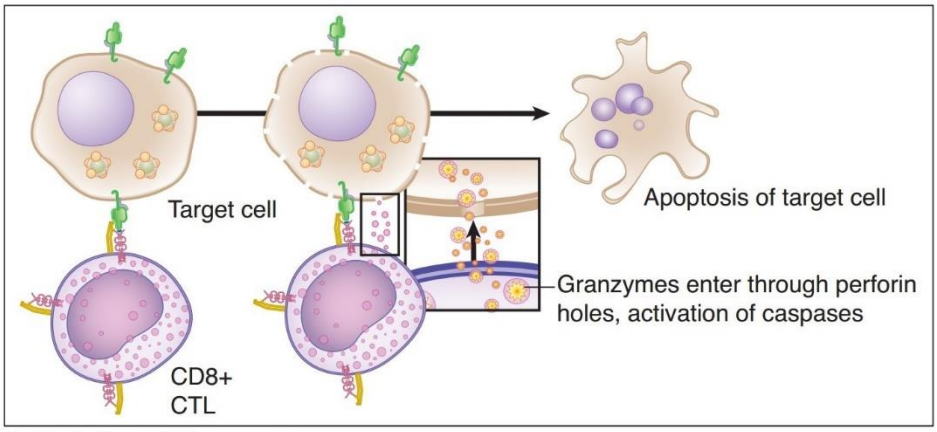
- The Th1 cell secretes IL-2 which acts on CD8+ cells to enhance their differentiation and cloning.

- Interferons produced in the area will increase the expression of MHC molecules to make targets more susceptible to killing.

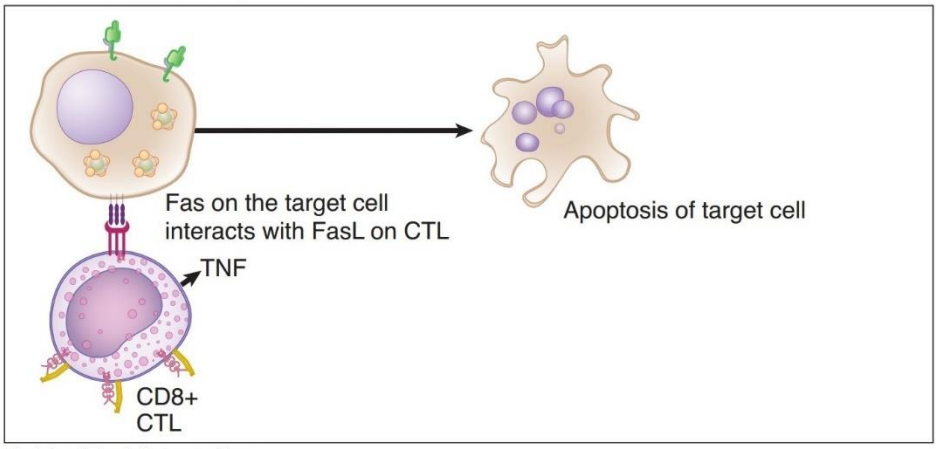
- <u>The death of the target may be mediated in distinct fashions:</u>

A. First, perforin present in the CTL granules creates pores in the membrane of the target cell through which granzymes (serine proteases) enter the target, inducing the activation of caspases, which activate the "death domain".

B. Furthermore, activated CTLs express a membrane protein called Fas ligand (FasL), which may bind to its complementary structure on the target, Fas. When this occurs, caspases are induced and death results.



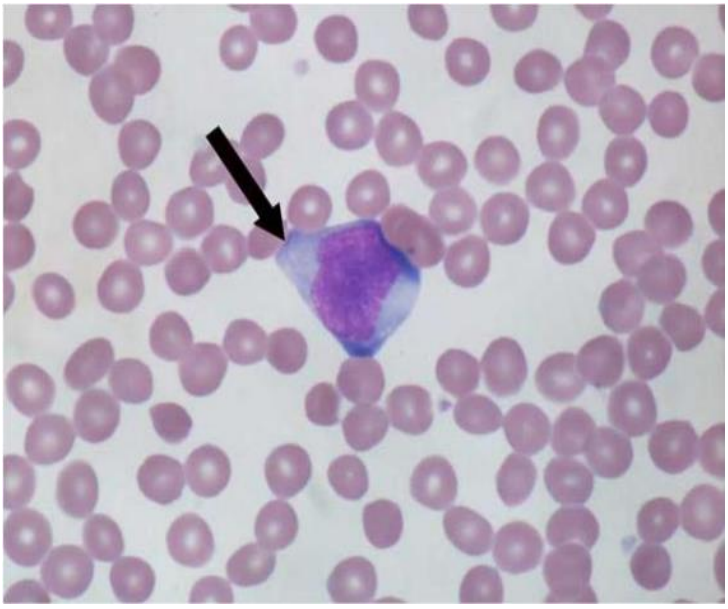
A: Perforin and Granzymes



B: Fas/FasL Interaction

❖ N.B:

- Infectious mononucleosis (IM) is a disease characterized by sore throat, malaise, lymphadenopathy, myalgias, splenomegaly, and fever.
- The causative agent in IM is the Epstein-Barr virus.
- After infecting the pharyngeal mucosa and tonsillar crypts, the virus gains access to the bloodstream where it preferentially infects B-lymphocytes by binding to the CD21 cell surface receptor.
- These reactive (atypical) CD8+ T-lymphocytes may be seen on peripheral blood smear in IM.
- They classically appear as cells much larger than quiescent lymphocytes with abundant cytoplasm, an eccentrically placed nucleus, and a cell membrane that appears to conform to the borders of neighboring cells.
- Atypical lymphocytes observed in the peripheral blood smears of patients with infectious mononucleosis represent activated CD8+ cytotoxic T-lymphocytes.



Activation of natural killer cells

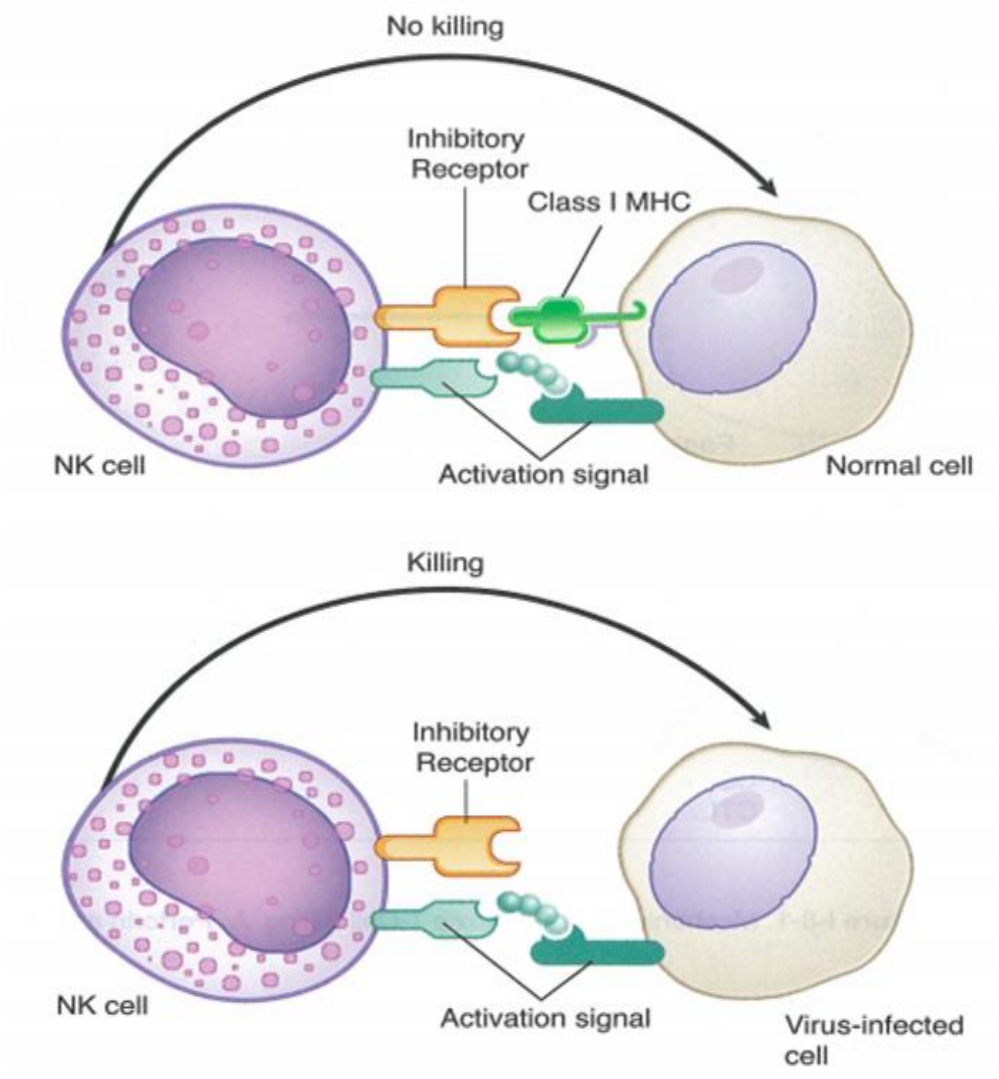
- Another cell-mediated effector mechanism enhanced by the action of Th1 cells is NK cell killing.
- NK cells are the only lymphocyte members of the innate branch of the immune response.
- They exhibit the capacity to kill cells infected with some viruses and tumor cells, and they kill via the same mechanisms of inducing apoptosis observed with CTLs (granzymes, perforin).
- NK activity is increased in the presence of interferons (IFNs stimulated during viral infections) and IL-12 (produced by phagocytic cells during the induction of Th1 responses).

- NK cells employ two categories of receptors (One delivers an activation signal, and one delivers an inhibitory signal):

A. The activation signals seem to be received from binding of lectins possibly conserved among many groups of common pathogens.

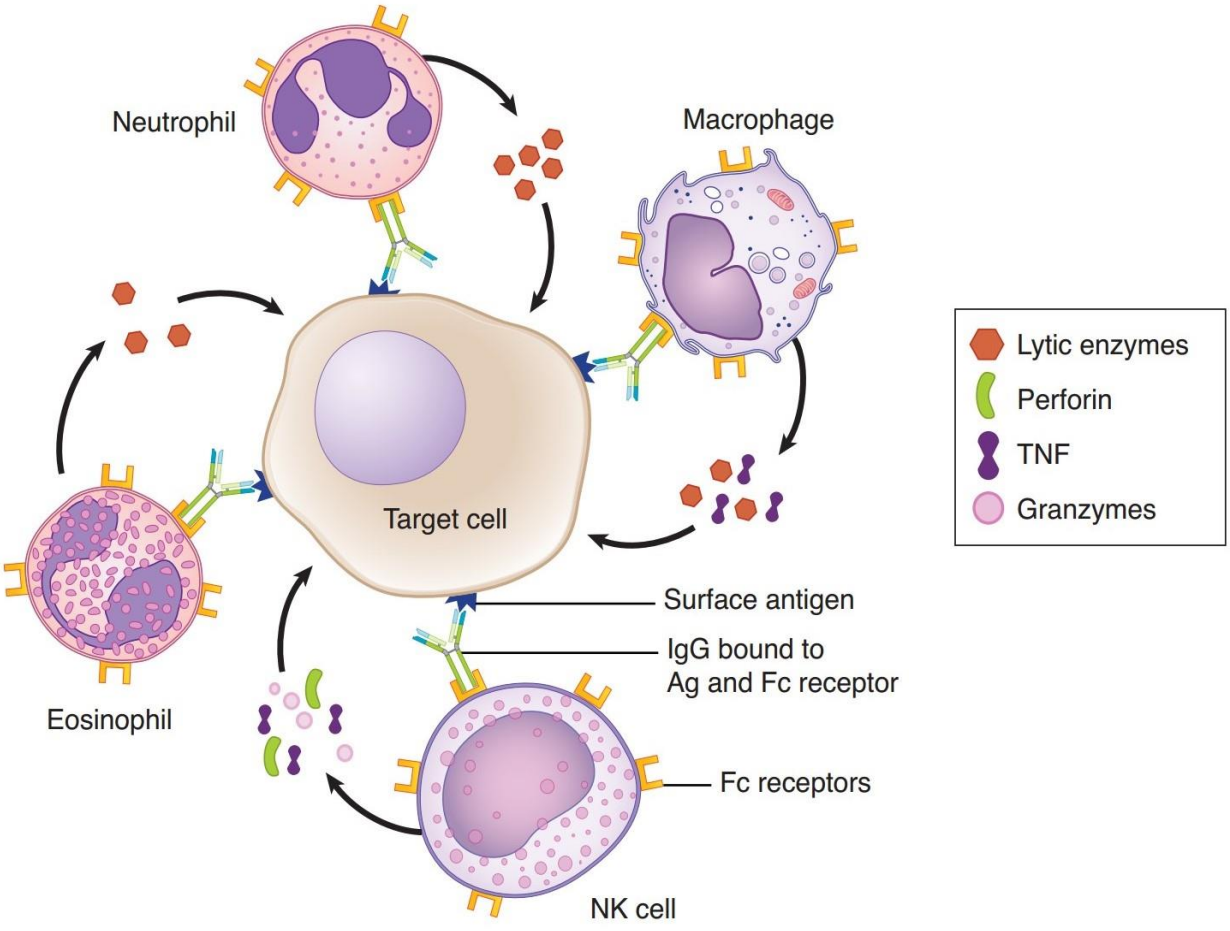
B. The inhibitory molecules on the NK cell seem to bind MHC class I antigens. Thus, a cell with normal MHC class I antigens will be protected from killing. In the absence of the MHC class I inhibitory signal, the NK cell will kill the target cell.

- MHC class I antigen expression may be downregulated during virus infections, and these antigens may be lost among tumor cells, which are genetically unstable and may delete portions of their genome.



Antibody-dependent cell-mediated cytotoxicity

- A final mechanism of cell-mediated cytotoxicity that bridges humoral and cell-mediated effector systems in the body is antibody-dependent cell-mediated cytotoxicity (ADCC).
- A number of cells with cytotoxic potential (NK cells, macrophages, neutrophils, and eosinophils) have membrane receptors for the Fc region of IgG.
- When IgG is specifically bound to a target cell, the cytotoxic cells can bind to the free Fc "tail" and subsequently cause lysis of the target cell.
- Although these effectors are not specific for antigen, the specificity of the idiotype of the antibody directs their cytotoxicity.



❖ Overview of cell surface proteins:

- MHC I present on all nucleated cells (not mature RBCs).

T cells	- TCR (binds antigen-MHC complex) - CD28 (binds B7 on APC) - CD3 (associated with TCR for signal transduction)
Helper T cells	CD4, CD40L
Cytotoxic T cells	CD8
Regulatory T cells	CD4, CD25
B cells	- Ig (binds antigen) - CD19, CD20, CD21 (receptor for Epstein-Barr virus), CD40 - MHC II, B7 - Must be 21 to drink Beer in a Barr.
Macrophages	- CD14, CD40 - MHC II, B7 - Fc and C3b receptors (enhanced phagocytosis)
NK cells	CD16 (binds Fc of IgG), CD56 (unique marker for NK)
Hematopoietic stem cells	CD34

Interferons

- Examples: IFN-α, IFN-β, IFN-γ

- Mechanism of action:

- A part of innate host defense, interferons interfere with both RNA and DNA viruses.

- Cells infected with a virus synthesize these glycoproteins, which act on local cells, priming them for viral defense by downregulating protein synthesis to resist potential viral replication and by upregulating MHC I expression to facilitate recognition of infected cells and enhance the activity of T cytotoxic and natural killer cells. Also play a major role in activating antitumor immunity.

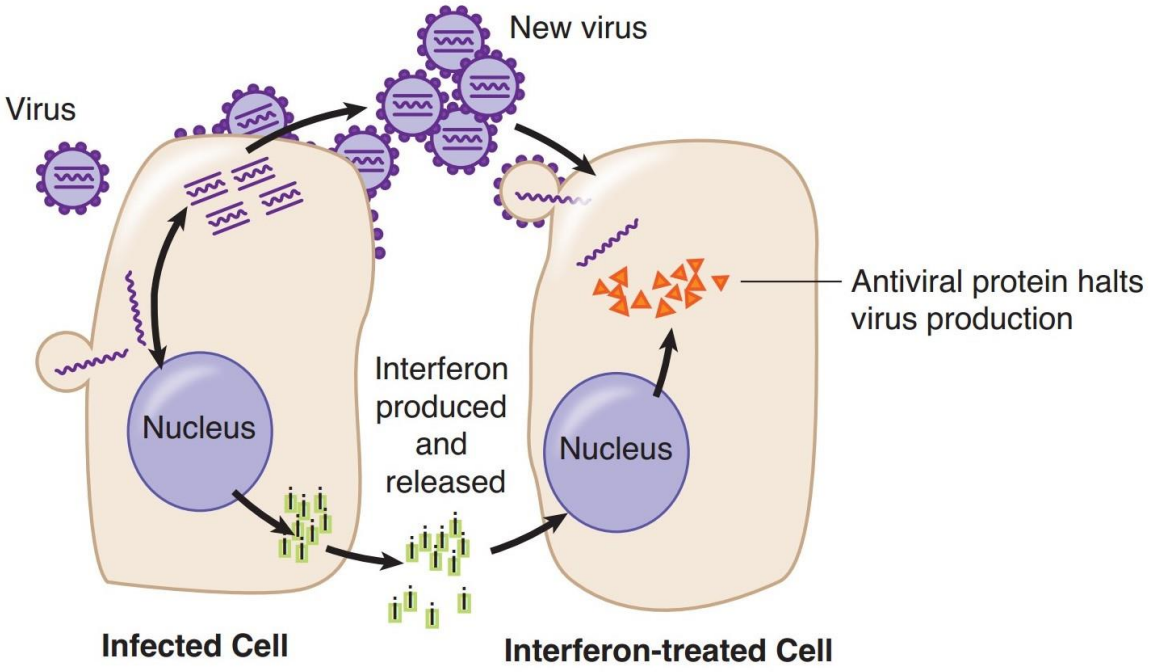
- Clinical use:

- IFN-α: chronic hepatitis B and C, Kaposi sarcoma, hairy cell leukemia, condyloma acuminatum, renal cell carcinoma, malignant melanoma.

- IFN-β: multiple sclerosis.

- IFN-γ: chronic granulomatous disease.

- Adverse effects: Flu-like symptoms, depression, neutropenia, myopathy, interferon-induced autoimmunity.


New virus
Virus
Antiviral protein halts virus production
Interferon produced and released
Nucleus
Nucleus
Infected Cell
Interferon-treated Cell

Cytokines

- Cytokines are peptide or glycoprotein mediators that are produced by cells of the immune system and have an effect on the behavior and properties of many cells.

- Many different and overlapping names have been given to the various cytokines:

- Cytokines produced by lymphocytes are often called lymphokines.

- Many cytokines are given the name interleukin (IL), followed by a number.

- Chemokines are cytokines that are involved in the migration and activation of cells, especially phagocytic cells.

- Interferons are cytokines of inducing body cells to resist viral replication, but they have other important functions, as well.

❖ Important cytokines:

1. Secreted By macrophages:

A. IL-1:

- Also called osteoclast-activating factor.
- Causes fever, acute inflammation.
- Activates endothelium to express adhesion molecules.
- Induces chemokine secretion to recruit WBCs.

B. IL-6: Causes fever and stimulates production of acute phase proteins.

C. IL-8: Major chemotactic factor for neutrophils.

D. IL-12:

- Induces differentiation of T cells into Th1 cells.
- Activates NK cells.

E. TNF-α:

- Mediates septic shock.
- Activates endothelium.
- Causes WBC recruitment, vascular leak.
- Causes cachexia in malignancy.
- Maintains granulomas in TB.

2. Secreted By All T cells:

A. IL-2: Stimulates growth of helper, cytotoxic, and regulatory T cells, and NK cells.

B. IL-3:

- Supports growth and differentiation of bone marrow stem cells.
- Functions like GM-CSF.

3. From Th1 cells:

▪ Interferon-γ:

- Secreted by Th1 and NK cells in response to IL-12 from macrophages.
- Stimulates macrophages to kill phagocytosed pathogens.
- Increases MHC expression and antigen presentation by all cells.

4. From Th2 cells:

A. IL-4:

- Induces differentiation into Th2 cells.
- Promotes growth of B cells.
- Enhances class switching to IgE and IgG.

B. IL-5:

- Promotes differentiation of B cells.
- Enhances class switching to IgA.
- Stimulates growth and differentiation of eosinophils.

C. IL-10:

- Modulates inflammatory response.
- Decreases expression of MHC class II and Th1 cytokines.
- Inhibits activated macrophages and dendritic cells.
- Also secreted by regulatory T cells.

❖ N.B:

1. TGF-β and IL-10 both attenuate the immune response.

▪ Of the cytokines released in the setting of tissue injury, TGF-B and IL-10 are thought to down-regulate local cytokine production and inflammatory reactions contributing to the systemic acute phase response.

2. Acute-phase reactants are Factors whose serum concentrations change significantly in response to inflammation; produced by the liver in both acute and chronic inflammatory states.

▪ Notably induced by IL-6.

❖ Mnemonic:

▪ "Hot T-bone stEAK":

- IL-1: fever (hot).
- IL-2: stimulates T cells.
- IL-3: stimulates bone marrow.
- IL-4: stimulates IgE production.
- IL-5: stimulates IgA production.
- IL-6: stimulates aKute-phase protein production.

❖ Cytokines available in recombinant form:

Cytokine	Clinical Uses
Aldesleukin (IL-2)	↑ Lymphocyte differentiation and ↑ NKs Used in renal cell cancer and metastatic melanoma
Oprelvekin (Interteukin- 11)	↑ Platelet formation → used in thrombocytopenia
Filgrastim (G-CSF)	↑ Granulocytes: used for marrow recovery
Sargramostim (GM-CSF)	↑ Granulocytes and macrophages → used for marrow recovery
Epoetin alfa (Erythropoietin)	Anemias, especially associated with renal failure
Thrombopoietin	Thrombocytopenia
Interferon-α	Hepatitis B and C, Kaposi sarcoma, leukemias, melanoma
Interferon-β	Multiple sclerosis
Interferon-γ	Chronic granulomatous disease

Vaccination and Immunotherapy

- Immunity to infectious organisms can be achieved by active or passive immunization.
- The goal of passive immunization is transient protection or alleviation of an existing condition, whereas the goal of active immunization is the elicitation of protective immunity and immunologic memory.
- Active and passive immunization can be achieved by both natural and artificial means.

❖ Passive vs. active immunity:

	Passive	Active
Means of Acquisition	Receiving preformed antibodies	Exposure to foreign antigens
Onset	Rapid	Slow
Duration	Short span of antibodies (half-life = 3 weeks)	Long-lasting protection (memory)
Examples	Natural: IgA in breast milk, maternal IgG crossing placenta. Artificial: antitoxin, humanized monoclonal antibody	Natural: recovery from infections. Artificial: vaccines, toxoid.
Notes	▪ After exposure to Tetanus toxin, Botulinum toxin, HBV, Varicella, or Rabies virus, Diphtheria toxin, unvaccinated patients are given preformed antibodies (passive) "To Be Healed Very Rapidly before Dying". ▪ Combined passive and active immunizations can be given for hepatitis B or rabies exposure	

- Vaccination Induces an active artificial immune response (humoral and/or cellular) to specific pathogens.

Vaccine type	Description	Pros/cons	Examples
Live attenuated Vaccine	- Microorganism loses its pathogenicity but retains capacity for transient growth within inoculated host. - Induces cellular and humoral responses. - MMR and varicella vaccines can be given to HIV ⊕ patients without evidence of immunity if CD4 cell count ≥ 200 cells/mm3.	- Pro: induces strong, often lifelong immunity. - Cons: may revert to virulent form. - Often contraindicated in pregnancy and immunodeficiency	Adenovirus (nonattenuated, given to military recruits), Typhoid (Ty21a, oral), Polio (Sabin), Varicella (chickenpox), Smallpox, BCG, Yellow fever, Influenza (intranasal), MMR, Rotavirus "Attention Teachers! Please Vaccinate Small, Beautiful Young Infants with MMR Regularly!"

Inactivated or killed Vaccine	- Pathogen is inactivated by heat or chemicals. - Maintaining epitope structure on surface antigens is important for immune response. - Mainly induces a humoral response.	- Pro: safer than live vaccines. - Cons: weaker immune response; booster shots usually required.	Rabies, Influenza (injection), Polio (Salk), hepatitis A ("R.I.P. Always").
Subunit	- Includes only the antigens that best stimulate the immune system.	- Pros: lower chance of adverse reactions. - Cons: expensive, weaker immune response.	HBV (antigen = HBsAg), HPV (types 6, 11, 16, and 18), acellular pertussis (aP), Neisseria meningitidis (various strains), Streptococcus pneumoniae, Haemophilus influenzae type b.
Toxoid	- Denatured bacterial toxin with an intact receptor binding site. - Stimulates the immune system to make antibodies without potential for causing disease.	- Pros: protects against the bacterial toxins. - Cons: antitoxin levels decrease with time, may require a booster.	Clostridium tetani, Corynebacterium diphtheriae

- Thymus-independent antigens:

- Antigens lacking a peptide component (lipopolysaccharides from gram-negative bacteria); cannot be presented by MHC to T cells.

- Streptococcus pneumoniae, Neisseria meningitidis, and Haemophifus influenzae are encapsulated bacteria whose polysaccharide capsule components can be covalently bound to protein carriers and used as vaccine antigens.

- The protein carriers convert the polysaccharides from T-cell independent to T-cell dependent antigens.

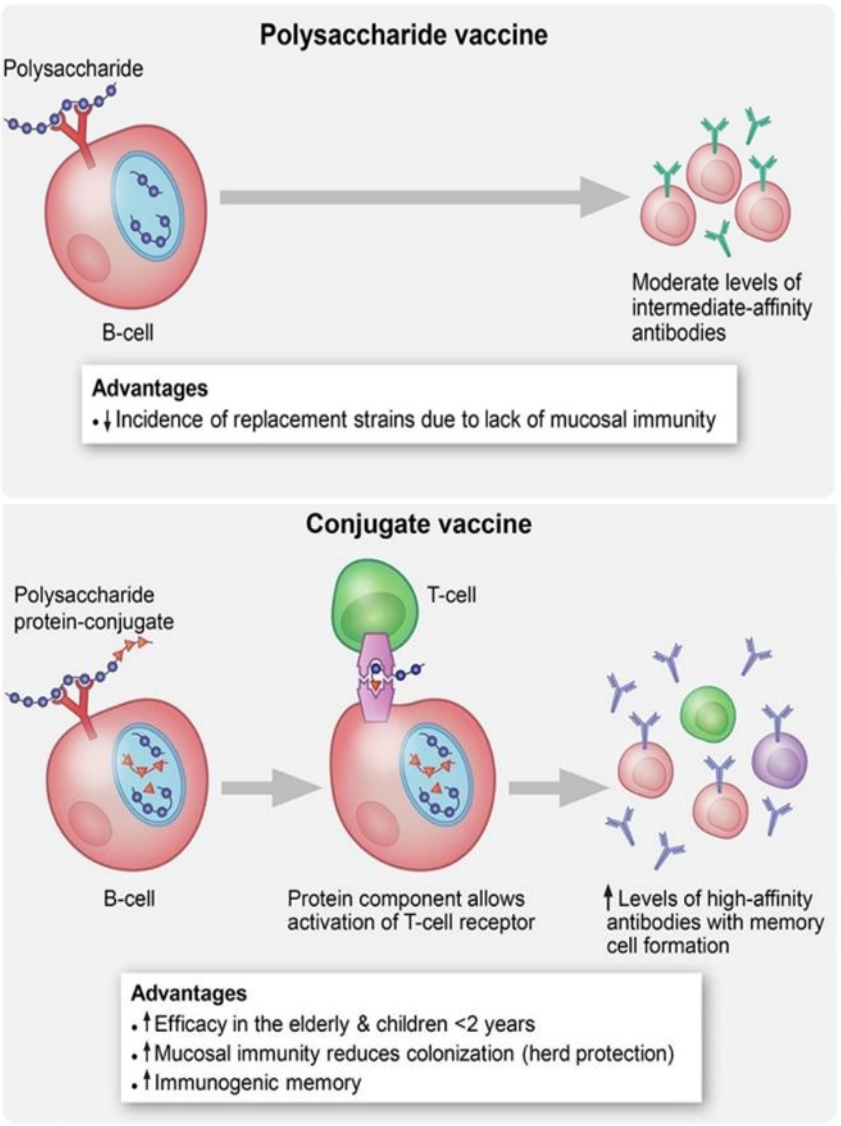
- Approved carrier proteins include mutant nontoxic diphtheria toxin, Neisseria meningitidis outer membrane protein complex, and tetanus toxoid.

- The Haemophilus influenzae type b (Hib) vaccine contains bacterial capsular polysaccharide conjugated with diphtheria toxoid.

- Thymus-dependent antigens:

- Antigens containing a protein component (diphtheria vaccine).

- Class switching and immunologic memory occur as a result of direct contact of B cells with Th cells (CD40–CD40L interaction).



❖ N.B:

1. One general principle of vaccination dictates that local secretory antibody synthesis is best promoted when specific mucosal surfaces are directly stimulated by antigen.

- When both live and killed vaccines are applied to a mucosal surface, the live attenuated viral vaccines appear more effective of the two in generating prolonged mucosal IgA secretion.
- The live vaccines are thought to colonize the natural site of viral entry, producing a greater and more prolonged immune response there.
- When a live attenuated vaccine (the Sabin oral polio vaccine) is applied to mucosal surfaces, it appears to promote more prolonged synthesis and secretion of local mucosal IgA than does a killed vaccine (the Salk inactivated polio vaccine).
- This increase in mucosal IgA offers immune protection at the normal site of viral entry.

2. The influenza vaccine is thought to prevent serious cases of the flu by increasing the host circulating antibodies against the hemagglutinin (HA) of the selected viral strains.

- Upon subsequent exposure to live influenza virus, these antibodies interfere with the binding of the HA to the sialic acid - containing oligosaccharides of host cell plasma membrane glycoprotein receptors. The live virus is prevented from entering cells via receptor-mediated endocytosis.

Diseases Caused by Immune Responses: Hypersensitivity and Autoimmunity

- Hypersensitivity diseases are conditions in which tissue damage is caused by immune responses.
- They may result from uncontrolled or excessive responses against foreign antigens or from a failure of self-tolerance, in which case they are called autoimmune diseases.
- Hypersensitivity diseases are classified on the basis of the effector mechanism responsible for tissue injury.
- Four types (ABCD): Anaphylactic and Atopic (type I), AntiBody-mediated (type II), Immune Complex (type III), Delayed (cell-mediated, type IV).
- Types I, II, and III are all antibody mediated.

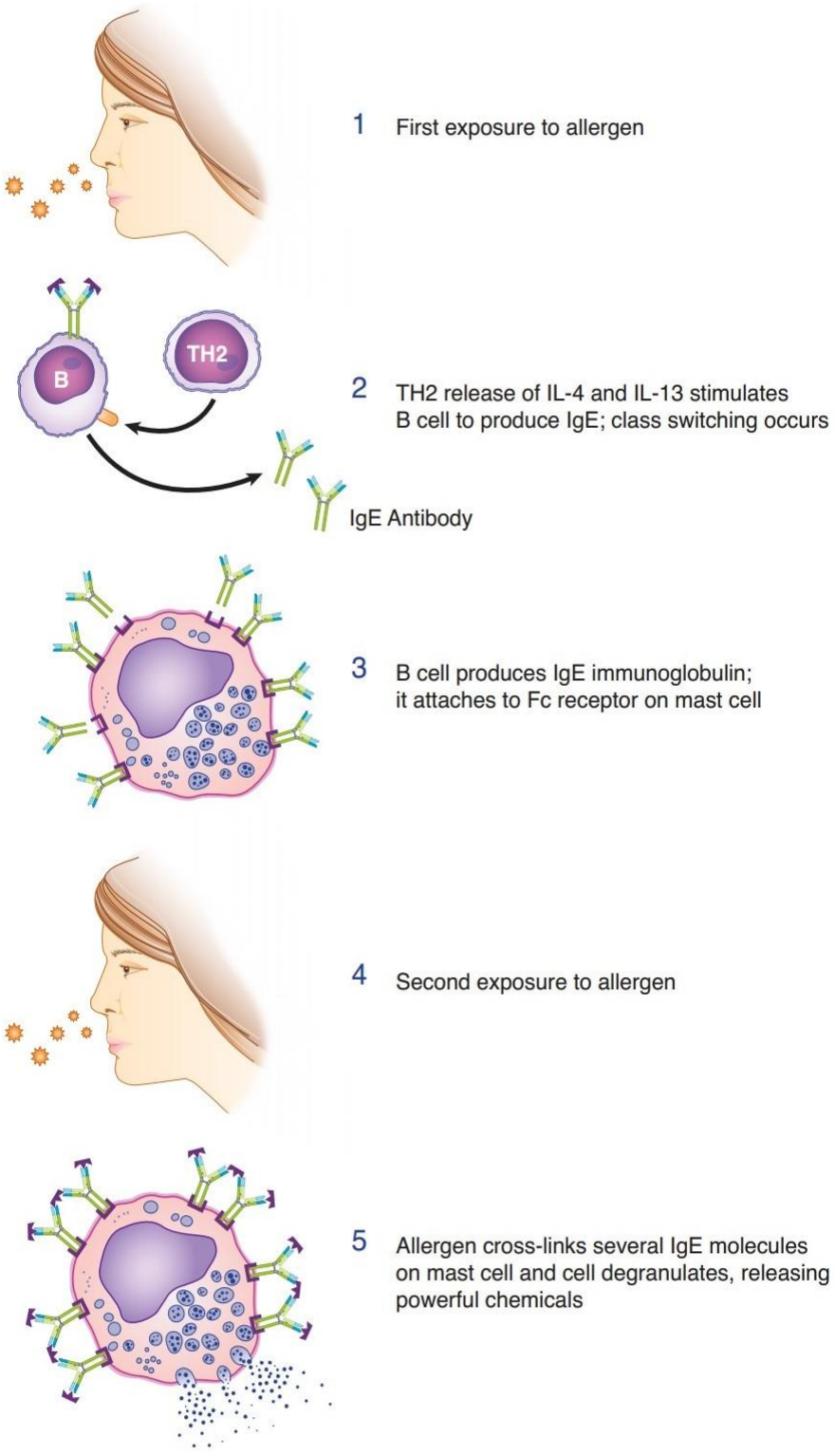
Type I (immediate) hypersensitivity

- This is the only type of hypersensitivity mediated by IgE antibodies.
- The effector cells of the immediate hypersensitivity reaction are mast cells, basophils, and eosinophils.
- First contact with antigen is asymptomatic. Upon initial exposure (first contact) to allergen, a patient who will eventually develop an allergic or anaphylactic response will undergo antibody class switching to IgE in B lymphocytes specific for these allergens.
- IgE produced by B-lymphocytes and plasma cells binds to IgE Fc receptors on basophils in the blood and mast cells in the tissues.
- Re-exposure (second time contact) to these allergens will result in cross-linking of IgE molecules on the surface of basophils and mast cells with resultant degranulation and release of histamine, proteases, heparin, leukotrienes and prostaglandins.
- Cross-linking of two IgE molecules on the surface of the cell by one molecule of allergen is required to cause degranulation.
- Two phases:

- Immediate (minutes): antigen crosslinks preformed IgE on presensitized mast cells → immediate degranulation → release of histamine (a vasoactive amine) and tryptase (a marker of mast cell activation).
- Late (hours): chemokines (attract inflammatory cells like eosinophils) and other mediators (leukotrienes) from mast cells → inflammation and tissue damage.

- This degranulation can cause a response as mild as an urticaria wheal, or as severe as anaphylaxis.

- The agents released by mast cells and basophils can cause systemic vasodilatation, increases in vascular permeability, and bronchoconstriction leading to the hemodynamic and respiratory instability characteristic of anaphylactic shock, and without prompt treatment with epinephrine this condition will lead to death.

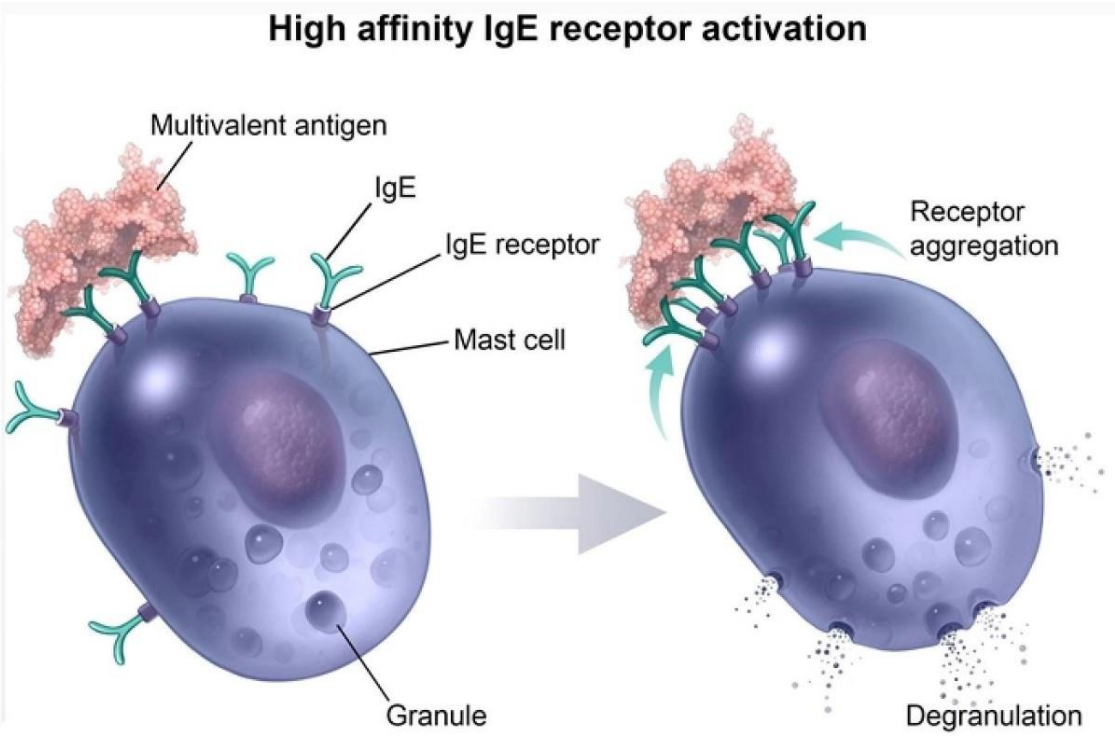


❖ Examples of type I hypersensitivity disorders:

- Allergic and atopic disorders (allergic rhinitis, hay fever, eczema, hives, asthma).
- Anaphylaxis (bee sting, some food/drug allergies).

❖ N.B:

- The high-affinity IgE receptor is found on mast cells and basophils and plays a primary role in mediating the allergic response.
- The receptor normally binds the Fc portion of circulating IgE, coating the cell with various antigen-specific IgE molecules.
- When a multivalent antigen comes in contact with the cell, multiple IgE antibodies become cross-linked, resulting in aggregation of the high-affinity IgE receptors on the mast cell surface.
- This clumping of receptors leads to the activation of non-receptor tyrosine kinases, triggering an intracellular cascade that ultimately results in mast cell and basophil degranulation.



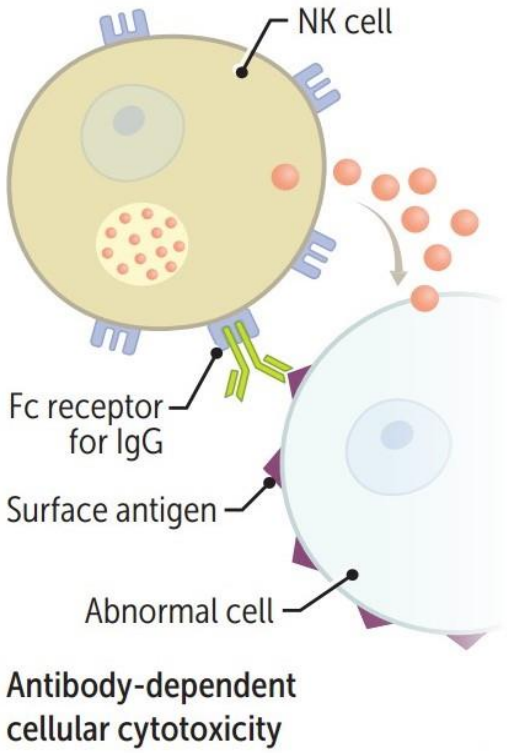
Type II hypersensitivity

- Antibodies bind to cell-surface antigens → cellular destruction, inflammation, and cellular dysfunction.

- Cellular destruction (cytotoxic):

- Cell is opsonized (coated) by antibodies, leading to either:
  - Phagocytosis and/or activation of complement system.
  - NK cell killing (antibody-dependent cellular cytotoxicity).

- Examples:
  - Autoimmune-hemolytic anemia.
  - Immune thrombocytopenia.
  - Transfusion reactions.
  - Hemolytic disease of the newborn.



- Inflammation:
- Binding of antibodies to cell surfaces → activation of complement system and Fc receptor-mediated inflammation.

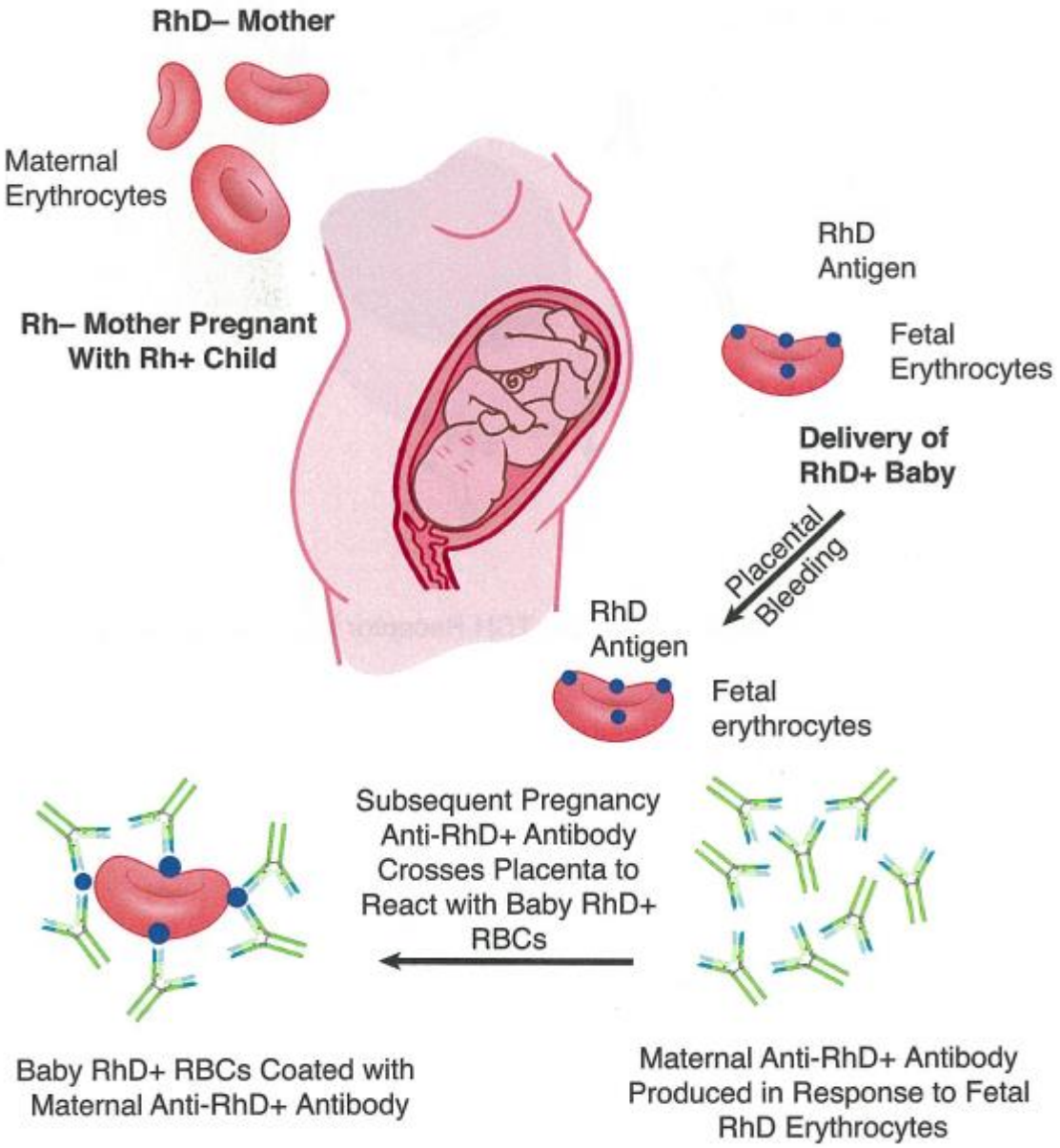
- Examples:
  - Goodpasture syndrome.
  - Rheumatic fever.
  - Hyperacute transplant rejection.

- Cellular dysfunction (non-cytotoxic):
- Antibodies bind to cell surface receptors → abnormal blockade or activation of downstream process.

- Examples:
  - Myasthenia gravis.
  - Graves disease.
  - Pemphigus vulgaris.

❖ Hemolytic disease of the newborn (erythroblastosis fetalis):

- An important example of type II hypersensitivity is HDNB, also known as erythroblastosis fetalis.
- The Rh (D) antigen is a blood group antigen present on the erythrocytes of Rh-positive individuals.
- About 85% of people are Rh+.
- If a pregnant woman is Rh- and the father is Rh+, there is a chance that the fetus will also be Rh+.
- This situation will pose no problem in the first pregnancy, as the mother's immune system will not usually encounter fetal blood cell antigens until placental separation at the time of birth.
- At that time, however, Rh+ fetal red blood cells will enter the maternal circulation and stimulate a T-dependent immune response, eventually resulting in the generation of memory B cells capable of producing IgG antibody against RhD.
- In a subsequent pregnancy with another Rh+ fetus, this maternal IgG can be transported across the placenta, react with fetal Rh+ red cells, and activate complement, producing hemolytic disease.
- Hemolytic disease of the newborn can be prevented by treating the Rh- mother with RhoGAM™, a preparation of human anti-RhD IgG antibody, at 28 weeks of gestation and again within 72 hours after birth.
- RhoGAM binds fetal erythrocyte surface Rh antigens within the maternal circulation, preventing their interaction with the maternal immune system and thus preventing sensitization.
- Fetal erythrocytes coated by this antibody are then sequestered and eliminated by the mother's spleen.
- The clinical picture in the affected infant includes severe anemia, hepatosplenomegaly, jaundice (possibly leading to kernicterus) and possible fetal demise.



❖ N.B:

1. With maternal blood types A and B, erythroblastosis fetalis and hemolytic disease of the newborn do not occur, as the naturally occurring antibodies (anti-A and B) are of the IgM type and cannot cross the placenta.

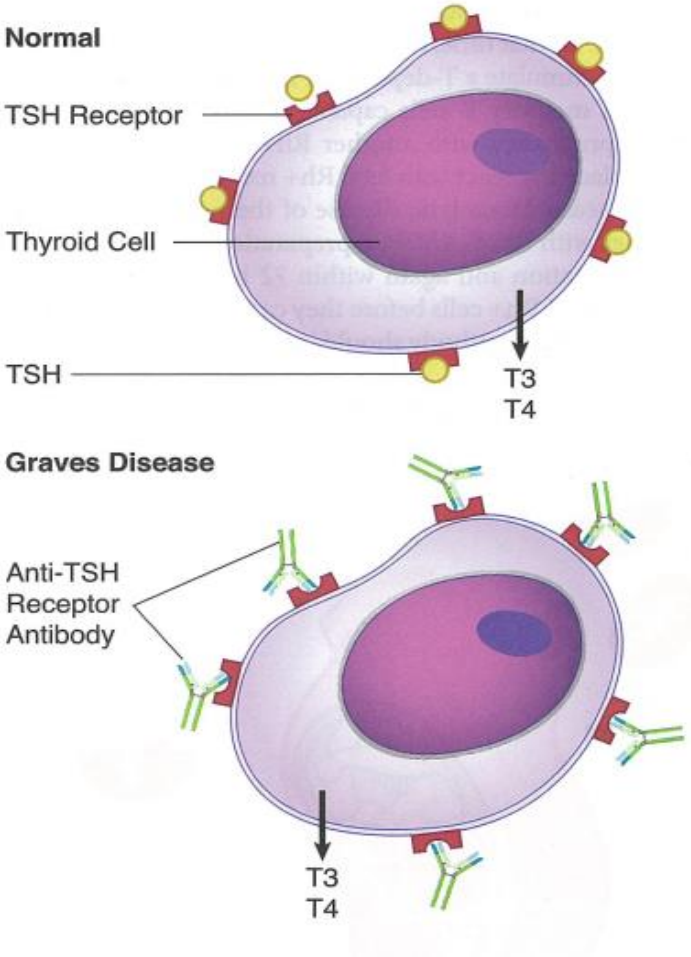
- In contrast, in type O mothers, the antibodies are predominantly IgG and can cross the placenta to cause fetal hemolysis.
- The association of a type A or B fetus with a type O mother occurs in approximately 15% of pregnancies; however, HDN occurs in only 3% of these pregnancies.
- Unlike Rh disease, ABO disease can occur with the first pregnancy because anti-A and anti-B antibodies are formed early in life from exposure to A- or B-like antigens present in foods, bacteria, and viruses.

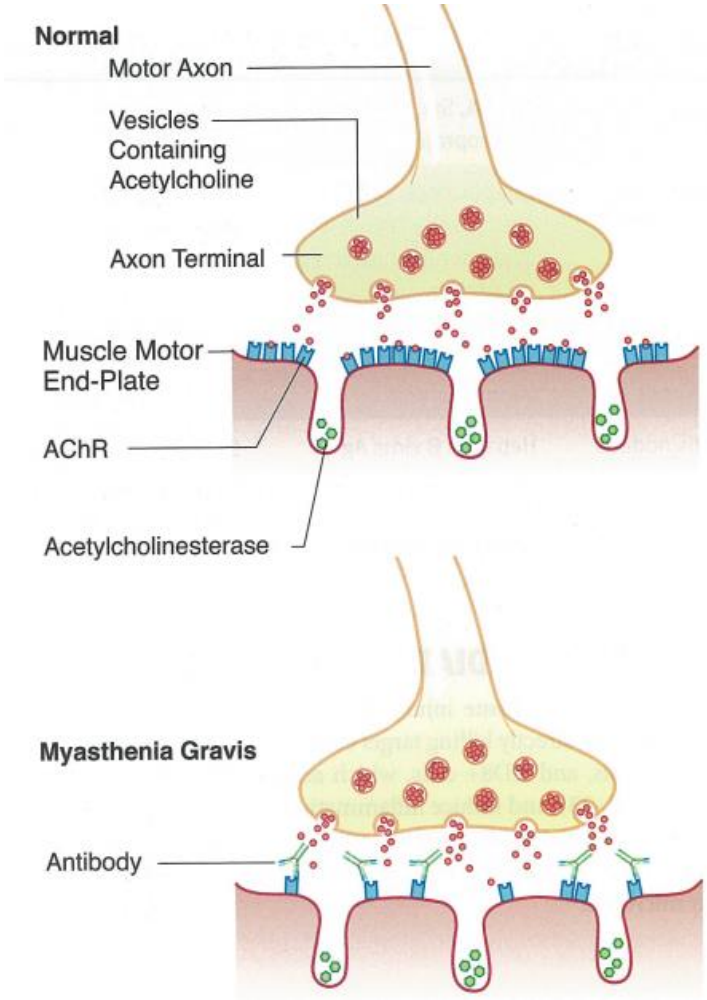
Blood Group	Antigen on RBC	Antibodies in serum	Genotypes
A	A antigen	Anti-B (IgM)	AO or AA
B	B antigen	Anti-A (IgM)	BO or BB
AB	A and B antigen	None	AB
O	None	Anti-A and Anti-B (usually IgG)	OO

2. The acute hemolytic transfusion reaction is an example of an antibody-mediated (Type II) hypersensitivity reaction.
- Resuscitation following motor vehicle accidents often includes blood transfusion in serious cases.
- Acute hemolytic reactions occur within minutes of starting a blood transfusion and are due to ABO incompatibility between the donor and recipient.
- Anti-ABO antibodies in the recipient bind the corresponding antigens on transfused donor erythrocytes.
- These antigen-antibody complexes activate complement, resulting in the production of the C3a and C5a complement components (anaphylatoxins) as well as the membrane attack complex (C5 through C9).
- Anaphylatoxins cause vasodilatation and symptoms of shock, while the membrane attack complex leads to red blood cell lysis.
- Acute transfusion reactions can be fatal.
- The symptoms include chills and shortness of breath. Patients may also experience fever, hypotension, disseminated intravascular coagulation (DIC), renal failure and hemoglobinuria (dark urine).
- Treatment includes immediate cessation of the transfusion and supportive measures.

3. Goodpasture syndrome involves autoantibodies against basement membrane collagen of glomerular and alveolar epithelia (Type II hypersensitivity).
- These antibodies cause inflammatory destruction of the basement membrane in lung alveoli and in renal glomeruli.

4. Myasthenia gravis (MG) results from an autoimmune type II, antibody mediated, hypersensitivity reaction against skeletal myocyte surface acetylcholine receptors.
- Extraocular muscle weakness is a common presenting symptom of myasthenia gravis, an autoimmune disease caused by autoantibodies against the acetylcholine receptors on the postsynaptic membrane of the neuromuscular junction.
- Normally, acetylcholine binding causes opening of these ligand-gated sodium channels, and the subsequent influx of sodium ions generates an excitatory postsynaptic potential (EPSP).
- In order to produce a self-propagating muscle action potential, there must be a sufficient number of activated acetylcholine receptors.


Normal
TSH Receptor
Thyroid Cell
TSH
T3
T4
Graves Disease
Anti-TSH
Receptor
Antibody
T3
T4


Normal
Motor Axon
Vesicles
Containing
Acetylcholine
Axon Terminal
Muscle Motor
End-Plate
AChR
Acetylcholinesterase
Myasthenia Gravis
Antibody

Type III (immune complexes) Hypersensitivity

- Immune complex: antigen-antibody (mostly IgG) complexes activate complement, which attracts neutrophils; neutrophils release lysosomal enzymes.

- Can be associated with vasculitis and systemic manifestations.

- Examples:

- SLE.
- Polyarteritis nodosa.
- Poststreptococcal glomerulonephritis.
- Serum sickness.
- Arthus reaction.

- Serum sickness:

- The prototypic immune complex disease. Antibodies to foreign proteins (serum antitoxin) are produced and 1-2 weeks later, antibody-antigen complexes form and deposit in tissues → complement activation → inflammation and tissue damage.

- Serum sickness-like reactions are associated with some drugs (monoclonal antibodies) and infections (hepatitis B).

- Sulfonamides are included among the list of drugs which can provoke this condition.

- This often results in hypocomplementemia, including a decreased serum C3 level.

- Associated findings include fever, urticaria, arthralgias, glomerulonephritis, lymphadenopathy, and a low serum C3 level 5-10 days after intravascular exposure to antigen.

- Arthus reaction:

- A local subacute antibody mediated hypersensitivity reaction.

- Intradermal injection of antigen into a presensitized (has circulating IgG) individual leads to immune complex formation in the skin.

- Characterized by edema, necrosis, and activation of complement.

❖ N.B:

- Post-streptococcal glomerulonephritis is mediated by a type III (immune complex) hypersensitivity reaction.
- During infection, antibodies form against antigens expressed by nephritogenic strains of group A β-hemolytic streptococci (Streptococcus pyogenes).
- These anti-streptococcal antibodies combine with streptococcal antigens to form immune complexes that are deposited along the glomerular basement membrane (type III hypersensitivity.
- These deposits can then be visualized as electron-dense subepithelial "humps" on electron microscopy and as granular depositions within the mesangium and glomerular capillary walls on IgG and C3 immunofluorescence.
- Patients with post-streptococcal glomerulonephritis present with edema, hematuria, and an antecedent history of streptococcal infection (impetigo, cellulitis, pharyngitis).

Type IV (T-cell mediated) Hypersensitivity

- Two mechanisms, each involving T cells (no antibody involved):

- Inflammatory reaction: effector CD4 T cells recognize antigen and release inflammation-inducing cytokines (interferon-γ).
- Direct cell cytotoxicity: CD8+ cytotoxic T cells kill targeted cells.

- Response does not involve antibodies (vs types I, II, and III).

- These reactions are referred to as "delayed" responses because, unlike reactions mediated by antibody that occur minutes after antigen exposure (ABO blood group incompatibility, hyperacute rejection, erythroblastosis fetalis), delayed reactions occur one to two days following antigen exposure (this is why you need to wait 48 to 72 hours for your annual PPD test to be read!).

- Examples:

- Contact dermatitis (poison ivy, nickel allergy).
- Graft-versus-host disease.

- Tests:

- PPD for TB infection.
- Patch test for contact dermatitis.
- Candida skin test for T cell immune function.

- 4T's: T cells, Transplant rejections, TB skin tests, Touching (contact dermatitis).

❖ N.B:

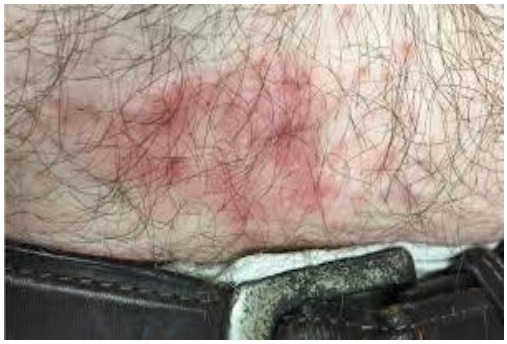
▪ Contact dermatitis Vs. Atopic dermatitis

1. Atopic dermatitis (Eczema):

- Often associated with atopic diseases (asthma, allergic rhinitis).
- It's a type I hypersensitivity reaction.

2. Contact dermatitis:

- Often associated with poison ivy and nickel allergy.
- It's a type IV hypersensitivity reaction.



❖ Hypersensitivity disorders:

Reaction	Examples	Presentation
Type I	- Allergic and atopic disorders (rhinitis, hay fever, eczema, hives, asthma) - Anaphylaxis (bee sting, some food/drug allergies)	Immediate, anaphylactic, atopic
Type II	- Acute hemolytic transfusion reactions - Autoimmune hemolytic anemia - Bullous pemphigoid - Erythroblastosis fetalis - Goodpasture syndrome - Graves disease - Guillain-Barré syndrome - Idiopathic thrombocytopenic purpura - Myasthenia gravis - Pemphigus vulgaris - Pernicious anemia - Rheumatic fever	Disease tends to be specific to tissue or site where antigen is found
Type III	- Arthus reaction (swelling and inflammation following tetanus vaccine) - SLE - Polyarteritis nodosa - Poststreptococcal glomerulonephritis - Serum sickness	Can be associated with vasculitis and systemic manifestations
Type IV	- Contact dermatitis (poison ivy, nickel allergy) - Graft-versus-host disease - Multiple sclerosis - PPD (test for M. tuberculosis)	Response is delayed and does not involve antibodies (vs. types I, II, and III)

❖ Blood transfusion reactions:

Type	Pathogenesis	Clinical presentation	Timing
Allergic/Anaphylactic reaction	Type I hypersensitivity reaction against plasma proteins in transfused blood. IgA deficient individuals must receive blood products without IgA.	Urticaria, pruritus, fever, wheezing, hypotension, respiratory arrest, shock.	Within minutes to 2-3 hours
Acute hemolytic transfusion reaction	Type II hypersensitivity reaction. Intravascular hemolysis (ABO blood group incompatibility) or extravascular hemolysis (host antibody reaction against foreign antigen on donor RBCs).	Fever, hypotension, tachypnea, tachycardia, flank pain, hemoglobinuria (intravascular hemolysis), jaundice (extravascular).	Within 1 hour
Febrile nonhemolytic transfusion reaction	Two known mechanisms: most likely induced by cytokines that are created and accumulate during the storage of blood products. or associated with type II hypersensitivity reaction with recipient antibodies directed against donor HLA and WBCs.	Fever, headaches, chills, flushing. Reaction prevented by leukoreduction of blood products.	Within 1-6 hours
Transfusion-related acute lung injury	Donor anti-leukocyte antibodies against recipient neutrophils and pulmonary endothelial cells.	Respiratory distress and noncardiogenic pulmonary edema.	Within 6 hours

Autoantibodies

AUTOANTIBODY	ASSOCIATED DISORDER
Anti-ACh receptor	Myasthenia gravis
Anti-presynaptic voltage-gated calcium channel	Lambert-Eaton myasthenic syndrome
Anti-β_2 glycoprotein I	Antiphospholipid syndrome
Antinuclear (ANA)	Nonspecific screening antibody, often associated with SLE
Anticardiolipin, lupus anticoagulant	SLE, antiphospholipid syndrome
Anti-dsDNA, anti-Smith	SLE
Anti-histone	Drug-induced lupus
Anti-U1 RNP (ribonucleoprotein)	Mixed connective tissue disease
Rheumatoid factor (IgM antibody against IgG Fc region), anti-CCP (more specific)	Rheumatoid arthritis
Anti-Ro/SSA, anti-La/SSB	Sjögren syndrome
Anti-Scl-70 (anti-DNA topoisomerase I)	Scleroderma (diffuse)
Anticentromere	Limited scleroderma (CREST syndrome)
Antisynthetase (eg, anti-Jo-1), anti-SRP, anti-helicase (anti-Mi-2)	Polymyositis, dermatomyositis
Antimitochondrial	1° biliary cholangitis
Anti-smooth muscle	Autoimmune hepatitis type 1
MPO-ANCA/p-ANCA	Microscopic polyangiitis, eosinophilic granulomatosis with polyangiitis (Churg-Strauss syndrome), ulcerative colitis
PR3-ANCA/c-ANCA	Granulomatosis with polyangiitis (Wegener)
Anti-phospholipase A_2 receptor	1° membranous nephropathy
Anti-hemidesmosome	Bullous pemphigoid
Anti-desmoglein (anti-desmosome)	Pemphigus vulgaris
Antimicrosomal, antithyroglobulin, antithyroid peroxidase	Hashimoto thyroiditis
Anti-TSH receptor	Graves disease
IgA anti-endomysial, IgA anti-tissue transglutaminase, IgA and IgG deamidated gliadin peptide	Celiac disease
Anti-glutamic acid decarboxylase, islet cell cytoplasmic antibodies	Type 1 diabetes mellitus
Antiparietal cell, anti-intrinsic factor	Pernicious anemia
Anti-glomerular basement membrane	Goodpasture syndrome

Immunodeficiency Diseases

- If individuals experience defects in the functioning of any of the components of the immune system, clinical manifestations are common.

B-cell disorders

A. X-linked (Bruton) agammaglobulinemia:

- Defect:

- It is a B-cell immunodeficiency disorder in which the Bruton tyrosine kinase (BTK) gene codes for a defective version of this critical signal transduction molecule.

- Normal Bruton tyrosine kinase function is necessary for the proper maturation of B-cells.

- Defect in BTK, a tyrosine kinase gene → no B-cell maturation.

- X-linked recessive (↑ in Boys).

- Presentation:

- This is an X-linked condition that results in a deficiency of all forms of antibody and low B cell counts due to a defect in B lymphocyte maturation.

- T cell numbers and function are intact.

- Recurrent bacterial and enteroviral infections after 6 months (↓ maternal IgG).

- They are deficient in all types of immunoglobulins, including IgA (which predisposes to recurrent lower respiratory tract infections and Giardia lamblia infection).

- Findings:

- Absent B cells in peripheral blood, ↓ Ig of all classes.

- Germinal centers contained within lymphoid follicles are where B lymphocytes normally go to proliferate and undergo somatic hypermutation after exposure to antigen. Without B cells, there would be no lymphoid follicles or germinal centers in the lymph nodes.

- Live vaccines contraindicated.

B. Selective IgA deficiency:

- Defect:

- Selective IgA deficiency is the most commonly occurring primary immunodeficiency.

- It is thought to occur due to failure of B-cells to switch from IgM to IgA production.

- Presentation:

- Most commonly these patients are asymptomatic, but classically this immunodeficiency predisposes to recurrent sinopulmonary and GI tract infections due to the absence of secretory IgA.

- Recurrent otitis media, sinusitis, bronchitis or pneumonias are caused by encapsulated bacteria, such as H. influenzae or S. pneumoniae.

- Gastrointestinal infections manifest as recurrent acute or chronic diarrhea due to viral, bacterial, and G. lamblia infections.

- ↑ Atopy and Autoimmune diseases.

- When transfused with blood or blood products containing small amounts of IgA these patients may develop potentially fatal anaphylactic reactions. Gamma-globulin preparations should not be used for treatment of these patients as it may increase the synthesis of anti-IgA antibodies because the patient's body recognizes it as a foreign.

- Findings: ↓ IgA with normal IgG, IgM levels.

C. Common variable immunodeficiency:

- Defect: Defect in B-cell differentiation. Many causes.

- Presentation: Onset in late teens. Can be acquired in 20s-30s; ↑ risk of autoimmune disease, bronchiectasis, lymphoma, sinopulmonary infections.

- Findings: ↓plasma cells, ↓ immunoglobulins.

D. Transient hypogammaglobulinemia of infancy:

- Defect: Delayed onset of normal IgG synthesis.

- Presentation: Detected in 5th to 6th month of life, resolves by 16-30 months; ↑susceptibility to pyogenic bacteria.

- Findings: ↓IgG in early life of infancy.

T-cell disorders

A. Thymic aplasia (DiGeorge syndrome):

- Defect: 22q11 microdeletion; maldevelopment of the third and fourth pharyngeal pouch derivatives → absent thymus and parathyroid gland.

- Presentation:

- The immunodeficiency results from aplasia of the thymus leading to an extreme deficiency in the number of mature T-lymphocytes.

- T-lymphocytes are synthesized in the bone marrow, but they require processing in the thymus in order to mature and be effective in the body.

- T-cell immunodeficiencies such as DiGeorge syndrome predispose patients to recurrent infections by viral, fungal, protozoan and intracellular bacterial pathogens.

- Other classic clinical associations with DiGeorge syndrome are:
  - Tetany resulting from hypocalcemia due to parathyroid gland aplasia.
  - Aortic arch abnormalities (teratology of fallot, truncus arteriosus).
  - Distorted facies due to aberrant formation of the mandible and palate (frequently with a cleft palate), and low-set ears.

- Findings:

- ↓ T cells, ↓PTH, ↓ Ca.

- Absent thymic shadow on CXR.

- The paracortex is the region of the lymph node populated primarily by T lymphocytes. In DiGeorge syndrome, this region is poorly developed due to a deficiency of mature T lymphocytes.

- CATCH-22: Cardiac defects [tetralogy of Fallot, truncus arteriosus]), Abnormal facies, Thymic hypoplasia → T-cell deficiency (recurrent viral/fungal infections), Cleft palate, Hypocalcemia 2° to parathyroid aplasia → tetany.

B. IL-12 receptor deficiency:

- Defect: ↓ Th1 response. Autosomal recessive.

- Presentation: Disseminated mycobacterial and fungal infections; may present after administration of BCG vaccine (live attenuated vaccine).

- Findings: ↓ IFN-γ.

C. Autosomal dominant hyper-IgE syndrome (Job syndrome):

- Defect:

- Th1 cells cannot make IFN-γ.

- Deficiency of Th17 cells due to STAT3 mutation → impaired recruitment of neutrophils to sites of infection.

- Presentation:

- Learn the ABCDEF's to get a Job: Cold (noninflamed) staphylococcal Abscesses, retained Baby teeth, Coarse facies, Dermatologic problems (eczema), ↑ IgE, bone Fractures from minor trauma.

- ↑IgE is due to ↓ IFN-γ → no inhibition of Th2 differentiation →↑ IL4 → ↑ IgE.

- Findings: ↑IgE, eosinophils.

D. Chronic mucocutaneous candidiasis:

- Defect:

- T-cell dysfunction.

- Heterogeneous group of immune system defects → impaired cell-mediated immunity against Candida sp. Classic form caused by defects in AIRE.

- Presentation: Noninvasive Candida albicans infections of skin and mucous membranes.

- Findings:

- Absent in vitro T-cell proliferation in response to Candida antigens.

- Absent cutaneous reaction to Candida antigens.

❖ N.B:

1. Candida albicans is a component of the normal human skin and mucous membrane flora.

- Candida does not cause disseminated infections in healthy people, but can induce a number of serious diseases when the immune system is weakened.
- Candida is, therefore, an opportunistic pathogen.
- Host immune defense against Candida is provided by T-lymphocytes and neutrophils.
- These two components of the antifungal defense have distinct functions:

A. T-lymphocytes (in particular Th cells):

- They are important for the prevention of superficial Candida infection.
- Individuals with deficient T-cell function are susceptible to localized Candida infections such as oral thrush, cutaneous candidiasis, and Candida vulvovaginitis.
- The role of T-lymphocytes in host defense from Candida can be seen in HIV-positive patients.
- These individuals have frequent superficial Candida infections, but rarely suffer from disseminated candidiasis.

B. Neutrophils:

- They prevent the hematogenous spread of Candida.
- Disseminated candidiasis is more likely to occur in neutropenic patients and those with inherited impairments of phagocytosis.
- If HIV-positive patients also have neutropenia, they are at risk for both localized and disseminated candidiasis.
- Examples of disseminated disease caused by Candida species include right-sided endocarditis, liver and kidney abscesses, and candidemia.

▪ For this reason, localized candidiasis is common in HIV-positive patients, while neutropenic individuals are more likely to have systemic disease.

2. The candida skin test gauges the delayed-type (type IV) hypersensitivity reaction.

▪ Failure to generate a response to this test is referred to as anergy.

▪ The active cells in the cell-mediated response are macrophages, CD4 and CD8 T-lymphocytes and NK cells.

E. MHC Class I Deficiency:

▪ Defect:

- A recessively inherited deficiency in the production of MHC class I molecules has been described in rare individuals.

- Some of these cases result from the failure of TAP molecules to transport MHC I molecules to the surface of the cell, and others are due to the production of aberrant or nonfunctional MHC I molecules themselves.

- Other causes may be a genetic mutation of B_2 macroglobulin which is important for the proper folding of MHC class I.

▪ Presentation:

- These patients, as anticipated, suffer from profound deficiencies of CD8 T cells, although numbers of CD4 T cells are normal.

- This is because MHC class I expression in the thymus is essential to the development of committed CD8 cells.

- Patients are susceptible to multiple, recurrent viral infections, but interestingly, not all viral infections appear to be involved.

- It may be that they are able to compensate in the case of some specific viral infections, by using NK cells to control those infections, whereas other viruses require killing by CD8+ cells alone.

B- and T-cell disorders

A. Severe combined immunodeficiency (SCID):

- Defect:

- Several types including defective IL-2R gamma chain (most common, X-linked).

- Gamma chain is a protein that is shared by the receptors for many interleukins, these interleukins and their receptors are involved in the development and differentiation of T and B cells.

- Adenosine deaminase deficiency is the second most cause of SCID.

- Adenosine deaminase is present in all cells of the human body, and it functions to deaminate adenosine to inosine as an initial step in the elimination of excess adenosine from the cell.

- Adenosine accumulation is toxic to lymphocytes and leads to widespread death of both T and B lymphocytes with resultant combined cellular and humoral immunodeficiency.

- Because both humoral and cell-mediated immunity are deficient in these patients, they are vulnerable to increased infections by bacteria, viruses and fungi.

- Presentation: Patients with SCID present with recurrent infections caused by bacteria, viruses, fungi, and opportunistic pathogens as well as failure to thrive, thrush and chronic diarrhea within the first year of life.

- Findings:

- ↓ T-cell receptor excision circles (TRECs).

- Absence of thymic shadow (CXR), germinal centers (lymph node biopsy), and T cells (flow cytometry).

- Treatment:

- Bone marrow transplant (no concern for rejection).

- Retroviral gene therapy for this illness is promising. Retroviral vectors are used to "infect" patient hematopoietic stem cells with the genetic code for adenosine deaminase thereby resulting in production of this enzyme by all daughter cells of that stem cell.

B. Ataxia-telangiectasia:

- Defect:

- It is an autosomal recessive condition that occurs due to mutation of ATM gene. ATM (Ataxia Telangiectasia Mutated) gene is responsible for DNA break repair.

- Defects in ATM gene → failure to repair DNA double strand breaks → cell cycle arrest.

- DNA in patients with ataxia-telangiectasia is hypersensitive to X-ray radiation that causes multiple chromosomal breaks.

▪ Presentation:

- Cerebellar ataxia, telangiectasias (abnormal dilatations of capillary vessels), and increased risk of sinopulmonary infections (due to IgA deficiency) constitute a characteristic triad of ataxia telangiectasia.

- Cerebellar atrophy leads to the ataxia that occurs in the first years of life.

- Oculocutaneous telangiectasia is another manifestation but is usually delayed.

- The risk of cancer in these patients is increased significantly because of inefficient DNA repair (↑ risk of lymphoma and leukemia).

▪ Findings: ↓ IgA, IgG, and IgE. Lymphopenia, cerebellar atrophy.

C. Hyper-IgM syndrome:

▪ Defect:

- Genetic deficiency in the CD-40 T-lymphocyte ligand that is essential in inducing B-cells to switch classes.

- Therefore, Th cells from these patients will fail to express functional CD40L on their membrane and will thereby fail to give the costimulatory signal necessary for the B-cell response to T-dependent antigens, so only IgM antibodies are produced.

- The B-cell response to T-independent antigens is unaffected.

▪ Presentation: Severe pyogenic infections early in life; opportunistic infection with Pneumocystis, Cryptosporidium, CMV.

▪ Findings:

- Normal or ↑ IgM. ↓↓ IgG, IgA, IgE.

- These patients fail to make germinal centers during a humoral immune response.

D. Wiskott-Aldrich syndrome:

▪ Defect:

- Mutation in WAS gene (X-linked recessive); leukocytes and platelets unable to reorganize actin cytoskeleton → defective antigen presentation.

- It results from a mutation on the X-chromosome and, therefore, is only present in males as an X-linked disorder.

- Presentation:
- The Wiskott-Aldrich syndrome consists of the triad of eczema, thrombocytopenia, and recurrent infections due to combined B-lymphocyte and T-lymphocyte deficiency.

- WATER: Wiskott-Aldrich → Thrombocytopenia, Eczema, Recurrent (pyogenic) infections.

- Infections worsen as the patient ages and become most apparent initially after transplacental maternal IgG and maternal mucosal IgA derived from the colostrum are degraded at approximately 6 months of age.

- Treatment is with an HLA-matched bone marrow transplantation.

- Findings: ↓ to normal IgG, IgM. ↑ IgE, IgA. Fewer and smaller platelets.

E. Bare Lymphocyte Syndrome/MHC class II deficiency:

- Defect: results from defects in the transcription factors required to coordinate their expression on the cell surface.

- Presentation:

- Because MHC class I antigens are expressed normally, they do have CD8 cells, although their function is diminished by the absence of Th1 cell cytokines.

- Immune problems tend to appear early in infancy and present as a mild form of severe combined immunodeficiency (SCID) with increased susceptibility to pyogenic and opportunistic infections.

- However, these defects can be distinguished from true SCID in that these patients will have T cells that can respond to nonspecific T-cell mitogens, such as phytohemagglutinin.

- They do not develop graft versus-host disease when given HLA-mismatched bone marrow transplants because they do not express the MHC class II molecules against which such grafted cells can react.

Phagocyte dysfunction

A. Leukocyte adhesion deficiency (type 1):

- Defect:

- LAD results from the absence of CD 18. Autosomal recessive.

- This leads to the inability to synthesize the beta-2 integrins LFA 1, affecting tight adhesion and transmigration of inflammatory cells to the site of inflammation.

- Integrins are essential for the migration of leukocytes from the vascular space to the tissues where they exert their effect.

- Presentation:

- Recurrent bacterial skin and mucosal infections, absent pus formation, impaired wound healing, delayed separation of umbilical cord (> 30 days).

- The first indication of this defect is often omphalitis, a swelling and reddening around the stalk of the umbilical cord.

- Findings:

- ↑ Neutrophils. Absence of neutrophils at infection sites.

- These patients frequently have abnormally high numbers of granulocytes in their circulation, but migration into sites of infection is not possible, so abscess and pus formation do not occur.

B. Chédiak-Higashi syndrome:

- Defect:

- Defect in lysosomal trafficking regulator gene (LYST).

- Microtubule dysfunction in phagosome-lysosome fusion; autosomal recessive.

- This causes abnormal giant lysosomal inclusions that are visible on light microscopy of a peripheral blood smear.

- Presentation:

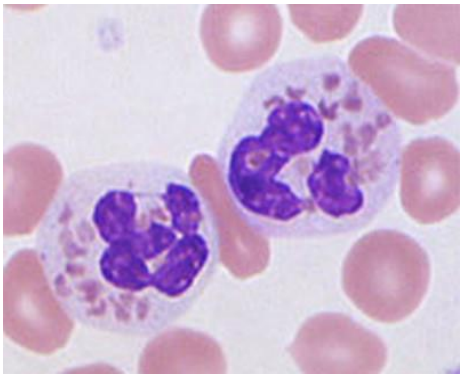
- Recurrent pyogenic infections by staphylococci and streptococci, partial albinism, peripheral neuropathy, progressive neurodegeneration, infiltrative lymphohistiocytosis.

- PLAIN: Progressive neurodegeneration, Lymphohistiocytosis, Albinism (partial), recurrent pyogenic Infections, peripheral Neuropathy.

- Findings:

- Giant granules in granulocytes and platelets.

- Pancytopenia. Mild coagulation defects.



C. Chronic granulomatous disease:

- Defect:

- Chronic granulomatous disease (CGD) is most frequently an X-linked disorder resulting from a deficiency of NADPH oxidase.

- Deficiency of this enzyme leads to an inability of neutrophils to form the oxidative burst to kill organisms in their phagolysosomes.

- Organisms that produce catalase are ineffectively killed by these defective neutrophils while organisms that do not produce catalase can still be killed due to accumulation of bacterial hydrogen peroxide within the phagosome.

- Presentation:

- ↑ susceptibility to catalase ⊕ organisms (Need PLACESS): Nocardia, Pseudomonas, Listeria, Aspergillus, Candida, Burkholderia Cepacia, E. coli, S. aureus, Serratia.

- These organisms are all catalase positive.

- Catalase decomposes H_2O_2 ($2\ H_2O_2 \rightarrow O_2 + 2\ H_2O$).

- Findings:

- Abnormal dihydrorhodamine (flow cytometry) test (↓ green fluorescence).

- Nitroblue tetrazolium dye reduction test is ⊖ (patient's neutrophils fail to turn blue upon nitroblue tetrazolium testing this is the hallmark test for CGD)

- The nitroblue tetrazolium test is carried out by adding nitroblue tetrazolium to a sample of patient neutrophils.

- Properly functioning neutrophils are able to produce reactive oxygen species such as superoxide, and these chemicals are able to reduce nitroblue tetrazolium, leading to formation of a dark blue pigment within the cells.

- Cells from patients with CGD are unable to reduce nitroblue tetrazolium because they cannot produce reactive oxygen species due to a genetic defect resulting in NADPH oxidase deficiency.

❖ N.B:

- Glucose-6-phosphate dehydrogenase (G6PD) deficiency → There is deficiency of essential enzymes in hexose monophosphate shunt → same symptoms as CGD with associated anemia.

❖ Infections in immunodeficiency:

Pathogen	↓ T cellS	↓ B cellS	↓Granulocytes	↓Complement
Bacteria	Sepsis	Encapsulated (Please SHINE my SKiS): Pseudomonas aeruginosa, Streptococcus pneumoniae, Haemophilus Influenzae type b, Neisseria meningitidis, Escherichia coli, Salmonella, Klebsiella pneumoniae, Group B Streptococcus	Some Bacteria Produce No Serious granules: Staphylococcus, Burkholderia cepacia, Pseudomonas aeruginosa, Nocardia, Serratia	Encapsulated species with early component deficiencies Neisseria with late component (MAC) deficiencies
Viruses	CMV, EBV, JCV, VZV, chronic infection with respiratory/GI viruses	Enteroviral encephalitis, poliovirus (live vaccine contraindicated)	N/A	N/A
Fungi/parasites	Candida (local), PCP, Cryptococcus	GI giardiasis (no IgA)	Candida (systemic), Aspergillus, Mucor	N/A

❖ N.B:

- B-cell deficiencies tend to produce recurrent bacterial infections, whereas T-cell deficiencies produce more fungal and viral infections.

Transplantation Immunology

- Transplantation is the process of taking cells, tissues, or organs (a graft) from one individual (the donor) and implanting them into another individual or another site in the same individual (the host or recipient).

- Several different types of grafts are used in medicine:

- Autograft: From self.
- Syngeneic graft (isograft): From identical twin or clone.
- Allograft: From nonidentical individual of same species.
- Xenograft: From different species.

Mechanisms of graft rejection

- The recognition of transplanted cells as self or foreign is determined by the extremely polymorphic genes of the major histocompatibility complex, which are expressed in a codominant fashion.

- This means that each individual inherits a complete set or haplotype from each parent and virtually assures that two genetically unrelated individuals will have distinctive differences in the antigens expressed on their cells.

- The net result is that all grafts except autografts are ultimately identified as foreign invading proteins and destroyed by the process of graft rejection.

- Even syngeneic grafts between identical twins can express recognizable antigenic differences due to somatic mutations that occur during the development of the individual. For this reason, all grafts except autografts must be followed by some degree of lifelong immunosuppression of the host to attempt to avoid rejection reactions.

- Four different classes of allograft rejection phenomena are classified according to their time of activation and the type of effector mechanism that predominates:

1. Hyperacute rejection:

- Onset: Within minutes.

- Pathogenesis:

- Hyperacute rejection is an antibody-mediated reaction that is caused by preformed antibodies (type II hypersensitivity reaction) within the recipient that are directed against donor antigens and activate complement.

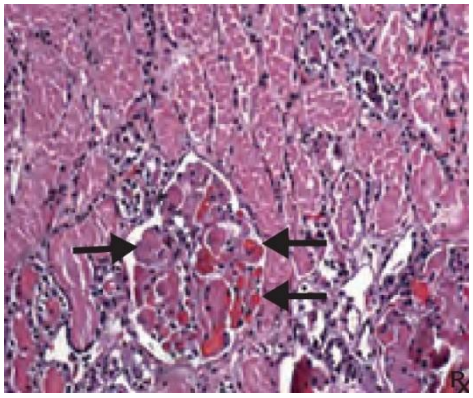
- Examples of such mismatches include ABO blood group antibodies and anti-HLA antibodies.

- Classically, this form of transplant rejection is diagnosed by the surgeon in the operating room because upon anastomosis of the donor and recipient blood vessels and initial perfusion of the organ, the organ immediately becomes cyanotic and mottled.

- Blood flow through the new organ ceases immediately due to fibrinoid necrosis of the small vessels of the organ in addition to the rapid formation of extensive thrombosis within the transplanted organ.

▪ Features:
- Widespread thrombosis of graft vessels (arrows within glomerulus) → ischemia/necrosis.

- Graft must be removed.



2. Acute rejection:

▪ Onset: Weeks to months.

▪ Pathogenesis:
- Cellular: CD8 T cells and/or CD4 T cells activated against donor MHCs (type IV hypersensitivity reaction).

- Humoral: similar to hyperacute, except antibodies develop after transplant.

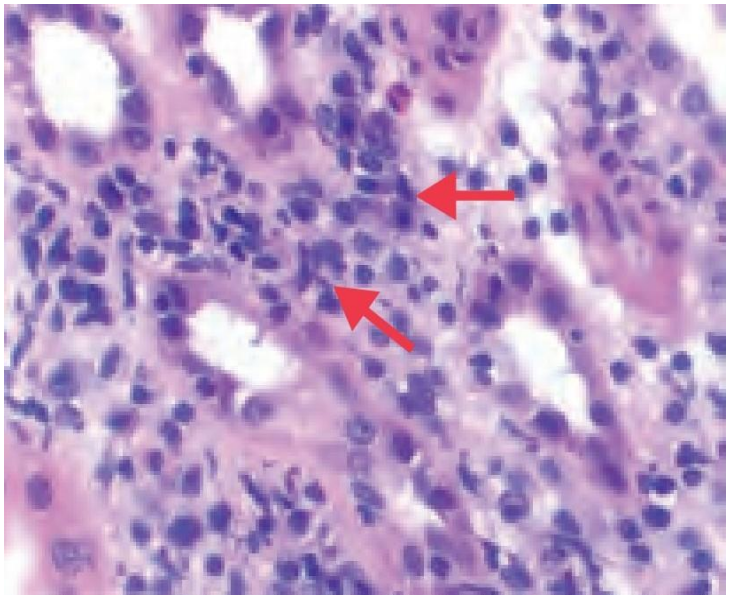
- Acute rejection occurs in approximately 2 of every 5 hearts transplanted and in the large majority of cases occurs by the cell-mediated pathway.

- In rare cases, acute rejection is due to anti-donor host antibodies, and cases of humoral rejection are diagnosed by direct immunofluorescence.

- The clue that indicates acute rejection is the timeframe of symptoms because acute rejection usually occurs one to four weeks following transplant and the histopathology showing a dense infiltrate of mononuclear cells (this is typical histopathology in acute rejection).

▪ Features:
- Vasculitis of graft vessels with dense interstitial lymphocytic infiltrate.

- Treatment with immunosuppressive drugs is aimed primarily at preventing this form of rejection.



3. Chronic rejection:

- Onset: Months to years.

- Pathogenesis:

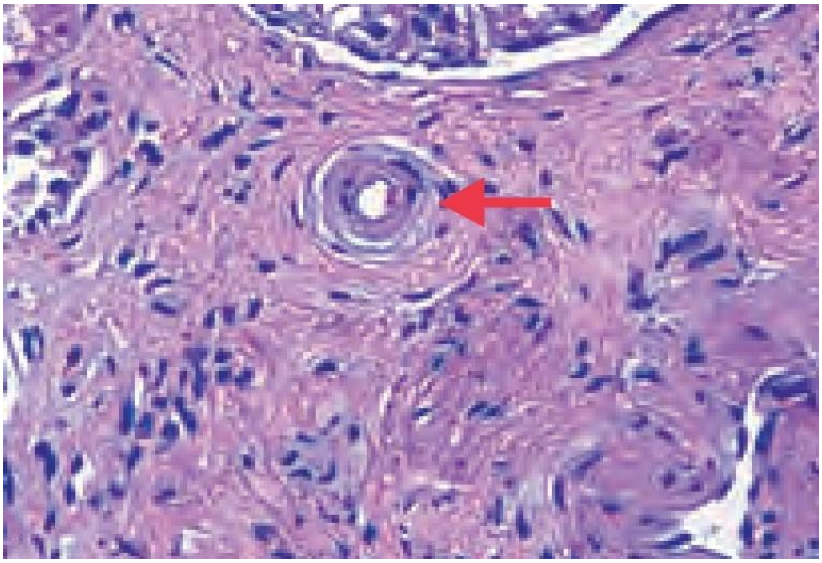
- CD4+ T cells respond to recipient APCs presenting donor peptides, including allogeneic MHC.

- Both cellular and humoral components (type II and IV hypersensitivity reactions).

- Features:

- Recipient T cells react and secrete cytokines that induces proliferation of vascular smooth muscle and parenchymal fibrosis and causes an obliterative intimal smooth muscle hypertrophy and fibrosis of cortical arteries (obliterative vascular fibrosis).

- Dominated by arteriosclerosis.



4. Graft-versus-host disease (GVHD):

- Onset: Varies.

- Pathogenesis:

- Graft-versus-host disease (GVHD) is a condition that most commonly occurs after allogeneic bone marrow transplantation, transplantation of organs rich in lymphocytes (such as liver), or transfusion of non-irradiated blood.

- The host is generally severely immunodeficient due to the primary disease process or as a result of immunosuppressive medications.

- Immunocompetent T-cells within the donor tissue recognize host MHC antigens as foreign and attack them (Type IV hypersensitivity reaction). Both donor CD4 and CD8 cells participate in destroying host cells.

- Any organ may be a target of GVHD, but the skin, liver, and GI tract are generally affected most severely:
  - Liver damage manifests with jaundice and increased levels of ALT, AST, alkaline phosphatase, and bilirubin.
  - GI tract involvement causes diarrhea, intestinal bleeding, abdominal cramping, and/or ileus.
  - In severe cases, there may be skin desquamation.

- Features:

- Maculopapular rash, jaundice, diarrhea, hepatosplenomegaly.

- Usually in bone marrow and liver transplants (rich in lymphocytes).

- Potentially beneficial in bone marrow transplant for leukemia (graft-versus-tumor effect).

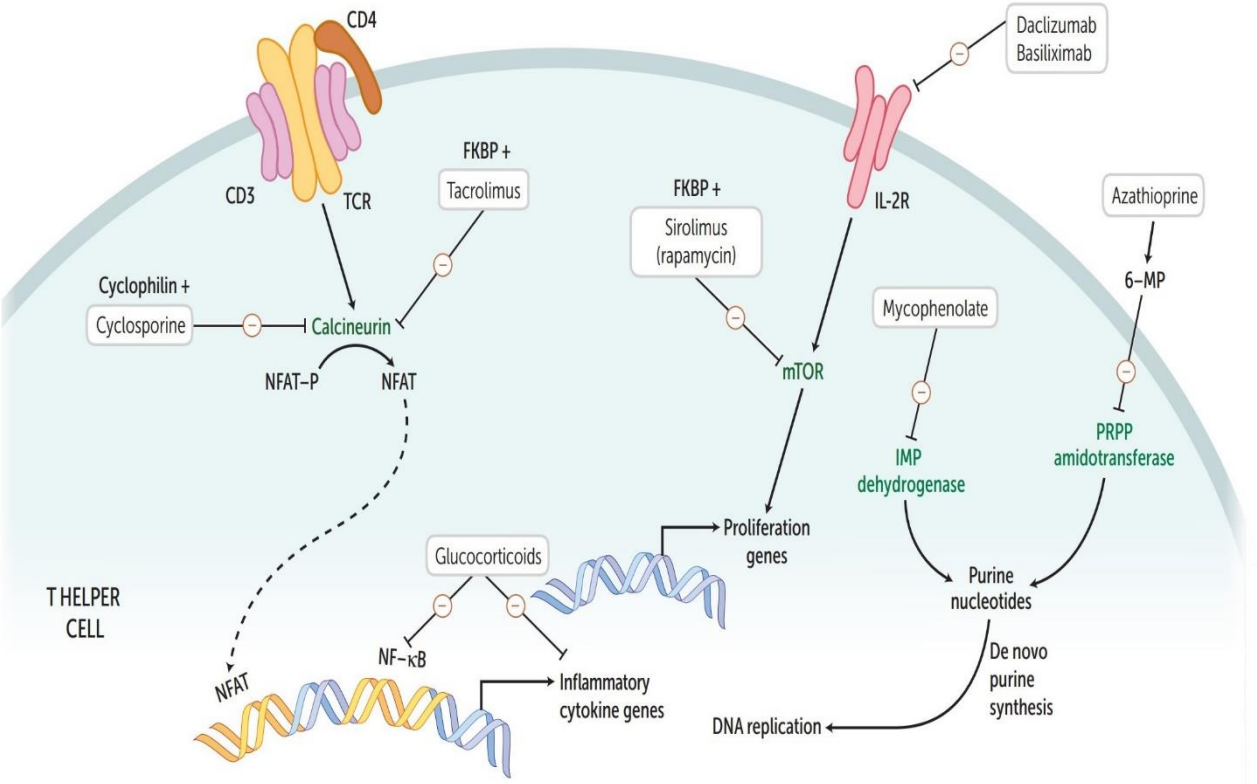
- Irradiate blood products prior to transfusion for immunocompromised patients to prevent GVHD.

CHAPTER 8

Immunosuppressant drugs

Immunosuppressant

- Agents that block lymphocyte activation and proliferation.
- Reduce acute transplant rejection by suppressing cellular immunity.
- Frequently combined to achieve greater efficacy with ↓ toxicity.
- Chronic suppression ↑ risk of infection and malignancy.



Cyclosporine

- Mechanism of action:
  - Calcineurin inhibitor, binds cyclophilin.
  - Blocks T-cell activation by preventing IL-2 transcription.
- Clinical uses: Psoriasis, rheumatoid arthritis.
- Side effects: Nephrotoxicity, hypertension, hyperlipidemia, neurotoxicity, gingival hyperplasia, hirsutism.

Tacrolimus (FK506)

- Mechanism of action:

- Calcineurin inhibitor; binds FK506 binding protein (FKBP).
- Blocks T-cell activation by preventing IL-2 transcription.

- Clinical uses: Transplant rejection prophylaxis.

- Side effects:

- Similar to cyclosporine, ↑ risk of diabetes and neurotoxicity; no gingival hyperplasia or hirsutism.
- Both calcineurin inhibitors are highly nephrotoxic, especially in higher doses or in patients with decreased renal function.

❖ N.B:

1. Both calcineurin inhibitors inhibit IL2 transcription, but with 2 different mechanisms:

- Cyclosporine bind to cyclophilin but tacrolimus bind FK-binding protein → ↓ calcineurin (cytoplasmic phosphatase) → ↓ activation of T-cell transcription factors → IL-2, IL-3, and interferon-γ.

2. In normal T cells, calcineurin is a protein phosphatase that is activated upon stimulation of the appropriate cell receptor.

- Once activated, the calcineurin then dephosphorylates NFAT (nuclear factor of activated T cells), which allows it to enter the nucleus and bind to an interleukin-2 (IL-2) promoter. Interleukin-2 stimulates the growth and differentiation of T cells and is therefore an important component of the immune response.
- Cyclosporine and tacrolimus, two of the more commonly used immunosuppressants in kidney transplant patients, inhibit calcineurin activation.

Sirolimus (Rapamycin)

- Mechanism of action:

- mTOR inhibitor; binds FKBP.
- Blocks T-cell activation and B-cell differentiation by preventing response to IL-2.

- Clinical uses: Kidney transplant rejection prophylaxis. Also used in drug-eluting stents.

- Side effects: "PanSirtopenia" (pancytopenia), insulin resistance, hyperlipidemia, not nephrotoxic (Kidney "sir-vives"), Synergistic with cyclosporine.

Daclizumab, basiliximab

- Mechanism of action: Monoclonal antibodies, block IL-2R.
- Clinical uses: Kidney transplant rejection prophylaxis.
- Side effects: Edema, hypertension, tremor.

Azathioprine

- Mechanism of action:
  - Antimetabolite. Precursor of 6- mercaptopurine.
  - Inhibits lymphocyte proliferation by blocking nucleotide Synthesis.
  - Pronounce "azathiopurine" → inhibits purine synthesis.
- Clinical uses: Transplant rejection prophylaxis, rheumatoid arthritis, Crohn disease, glomerulonephritis, other autoimmune conditions.
- Side effects: Pancytopenia.

❖ N.B:

- 6- MP degraded by xanthine oxidase, toxicity ↑ by allopurinol (xanthine oxidase inhibitor).

Mycophenolate, Mofetil

- Mechanism of action: Reversibly inhibits IMP dehydrogenase, preventing purine synthesis of B and T cells.
- Clinical uses: Lupus nephritis.
- Side effects:
  - GI upset, pancytopenia, hypertension, hyperglycemia. Less nephrotoxic and neurotoxic.
  - Associated with invasive CMV infection.

Glucocorticoid

- Mechanism of action:

- Inhibit NF-κB.
- Suppress both B- and T-cell function by ↓ transcription of many cytokines.

- Clinical uses: Transplant rejection prophylaxis (immunosuppression), many autoimmune disorders, inflammation.

- Side effects:

- Hyperglycemia, osteoporosis, central obesity, muscle breakdown, psychosis, acne, hypertension, cataracts, avascular necrosis.

- Can cause iatrogenic Cushing syndrome.

Recombinant cytokines and clinical uses

CYTOKINE	AGENT	CLINICAL USES
Bone marrow stimulation		
Erythropoietin	Epoetin alfa (EPO analog)	Anemias (especially in renal failure)
Colony stimulating factors	Filgrastim (G-CSF), Sargramostim (GM-CSF)	Leukopenia; recovery of granulocyte and monocyte counts
Thrombopoietin	Romiplostim (TPO analog), eltrombopag (TPO receptor agonist)	Autoimmune thrombocytopenia Platelet stimulator
Immunotherapy		
Interleukin-2	Aldesleukin	Renal cell carcinoma, metastatic melanoma
Interferon	IFN-α	Chronic hepatitis C (not preferred) and B, renal cell carcinoma
	IFN-β	Multiple sclerosis
	IFN-γ	Chronic granulomatous disease

❖ N.B:

- Interleukin-2 (IL-2) is produced by helper T cells and stimulates the growth of CD4+ and CD8+ T cells and B cells. IL-2 also activates natural killer cells and monocytes.
- The increased activity of T cells and natural killer cells is thought to be responsible for Il-2's anti-cancer effect on metastatic melanoma and renal cell carcinoma.
- IL-2 (aldesleukin) is currently used as immunotherapy for metastatic melanoma and renal cell carcinoma.
- The IL- 2-induced immune response against renal cell carcinoma results in tumor regression in approximately 10% of patients.

Therapeutic antibodies

AGENT	TARGET	CLINICAL USE	NOTES
Cancer therapy			
Alemtuzumab	CD52	CLL, MS	"Alymtuzumab"—chronic lymphocytic leukemia
Bevacizumab	VEGF	Colorectal cancer, renal cell carcinoma, non-small cell lung cancer	Also used for neovascular age-related macular degeneration, proliferative diabetic retinopathy, and macular edema
Rituximab	CD20	B-cell non-Hodgkin lymphoma, CLL, rheumatoid arthritis, ITP, MS	Risk of PML in patients with JC virus Ri2Ximab
Trastuzumab	HER2	Breast cancer, gastric cancer	HER2—"tras2zumab"
Autoimmune disease therapy			
Adalimumab, infliximab	Soluble TNF-α	IBD, rheumatoid arthritis, ankylosing spondylitis, psoriasis	Etanercept is a decoy TNF-α receptor and not a monoclonal antibody
Eculizumab	Complement protein C5	Paroxysmal nocturnal hemoglobinuria	
Natalizumab	α4-integrin	MS, Crohn disease	α4-integrin: WBC adhesion Risk of PML in patients with JC virus
Ustekinumab	IL-12/IL-23	Psoriasis, psoriatic arthritis	
Other applications			
Abciximab	Platelet glycoproteins IIb/IIIa	Antiplatelet agent for prevention of ischemic complications in patients undergoing percutaneous coronary intervention	ABC is as easy as 123
Denosumab	RANKL	Osteoporosis; inhibits osteoclast maturation (mimics osteoprotegerin)	Denosumab helps make dense bones
Omalizumab	IgE	Refractory allergic asthma; prevents IgE binding to FcεRI	
Palivizumab	RSV F protein	RSV prophylaxis for high-risk infants	PaliVIzumab—VIrus