# PART II

## MICROBIOLOGY

The human body plays host to a vast number of microorganisms. This brief introductory chapter describes the normal microbial flora that colonize different anatomical sites, as well as the variety of factors that can confer virulence to selected microorganisms.

## NORMAL MICROBIAL FLORA

A. **Properties.** Normal microbial flora describes the population of microorganisms that usually reside in the body. The microbiologic flora can be defined as either **"resident flora,"** a relatively fixed population that will repopulate if disturbed or **"transient flora"** that are derived from the local environment. These microbes usually reside in the body without invasion and can even prevent infection by more pathogenic organisms, a phenomenon known as ***bacterial interference.*** *The flora have commensal functions such as vitamin K synthesis. However, they may cause invasive disease in immunocompromised hosts or if displaced from their normal area.*

B. **Location.** Microbial flora differ in composition depending on their *anatomical locations and microenvironments. The distribution of normal* microbial flora is summarized in Table 1-1.

NOTE

*Normal flora prevent pathogen colonization by occupying the receptor sites.*

**NOTE:**

NBDE commonly asks about normal oral flora. The most common are the viridans streptococci (Gram-positive, facultatively anaerobic cocci). Strep mutans in particular is the major cause of caries.

*Table 1-1. Major species of microbial flora found in different anatomical locations.*

| Location | Major Organisms of the Normal Flora |
|---|---|
| **Skin** | *Propionibacterium acnes, Staphlococcus epidermidis,* diptheroids; transient colonization by *Staphlococcus aureus* |
| **Oral cavity** | Viridans *Streptococci, Branhamella* species, *Prevotella melaninogenicus, Actinomyces* species, *Peptostreptococcus* species, other anaerobes |
| **Nasopharynx** | Oral organisms; transient colonization by *S. pneumoniae, Haemophilus* species, *N. meningitidis* |
| **Stomach** | Rapidly becomes sterile |
| **Small intestine** | Scant |
| **Colon** | *Bacteroides* species, *Clostridium* species, *Fusobacterium* species, *E. coli, Proteus* species, *Pseudomonas aeruginosa, Enterococcus* species, other bacteria and yeasts |
| **Vagina** | Childbearing years: *Lactobacillus* species, yeasts, *Streptococcus* species<br>Prepuberty/Postmenopause: colonic and skin flora |

## MICROBIAL VIRULENCE FACTORS

Microbial virulence factors are gene products required for a microbial pathogen to establish itself in the host. These gene products are located on the bacterial chromosome or on mobile genetic elements such as plasmids or transposons. **Primary pathogens** express virulence factors that allow them to cause disease in the normal host. **Opportunistic pathogens** are environmental organisms or normal flora that lack the means to overcome normal host-defense mechanisms. They cause disease only when the normal host defenses are breached or deficient. Virulence factors can be divided into several categories.

A. **Enzyme production** can be of several types depending on the needs of the organism, its requirements for survival, and the local environment.
   1. **Hyaluronidase** breaks down hyaluronic acid to aid in the digestion of tissue.
   2. **Protease** digests proteins to enhance the spread of infections.
   3. **Coagulase** allows coagulation of fibrinogen to clot plasma.
   4. **Collagenase** breaks down collagen (connective tissues).

B. **Toxins**
   1. Exotoxins are heat-labile proteins with specific enzymatic activities produced by many **Gram-positive** and **Gram-negative** organisms. Exotoxins are released extracellularly and are often the sole cause of disease.
      a. Some toxins have several domains with discrete biologic functions that confer maximal toxicity. An example is **A-B exotoxin,** where

the B subunit binds to host tissue cell glycoproteins and the A subunit enzymatically attacks a susceptible target.

b. Many toxins are **ADP-ribosylating toxins**.

2. **Endotoxin** is the **heat-stable lipopolysaccharide** moiety found in the outer membrane of **Gram-negative organisms.** When released by cell lysis, the **lipid A portion** of lipopolysaccharide can induce septic shock characterized by fever, acidosis, hypotension, complement consumption, and **disseminated intravascular coagulation** (DIC).

C. **Surface components** may protect the organism from immune responses such as phagocytosis or aid in tissue invasion. For example, the **polysaccharide capsules** of *H. influenzae* type b and the acidic polysaccharide capsule of *Streptococcus pneumoniae* interfere with phagocytosis. Other surface proteins, such as adhesins or filamentous appendages (fimbriae, pili), are involved in adherence of invading microorganisms to cells of the host.

*IN A NUTSHELL*

| ***Exotoxin*** | ***Endotoxin*** |
|---|---|
| *Protein* | *Lipopolysaccharide* |
| *Gram$^+$ and Gram$^-$* | *Gram$^-$* |
| *Released extracellularly* | *Part of outer membrane; not secreted* |
| *Heat labile* | *Heat stabile* |

This chapter reviews the essential features of bacterial structure with an emphasis on the differences between Gram-positive and Gram-negative organisms. Also discussed are important concepts relating to bacterial growth, metabolism, sporulation, and the methods by which bacteria transfer genetic information.

## CLASSIFICATION AND IDENTIFICATION OF BACTERIA

A. **General properties** can differentiate prokaryotic (single-celled) organisms from higher eukaryotic organisms. The most distinguishable features include:

1. Prokaryotes have **70S ribosomes**, while eukaryotes have **80S ribosomes**. This difference allows some classes of antibiotics to specifically target prokaryotic protein biosynthesis.
2. Prokaryotes have a **naked, single, circular chromosome** of double-stranded DNA that replicates bidirectionally. **There is no true nucleus.** Prokaryotes lack a nuclear membrane, and their DNA does not have basic proteins (histones) associated with it.
3. Prokaryotes **lack membrane-bound organelles** such as mitochondria.
4. The **cell wall** of most bacteria is a unique, rigid, **peptidoglycan layer** that allows the characteristic Gram-positive and Gram-negative staining of bacteria. **Gram stain** (positive vs. negative) is based on biochemical retention of dyes by the cell's outermost layer; Gram-positives have a larger amount of peptidoglycan. **Acid-fast staining** is based on the ability to resist acid decolorization due to a high content of waxes in the cell wall. Mycobacteria species are acid fast and Nocardia species are partially acid fast.
5. **Flagella** are responsible for **bacterial motility.**

NOTE

*The mycoplasmas (including ureaplasma) are the only bacteria that do not have cell walls. Chlamydia have cell walls that lack muramic acid. Both mycoplasma and chlamydia are therefore resistant to beta-lactam antibiotics.*

6. **Pili or fimbriae** are used for attachment to host cells and are required for conjugation in Gram-negative bacteria. These structures convey adhesive properties to bacteria (adhesin).

B. **Classification of bacteria** is defined arbitrarily by the biochemical characteristics and/or phenotypic features that allow look-alike organisms to be differentiated from each other. The genus and species of an isolate can be determined by morphology and Gram stain and by biochemical and nutritional traits. Some specific examples include:

1. **Biochemical characteristics** such as substrate specificity (e.g., *B. pertussis* grows specifically on Bordet-Gengou agar), the ability to ferment specific sugars (e.g., *E. coli* are lactose fermentors), and the production of unique metabolic products (e.g., *Mycobacterium tuberculosis* produces niacin).
2. **Serologic reactivity**—the identification with specific antibodies in diagnostic immunoassays.
3. **Bacteriophage typing** is useful epidemiologically in tracing the source of epidemics.
4. **Animal pathogenicity**
5. **Antibiotic sensitivity**

## BACTERIAL STRUCTURE

A. The **cell envelope** is defined as all layers that enclose the cytosol of a bacterium. It is the main structural feature that differentiates Gram-positive from Gram-negative bacteria. Gram-positive bacteria have a smooth or finely patterned surface consisting of the cytoplasmic membrane, peptidoglycan layer, and sometimes an outer capsule. Gram-negative bacteria have a complex cell envelope consisting of a cytoplasmic membrane (inner membrane), **periplasmic space** containing peptidoglycan, an **outer membrane,** and sometimes a capsule.

1. **Capsule production** is usually correlated with virulence because capsules are antiphagocytic. Most capsules are carbohydrate in nature.
2. The **slime layer** is easily washed off and is less adherent than capsules.
3. The **bacterial cell wall** is a structure unique to prokaryotes and is a major site of antibiotic attack (e.g., penicillin blocks peptidoglycan synthesis).
   a. **Gram-positive bacteria** are composed of a thick layer of peptidoglycan, **lipoteichoic acids,** polysaccharides, and sometimes, **teichoic acid.** The surface proteins bind to extracellular material.
   b. **Gram-negative bacteria** have a trilayered outer membrane anchored to the cell membrane by **lipoprotein. Endotoxin** (lipopolysaccharide, somatic O antigen, and core polysaccharide)

### IN A NUTSHELL

***Gram-positives:***
- *Thick peptidoglycan cell wall overlays cell membrane*
- *No outer membrane*
- *Cell wall contains lipoteichoic acids and some have teichoic acid (virulence factor)*

***Gram-negatives:***
- *Thin peptidoglycan cell wall overlays cell membrane*
- *Also have an outer membrane*
- *Space between cell membrane and outer membrane includes peptidoglycan and is called the periplasmic space*
- *Lipoprotein in the periplasmic space connects the outer membrane to the cell membrane*
- *Lipopolysaccharide (LPS) is a component of the outer membrane:*
  *Lipid A = toxic portion*

in the outer membrane is unique to Gram-negative bacteria. **Protein porin channels** allow the flow of extracellular material through the cell's outer membrane.

4. The **periplasm** is the space between the plasma membrane and the outer membrane. It contains proteins, peptidoglycan, hydrolytic enzymes, and plasmid-controlled penicillinases. The periplasmic space is important in osmoregulation of the cell.

B. **Plasma (cell) membrane** functions primarily as an osmotic barrier. Its composition is 60–70% protein, 30–40% lipid, and small amounts of carbohydrate. If present, the **bacterial electron transport chain** is located within the cytoplasmic membrane. Structures associated with the plasma membrane are membrane polyribosome-DNA aggregates and **mesosomes.** Mesosomes are convoluted structures of cell membrane important in cell division.

C. **Cytoplasmic structures**

1. The **nucleoid region** of bacteria consists of a circular chromosome of double-stranded DNA that lacks introns, histones, and a nuclear membrane. In contrast, eukaryotic nuclei contain all of the above.
2. **Ribosomes** consist of 70% RNA and 30% protein. 70S ribosomes (monomers) are attached to messenger RNA. The 70S complex can be broken down into subunits of 30S and 50S.
3. **Polyamines** (e.g., putrescine) are located mainly in ribosomes and prevent dissociation of the 70S ribosome.
4. **Cytoplasmic granules** accumulate food reserves such as glycogen, lipids in the form of poly-β-hydroxybutyrate, and phosphate in the form of volutin granules.
5. **Spores** (endospores) are found in Bacillus and Clostridium species. Spores promote the survival of the organism under adverse environmental conditions because they **resist heat and drying.** Spores are highly dehydrated and refractile. They convert into vegetative cells via germination when conditions of the environment are more favorable.

## BACTERIAL GROWTH

How rapidly an infection can progress before host defenses respond can determine the severity of disease. In a closed system, growth is dependent upon the availability of nutrients, the external environment (e.g., temperature), and the growth rate of the specific species. Figure 2-1 depicts a typical bacterial growth curve.

*NOTE*

*Because the spores of Bacillus sp. are resistant to the most extreme environmental conditions, they are used to check the effectiveness of autoclaves in instrument sterilization.*

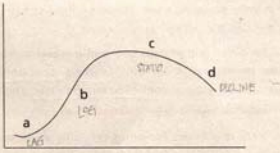


*Figure 2-1. Growth curve in a closed system. a = lag phase; b = exponential or log phase; 3 = stationary phase; d = phase of decline.*

**A. Bacterial growth in a closed system**

1. The **lag phase** is a period of **no growth** when the organisms are adapting to a new environment.
2. The **exponential or log phase** describes the **steady state of growth**, typically at the organism's fastest rate. It continues until the nutrients are depleted or toxic waste products accumulate. Many antibiotics, especially those that target cell wall synthesis, are maximally effective during this phase.
3. The **stationary phase** occurs when **nutrients are exhausted or toxins accumulate**. Cell number is stable (cell loss = cell formation). Different outcomes can occur depending on the species of bacteria. Some bacteria stop growing but remain viable for long periods of time. Some organisms cannot maintain a viable, nongrowing state. When they reach the stationary phase, they immediately start to die. Other organisms, if conditions do not improve, start forming spores.
4. The **phase of decline** is observed when the **death rate increases**, due to cell starvation or sensitivity to toxins.

## SURVIVAL IN OXYGEN

Survival in oxygen is an important parameter used to classify bacteria. **All bacteria produce the superoxide ion** ($O_2^-$) **in the presence of oxygen**. Three enzymes are important to detoxify this ion. **Superoxide dismutase** converts the superoxide ion to hydrogen peroxide. **Catalase** or **peroxidase** then metabolizes the hydrogen peroxide to water and oxygen. **Obligate anaerobes** (strict anaerobes) lack these enzymes or have such low levels that an oxygen environment is toxic for them. Examples of obligate anaerobes are **clostridium** and **bacteriodes**. **Facultative organisms** grow with or without oxygen. Whether they ferment or respire is an independent issue.

NOTE

*Oxygen is toxic to obligate anaerobic organisms because they lack superoxide dismutase.*

## ENERGY PRODUCTION

Energy production requires a source of carbon. Some bacteria are very versatile, requiring only a few essential nutrients. Many pathogenic bacteria have become so host-adapted that they have lost much of their metabolic machinery. They have many additional requirements and are termed **fastidious bacteria.**

A. **Siderophores** are iron- ($Fe^{3+}$) chelating compounds, which are essential for growth of many pathogenic bacteria. The siderophore is a low-molecular weight material, such as a **catechol** that is excreted from the cell. This molecule binds iron and is then bound to a cell wall or outer membrane protein and transported into the cytosol of the cell, where it is utilized in energy metabolism.

B. **Mechanisms of energy production**

1. **Fermentation** is the **anaerobic** degradation of glucose to obtain ATP.
   a. It is much less efficient than respiration for generating energy. It can be used by obligate anaerobes and facultative organisms. End products of fermentation differ depending upon the organism and are often useful in identification.
   b. Most obligate anaerobes and all *Streptococcus* species use fermentation. *Streptococcus* species can only ferment because they cannot make cytochromes or catalase. However, they do have **superoxide dismutase** and **peroxidase**, so they can survive in an oxygen environment.
2. **Respiration** completely oxidizes organic fuels, and requires an **electron transport chain** to drive the synthesis of ATP.
   a. Respiration produces almost twenty times as much ATP as fermentation. It requires a terminal electron acceptor. The usual electron acceptor is oxygen; however, alternate electron acceptors, such as nitrate and fumarate, are used by some organisms.
   b. Given a choice, bacteria will opt for respiration over fermentation. However, bacteria differ in their intrinsic ability to use fermentation or respiration.
      (1) Strict or **obligate aerobes** only respire and must use oxygen as a terminal electron acceptor. *Mycobacterium tuberculosis* is a good example of an obligate aerobe.
      (2) Some bacteria only ferment.
      (3) The majority of bacteria use the most versatile strategy, fermentation or respiration, depending upon the conditions.

## SPORULATION

A. A **spore** is a dormant structure capable of surviving prolonged periods of unfavorable environmental forces. Spores are capable of re-establishing the vegetative stage of growth when environmental factors become more favorable. Spores are resistant to radiation, drying, and disinfectants. Thermal resistance to denaturation is due to the high content of **calcium** and **dipicolinic acid** in the core. **Spore formation** is observed in *Bacillus* and *Clostridium* **species.**

B. **Initiation of sporulation** is related to the **guanosine triphosphate pool**. Regulation is by means of negative feedback. In addition, the availability of carbon and nitrogen sources are important to sporulation activity.

C. **Germination and outgrowth** occur when environmental and nutritional factors allow for renewed cell growth.

1. **Vegetative growth** is triggered by the exposure to stimulants such as glucose, nucleic acids, and amino acids.
2. **Activation of autolysin** results in autolysis of the cortex. The synthesis of protein and structural components follows. The spore core membrane develops into the cell wall.

## GENETIC TRANSFER

Genetic transfer refers to three principal mechanisms that result in the movement of genetic material into a host organism.

A. **Transformation** is the **uptake and integration of naked DNA** from the environment.

1. Once inside the cell, homologous recombination with the chromosome of the recipient must occur for the transformation to be successful.
2. Transformation can be induced in the laboratory with salt and heat shock. This technique is used to force cells to take up plasmids carrying genes of interest.
3. There are natural transformers among both the Gram-positive and Gram-negative bacteria. The medically important natural transformers are *Streptococcus* species, *Haemophilus* species, *Neisseria gonorrhea*, and *Helicobacter pylori*.

B. **Transduction** is the **phage-mediated transfer of bacterial DNA**. There are two kinds of transduction: generalized transduction and specialized transduction.

1. In **generalized transduction,** bacterial DNA is mistakenly packaged into an empty phage head. This is a very low frequency event, but any gene can be transferred. Once inside the recipient cell, homolo-

**NOTE**

*The gene for diphtheria toxin is carried on a lysogenic bacteriophage.*

gous recombination must occur for the transduction of information to be successful.

2. During **specialized transduction,** a lysogenic bacteriophage that is integrated into the bacterial chromosome excises itself, accidentally taking some chromosomal DNA. When the phage replicates, any bacterial gene that it has picked up is also replicated. These genes will be carried into cells that the progeny viruses infect. Specialized transduction occurs at a higher frequency than generalized transduction.

C. **Conjugation** is the **direct transfer of bacterial DNA between organisms.** It requires **cell-to-cell contact.** It is the **most important mechanism** for widespread transfer of genetic information between bacteria.

1. Most conjugation is **plasmid-mediated.** A plasmid is an **extrachromosomal piece of circular DNA that can replicate itself.** It often carries genes, such as those that encode resistance to antibiotics, and virulence factors, such as enterotoxins or adhesins. Plasmids vary in size, copy number per cell, and host range.
2. If a plasmid can exist only within a single species, it is called a **narrow-host-range plasmid.** If it can transfer between different genera of organisms, it is called a **broad-host-range plasmid.**
3. All plasmids can replicate themselves within the appropriate host, but not all plasmids can transfer themselves.
   a. A **conjugative plasmid** codes for the genes involved in transfer between cells.
   b. A **nonconjugative,** or mobilizable, plasmid requires the help of a conjugative plasmid to transmit itself to another bacteria.

D. **Insertion sequences** are small (1,000 bp) **pieces of DNA that code for the enzyme transposase,** which allows them to **jump into and out of DNA.**

1. A **transposon** consists of two insertion sequences flanking an antibiotic resistance gene.
2. Transposons and insertion sequences can insert into target DNA without significant homology at the site of insertion.
3. This ability to move between chromosomes, plasmids, or bacteriophages is an efficient mechanism for moving genes through a bacterial population. In fact, transposons are frequently associated with the formation of multiple-drug resistance plasmids.

## DENTAL CLINIC MICROBIOLOGY

A. **Sterilization and disinfection**

Dental instruments are cleaned and processed to remove pathogenic microorganisms. By far, the most common processing technique is **sterilization**, in which all life forms (bacteria, fungi, viruses and bacterial

*IN A NUTSHELL*

- ***Transformation*** → *Uptake and integration of naked DNA fragments*
- ***Transduction*** → *Phage-mediated exchange of information*
- ***Conjugation*** → *Direct bacteria-to-bacteria transfer of DNA (bacterial sex)*

spores) are killed. By contrast, **disinfection** refers to killing of pathogenic organisms and most microorganisms in general. Disinfection will not kill bacterial spores, and disinfected instruments are not sterile.

B. **Sterilization methods**

1. **Autoclave** (steam)

   Autoclave uses steam under pressure. Typical temperatures and times are **121° for 20–30 minutes** depending on load types.

2. **Dry heat** (driclave)

   Dry heat requires both greater temperature and more time. Typical temperatures and times are **160° for 1–2 hours** depending on load type.

3. **Ethylene oxide** (chemiclave)

   Ethylene oxide requires times of **8–12 hours**.

4. **Miscellaneous**

   **"Cold sterilization"** using long-term disinfectants is a misnomer. Spores are not killed by long disinfectant soaking, unless extremely long time periods are used. **(Example: glutaraldehyde 12–15 hours).**

   All instruments must be cleaned before sterilization. Proteinaceous material, in particular, blocks heat from reaching microorganisms (example: blood, tissue). Other factors interfering with sterilization are overpacked autoclaves and use an incorrect cycle (an exposure time which is too short).

C. **Disinfectant/Antiseptic/Sterilization**

Disinfectants, antiseptics and heat kill by a variety of actions (Table 2-1).

*Table 2-1.*

| Material, Method | Action |
|---|---|
| Steam Heat/most disinfectants | Protein denaturation |
| Ethylene oxide | Alkylation of proteins |
| Dry Heat | Protein denaturation/desiccation |
| Glutaraldehyde | Alkylation/protein precipitation |
| Detergent | Membrane disruption |
| Soap | Emulsification of fat/debris removal |
| Chlorhexidine | Membrane disruption |
| Mercury compounds | Protein precipitation |

DISINFECTION – does not kill bacterial Spores

DISINFECTED INSTRUMENTS are NOT sterile!

BOILING POINT OF $H_2O$ = 212°F

100°C → temp. of boiling $H_2O$ sufficient 4 disinfection BUT NOT STERILIZATION

121°C → typical steam autoclave temp.

PSI → pounds per square inch of PRESSURE

single most effective infection control

D. **Disinfectant guidelines**

1. Must be EPA registered
2. Should kill "benchmark" organism *Mycobacterium tuberculosis*
3. Should have the ADA seal of approval for use on dental instruments.

E. **Sterilization monitors**

Sterilizers need to be checked to insure that they are adequately sterilizing instruments. There are two types of monitors:

1. Process Indicators—show that sufficient temperature was reached in that load. Often a color change strip or sections of autoclave bag. Does **not** show sterilization, only presence of high temperature for period of time.
2. Biological Monitors—Spore strips of spore-forming *Bacillus* sp are loaded with instrument load. Spores are cultured following autoclaving cycle. Negative culture is expected. Usually a "test strip" is autoclaved, while a "control strip" is not (to show viability of spores).

   Current requirement in most jurisdictions is **weekly** biologic monitoring.

F. **Universal precautions**

Developed in the dental field following dental-related hepatitis B clusters in the 1970s, "universal precautions" is both a philosophy and a set of procedures. All patients are assumed to be potentially infectious and equivalent, sufficient disinfection/sterilization/cleaning procedures, etc. are completed for all patients regardless of the perceived or recorded health status of the patient. Preparation of rooms, instruments, and materials depends on **procedure, not patient**.

1. Procedures included in universal precautions:
   a. Sterilization of most instruments.
   b. Disinfection of some instruments and "touch and splash" surfaces
   c. Barrier methods (gloves, masks, face shields, plastic chair covers, light handle covers)
   d. Disposable instruments (trays, prophy angles, etc.)

*NOTE*

*Disinfectants are used on materials and surfaces, whereas antiseptics have a similar function but are used on live tissue.*

There are two medically important genera of Gram-positive cocci—Staphylococci and Streptococci. Both are nonmotile and do not form spores. Staphylococci are catalase-positive, whereas Streptococci are catalase-negative.

## STAPHYLOCOCCUS

A. **Genus characteristics and classification**

1. Staphylococci are Gram-positive cocci that divide perpendicular to the last plane of division, forming clumps or clusters. Depending on the age of the culture, they can be observed singly, in pairs, in short chains, or in grape-like clusters.
2. They are hardy organisms because they are relatively resistant to heat and drying.
3. Metabolically, the Staphylococci are **facultative organisms** and possess both **superoxide dismutase** and **catalase**.
4. Clinically, the most important distinction is between *S. aureus* and all other species that are nonpathogenic members of the normal flora. The **coagulase test** is a simple way to differentiate *S. aureus* from the coagulase-negative Staphylococci.
5. While there are six species of coagulase-negative Staphylococci, the most numerous species on the skin is *S. epidermidis*. The other coagulase-negative species of clinical relevance is *S. saprophyticus*.

B. ***Staphylococcus aureus*** is a common infectious agent of humans, and tends to cause localized or toxin-mediated disease.

1. *Staphylococcus aureus* should not be considered normal flora, but it does transiently colonize the nasopharynx, skin, and vagina of up to 30% of the population.
2. Table 3-1 summarizes the conditions commonly caused by *S. aureus*.

*Table 3-1. Common conditions caused by Staphylococcus aureus.*

| |
|---|
| **Direct infection** |
| **Skin:** Folliculitis, furuncles, carbuncles, abscesses, cellulitis, wound infection |
| **Deep infection:** Osteomyelitis (often post-trauma and/or surgery) |
| **Systemic infections secondary to above** |
| Osteomyelitis, endocarditis, lung abscesses, pneumonia |
| **Toxin-mediated disease** |
| Food poisoning, scalded skin syndrome, bullous impetigo, toxic shock syndrome |

3. *Staphylococcus aureus* does not produce a single factor that is necessary for virulence. The **best host defense against infections are PMNs**. No protective immune response is raised, so one can get infections again and again. Multiple virulence factors include: protein A, binding protein, coagulase, DNAse, staphylokinase, hyaluronidase, lipase, and various exotoxins (including hemolysins). *S. aureus* is generally **resistant to penicillin and other β-lactase antibiotics**.
4. **Treatment.** The drug of choice is a **penicillinase-resistant penicillin** (e.g., methicillin, nafcillin, or oxacillin) or **a first generation cephalosporin**. **Methicillin-resistant *S. aureus*** (**MRSA**) requires treatment with **vancomycin**.

C. ***Staphylococcus epidermidis*** is most commonly a **nosocomial pathogen**.

1. The major virulence factor it produces is a **viscous exopolysaccharide biofilm (slime)**.
2. When foreign bodies like IVs, catheters, and prosthetic valves are inserted into the host, *Staphylococcus epidermidis* can grow on their surface, embedded in the biofilm. This biofilm makes it difficult for the immune system to destroy the organism.
3. **Treatment** is with **vancomycin.**

D. ***Staphylococcus saprophyticus*** UTI

1. Causes **urinary tract infections** in sexually active women.
2. **Treatment** is with **penicillin**.

## STREPTOCOCCUS

A. **Genus characteristics**

1. Streptococci are Gram-positive cocci that form **chains.**
2. Metabolically, the Streptococci are **aerotolerant anaerobes** because they derive energy from fermentation only (lack cytochromes).
   a. The principal end product of fermentation is lactic acid, and perhaps because of this they are more acid-tolerant than most bacteria.

aerobic

b. Although Streptococci can live in conditions where oxygen is present, they **lack catalase**. Catalase is a cytochrome-containing enzyme that degrades hydrogen peroxide to oxygen and water. This feature is useful for differentiating the Streptococci from other Gram-positive cocci such as the Staphylococci.

c. Almost all medically important Streptococci are **auxotrophs**, meaning they require one or more vitamins, amino acids, or nucleic acids for growth, and therefore are not free-living in the environment.

B. **Classification.** The most common classification scheme for the Streptococci is based upon their reaction in blood agar.

1. **α-Hemolysis**—the red blood cells surrounding the colonies are intact, but there is partial breakdown of the heme, resulting in a green (**viridans**) pigment.

2. **β-Hemolysis**—the red blood cells surrounding the colonies are completely lysed. Beta-hemolytic Streptococci are also classified serologically into Lancefield groups (A-O) based on their cell wall carbohydrate. Clinically, the most important group is Group A.

3. **γ-Hemolysis**—no hemolysis or color change of the red blood cells is detected.

C. **α-Hemolytic Streptococci** can be distinguished from each other by their inhibition or growth in the presence of **optochin or bile.** This group includes *Streptococcus pneumoniae* and the Viridans Streptococci.

1. ***Streptococcus pneumoniae***, also known as **pneumococci**, grow in pairs or short chains.

a. **Transmission.** *Streptococcus pneumoniae* is spread person-to-person through **aerosol droplets.** 20–40% of normals are transiently colonized in their nasopharynx.

b. **Clinical manifestations.** It is the most common cause of **bacterial pneumonia** and also causes **otitis media, sinusitis, bronchitis,** and **bacteremia.** It is the most common cause of **meningitis** in the elderly.

c. **Risk factors** for infection due to *Streptococcus pneumoniae* include poverty, a debilitated state of health, the absence of a spleen, and certain diseases such as sickle cell anemia, Hodgkin's disease, multiple myeloma, and AIDS.

d. The most important **virulence factor** of *Streptococcus pneumoniae* is its **carbohydrate capsule.**

e. **Prevention.** A **vaccine** of 23 of the polysaccharide antigens exists. It should be given to the elderly, those undergoing splenectomy, and those with a condition predisposing them to *Streptococcus pneumoniae* disease.

### IN A NUTSHELL

***S. pneumoniae:***

- *Alpha hemolytic; inhibited by optochin and bile*
- *Virulence conferred by polysaccharide capsule*
- *Clinical correlations:*
  - *Pneumonia (especially middle-aged and older adults)*
  - *Otitis media*
  - *Sinusitis*
  - *Meningitis (#1 cause in elderly)*
  - *Bacteremia*
- *Certain populations are especially susceptible: elderly, smokers, alcoholics, children, asplenics*
- *Vaccine available*
- *Treatment with penicillin but resistance on the rise*

f. **Treatment.** In the past, all *Streptococcus pneumoniae* were sensitive to **penicillin**. Penicillin resistance due to transformation with DNA from nonpathogenic Streptococci in the oral pharynx is becoming a problem. These cases can be treated with vancomycin or erythromycin.

2. **Viridans Streptococci** are normal oral flora.
   a. They produce dextran, a substance that allows them to adhere to many surfaces.
   b. They are the major cause of **subacute endocarditis** (e.g., ***S. sanguis***) in those with abnormal heart valves. ***Streptococcus mutans*** causes **dental caries.**
   c. Treatment of choice is penicillin.

D. **β-Hemolytic Streptococci** are further subdivided into groups A through D and F and G based on antibodies to a heat-stable, acid-stable carbohydrate in their cell walls. This antigen is called C carbohydrate or the Lancefield antigen.

1. **Group A Streptococci (GAS)** contains only one species, ***Streptococcus pyogenes,*** but it is the most important Streptococcal pathogen.
   a. It can be distinguished from the other β-hemolytic Streptococci because it is **inhibited by the antibiotic bacitracin.**
   b. **Clinical manifestations** of *S. pyogenes* are characterized as suppurative and nonsuppurative.
      (1) **Suppurative** complications of **pharyngitis** include otitis media, peritonsillar cellulitis, peritonsillar and retropharyngeal abscesses, and bacteremic metastatic spread. Other suppurative infections include **erysipelas** (skin infection), **pyoderma (impetigo), scarlet fever,** cellulitis, lymphangitis, perianal cellulitis, puerperal sepsis, meningitis, pneumonia, and empyema.
      (2) **Nonsuppurative** sequelae occur weeks after initial infection. Inflammation occurs in organs not originally infected. These sequelae include **acute glomerulonephritis,** in which edema, hypertension, and hematuria occur after pharyngeal or skin infection. Also classified within this group is **rheumatic fever,** occurring 7–28 days after pharyngitis and results in fever, carditis, and polyarthritis.
   c. **Transmission and epidemiology.** Group A Strep is an obligate human parasite spread person-to-person by respiratory secretion via droplets, direct contact with the skin, or fomites. Pharyngitis is most common in winter and spring, with the highest incidence among preadolescents. Contaminated milk or eggs have also been the cause of food-borne epidemics of pharyngitis. Impetigo-like skin infections are most prevalent in summer and are often due to the infection of insect bites.

### NOTE

*Dextrans are involved in the ability of dental plaque (especially **S. mutans**) to adhere to enamel.*

### IN A NUTSHELL

***S. pyogenes (Group A):***
- *Beta hemolytic; sensitive to bacitracin*
- *Many virulence factors, including M protein, capsule, F protein, hyaluronidase, Streptolysins O and S, and erythrogenic toxins*
- *Clinical correlations*
  - *Pharyngitis (strep throat)*
  - *Scarlet fever*
  - *Skin: erysipelas, cellulitis, impetigo, necrotizing fasciitis, pyoderma*
  - *Secondary diseases: rheumatic fever and acute glomerulonephritis*
- *Treatment with penicillin*

### IN A NUTSHELL

***S. agalactia (Group B):***
- *β-hemolytic; resistant to bacitracin*
- *Antiphagocytic capsule*
- *Causes meningitis, sepsis, and pneumonia in neonates (acquired during passage through vaginal canal where organisms are part of normal flora)*

***Enterococci:***
- *Variable hemolysis*
- *Normal fecal flora*
- *Cause urinary tract infections in hospitalized patients; rare cause of subacute endocarditis*

d. **Virulence factors.** The most important virulence factor to remember is **M protein.**

2. **Group B Streptococcus** ***(Streptococcus agalactiae)*** is part of normal vaginal and intestinal flora in 25% of a given population.
   a. In contrast to *S. pyogenes, S. agalactiae* are **resistant to bacitracin.**
   b. The major virulence factor is an **antiphagocytic polysaccharide capsule.**
   c. **Infants** are more susceptible to disease from the organism than adults. They aspirate the organism during **passage through the birth canal,** and if they lack passive resistance from maternal IgG antibody, disease may ensue.
   d. **Clinical manifestations** include **pneumonia, sepsis,** and **meningitis.** Group B Streptococci and *E. coli* are the major causes of these diseases in the neonatal population (under 1 month of age).
   e. **Treatment** for *S. agalactiae* is a penicillinase-resistant synthetic penicillin.

3. **Enterococcus,** formerly Group D Streptococcus, include the important pathogens ***Enterococcus faecalis*** and ***Enterococcus faecium.***
   a. Both organisms are part of the **normal fecal flora.** These organisms were classified as Group D, β-hemolytic Streptococci; however, hemolysis is not a consistent feature because it is encoded on a plasmid.
   b. These organism can cause infection when they spread to the **urinary tract.** When the intestine is disrupted, they are one of the organisms found in an abscess. Enterococcus species also cause approximately 10% of cases of **subacute endocarditis.**
   c. Unlike the Streptococci, Enterococci exhibit penicillin tolerance because they are inhibited, but not killed, by the antibiotic. Recently, vancomycin-resistant Enterococci have appeared.

**CLINICAL CORRELATE**

*Patients with heart murmurs must be premedicated to avoid bacterial endocarditis.*

→ causes SEPTICEMIA in infants as well as BOVINE MASTITIS

The four most important genera of Gram-positive bacilli are: Listeria, Corneybacterium, Bacillus, and Clostridium. Listeria and Corynebacterium do not form spores, whereas Bacillus and Clostridium do.

## LISTERIA MONOCYTOGENES

A. **Characteristics**

1. *L-monocytogenes* is a small Gram-positive coccobacillus that does not form spores.
2. Microscopically, the organism resembles nonpathogenic members of the Corynebacterium genus (**"diphtheroids"**), which are part of normal skin flora.
3. Unlike the diphtheroids, *Listeria monocytogenes* is **motile at room temperature** and **produces β-hemolysis on blood agar.**

B. **Transmission.** Listeria is a **facultative intracellular pathogen**; it **infects phagocytic cells.** It also produces ***listeriolysin O,*** which is a beta-hemolysin similar to streptolysin O. Listeria is most often acquired through ingestion of **contaminated meat or unpasteurized dairy products.**

C. **Risk factors.** Groups at risk of serious disease from *Listeria monocytogenes* include **neonates, pregnant women, immunosuppressed patients, and alcoholics.**

D. **Treatment** is **ampicillin** with **sulfamethoxazole/trimethoprim** as an alternative.

## CORNEYBACTERIUM DIPHTHERIAE

A. **Characteristics.** *C. diphtheria* is a **nonmotile, club-shaped, nonspore-forming,** Gram-positive rod.

SPORE FORMING
1. bacillus
2. clostridium

NON-SPORE FORMING
1. listeria
2. corynebacterium

B. **Diphtheria toxin** is responsible for the clinical symptoms.
   1. A **lysogenic phage encodes diphtheria toxin.**

C. **Clinical manifestations** of diphtheria include **upper respiratory infection** resulting in a **tonsillar grayish pseudomembrane** that may spread to the pharynx and larynx and can compromise the airway. Although the organism is not invasive, the toxin is **absorbed systemically** and acts on other tissues. Diphtheria toxin is especially toxic to the heart and can cause cardiac failure.

D. **Treatment** consists of **antitoxin** and **erythromycin** and should be administered as soon as possible. ASAP!

E. **Prevention.** A vaccine containing **diphtheria toxoid** is administered during the first year of life. Boosters are required every ten years.

## BACILLUS

Bacillus species are a group of large Gram-positive rods that produce **spores.**

A. ***Bacillus anthracis*** is the major pathogenic species.
   1. Spores produced by the organism persist in soil or in products from infected herbivores for many years.
   2. Encoded on a plasmid are an **antiphagocytic capsule** and three virulence factors that act in concert. What is commonly called anthrax toxin is actually a combination of these three toxins:
      a. **Protective antigen (PA)**
      b. **Lethal factor**
      c. **Edema factor**
   3. **Transmission.** Infection commonly occurs through **skin cuts or abrasions**, although the organism may also be inhaled.
   4. **Clinical manifestations** of anthrax may be **cutaneous** or **systemic.**
      a. **Cutaneous anthrax** accounts for 95% of all infections. The characteristic presentation is papules that develop into ulcers with necrotic centers. Regional lymphadenopathy can occur. **Edema** is a major complication, and this infection may be fatal in 20% of untreated cases.
      b. **Systemic anthrax** is acquired through the respiratory (inhalation anthrax-Woolsorter's disease) or gastrointestinal route and results in lymphadenopathy and septicemia. It is almost **always fatal.**
   5. **Treatment and prevention**
      a. The drug of choice for *Bacillus anthracis* infection is **penicillin.**
      b. Anthrax is controlled through prevention and clean-up of contaminated areas.
      c. In the United States, a killed vaccine is available for individuals with a high risk of exposure and for livestock.

**NOTE**

*The antiphagocytic capsule of B. anthracis is unique in that it is composed of D-glutamate, not polysaccharide.*

B. ***Bacillus cereus*** produces two enterotoxins and usually grows in foods, especially in cereal grains such as rice.

1. The principal **clinical manifestation** is **food poisoning** of two types:
   a. A short incubation food poisoning (1–6 hours; emetic type) causes severe nausea and vomiting.
   b. Food poisoning of long incubation (10–24 hours; diarrheal type) is observed as abdominal cramps and diarrhea.
2. **Treatment and prevention** include support for food poisoning, such as as **administration of fluids**. If an antibiotic is indicated, vancomycin can be used.

## CLOSTRIDIA

Clostridia are characterized as large, **obligate anaerobic, spore-forming** rods.

A. **Characteristics**

1. Clostridia are usually found in the **soil or human GI tract**.
2. The four major pathogenic species, *Clostridium perfringens, C. difficile, C. tetani,* and *C. botulinum,* produce many toxins and destructive enzymes, including collagenase, protease, hyaluronidase, and lecithinase.
3. Most infections are mixed, that is, an aerobic organism grows first and reduces the environment, thus allowing the anaerobic Clostridia to grow.

B. ***Clostridium perfringens*** is a fast growing, nonmotile organism found in the **soil and the intestine**.

1. **Characteristics**. There are five types, A through E, based on the production of **four toxins, alpha, beta, epsilon, and iota.** All strains produce **alpha toxin**, a calcium-dependent phospholipase C that is also known as **lecithinase**. It causes the lysis of erythrocytes and other cells.
2. **Transmission** of *C. perfringens* occurs through infection of disrupted skin, bowel, or other epithelial tissues. Infection may be due to traumatic injury, surgery, or septic abortion. The organism is part of the normal intestinal flora. Spores are frequently **found in soil**.
3. **Clinical manifestations** take on several forms depending on the site and severity of infection. A diffusely spreading organism may cause **cellulitis and fasciitis and/or bacteremia**.
   a. **Gas gangrene** or myonecrosis is a life-threatening illness characterized by **muscle and connective tissue necrosis**. Gas, an end product of fermentation, forms in the muscle tissue and causes **crepitation**. Approximately 80% of the cases of gas gangrene are caused by *C. perfringens.*

PATHOGENIC SP.
C. difficile –
C. perfringes – GAS GANGRENE
C. tetani – TETANUS
C. botulinum – BOTULISM

TOXIC ENZYMES
1. collagenase
2. protease
3. hyaluronidase
4. lecithinase

C. perfringes
C. novyi
C. septicum
– causes Clostridial tx inf. & bact.

C. tetanosum – part of a normal flora in many in

b. **Food poisoning** can be caused by *C. perfringens*. It is thought to be the third most common cause of bacterial food-borne epidemics, after *Staphylococcus aureus* and Salmonella. The infection is characterized by abdominal pain and diarrhea for 12–24 hours; systemic effects are uncommon.

c. **Skin and soft-tissue infections** are typically localized infections. Tissue infection is characterized by crepitant cellulitis, stump infection in amputees, perirectal abscesses, diabetic foot ulcers, and decubitus ulcers.

d. **Suppurative infection** is usually polymicrobial in origin. Intra-abdominal infection may cause **bowel perforation** and **emphysematous cholecystitis**. Pelvic infection may be seen as tubo-ovarian and pelvic abscesses and as septic abortion.

4. **Treatment** for gas gangrene consists of **surgical debridement plus penicillin**. Food poisoning due to the enterotoxin of type A is usually self-limiting, and hence is not treated with antibiotics.

produces a CYTOPATHIC TOXIN
s/c causes COLITIS
585: a. watery diarrhea
b. abdominal cramping

C. ***Clostridium difficile***

1. *Clostridium difficile* is a component of the normal bowel flora in a small percentage of adults.
2. The organism can produce **two heat-labile toxins, enterotoxin (exotoxin A)** and **cytotoxin (exotoxin B)**.
3. **Clinical manifestations.** *C. difficile* causes over 25% of **antibiotic-associated diarrhea** and 95% of the cases of **pseudomembranous colitis**.
   a. Two antibiotics that commonly precipitate pseudomembranous colitis are **clindamycin** and **ampicillin**.
   b. **Pseudomembranous colitis** presents with a history of nausea, vomiting, abdominal pain, and voluminous green diarrhea.
   c. Proctoscopy demonstrates pseudomembranes with an erythematous mucosa.
   d. Diagnosis is confirmed by demonstrating toxin in the stool.
4. **Treatment.** Oral **vancomycin** or **metronidazole** are the current treatments.

D. ***Clostridium tetani***

1. **Characteristics**
   a. Spores are abundant in the soil. When they are inoculated into a site of injury, a drop in the redox potential leads to germination. In 50% of cases, there is no history of a wound.
   b. The organism produces **tetanospasmin**, a plasmid-encoded **neurotoxin** that blocks the normal inhibition of spinal motor neurons. It prevents the release of the inhibitory neurotransmitters glycine and gamma-amino butyric acid, leading to **spastic paralysis**. Death can occur from respiratory failure.

2. **Clinical manifestations.** Tetanus has four clinical presentations:
   a. **Local infection** can cause persistent local muscle contraction. It has low mortality and is noninvasive.
   b. **Cephalic infection** is rare in presentation. It can follow chronic otitis media and can progress to the generalized form.
   c. **Generalized tetanus infection** is the most recognized form. It is painful and presents with **"lockjaw"** progressing to opisthotonos. Mortality is approximately 60%.
   d. **Neonatal tetanus** infects the umbilical stump. It is the major cause of infant mortality in developing countries.
3. **Treatment** for tetanus includes surgical debridement of the wound, the use of human **tetanus antitoxin, respiratory support** (if required), and **muscle relaxants** (curare-like drugs). The antibiotic treatment of choice is **metronidazole.**
4. **Prevention.** Since the same toxin is found in all strains, tetanus can be prevented by **immunization** with tetanus toxoid. Booster shots are required every ten years. In contrast to diphtheria, a patient who survives tetanus is not immune to the disease because the minute amount of toxin necessary to cause disease is not sufficient to elicit an immune response.

E. ***Clostridium botulinum*** is found ubiquitously in soil.

1. **Characteristics**
   a. It produces a powerful, **heat-labile neurotoxin** that is usually ingested pre-formed with **improperly canned food.** The toxin **blocks the release of acetylcholine** from motor neurons in the peripheral nervous system, producing **flaccid paralysis.**
   b. There are seven immunologic types of toxins, some of which are phage-encoded.
2. **Clinical manifestations**
   a. There are a constellation of clinical signs associated with *C. botulinum* intoxication.
      (1) Dilated unreactive pupils (bulbar paralysis)
      (2) Descending weakness or paralysis usually starting with the cranial nerves
      (3) Progressive respiratory weakness
      (4) Absence of fever
      (5) Dry mucous membranes
      (6) Unexplained postural hypotension
   b. **Infant botulism** is also called **floppy baby syndrome.** When an infant is colonized by the organism, extremely low levels of toxin can be produced, leading to failure to thrive and eventually to progressive muscular weakness and poor motor development. Older children and adults do not seem to be afflicted. Infant bot-

## IN A NUTSHELL

***Virulence factors of Gram+ bacilli:***

| | |
|---|---|
| ***Corneybacterium diphtheriae*** → | *Phage-encoded toxin that inactivates EF-2 by ADP ribosylation. Protein synthesis inhibited in host.* |
| ***Bacillus anthracis*** → | *Antiphagocytic capsule* + *Anthrax toxin* [ *Protective antigen; Lethal factor; Edema factor (adenylate cyclase)* ] |
| ***Bacillus cereus*** → | *2 enterotoxins* |
| ***Clostridium perfringens*** → | *4 toxins; key one is alpha toxin (lecithinase)* |
| ***Clostridium difficile*** → | *Enterotoxin (exotoxin A) Cytotoxin (exotoxin B)* |
| ***Clostridium tetani*** → | *Plasma-encoded neurotoxin (tetanospasmin) blocks release of inhibitory neurotransmitters—spastic paralysis* |
| ***Clostridium botulinum*** → | *Neurotoxin blocks release of acetylcholine—flaccid paralysis* |

ulism is the most common form in the U.S. and is the reason why infants should not be fed honey.

c. In contrast to tetanus, the autonomic dysfunction that results from botulism is not life-threatening if the patient is intubated and appropriate ventilator management is used.

3. **Treatment** for botulism toxin poisoning includes respiratory support and human antitoxin treatment.

# Bacteriology: Gram-Negative Organisms 5

The Gram-negative bacteria are a very heterogeneous group with a large number of medically relevant species. This chapter reviews the important Gram-negative cocci (Neisseria), as well as the various groups of Gram-negative bacilli (enteric organisms, zoonotic organisms, and respiratory organisms).

## GRAM-NEGATIVE COCCI

A. **Neisseria** — able to utilize GLUCOSE

1. **Genus characteristics**
   a. Neisseria are nonmotile, nonspore-forming, Gram-negative cocci. They are characteristically arranged in pairs (**diplococci**) with flattened adjacent sides facing each other. On Gram stain, they resemble coffee beans.
   b. Eight species have been identified. Two of these, ***N. meningitidis*** and ***N. gonorrhoeae,*** are pathogenic for humans.
2. **Isolation of the pathogenic Neisseria.** The pathogenic Neisseria are fastidious organisms. They are very susceptible to heat, cold, and drying, so specimens require special handling.

B. ***N. meningitidis*** (meningococcus) — able to utilize MALTOSE

NEiM
Meningitidis = glucose + maltose

1. **Features**
   a. The key virulence factor for *N. meningitis* is its **antiphagocytic capsule.** The capsule is the basis of serotyping and serves as the antigen for vaccines.
   b. Endotoxin and IgA protease are also important virulence factors.
2. **Transmission** of meningococcus is via **respiratory droplets.** The carriage rate in adult nasopharyngeal tissue is approximately 10–30%. Most carriers are asymptomatic. It enters the upper respiratory tract and may eventually disseminate. The risk of disseminated disease is

greatest in individuals with **late complement (C6, C7, and C8) deficiencies.**

3. **Clinical manifestations**
   a. **Meningitis,** usually of sudden and fulminant onset
   b. **Meningococcemia,** a vasculitic purpura causing **disseminated intravascular coagulation** (DIC)
   c. Meningococcus is responsible for **Waterhouse-Friderichsen syndrome,** characterized by coagulopathy, hypotension, adrenal cortical necrosis, and sepsis, which is usually fatal.
4. **Diagnosis** is made by identifying Gram-negative cocci in the spinal fluid and confirmed by culture of the CSF or blood and demonstration of oxidase production and maltose fermentation.
5. **Treatment and prevention**
   a. **Penicillin G** is the antibiotic of choice.
   b. Carriers and close contacts can be treated prophylactically with **rifampin.**
   c. A quadrivalent vaccine exists to capsule types A, C, Y, and W135. The vaccine does not protect against type B, which has a capsule that is not immunogenic.

C. ***N. gonorrhoeae*** (gonococcus) → uses only GLUCOSE

1. **Features**
   a. Many strains produce β-lactamase
2. **Transmission** of the gonococcus is via **venereal contact,** but may also involve fomites. It has an increased incidence of infection in sexually active young adults (15–30 years old), nonwhites, low socioeconomic groups, and urban settings.
3. **Clinical manifestations** are primarily associated with the urogenital tract, although disseminated disease can occur.
   a. **Acute urethritis and gonorrhea** in **men** is observed as a yellow **purulent discharge with dysuria.** Ninety percent of men are symptomatic. Common complications of acute urethritis include urethral stricture, epididymitis, and prostatitis. Proctitis is especially common in male homosexuals.
   b. **Gonorrhea in females** is asymptomatic in 20–80% of cases, most likely due to the inability to observe a discharge. Complications include pelvic inflammatory disease (15–20% of cases), generalized peritonitis, and infertility.
   c. **Disseminated disease** can be observed as meningitis, subacute bacterial endocarditis, and **arthritis.** — SABE
   d. **Ophthalmia neonatorum** results from maternal transmission to the infant during birth. Ophthalmic tetracycline, erythromycin, or silver nitrate is given to the neonate for prevention. Neonatal infection may cause gonococcal arthritis.

e. **Pharyngitis.** Diagnosis is established by identifying Gram-negative cocci in PMNs from the infected site and is confirmed by culture. This may occur in neonates but is much more common in individuals who engage in oral sex.

4. **Diagnosis** of gonococcal urethritis in **males** is made by the observation of Gram-negative diplococci and neutrophils in the urethral exudate. In **females**, culture and biochemical testing is required due to the presence of other Gram-negative coccal forms as part of normal vaginal flora.

5. **Treatment and prevention**
   a. **Ceftriaxone** is the antibiotic of choice for treating infection. *N. gonorrhoeae* is no longer susceptible to penicillin because of two mechanisms: **a plasmid-mediated β-lactamase** and **chromosomally mediated decreased affinity of penicillin-binding proteins.**
   b. Because combined infections are so common, treatment regimen should include an antichlamydial agent (e.g., doxycycline).
   c. Safe sexual practices can decrease the incidence of gonorrhea.

*IN A NUTSHELL*

| | ***Gonococcus*** | ***Meningococcus*** |
|---|---|---|
| ***Spread*** | *Venereal; passage through birth canal* | *Aerosol* |
| ***Vaccine*** | *No* | *Yes* |
| ***Treatment*** | *Ceftriaxone* | *Penicillin* |

## GRAM-NEGATIVE BACILLI: ENTEROBACTERIACEAE

The family Enterobacteriaceae includes many Gram-negative, nonspore-forming, facultative bacilli with simple growth requirements. The majority of Enterobacteriaceae are **normal gastrointestinal flora.** In the gastrointestinal tract they exist in a symbiotic relationship with the host. They synthesize vitamin K and deconjugate bile salts and sex hormones for recirculation to the liver. They also prevent the colonization of intestinal mucosa by primary pathogens. Colonization is inhibited by colicin/bacteriocin synthesis and receptor competition. The normal flora of Enterobacteriaceae, which include Escherichia, Citrobacter, Klebsiella, Enterobacter, Serratia, and Proteus, generally lack the virulence factors of the pathogenic species. They act as opportunistic pathogens when they breach the normal anatomic barriers or when the host is severely immunocompromised. Opportunistic Enterobacteriaceae are the most common cause of intra-abdominal sepsis and urinary tract infections. A subset of Enterobacteriaceae are considered primary pathogens because they contain virulence factors capable of overcoming normal host defences. They are not part of the normal flora. This subset includes *Shigella* species, *Salmonella* species, *Yersinia* species, and some strains of *Escherichia coli.*

A. **General characteristics.** There is no universally accepted taxonomic classification for this group.

B. **Physiology**

1. The Enterobacteriaceae are facultative organisms; they can ferment or respire, depending upon the conditions.

2. Enterobacteriaceae are easily destroyed by heat and by common disinfectants or germicides. They are sensitive to drying or desiccation. In contrast, they survive best in a high-moisture environment. Respiratory care or anesthesia equipment are common sources of nosocomial infections. Contaminated ice machines or water supplies may harbor these organisms, causing epidemics.

C. **Antigenic structure**

1. **K antigens** (Vi antigen) are acidic **polysaccharide capsules.** They are antiphagocytic by blocking the access of complement or antibodies to the organism.
2. **H antigens** are the flagellar proteins and are present only in motile organisms. H antigen may exist in one of two phases in Salmonella.
3. **O antigen,** or somatic antigen, is the name for repeating oligosaccharide units found in the endotoxin in the outer membrane of Enterobacteriaceae.

D. **Pathogenicity** is due primarily to **endotoxin** (also called LPS; the lipopolysaccharide portion of the cell wall) present in all Enterobacteriaceae.

1. Toxicity lies in the lipid A portion. LPS may cause **endotoxic shock** when the enteric bacilli enter the bloodstream (septic shock). Endotoxic shock is characterized by blood pools forming in the microcirculation, resulting in hypotension. Vital organs lack adequate blood supply, leading to decreased tissue perfusion, acidosis, ischemia, and cellular hypoxia. DIC may also occur to further compromise the patient.
2. **Enterotoxins** are produced by some members of the family (some *E. coli*, Shigella) that exert their toxic effects on the small intestine. Enterotoxins cause secretion of fluid into the lumen, resulting in secretory diarrhea.
3. **Pili,** or fimbriae, promote adherence to tissues.

E. **Shigella**

1. **Characteristics**
   a. Shigella is an **obligate** human pathogen. There are four species of Shigella: *S. dysenteriae, S. flexneri, S. sonnei,* and *S. boydii.*
   b. It is a slender, **nonmotile** organism.
2. **Transmission.** The primary mode of transmission is **fecal-oral.** These organisms are not killed by stomach acid; therefore, only a small number of organisms (< 100) are needed to cause disease.
3. **Pathogenesis.** The site of disease is in the colon, where Shigella invades and superficially destroys the intestinal mucosa. Virulence factors of Shigella include **adhesins, invasins,** and **toxins.** Most are encoded on plasmids. Finally, like all Gram-negative bacteria, Shigella have **endotoxin.** This endotoxin increases the local inflammatory

### IN A NUTSHELL

***All Enterobacteriaceae:***
- *Gram-negative bacilli*
- *Facultative anaerobes*
- *Have endotoxin*
- *Found in GI tract (exception: Y. pestis)*

***Some Enterobacteriaceae:***
- *Motile (all except Klebsiella and Shigella)*
- *Have polysaccharide capsule (Klebsiella, Salmonella)—K antigen*

### NOTE

*Shigella and Salmonella typhi are the only Enterobacteriaceae that are human-only pathogens.*

response, but seldom causes septic shock because the organisms do not invade beyond the submucosa.

4. **Clinical manifestations** (shigellosis)
   a. **Bacillary dysentery** characterized by abdominal cramps and diarrhea. The feces contain blood, **polymorphonuclear leukocytes,** and mucus.
   b. One to four weeks after the disease, a **carrier state** may be set up if the organism is not cleared. Carriers experience long-term, recurrent bouts of disease.
5. **Treatment and prevention** includes hydration and electrolyte replacement.
   a. Fluoroquinolones are now considered antibiotics of choice. Alternatively, ampicillin, tetracycline, or trimethoprim-sulfamethoxazole decreases the duration of symptoms and development of the carrier state.
   b. Prevention is facilitated through personal hygiene, proper garbage disposal, and water purification.

F. ***E. coli*** are motile enterobacteria. The GI tract is a natural reservoir for *E. coli*.

1. **Pathogenicity** in strains causing neonatal meningitis or uropathogenic strains is related to the presence of the **K1 capsular antigen,** which inhibits phagocytosis. Nephropathogenicity is associated with plasmid-mediated **hemolysin** production.
2. **Enterotoxigenic *Escherichia coli*** (ETEC) is a major cause of infant death in developing countries and is the most common cause of **"traveler's diarrhea."**
   a. More than 100 serotypes of *E. coli* cause this noninflammatory, secretory diarrhea, which is similar to cholera but less severe.
   b. The organism is acquired through the ingestion of fecally-contaminated food or water.
   c. Disease is noninvasive and occurs in the small intestine.
   d. Major virulence factors are plasmid-encoded, including enterotoxins and pili-termed colonization factor antigens (CFA) that act as adhesins.
3. **Enteroaggregative *Escherichia coli*** (EAggEC) causes persistent **watery diarrhea in children and traveler's diarrhea.**
   a. The organism is acquired by ingestion.
   b. Virulence factors include pili, an enterotoxin, and a cytotoxin.
4. **Enteropathogenic *Escherichia coli*** causes **noninflammatory diarrhea.**
   a. **Infants,** especially in the developing world, are very susceptible.
5. **Enterohemorrhagic *Escherichia coli*** (EHEC) causes **bloody diarrhea,** similar to dysentery.

**CLINICAL CORRELATE**

*An examination of a fecal sample for PMNs is a key component of the workup of infectious diarrhea. If they are present, you are dealing with an invasive organism such as Shigella, Salmonella, or Campylobacter. If there are no PMNs, you are more likely to be dealing with a toxin-producer such as E. coli or V. cholerae.*

**NOTE**

*An enterotoxin is a protein exotoxin that is released locally in the intestine.*

**NOTE**

*LT is a classic A-B toxin. The B portion binds to the epithelium in the small intestine. The A portion is enzymatically active and ADP ribosylates the adenylate cyclase regulatory protein, thus increasing cAMP levels in the cell.*

*IN A NUTSHELL*

***E. coli:***

- *Most common cause of UTIs*
- *Most common cause of traveler's diarrhea*
- *Causes neonatal pneumonia, sepsis, and meningitis*

6. **Enteroinvasive *Escherichia coli* (EIEC)** causes dysentery that is clinically indistinguishable from dysentery due to Shigella.
7. **Other clinical manifestations of *E. coli* infection**
   a. *E. coli* is the most common causative organism in **urinary tract infections (UTIs).**
   b. **Neonatal pneumonia** is usually the result of nosocomial infection due to aspiration during the birth process. Sepsis may also occur.
   c. **Neonatal meningitis** is also caused by exposure during birth. It is a serious infection resulting in 40–80% mortality.
8. **Treatment and prevention.** Antibiotic treatment is based on the site, severity, and sensitivity of the infectious organism.
   a. UTI is usually treated with Bactrim (sulfa + TMP) or a fluoroquinolone.
   b. Pneumonia, meningitis, and sepsis are commonly treated with a third-generation cephalosporin such as cefotaxime and/or an aminoglycoside.
   c. Diarrheal syndrome is usually treated with fluids and electrolytes only. Bismuth subsalicylate inactivates and binds enterotoxins.

G. **Salmonella.** Salmonella are **motile.**

1. **Nontyphoidal Salmonella** infections cause **inflammatory diarrhea** with fever and variable septicemia.
   a. Infection is acquired through the ingestion of eggs, chicken, and other contaminated food or water.
   b. In contrast to Shigella, a large inoculum (> 1 million cells) is needed to survive gastric acid and cause disease.
   c. Disease is more severe in children under the age of ten.
   d. Known virulence factors include antiphagocytic capsules, outer membrane protein adhesins, and LPS. LPS and outer membrane proteins prevent complement-mediated killing during bacteremia. Also, the organism can grow within macrophages.

*NOTE*

*Unlike the other Salmonellae, S. typhi inhabit only the human colon.*

2. **Typhoidal Salmonella** infections are caused by **Salmonella typhi** and **Salmonella paratyphi** A, B, and C1. **Typhoid** or **enteric fever** is a progressive, subacute, febrile-wasting illness. It is common in developing countries, and the most severe manifestations occur in young children.
   a. **Transmission** is by ingestion of a large inoculum of bacteria in food or water contaminated by human feces.
   b. The intestine shows local inflammation with mucosal ulcerations and perforations. The organisms spread to the bloodstream and the reticuloendothelial system.
   c. LPS and other outer membrane proteins inhibit complement-mediated killing. **Vi antigen,** the polysaccharide capsule, inhibits phagocytosis and may contribute to the resistance of *S. typhi* to complement-mediated killing.

d. Clinically, there are three phases of typhoid fever.
   (1) The **first week** is expressed as fever, lethargy, constipation, and pain.
   (2) The **second week** is when **bacteremia** occurs. Biliary and organ involvement arise along with a sustained fever with temperature/pulse dissociation (i.e., high fever, low pulse rate), abdominal pain, *rose* spots, and diarrhea. This is when the GI tract is reinfected.
   (3) With the **third week,** exhaustion and fever improve unless other complications arise. Complications of typhoid fever include relapse (20%), severe bleeding, thrombophlebitis, abscess formation, pneumonia, cholecystitis (in acute and chronic colonization), and death (2–10%).

e. **Treatment.** While chloramphenicol is still a first-choice drug for typhoid fever, the emergence of drug resistance has caused a shift to third-generation cephalosporins (e.g., ceftriaxone) and quinolones (e.g., ciprofloxacin) in developed countries.

*CLINICAL CORRELATE*

*Salmonella causes osteomyelitis in sickle-cell patients.*

H. **Common opportunistic Enterobacteriaceae**

1. **Genus Klebsiella** are nonmotile, lactose-fermenting rods. The colonies appear large and mucoid due to the presence of the **large capsule.** The major pathogen, ***Klebsiella pneumoniae,*** causes **severe lobar pneumonia** in individuals with underlying conditions such as alcoholism, diabetes, and chronic obstructive pulmonary disease.

*MNEMONIC*

***The A's of Klebsiella:***

- *Alcoholics*
- *Aspiration pneumonia*
- *Abscesses in the lungs*

*Klebsiella pneumonia is sometimes characterized by thick, bloody sputum classically described as "currant jelly" sputum.*

2. **Genus Proteus** are highly **motile** organisms that cause **urinary tract infections.** Proteus species **produce urease,** which raises the urinary pH to levels that promote the production of **struvite.** These **stones** obstruct urinary flow and serve as a hiding place for the organism. Up to 10% of urinary tract infections are due to this organism.
3. Other *genera* of Enterobacteriaceae that are normal flora, but that can cause opportunistic infections, include Citrobacter (pyelonephritis), Enterobacter (pneumonia), and Serratia (pneumonia and UTIs).

## GRAM-NEGATIVE BACILLI: ADDITIONAL ENTERIC ORGANISMS

A. **Vibrio** have a highly characteristic comma-shape morphology. They are naturally found in both fresh and salt water and in several cold-blooded animals.

1. ***Vibrio cholerae*** are the most clinically important members of this group.
   a. **Pathogenicity** is related to the presence of a pilus that mediates adherence to the small intestine epithelium. It is a **noninvasive**

infection with clinical effects mediated by the **enterotoxin** (choleragen).

b. **Transmission** is via fecal-oral spread from contaminated water and food.

c. **Clinical manifestations** of **cholera** are severe, **watery ("rice water") diarrhea** (20 liters/day) with the loss of sodium, chloride, potassium, and bicarbonate.

d. **Treatment and prevention** includes rapid rehydration and electrolyte replacement. The antibiotic of choice is either **tetracycline** or **doxycycline.**

2. ***Vibrio parahemolyticus*** structurally resembles *V. cholerae* as a comma-shaped organism.

a. **Clinical manifestations** are primarily diarrhea, attributed to ingestion of **raw or improperly handled seafood (especially shellfish).** The incubation period is 12–24 hours. Symptoms include explosive watery diarrhea (may be bloody), headache, abdominal cramps, fever, and vomiting.

b. **Treatment and prevention.** Mild disease is usually self-limiting, subsiding within 2–4 days with no treatment required. Organisms are usually sensitive to chloramphenicol, tetracycline, and cephalosporins. Adequate refrigeration of raw and cooked seafood aids in prevention.

B. **Campylobacter and Helicobacter.** Campylobacter are small, curved Gram-negative rods. The natural reservoirs are many domestic animals.

1. ***C. jejuni*** is spread person-to-person via the fecal-oral route.

a. It is a primary pathogen that causes **enterocolitis,** an invasive enteritis with bloody diarrhea, crampy abdominal pains, malaise, and fever.

b. Inflammatory proctitis is a clinical manifestation in homosexuals.

c. Reactive arthritis may follow in individuals who have HLA-B27.

d. It is gut flora of many domestic animals, including chickens, pigs, sheep, goats, and cattle.

e. The antibiotic treatment of choice is either **erythromycin** or ciprofloxacin.

2. ***C. fetus*** is an opportunistic pathogen that causes bacteremia and metastatic infections in **immunocompromised patients.**

3. ***Helicobacter pylori*** is a spiral-shaped, motile rod that **produces urease.**

a. Its reservoir in nature is unknown and is possibly in humans only.

b. *H. pylori* exhibits age-dependent colonization rates and familial clustering. For example, it is present in the gastric mucosa of fewer than 20% of people less than 30 years old but increases to greater than 50% of people over 60 years old.

c. It lives in the gastric mucus in close proximity to the gastric epithelial cells.

**NOTE**

*Aeromonas and Plesiomonas are two other genera belonging to the Vibrionaceae family. Aeromonas hydrophilia live in water and can cause gastroenteritis and wound infections. Plesiomonas shigelloides is also associated with water sources and causes gastroenteritis.*

**IN A NUTSHELL**

***H. pylori*** *and* ***proteus*** *both produce urease and create an alkaline environment.*

d. Colonization may be asymptomatic; however, there is a high association with idiopathic chronic and acute **antral gastritis**, as well as **duodenal ulcer** (90%).
e. Treatment consists of bismuth salts, metronidazole and tetracycline, or amoxicillin.

C. **Pseudomonas** is a genus of Gram-negative rods that are widespread in soil and water. They are also a minor component of the bowel flora. Humans are very resistant to infection with Pseudonomas species. More than one host defense must be breached for infection to occur. The major host defenses are intact body surfaces, normal bacterial flora, complement lysis, and killing by polymorphonuclear leukocytes.

1. ***Pseudomonas aeruginosa*** is the most important member of the genus. It is an important nosocomial infection in **immunocompromised** and **chronically ill patients.**
   a. It possesses many virulence factors, and no single factor is decisive for virulence. Virulence factors include:
      (1) Pili-adhesin
      (2) Slime layer capsule-antiphagocytic alginate capsule; seen mainly in cystic fibrosis patients
      (3) Endotoxin
      (4) Exotoxins A and S inhibit protein synthesis.
      (5) Many enzymes (e.g., gelatinase, collagenase, phospholipase C, elastase); elastase is an important virulence factor because it allows the organism to invade blood vessels.
      (6) R factors
   b. **Risk groups** include immunocompromised patients and hospitalized patients with underlying disease. Groups at particular risk are radiation treatment patients, **burn patients,** patients with metastatic or metabolic disease, patients on prolonged immunosuppressive or antimicrobial treatment, patients with prior instrumentation or manipulation, and patients with **cystic fibrosis.**
   c. **Clinical manifestations**
      (1) Wound and burn infections
      (2) Ecthyma gangrenosum—skin lesion with vascular invasion leading to hemorrhage and necrosis.
      (3) Ear infections—otitis externa (swimmer's ear, mild); in diabetics—malignant otitis externa
      (4) Pulmonary infections—especially in cystic fibrosis patients and the immunocompromised
      (5) Corneal infections in contact wearers
      (6) Urinary tract infections
   d. **Treatment** will include antipseudomonal penicillin (e.g., ticaricillin, piperacillin) and aminoglycoside (e.g., tobramycin).

## RESPIRATORY PATHOGENS

A. **Haemophilus**

1. **Characteristics.** Haemophilus are small pleomorphic coccobacilli.
   a. Many species of Haemophilus are normal flora of the human pharynx.
   b. The major pathogen of the group is ***Haemophilus influenzae.***
   c. **Serotyping** in *Haemophilus influenzae* is based on the **polysaccharide capsules.** Disease due to *Haemophilus influenzae* is caused chiefly by **serotype b**. This capsule type prevents phagocytosis of the organism.
2. **Clinical manifestations.** Diseases caused by *Haemophilus influenzae* type b include **meningitis, otitis media,** and **epiglottitis.** Primarily children under the age of 5 are affected.
   a. **Meningitis.** Until the development of a vaccine, *Haemophilus influenzae* type b was the most common cause of bacterial meningitis in the 3-month to 6-year age group.
   b. **Acute epiglottitis** occurs with rapid onset and can compromise the airway.
   c. Nontypable strains cause pneumonia and otitis media. ***Haemophilus ducreyi*** is the cause of the venereal disease **chancroid.** It is characterized by painful, nonindurated, ragged ulcers confined to the genitalia and perianal areas.
3. **Treatment.** Recommended treatment for *Haemophilus influenzae* includes cefotaxime or ceftriaxone for meningitis or epiglottitis, and amoxicillin plus clavulanate for nonlife-threatening illnesses. Resistance due to β-lactamases is encountered in greater than 30% of isolates.
4. **Prevention.** A vaccine now exists for *Haemophilus influenzae* type b.

B. **Bordetella**

1. **Characteristics.** Bordetella are very small, Gram-negative fastidious coccibacilli. They are strict aerobes.
   a. ***B. pertussis*** causes **whooping cough,** and *B. parapertussis* causes a relatively mild disease, parapertussis.
   b. **B. pertussis** contains many virulence factors.
      (1) Attachment to the host is mediated by pili, also called fimbriae or filamentous hemagglutinins.
      (2) Toxins include pertussis toxin, adenylate cyclase toxin, tracheal cytotoxin, and lipopolysaccharide.
   c. **Clinical manifestations. Whooping cough** is the clinical manifestation of *B. pertussis* infection. This disease is highly communicable by the respiratory route, and humans are the only known reser-

### IN A NUTSHELL

*Pertussis toxin, cholera toxin, and the heat-labile toxin of E. coli (LT) all work by ADP-ribosylation of G protein and the consequent increase in cAMP. There is a difference, however: cholera toxin and E. coli heat-labile toxin activate the stimulatory unit of G protein ($G_s$); pertussis toxin activates the inhibitory unit of G protein ($G_i$).*

voir. Whooping cough develops in three stages following an incubation period of 7–10 days.

(1) **Catarrhal** or prodromal state occurs as a mild upper respiratory infection.

(2) **Paroxysmal cough,** followed by the characteristic whoop on inspiration, then develops.

(3) **Convalescence** is characterized by a slow decline in the "whoop." This stage lasts for months.

d. **Treatment and prevention. Erythromycin** is the drug of choice for *Bordetella pertussis* infection. However, antibiotic treatment has no effect on the course of disease if treatment is begun after the catarrhal stage. The best protection is **immunization.** There are two vaccines for pertussis, a killed-cell vaccine and an acellular vaccine.

C. **Legionella** is a genus of Gram-negative bacteria that has over 40 species. The most important is ***Legionella pneumophila,*** a facultative intracellular parasite that uses the complement receptor to infect macrophages and then multiplies within the phagosome. It causes a wide spectrum of disease in humans.

1. **Characteristics**
   a. *Legionella pneumophila* is widely distributed in **aquatic environments.**
   b. It is an aerobic organism that **requires iron and cysteine** for growth.
2. **Transmission** is by aerosols.
   a. The source for humans is often **air-conditioning equipment,** respiratory treatment devices, humidifiers, shower heads, or whirlpools.
   b. It is not transmitted person-to-person.
   c. Risk factors include immunocompromised status, alcohol abuse, and cigarette smoking.
3. **Clinical manifestations.** Disease caused by *Legionella pneumophila* varies greatly in its intensity. Infection can be asymptomatic.
   a. **Pontiac fever** is a mild, febrile illness without pneumonia caused by *Legionella pneumophila.*
   b. It causes a mild, atypical pneumonia that may progress to Legionnaires' disease.
   c. **Legionnaires' disease** is a severe, often fatal, pneumonia.
4. **Treatment.** The drug of choice for *Legionella pneumophila* is **erythromycin.**

### CLINICAL CORRELATE

*We have now reviewed all three elements of the DPT vaccine:*

- *Diphtheria*
- *Pertussis*
- *Tetanus*

Obligate anaerobes are bacteria that require a reducing environment. They cannot survive in an environment that contains oxygen because they are unable to detoxify the superoxide anion.

## GENERAL CHARACTERISTICS

### A. Morphology and physiology

1. This group includes Gram-positive and Gram-negative cocci, bacilli, and coil-shaped spirochetes that are often pleomorphic.
2. What unites these diverse organisms is the inability to detoxify the superoxide ion. The most essential enzyme in this process is **superoxide dismutase**, which catalyzes the conversion of the superoxide ion to hydrogen peroxide. The hydrogen peroxide can be further processed to water and oxygen through the action of **catalase or peroxidase**. Almost all obligate anaerobes lack these enzymes and hence require a reducing environment.
3. The greatest natural defense against anaerobic infection is healthy tissue that has a high oxidation-reduction potential and hence will not support the growth of the anaerobes.
4. Obligate anaerobes are normal inhabitants of anaerobic niches in the oral cavity, the vagina, and the gut. They can cause opportunistic infections in tissues adjacent to their normal habitat when they are displaced due to tissue injury or vascular compromise.

### B. Pathology

1. The primary pathology is frequently **purulent abscess** formation.
2. Culture of the abscess most often reveals a polymicrobial infection with multiple facultative and anaerobic species.

*IN A NUTSHELL*

*Obligate anaerobes lack superoxide dismutase. Therefore, they cannot survive in an environment that contains oxygen.*

C. **Treatment.** Treatment includes **surgical drainage** plus antibiotics. Penicillin G, clindamycin, metronidazole, and chloramphenicol are usually the most effective antimicrobials. Cefoxitin is also effective against most anaerobes.

## ANAEROBIC GRAM-NEGATIVE BACILLI

A. **Bacteroides** are the **primary organisms of the colon,** accounting for 30% of fecal isolates. Bacteroides are the most frequent cause of anaerobic infections, and *Bacteroides fragilis* is the most common clinical infectious isolate.

1. ***Bacteroides fragilis*** is a Gram-negative, nonspore-forming, nonmotile bacilli.
   a. It inhabits the intestinal and genital tracts.
   b. It has four major virulence factors.
      (1) Unlike other group members, *B. fragilis* contains a **polysaccharide capsule** that is antiphagocytic and chemotactic. Capsular antigen alone can produce an abscess.
      (2) Since it is a Gram-negative organism, *Bacteroides fragilis* contains **endotoxin** (although it is much less toxic than the endotoxin produced by *E. coli*).
      (3) It can produce a small amount of superoxide dismutase, allowing it to survive oxygen exposures for long periods of time.
      (4) *Bacteroides fragilis* contains β-**lactamases** that confer resistance to penicillin.
   c. **Clinical manifestations** include intra-abdominal infections, including abscesses and peritonitis. It is a primary cause of Gram-negative bacteremia.
   d. **Treatment** consists of **metronidazole** with clindamycin or chloramphenicol alternatives. Surgical debridement and drainage may be necessary to eradicate the organisms.

2. ***Prevotella melaninogenicus,*** formerly ***Bacteroides melaninogenicus,*** is a small coccobacilli that can be found primarily in the oral pharynx.
   a. On blood agar, it forms **black pigmented colonies.**
   b. The virulence factors of *Prevotella melaninogenicus* include an antiphagocytic capsule and collagenase.
   c. *Prevotella melaninogenicus* is an important agent in oral and pulmonary infections.

B. **Fusobacteria** are pleomorphic, Gram-negative rods with tapered ends. They normally inhabit the mouth, gastrointestinal tract, and female genital tract.

1. Fusobacteria do not have a capsule but do possess an extremely potent endotoxin.

*CLINICAL CORRELATE*

*Bacteroides are part of the normal gastrointestinal flora. They can cause disease when there is a break in the surface of the mucosa.*

*NOTE*

*Prevotella (Bacteroides) melaninogenicus is suspected to be a primary factor in adult chronic periodonitits.*

*NOTE*

*Fusobacteria are often found in diseased periodontal pockets.*

2. ***Fusobacterium nucleatum*** is the most common isolate. It is an important agent in oral infections, lung abscesses, and other pleuropulmonary infections.
3. ***Fusobacterium necrophorum*** is often found in liver abscesses. It contains both a leukocidin and a hemolysin.
4. Most isolates of Fusobacteria are susceptible to penicillins, cephalosporins, and clindamycin.

## ANAEROBIC GRAM-POSITIVE BACILLI

A. **Spore-forming.** Clostridium species were discussed previously in the Gram-Positive Bacilli chapter.

B. **Nonspore-forming**

1. **Propionibacteria** normally inhabit the skin. These organisms may infect shunts and prosthetic devices and are a cause of **acne.**
2. **Actinomyces**—see next chapter.

## ANAEROBIC COCCI

A. **Anaerobic Gram-positive cocci**

1. **Peptostreptococci** are the only genus of anaerobic cocci that are important in clinical infections.
   a. They are almost always isolated from a **mixed infection.**
   b. As a group, they are frequently recovered from cutaneous, oral, respiratory, and female genital tract infections.
   c. Peptostreptococci are sensitive to penicillin, cephalosporins, clindamycin, and metronidazole.

B. **Anaerobic Gram-negative cocci. Veillonella species** are small bacteria that resemble the *Neisseria* species. Part of normal mouth, nasopharynx, and vaginal flora, they are rarely a cause of infection. They may be confused with *Neisseria* on Gram stain.

The mycobacteria are acid-fast bacilli notable for the high-lipid content of their cell wall. They are responsible for tuberculosis (*Myobacterium tuberculosis*), disseminated disease in AIDS patients (*M. avium-intracellulare*), and leprosy (*M. leprae*). Actinomycetes are Gram-positive organisms with a characteristic branching filament growth pattern that resembles fungi. These organisms are abundant in soil. The Actinomycetes include two genera of clinical relevance: Actinomyces and Nocardia.

## MYCOBACTERIA

### A. *Mycobacterium tuberculosis*

1. **Characteristics**
   a. **Obligate aerobes** and **acid-fast bacilli (AFB)**. Once stained, *M. tuberculosis* resist decolorization with an acidic alcohol rinse (hence the name acid-fast, based on ability to retain the stain). This property is dependent on the **waxy lipid cell wall,** which includes **mycolic acids.**
   b. The cell wall contains lipoproteins or glycolipoproteins essential for tuberculin activity and confers the ability to induce **Type IV hypersensitivity reactions** (DTH, delayed-type hypersensitivity).
   c. *M. tuberculosis* is **slow-growing,** requiring 20–60 days before growth can be visualized; its doubling time is 18 hours.
2. **Antigenicity**

   **Purified-protein derivative (PPD)** of the cell wall is the preferred antigenic material for **skin testing.**

**BRIDGE TO IMMUNOLOGY**

*Type IV hypersensitivity reactions are discussed in detail in the Basic Immunology chapter of the Microbiology/Immunology review book.*

3. **Pathogenicity**
   a. **Cord factor** is associated with virulence and the characteristic serpentine grouping pattern that is seen in virulent strains. Cord factor inhibits polymorphonuclear leukocytic migration, elicits granuloma formation, and attacks mitochondrial membranes.
   b. **Sulfatides** act synergistically with cord factor and protect the microorganism from attack by the hydrolytic enzymes of the lysosome.
4. **Epidemiology.** *M. tuberculosis* is found only in humans. It is more common in lower socioeconomic groups. There has been a rapid, recent rise in cases in the U.S., partially related to the AIDS epidemic and to immigration patterns.
5. **Transmission**
   a. Occurs primarily through **droplet nuclei** inhalation.
   b. The most infectious person is one with untreated cavitary pulmonary tuberculosis who is actively expelling bacilli.
   c. The risk of infection is much greater than the risk of disease (i.e., more people are infected than have clinical disease) because the disease may be related to weakened immune responses.
6. **Pathogenesis** is dependent on immunologic responses. First, delayed-type hypersensitivity (a T-cell immune response) occurs within 3–4 weeks after infection and correlates with positive tuberculin reaction. Acquired cellular immunity is associated with "resistance" or protection from reinfection.
   a. **Primary infection**
      (1) **Exudative type** occurs when the organism is inhaled and spreads via alveolar macrophages, to the hilar lymph nodes. Hematogenous dissemination may occur at this time; however, signs of infection are minimal and immune-competent hosts will successfully limit the organism to the pulmonary location.
      (2) **Productive type** is characterized by the tubercle forms (with or without caseation) and depends on the host's immune response.
      (3) The primary site of infection forms **calcified lesions** (referred to as **Ghon complex**). Delayed-type hypersensitivity develops and infection becomes quiescent in pulmonary as well as metastatic sites (the PPD test is now positive). Immunocompromised or debilitated patients may have progressive primary disease from local sites or more distant sites without the disease becoming quiescent.
   b. **Secondary infection (reactivation)** is usually localized, particularly in **lung apices,** due to the higher oxygen tension ($PO_2$). Tubercle formation occurs histologically with **caseation, necrosis,** and **fibrosis.** Secondary infection results from either a breakdown of quies-

**CLINICAL CORRELATE**

*The primary lung infection is usually found subadjacent to the pleura in the lower part of an upper lobe or in the upper part of a lower lobe of one lung. This localization reflects the areas receiving the greatest flow of air.*

cent foci or from new infection, despite the acquisition of cellular (T-cell) immunity.

7. **Clinical features of tuberculosis.** The clinical presentation may include nonspecific constitutional symptoms such as fatigue, weight loss, anorexia, weakness, fever, and night sweats.
   a. **Pulmonary TB.** Eighty-five percent of cases are pulmonary, although infection may involve any organ of the body. Pulmonary disease may present with cough, hemoptysis, and pneumonitis.
   b. **Miliary or disseminated masses** develop anywhere, but some sites are favored, signifying an advanced stage of the disease. These favored sites include bone and joints (osteomyelitis), meninges (meningitis), kidneys, peritoneum, and lymph nodes.
8. **Diagnosis**
   a. **Abnormal chest x-ray**
   b. **Acid-fast bacteria (AFB)** in sputum; culture of *M. tuberculosis*.
   c. **Skin testing**
      (1) PPD-S, the designated standard (purified-protein derivative), is injected under the skin. Tuberculin tests should be read from 48–72 hours after intradermal injection.
      (2) The interpretation is based on **diameter of induration** and recorded in millimeters: 10 mm or greater in diameter of induration is considered positive for *M. tuberculosis* infection (but not necessarily active disease); 5–10 mm is considered as doubtful significance.
9. **Treatment.** Treatment should be for a period of 6–9 months with a combination of **at least three antituberculous drugs.** If the patient is HIV-positive, treatment should be continued for a longer period (9–12 months). The emergence of drug-resistant strains, particularly in large metropolitan areas, is a growing problem that can best be controlled by sensitivity testing of the isolate from the patient. When resistance is detected, additional drugs (ethionamide, streptomycin, ciprofloxicin) may be added to the regimen.
10. **Prevention**
    a. **INH prophylaxis** of household contacts of patients with newly diagnosed active disease or treatment of recently converted (within the past two years) PPD-positive individuals.
    b. **BCG immunization.** This treatment is given only to PPD-negative individuals in countries where incidence of tuberculosis is high. It is used to establish cell-mediated immunity to TB, although it must be remembered that individuals will become skin test-positive for PPD (thus eliminating its utility as a diagnostic tool).

### NOTE

*Eighty percent of pulmonary TB cases in adults are due to reactivation of an infection acquired years or even decades earlier.*

### CLINICAL CORRELATE

*Emergence of multiple-drug-resistant strains has prompted the Centers for Disease Control to recommend four drug regimens. Currently, the drugs considered "first line" include:*

- *Isoniazid*
- *Rifampin*
- *Ethambutol*
- *Pyrazinamide*

B. ***Mycobacterium bovis***

1. ***M. bovis*** is the etiologic agent of **tuberculosis in cattle.** It can also cause human TB, usually through the ingestion of unpasteurized, contaminated milk.
2. The most common clinical manifestations are lesions in the cervical and mesenteric lymph nodes with possible dissemination to the bones and joints. Pulmonary TB is also possible through the inhalation of infected droplets (for example, by dairy farmers).
3. *M. bovis* will result in a positive PPD skin test.
4. The BCG vaccine is derived from a live, attenuated *M. bovis* strain.

C. ***Nontuberculous mycobacteria* ("atypical").** The nontuberculous mycobacteria encompass approximately 20 species, including *M. kansasii, M. marinum, M. scrofulaceum, M. fortuitum-chelonei,* and most importantly, *M. avium-intracellulare.* They cause mycobacteriosis. The agents of mycobacteriosis are not spread from human to human; they are environmental agents.

1. **General characteristics of non-TB mycobacteria.** There is no known primary animal host. The organisms usually occur as natural inhabitants of the soil.
2. **Clinical manifestations**
   a. **Pulmonary disease** is usually found in older white men with chronic bronchitis and emphysema. Pathogens include *M. kansasii, M. avium-intracellulare,* and *M. fortuitum-cheloni* complex.
   b. **Lymphadenitis** is caused by *M. scrofulaceum* and most commonly occurs in children.
   c. **Cutaneous lesions** are caused by *M. marinum* when this organism contaminates an open wound; the disease is called "swimming pool" granuloma.
   d. **Disseminated disease** can arise from *M. kansasii* or from *M. avium-intracellulare,* particularly in patients with acquired immunodeficiency syndrome (AIDS).
3. **Treatment.** Many of the mycobacteria are resistant to the usual antituberculosis drugs. Antibiotic regimens may require as many as six drugs, including rifampin (which is quite effective against *M. kansasii*) and clarithromycin (effective against *M. avium-intracellulare* complex). Surgical resection is also recommended on occasion.

D. ***Mycobacterium leprae***

1. **Species characteristics**
   a. Cannot grow *in vitro* on any culture medium.
   b. Organisms are acid fast and induce a delayed-type hypersensitivity in patients.

2. **Leprosy (Hansen's disease)**
   a. Leprosy is endemic in Africa, South and Southeast Asia, and South America. Small endemic areas are found in the U.S. (Hawaii, Texas, California, Louisiana, and Florida).
   b. **Transmission** is through contact with organisms from nasal secretions or ulcer exudates from infected individuals. Lesions classically involve the **cooler regions of the body,** including the skin of nasopharynx, cartilage, eyes, testicles, and larynx. Incubation period for the infection to progress to clinical disease is 5–7 years.
   c. **Disease forms**
      (1) **Tuberculoid leprosy** is indolent and nonprogressing. Clinical findings show mature **granuloma in the dermis,** which also contains epithelioid cells, giant cells, and lymphocytes (predominantly $T_{DTH}$ cells).
      (2) **Lepromatous leprosy** is a progressive and invasive disease. Pathologic examination shows foamy histiocytes with an absence of epithelioid and giant cells. Cell-mediated immunity is suppressed **(negative lepromin skin test)** and T8 (suppressor cells) lymphocytes infiltrate the skin lesions. Numerous acid-fast organisms are present in the lesions. Schwann cells are infected, but less nerve damage occurs in comparison with tuberculoid leprosy. Skin lesions are of an invasive and nodular nature.
   d. **Immunity** to *M. leprae* is mediated primarily by $CD4^+$ T cells. The disease is marked by a low infectivity rate and occurs more frequently in individuals with defective cell-mediated immunity.
   e. **Treatment** requires long-term (3–5 years) antibiotic treatment to eradicate the organism. Tuberculoid leprosy responds to **dapsone** plus **rifampin.** Lepromatous leprosy is treated with dapsone plus rifampin and **clofazimine.** Close contacts should also be treated.

## ACTINOMYCETES

Actinomycetes (Actinomyces, Nocardia, Streptomyces) are characterized by their filamentous form.

A. **Actinomyces.** Several species from this genus can cause actinomycosis. The most important species are ***A. israelii*** and ***A. naeslundii***.

1. **Species characteristics**
   a. *A. israelii* are anaerobic, nonacid fast, **Gram-positive bacilli.**
   b. They are part of the **normal oral flora** (not found in soil) and are usually pathogenic only after oral trauma.
2. **Clinical manifestations (termed actinomycosis)**
   a. **Cervicofacial actinomycosis** causing lower jaw involvement following the development of dental caries (about 50% of actinomycotic infections) or after dental work. **Pyogenic abscesses** may

*CLINICAL CORRELATE*

*Actinomyces infections with "sulfur granules" can be mistaken for an abscessed tooth with a sinus tract.*

**NOTE**

*Common extension of oral infections will occur to the skin where characteristic "sulfur granules" can be seen.*

develop with swelling, tenderness, and erythema. **Granules** consist of Gram-positive mycelial filaments surrounded by eosinophils and leukocytes. **Osteomyelitis** (bone infection) is a frequent occurrence. Chronic, poorly healed oral cavity abscesses will spread by direct extension. Fortunately, the incidence of oral actinomycosis is declining due to improved oral hygiene practices.

b. **Thoracic actinomycosis** is caused by the extension from cervicofacial infection in approximately 20% of cases.

c. **Abdominal actinomycosis** is due to traumatic perforation of the intestinal mucosa, such as a ruptured appendix or perforated ulcer.

d. **Pelvic actinomycosis** arises in women with intrauterine devices.

3. **Diagnosis** is made by examination of secretions or tissues for granules. Crushed **"sulfur granules"** contain Gram-positive, nonacid-fast rods.

4. **Treatment** consists of penicillin or ampicillin for several weeks. Infection may require **surgical drainage** since antibiotics achieve poor penetration through abscesses.

B. **Nocardia.** ***N. asteroides*** is the most common isolate found in soil and aquatic environments.

1. **Characteristics.** Nocardia are aerobic, Gram-positive, **partially acid-fast** filamentous organisms.

2. **Clinical manifestations** (nocardiosis)

a. Half of patients have underlying disease. It is an opportunistic infection in patients with hematologic malignancies. Seventy-five percent of cases occur in males.

b. Infection begins as a **chronic lobar pneumonia** following inhalation of the organism and may be subclinical.

c. The central nervous system is the most common site of metastatic infection, occurring hematologically. The kidneys and skin may also become involved (in metastatic infection). **Abscess formation** is the most common pathologic finding.

3. **Treatment** is usually with sulfonamides and may require surgical drainage of abscesses.

**IN A NUTSHELL**

- *Actinomyces are anaerobes; Nocardia are not.*
- *Nocardia are partially acid-fast; Actinomyces are not.*

# Bacteriology: Rickettsiae and Chlamydiae 8

Rickettsia and Chlamydia are obligate intracellular parasites. Rickettsial diseases include Rocky Mountain spotted fever, typhus, cat scratch disease, and Q fever. Chlamydia are responsible for ocular trachoma and are the number one cause of sexually transmitted diseases in the United States.

## RICKETTSIACEAE

A. **General characteristics and physiology**

1. Rickettsiaceae are a family of small, pleomorphic Gram-negative coccobacilli.
2. The Rickettsiaceae family comprises four genera: Rickettsia, Bartonella, Coxiella, and Ehrlichia. The members of the family share several unique characteristics.
   a. All members are **transmitted by arthropods,** except Bartonella (where direct contact is the mode of transmission); *Coxiella burnetii* is acquired by humans primarily through aerosol inhalation, although it has an arthropod vector in its natural hosts.
   b. Most species are **obligate intracellular parasites** of endothelial cells (Rickettsia) or leukocytes (other genera).
   c. Most cause zoonotic disease with humans as an accidental host. **Chlamydia trachomatis** is the exception; it is a human pathogen with no reservoirs.
   d. **All are susceptible to tetracyclines** (e.g., doxycycline).

B. **Rickettsial diseases**

1. **Rocky Mountain spotted fever (RMSF)** is caused by ***R. rickettsii*** and accounts for 95% of rickettsial diseases in the U.S.
   a. **Epidemiology.** RMSF is found throughout the United States, particularly in the south-central and eastern portions of the country.

b. **Transmission.** The arthropod vectors of *R. rickettsii* are various species of **ticks.** The bite of the infected tick transmits the organism. The **reservoirs** for the agent include **rodents, dogs,** and **ticks.**

2. **Epidemic typhus** (louse-borne typhus) is caused by ***Rickettsia prowazekii.***
   a. **Transmission.** The agent is transmitted by the **human body louse.** *Rickettsia prowazekii* are found within the louse feces and enter individuals via skin excoriations. The louse is the vector, but not a reservoir, for *Rickettsia prowazekii* (humans are the primary reservoir).
   b. **Brill-Zinsser disease** is the recurrent form of *R. prowazekii* infection. *R. prowazekii* can remain dormant in lymph nodes.
3. **Endemic** or **murine typhus** is caused by ***R. typhi*** cycled by the rat and its ectoparasites (through feces).
   a. **Transmission.** The **rat flea** is the most common vector. It becomes infected when it feeds on the reservoir—the rat—during the acute part of the rat's illness. Organisms are passed in the flea feces and can be scratched in at the site of the bite.
   b. **Clinical manifestations** are similar to but less severe than in epidemic typhus.
4. **Scrub typhus** is caused by ***R. tsutsugamushi.*** It occurs endemically in Asia.
   a. **Transmission.** *R. tsutsugamushi* is transmitted by **mites** (chiggers). Because there is transovarial passage of the organism, the mite is a reservoir as well as the vector. Other reservoirs include rats, field mice, and shrews. Humans are accidental hosts.
5. **Q fever** is caused by ***Coxiella burnetii.***
   a. **Transmission.** Q fever does not have an arthropod vector in the human disease cycle. It is spread mainly by inhaling infected dusts, by handling infected hides or tissues, or by drinking milk contaminated with *Coxiella burnetii.* The organism is widely distributed in nature. Reservoirs include ticks, small wild animals, sheep, cows, and goats.
   b. **Clinical features**
      (1) Q fever is characterized by vague symptoms such as fever and chills, headache, malaise, and myalgia. It is unique among the rickettsial diseases in that it **does not cause a skin rash.**
      (2) Many cases are asymptomatic or present with a self-limited febrile illness.
      (3) The classic presentation of the disease is as a **pneumonia** with fever and no pulmonary symptoms. Atypical pneumonia and a rapidly progressive pneumonia also occur.
      (4) Chronic Q fever can manifest as myocarditis or hepatitis.

6. **Human ehrlichiosis** is caused by ***Ehrlichia chaffeensis.*** It has a **tick vector.** The clinical course can range from asymptomatic to fatal. The classic presentation includes fever, headache, and myalgia. Important laboratory features are leukopenia, thrombocytopenia, and anemia. Fewer than half of patients develop a rash.
7. *Bartonella henselae* causes **cat scratch disease** (a benign local lymphadenopathy that follows contact with cats) and **bacillary angiomatosis.** The latter occurs in AIDS patients and is characterized by proliferative vascular lesions in the dermis (resembles Kaposi's sarcoma) and internal organs.

## CHLAMYDIAE

A. **General characteristics and physiology**

1. Chlamydiae **are obligate intracellular parasites** infecting birds and mammals.
2. They possess a Gram-negative envelope that contains a genus-specific lipopolysaccharide but **lacks muramic acid.**
3. Their genome is very small, so they lack the capacity to carry out many metabolic pathways, including the production of ATP. This makes them dependent upon their host for energy.
4. Three species of Chlamydia are pathogenic for humans: ***C. trachomatis, C. psittaci,*** and ***C. pneumoniae*** (TWAR).
5. The **life cycle** of Chlamydia contains morphologically distinct infectious and reproductive forms. The infectious form is known as the **elementary body (EB).** It is incapable of multiplication. The intracellular form capable of binary fission is called the **reticulate body (RB).** This form is not infectious.

B. ***Chlamydia trachomatis.*** *Chlamydia trachomatis* is transmitted by fomites, **sexually, or perinatally.** It only infects humans. Infected cells develop oval **vacuolar inclusions** that contain glycogen and hence will stain with iodine.

C. ***Chlamydia psittaci.*** *Chlamydia psittaci* is transmitted by inhalation of the organisms from **infected birds** and their droppings.

1. **Risk factors.** Anyone coming in contact with an infected bird is at risk, but those who have close contact with birds such as veterinarians, pet store employees, poultry farmers, or bird handlers are at an increased risk.
2. **Clinical features.** The disease has a 5-to-15-day incubation period. Severity of the illness ranges from asymptomatic disease to a fatal systemic illness with severe **pneumonia.**
   a. **Pulmonary symptoms** include a nonproductive cough and rales.

### NOTE

***Q fever is an outlier among the Rickettsiaceae:***

- *It does not have an arthropod vector.*
- *It does not cause a rash.*

### IN A NUTSHELL

| Organism | Vector | Disease |
|---|---|---|
| ***R. rickettsii*** | *Tick* | *Rocky Mountain spotted fever (think rash – palms/soles moving to trunk)* |
| ***R. prowazekii*** | *Louse* | *Epidemic typhus (think rash – trunk spreading to extremities)* |
| ***R. typhi*** | *Flea* | *Endemic (murine) typhus* |
| ***R. tsutsugamushi*** | *Mite* | *Scrub typhus* |
| ***E. chaffeenis*** | *Tick* | *Ehrlichiosis* |
| ***C. burnetii*** | *None* | *Q fever (no rash!)* |
| ***B. henselae*** | *None* | *Cat scratch fever and bacillary angiomatosis* |

### IN A NUTSHELL

*Birds + pneumonia → think* ***C. psittaci***

b. **CNS manifestations** are characterized by severe frontal headaches. Toxic encephalitis may occur, leading to death.

D. ***Chlamydia pneumoniae.*** *Chlamydia pneumoniae* (formerly TWAR) is a human-only pathogen that is believed to be transmitted by inhalation. This organism is a cause of pharyngitis, bronchitis, and a relatively mild atypical pneumonia, although life-threatening infections have occurred.

E. **Treatment.** The drugs of choice for Chlamydia infections are tetracyclines (doxycyline), or erythromycins.

*NOTE*

*Epidemiologic studies have also associated atherosclerosis and coronary artery disease with antibody to Chlamydia pneumoniae, and this organism has been found in plaques of coronary arteries. However, its role in the pathogenesis of atherosclerosis is still unknown.*

The spirochetes are motile, helically coiled organisms that divide by transverse fission. Spirochetes contain an axial fibril, an outer sheath, a protoplasmic cylinder (cell wall and membrane), and cytoplasm. There are three genera of spirochetes that can cause disease in humans: Treponema (syphilis, yaws, pinta, bejel), Borrelia (Lyme disease, relapsing fever), and Leptospira (leptospirosis). This chapter focuses primarily on the two most clinically relevant spirochetes: *Treponema pallidum,* the etiologic agent of syphilis, and *Borrelia burgdorferi,* the tick-borne cause of Lyme disease.

## TREPONEMA

A. ***Treponema pallidum*** is the most important species of spirochetes. It has a capsule-like outer coat and a tapered end. Organisms are highly motile and constantly rotate about an axial filament. An important characteristic is that *T. pallidum* **does not grow on artificial media** and therefore cannot be cultured in the laboratory. *T. pallidum* is the etiologic agent of **syphilis**.

1. **Transmission and epidemiology**
   a. Syphilis is primarily a sexually transmitted disease. It can also be transmitted across the placenta and rarely, from blood transfusions.
   b. Risk groups include individuals with multiple sex partners and infants born to infected mothers.

2. **Clinical manifestations**
   a. **Primary syphilis** arises within 2–10 weeks after exposure. Organisms spread locally from the site of inoculation to the lymph nodes and bloodstream. A **chancre** forms at the site of inoculation. Initially, the chancre is a firm, **painless** reddish lesion with a raised border. The center usually erodes into an ulcer that heals within 3–6 weeks without scarring. The chancre contains numer-

ous spirochetes. Symptoms may be overlooked in females owing to poor visualization of the genitourinary tract. Lymph nodes may be enlarged but nontender. If untreated, disease progresses to the secondary stage.

b. **Secondary syphilis** occurs 1–3 months after primary syphilis. Symptoms represent disseminated disease and commonly include **rash,** fever, sore throat, headache, and generalized lymphadenopathy (especially in the epitrochlear region). White patches occur on mucous membranes (condylomas). **Condyloma lata** occur in moist areas, including the anus, vagina, axilla, and mouth. These lesions are highly infectious and are teaming with treponema.

c. **Latent syphilis** develops in 30–40% of infected individuals. Mucocutaneous relapses are most common, with the lesions remaining infectious. After two years, contagious lesions rarely develop.

d. **Tertiary (late) syphilis** arises in approximately 30% of untreated cases of primary syphilis.
   (1) **Benign tertiary syphilis** is marked by indolent **granulomatous lesions (gummas)** in the skin, mucocutaneous tissue, liver, or skeletal system. Spirochetes are rarely seen.
   (2) **Cardiovascular syphilis** is manifested as aortitis and occurs 10–20 years after untreated primary disease. Medial necrosis can occur with the destruction of elastic tissue. Saccular or fusiform aneurysms may develop and rarely dissect.
   (3) **Neurosyphilis.** Can occur more than 20 years after the initial infection. One or more CSF abnormalities may be apparent. Other neurologic symptoms are **"Argyll-Robertson pupils"** and **tabes dorsalis.** In the former condition, the pupil constricts on accommodation but fails to react to light. Tabes dorsalis is observed as a wide-based gait with long "slapping" motions of legs upon ambulation.

e. **Congenital syphilis** results from the transplacental transmission of spirochetes to the developing fetus. It most commonly occurs if the mother has primary or secondary disease. There is approximately 25% mortality if left untreated, with a high incidence of spontaneous abortions and stillbirths. Adequate treatment of the mother will prevent disease in the fetus. Early manifestations in the newborn include hepatosplenomegaly, hemolytic anemia, pneumonia, skin lesions, and snuffles (obstructed nasal breathing). Late manifestations may include Hutchinson's triad (abnormal teeth, interstitial keratitis, eighth-nerve deafness), saddlenose deformity, mulberry molars, and central nervous system and developmental abnormalities.

**CLINICAL CORRELATE**

*Syphilis infection at birth can cause Hutchinson's incisors in offspring.*

3. **Serology diagnostics** use two methods of analysis: antigen nonspecific and antigen specific. While **darkfield microscopy** can be used to visualize organisms from lesions, these serologic tests are the most common method of diagnosis.
   a. **Nontreponemal tests** use **cardiolipin** as the antigen and can be performed as a complement fixation (CF) test or as a flocculation test (called **VDRL** or **RPR assays**).
   b. Fluorescent antibody tests (**fluorescent treponemal antibody, FTA**) are specific for treponemal antigens. The assay detects specific antibodies and is used for the confirmation of positive nontreponemal tests. Once positive, it remains positive for life and therefore cannot be used to monitor therapeutic response.
4. **Treatment and prevention**
   a. **Penicillin** is still the drug of choice because all treponemes are highly sensitive organisms.
   b. Spread can be minimized through use of safe sexual practices.

## BORRELIA

A. **General characteristics**
   1. Borrelia are transmitted by **arthropods** to humans.
   2. Borrelia are coarse, irregular coils that are very flexible and motile.

B. **Lyme disease**
   1. **Transmission and epidemiology.** Lyme disease is caused by ***Borrelia burgdorferi.***
      a. The organism resides in **tick vectors (Ixodes) that have fed on infected deer or mice reservoirs.**
      b. The highest incidence of disease is found during the spring and summer when the nymph and adult stages of the tick require blood meals.
      c. The disease was first described in Lyme, Connecticut, and is now found throughout the U.S. and in Europe and Australia.
      d. It is not uncommon for the patient to have no memory of a tick bite.
   2. **Clinical manifestations**
      a. The hallmark initial finding is **erythema chronicum migrans** (a red macule, progressing to annular erythema with central clearing—"bull's-eye") at the site of the tick bite. The rash occurs within the first 10 days of infection and fades within 3–6 weeks. Infection, however, is still active. Constitutional symptoms include fever, headache, malaise, myalgias, adenopathy, and mild meningeal irritation. These early symptoms typically last for approximately four weeks.

b. Untreated infection may lead to late neurologic and cardiac disease.
   (1) **Neurologic symptoms** include severe headaches, meningitis, cranial nerve palsies, and painful peripheral neuropathies. These symptoms resolve after several months.
   (2) **Cardiac symptoms** include fluctuating cardiac arrhythmias (resolving after several weeks), myocarditis, and pericarditis.
c. Late-stage disease is marked by arthritis, occurring weeks to years after disease onset.

3. **Diagnosis** of Lyme disease is made from the clinical picture and a history of tick bite or exposure, in combination with laboratory identification by serology.
4. **Treatment** consists of doxycycline for early disease; also amoxicillin, cephalosporins, and erythromycin.

C. **Relapsing fever** is named for its numerous antigenic shifts and variations. It is caused by *B. recurrentis* and is transmitted by the **human body louse.**

1. **Clinical manifestations**
   a. The incubation period is approximately one week (3–10 days) and is marked by the acute onset of high fever, severe headache, myalgia, photophobia, cough, and meningismus. Symptoms are associated with spirochetes in the bloodstream.
   b. The initial fever lasts 3–6 days and can be associated with hemorrhage, rash, and neurologic manifestations.
   c. The initial fever is followed by an afebrile period of 4–10 days.
   d. Subsequent fevers are less intense and last a shorter period of time. Antigenic shifts occur during this time. The afebrile times allow a new virulent variant to emerge and cause disease.
2. **Treatment.** Treatment with tetracycline or erythromycin rapidly leads to recovery.

Mycoplasmataceae are the smallest free-living organisms. They are prokaryotic cells resembling Gram-negative bacteria, but they lack the cell wall. The family contains the genera **Mycoplasma** and **Ureaplasma.** The organisms are classified into one or the other genera based on the ability to hydrolyze urea. The species of medical importance in humans are *Mycoplasma pneumoniae, Mycoplasma hominis,* and *Ureaplasma urealyticum.* Disease in humans involves the respiratory and urogenital tracts.

## MYCOPLASMATACEAE

A. **General characteristics and physiology**

1. Morphologically, Mycoplasmataceae are filamentous and pleomorphic. They **lack cell walls** and are therefore penicillin-resistant.
2. They are facultative organisms that are mainly fermentative.
3. These organisms are unique among the prokaryotes because they **require sterols** for growth and because their cell membranes contain cholesterol.

B. ***Mycoplasma pneumoniae***

1. **Epidemiology and transmission.** *Mycoplasma pneumoniae* is found throughout the world. Transmission occurs via aerosol droplets. Clinical illness is more likely in ages 5–20.
2. **Pathogenesis.** Once in the upper respiratory tract, *Mycoplasma pneumoniae* are found among the cilia of the epithelial cells.
3. **Clinical manifestations.** *M. pneumoniae* is the most common cause of **pneumonia in young adults** (walking pneumonia).

a. Frequent clinical manifestations of this atypical pneumonia include **nonproductive cough,** low-grade fever, and an insidious headache.
b. Nonpurulent otitis media or **bullous myringitis** occur in about 20% of patients with mycoplasmal pneumonia. The presence of these conditions concomitantly with pneumonitis in a teenager is a strong indication of *M. pneumoniae* infection.

4. **Diagnosis** is primarily based on clinical findings and serology.
5. **Treatment** consists of macrolides (e.g., **erythromycin** or azithromycin), tetracyclines, or fluoroquinolones. Since the family Mycoplasmataceae lacks a cell wall, all members are resistant to the β-lactam antibiotics.

C. ***Mycoplasma hominis***

1. Sexually transmitted agent
2. A major source of infection in postpartum women.
3. Clinical manifestations include postabortal and postpartum fevers and bacteremia, as well as pelvic inflammatory disease.
4. Treatment with tetracyclines; in contrast to other Mycoplasma, it is resistant to macrolides.

D. ***Ureaplasma urealyticum***

1. Sexually transmitted agent
2. Unlike other Mycoplasma, it produces urea
3. A minor cause of nongonococcal urethritis
4. Treated with tetracycline or erythromycin

IN A NUTSHELL

***M. pneumoniae:***

- *Lack cell walls*
- *Transmitted via respiratory droplets*
- *Cause atypical pneumonia—#1 cause of pneumonia in young adults*
- *Cold agglutinins (IgM) used in presumptive diagnosis*
- *Treatment: erythromycin, tetracycline, or fluoroquinolones*

Viruses are the smallest agents of infection, ranging from 20–300 nm in diameter and consisting of either RNA or DNA surrounded by a protective protein shell (capsid). The protein shell may be surrounded by an envelope containing lipid and protein. Virus multiplication occurs only within host cells, and is accomplished by the separate synthesis and subsequent assembly of component parts. Some viruses have the unique capacity to become latent and even to integrate their genomes into the host cells. In this situation, the integrated viral genome is replicated as part of the host genome and is transmitted to each daughter cell without production of infectious virus.

## CLASSIFICATION AND IDENTIFICATION

Classification and identification of viruses is made on common characteristics shared by viral families (such as the presence of single- or double-stranded viral nucleic acids, DNA or RNA).

A. **Morphology** is based on structures and components (nucleic acids, envelope, etc.) common to the viruses and is a basis for viral classifications. See Figure 11-1.

1. **Terminology**
   a. **Virion** describes the complete infectious virus particle.
   b. **Capsid** is the **protein shell** that encloses and protects the nucleic acid genome (either RNA or DNA). The individual protein units are called **capsomeres.** These structures protect the viral genome from destruction in the extracellular environment. Capsids also control the host range and cell tropism of the naked viruses because they are the molecules that adsorb to the cell surface.

### IN A NUTSHELL

***Viruses:***

- *Smallest infectious agents*
- *Only one type of nucleic acid (DNA or RNA)*
- *No metabolic activity outside of living cells; obligate intracellular parasite*

c. **Nucleocapsid** denotes the protein shell plus the nucleic acid.
d. **Peplomeres** are the protein spikes found in the envelope of some viruses.

2. **Nucleocapsids** have characteristic symmetry that is usually **helical** or **icosahedral** (see Figure 11-1).

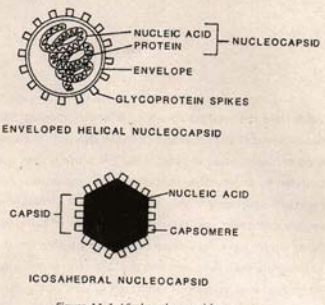


*Figure 11-1. Viral nucleocapsids.*

a. A **helical nucleocapsid** is marked by an extended nucleic acid cavity surrounded by helically arranged proteins with an outer lipid envelope. Examples of viruses with helical structure include ortho- and paramyxoviruses and rhabdoviruses.
b. **Icosahedral symmetry** is marked by condensed nucleic acids forming a central portion of cuboidal nucleocapsid structure. The icosahedral structure may be enveloped or naked. Examples of icosahedral symmetry include parvovirus, adenovirus, herpesvirus, and picornavirus.

3. **Envelopes** are lipid-containing structures surrounding some viral particles.
   a. Envelopes are derived from nuclear or plasma cell membranes acquired during viral maturation. The viral envelope is usually acquired when the viral nucleocapsid buds through the host's membrane.
   b. Envelopes lack rigidity and appear heterogeneous by electron microscopy.

c. **Viral glycoprotein** peplomeres are contained in the viral outer envelope. Glycoproteins serve an important role in antigenic structure and host immune responses. These viral attachment proteins (VAP) mediate viral binding (see below) and entry into host cells.

d. **Lipids** are the major component of the viral envelope. They are a complex mixture of phospholipids, glycolipids, and neutral lipids that are a part of the host cell membrane in which the virus multiplied.

4. **Virus classification** is based on nucleic acid composition such as the presence of single-stranded or double-stranded DNA or RNA. **Positive-sense RNA (+RNA)** serves directly as mRNA, while **negative-sense RNA (–RNA)** must use an RNA polymerase to synthesize a complementary positive strand to serve as mRNA.

5. **Viral proteins** are important in the initial contact with the host cell; they dictate which cells will be infected. Viral proteins also determine the antigenic structure of a virus and protect the genome against host nucleases.

a. **Hemagglutinins** are viral proteins that agglutinate red blood cells. The hemagglutinin of influenza virus promotes attachment of the virus to host cells. It can also function in the fusion of the virion with the host cell membrane. They are excellent vaccine antigens.

b. Enzymes serve several biologic functions for the virus.

(1) **Neuraminidase** acts to hydrolyze sialic acid. It helps release virus particles from the cells in which they were formed to further propagate infectivity.

(2) **RNA polymerase** is required for viral replication of negative-sense RNA viruses. This enzyme must be brought into the cell as a part of the virion.

(3) **Reverse transcriptase** in retroviruses transcribes single-stranded RNA into double-stranded DNA. Transcribed DNA can then be integrated into the host genome by an **integrase** enzyme.

B. **Replication.** Viruses are dependent on the host cell to provide the synthetic mechanisms and metabolic machinery for replication.

1. The **replication cycle** of the virus may either lyse the host cell or form a stable interaction that allows the host cell to survive. A stable interaction causes the viral genome to become incorporated into the host cell. The virus may be latent (e.g., herpesvirus) or it may continue to multiply without causing overt disease. The cycle of viral replication has discrete stages:

a. **Adsorption,** or attachment, is the first step. It is highly specific, involving the attraction and physical interaction of viral surface proteins (capsomere or peplomere units) with a host cell surface

### NOTE

*Antibody to the gp120 glycoprotein of HIV is used to monitor the course of HIV infection. The level of the capsomere protein p24 is used to determine the virus load in the blood.*

### IN A NUTSHELL

- *All RNA viruses have single-stranded RNA except for Reoviruses (ds)*
- *All RNA viruses are enveloped except Reoviruses, Caliciviruses, and Picornaviruses*
- *All DNA viruses have double-stranded DNA except Parvoviruses (ss); Hepadnavirus has ss regions in the DNA*
- *All DNA viruses have an icosahedral nucleocapsid except Poxviruses*
- *All viruses with helically symmetrical nucleocapsids are RNA viruses*

receptor. Adsorption is **host-specific** and **tissue-specific** (e.g., poliovirus attaches primarily to CNS and GI tract cells).

b. **Penetration and uncoating** follows the adsorption step and is typically mediated by receptor-specific endocytosis. The virus usually loses its coat or envelope directly after penetration. Uncoating separates the capsid (and envelope) from the nucleic acids.

c. The **synthetic period** begins when uncoating is complete. Its time course is variable depending upon the virus. Specific mRNA must be transcribed from viral nucleic acid by host cell mechanisms. Macromolecular synthesis begins with an eventual accumulation of viral components (including empty capsids or nonenveloped nucleocapsids). Minus-strand RNA viruses, double-stranded RNA viruses, and DNA viruses initiate nucleic acid synthesis to produce mRNA, whereas positive-sense RNA viruses can immediately initiate protein synthesis. This stage is marked by an orderly sequence of synthetic events (with some proteins synthesized early and others later in the synthetic processes).

d. **Production of viral proteins**

(1) In **positive-sense RNA viruses** like polio, the viral RNA (mRNA) is read directly by the host cell ribosome, and enzymes for RNA synthesis are produced. Other proteins that are synthesized at this time will inhibit host biosynthetic processes, while still others will serve as capsid (structural) proteins.

(2) In other viral groups, the viral genome ( –RNA, double-stranded RNA, or DNA) must first synthesize the messenger RNA molecules. They do this by an RNA-dependent RNA polymerase (transcriptase) that is contained in the virion and encoded by the viral genome in the case of RNA viruses; or by transcription of the viral DNA to synthesize the mRNAs necessary for protein synthesis. Some of the proteins will be structural units (capsomeres, peplomeres) and others will be enzymes necessary for progeny DNA synthesis (DNA polymerase).

e. **Replication of viral genome (nucleic acid)**

(1) **Plus-stranded RNA viruses** can immediately begin protein synthesis without nucleic acid replication or transcription events. RNA synthesis will occur when sufficient amounts of RNA polymerase are formed (using the host cell machinery for synthesis). A minus-strand copy is made from the parental strand RNA that serves as the template (replicative intermediate) for the transcription of plus-strand progeny.

(2) **Minus-strand and double-stranded RNA** viruses must first synthesize mRNA for the eventual translation into viral proteins. The minus-strand acts as a negative template for synthesis of mRNA. These viral genomes carry RNA-dependent RNA polymerase required to synthesize mRNA from the (–) strand.


### IN A NUTSHELL

*Positive-sense RNA viruses* ***are*** *mRNA and can directly encode all the proteins needed for replication. Other viruses require enzymes such as RNA-dependent or DNA-dependent RNA polymerases to produce mRNA for viral replication.*

### NOTE

- *All DNA viruses replicate entirely in the nucleus except for poxviruses.*
- *All RNA viruses replicate entirely in the cytoplasm except for influenza viruses and retroviruses.*

(3) Double-stranded RNA viruses (REO and Rota) synthesize a positive strand of RNA from the negative strand of the parent. This acts both as mRNA and as the replicative intermediate to make the negative-sense RNA that will be assembled with a complimentary positive strand to make the progeny genome.

(4) Retroviruses use the negative strand of the DNA intermediate to make positive-sense progeny RNA.

(5) Double-stranded DNA viruses replicate by the same process employed by the host cell; each strand serves as the template for synthesis of the complimentary DNA copy. Hepatitis B virus contains a viral RNA-dependent DNA polymerase (a reverse transcriptase) that uses the viral mRNA as a template to synthesize the missing portion of the viral genome, which is then duplicated by host cell DNA polymerase.

(6) Single-stranded DNA viruses (parvovirus) synthesize a double-stranded intermediate to use as the replicative template for the single-stranded DNA progeny.

f. **Viral assembly** occurs toward the end of the synthetic period. Complete intracellular virus assembly begins. The viral genomes and capsid polypeptides assemble, forming infectious viral offspring.

g. **Release** of the complete nucleocapsid is the final stage of the replication cycle.

(1) Enveloped viruses are released gradually by a budding process. Nucleocapsids bud through virally altered membrane patches, thus gaining viral specific glycoproteins.

(2) Poxviruses and naked capsid viruses burst out rapidly from the cell, causing the cell to disintegrate.

*IN A NUTSHELL*

***Viral growth cycle:***

- *Attachment of virus to cell*
- *Penetration of cell*
- *Uncoating of viral genome*
- *Transcription of genome into mRNA*
- *Translation into proteins*
- *Replication of viral genome*
- *Assembly of particles into new viruses*
- *Release of virus*

## DNA VIRUSES

### A. Adenoviruses

1. **Characteristics.** Adenoviruses are medium-sized, linear, **double-stranded DNA** viruses with a **naked icosahedral nucleocapsid**. They contain fibers that serve as viral attachment sites. There are 41 adenovirus serotypes, with approximately one-third accounting for the bulk of human disease.

2. **Transmission** usually occurs person-to-person through **respiratory and ocular secretions** (usually infecting mucous membranes or lymphoid tissue). Humans are the only known host.

*MNEMONIC*

*To remember which viruses are RNA vs. DNA, just memorize the DNA viruses (there are fewer) and know that all the rest are RNA viruses. To help remember the DNA viruses, think about how HHAPPP you will be once you finish studying. The tough part is recalling that the PPP represents papova-parvo-pox (picorna and paramyxo are RNA viruses).*

a. **T helper cells are CD4 positive.** There are two distinct subsets of T helper cells with different functions. One function is to stimulate B lymphocytes to proliferate and differentiate into antibody-producing cells. A second function of $CD4^+$ T cells is to promote cytotoxic T-cell ($CD8^+$ cells) responses.
   (1) Activation and proliferation of T helper cells depends on corecognition of specific antigenic peptides and MHC class II molecules on antigen-presenting cells such as macrophages or dendritic cells.
   (2) Activated T helper cells produce **lymphokines**, differentiation factors, and inflammatory cytokines.
b. **Cytotoxic T cells are also CD8 positive.** They lyse cells carrying specific antigens such as virus-infected cells and tumor cells. Cytotoxic T cells act by recognizing foreign antigen and MHC class I molecules with their T-cell receptor.
c. Suppression is a function of both CD8 and CD4 cells.

3. **Natural killer (NK) cells** represent 10–15% of lymphocytes in peripheral circulation.
   a. NK cells kill certain tumor cells (without damaging normal tissues) and defend against viral infection. Unlike T cells, NK cells recognize foreign antigen that does not have to be presented on **MHC molecules.**
   b. NK cells mediate **antibody-dependent cellular toxicity (ADCC).** This function allows NK cells to kill opsonized, or antibody-coated, target cells.
   c. NK cells are activated by cytokines such as interferon gamma.

## LYMPHORETICULAR SYSTEM

The lymphoreticular system (Figure 1-1) comprises the primary lymphoid organs, in which hematopoiesis and lymphopoiesis occurs, and secondary lymphoid organs, in which immune responses occur. **Primary or "central" lymphoid organs** in children and adults include the **bone marrow** and **thymus;** in the fetus, the spleen and liver are also primary organs. Hematopoiesis occurs in the bone marrow, producing mature erythrocytes, platelets, monocytes, granulocytes and B cells, as well as precursors for T cells, NK cells, dendritic cells, and mast cells. The T cells finish maturation in the **thymus,** whereas the rest finish maturation in the periphery. The major **secondary** or **"peripheral" lymphoid organs** and tissues include the **lymph nodes, spleen,** and the **mucosa-associated lymphoid tissue system (MALTS).** MALTS includes the **gut-associated lymphoid tissue (GALT), bronchus-associated lymphoid tissue (BALT),** and submucosal lymphoid tissues of the genitourinary tract. The purpose of secondary lymphoid organs is to trap and present antigen to circulating lymphocytes in stimulating adaptive immune responses. These secondary tissues or organs protect all surfaces and fluids

### NOTE

*There are two subsets of helper T cells. Th1 releases IL-2 and IFN-γ, whereas Th2 releases other interleukins (e.g., 4, 5, 6, 10). Th1 cells stimulate proliferation and cytoxic responses, whereas Th2 cells stimulate B-cell maturation, differentiation, and class switching.*

### IN A NUTSHELL

- *T helper cells → $CD4^+$*
- *Cytotoxic T cells → $CD8^+$*

*Another T-cell subset is the cell responsible for the delayed-type hypersensitivity ($T_{DTH}$) cell, which has CD4 in its membrane.*

### NOTE

*ADCC may be medicated by NK cells, eosinophils or neutrophils, all of which have Fc receptors.*

3. **Clinical manifestations**
   a. **Acute respiratory disease** that may induce a latent infection in tonsils, adenoids, and other lymphoid tissues. Most infections are acute and self-limited. Influenza-like illness occurs in the late fall and winter, characterized by pharyngitis, fever, cough, and malaise.
   b. Acute febrile pharyngitis may be seen in infants and children.
   c. Conjunctivitis ("pink eye")
   d. Diarrhea and gastroenteritis
   e. Pneumonia and pharyngoconjunctivitis are less frequent complications, except in the military, where frequent epidemics may occur.

4. **Diagnosis** is made by clinical presentation, culture of stool, urine, throat swabs, or conjunctival scrapings. Serology can be performed by ELISA or complement fixation techniques.

5. **Treatment and prevention**
   a. Treatment is supportive.
   b. A vaccine consisting of live, nonattenuated viruses is used in the military. An asymptomatic intestinal infection results that induces respiratory tract immunity.

**CLINICAL NOTE**

*HPV have oral manifestations of a pedunculated growth usually on the hard palate.*

B. **Papovavirus** family includes Papilloma, Polyoma, and Vacuolating viruses.

1. **Characteristics**. Papovaviruses are small **double-stranded, circular, DNA** viruses with a **naked icosahedral nucleocapsid**.

2. **Human papillomaviruses** (HPV) are distributed worldwide. They cause skin warts (prevalent in children) and genital warts (condyloma acuminata) and are the most common cause of **viral** STD.
   a. **Transmission** occurs via contact with warts.
   b. In addition to warts, HPV has also been implicated in benign laryngeal papillomas.
   c. A growing number of serotypes are associated with penile, laryngeal, and especially, **cervical cancer.**
   d. **Treatment** includes removal by electrocautery, cryotherapy, or chemicals. Recurrence is common.

**CLINICAL NOTE**

*For papovavirus, oral treatment is excision. Secondary healing is usually sufficient.*

3. **BK virus** is distributed widely in the human population, with 75% serologic positivity in children aged 6 and older. Infection remains latent. Disease (virus in urine) is limited to **patients undergoing immunosuppressive** treatment and is thought to be a reactivation of infection acquired in childhood.

4. **JC virus** is a polyoma virus associated with **human progressive multifocal leukoencephalopathy.**

C. **Herpesviruses**

1. **Characteristics**. Herpesviruses are large, **double-stranded, DNA** viruses with an **enveloped icosahedral nucleocapsid**. Herpesviruses are characterized by **latent infections** with recrudescence of disease (increasingly prevalent in immunocompromised patients).

2. **Herpes simplex virus, types 1 and 2 (HSV-1 and HSV-2),** cause **oral and genital lesions** by infecting epithelial cells. Upon the resolution of acute illness, latent infections are commonly found in **neurons**. Humans are the only known host. Direct contact with the infected lesion or secretions are necessary for transmission.
   a. **HSV-1** is typically acquired early in life. It is usually associated with **oral lesions ("fever blisters")** and with neurologic disease. HSV-1 is the leading cause of **sporadic encephalitis** in the U.S. Note that HSV-1 can cause genital lesions as well.
   b. **HSV-2** is acquired after the onset of sexual activity. It is associated with **genital lesions** and with neurologic disease. It is transmitted through sexual contact.
   c. **Primary infection** may be asymptomatic or characterized by **vesicular lesions** with edema, leading to ulceration and crusting. The lesions heal without scarring. Infection is characterized by **primary gingivostomatitis (HSV-1)** or by **primary herpes genitalis (HSV-2).** Neonatal herpes may be acquired *in utero* or during birth.
   d. **Recurrent infection** occurs at the site of primary infection. It involves the activation of latent virus from neurons of cervical or sacral ganglia. Stresses that lead to reactivation include hormonal changes (menses), fever, sunlight, physical trauma, and immune suppression.
   e. **Diagnosis** is made by identification of the clinical lesions as well as with viral isolation by tissue culture. The **Tzanck smear** will demonstrate **multinucleated giant cells** on the stains of scraped lesions (differential includes varicella-zoster virus). Immunofluorescent stain of the lesions will demonstrate viral **intranuclear inclusion bodies**. The latter test is preferred.
   f. **Antiviral treatment** with **acyclovir** is instituted upon diagnosis in serious infections.

3. **Varicella-zoster virus (VZV)** is isolated from patients with **chickenpox** and **shingles**. Shingles is reactivation of latent varicella infection.
   a. **Chickenpox** (varicella), caused by herpes zoster virus, is a mild self-limited illness in children expressed as fever followed by a **macular then papular eruption** on skin and mucous membranes. Chickenpox usually occurs in epidemics and is highly contagious. The virus is spread by **respiratory secretions** with approximately a 2-week incubation period. The papules are pruritic and become vesicular on skin and mucous membranes (all stages of the lesions

*NOTE*

*Dentists are often asked by parents to diagnose primary gingivostomatitis in young patients. Painful oral lesions and fever will be present in a young patient (usually < 4 years). The disease is self-limiting.*

are found simultaneously). Disease is more severe in adults, with **pneumonia common in immunocompromised patients**. Encephalitis is a rare but severe complication.

b. **Shingles** is a recurrent infection, usually in adults, that may be activated by trauma, neoplasm, drugs, or immunosuppression. Shingles occur from virus that remains latent in the sensory ganglia of spinal or cranial nerves. Severe **dermatomal pain** occurs with **vesicular eruption**, fever, and malaise. Pain and neuralgia may precede dermal eruption by 1–3 days. The pain may persist for months (postherpetic neuralgia), especially in older patients.

c. **Diagnosis** is primarily made by serologic assay and by the history of exposure to the virus. Scrapings of lesions stained with fluorescein-labeled antivaricella antibodies will reveal multinucleated giant cells with viral intranuclear inclusions.

d. **Treatment and prevention**

(1) Varicella infections can be treated with acyclovir.

(2) Immunosuppressed patients can be treated prophylactically with human varicella-zoster immunoglobulin (VZIG).

(3) An **attenuated varicella vaccine** was approved for use in the U.S. in 1995. Children should be vaccinated between 12 and 18 months of age because maternal antibody can interfere with attenuated viral vaccine efficacy.

4. **Epstein-Barr virus** is the etiologic agent of **infectious mononucleosis (IM).** IM is spread by saliva and respiratory secretions and is initiated in the oropharynx. EBV replicates in epithelial cells prior to infecting the B lymphocytes. EBV infection is associated with **Burkitt's lymphoma** and **nasopharyngeal carcinoma** in particular populations. It is also the etiologic agent of **hairy oral leukoplakia** in immunocompromised hosts.

a. **Clinical manifestations** last 2–4 weeks and include fatigue, malaise, tender lymphadenopathy, pharyngitis, fever, headache, and splenomegaly. Hepatitis and meningitis are less frequent clinical symptoms. **Atypical lymphocytes** with a foamy cytoplasm (Downey cells) are noted on peripheral blood smear.

b. **Nonspecific serologic responses** to EBV infection are due to the viral infection of B lymphocytes. Heterophile antibodies that arise will agglutinate sheep and horse red blood cells (as measured by the classic Paul-Bunnell test).

c. **Specific antibodies** also arise to the viral capsid proteins. IgM class antibodies indicates recent infection, while IgG antibodies persist for more than one year.

d. **Diagnosis.** Ten percent or more of patients fail to make heterophile antibodies. In these individuals diagnosis is made by ELISA assay, isolation of virus, and/or nucleic acid hybridization technology.

### CLINICAL CORRELATE

*Due to severe splenomegaly, patients with mononucleosis may undergo splenic rupture during physical exam.*

### IN A NUTSHELL

***Epstein-Barr virus:***

- *Herpes virus*
- *Causes infectious mononucleosis*
- *Hairy oral leukoplakia in immunocompromised patients*
- *Associated with Burkitt's lymphoma and nasopharyngeal carcinoma*
- *Heterophile antibodies; Monospot test*
- *Atypical lymphocytes*

e. **Treatment** is supportive. Acyclovir can be used in severely ill patients.

5. **Cytomegalovirus (CMV)**
   a. CMV infection of **nonimmunocompromised** (normal) humans elicits a mononucleosis illness that is clinically indistinguishable from EBV-mononucleosis. The majority of human infections are subclinical (with no overt symptoms) but may lead to life-long latent infection. CMV has a 4–8 week incubation period. Less common clinical sequelae include pneumonia, hepatitis, myocarditis, and meningoencephalitis.
   b. Illness in **immunosuppressed patients** (cancer, transplant [especially kidney], chemotherapy-induced, and AIDS patients) is often due to the reactivation of previously acquired infection. Infection in the immunosuppressed is more severe and is marked by fever, adenopathy, leukopenia, hepatosplenomegaly, and myalgias. Interstitial pneumonia, hepatitis, and gastrointestinal ulceration may also arise in immune compromised individuals. **CMV retinitis** is seen in AIDS patients.
   c. **Congenital disease** arises when the fetus acquires CMV virus transplacentally, causing **cytomegalic inclusion disease.** Infection can be acquired during any trimester. A severe fatal form of the illness is characterized by large intranuclear inclusion bodies in salivary glands, kidneys, brain, liver, and lungs. Periventricular calcifications are seen in the CNS; the infant will be deaf and have hemorrhagic cutaneous lesions (blueberry muffin baby).
   d. **Antiviral treatment** is instituted in severe disease with **ganciclovir.** Mononucleosis in normal patients is usually self-limiting without antiviral treatment.

6. **Human herpesvirus-6** (HHV-6) is a lymphotrophic human herpesvirus. It is thought to be the etiologic agent of pediatric "sixth disease," or **roseola infection** (exanthem subitum).

D. **Poxviruses**

1. **Characteristics.** Poxviruses are the largest of all the viruses. They are linear **double-stranded, DNA-enveloped** viruses that **replicate entirely in the cytoplasm of infected cells.** They appear ovoid or brick-like in shape. The virion contains several enzymes for replication, including both DNA and RNA polymerases.

2. **Variola virus (smallpox virus)** is confined to humans and is spread by direct person-to-person contact. Smallpox is now officially considered eradicated after years of worldwide vaccination.

3. **Molluscum contagiosum** causes umbilicated **wart-like skin lesions** and satellite nodules on the periphery of the parent nodule. In children,

the lesions are more commonly found on the trunk, face, or limbs. The sexually transmitted virus will be expressed as genital lesions.

The disease is typically benign and self-limiting but may require up to three years to resolve (particularly genital lesions). The lesions may be numerous in immunocompromised (especially AIDS) patients.

4. **Cowpox** is an occupational disease transmitted to humans by contact with infected cow udders. Infection is usually restricted to fingers and hands and is self-limited. The disease is usually milder in vaccinated individuals.

E. **Hepadnaviruses** are DNA viruses that include the **hepatitis B virus (HBV),** which is discussed in the separate section below on hepatitis viruses.

F. **Parvoviruses** are small single-stranded DNA viruses. **Serotype B19** is the only human pathogen in the family. It causes **erythema infectiosum** ("fifth disease") in children (characterized by slapped cheek rash), aplastic crisis in individuals with chronic hemolytic diseases such as thalassemia and sickle cell (the virus infects premature RBCs and kills them), and fetal infections that may cause hydrops fetalis or stillbirth of fetuses that are profoundly anemic.

*NOTE*

*Other erythemas are measles, rubella, scarlet fever, roseola, and exanthem subitum (HHV-6).*

## RNA VIRUSES

A. **Picornaviruses** are small, positive, **single-stranded RNA** viruses with a **naked** nucleocapsid. The RNA genome is **positive sense** (can serve as mRNA), and replication of picornaviruses occurs in the cytoplasm of the host cell. The picornaviruses can be divided into enteroviruses (poliovirus, coxsackie A and B, echovirus, and enterovirus) and rhinoviruses. Enteroviruses are acid-resistant and are able to survive in the GI tract; rhinoviruses are acid-sensitive.

*NOTE*

*Polioviruses are polycistronic, which means that the RNA is read as large polyprotein messages (i.e., no stop codons in the genome). The individual proteins are produced by the action of a viral protease that "chops up" the polyprotein product of translation. Similar replicative processes occur in HIV; the newest AIDS drugs being used today are protease inhibitors.*

1. **Polioviruses** bind to receptors in the gut and on neurons. Disease occurs only in primates, with the majority of infections expressed as subclinical disease.
   a. **Transmission.** Poliovirus is excreted in the feces and transmission occurs primarily by person-to-person contact and via contaminated water sources.
   b. **Pathogenesis.** Poliovirus is ingested, replicates in oropharyngeal and intestinal mucosa, and drains to the cervical and mesenteric lymph nodes. Transient viremia ensues, and the virus then spreads systemically. CNS involvement may lead to the destruction of motor neurons in the spinal cord, resulting in flaccid paralysis.
   c. **Prevention.** Live attenuated virus vaccine (oral; OPV; Sabin) or the killed virus vaccine (IPV; Salk vaccine) are used to induce immunity. Both vaccines induce serum antibodies; only the oral vaccine induces gut immunity and sIgA synthesis.

*CLINICAL CORRELATE*

*Polio vaccination schedule: currently IPV is given at the first two visits to the pediatrician (less than 4 months of age) together with DPT, Hib, and Hep B. This is followed by two doses of OPV. A booster may be given when starting school.*

2. **Echoviruses** (Enteric Cytopathic Human Orphan) consist of 32 serotypes recognized by viral capsid antigen differences. All echoviruses infect the gastrointestinal tract, although not all cause human disease.
   a. **Transmission and epidemiology.** Echoviruses are acquired by ingestion or inhalation. The initial infection occurs in the throat, followed by gastrointestinal tract infection. The incidence of clinical disease is increased in the summer months.
   b. **Clinical manifestations.** Disease is observed as aseptic meningitis, fever, rash, enteritis, common colds, and/or acute hemorrhagic conjunctivitis. Less common symptoms include paralysis, pleurodynia, encephalitis, myocarditis, and respiratory illness.

3. **Coxsackieviruses** are divided into A and B groups according to their pathogenesis in suckling mice. **Coxsackie A** causes diffuse myositis of skeletal muscle; **Coxsackie B** causes focal necrosis of skeletal muscle and degeneration of brain and other tissues.
   a. **Transmission and epidemiology.** Epidemics occur in the summer and fall. Coxsackie is transmitted by nasopharyngeal secretions and by the fecal-oral route. Coxsackie infection is usually an asymptomatic or benign illness with the following exceptions:
   b. **Clinical manifestations**
      (1) **Coxsackie A** may cause **herpangina**, with headache, sore throat, dysphasia, stiff neck, fever, anorexia, and abdominal pain. Discrete vesicles are seen in the oropharynx. It is also the etiologic agent of hand-foot-and-mouth disease.
      (2) **Coxsackie B** may cause **myocarditis**, pericarditis, and **pleurodynia.**
      (3) Both viral groups may cause meningitis in humans.

4. **Enterovirus 72** is the etiologic agent of **hepatitis A.** It is discussed in the section on hepatitis viruses. Other enteroviruses are associated with exanthema and aseptic meningitis.

5. **Rhinoviruses** are associated most frequently with the **common cold.**
   a. **Transmission and epidemiology.** Rhinoviruses infect human hosts only and are commonly isolated from the nose and throat. The incubation period lasts 2–4 days, with clinical illness lasting up to a week. Over 100 serotypes have been identified, each conferring type-specific immunity.
   b. **Clinical manifestations** include upper respiratory tract irritation, headache, nasal discharge, cough, malaise, chills, and myalgia. Fever is usually limited or nonexistent, as is cervical lymphadenopathy.
   c. **Treatment and prevention.** Treatment is supportive. The large number of serotypes makes a vaccine impractical.

B. **Orthomyxoviruses** include **influenza viruses A, B, and C.** Orthomyxoviruses are medium-sized, negative-sense, **single-stranded, segmented RNA** with an **enveloped nucleocapsid.**

1. **Structure.** Influenza viruses are composed of eight separate segments of RNA.

2. **Classification** into types A, B, and C is based on NP protein and M protein antigens.

3. **Antigenic variation** is an important mechanism for the virus to evade immune system destruction and is the reason for yearly change in vaccine composition.

4. **Transmission** is by inhalation. Epidemics occur with antigenic drifts, whereas pandemics arise from antigenic shifts.

5. **Clinical manifestations.** Influenza type C causes symptoms of the common cold with an incubation period of 1–4 days. The symptoms of Influenza types A and B are more severe and include:
   a. Fever, chills, myalgia, and lassitude, all occurring abruptly
   b. Sore throat, headache, nasal congestion, and dry cough
   c. A potential progression to viral pneumonia or secondary bacterial infection (especially Staphylococcus). These symptoms usually occur in the elderly and debilitated.
   d. Influenza B is one of the many viruses associated with **Reye's syndrome.**

6. **Treatment and prevention**
   a. **Treatment** is effective only in type A infection and consists of **amantadine** or **rimantadine,** which decreases the duration of symptoms.
   b. **Vaccines** composed of inactivated virus are designed to elicit immunity against the existing serotypes in the population. Vaccines change from year to year based on the particular serologic determinants of the virus.

C. **Paramyxoviruses** are **negative-sense RNA** viruses with an **enveloped nucleocapsid.** Paramyxoviruses are genetically stable (no antigenic shifts or drifts). Initial infection is via the respiratory tract. These viruses are the most common cause of **respiratory infections in children.**

1. **Parainfluenza viruses** are ubiquitous and are spread by aerosolized droplets. Parainfluenza is the etiologic agent of **croup.**
   a. Adults usually have neutralizing antibody to all four major serotypes. However, reinfection may occur despite the presence of neutralizing antibody. Reinfections are typically mild compared with the primary infection, which usually occurs during the first 6 years of life.

b. **Clinical manifestations** are usually associated with febrile illness (viremia is uncommon). They include **laryngo-tracheobronchitis (croup),** or an obstruction due to swelling of larynx and trachea, **bronchiolitis** and pneumonia.
c. **Treatment** is symptomatic; steam/nebulized air reduces symptoms.

3. **Measles virus (rubeola)** is a highly contagious childhood infection characterized by **fever and maculopapular exanthem.** Rubeola is transmitted by respiratory secretions. The virus multiplies in the oropharynx then spreads to lymphoid tissue followed by further viral replication throughout the reticuloendothelial system. Infection leads to permanent immunity because only one antigenic type exists.
   a. **Clinical manifestations. Koplik spots,** bluish-white specks on a red base found on buccal mucosa, are pathognomonic for measles. Other clinical symptoms include an abrupt onset of anorexia, nausea, fever, malaise, coryza, conjunctivitis, and cough. A maculopapular, erythematous rash, lasting about 5 days, originates on the face and may spread to the torso.
   b. **Complications** of measles infection include **encephalo-myelitis** (1 in 1,000 cases), pneumonia (in immunodeficient patients), otitis media, and **subacute sclerosing panencephalitis (SSPE).** SSPE is a fatal disease developing many years after acute infection. Measles infections are also thought to precede the onset of multiple sclerosis. These complications are thought to be due to an autoimmune response against nervous tissue that is induced by the measles virus.
   c. **Treatment and prevention**
      (1) **A live, attenuated vaccine** is available as part of the measles-mumps-rubella vaccine, which is administered at **15 months** and again at **entry to school.**
      (2) Unvaccinated children exposed to measles can be treated with pooled serum globulin because most donors have measles antibodies in their serum.

4. **Mumps virus** causes an acute contagious, nonsuppurative **parotitis** (either unilateral or bilateral). Less frequent clinical sequelae include **orchitis,** occurring in 20–35% of postpubertal males, and aseptic meningitis. However, as many as one-third of cases are asymptomatic. Mumps is prevented by **immunization with live attenuated virus** as part of the measles-mumps-rubella (MMR) vaccine.

5. **Respiratory syncytial virus (RSV)** is the primary cause of lower respiratory tract infections in **infants.**
   a. **Transmission** occurs via aerosolized droplets; it can also be spread by fomites.
   b. RSV replicates in the upper respiratory tract and persists in older children and adults. In infants and young children, the virus

**CLINICAL CORRELATE**

*Children suffering from croup often have a characteristic "barking" cough.*

**NOTE**

*Koplik spots = measles = paramyxovirus*

**NOTE**

*MMR vaccine is given at 15 months to avoid interference with viral replication by traces of maternal antibody.*

*IN A NUTSHELL*

***Paramyxoviruses:***

- *Parainfluenza*
- *Measles*
- *Mumps*
- *RSV*

spreads to the lower respiratory tract, causing bronchitis and pneumonia.

c. The spectrum of disease ranges from common cold-like symptoms to severe lower respiratory illnesses (especially in infants). Impaired immune functions will allow recurrent and prolonged infections with RSV.

d. **Treatment** includes supportive care and **aerosolized ribavirin** in more severe cases. Virus-specific immunoglobulin injections are also being employed in the treatment of severe disease.

D. **Togaviruses** elicit diseases that range from febrile illness to encephalitis or severe bleeding disorders. Togaviruses contain positive, **single-stranded RNA** in an **enveloped nucleocapsid** with glycoprotein hemagglutinins in the envelope.

1. **Alphaviruses** multiply in many arthropods **(arthropod-borne)** and vertebrates. They are zoonotic agents spread to man by insect vectors. Alphaviruses include **encephalitis viruses**.
   a. **Eastern equine encephalitis (EEE)** virus causes a severe disease with 50–70% mortality. The illness is marked by the abrupt onset of headache, fever, and nuchal rigidity, with nausea, vomiting, and drowsiness. The individual may be left with neurologic deficits.
   b. **Western equine encephalitis** virus causes less severe disease and is seen more in children.

2. **Rubivirus** or **rubella** (3-day measles) is the virus causing German measles. It is the **only togavirus not transmitted by an arthropod vector.**
   a. **Rubella** consists of a single antigenic type that resembles measles. However, rubella is an infection of shorter duration and the disease is much less severe. The virus infects the upper respiratory tract and then spreads throughout the body via a viremia.
   b. A **morbilliform rash** occurs 2–3 weeks postinfection.
   c. **Congenital rubella.** Rubella virus may be **transmitted across the placental barrier.** This has serious consequences if it occurs in the first trimester. If the fetus survives, neurologic and other congenital abnormalities are common, including mental retardation, heart abnormalities, blindness, motor abnormalities, and encephalitis.
   d. Live **attenuated virus vaccination** is effective for prevention of infection (as part of MMR [measles-mumps-rubella]).

E. **Flaviviruses** are very similar to togaviruses and specifically resemble alphaviruses. They are arthropod-borne viruses.

1. **Yellow fever** is a **mosquito-borne** flavivirus infection with an incubation period of 3–6 days.
   a. Two viral forms exist: urban (human reservoir) and jungle (monkey reservoir) types; both may result in severe disease affecting liver and kidney (with icterus and hemorrhage).
   b. Yellow fever is characterized by the acute onset of fever, jaundice, proteinuria, vomiting (vomitus is characteristically black), and the hemorrhage of internal organs with necrosis. Severe symptoms may lead to hypovolemic shock and death.
   c. A safe and effective attenuated vaccine (strain 17D) is available.

2. **Dengue fever** is also a **mosquito-borne** illness characterized by fever, rash, arthralgia, and lymphadenopathy. Only the more severe form involves hemorrhagic manifestations. Several antigenic serotypes are recognized and clinical complications may result in death (10% fatality rate). Dengue fever occurs primarily in the tropics.

F. **Bunyaviruses** are segmented **(–) single-stranded RNA** viruses with an **enveloped nucleocapsid.** Bunyaviruses are transmitted by **arthropod vectors (mosquitos)** and humans are only accidentally infected hosts.

1. **California encephalitis viruses** cause an abrupt fever and severe bifrontal headache. Other sequelae include CNS involvement requiring prolonged convalescence but with low morbidity and mortality.

2. **Hantavirus** causes hemorragic fever and acute respiratory distress syndrome with a high case fatality rate. They are natural pathogens of rodents. Humans become infected by inhalation of infectious urine or feces.

G. **Rhabdoviruses** include only one significant human pathogen, **Rabies virus.** Rabies is a **bullet-shaped** enveloped virus with **single-stranded (–) RNA.** It has a **nucleocapsid** with protruding glycoprotein spikes; the virus replicates in host cell cytoplasm. The rabies virus is of a single immunologic type eliciting neutralizing antibodies against the surface glycoproteins.

1. **Transmission** is dependent on the persistence of the virus in a wild animal reservoir (e.g., skunks, raccoons, bats, foxes), making this virus practically impossible to eradicate.

2. **Pathogenesis.** The virus enters through breaks in the skin produced from the **bite of a rabid animal.** The virus replicates in the muscle and connective tissue with an incubation period of 2–16 weeks. The virus moves through the axoplasm of peripheral nerves to the CNS, including the basal ganglia. Rabies may also inhabit the salivary glands and

*NOTE*

*Negri bodies = rabies = rhabdovirus*

other tissues along its retrograde movement through the peripheral nerve pathways. **Negri bodies,** the cytoplasmic viral inclusions in neurons of the hippocampus, are pathognomic for the infection.

3. **Clinical manifestations.** The clinical course is described in four discrete phases: prodrome, sensory, excitement, and paralytic. Prodrome symptoms include paresthesia at wound site, irritability (including fever and a change in mood or temperament), and a flu-like illness. Pharyngeal spasms may arise, resulting in drooling (hydrophobia). Terminal disease involves seizures, coma, and, eventually, death.

4. **Diagnosis** is confirmed by the recovery of virus from saliva, by serology (direct immunofluorescence), or by immunofluorescence of Negri bodies in neural tissue (especially Ammon's horn).

5. **Treatment and prevention**
   a. **Vaccines** consist of inactivated virus from infected human diploid cells. Pets and high-risk individuals should be vaccinated, as should anyone with a history of being bitten by an animal that is possibly rabid.
   b. **Human rabies immunoglobulin (HRIG)** should be given immediately in cases of probable infection.

H. **Retroviruses** are diploid **(+) single-stranded RNA,** enveloped viruses associated with tumors, oncogenesis, and immunodeficiency disease (AIDS).

1. Retroviruses contain two copies of single-stranded RNA, and viral-encoded **reverse transcriptase** produces double-stranded DNA from the RNA.

2. The viral genome encodes three groups of proteins: **Pol protein** (reverse transcriptase and integrase), **Env protein** (type-specific envelope proteins), and **Gag protein** (type-specific viral core proteins).

3. This viral family includes **human T-cell leukemia viruses (HTLV I, II)** and **human immunodeficiency virus** (HIV). HTLV viruses are categorized as oncoviruses, whereas HIV is considered a lentivirus and is not oncogenic.

4. **Oncoviruses.** These viruses encode oncogenes that promote cell growth. The most important oncogenic retrovirus is **human T-lymphotropic virus 1 (HTLV 1)**. This virus infects CD4+ T cells (helper and delayed-type hypersensitivity). Infection can progress to **acute T-cell lymphocytic leukemia (ATLL)** (aka adult T-cell leukemia). **HTLV II** causes hairy cell leukemia.

5. **HIV is the etiologic agent of acquired immunodeficiency syndrome (AIDS).** AIDS was initially recognized as a clinical syndrome in 1981 followed by the isolation of HIV in 1983. HIV is a nononcogenic retrovirus that infects millions of people worldwide. HIV infects helper T

cells via attachment to cell surface proteins (termed CD4), resulting in severe immunodeficiency. The virus also infects macrophages via an interaction with CD4 and CCR5 on the macrophage membrane. Patients are prone to opportunistic infections, malignancies, and wasting syndromes. Total CD4 T-cell counts are directly correlated with the degree of disease and infection. Opportunistic infections typically occur when the CD4 count drops below 200.

6. **HIV is classified as a lentivirus** (nononcogenic, cytocidal, retrovirus). HIV has a cylindrical, conical capsule that contains two positive, single-stranded RNA genomes with a glycoprotein envelope. The **gp120 glycoprotein spike** protrudes from this envelope and is the ligand for the CD4 molecule. Reverse transcriptase enzyme, critical to its replication, is inside the virion particle. The envelope proteins demonstrate considerable variation from virion to virion. This property makes it difficult for immune responses to clear the virus and complicates the development of a vaccine. Besides HIV, the lentivirus subfamily also includes SIV (simian), FIV (feline), and BIV (bovine) immunodeficiency viruses. Although this viral family shares common features (such as genes for reverse transcriptase), these viruses are infectious only in their respective species.

*BRIDGE TO IMMUNOLOGY*

*HIV is discussed in detail in the HIV and AIDS chapter of this book.*

*NOTE*

*The high level of antigenic variation among the HIV proteins is the result of inaccuracies in the action of reverse transcriptase, leading to incorporation of incorrect nucleotides.*

## HEPATITIS VIRUSES

Hepatitis viruses include both DNA (hepatitis B) and RNA (hepatitis A, C, D, E, and G) viruses.

HEP. A
- CAUSES INFECTIOUS HEP.
- SPREAD THRU FECAL/ORAL ROUTE

SOURCE : a. CONTAM. $H_2O$
b. SHELLFISH
c. FOOD IN d RESTAURANT
d. CAMP
e. MILITARY BASE

A. **Hepatitis A virus (HAV)** is a **picornavirus.**

1. **Transmission.** Hepatitis A is transmitted **fecal-orally** after a 15-to-40-day incubation period. Hepatitis A is associated with epidemic and endemic spread of infection (usually occurring as familial or institutional outbreaks). Most childhood infections are asymptomatic, but adult disease can be severe.

2. There are typically no extrahepatic manifestations, and there is **no chronic hepatitis or carrier state.** Infection is not associated with either cirrhosis or hepatic carcinoma. Fulminant hepatitis occurs only in 1–4% of clinical infections.

3. **Diagnosis** is made by the presence of **anti-hepatitis A IgM.**

4. There is a killed virus vaccine against hepatitis A that is recommended for individuals traveling to endemic areas, food handlers, and day-care workers. Pooled gamma globulin can be administered to travelers if there is insufficient time for active immunization.

HEP. B → many active or inactive chronic

→ "Dane Particle"

B. **Hepatitis B virus (HBV)** is an **enveloped double-stranded DNA virus** with single-stranded segments. It is classified as a **hepadnavirus.**

1. Characteristic viral antigens include:
   a. **Surface antigen (HBsAg)** is found on the surface of the virion. Its presence in serum **indicates active viremia** (infectivity).
   b. **Core antigen (HBcAg)** is found in the capsid.
   c. **E antigen (HBeAg)** is another epitope found in the capsomere proteins.

helps in testing & dis. or staging & level of infectivity

2. **Antibodies to HBV**-specific antigens are diagnostic markers of disease activity.
   a. **Antibodies to the surface antigen (HBsAg)** are considered protective antibodies and are detected after the disappearance of the virus.
   b. **Antibodies to HBcAg** are not protective. They are detected just after the appearance of HBsAg and are used to confirm infection when HBsAg and anti-HBsAg are absent (window phase).
   c. **Antibodies to HBeAg** are associated with a low risk of infectivity.

3. **Transmission** is parenteral or sexual. The majority of affected patients recover from the illness.

4. **Clinical manifestations** include: anorexia, nausea, vomiting, headache, fever, abdominal pain, dark urine, and sometimes jaundice.
   a. Liver function tests will indicate **elevated transaminases, hyperbilirubinemia, and elevated alkaline phosphatase.**
   b. Pathology is observed as the accumulation of inflammatory cells and parenchymal necrosis in liver.
   c. Extrahepatic manifestations include arthralgia, arthritis, nephritis, and dermatitis.
   d. Ten to fifteen percent continue to carry the virus and may develop **chronic persistent hepatitis or chronic active hepatitis** with subsequent fibrosis, cirrhosis, and hepatocellular carcinoma.

5. **Prevention. Recombinant HBsAg vaccine** is available and is recommended for all individuals at risk (e.g., health care workers). It is also now part of the protocol for **childhood immunizations.** Children should receive three doses: one at birth (up to 2 months), one at 2–4 months, and a final dose at 6–18 months. Children born of hepatitis B carriers should be injected at birth with hepatitis B-specific immunoglobulins (HBIG).

C. **Hepatitis C virus (HCV)** is a **positive-sense, single-stranded RNA virus** classified as a **flavivirus.** It is associated with **post-transfusion hepatitis.** HCV has been characterized as the etiologic agent for the majority (> 50%) of non-A–non-B (NANB) hepatitis infections. Screening of blood for HCV has significantly reduced the incidence of transfusion-related hepatitis. HCV develops a chronic carrier in nearly 50% of patients and is associated with cirrhosis and hepatocellular carcinoma.

**NOTE**

*Hepatitis B vaccine is standard prevention for all health care workers exposed to blood and body fluids in their normal work. Doses are usually: initial, one month and six months (approximately). The recombinant DNA yeast source of the vaccine ensures no possibility of exposure to other viruses from the vaccination.*

**NOTE**

*Currently, there is no standard for the use of Hep A or Hep C vaccine in health care workers.*

D. **Hepatitis D (delta agent)** is a defective RNA virus that can replicate only in cells concurrently infected with hepatitis B. This virion **requires the presence of hepatitis B** enzymes to replicate. It occurs in Italy and in the Near East.

E. **Hepatitis E** is caused by a single-stranded RNA virus that is similar to Norwalk agent (calicivirus). Clinically, it causes a disease similar to hepatitis A, but it can become fulminant in pregnant women (20% mortality). It is spread via the fecal-oral route. It occurs primarily in the Far East.

*NOTE*

*Not surprisingly, yet another hepatitis virus was recently identified (1996). Hepatitis G (HGV) is an RNA virus associated with both acute and chronic hepatitis. It has a global distribution and is transmissible by transfusion. It appears to be distantly related to hepatitis C.*

# Mycology

12

Fungi are eukaryotic organisms that can be classified as either yeast or molds based on their morphology and their mode of reproduction. The simplest way to review medically important fungi is to divide them into groups based on their clinical presentation: cutaneous mycoses, subcutaneous mycoses, systemic mycoses, and opportunistic mycoses.

## INTRODUCTION

A. **Morphology.** Fungi are eukaryotes possessing a cell wall (composed of glucose and mannose polymers called **chitin**) and a cell membrane (containing **ergosterol**). Capsules are found only with *Cryptococcus neoformans*. The fungi are divided into yeasts or molds based on shape and mode of reproduction.

1. **Yeasts** have round or oval morphology and reproduce by budding.
2. **Molds** have tubular structures called **hyphae**. Molds grow by branching and longitudinal extension to form **mycelial structures.** Hyphae can be either **septate** (divided into nucleated cells) or **nonseptate** (coenocytic).
3. **Dimorphic fungi** grow in the host as a yeast-like form but grow as molds at room temperature *in vitro*.

B. **Reproduction** can be sexual and/or asexual. **Asexual spores** form through mitosis. This form of reproduction is referred to as an "imperfect" state. Most pathogenic fungi are found only in the imperfect state.

C. **Immunity.** The T-cell response is protective in fungal diseases; antibodies are not, although they may have some role in serodiagnosis.

## OPPORTUNISTIC MYCOSES

The opportunistic fungi are those that cause disease in immunocompromised patients (AIDS, chemotherapy, transplants). Unlike the systemic mycoses, these fungi are sometimes normal flora and are not limited to certain geographic regions.

A. **Candida**. Candidal infections may be cutaneous or systemic.
   1. ***C. albicans*** is the major pathogenic species.
   2. Candida are normal inhabitants of mucocutaneous body surfaces, soil, hospital environments, and some foods. *C. albicans* colonizes normal skin, and most diseases are endogenous in origin. Occasional person-to-person spread occurs (e.g., newborn thrush).
   3. **Invasive disease** results from host alterations, leading to a change in the commensal status of the organism. Factors important in the invasiveness of Candida include predisposing illnesses (such as diabetes mellitus), damaged mucosal surfaces (such as those caused by indwelling catheters), depression of the immune status (as in immunocompromised individuals), and the use of steroids or antibiotics.
   4. **Clinical manifestations** depend on the site of infection.
      a. **Oropharyngeal infection** (includes **thrush**) is observed as discrete or confluent **white patches** on the tongue and buccal mucosa. Microscopic examination of scraping reveals pseudohyphae. Treatment consists of nystatin suspension, oral ketoconazole, or mycelex troches.
      b. **Vaginal infection** is frequently seen in diabetes mellitus, antibiotic treatment, and in pregnancy. It is associated with thick yellow-white discharge and intense pruritus. Nystatin suppositories are beneficial. Imidazole drugs (topical or oral) are also therapeutic.
      c. **Gastrointestinal colonization** causes disease in malnourished or immunocompromised persons or in persons undergoing prolonged intra-abdominal surgical procedures. Clinical presentation may include diffuse ulcerative and erosive **esophagitis**, gastritis, or multiple superficial ulcerations of the small and large intestine. Stool and throat cultures may not be diagnostic because of frequent colonization by Candida. Treatment consists of nystatin, ketoconazole, and low-dose amphotericin B.
      d. **Invasive, disseminated infection** occurs, particularly in patients with leukemia, lymphoma, and AIDS. The GI tract is probably the most common portal of entry. Symptoms include fever, shock, hypotension, and prostration. Renal infection occurs usually from hematogenous spread and may result in renal failure. Endocarditis occurs in drug abusers.

**NOTE**

*Candida found in your dental patients may indicate AIDS, other immunosuppression, recent antibiotic use, or other etiologies.*

**CLINICAL NOTE**

*Oral candida infections can be wiped off by gauze as a form of diagnosis.*

e. **Chronic mucocutaneous candidiasis** represents extensive cutaneous disease that is refractory to treatment and can be disfiguring.

5. **Diagnosis** is made by demonstration of fungal pseudohyphae in the tissue or by culture and biochemical identification (e.g., urease positive).

6. **Treatment.** Systemic treatment includes ketoconazole or fluconazole; amphotericin B is used less frequently. Flucytosine is also valuable in systemic disease. Cutaneous involvement usually responds to topical miconazole.

B. **Cryptococcosis** is due to infection with ***Cryptococcus neoformans.*** *C. neoformans* is an encapsulated yeast that reproduces by budding. *C. neoformans* is found worldwide in avian feces (particularly pigeon droppings), in soil, fruits, milk, and wood products. Immunosuppression due to malignancy or AIDS predisposes to this disease.

1. **Pulmonary disease** is common since this is the primary portal of entry. Disease is usually transient and not severe if the patient is otherwise healthy.

2. **Disseminated disease** arises most often in immunocompromised individuals.
   a. **Central nervous system** involvement includes **meningitis** or lesions that occupy the cerebral white and gray matter space.
   b. **Treatment** consists of **amphotericin B** alone or in combination with **5-fluorocytosine.**

C. **Aspergillosis** is a disease arising from several species of ubiquitous molds. ***A. fumigatus*** is the most common species. Organisms are normal inhabitants of the soil, and spores are readily disseminated in the air.

1. **Allergic bronchopulmonary aspergillosis** is marked by a **hypersensitivity reaction** to the fungal antigens. Inhalation of conidia or mycelial fragments may elicit an IgE-mediated hypersensitivity reaction causing bronchospasm.
   a. **Clinical manifestations** include episodic wheezing, fixed or transient pulmonary infiltrates, fever, and peripheral **eosinophilia.**
   b. **Treatment.** No treatment is required if the disease is mild. Corticosteroids, however, are helpful in reducing symptoms.

2. **Aspergillomas (fungus balls)** are the result of colonization of pulmonary cavities (usually secondary to tuberculosis or sarcoidosis). Patients may be asymptomatic, but **hemoptysis** occurs in the majority of cases. Surgery is indicated for massive hemoptysis.

3. **Invasive aspergillosis** usually occurs as an opportunistic infection in immunocompromised patients, with iatrogenic neutropenia.
   a. **Pulmonary involvement** is present in 90–95% of cases. Invasive aspergillosis presents as an unremitting fever and pulmonary infil-

*NOTE*

*Think cryptococcus if:*

- *The patient is immunocompromised*
- *Meningeal signs*

trate despite treatment with broad-spectrum antibiotics. Necrotizing bronchopneumonia is common.

b. Extrapulmonary dissemination to the esophagus, brain, or GI tract (with GI bleeding) occurs in about 25% of cases.

c. **Diagnosis** must demonstrate tissue invasion. **Septate hyphae** will be seen in biopsy specimens.

d. **Treatment.** Surgical removal of the aspergilloma may be necessary. Amphotericin B is the therapeutic standard, although itraconazole may be a valuable alternative.

D. **Zygomycosis (mucormycosis)** is most often caused by organisms from the genera **Rhizopus and Mucor**. These molds are ubiquitous on decaying vegetable matter in soil. They have **nonseptate hyphae.**

1. Individuals predisposed to invasive zygomycosis disease include those with **diabetic ketoacidosis**, leukemia, and lymphoma, antibiotic and steroid use, and infants and children with malnutrition.

2. **Clinical manifestations**

a. **Rhinocerebral disease** is the most common presentation. It typically occurs in **diabetics with ketoacidosis** and is an infection of the nasal mucosa, palate, sinuses, and/or orbit, whereby progressive neurologic deficits ensue as the organism invades to the base of the brain.

b. **Pulmonary disease** is usually the consequence of inhalation of spores in a patient with leukemia or lymphoma.

3. **Treatment** involves control of underlying disease such as diabetes, surgical debridement, and amphotericin B.

E. ***Pneumocystis carinii*** was originally thought to be a protozoa because of its morphologic stages and sensitivity to antiprotozoal drugs. However, rRNA homologies suggest that it is a fungus.

1. **Clinical manifestations.** In individuals with normal cell-mediated immunity, infection is asymptomatic. Defects in cell-mediated immunity, such as AIDS, cause trophozoites to invade the alveoli and cause **interstitial pneumonia.**

2. **Treatment** for pneumonia consists of **trimethoprim-sulfamethoxazole** or **pentamidine.** Steroids are indicated for severe pneumonitis. Prophylactic and suppressive treatment consists of trimethoprimsulfamethoxazole, with dapsone or pentamidine as a second choice.

*IN A NUTSHELL*

***Fungi forms in vivo:***

- *Coccidioides* → *spherules*
- *Histoplasma* → *intracellular yeast*
- *Blastomyces* → *broad-based buds*
- *Cryptococcus* → *large capsule*
- *Candida* → *pseudohyphae*
- *Aspergillus* → *branching septate hyphae*
- *Mucor/Rhizopus* → *nonseptate hyphae*

The immune system is an intricate collection of organs, tissues, cells, and soluble factors that allow individuals to defend against harmful agents such as viruses, bacteria, and tumor cells. The immune system includes the primary or central lymphoid organs in which the leukocytes develop, the secondary or peripheral lymphoid organs and tissues in which immune responses occur, and the leukocytes circulating in the blood. The first two sections will review the cells and organs that comprise the immune system. The third section will present the basic characteristics of immune responses, including antigens, antibodies and T-cell receptors, and mechanisms of regulation. The fourth section will examine T-cell and B-cell activation, the cellular interactions necessary for cell-mediated (e.g., T cell) and humoral (e.g., antibodies) responses and the cytokines that influence these processes. The fifth section will discuss complement and inflammation, and the last section will examine important immunological laboratory methods.

## CELLS OF THE IMMUNE SYSTEM

Leukocytes include lymphocytes (B cells, T cells, and large granular lymphocytes or NK cells), mononuclear phagocytes (monocytes and macrophages), polymorphonuclear granulocytes (neutrophils, eosinophils, and basophils), mast cells, and dendritic cells. All leukocytes, as well as erythrocytes and platelets, initially differentiate in the adult bone marrow (described below), and most complete maturation there, although T cells finish their maturation in the thymus.

A. **Monocytes and macrophages** control infections that are not susceptible to neutrophil attack. They are derived as follows: stem cell → monoblast → promonocyte → circulating monocyte → tissue macrophage. Macrophages have a long life span compared with neutrophils.

NOTE

*Cells of the immune system mature in the bone marrow (B cells and phagocytic cells) or in the thymus (T cells).*

1. **Function of monocytes and macrophages.** These cells play a central role in cell-mediated immunity. They ingest particles via pinocytosis or phagocytosis and modulate inflammation via secretion of mediators. They also act as cells that process and present antigens to T lymphocytes (antigen-presenting cells).
   a. **Macrophages secrete over 100 mediators,** including interleukins 1, 8, and 12, collagenase, elastase, lipase, proteases, prostaglandins, leukotrienes, thromboxanes, lysozymes, and interferons. They also secrete complement components.
   b. **Macrophages have Fc receptors and class II MHC molecules** on the cell surface that mediate their biologic functions. The Fc receptors allow the uptake of immune complexes (Ig complexed to antigen) and class II molecules present antigenic peptides to T cells.
   c. **Circulating monocytes differentiate into tissue macrophages** with specific names. For example, tissue macrophages present within the sinusoids of the liver are called **Kupffer cells.** Macrophages found in the lung are termed **alveolar macrophages;** in the brain, **microglial cells.**
      (1) Kupffer cells encounter antigens first from intestinal lumen absorption and serve to clear particulate and soluble matter from portal circulation. They phagocytose bacterial endotoxin, soluble immune complexes, activated clotting factors, and microorganisms.
      (2) Alveolar macrophages destroy inhaled antigens and microbes.
2. **Morphology of macrophages**
   a. **Epithelioid cells,** usually found in granulomas, are derived from blood monocytes and are activated by an immune response to antigen.
   b. **Multinucleated giant cells** are formed by the fusion of macrophages.
3. **Macrophage activation.** Macrophages are stimulated by lymphokines (mostly interferon gamma) to kill microorganisms and tumor cells.
   a. Activated macrophages have increased lysosomal hydrolytic enzymes and an increased chemotactic response. C5a and various cytokines from lymphocytes, neutrophils, and fibroblasts are chemoattractants for activated macrophages.
   b. Antigen coated with appropriate complement proteins (e.g., C3b) and antibody is more readily phagocytosed. This process is called **opsonization.**
   c. Morphologic changes occur during activation and include increases in size, number of pseudopods, and pinocytotic vesicles.
   d. Macrophages experience a respiratory burst during phagocytosis via the **hexose-monophosphate shunt** pathway. This is a source of energy needed for cell membrane synthesis and also generates toxic oxygen metabolites such as singlet oxygen, superoxide anion, and hydrogen peroxide.

*NOTE*

- *Macrophages and neutrophils both phagocytize bacteria that are coated with antibody and complement. The C3b fragment of complement binds to bacteria opsonized by antibody, then binds to receptors on phagocytic cells and signals them to phagocytize the organisms.*
- *The Fc receptors on macrophages are also useful for opsonization of bacteria by antibody. They react with the Fc region of IgG antibody molecules and hold the microbe close to the phagocytic cell membrane, thus facilitating the engulfment process.*

4. **Macrophages present antigens to T lymphocytes.** Antigen undergoes phagocytosis or pinocytosis. Once in the cytoplasm of the macrophage, the antigen is degraded into small peptides. The peptides are then noncovalently bound to class II MHC molecules in an endosomal vesicle, and the complex is then transported to the cell surface, where it stimulates class II–restricted antigen-specific helper T cells (CD4).

B. **Dendritic cells** are present in peripheral blood and lymphoid organs. Their primary function is to digest and process antigen for presentation to T cells. Dendritic cells include Langerhans' cells of skin, veiled cells in afferent lymphatics, and interdigitating reticulum cells in spleen and lymph nodes.

C. **Granulocytes or polymorphonuclear leukocytes.** There are three types of granulocytes: neutrophils, eosinophils, and basophils.

1. **Neutrophils (polymorphonuclear leukocytes, PMNs)** represent 60% of circulating blood leukocytes. Circulating neutrophils have a receptor for the Fc region of IgG ($Fc\gamma R$) and C3b.
   a. Neutrophils have a multilobulated nucleus with dense chromatin and cytoplasmic lysosomes containing peroxidases and acid hydrolases.
   b. They reach the tissues by diapedesis, inserting pseudopodia between the endothelial cells and dissolving the basement membrane so that movement through blood vessel wall occurs.
   c. Neutrophils are the first cells to arrive at acute inflammatory sites. They actively kill bacteria; their half-life is about 10 hours in the blood and three days in tissues.
   d. Cytoplasmic granules contain digestive enzymes. These include azurophilic granules that contain myeloperoxidase and specific granules that contain lactoferrin.
   e. Neutrophils phagocytize and then kill the organism by generation of $H_2O_2$ and toxic oxygen radicals and via the action of granule-derived enzymes.

2. Eosinophils represent 1–3% of circulating leukocytes.
   a. Approximately 50% of circulating eosinophils have receptors for complement.
   b. Eosinophils have a bilobed nucleus and contain crystalloid granules staining red with Giemsa.
   c. Eosinophil chemotactic factors include histamine, C5a, $LTB_4$, PAF, and ECF-A (eosinophil chemotactic factor of anaphylaxis).
   d. They are functionally important in late inflammatory reactions, particularly in **parasitic infections** and **allergy.**
   e. Some important contents of eosinophils and their functions include:
      (1) **Histaminase** degrades histamine.

*IN A NUTSHELL*

*Macrophages process **exogenous** antigens and present the epitopes in a groove of the class II MHC molecules. CD4 T cells bearing receptors specific for the epitope will react with the epitope/MHC complex and be triggered to release lymphokines. **Endogenous** antigens are similarly presented to CD8 cells on MHC1, also present on macrophages and their relatives.*

*IN A NUTSHELL*

*PMNs kill microbes via:*
- *Toxic oxygen metabolites*
- *Digestive enzymes present in lysosomal granules*

*CLINICAL CORRELATE*

*Eosinophilia is a hallmark of:*
- *Atopic allergies*
- *Worm infestations*

*It can also be seen in collagen vascular diseases, neoplastic disorders, and any skin rash.*

**NOTE**

*Eosinophil granule contents that help control allergic reactions include histaminase and aryl sulfatase.*

(2) **Pyrogen** produces fever.
(3) **Peroxidase** kills microorganisms.
(4) **Aryl sulfatase** degrades leukotrienes $C_4$, $D_4$, and $E_4$.
(5) **Major basic proteins** are toxic to worms.

3. **Basophils** represent 1% of circulating leukocytes and are the smallest type of granulocytes.
   a. They contain abundant granules with RNA, mucopolysaccharides, and hypersensitivity mediators, such as histamine.
   b. They have receptors for the Fc portion of IgE.
   c. IgE binding promotes degranulation as it does in the tissue mast cells. Histamine and other mediators are released during degranulation and are responsible for the symptoms seen in atopic allergies.

D. **Lymphocytes,** which include B and T lymphocytes, represent 30% of circulating leukocytes. Lymphocytes have a high nucleus-to-cytoplasm ratio and are distinguished by their antigen receptors and cell surface markers.

1. **B lymphocytes.** B lymphocytes differentiate into **plasma cells,** which secrete large amounts of immunoglobulin.
   a. There are two major subsets of B lymphocytes (from mouse models). **CDb 5–positive cells** produce IgM antibody to soluble polysaccharides and self antigens. They are stimulated by nonspecific lymphokines from T-helper cells. **CDb 5–negative cells** produce IgG, IgA of IgE antibody to protein antigens, cellular antigens, and bacterial lipopolysaccharides. These cells require direct physical interaction with specific helper T cells.
   b. **Memory B cells,** generated after the primary exposure to an antigen, secrete antibody with increased affinity for its antigen.
   c. **Mature B cells** have surface IgM and IgD that bind antigen, causing the B cell to become active and secrete immunoglobulins.
   d. B cells respond to two types of antigens:
      (1) **T-cell–independent antigens** stimulate B cells to secrete immunoglobulin in the absence of $CD4^+$ helper T cells.
      (2) **T-cell–dependent antigens** (most all protein antigens) regulate B- and T-cell interaction before immunoglobulin is produced. The T cell then drives B cells to secrete antibody via direct contact and by lymphokine secretion.

**NOTE**

*T-cell-independent antigens induce IgM antibody only and do not cause immunologic memory (anamnesis). Much of this antibody is secreted by CD5 B cells.*

2. **T lymphocytes.** Two major types of T cells exist and are classified based on the expression of the cell surface proteins CD4 or CD8. Cellular differentiation (CD) proteins are present on many cells of the body. They reflect the function of the cell and are thus "markers" for particular cells. For example, CD3 is found in the membrane of all mature T cells, whereas CD4 and CD8 proteins are found on certain subsets of these mature T cells.

(3) Double-stranded RNA viruses (REO and Rota) synthesize a positive strand of RNA from the negative strand of the parent. This acts both as mRNA and as the replicative intermediate to make the negative-sense RNA that will be assembled with a complimentary positive strand to make the progeny genome.

(4) Retroviruses use the negative strand of the DNA intermediate to make positive-sense progeny RNA.

(5) Double-stranded DNA viruses replicate by the same process employed by the host cell; each strand serves as the template for synthesis of the complimentary DNA copy. Hepatitis B virus contains a viral RNA-dependent DNA polymerase (a reverse transcriptase) that uses the viral mRNA as a template to synthesize the missing portion of the viral genome, which is then duplicated by host cell DNA polymerase.

(6) Single-stranded DNA viruses (parvovirus) synthesize a double-stranded intermediate to use as the replicative template for the single-stranded DNA progeny.

f. **Viral assembly** occurs toward the end of the synthetic period. Complete intracellular virus assembly begins. The viral genomes and capsid polypeptides assemble, forming infectious viral offspring.

g. **Release** of the complete nucleocapsid is the final stage of the replication cycle.

(1) Enveloped viruses are released gradually by a budding process. Nucleocapsids bud through virally altered membrane patches, thus gaining viral specific glycoproteins.

(2) Poxviruses and naked capsid viruses burst out rapidly from the cell, causing the cell to disintegrate.

*IN A NUTSHELL*

***Viral growth cycle:***

- *Attachment of virus to cell*
- *Penetration of cell*
- *Uncoating of viral genome*
- *Transcription of genome into mRNA*
- *Translation into proteins*
- *Replication of viral genome*
- *Assembly of particles into new viruses*
- *Release of virus*

## DNA VIRUSES

### A. Adenoviruses

1. **Characteristics.** Adenoviruses are medium-sized, linear, **double-stranded DNA** viruses with a **naked icosahedral nucleocapsid**. They contain fibers that serve as viral attachment sites. There are 41 adenovirus serotypes, with approximately one-third accounting for the bulk of human disease.

2. **Transmission** usually occurs person-to-person through **respiratory and ocular secretions** (usually infecting mucous membranes or lymphoid tissue). Humans are the only known host.

*MNEMONIC*

*To remember which viruses are RNA vs. DNA, just memorize the DNA viruses (there are fewer) and know that all the rest are RNA viruses. To help remember the DNA viruses, think about how HHAPPP you will be once you finish studying. The tough part is recalling that the PPP represents papova-parvo-pox (picorna and paramyxo are RNA viruses).*

*IN A NUTSHELL*

***Primary lymphoid organs:***
- *Bone marrow*
- *Thymus*

***Secondary lymphoid organs:***
- *Lymph nodes*
- *Spleen*
- *Tonsils*
- *Mucosa-associated lymphoid tissue (MALT)*
  - *Gut-associated lymphoid tissue (GALT)*
  - *Bronchus-associated lymphoid tissue (BALT)*

*NOTE*

*Bone marrow is the source of pluripotent stem cells and the site of B-cell maturation.*

of the body. The extracellular fluid, or lymph, is filtered through lymph nodes and the tissues of MALT while the primary filter for the blood is the spleen and to some extent, the liver.

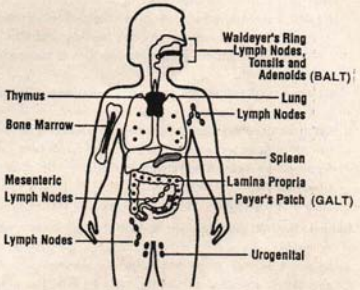


*Figure 1-1. The lymphoreticular system.*

A. **Bone marrow structure and function.** Bone marrow is a primary organ because it is the site of hematopoiesis and B-cell maturation, as well as the site of origin of the stem cells involved in T-cell production. It can also be considered a secondary organ because it is an important site for plasma cells to secrete antibody into the blood. It contains activated T cells as well.

1. **Bone marrow structure.** The bone marrow is a very large tissue composing 3–5% of body mass in humans. It is found in the long bones of the body, in the cranium, ribs, and iliac crest. There are two functional components of approximately equal size: the vascular and adipose portion and the hematopoietic portion. The latter is involved in the formation of blood cells, which are all derived from a single progenitor stem cell.
2. **Hematopoietic cell differentiation** (Figure 1-2). All blood cells are derived from pluripotent hematopoietic stem cells that differentiate into myeloid and lymphoid progenitor cells.

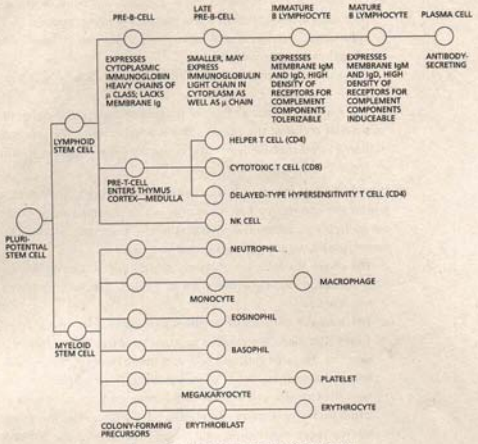


*Figure 1-2. Hematopoietic cell differentiation.*

a. Progenitor cells are acted upon by appropriate stimuli in certain anatomic sites, such as primary lymphoid organs (thymus and bone marrow) and secondary lymphoid organs (lymph nodes). Stimuli include colony-stimulating factors, erythropoietin, thymosin, and antigen (both self and foreign).

b. After maturation in the thymus or bone marrow, lymphocytes leave these sites and migrate to spleen, lymph nodes, and MALT (secondary lymphoid organs) where further development occurs under the influence of antigens and cytokines.

B. **Thymus structure and function.** Precursors of thymic (T) lymphocytes travel from the bone marrow to the thymus, where early differentiation and maturation take place, including T-cell receptor expression and clonal deletion of autoreactive T cells. T lymphocytes leave the thymus to seed the secondary lymphoid organs in thymic-dependent regions, where they can be activated and undergo their final maturation into effector T cells that function in immune responses.

1. **Development of the thymus.** The thymus is derived from the third and fourth pharyngeal pouches and is found in the mediastinum.

a. The development of the thymus begins with epithelial (endodermal) outgrowths of the third and fourth pharyngeal pouches.
b. Subsequently, this epithelial reticulum is infiltrated by pre–T-cells and other mesodermal elements.
c. The thymus reaches its maximum weight at puberty and then slowly involutes.
d. There is a significant amount of programmed cell death (apoptosis) that occurs in the thymus. This is a reflection of the elimination of autoreactive lymphocytes during differentiation in this organ.

2. **Organization of the thymus.** The **stroma** of the thymus consists of a prominent connective tissue capsule, which invaginates into the parenchyma as septa and divides the thymus into lobules. The parenchyma is organized into a cortex and medulla.
   a. **The cortex** is composed of tightly packed differentiating thymocytes surrounded by a meshwork of epithelial reticular cells and macrophages.
   b. **The medulla** consists of epithelial reticular cells and mature T cells. The medulla exhibits a paler staining than the cortex as a result of the large reticular cells and the presence of larger lymphocytes, which are not as densely packed. The medulla also contains the characteristic **Hassal's corpuscles,** which are composed of concentrically arranged dead and dying reticular cells, macrophages, neutrophils, and nuclear material whose origin is unknown.
3. **Blood supply.** Arteries from the connective tissue capsule and septa enter the thymus at the level of the corticomedullary junction. These arteries branch to give off capillaries, which loop up to the periphery and turn back to the medulla to form venules, which leave the septa.
   a. **Thymic capillaries** have a nonporous endothelium with a thick basement membrane.
   b. **Epithelial reticular cells** surround thymic vessels and constitute an incomplete barrier, which separates the blood from the thymocytes. The thymic reticulum is composed of branching epithelial cells that are joined one to another by desmosomes.
4. **The thymus** is large at birth in relation to other organs; it increases in size until puberty, when involution begins. **Thymectomy** in young animals results in poor development of the other lymphoid tissues and the absence of cell-mediated immunity. This can be reversed by a thymic graft. Congenital absence of the thymus (i.e., **DiGeorge's syndrome**) results in poor development of peripheral lymphoid tissues and the absence of cell-mediated immunity.
5. **Thymosin,** a family of lymphokines that stimulate thymus-dependent zones in the peripheral lymphoid tissues, is produced by thymic epithelium.

### IN A NUTSHELL

***Thymus cortex:***
- *Darker peripheral zone*
- *Extensive population of immature T cells, epithelial reticular cells, and macrophages*

***Thymus medulla:***
- *Lighter central zone*
- *Larger number of epithelial reticular cells, and mature large- and medium-sized T cells*
- *Hassal's corpuscles*

### CLINICAL CORRELATE

*DiGeorge's syndrome is the absence or atrophy of the thymus, resulting in reduced level of B cells and severe decrease in T cells. Afflicted individuals have compromised B-cell immunity and no T-cell immunity. DiGeorge's syndrome is often accompanied by hypoplasia of the parathyroid glands with resultant tetany. Early death from severe infection often occurs.*

C. **Lymphatics.** More plasma is filtered from capillary beds into the tissues of the body than is reabsorbed back into the venous end of those capillary beds. This excess fluid moves through the tissues or organs as **interstitial fluid,** and is then collected in small lymphatic vessels throughout the tissues as **lymph.** These small lymphatics fuse into larger afferent lymphatics that enter lymph nodes. The efferent lymphatic leaving one node may become the afferent lymphatic entering another node in a cluster. Eventually, most efferent lymphatics fuse into the large **thoracic duct** that extends the length of the thorax and empties into the left subclavian vein, returning the lymph fluid to the blood.

D. **Lymph nodes** are highly organized secondary organs that are the most common site for an adaptive immune response because they filter the lymph that washes through the body tissues. During an infection, lymph nodes may increase two to five times in size. They are encapsulated, kidney-shaped structures that have a concave side with a hilum. The blood vessels and nerves enter and exit at the hilum, and the efferent lymphatic exits from this region. Beneath the stromal capsule, the parenchyma is organized into a cortex and medulla.

1. The **stroma** consists of a dense connective tissue capsule that surrounds each lymph node and sends collagenous trabeculae into the node to divide its parenchyma into incomplete compartments.
   a. Reticular cells produce reticular fibers that anastomose with the trabeculae and form an extensive network.
   b. The delicate reticulum filters the lymph and suspends the lymphocytes and macrophages.
   c. This stromal organization and function facilitates cell-to-cell and antigen-receptor interactions.
2. **Cortex.** Beneath the capsule (except at the hilum) lies a cortex, which is composed of lymphatic nodules (B cells) in a diffuse lymphatic tissue network (T cells) that intermingles with the subcapsular and peritrabecular sinuses (macrophageal).
   a. **Lymphatic sinuses** are the lymphatic passageways within lymph nodes. They are lined with flat endothelial cells in the area of the capsule and are partially lined by reticular cells, fibers, and macrophages elsewhere. The sinuses receive lymph brought by afferent vessels and transport it toward the medulla.
   b. **Germinal centers** may be present in the nodules of the cortex. They are composed mostly of B cells, some T cells, and macrophages and are transient structures in which new antigens are localized and processed. Antigen stimulation increases the number and development of germinal centers. In germinal centers B cells develop into plasma cells in response to specific antigens.

*CLINICAL CORRELATE*

*Each node receives lymph from a defined and limited region of the body. Neoplasms can metastasize via these nodes. For example, a common metastatic site for breast cancer is the axillary nodes.*

3. The **medulla** of the lymph node occupies the center of the organ. It contains medullary cords composed of lymphoid tissue that extend from the cortex. **Medullary sinuses,** like those in the cortex, transmit the lymph toward the hilum, where it exits through the efferent lymphatic.
4. **Function.** Lymph nodes serve as filters clearing lymph of foreign particles before circulating to other areas of the body.
   a. Lymph enters the node via afferent lymphatic vessels, which are located on the convex side of the organ.
   b. From the afferent lymphatics, lymph passes through the subcapsular sinus to the peritrabecular sinuses. It then enters the medullary sinuses and exits the lymph node via the efferent lymphatic vessels at the hilum.

**CLINICAL CORRELATE**

*Patients with sickle-cell disease undergo gradual splenic infarction. This predisposes them to septicemia, particularly those caused by Streptococcus pneumoniae. Other opportunists include Salmonella, Meningococci, and H. influenzae.*

E. The **spleen** is a peripheral lymphoid organ in the upper left quadrant of the abdominal cavity, which acts as a filter for blood, clears old and defective erythrocytes (RBCs), and provides protection from blood-borne pathogens.

1. The **stroma** of the spleen consists of the following:
   a. A dense connective tissue capsule that contains smooth muscle cells.
   b. Trabeculae that branch off of the capsule and partially partition the parenchyma of splenic pulp.
   c. A delicate meshwork of reticular connective tissue that filters the blood.
2. Splenic **parenchyma** consists of **white and red pulp.**
   a. **White pulp** consists of lymphatic tissue arranged in sheaths around arterioles (T cells) and in nodules (B cells). Antigenic stimulation increases the amount of white pulp.
      (1) The **periarteriolar lymphocyte sheaths (PALS)** are accumulations of diffuse lymphatic tissues that are rich in T cells. The marginal zone is occupied mostly by B cells.
      (2) B lymphocytes cluster peripherally in the PALS to form primary follicles. After antigenic stimulation, these follicles develop into secondary follicles with germinal centers containing rapidly dividing B cells.
      (3) At the **marginal zone,** dendritic cells trap and process antigen and migrate to PALS to present it to antigen-specific cells of the immune system.
   b. **Red pulp** consists primarily of erythrocyte-filled sinusoids and macrophages in a reticular fiber network; most filtration occurs here.
      (1) The sinuses vary in size and are separated by pulp (i.e., Billroth) cords.

**NOTE**

*In the splenic white pulp, B and T lymphocytes are segregated—the central portion of PALs are rich in T cells, whereas the marginal zone and nodules are populated by B cells.*

(2) RBCs and platelets are exposed to macrophages in the pulp cords; macrophages phagocytize worn-out or damaged cells.
(3) Splenic sinusoids are lined by loosely arranged endothelial cells, which have numerous fenestrations. These sinusoids are surrounded by a poorly developed basal lamina.
(4) Phagocytosis is carried out primarily by macrophages located outside the sinusoids. The macrophages extend finger-like projections into the sinusoids, which push through the discontinuous basement membrane between the endothelial cells.

3. **Blood supply.** Arteries from the hilum of the spleen pass along trabeculae to enter the periarteriolar sheath.
   a. These vessels branch like brush bristles to form **penicillar arteries,** which continue in capillaries and open blindly in the red pulp connecting directly to venous sinusoids.
   b. The **venous sinusoids** are specialized, large caliber spaces, which are bordered by elongated endothelial cells and held in place by circularly arranged reticular fibers.
   c. Venous sinusoids drain into veins that eventually exit at the hilum.

F. **Gut-associated lymphoid tissue (GALT)** is not encapsulated and is present in the submucosa and lamina propria. It is the site of immune responses to injested microbes and some food antigens.

1. **Structure.** Lymphoid tissue in GALT includes the large follicular aggregates in the small intestine called **Peyer's patches,** the **lamina propria** beneath the mucosal epithelia in the villi, and the **intraepithelial lymphocytes (IELs)** found between mucosal epithelial cells along the surface of the villi.

2. **Function**
   a. The lymphoid tissues lining the intestinal tract are well exemplified by the Peyer's patches. Structurally unique antigen-presenting cells called **M cells** are located in mucous membranes. They endocytose microbes and antigens and present specific epitopes to T lymphocytes located between follicles in the lamina propria. With T cell help, the B cells become activated and form germinal centers where they differentiate into IgA-secreting plasma cells.
   b. The IgA dimers react with a polyimmunoglobulin receptor on intestinal epithelial cells. The dimer (held together by the J chain) is internalized and crosses the epithelial cell cytosol. It is then proteolytically cleaved from the poly-Ig receptor and excreted into the lumen of the intestine. A small portion of the Ig receptor remains attached to the dimer; this is called the **secretory component.** The function of this small peptide is to protect the antibody molecule from enzymatic hydrolysis in intestinal fluid.

G. **Bronchus-associated lymphoid tissue (BALT)** includes the lymphoid tissue beneath the respiratory mucosa and the aggregates of nodular lymphat-

*CLINICAL CORRELATE*

*Peyer's patches and other lymphoid tissues will be hypoplastic in individuals with Bruton's hypogammaglobulinemia. Lymphoid tissue is almost totally absent in patients with severe combined immunodeficiency.*

ic tissue called **tonsils**. These are structurally similar to lymph nodes and contain deep crypts that allow antigen to be trapped, degraded, and processed by antigen-presenting cells, such as macrophages and dendritic cells.

1. **Organization of the tonsils.** Tonsils are peripheral lymphoid organs composed of aggregates of nodular and diffuse lymphatic tissues that protect epithelial surfaces. The lymphoid tissues are located beneath the epithelium in the underlying connective tissues.
   a. Lymphatic nodules contain aggregrates of B lymphocytes, which differentiate into plasma cells to produce antibodies for the humoral immune response.
   b. T cells are found primarily in diffuse lymphatic tissues.
2. **Types of tonsils.** Three important examples of tonsillar tissue are the **palatine tonsils,** the **lingual tonsils,** and the **pharyngeal tonsil.**
   a. **Palatine tonsils** are located bilaterally in the oropharynx.
      (1) They are composed of dense lymphoid tissue, which forms a band of lymphatic nodules with germinal centers. These are intermingled with diffuse lymphatic tissue beneath the stratified squamous epithelium.
      (2) A dense connective tissue capsule often separates the tonsil from subjacent tissues.
      (3) Each tonsil has numerous epithelial invaginations, or **crypts,** which contain desquamated epithelial cells, lymphocytes, and bacteria in their lumina.
   b. **Lingual tonsils** are smaller and more numerous aggregates of lymphoid tissues located at the base of the tongue.
      (1) They are covered by the stratified squamous epithelium on the dorsum of the tongue.
      (2) Each aggregate possesses a single crypt.
   c. **The pharyngeal tonsil** is an unpaired accumulation of lymphoid tissue located on the posterior wall of the nasopharynx.
      (1) It is usually covered by a pseudostratified columnar epithelium with cilia and goblet cells; however, the epithelium can be obscured by an infiltration of lymphocytes or by metaplasia (seen in smokers). The pharyngeal tonsils, or hypertrophic pharyngeal tonsils, are often referred to as **adenoids.**
      (2) Instead of forming crypts, the overlying epithelium occurs in a series of folds.

**CLINICAL CORRELATE**

*Tonsillitis involves the formation of abcesses in the crypts.*

H. **Lymphocyte recirculation**

1. Lymphocytes are capable of high levels of recirculation, continuously moving from the blood out into the tissues and then returning via the lymphatics.
2. The lymphocytes bear in their membranes **selectins** that interact specifically with **addressins** that are found in the lymphatic vascula-

ture, particularly in the postcapillary venules. It is here that specialized cells with a plump cuboidal shape are found; these are called high endothelial venules due to the height in the region caused by the cuboidal cells.

3. As T cells home to lymph nodes, they make contact with epitopes presented in the groove of class II MHC molecules located on the surface of antigen-presenting cells. This dynamic leads to T-cell help and B-cell triggering.
4. Once the triggering has occurred, the committed lymphocytes stay trapped in peripheral lymphoid tissues where they differentiate to mature effector cells in the case of T cells, and plasma cells in the case of B cells.
5. Different populations of lymphocytes express different homing receptors; some B cells express an integrin that reacts with vascular addressins in MALT, whereas T cells express a selectin for addressins in peripheral lymphoid tissues (hence T cells predominate in lymph nodes and B cells in Peyer's patches).

*NOTE*

*Lymphocyte homing is controlled by selectins in the lymphocyte membrane that react specifically with addressins found in the lymphatic vasculature.*

## THE IMMUNE RESPONSE

Immune cells and the responses they generate can be categorized into innate (natural) and acquired. The innate responses are necessary for immediate reponse to a pathogen and for establishing a local inflammation to recruit circulating phagocytes and, later, activated T cells and B cells. The T and B lymphocytes make the acquired response and are responsible for many of the classic characteristics of immunity, including memory, antigen specificity, and tolerance to self. The characteristics of antigen presentation and recognition by T cells and B cells will be discussed below.

A. **Natural versus acquired immunity.** The immune system is composed of cells that defend against foreign invaders by both nonspecific mechanisms (termed natural or innate immunity) and antigen-specific mechanisms (known as acquired immunity). The nonspecific response is typically observed first since no prior exposure to antigen is necessary. This response may not be sufficient to clear the foreign antigen, and an antigen-specific mechanism produced by B and T cells may be required.

1. **Natural immunity** (also known as innate immunity) is present at birth in all individuals. This response does not increase upon repeated exposure to a given antigen.
   a. The first line of defense is intact skin and mucous membranes.
   b. Natural immunity allows elimination of foreign substance without previous exposure.
   c. This immunity is effected by either natural antibodies or natural cytotoxic cells, including macrophages, neutrophils, eosinophils, and natural killer cells.

*IN A NUTSHELL*

***Natural immunity:***
- *Present at birth*
- *Composed of skin, mucous membranes, secretions such as saliva and tears, phagocytic cells, and NK cells*

***Acquired immunity:***
- *Developed in response to immunogen exposure*
- *Comprised of antibodies (IgG, IgA, etc.) and sensitized lymphocytes (B and T cells)*

2. **Acquired immunity** is established late in fetal life, and its development continues during childhood. Continued exposure to foreign antigen will stimulate acquired immunity.
   a. Responses are specific to individual antigens because of antigen specific recognition by surface antibody on B cells and by T-cell receptors (TCRs) on T cells.
   b. Individual lymphocytes recognize their appropriate antigens (discussed below) and are activated to proliferate into a **clone of effector cells and memory cells** with the same antigen specificity (clonal expansion).
   c. May have **anamnestic response**, in which subsequent response to previously recognized antigen is more avid and often magnified (this is a result of the production of memory T and B cells).
   d. Antigen is eliminated due to specific antigen recognition and effector functions (discussed below).
3. **Self-tolerance** describes the absence of immune responses to one's own tissue antigens. It is necessary to prevent autoimmune responses. Mechanisms of self-tolerance include the following:
   a. **Clonal deletion**, the elimination of clones of developing T cells with receptors against self-antigens, occurs during cell maturation in the thymus.
   b. **Suppression of peripheral T and B lymphocytes** bearing receptors for self-antigens is maintained by the lack of costimulatory signals when naive cells encounter antigen.

B. **Antigens and antibodies.** An **antigen** is any substance that can be specifically bound by an antibody or T-cell receptor, whereas an **immunogen** is that which induces an immune response. Because antibodies and TCRs recognize different types of antigens and antibody production requires helper T-cell activation and assistance, immunogens typically possess both antibody and T-cell reactive regions.

1. An **epitope** or **antigenic determinant** is a specific site on the antigen that is recognized by the immune system, i.e., the part of the antigen to which an antibody binds.
   a. Most antigens express many epitopes.
   b. B-cell epitopes usually occur at the hydrophilic surface, whereas T-cell epitopes may be embedded within the protein.
2. **Antibodies** are able to bind to epitopes on a wide variety of molecules, including proteins, carbohydrates, nucleic acids, and small organic molecules. **T-cell receptors (TCRs)** are only able to recognize peptides bound to **major histocompatiblity (MHC)** proteins on the surface of a cell (discussed below).

**NOTE**

*Thymus-independent antigens do **not** induce memory cell production.*

3. Many macromolecules can be antigenic.
   a. **Proteins** (glycoproteins, lipoproteins, or nucleoproteins) are the most common form of antigen and are usually very good immunogens.
      (1) Their antigenicity is based on amino acid composition, three-dimensional conformation, and/or biochemical properties (such as charge, etc.).
      (2) Peptides derived from processed proteins and bound to cell-surface MHC proteins are the only type of antigen that T-cell receptors can recognize.
   b. Large, repetitive **polysaccharides** can activate B cells with little or no helper T aid (but cannot be recognized alone by TCRs) and are therefore considered T-cell–independent antigens.
   c. **Nucleic acids** can be recognized by antibodies but are poor immunogens because they cannot act as T-cell antigens unless they are bound to a specific protein carrier.
   d. **Lipids** are usually not immunogenic. When lipids are coupled to protein antigens, they tend to induce T-cell mediated delayed hypersensitivity rather than antibody production.
   e. **Haptens** are small molecules that can act as an antibody epitope but will not induce immune responses because they are not recognized as T-cell antigens. When combined with a carrier protein, the hapten-carrier complex can produce hapten-specific and carrier-specific antibodies since the helper T cell can recognize carrier peptides. Many allergens (e.g., penicillin) are haptens.

C. **Major histocompatibility complex (MHC).** The MHC is a collection of highly polymorphic genes encoding the proteins that regulate immune responses. These genes include, most notably, the **class I** and **class II** cell surface proteins and the **class III genes** that encode complement proteins. In humans, the MHC genes are termed **HLA (human leukocyte antigens)** and are found on the short arm of chromosome 6. The HLA proteins are glycoproteins present on cell surfaces that enable T cells to recognize and bind antigenic peptides, i.e., they function in immune recognition.

1. **HLA class I antigen.** Class I proteins are membrane glycoproteins on the surface of all nucleated cells and platelets. They bind peptides processed from protein synthesized in the cell's cytosol and are necessary for antigen recognition by $CD8^+$ cytotoxic T lymphocytes (CTLs). In humans, the three types of class I genes are referred to as **HLA-A, HLA-B,** and **HLA-C** antigens. **Cytotoxic T cells** recognize viral, intracellular bacterial, parasitic, or tumor antigens in association with class I molecules. CD8 on the surface of these cells recognizes a nonpolymorphic region of the class I MHC molecule.

NOTE

***Carrier effect:***
*Poorly immunogenic or nonimmunogenic molecules acquire immunogenicity when they are chemically linked to proteins that serve as carriers and impart the diversity and T-cell reactivity needed.*

IN A NUTSHELL

***Class I MHC:***
- *All nucleated cells*
- *Human class I genes = HLA-A, HLA-B, and HLA-C*
- *Cytotoxic T cells (CD8) recognize class I MHC on infected cells (viruses, intracellular bacteria, parasites, tumor antigens)*

***Class II MHC:***
- *Limited to cells that interact with helper T cells = antigen-presenting cells (B cells, monocytes, macrophages, Langerhans cells, dendritic cells, thymic epithelium)*
- *Human class II genes = HLA-DR, HLA-DQ, and HLA-DP*
- *Helper T cells (CD4) bind to class II MHC on antigen-presenting cells*

2. **HLA class II antigen**. HLA class II proteins are expressed on a more restricted set of cells, including antigen-presenting cells (dendritic cells, Langerhans cells, activated macrophages), B cells, and thymic epithelial cells involved in T-cell maturation. These proteins bind peptide epitopes processed from endocytosed molecules and are necessary for **antigen recognition by T helper cells (Th)** and **T cells that mediate delayed-type hypersensitivity ($Th_{DT}$).** Class II genes encode for cell surface glycoproteins with two polypeptide components called α and β. In humans, the class II genes include **HLA DR, DQ,** and **DP. CD4 cells** recognize viral, bacterial, parasite, or injected proteins in association with class II.
3. **HLA class III antigen.** Class III genes encode for complement components or regulators of serum complement component levels. C2 and factor B are encoded by class III MHC genes.
4. **HLA disease associations.** Many diseases are associated with increased frequency of certain HLA antigens. Ankylosing spondylitis is typically associated with HLA-B27; other diseases are also associated with specific HLA antigens: rheumatoid arthritis (DR4), Sjögren's syndrome (DR3), and insulin-dependent diabetes mellitus (DR3 and DR4).
   a. Most of the HLA-associated diseases are of unknown etiology and have associated immunologic abnormalities.
   b. Tissue typing for organ transplantation involves matching the HLA class I and class II antigens.

D. **Antibodies.** Antibodies act as antigen-specific receptors on B cells, and when secreted by plasma cells, mediate humoral responses. Antibodies compose approximately 20% of plasma proteins. They are produced by B cells in response to the introduction of foreign substances (i.e., antigens) into the body. Antibodies are bifunctional: they bind epitopes on antigens, thereby directly attacking the antigen, and they stimulate other biologic phenomena such as activating complement and binding Fc receptors on other lymphoid cells.

1. **Structure.** Antibodies consist of four polypeptide chains—**two identical light chains** and **two identical heavy chains**—all bound together by disulfide bonds (see Figure 1-3). Heavy and light chains have **hypervariable regions** at the amino terminus (responsible for antigen-binding specificity) and **constant regions** at the carboxy terminus. There are intrachain disulfide links that divide each chain into subunits of 110 amino acids. Thus, light chains have two domains referred to as the variable and constant domains, whereas heavy chains have four or five domains—one variable domain and three or four constant ones.
   a. **Light chains.** The molecular weight of each light chain is approximately 23 kD and is composed of 220 amino acids. There are two subtypes of light chains, kappa and lambda.

**NOTE**

*MHC class III proteins have nothing to do with transplant acceptance or rejection. They are proteins involved in innate immunity.*

(1) **Kappa chains** differ from **lambda chains** on the basis of structural differences in the constant region. The genes coding for each are not on the same chromosome.
(2) Each antibody molecule has either two kappa or two lambda chains.
(3) A specific immunoglobulin always has identical kappa or lambda chains.
(4) The ratio of chain subtypes is constant within a species.

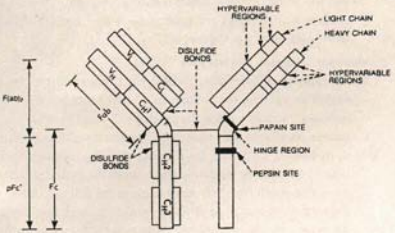


*Figure 1-3. Schematic structure of the immunoglobulin molecule.*

b. **Heavy chains.** The molecular weight of each heavy chain is 50–75 kD and is composed of 450–580 amino acids.
(1) There are **five different isotypes** corresponding to the individual heavy chain gene utilized. Structural differences in the constant regions of the various types of heavy chains account for their different biologic properties.
(a) IgG has gamma (γ) heavy chains.
(b) IgA has alpha (α) heavy chains.
(c) IgM has mu (μ) heavy chains.
(d) IgE has epsilon (ε) heavy chains.
(e) IgD has delta (δ) heavy chains.
(2) Heavy chains are further divided into subclasses based on the number of interchain disulfide bridges. For example, IgG1 and IgG4 have two disulfide bonds, IgG2 has four disulfide bonds, and IgG3 has 15 disulfide bonds.
(3) Genes coding for heavy chains are on the same chromosome.

2. **Antigen-binding sites** are found in the variable region located in the 110 amino-acid segment of the amino terminal end of the heavy and light chains.

*IN A NUTSHELL*

| ***Isotype*** | ***Ig function*** |
|---|---|
| *IgG →* | • *Opsonization*<br>• *Placental passage*<br>• *Complement activation* |
| *IgA →* | • *Mucosal (secretory) immunity* |
| *IgM →* | • *Complement activation* |
| *IgE →* | • *Basophil and mast cell sensitization* |
| *IgD →* | • *Antigen triggering of B cells* |

a. The variability of amino acid sequence confers antigen-binding specificity to individual immunoglobulin molecules. This site has both framework and hypervariable or complimentarity determining subregions. **Framework subregions** are relatively conserved amino acid sequences that preserve the three-dimensional structure of the variable region and stabilize the hypervariable regions. **Hypervariable regions** are folded to form the antigen-binding site (also called the paratope).
b. Different structurally related antigens may be cross-reactive and bound by the same antigen-combining site on different antibodies. This is important in diseases such as rheumatic fever, where streptococcal antigens are structurally related and cross-reactive with heart tissue (cardiac myosin).

3. **Idiotypes, allotypes, and isotypes**
   a. **Idiotype** is the term used to describe the area of the variable region responsible for antigen specificity. These are the unique epitopes that are found in the paratope of the antibody.
   b. **Isotype** is the term used to describe the subclasses of immunoglobulins (G-A-M-E-D) that are distinguished by unique constant regions encoded by the heavy chain gene. The individual isotypes have unique effector mechanisms such as the ability to bind complement or mediate hypersensitivity responses (described below).
   c. **Allotype** is the protein product of an allele that may be detected as an antigen by another member of the same species. It involves different alleles at a specific site in the constant region of the heavy chain.
4. **Immune serum** contains a heterogeneous population of antibodies with varying affinity for antigens; the average affinity increases as the period of time after immunization lengthens due to somatic mutations.
5. **Monoclonal antibodies** are antibodies that are specific for a single epitope determinant. They are produced experimentally by **hybridomas,** created from the fusion of activated B lymphocytes to plasmacytoma cells that are grown in tissue culture.

E. **Properties of immunoglobulin subclasses.** As previously noted, there are five immunoglobulin subclasses: IgG, IgA, IgM, IgE, and IgD (remember them by "G-A-M-E-D"). They can be distinguished by the type of heavy chain they possess and by their distinct biologic functions.

1. **IgG** provides the major defense against bacteria and toxins. There are four subclasses, varying by heavy chain isotype (G1, G2, G3, G4).
   a. IgG is important in the secondary immune response to antigen and provides long-lasting immunity.

NOTE

***Monoclonal antibody:***

*B cell + plasmocytoma cell = hybridoma. The hybridoma secretes antibody identical to the one produced by the B cell. The plasmacytoma gives the hybridoma its immortality.*

IN A NUTSHELL

***Major functions of IgG:***

- *Passive immunity in fetus*
- *Opsonization*
- *Complement activation*

b. IgG is the **only class of immunoglobulin that crosses the human placenta** into fetal circulation; it is responsible for protecting the newborn during the first 4–6 months of life.
c. Three subclasses of IgG fix complement (IgG3 > IgG1 > IgG2).

2. **IgM** is important in the primary immune response to antigen because it is usually the first antibody detected in the serum after exposure to a virus or specific antigen.
   a. Circulating IgM is a **pentamer** of five immunoglobulin molecules (10 heavy μ chains and 10 light chains) and one disulfide-linked J chain.
   b. IgM **fixes complement** very efficiently because each circulating molecule has ten Fc sites.
   c. Agglutination of antigens via the pentameric structure of IgM allows the cross-linking of antigens.
   d. **Isohemagglutinins, rheumatoid factors,** and **heterophile antibodies** are all IgM.
3. **IgA** has an important barrier function on mucosal surfaces and functions in the **secretory immune response.**
   a. The serum form is usually monomeric, consisting of two heavy chains (α1, α2) and two light chains.
   b. The **secretory form (sIgA)** is found in tears, colostrum, saliva, milk, and other secretions.
      (1) It is usually dimeric, occasionally trimeric, joined by a polypeptide J chain.
      (2) sIgA contains a 70,000-dalton secretory component (T piece) added to polymeric IgA by epithelial cells as it passes through to the luminal side.
      (3) The secretory component confers stability to the molecule, making it less susceptible to proteolysis in the gastrointestinal tract.
      (4) It is produced by plasma cells in the lamina propria of the gastrointestinal and respiratory tracts.
4. **IgD** functions as a cell surface antigen receptor in naive B lymphocytes. IgD is very susceptible to proteolysis. IgD functions primarily as a receptor for antigen on B cells and stimulates B-cell proliferation. There are no subclasses of IgD.
5. **IgE,** also called the "reaginic antibody," is associated with **allergic response** and **immediate hypersensitivity.**
   a. The Fc region of IgE binds to the surface of **basophils** and **mast cells.** When antigen cross-links two IgE molecules, mast cells degranulate and release leukotrienes, histamine, eosinophil chemotactic factors, and heparin, which results in an intermediate hypersensitivity reaction. The half-life of IgE is increased when it is bound to cell surfaces.
   b. IgE offers some protection against metazoan parasites, mainly via the release of ECF-A and the induced inflammatory response at

*IN A NUTSHELL*

***Major features of IgM:***
- *First antibody synthesized*
- *Found in B-cell membrane*
- *Pentamer with J chain*
- *Very efficient activator of complement*

*IN A NUTSHELL*

***Major features of IgA:***
- *Secretory immunity*
- *Often seen as dimer with J chain and secretory piece (sIgA)*

*IN A NUTSHELL*

***Major features of IgD:***
- *Very low concentration in plasma*
- *High levels in membrane of mature B cell*
- *Functions in antigen recognition by B cell*

*IN A NUTSHELL*

***Major features of IgE:***
- *Very, very low concentrations in plasma*
- *Homocytotropic for basophils and mast cells*
- *Reaction with antigen causes release of histamine and production of leukotrienes*
- *Immediate hypersensivity*

the site of worm infestation. Eosinophils degranulate in the area and release major basic protein (MBP). This protein has been shown to have a direct toxic effect upon schistosomes and, presumably, acts as a general metazoan poison.

F. **T-cell receptors (TCR)** act as antigen-specific receptors, allowing T cells to function in cell-mediated responses. They do not recognize antigen alone, but only in the context of MHC molecules.

1. Their expression is interwoven with T-cell maturation in the thymus. In the thymus, T cells mature and undergo positive or negative selection. Negative selection deletes autoreactive cells. T cells develop immunologic specificity in a manner similar to that employed by B cells; that is, they use genetic recombination among variable, joining, and diversity genes to achieve their immunologic repertoire. The gene pools are different than those for B-cell (and antibody) diversity, but the processes are very similar.

*NOTE*

*The T-cell receptor has a function of epitope recognition similar to that of an antibody. It also has a structure and genetic composition similar to the Fab portion of antibody molecules.*

G. **Types of immune responses.** Immune responses usually involve some level of inflammation and are an acquired or adaptive response of the body. An extracellular vaccine or pathogen will stimulate a humoral response, whereas an intracellular pathogen will stimulate both humoral and cell-mediated responses.

1. **Infection at a tissue site causes inflammation,** which enhances antigen delivery by follicular dendritic cells and macrophages to lymphocytes in nearby secondary lymphoid organs and recruits circulating phagocytes and NK cells to attempt to resolve the infection. Activation and clonal expansion of antigen-specific lymphocytes in the secondary organ requires several days to generate a large number of effector lymphocytes and some memory lymphocytes. The effector lymphocytes then circulate through the blood and extravasate at site of infection due to upregulation of adhesin molecules on endothelial cells and locally produced cytokines (IL-8)

*NOTE*

*Adjuvants are immunostimulants that increase the immune response by their inflammatory action. They also prolong contact with the immunogen by acting as a depot of antigen deposition.*

2. **Humoral immune responses** are generated against most antigens and require the secretion of antibody by plasma cells (activated B cells) in response to a specific immunogenic stimulus.
   a. The **primary immune response** is the first response to antigenic exposure. During the initial lag phase, lasting the first 3–5 days, no free antibody can be measured. During this phase, T helper cells and B cells are being activated and activated B cells are differentiating into antibody-secreting plasma cells (the specifics of T- and B-cell activation are discussed later). Thereafter, an exponential (log) phase-rise in detectable antibody in the circulation occurs.
      (1) IgM is primarily produced. After the first IgM response, class switching occurs and the level of IgM usually declines.

*IN A NUTSHELL*

*Acquired immunity can be divided into two broad categories:*

- ***Humoral, or antibody, responses***
  - *Primary response is always IgM*
  - *Secondary response is a result of isotype or class switching and results in synthesis of IgG, IgA, and/or IgE production.*
- ***Cellular responses** involve thymus-derived lymphocytes and culminate in production of helper cells, cytotoxic cells, and delayed-type hypersensitivity cells.*

(2) Later in the response, higher affinity IgG become detectable; in response to an oral or mucosal antigen, sIgA becomes detectable in secretions as well.

b. The **secondary immune response** is an anamnestic response to previously encountered antigen. Memory B and T cells are responsible for this phase.

(1) In a secondary response, high-affinity IgG class antibody levels rise more rapidly; this requires less antigen to elicit a response. The ability to respond may persist for years due to the presence of long-lived memory B cells and T cells. This anamnestic response explains the efficacy of booster injections of vaccines.

(2) Similarly, a secondary response to an oral antigen rapidly generates higher levels of IgA, much of which is transcytosed across the mucosal epithelial cells onto the external mucosal surface.

(3) Secondary responses to large extracellular particles, such as some helminths, pollens, or enzymes (e.g., those found in cat saliva), may produce unusually high levels of IgE. IgE may be important in the response to helminths, but IgE made in response to pollens and enzymes can lead to allergies.

3. **Cellular immune response.** Effector (activated) T helper cells have multiple functions, including helping activate B cells for most humoral responses, helping activate CTLs, and acting as effector cells in cell-mediated responses such as the killing of virally infected cells and some tumor cells.

a. **T-helper cell activation** requires presentation of foreign peptides on HLA class II proteins on antigen-presenting cells.

b. T-helper cells secrete cytokines that lead to the differentiation and proliferation of lymphocytes.

c. T cells are the major cell type needed for cell-mediated responses. Effector $CD4^+$ (helper) cells can secrete cytokines that activate innate cells, e.g., NK cells (to help kill virally infected or tumor cells), or macrophages and neutrophils. Excessive activation of macrophages causes the macrophages to release inflammatory cytokines that can cause delayed-type hypersensitivity.

H. **Regulation.** All immune responses must be regulated. Antigen presentation can affect the strength of the response, as can immune suppression. Anti-idiotypic antibodies may play a role in downregulating normal humoral responses.

1. **Immunologic tolerance** describes the specific depression of immune responses induced by previous antigen exposure; the opposite of immunity. The degree of immune tolerance may be partial or complete. That is, one may elicit tolerance to one epitope but not to

*CLINICAL CORRELATE*

*Some allergies can be controlled by the ingestion of the allergen. This has proven to be of value in some patients with pollen allergies (they eat honey).*

another on a specific molecule. Important factors in tolerance induction include:

a. **The form of antigen**
b. **Route of exposure**
c. **The age of the recipient**
d. **Dosage of antigen**

2. **Immune suppression** is described as the active immunologic unresponsiveness resulting from interaction of normal cells with a suppressor cell population or by physical or chemical agent interactions with the immune system.
   a. **Suppression by $CD8^+$ cells.** These cells affect the functions of T-helper cells ($CD4^+$), B lymphocytes, and monocytes or macrophages.
   b. **Physical immune suppression**
      (1) **X-ray and ultraviolet radiation** can suppress immune responses by the elimination of lymphocytes. B cells are more sensitive to radiation than are T cells. Bone marrow and lymphoid tissues are especially sensitive.
      (2) **Surgical intervention** can be utilized by the removal of lymphoid tissue such as the thymus, spleen, and lymph nodes.
      (3) **Chemical agents** can successfully suppress immune responses. **Corticosteroids** can cause peripheral blood lymphopenia, inhibit RNA and DNA synthesis, decrease macrophage responses, decrease monocyte chemotaxis, and decrease IgG responses. **Purine or pyrimidine analogs** (e.g., azathioprine) inhibit IgG response. Folic acid **antagonists** interfere with DNA and protein synthesis in lymphocytes. **Alkylating agents** (e.g., cyclophosphamide) reduce the number of lymphocytes in the spleen.

*CLINICAL CORRELATE*

*Frequently administered low doses of antigen may be the reason that large organ transplants (e.g., liver) survive well in most recipients.*

*CLINICAL CORRELATE*

*A serious consequence of immunosuppression is opportunistic infections. Recurrence of CMV infection in bone marrow transplant recipients is a major cause of morbidity.*

## CELLULAR INTERACTIONS IN IMMUNE RESPONSES

A. **Antigen processing.** The mechanism whereby antigen is internalized and re-expressed on the antigen-presenting cell (APC) membrane associated with MHC class I or class II molecules is called **antigen processing.** There are two separate routes that dictate with which MHC molecule the peptide will associate.

   1. The **exogenous route** of antigen processing occurs when a cell takes in foreign antigen by phagocytosis or pinocytosis. The antigen is engulfed and is found in endosomes inside the cells. Proteolytic enzymes digest the antigen into small peptide fragments. The endosomes fuse with exocytic vesicles that bear class II molecules on the interior of their membranes and the peptide epitopes then bind in the antigen-binding groove of the **class II MHC.** The endosome then

travels to the cellular membrane and fuses with the membrane, thereby expressing the MHC-peptide on the surface of the cell.

2. Many antigens are synthesized within the cell. Examples of such antigens include tumor antigens and viral proteins. These proteins are processed by the **endogenous route** for antigen processing. These proteins are synthesized in the cytoplasm of the cell, and some are broken down into peptide fragments. They are transported to the lumen of the endoplasmic reticulum, through the TAP peptide transporter complex, where they associate with **class I MHC molecules.** The MHC I-peptide complex is transported through the Golgi to the cell surface.

B. **The T-cell receptor and its interaction with antigen**

1. The T-cell receptor is antigen (epitope) specific. It binds to the epitope located in a groove in the appropriate class I or II MHC molecule on the surface of the APC.

2. The TCR is noncovalently linked to the CD3 molecule, and engagement of the TCR by antigen stimulates CD3 to transmit biochemical signals into the interior of the cell. These signals are required to trigger the T cell. When peptides are presented in association with MHC molecules, the antigen-binding region of the TCR recognizes processed peptide in association with the MHC molecule. Additionally, the CD4 molecule binds to a different region of the MHC molecule. CD8 molecules can bind to class I MHC, while CD4 molecules cannot. The need for CD4 to bind to MHC II and CD8 to bind to MHC I explains why helper cells ($CD4^+$) respond to processed antigens associated with class II, whereas cytotoxic T cells ($CD8^+$) respond to antigens associated with class I MHC molecules.

C. The **T cell–APC interaction.** We have already discussed the physical interaction of the TCR with the MHC class I or class II peptide complex. However, for the T cell to become activated, other molecules on the cell surface of the T cell and APC must interact. These accessory molecules are collectively known as **adhesins** and further strengthen the interaction between the T lymphocyte and the antigen-presenting cell.

D. **Requirements for activation of cytotoxic T cells (Tc).** While T-helper cells can be fully activated by the T cell-APC interaction, cytotoxic T cells cannot. This is primarily because these cells do not secrete enough IL-2 to support their own growth. For this reason Tc require activated Th1 cells to supply the IL-2 that they need. While the tumor, graft, or virally infected cell itself is able to supply the Tc with peptide associated with MHC class I molecules, antigen-presenting cells must be able to take up some of the soluble antigens and present this antigen to helper T cells. The helper T cells can then supply the IL-2, which is needed by the Tc before it will develop into a fully functional cytotoxic T cell.

*IN A NUTSHELL*

- *Endogenous antigens synthesized within the cell, bind with class I MHC molecules, and are presented on the cell surface.*
- *Exogenous antigens are internalized, digested, and fragments are complexed with class II MHC molecules to be presented on the cell surface.*

*IN A NUTSHELL*

***Summary of interleukin role in cell activation:***

- *IL-1 produced by macrophages activates Th cells.*
- *IL-2 produced by Th1 cells is a signal for proliferation.*
- *IL-4,5,6 produced by Th2 cells are signals for maturation and class switching.*

*IN A NUTSHELL*

***Interleukins:***

- *IL-1→* • *Stimulates other cells to proliferate, activate, and chemotax* • *Stimulates IL-2 secretion* • *Pyrogenic (fever inducing)*
- *IL-2 →* • *Produced by activated T cells* • *Stimulates T cells (helper, cytotoxic and natural killer cells)* • *Stimulates B cells*
- *IL-3 →* • *Secreted by activated T cells* • *Stimulates bone marrow stem cells*
- *IL-4 →* • *Secreted by activated helper cells and mast cells* • *Stimulates B cells* • *Increases IgG and IgE*
- *IL-5 →* • *Secreted by activated helper cells* • *Promotes B-cell proliferation* • *Increases IgA and increases synthesis of eosinophils*
- *IL-6 →* • *Stimulates production of acute phase reactants* • *Stimulates B cells*
- *IL-7 →* • *Stimulates pre-B and pre-T cells*
- *IL-8 →* • *Stimulates chemotaxis and adhesion of neutrophils*
- *IL-10 →* • *Inhibits cytokine release from macrophages* • *Inhibits interferon synthesis by Th1 cells*
- *IL-12 →* • *Activates natural killer cells* • *Induces Th → Th1* • *Increases CTL and DTH cell numbers*

E. **Requirements for activation of B cells**

1. When B cells are triggered to make antibody they require the interaction of native (unprocessed) antigen with their antigen receptor (membrane immunoglobulin). However, for class switching to occur, activated T cells must supply necessary Th2 cytokines, including IL-4, IL-5, IL-6, IL-10. B cells also need some IL-2, which may be supplied by a Th1 cell.
2. Some antigens, notably polysaccharides, can stimulate B cells without the need for T cell help. These antigens are said to be "thymus independent." The antibodies made in such responses are IgM only; also, no immunologic memory is induced. This is due to the fact that helper T cells do not respond to polysaccharides because they cannot be presented in MHC II.
3. Protein antigens are "thymus dependent." Immunoglobulins made to thymus-dependent antigens will include all classes of immunoglobulin since class switching will occur in these responses due to the presence of Th2-derived cytokines.

F. **Cytokines.** Cytokine is a term used to describe a collection of proteins that regulate immunologic and inflammatory response to injury. **Lymphokines** are cytokines produced by lymphocytes. **Monokines** are cytokines produced by monocytes or macrophages. Cytokines specifically modulate responses to antigen in several ways, including the regulation of cell growth and the control of differentiation of immunologic cells. Cytokines also mediate intercellular signaling that controls activation or suppression of immune cells. Normally, resting cells do not secrete cytokines; cells must be stimulated to produce them. Cytokines have both **autocrine effects** (i.e., exerts a local effect on the same cell that produces the cytokine) and/or **paracrine effects** (exerts an effect over a distance upon other cells). Cytokines are typically very potent agents that can act on their target cells at very low concentrations. Also, a single cytokine can have multiple functions and many cytokines have similar effects. Cytokines often influence the synthesis of other cytokines, leading to cascades with both positive and negative regulatory mechanisms.

## COMPLEMENT AND INFLAMMATION

Inflammation is an integral part of all immune responses. One of the major mechanisms for initiating inflammation is activation of the complement cascade, which also produces powerful opsonins, chemoattractants, and anaphylatoxins, and can directly mediate killing through cell lysis.

A. **Complement.** Historically, complement was the term used to describe the activity in serum which, when combined with specific antibody, would cause bacterial lysis. Complement is now known to be a system of proteins that interact to play a role in humoral immunity and inflammation.

Complement is a complex group of proteins and glycoproteins found in blood and tissue fluids. Complement can directly opsonize foreign material for phagocytosis after antibody activation (C3 component) and can participate directly in killing some cells and microorganisms. Finally, peptide fragments from the complement proteins can regulate inflammation.

1. **Complement activation.** There are two pathways of complement activation, the **classical pathway** and the **alternative pathway.** The end result of either pathway is the formation of a **membrane attack complex (MAC),** which is a lipid-soluble pore structure that causes osmotic lysis of cells.
   a. The **classical pathway** is the main antibody-directed mechanism for complement activation. It is the most rapid and efficient pathway.
      (1) Complement recognizes antigen-antibody complexes. The antibody must be **IgG or IgM,** but IgM is more efficient than IgG.
      (2) Antigen-antibody complexes bind to C1, which then activates the cascade.
   b. The **alternative pathway** of complement activation occurs as a result of binding directly to the surface of an infectious organism. This pathway is slow and less efficient than the classic pathway.
      (1) The alternative pathway primarily recognizes bacteria and depends upon the interaction of a small amount of preformed C3b with protective surfaces.
      (2) It is important to remember that both pathways lead to lysis by the terminal complement components (C8 and C9); however, they are initiated by different complement components.
2. **Classic pathway.** The reaction occurs in the following order: **C1q, C1r, C1s, C4, C2, and C3**. Each component of the classic pathway has unique biologic functions.
3. **Alternative pathway.** There are several key components of the alternative complement pathway.
   a. There are several initiators of the alternative pathway.
      (1) Lipopolysaccharides, the cell wall components of Gram-negative bacteria
      (2) Bacterial and plant polysaccharides
      (3) Cell membrane constituents
      (4) Aggregated IgA, IgG, IgE, and IgM
      (5) Cobra venom factor
      (6) Endotoxins that complex with Factor B to form C3 convertase
4. **The membrane attack complex (MAC)** is formed from the reactions among C5, C6, C7, C8, and C9. It leads to lysis of cells.
5. The products of the complement cascades have several biologic roles including viral neutralization, lysis of infected cells, and direct

### CLINICAL CORRELATE

*The alternative pathway protects the body from pathogens in the absence of antibody. The bacterial surface itself activates the cascade.*

### IN A NUTSHELL

***Biologically important C proteins:***

- *C2a, C4a = weak anaphylatoxins*
- *C3a, C5a = strong anaphylatoxins*
- *C5a = potent chemotaxin*
- *C3b = potent opsonin*

### CLINICAL CORRELATE

*Deficiencies in complement components predispose patients to certain diseases:*

| | | |
|---|---|---|
| *C3 deficiency* | → | *Increased susceptibility to pyogenic infections* |
| *C2 deficiency* | → | *Increased incidence of connective tissue disorders* |
| *C5-8 deficiency* | → | *Recurrent Neisseria infections (meningococcal, gonococcal)* |
| *C1 esterase inhibitor deficiency* | → | *Hereditary angioneurotic edema* |

### IN A NUTSHELL

*The inflammatory response can be triggered by local tissue damage that results in enzyme activation or by mast cell degranulation caused by anaphylatoxin interaction with specific receptors (e.g., C3aR, C5aR) on the cytoplasmic membrane. Allergen reacting with cell-bound IgE can also trigger degranulation.*

pathogen lysis. Complement activation can also mediate immune adherence, the focusing of antigen on macrophage or lymphocyte surfaces, and promoting phagocytosis. Finally, biologic responses such as anaphylaxis, kinin activity, smooth muscle contraction, vasodilatation, and chemotaxis are mediated by individual complement components.

B. **Inflammation** is a pathologic state initially characterized by **pain, redness, heat,** and **swelling.** These features of inflammation are due to vascular permeability changes leading to an infiltration of leukocytes, primarily *neutrophils and macrophages, although small numbers of* eosinophils and basophils can also be found. Lymphocytes can also enter sites of inflammation, where the release of cytokines can enhance the inflammatory response. A variety of compounds or chemicals can elicit inflammatory responses, and a variety of chemotactic factors are responsible for the migration of cells to sites of inflammation.

1. **Vasoactive and smooth muscle constrictors**
   a. **Histamine**
      (1) Histamine is stored as granules in **mast cells, basophils,** and **platelets,** with higher concentrations found in the intestine, lung, and skin.
      (2) Histamine is released from mast cells when antigen contacts IgE bound on the mast cell. It may also be released by nonimmunologic mechanisms, e.g., trauma or cold.
      (3) Histamine interacts with target-cell receptors H1, H2, and H3. H1 causes contraction of smooth muscle, increases vascular permeability, and elevates intracellular cyclic GMP. H2 increases gastric acid secretion, respiratory mucus production, and intracellular cyclic AMP. H3 is found in the central nervous system and functions in the negative feedback inhibition of histamine release and synthesis.
      (4) Histamine can be isolated from inflammatory sites in early inflammation, but its concentration dwindles within one hour.
   b. **Arachidonic acid products.** Arachidonic acid is derived from cell membrane phospholipids, following conversion from linoleic acid. It is degraded via two pathways, the cyclooxygenase pathway and the lipoxygenase pathway.
      (1) The **cyclooxygenase pathway** converts arachidonic acid to prostaglandin $PGG_2$. $PGG_2$ is converted to $PGH_2$, which is ultimately converted to $TxA_2$ (thromboxane $A_2$), other more stable prostaglandins ($PGF_2\alpha$, $PGE_2$, $PGD_2$), and prostacyclin ($CPGI_2$).
      (2) The **lipoxygenase pathway** produces the **leukotrienes,** including $LTC_4$, $LTD_4$, and $LTE_4$, which are collectively known as the **slow-reacting substance of anaphylaxis.**
   c. **Platelet-activating factor (PAF)** is derived from cell membrane lipid and is synthesized by basophils, neutrophils, monocytes, and epithelium.

(1) PAF activates platelets by initiating the release of platelet granule constituents, causing platelets to clump.
(2) PAF stimulates the synthesis of prostaglandins and leukotrienes.
(3) PAF also increases the adhesiveness of neutrophils for endothelial cells.

d. **Adenosine** is an inflammatory agent derived from mast cells after ATP breakdown. It interacts with A1 and A2 receptors on the cell membrane. The A2 receptor is associated with increased levels of intracellular cyclic AMP, an effect that is blocked by methylxanthine drugs.

2. **Chemotactic factors**
   a. **Eosinophil chemotactic factors.** There are six major eosinophil chemotactic factors, including histamine.
   b. **Neutrophil chemotactic factors.** There are six neutrophil chemotactic factors. Their primary role is to attract neutrophils to sites of inflammation. They include IL-1 and IL-8.
3. **Enzyme mediators**
   a. **Neutral proteases** are products of mast cells.
   b. **Acid hydrolases** are found in the lysosomes of many cell types. They degrade membrane components such as chondroitin sulfate.
4. **Proteoglycans** function in the storage and release of other vascular mediators.
   a. **Heparin** is an anticoagulant that modulates tryptase activity. **Tryptase,** the major protein of human lung mast cells, can activate C3 directly in the absence of heparin. It is stored in mast cell granules in association with histamine.
   b. **Chondroitin sulfates** are structural molecules that function as binding sites for other mediators within mast cells.
5. **Toxic oxygen molecules** are important compounds that function in killing microorganisms and in the activation of neutrophils, eosinophils, and mast cells. They include singlet oxygen superoxide anion, hydrogen peroxide, and hydroxyl radicals.
6. **Kinins** are polypeptides (e.g., fibrin-split peptides) formed from precursors in the plasma.
   a. Kinins are labile proteins with physiologic effects similar to histamine.
   b. **Bradykinin,** a major kinin, functions as a vasodilator by increasing capillary permeability and producing erythema and edema. It also causes smooth muscle contraction.

**CLINICAL CORRELATE**

*Eosinophils are important cells of the innate immune system; they augment acquired immunity to metazoans. They contain basic proteins in their granules that are toxic to worms.*

**CLINICAL CORRELATE**

*Enzyme activation during the complement cascade or as a part of the inflammatory response can activate the clotting system (Hageman factor, etc.) and lead to DIC.*

# HIV and AIDS



A. **Acquired immunodeficiency**. Acquired immunodeficiency syndrome is the defining infectious disease of our generation. AIDS is transmitted by contact with blood or other body fluids of an infected individual.

1. **Causative agent.** AIDS is caused by the **human immunodeficiency virus (HIV),** which is a C-type retrovirus belonging to the Lentivirus family. The RNA core is surrounded by a lipid envelope that is derived from the host plasma membrane. The viral membrane contains a transmembrane protein, gp160. This protein is commonly detected in Western blot analysis as two fragments—gp41 and gp120. The core proteins include **reverse transcriptase** and two nonglycosylated proteins designated p18 and p24.
2. **Risk groups** for HIV infection include homosexual males, intravenous drug abusers, children born to infected mothers, sexual partners of people in high risk groups, and recipients of infected blood products or secretions. The latter risk is now minimal given the excellent methods of screening blood products for potential contamination.
3. **Mechanism of transmission.** Modes of transmission of HIV include needle contamination among drug abusers, sexual contact, and exposure to blood during the birth process.
4. **Mechanism of infection.** AIDS is characterized by a **profound loss of $CD4^+$ T cells.** The virus binds to the CD4 molecule on the T cell via gp120. In addition to T cells, HIV can also infect other $CD4^+$ cells, such as macrophages and astrocytes in the brain. Having entered into the cell, the RNA is reverse transcribed and integrated within the host DNA. Exposure of the T cell to activating stimuli such as cytokines or

**IN A NUTSHELL**

***Hyper-IgE syndrome:***

- *IgE = > 3,000 Iμ/ml*
- *↓ Antibody responses*
- *↑ Respiratory infections, especially S. aureus*
- *Eosinophilia*
- *Dermatitis*
- *Growth retardation*

antigens results in activation of the virus by stimulating transcription of virally encoded genes. Once activated, the virus replicates within the cell. Extensive viral budding can lead to death of the T cell. In contrast to the T cell, the macrophage is more resistant to death from viral infection and appears to serve as an important viral reservoir.

5. **Cellular consequences of HIV infection**
   a. **T cells.** A central consequence of infection with HIV is a loss of $CD4^+$ helper T cells. However, in addition to a loss of $CD4^+$ T cells, there is also a decrease in the response of T cells to antigen and an impaired production of cytokines, such as IL-2 and IFN-$\gamma$.
   b. **B cells.** Although patients with early HIV infection appear to have polyclonal activation of B cells with circulating immune complexes in their plasma, they steadily lose the ability to mount an effective antibody response to new antigens.
   c. **Macrophages.** HIV can enter the macrophage through binding of gp120 to CD4 and a second membrane receptor, CCR5 (a chemokine receptor). Both circulating monocytes and macrophages serve as a reservoir for the virus.
6. **Natural history of HIV infection.** The Centers for Disease Control (CDC) has proposed four clinical subgroups for AIDS-infected individuals:
   a. **Group I.** This group of individuals is characterized by an acute infection. After the initial infection, the patient may develop a syndrome resembling infectious mononucleosis, characterized by rash, sore throat, fever, or even aseptic meningitis.
   b. **Group II.** This group of individuals is characterized by asymptomatic infection. The disease becomes clinically latent and can remain so for 7–10 years.
   c. **Group III.** This group is characterized by persistent generalized lymphadenopathy together with fever, rash, and fatigue. **AIDS-related complex (ARC)** represents a nonspecific cluster of signs and symptoms of AIDS that is not accompanied by a decrease in $CD4^+$ cells. A diagnosis of early ARC is made if the individual has one or two of the following symptoms: fatigue, fever, weight loss, persistent skin rash, oral hairy leukoplakia, herpes simplex, and oral thrush. Advanced ARC is determined if the individual has two or more of these symptoms.
   d. **Group IV.** This group is characterized by generalized disease, including neurologic opportunistic infections and secondary neoplasms. Ultimately, the disease progresses to the breakdown of the immune system with full-blown AIDS characterized by the development of secondary tumors and numerous opportunistic infections.

**NOTE**

*The loss of $CD4^+$ cells would lead one to anticipate a deficit in helper cell activity with reduced antibody synthesis, but this may not occur. The pathogens that predominate (i.e., fungi, viruses, and mycobacteria) in these patients are consistent with a T-cell defect.*

(1) Among the tumors common to AIDS is **Kaposi's sarcoma,** which can be found in individuals even before breakdown of the immune system.
(2) Non-Hodgkin's lymphomas are increasingly common tumors that are found in severely immunocompromised patients.
(3) HIV-infected individuals are also susceptible to opportunistic infection by protozoa (e.g., Cryptosporidia and *Toxoplasma gondii*), fungi (e.g., Cryptococcosis, Candidiasis, and *Pneumocystis carinii*), bacteria (e.g., *Mycobacterium avium-intracellulare* and *M. tuberculosis*), and viruses (e.g., cytomegalovirus [CMV], herpes simplex, and varicella zoster).
(4) Central nervous system (CNS) involvement is a common feature of AIDS (60%). It can result either from direct infection of the CNS by the virus or from opportunistic infections (e.g., toxoplasmosis).

7. **Diagnosis.** During primary illness, antibodies are not commonly detected. HIV antibodies usually appear six weeks to six months after exposure. Antibodies against the various viral proteins do not develop simultaneously. For example, antibodies against p24 (core) and gp41 (transmembrane proteins) are detected before those induced by pol gene products.
   a. Antibodies to HIV are detected by ELISA.
   b. Confirmatory tests are performed by Western blot.
8. **Treatment, prognosis, and prevention.** While the average incubation period for infected adults is 10 or more years, infants develop clinical disease much sooner after exposure. The treatment is aimed at retarding viral replication and preventing opportunistic infection. Patients with AIDS or AIDS-related complex (ARC) are treated with **nucleoside analogs,** such as zidovudine (3'-azido-3'-deoxythymidine or AZT) and with **protease inhibitors** (e.g., saquinavir). The most important treatment at this time is **prevention,** whether through education, counseling, or behavior modification. In addition, screening of potentially infected blood has virtually eliminated transmission by blood transfusion, and cloned antihemophilic factor (factor VIII) has reduced the risk of HIV transmission to hemophiliacs.

### *Clinical Correlate*

*HIV patients can present with a wide range of problems across organ systems. If you encounter an HIV (or high-risk group) patient on the exam, you should know that there is a high incidence of the following disorders in HIV patients:*

- *Oral thrush (Candida)*
- *Esophagitis (Candida, CMV, HSV)*
- *Diarrhea*
  - *Bacterial (Salmonella, Shigella, Yersinia, Campylobacter, Myobacterium avium-intracellulare, C. difficile)*
  - *Parasitic (Cryptosporidium, Isospora, Giardia, Entamoeba)*
  - *Viral (CMV colitis)*
  - *Fungal (Candida)*
- *Intestinal neoplasms: lymphoma, Kaposi's sarcoma*
- *Pneumonias (especially Pneumocystis carinii)*
- *Tuberculosis and atypical mycobacterial infection*
- *Fungal respiratory disorders: histoplasmosis, coccidioidomycosis*
- *Pulmonary neoplasms: Kaposi's sarcoma, lymphoma*
- *Hematologic problems: anemia, leukopenia, thrombocytopenia*
- *Neurologic disorders*
  - *Cryptococcal meningitis*
  - *Toxoplasmosis*
  - *Progressive multifocal leukoencephalopathy*
  - *CMV encephalopathy*
  - *CMV retinitis*
  - *AIDS dementia*
  - *CNS lymphoma*
- *Sexually transmitted diseases*
- *Skin*
  - *Shingles*
  - *Kaposi's sarcoma*
  - *Seborrheic dermatitis*
  - *Herpes simplex*
- *Disseminated infections*
  - *Cytomegalovirus*
  - *Mycobacterium avium-intracellulare*
  - *Histoplasmosis*