PATHOGENESIS

A microorganism is a pathogen if it is capable of causing disease; however, some organisms are frequently pathogens, whereas others cause disease rarely**.**

Opportunistic pathogens are those that rarely if ever cause disease in immunocompetent people but can cause serious infections in immunocompromised patients. These opportunists are frequently members of the body's normal flora. The origin of the term "opportunistic" refers to the ability of the organism to take the opportunity offered by reduced host defenses to cause disease.

Virulence is a quantitative measure of pathogenicity and is measured by the number of organisms required to cause disease. The infectious dose of an organism required to cause disease varies greatly among the pathogenic bacteria. For example, *Shigella* and *Salmonella* both cause diarrhea by infecting the gastrointestinal tract but the infectious dose of *Shigella* is less than 100 organisms whereas the infectious dose of *Salmonella* is on the order of 100, 000 organisms. The infectious dose of bacteria depends primarily on their **"virulence factors"** for example, whether their pili allow them to adhere well to mucous membranes, whether they produce exotoxins or endotoxins, whether they possess a capsule to protect them from phagocytosis, and whether they can survive various nonspecific host defenses such as acid in the stomach.

There are two uses of the word **"parasite".** Within the context of this chapter, the term refers to the parasitic relationship of the bacteria to the host cells; that is, the presence of the bacteria are detrimental to the host cells. Bacteria that are human pathogens can be thought of, therefore, as parasites. Some bacterial pathogens are **obligate intracellular parasites**, eg, *Chlamydia* and *Rickettsia*, because they can grow only within host cells. Many bacteria are facultative parasites because they can grow within cells, outside cells, or on bacteriologic media.

8.1 TYPES OF BACTERIAL INFECTIONS

Bacteria cause disease by two major mechanisms:

(1) toxin production and

(2) invasion and inflammation.

Toxins fall into two general categories: **exotoxins and endotoxins**.

Exotoxins are polypeptides released by the cell, whereas **endotoxins** are lipopolysaccharides, which form an integral part of the cell wall. **Endotoxins occur only in gram-negative rods and cocci**; **are not actively released from the cell**; **and cause fever, shock, and other generalized symptoms**. Both exotoxins and endotoxins by themselves can cause symptoms; the presence of the bacteria in the host is not required.

Invasive bacteria, on the other hand, grow to large numbers locally and induce an inflammatory response consisting of erythema, edema, warmth, and pain.

Many, but not all, infections are communicable, ie, are spread from host to host. For example, tuberculosis is communicable, as it is spread from person to person via airborne droplets produced by coughing; but botulism is not, as the exotoxin produced by the organism in the contaminated food affects only those eating that food. **If a disease is highly communicable, the term "contagious" is applied.**

An infection is epidemic if it occurs much more frequently than usual; it is **pandemic if it has a worldwide distribution**. An **endemic infection is constantly present at a low level in a specific population.**

In addition to infections that result in overt symptoms, many are **inapparent or subclinical** and can be detected only by demonstrating a rise in antibody titer or isolating the organism. Some infections result in a **latent state**, after which reactivation of the growth of the organism and recurrence of symptoms may occur. Certain other infections lead to a **chronic carrier state**, in which the organisms continue to grow with or without producing symptoms in the host. Chronic carriers, are an important source of infection of others and hence are a public health hazard.

The determination of whether an organism recovered from a patient is actually the cause of the disease involves an awareness of two phenomena: normal flora and colonization. Members of the normal flora are permanent residents of the body and vary in type according to anatomic site. When an organism is obtained from a patient's specimen, the question of whether it is a member of the normal flora is important in interpreting the finding. **Colonization** refers to the presence of a new organism that is neither a member of the normal flora nor the cause of symptoms. It can be a difficult clinical dilemma to distinguish between a pathogen and a colonizer, especially in specimens obtained from the respiratory tract, such as throat cultures and sputum cultures.

8.2 STAGES OF BACTERIAL PATHOGENESIS

Most bacterial infections are acquired from an external source, and for those, the stages of infection are as described below. Some bacterial infections are caused by members of the normal flora and, as such, are not transmitted directly prior to the onset of infection. A generalized sequence of the stages of infection is as follows:

1. Transmission from an external source into the portal of entry
2. Evasion of primary host defenses such as skin or stomach acid
3. Adherence to mucous membranes, usually by bacterial pili
4. Colonization by growth of the bacteria at the site of adherence
5. Disease symptoms caused by toxin production or invasion accompanied by inflammation
6. Host responses, both nonspecific and specific (immunity), during steps 3, 4, and 5
7. Progression or resolution of the disease

8.3 DETERMINANTS OF BACTERIAL PATHOGENESIS

8.3.1. Transmission

Although some infections are caused by members of the normal flora, most are acquired by transmission from external sources. Pathogens exit the infected patient most frequently from the respiratory and gastrointestinal tracts; hence, transmission to the new host usually occurs **via airborne respiratory droplets or fecal contamination of food and water**. Organisms can also be transmitted by **sexual contact, urine, skin contact, blood transfusions, contaminated needles, or biting insects**.

There are four important **portals of entry: respiratory tract, gastrointestinal tract, genital tract, and skin.**

Animals can also be an important source of organisms that infect humans. They can be either the source (reservoir) or the mode of transmission (vector) of certain organisms. Diseases for which animals are the reservoirs are called zoonoses.

8.3.2. Adherence to Cell Surfaces

Certain bacteria have specialized structures, eg, **pili, or produce substances, eg, capsules or glycocalyces**, that allow them to adhere to the surface of human cells, thereby enhancing their ability to cause disease. These adherence mechanisms are essential for organisms that attach to mucous membranes; mutants that lack these mechanisms are often nonpathogenic. For example, the pili of *Neisseria gonorrhoeae* and *Escherichia coli* mediate the attachment of the organisms to the urinary tract epithelium and the glycocalyx of *Staphylococcus epidermidis* and certain viridans streptococci allows the organisms to adhere strongly to the endothelium of heart valves.

Foreign bodies, such as artificial heart valves and artificial joints, predispose to infections. Bacteria can adhere to these surfaces, but phagocytes adhere poorly owing to the absence of selectins and other binding proteins on the artificial surface.

8.3.3. Invasion and Inflammation

One of the two main mechanisms by which bacteria cause disease is invasion of tissue followed by inflammation. Several enzymes secreted by invasive bacteria play a role in pathogenesis. Among the most prominent are:

1. **collagenase and hyaluronidase**, which degrade collagen and hyaluronic acid, respectively, thereby allowing the bacteria to spread through subcutaneous tissue; they are especially important in cellulitis caused by *Streptococcus pyogenes*;
2. **coagulase**, which is produced by *Staphylococcus aureus* and accelerates the formation of a fibrin clot from its precursor, fibrinogen (this clot may protect the bacteria from phagocytosis by walling off the infected area and by coating the organisms with a layer of fibrin);
3. **immunoglobulin A (IgA) protease**, which degrades IgA, allowing the organism to adhere to mucous membranes, and is produced chiefly by *N gonorrhoeae*, *Haemophilus influenzae*, and *Streptococcus pneumoniae*; and
4. **leukocidins**, which can destroy both neutrophilic leukocytes and macrophages.

In addition to these enzymes, several virulence factors contribute to invasiveness by limiting the ability of the host defense mechanisms, especially phagocytosis, to operate effectively.

(a) The most important of these **antiphagocytic factors** is the **capsule** external to the cell wall of several important pathogens such as *S pneumoniae* and *Neisseria meningitidis*. The polysaccharide capsule prevents the phagocyte from adhering to the bacteria; anticapsular antibodies allow more effective phagocytosis to occur (a process called opsonization). The vaccines against *S pneumoniae*, *H influenzae*, and *N meningitidis* contain capsular polysaccharides that induce protective anticapsular antibodies.

(b) A second group of antiphagocytic factors are the cell wall proteins of the gram-positive cocci, such as the **M protein of the group A streptococci (*S pyogenes*) and protein A of *S aureus*.** The M protein is antiphagocytic, and protein A binds to IgG and prevents the activation of complement.

Bacteria can **cause two types of inflammation**: **pyogenic and granulomatous**. In pyogenic (pus-producing) inflammation, neutrophils are the predominant cells. In granulomatous inflammation, macrophages and T cells predominate. The most important organism in this category is *Mycobacterium tuberculosis*.

Several bacterial and fungal pathogens typically invade, survive, and grow within reticuloendothelial cells. They are "**intracellular" pathogens** and commonly cause **granulomatous lesions.** The best-known of these bacteria belong to the genera *Mycobacterium, Legionella, Brucella*, and *Listeria*. The invasion of cells by bacteria is dependent on the interaction of specific bacterial surface proteins called invasins and specific cellular receptors belonging to the integrin family of transmem-brane adhesion proteins. The movement of bacteria into the cell is a function of actin microfilaments. Once inside the cell, these bacteria typically reside within cell vacuoles such as **phagosomes**. Some remain there, others migrate into the cytoplasm, and some move from the cytoplasm into adjacent cells through tunnels (**translocation**) formed from actin. Infection of the surrounding cells in this manner allows the bacteria to evade host defenses.

8.3.4. Toxin Production

The second major mechanism by which bacteria cause disease is the production of toxins. **Exotoxins** are produced by several **gram-positive and gram-negative bacteria**, in contrast to **endotoxins**, which are present **only in gram-negative bacteria**. The essential characteristic of exotoxins is that they are secreted by the bacteria, whereas endotoxin is a component of the cell wall. Exotoxins are polypeptides whose genes are frequently located on plasmids or lysogenic bacterial viruses (bacteriophages).

4.1 Exotoxins are among the most toxic substances known. For example, the fatal dose of tetanus toxin for a human is estimated to be less than 1 μg. Exotoxin polypeptides are **good antigens** and **induce the synthesis of protective antibodies called antitoxins**, some of which are **useful in prevention or treatment** of diseases such as botulism and tetanus. When treated with formaldehyde (or acid or heat), the exotoxin polypeptides are converted into **toxoids,** which are used in protective **vaccines** because they retain their antigenicity but have lost their toxicity.

Many exotoxins have an **A-B subunit structure**; the A (or active) subunit possesses the toxic activity, and the B (or binding) subunit is responsible for binding the exotoxin to specific receptors on the membrane of the human cell. Important exotoxins that have an A-B subunit structure include diphtheria toxin, tetanus toxin, botulinum toxin, cholera toxin, and the enterotoxin of *E coli*.

A. Gram-positive bacteria

The exotoxins produced by gram-positive bacteria have several different mechanisms of action and produce different clinical effects.

(1) Diphtheria toxin, produced by *Corynebacterium diphtheriae*, inhibits protein synthesis by ADP-ribosylation of elongation factor 2 (EF-2).

A single molecule of fragment A will kill a cell within a few hours. Other organisms whose exotoxins act by ADP-ribosylation are *E coli*, *Vibrio cholerae*, and *Bordetella pertussis*.

The tox gene, which codes for the exotoxin, is carried by a temperate bacteriophage. As a result, only *C diphtheriae* strains lysogenized by this phage cause diphtheria. (Nonlysogenized *C diphtheriae* can be found in the throats of some healthy people.) Regulation of exotoxin synthesis is controlled by the interaction of iron in the medium with a tox gene repressor synthesized by the bacterium. As the concentration of iron increases, the iron-repressor complex inhibits the transcription of the tox gene.

(2) Tetanus toxin, produced by *Clostridium tetani*, is a neurotoxin that prevents release of the inhibitory neurotransmitter glycine. When the inhibitory neurons are nonfunctional, the excitatory neurons are unopposed, leading to muscle spasms and a spastic paralysis. Tetanus toxin (tetanospasmin) is composed of two polypeptide subunits encoded by plasmid DNA. The toxin released at the site of the peripheral wound may travel either by retrograde axonal transport or in the bloodstream to the anterior horn and interstitial neurons of the spinal cord. Blockage of release of the inhibitory transmitter leads to convulsive contractions of the voluntary muscles best exemplified by spasm of the jaw and neck muscles ("lockjaw").

(3) Botulinum toxin, produced by *Clostridium botulinum*, is a neurotoxin that blocks the release of acetylcholine at the synapse, producing a flaccid paralysis. The toxin is encoded by the genes of a temperate bacteriophage. Approximately 1ug is lethal for humans; it is one of the most toxic compounds known.

(4) Two exotoxins are produced by *Clostridium difficile* both of which are involved in the pathogenesis of pseudomembranous colitis. Exotoxin A is an enterotoxin that causes watery diarrhea. Exotoxin B is a cytotoxin that damages the colonic mucosa and causes pseudomembranes to form. Exotoxin B can disaggregate actin filaments in the cytoskeleton.

(5) Multiple toxins are produced by *Clostridium perfringens* and other species of clostridia that cause gas gangrene. A total of 7 lethal factors and 5 enzymes have been characterized, but no species of *Clostridium* makes all 12 products. The best-characterized is the alpha toxin, which is a lecithinase that hydrolyzes lecithin in the cell membrane, resulting in widespread cell death. The other four enzymes are collagenase, protease, hyaluronidase, and deoxyribonuclease (DNase). The seven lethal toxins are a heterogeneous group with hemolytic and necrotizing activity.

Certain strains of *C perfringens* produce an enterotoxin that causes watery diarrhea. This enterotoxin acts as a superantigen similar to the enterotoxin of *S aureus*.

(6) Three exotoxins are produced by *Bacillus anthracis*, the agent of anthrax: edema factor, protective antigen, and lethal factor. Edema factor is an adenylate cyclase that requires protective antigen for its entry into human cells. The bacterial adenylate cyclase raises the cAMP concentration within the cell, resulting in loss of chloride ions and water and consequent edema formation in the tissue. The mode of action of lethal factor is unknown.

(7) Toxic shock syndrome toxin (TSST) is produced by certain strains of *Staphylococcus aureus*. TSST binds directly to class II major histocompatibility (MHC) proteins without intracellular processing. This complex interacts with the β-chain of the T cell receptor of many helper T cells. This causes the release of large amounts of interleukins, especially interleukin-1 and interleukin-2. They produce many of the signs

and symptoms of toxic shock. The staphylococcal enterotoxins that cause food poisoning have a similar mode of action.

(8) Erythrogenic toxin, produced by *Streptococcus pyogenes*, causes the rash characteristic of scarlet fever. Its mechanism of action is similar to that of TSST; ie, it acts as a superantigen (see above). The DNA that codes for the toxin resides on a temperate bacteriophage. Nonlysogenic bacteria do not cause scarlet fever, although they can cause pharyngitis.

B. Gram-negative bacteria:

The exotoxins produced by gram-negative bacteria also have several different mechanisms of action and produce different clinical effects.

(1) The heat-labile enterotoxin produced by *E coli* causes watery, nonbloody diarrhea by stimulating adenylate cyclase activity in cells in the small intestine. The resulting increase in the concentration of cyclic adenosine monophosphate (cAMP) causes excretion of the chloride ion, inhibition of sodium ion absorption, and significant fluid and electrolyte loss into the lumen of the gut. **The heatlabile toxin**, which is inactivated at 65 °C for 30 minutes, is composed of A-B subunits

In addition to the labile toxin, there is a **heat-stable toxin**, which is a polypeptide that is not inactivated by boiling for 30 minutes. The heat-stable toxin inhibits the reabsorption of sodium ions and causes diarrhea.

Verotoxin is an exotoxin produced by strains of *E coli* with the O157:H7 serotype. These strains cause **bloody diarrhea** and are the cause of outbreaks associated with eating undercooked hamburger in fast-food restaurants. The toxin is named for its cytotoxic effect on Vero (monkey) cells in culture.

(2) The enterotoxins produced by *V cholerae,* the agent of cholera, and *Bacillus cereus*, a cause of diarrhea, act in a manner similar to that of the heat-labile toxin of *E coli*.

(3) Pertussis toxin, produced by *Bordetella pertussis*, the cause of whooping cough, is an exotoxin that enhances adenylate cyclase activity. Pertussis toxin also causes the marked lymphocytosis seen on patients with pertussis. The toxin inhibits signal transduction by all chemokine receptors, resulting in an inability of lymphocytes to enter lymphoid tissue (spleen, lymph nodes) and consequently, in an increase in their number in the blood.

4.2 Endotoxins are integral parts of the cell walls of both gram-negative rods and cocci, in contrast to exotoxins, which are released from the cell. In addition, several other features distinguish these substances. **Endotoxins are lipopolysaccharides (LPS),** whereas **exotoxins are polypeptides**; the enzymes that produce the lipopolysaccharide are encoded by genes on the bacterial chromosome, rather than by plasmid or bacteriophage DNA, which usually encodes the exotoxins. **The toxicity of endotoxins is low in comparison with that of exotoxins.**

All endotoxins produce **the same generalized effects of fever and shock,** although the endotoxins of some organisms are more effective than those of others. Endotoxins are **weakly antigenic**, they induce protective antibodies so poorly that multiple episodes of toxicity can occur. No toxoids have been produced from endotoxins, and endotoxins are not used as antigens in any available vaccine.

The endotoxins of gram-negative bacteria are the **best-established causes of septic shock,** but surface molecules of gram-positive bacteria (which do not have endotoxins) can also cause septic shock (see below). Two features of septic shock are interesting:

(1) **Septic shock is different from toxic shock**. In septic shock, the bacteria are in the bloodstream, whereas in toxic shock, it is the toxin that is circulating in the blood. The clinical importance of this observation is that in septic shock, blood cultures are usually positive, whereas in toxic shock, they are usually negative.

(2) **Septic shock can cause the death of a patient even though antibiotics have killed the bacteria in the patient's blood,** ie, the blood cultures have become negative. This occurs because septic shock is mediated by **cytokines**, such as tumor necrosis factor and interleukin-1, that continue to act even though the bacteria that induced the cytokines are no longer present.

The toxic portion of the LPS is lipid A, which is composed of disaccharides with several fatty acids attached. β-hydroxymyristic acid is always one of the fatty acids and is found only in lipid A. The other fatty acids differ according to species. **The polysaccharide core** in the middle of the molecule protrudes from the surface of the bacteria and has the same chemical composition within members of a genus. **The repeat unit of sugars** on the exterior differs in each species and frequently differs between strains of a single species. It is an important antigen of some gram-negative rods **("O" or somatic antigen**) and is composed of three, four, or five sugars repeated up to 25 times. Because the number of permutations of this array is very large, many antigenic types exist. For example, more than 1500 antigenic types have been identified for *Salmonella*.

The biologic effects of endotoxin include:

1. **fever** due to the release by macrophages of endogenous pyrogen (interleukin-1), which acts on the hypothalamic temperature-regulatory center;
2. **hypotension, shock, and impaired perfusion of essential organs** owing to bradykinin-induced vasodilation, increased vascular permeability, and decreased peripheral resistance (nitric oxide, a potent vasodilator, also causes hypotension);
3. **disseminated intravascular coagulation** due to activation of the coagulation system through Hageman factor (factor XII), resulting in thrombosis, a petechial or purpuric rash, and tissue ischemia;
4. **activation of the alternative pathway of the complement cascade**, resulting in inflammation and tissue damage; and
5. **activation of macrophages**, **increasing their phagocytic ability**, and activation of many clones of B lymphocytes, increasing antibody production. (Endotoxin is a polyclonal activator of B cells, but not T cells.)

9. INFECTION. TYPICAL STAGES OF AN INFECTIOUS DISEASE

Infectious disease is the infection which entails the replication of organisms in the tissues of a host, with related development of overt clinical manifestations. If the infection provokes an immune response only, without overt clinical disease, there is an **inapparent or subclinical infection.**

Many of the organisms live on the skin, in the oral cavity, in the respiratory and GI, genital tracts, representing our **normal flora.** Microorganisms far outnumber humans. They are everywhere: in the soil, in fresh- and seawater, in the air. But, only a relatively few of them are capable of causing disease. Complex, dynamic interaction occurs between human hosts and pathogenic organisms. Whether an organism remains apart from a human host, becomes part of the normal flora, or invades the host and causes disease depends on these interactions.

The first line of host defense is the anatomic barrier of me skin and mucous membranes. Added to this defense in the respiratory tract are the cough reflex and the upward flew of mucus, propelled by the concerted beating of cilia. As a result of these interactions, few bacteria are usually found below the level of the larynx. In the GI tract, gastric acid plays a similar defensive role. A few diseases (e.g., staphylococcal food poisoning) are caused by toxins elaborated by microorganisms outside the host rather than by invasion of microorganisms. Most infections diseases, even some of those mediated by **toxins** (e.g., tetanus, toxic shock syndrome), result from **tissue invasion**, in which the first essential step is adherence of microorganisms to host cells (e.g.*Vibrio cholerae*).

With other organisms (e.g.. *Salmonella* spp. *Streptococcus pneumoniae*), **adherence** is only a first step, allowing **penetration of local tissue or dissemination to distant sites**. Whether an organism invades local tissue or disseminates depends on many microbial factors, such as toxins, enzymes, and nontoxic substances (eg, **staphylococcal protein A** or the **polysaccharide capsules** of various bacteria). Multiplication of bacteria in host tissue elicits an **acute inflammatory reaction** mediated by inflammatory cytokines. Both PMN leukocytes and monocyte-macrophages counteract this multiplication, constituting the next line of defense.

Finally, the **full power of the immune system** is brought into action to control production of both Ig and T-cell responses, thus inhibiting infection.

Natural defenses can be aided by administration of anti-infective substances, such as **antibiotics, antifungals, and antiviral agents**. However, the importance of the host response is underscored by the fact that when the host is severely impaired, such exogenous substances often are not effective.

There are **3 possible outcomes for these events**:

1. The multiplication of organisms, production of toxic bacterial products, and overwhelming inflammatory response impair the host to such an extent that the host dies.
2. A state of equilibrium is reached, with the establishment of chronic infection (slow multiplication) or latency (DNA integration of viral genome) that may last the lifetime of the host.
3. Host defense mechanisms, with or without the aid of exogenous substances such as antibiotics, eradicate the invading organism, inducing specific protective immunity.

9.1 PATHOGENICITY

The **relationship that exists between the human host and the microbial world** is complex. Each is in constant interaction with the other as well as with many additional influences, all of which affect the host-microbe relationship. **Factors** such as: nutrition, stress, genetic background, and other diseases present, all have an impact on the outcome of the meeting of human and microbe. The relationship is an equilibrium that is in constant motion and subject to change.

A pathogen is a microbe that can cause disease in a susceptible host. Years ago, relatively few organisms e.g., *Yersinia pestis* and *Bacillus anthracis*, were pathogenic in nearly all situations. Others, such as *Serratia marcescens*, were considered never to be pathogenic. Our understanding of host-parasite interactions and our ability to isolate, grow, and identify organisms have improved over the years. In addition, patient populations have changed owing to a longer life, highly invasive medical procedures,

transplants, and so on. As a result, organisms that are found as normal flora in the environment are being seen with increasing frequency in clinical settings.

Opportunistic Pathogens: are microorganisms that humans encounter, cause disease only if there is a significant change in host resistance or within the organism itself. They are usually part of the normal flora, but the classic pathogens may also be found among the opportunistic organisms when host defenses weaken. The infections caused by these organisms are opportunistic infections. Infections due to these organisms rarely occur in healthy individuals. When an infection is the result of medical treatment or procedures, it is termed an iatrogenic infection, or nosocomial infections (UTI - patients urinary catheters). Patients who are given immunosuppressive drugs because they have received a transplant are more susceptible to infection.

9.2 ***VIRULENCE***

Virulence is the relative ability of a microorganism to cause disease, or the degree of pathogenicity. It is usually measured by **the numbers of microorganisms necessary to cause infection** in the host. Those organisms that can establish infection with a relatively low infective dose are considered more virulent than those that require high numbers for infection. If a microorganism requires a relatively high infective dose but the disease it causes is often fatal, we tend to think of the microorganism as highly virulent. A number of organism characteristics or factors contribute to virulence: **capsules, toxins, enzymes, cell wall receptors, pili, and others**. These virulence factors allow the pathogen to evade or overcome host defenses and cause disease.

Virulence factors

1.Adherence

Most infectious agents must attach to host cells before infection occurs. In virtually all other cases, the bacterium, virus, or fungus requires adherence to the host cell before infection and disease progress (In some diseases due to exotoxins adherence is not important). The cell surface structures that mediate attachment are called **adhesins**. The host cells must possess the necessary receptors for the adhesins. If the host or the infectious agent undergoes a mutation that changes the structure of the adhesin or the receptor, adherence likely will not take place and the virulence of the infectious agent is affected. The main adhesins in bacteria are the **pili (fimbriae) and surface polysaccharides**. Pili enable bacteria to adhere to host cell surfaces.

1. Proliferation

In order to establish itself and cause disease, a pathogen must be able to replicate following attachment to host cells. Many host factors work to **prevent proliferation**. **Secretory antibody, lactoferrin, and lysozyme** have been mentioned previously. To be successful in establishing infection, infectious agents must be able to avoid or overcome these local factors. Several pathogens (*Haemophilus influenzae, N. gonorrhoeae, Neisseria meningitidis*) produce an **IgA protease** that degrades the IgA found at mucosal surfaces. Other pathogens (influenza virus, *Borrelia*) circumvent host antibodies by shifting key cell-surface antigens. The host produces antibodies against the "old" antigens, which are no longer effective.

An extremely important event in the life of an invading pathogen is **phagocytosis**. **Evasion of phagocytosis** is essential for most pathogens to be able to survive and multiply. The most common characteristic of bacteria that allows for such evasion is a **polysaccharide capsule**. Many of those possessing a capsule are highly virulent until its removal, at which point their virulence becomes extremely low. Some pathogens are able to survive phagocytosis. Those that do have developed several methods to prevent being killed. Some prevent fusion of phagosomes and lysosomes. Others have a resistance to the effects of the lysosomal contents, and still others escape into the cytoplasm.

3. Tissue Damage

Generally speaking, disease from infection is noticeable only if tissue damage occurs. This damage may be from toxins, either exotoxins or endotoxins, or from inflammatory substances that cause, immunologically mediated damage.

4. Exotoxins

Many of the bacterial exotoxins are highly characterized. Most are composed of two subunits: the first is nontoxic and serves to bind the toxin to the host cells; the second is toxic.

It is common for the toxin gene to be encoded by phage, plasmids, or transposons. Therefore, only those carry the extrachromosomal DNA coding for the toxin gene produce toxin.

5. Endotoxins

Gram-negative bacteria have endotoxins. Endotoxins are composed of the lipopolysaccharide portion of the cell wall. The toxicity is due to the lipid A portion of the lipopolysaccharide. The effects of endotoxin consist of **dramatic changes in blood pressure, clotting, body temperature, circulating blood cells, metabolism, humoral immunity, cellular immunity, and resistance to infection.** Endotoxin stimulates the fever centers in the hypothalamus. An increase in body temperature occurs within an hour after exposure. Exposure to endotoxin also causes hypotension. Severe hypotension occurs within 30 minutes following exposure. Septic or endotoxic shock is a serious and potentially life-threatening problem.

A severe infection with gram-negative bacteria can lead to **serious and often life-threatening problems**.

6. Invasion

Pathogens exhibit invasion to some degree or another. Invasion is the process of penetrating and growing in tissues. With some organisms, the invasion is localized and involves only a few layers of cells. With others, it involves deep tissues; the gonococcus, for example, is an invasive organism that may infect the fallopian tubes.

7. Dissemination

Dissemination is the spread of organisms to distant sites, i.e., organs and tissues (in *Salmonella*, dissemination is an important aspect of the disease, other organisms, such as *C. diphtheriae*, do not spread beyond their initial site of infection, yet the disease may produce is serious often fatal. Certain organisms that survive phagocytosis may be disseminated rapidly to many body sites, but the organisms themselves are not invasive. The phagocyte simply carries the organism, but the bacterium itself is incapable of penetrating tissues (*C.perfringens* is an example of a highly invasive organism that may not necessarily disseminate).

8. Transmission

The route by which a pathogen may be transmitted to a susceptible host is often explained by the characteristics of that pathogen. Some organisms may be naturally transmitted by more ,than one route, most have a limited number of routes. These routes can be characterized as: airborne, via food and water (ingestion), through close contact (include sexual transmission), through cuts and bites, and via arthropods; animal diseases that can infect humans are transmitted through animal contact (zoonoses).

8.1 Airborne Transmission

Respiratory spread of infectious disease is common. Often, the respiratory secretions are aerosolized by coughing, sneezing, and talking. Very small particles, referred to as droplet nuclei, are the residue from the evaporation of fluid from larger droplets and are light enough to remain airborne for long periods.

Pathogens that are spread through the air generally must be resistant to drying and inactivation by ultraviolet light. Some infectious agents may be transmitted by dust particles that have become airborne.

But, the body has a number of defenses against airborne infectious agents.

In order for a microorganism to cause disease, it must circumvent these defenses, penetrate the mucous layer, and attach to the epithelium. The host also produces **secretory IgA, lysozyme, alveolar macrophages.**

Respiratory tract infections (RTI) are the most common reason that patients of all ages seek medical attention. Although most **upper RTI** are self-limiting and can be treated by the patient with over-the-counter medications, some are more serious. Streptococcal sore throat, sinusitis, otitis media, acute epiglottitis, and diphtheria can be serious, even life-threatening. Viral diseases causing the common cold are usually not life-threatening but can result in much discomfort and time lost from work or school. All the diseases just mentioned can be spread via aerosols, some may also be transmitted via the fingers and

hands, especially true for the common cold-causing rhinovirus. The fingers and hands are contaminated with infectious nasal secretions because of hand-to-nose contact.

The infectious virus particles are passed from the infected individual to a susceptible recipient via hand-to-hand or hand-to-face contact. The recipient transmits the virus picked up from the hands of the infected individual by touching the face and nose. In this case, the disease is transmitted via the respiratory route, but it is not in the normal, classic manner of respiratory transmission.

Transmission may also result from contact with inanimate objects contaminated with the infectious agent. For example, a door knob is contaminated by the hand and fingers of an infected individual, and the virus is transmitted to a susceptible person's hand and fingers when that person opens the door. Control of such transmission is often as simple as frequent hand washing.

Lower RTI are less common but, more serious than those of the upper respiratory tract. The organisms causing these infections have managed to bypass host defenses, or the host defenses have been compromised (e.g., by alcoholism, heavy smoking), allowing the pathogen access to the deeper portions of the respiratory tract.

The most common microorganism causing lower respiratory tract infection of individuals over age 30 is *S. pneumoniae*. Among younger people, the common causes of pneumonia are *Mycoplasma* and viruses.

In **chronic lower RTI,** the survival of the infecting agent within phagocytes plays a role in the pathogenic mechanism. *M. tuberculosis,* the agent of tuberculosis, a chronic debilitating infection, is the classic example of an intracellular pathogen.

8.2 Transmission by Food and Water

Transmission of gastrointestinal infections (GEI) is usually a result of ingestion of contaminated food or water. In some situations, infection occurs via the fecal-oral route.

The digestive tract is colonized with vast numbers of different microorganisms. Under normal conditions, the normal flora maintains a harmless relationship with the host. Gastric enzymes and juices in the stomach act to prevent survival of most organisms, but many survive and. colonize the small intestine and colon.

Organisms: that can cause disease by means of a preformed toxin include *C. botulinum, B. cereus*, and *S. aureus*. The severity of disease ranges from a mild diarrhea to a rapidly fatal intoxication. **Food poisoning** by *B. cereus* and *S. aureus* is relatively common and is self- limiting. Botulism, although rare, can be life-threatening.

Other bacteria produce a toxin after infection of the intestinal tract. Generally, in order to be effective as a disease producer, an organism must survive, adhere to, and colonize the intestinal mucosa and either produce a toxin or invade deeper tissues. A commonly seen cause of diarrhea and intestinal infection is ***E. coli*** (normal intestinal flora);

Some strains of *E. coli* produce cytotoxins that cause alterations in the biochemical activity of the intestinal epithelial cells, resulting in problems with fluid and electrolyte control by the intestinal cells. These strains of ***E. coli,*** **- enterotoxigenic** (ETEC), are a common cause of "traveler's diarrhea" as well as other intestinal problems.

Probably the classic intestinal pathogen is ***Vibrio cholerae,*** the cause of cholera. This organism produces an enterotoxin that causes the outpouring of fluid from the cells into the lumen of the intestine. Massive amounts (up to 20 liters per day) can be lost.

Other intestinal pathogens are *C. difficile, Shigella, A. hydrophila, C. jejuni,* and *Salmonella.* The infective dose, severity, and incidence of disease vary with the agent.

8.3 Close Contact

All of the routes of transmission require what could be called close contact. Obviously, for a respiratory pathogen to be transmitted via aerosols, the susceptible host must be relatively close. Two prominent infections passed by **direct transfer of saliva** (e.g., kissing) are herpes simplex and infectious mononucleosis. **Skin-to-skin transfer** of infectious disease is not as common as some of the other routes, but diseases such as warts (human papillomavirus), syphilis, and impetigo result when material from infectious lesions inoculates a susceptible host's skin. The list of **sexually transmitted diseases** is a long

one (gonorrhea, herpes simplex, hepatitis, *Chlamydia*, syphilis, *Trichomonas*, and AIDS). A number of viruses (hepatitis, rotavirus, adenovirus, coxsackievirus) and parasites (*G. lamblia, E. histolytica*) also cause diarrheal disease.

8.4 Cuts and Bites

The classic example of a bite wound infection is rabies. In fact, human rabies is relatively rare. Of more concern with animal, and especially human, bites is infection by the normal flora of the mouth.

Zoonoses: The route of transmission known as zoonosis depends upon contact with animals or animal products. Certain diseases of animals may also infect humans in contact with them.

These diseases may be passed by animal bites (rabies), arthropod vectors (plague), contact with secretions (brucellosis), and contact with animal carcasses and products (tularemia, listeriosis).

9.3 STAGES OF ACUTE INFECTIONS

A typical acute infectious disease has four stages:

1. **the incubation period**, which is the time between the acquisition of the organism (or toxin) and the beginning of symptoms (this time varies from hours to days to weeks depending on the organism);
2. **the prodrome period**, during which nonspecific symptoms such as fever, malaise, and loss of appetite occur;
3. **the specific-illness period**, during which the overt characteristic signs and symptoms of the disease occur; and
4. **the recovery period**, during which the illness abates and the patient returns to the healthy state.

After the recovery period, some individuals become chronic carriers of the organisms and may shed them while remaining clinically well. Others may develop a latent infection, which can recur either in the same form as the primary infection or manifesting different signs and symptoms. Although many infections cause symptoms, many others are subclinical; ie, the individual remains asymptomatic although infected with the organism.

9.4 MANIFESTATIONS OF INFECTION

Fever

A. Normal basal body temperature is generally accepted to be 37°C (98.6°F), determined orally. The normal figure for any given time of day varies among individuals, so the normal oral temperature range is 36-37.8°C (96.8-100°F). Rectal temperatures are higher by approximately 0.6°C (1°F).

B. Diurnal variation. Temperature is lowest in the early morning and highest in the late afternoon or early evening (4:00-8:00 PM).

1. Diurnal variation may normally be as much as 1°C (1.8°F) or more in any given individual.

2. The diurnal rhythm is consistent in each person. Its absence may suggest the possibility of factitious fever (assuming no hypothalamic disorder exists).

C. Fever and hyperthermia are physiologically two distinct processes. Fever is said to exist whenever the body temperature rises above the peak normal range.

D. Lethal temperature ranges

Infections likely to be associated with **extreme fever are gram-negative bacteremia**, **Legionnaires' disease, abacteremic pyelonephritis, bacterial meningitis, viral encephalitis, typhoid fever, and malaria**. Noninfectious causes of extreme pyrexia are likely to be heat stroke, intracerebral hemorrhage, hemorrhagic pancreatitis, and the malignant hyperthermia associated with general anesthesia or with neuroleptic drugs.

Systemic responses

Infection induces a constellation of host responses in different organ systems.

Hematologic manifestations: leukocytosis with an increase in the total number of neutrophils (exageration of this phenomenon can result in leukemoid reactions, with release of immature leukocytes

into circulation), anemia develops despite adequate iron stores (may be acute, resulting from bleeding or destruction of red blood cells, or chronic), disseminated intravascular coagulation, thrombocytopenia.

Cardiac manifestations: range from tachycardia and increased cardiac output to failing myocardial function.

Respiratory manifestations: hyperventilation, commonly with a marked respiratory alkalosis.

Renal manifestations: sepsis produces manifestations ranging from minimal proteinuria to acute renal failure.

There may be also **hepatic manifestations** (e.g., cholestatic jaundice), gastrointestinal manifestations, neurologic manifestations and endocrinologic and metabolic manifestations.

9.5 EPIDEMIOLOGY

Epidemiology is the **study of the occurrence, distribution, and causes of disease and injury.**

Definitions: Several terms are helpful in describing the incidence and effect of disease in a population.

Carrier. A carrier is a person or animal who harbors and spreads a microorganism that causes disease but who does not become ill. There are two types of carriers. A casual carrier harbors the microorganism temporarily for a few days or weeks; examples of microorganisms maintained in this fashion are *C. diphtheriae*, *N. meningitidis*, and *S. pyogenes*. A chronic carrier remains infected for a relatively long time, sometimes throughout life; the typhoid bacillus may be carried chronically.

Endemic. When an organism or disease is constantly present in a population, that disease or organism is termed endemic. It is indigenous to a geographic area or population. Some microorganisms or diseases are endemic for one geographical area but not for another. Schistosomiasis is endemic to parts of the Middle East, but not for many other parts of the world. Cholera is endemic for large portions of the globe. In the United States, we have a more-or-less constant incidence of common colds, streptococcal pharyngitis, and gonorrhea.

Epidemic. When a disease affects a significantly large number of people at the same time in a geographic area, it is said to be an epidemic. The number of cases per given time is not the only measure. Ten cases of diphtheria in a short time could be considered an epidemic because diphtheria is not often seen. Ten cases of streptococcal pharyngitis during that same period are probably normal, however, because it is an endemic disease. A classic example of an epidemic is influenza. There are epidemics of varying degrees every year.

Periodically (in intervals of 10 to 20 years), there are **worldwide epidemics** of influenza affecting tens of millions of people; they are termed **pandemics**. Currently, cholera is in its seventh pandemic; it has spread throughout world regions and even continents.

Incidence Rate. The number of times a new event occurs in a given period is called the incidence rate. It is usually given as cases or infections per 1000 or 100,000 population. It allows a prevalence comparison between diseases or infections.

Incubation Period. The time between exposure to a pathogen and the onset of symptoms is the incubation period. This is often difficult to determine because individuals often have difficulty pinpointing the date or time of exposure. An individual may be infectious during the incubation period; this situation presents a difficult public health problem, because no symptoms are present to identify the infectious person but transmission of the organism is taking place.

Index Case. The first case of a disease, which serves as a source of infection, is the index case.

Morbidity Rate. The rate at which an illness occurs, the morbidity rate, is the number of cases of a disease in a specified population during a defined time interval. The morbidity rate can be a measure of the infectiousness of an organism.

Mortality Rate. The mortality rate is the number of deaths due to a disease in a population. Organisms that are highly virulent often have a high mortality rate.

Nosocomial Infections. An infection acquired during hospitalization is termed a nosocomial infection.

Reservoir. The reservoir is the source of an infection. It may be a person, an animal, or something in the environment.

Surveillance. Surveillance is the collection of data pertaining to disease occurrence. It is carried out at various levels (physician, city, county, state, federal, international) with a system for reporting to public health agencies.

10. NATURAL IMMUNITY AND PHYSIOLOGICAL DEFENSE MECHANISMS

Entry of a pathogenic microorganism into a susceptible host can be followed by invasion and colonization of tissues, circumvention of the host immune response, and injury to the host tissues.

The human immune system provides barriers against an enormous number of infectious agents in the environment. Some of these barriers are natural, or innate, and others are induced, or acquired.

Innate immunity is nonspecific. It consists of **natural barriers** to infection that are a part of normal body function. **Physical barriers** such as the skin and mucous membranes provide the first line of defense against infectious agents. **Phagocytic cells** such as neutrophils and macrophages are effective killers of microbes that penetrate the physical barriers. Phagocytes are the second innate line of defense against infectious agents. **Others mechanisms** implied in nonspecific resistance are inflammation and production of antimicrobial substances (other than antibodies).

Acquired immunity is an inducible, specific immunologic response to exposure to a **particular infectious agent**. It may be **humoral or cell mediated**. **Humoral immunity** is an immune state resulting from the production of **antibodies by bone marrow-derived (B) lymphocytes**. **Cell-mediated (cellular) immunity is an immune response primarily of thymus-derived (T) lymphocytes.** When an organism infects the body, the defence systems already in place may well be sufficient to prevent replication and spread of the infectious agent, thereby preventing development of disease. However, should innate immunity be insufficient to parry the invasion by the infectious agent, the "adaptative" immune system then comes into action, although it takes time to reach its maximum efficiency. When it does take effect, it eliminates the infective organism, allowing the recovery from disease.

The main feature distinguishing the adaptative immune system from the innate mechanism is that **specific memory** of infection is imprinted on the adaptative immune system, so that should be there a subsequent reinfection by the same agent, a particularly effective response comes into play with remarkable speed. It is worth emphasizing, however, that there is close synergy between the two systems with the adaptative mechanisms greatly improving the efficiency of the innate response.

Table : Comparison of innate and adaptative immune systems

	INNATE IMMUNE SYSTEM	ADAPTATIVE IMMUNE SYSTEM
SOLUBLE FACTORS	Lysozyme, complement, acute phase proteins, interferon, lactoferrin, transferrin, lactoperoxidase, β-lysin, chemotactic factors, properdin, defensins	Antibodies
CELLS	Phagocytes, NK cells	T lymphocytes

Table: Response to microbial infection

	INNATE IMMUNE SYSTEM	ADAPTATIVE IMMUNE SYSTEM
FIRST CONTACT	+	+
SECOND CONTACT	+ non specific no memory resistance not improved by repeated contacts	+ + + + specific memory resistance improved by repeated contacts

10.1 MECHANICAL BARRIERS

The skin and mucous membranes serve as barriers to the microorganisms. With the exception of a few organisms (e.g., papilloma virus, dermatophytes [”skin loving” fungi]), most microorganisms cannot establish infections without penetrating the skin or mucous membranes. The intact skin is one of the human body’s largest organs, comprising over 15% of the body’s dry weight. It consists of two distinct portions, the dermis and the epidermis. The dermis, the skin’s inner, thicker portion, is composed of connective tissue. The epidermis, the outer, thinner portion, is in direct contact with the external environment. The epidermis consists of layers of continuous sheets of tightly packed epithelial cells with little or no material between sheets. Langerhans cells of the epidermis participate in immunity by assisting helper T cells and suppressor T cells, respectively. **Free fatty acids** produced in sebaceous glands and by organisms on the skin surface**, lactic acid** in perspiration, and the **low pH** and relatively dry environment of the skin are all unfavorable for the survival of most organisms.

Lysozyme and lactoferrin are antimicrobial substances found in secretions at mucosal surfaces. Lysozyme induces lysis of bacteria through disruption of the linkage connecting N-acetylmuraminic acid and N-acetylglucosamine in the walls of gram-positive bacteria. Lactoferrin, an iron binding protein, competes with microorganisms for this substance.

Inhaled microorganisms in dust or droplets greater than 5 μm adhere to the mucosa lining the upper respiratory tract and are swept upward by cilia to the posterior pharynx and then expectorated or swallowed. Particles less than 5 μm are able to reach the lower airways but should be rapidly phagocytized by alveolar macrophages. However, cigarette smoke or other pollutants, as well as some bacteria and viruses (e.g., *Bordetella pertussis*, influenza virus), can interfere with this clearance mechanism by damaging the ciliated epithelial cells, thus rendering the patient susceptible to secondary bacterial pneumonia. Intubation or tracheostomy also decreases the normal defense mechanisms.

Besides the skin and mucous membranes, **several other mechanical factors** help protect certain epithelial surfaces. One such mechanism that protects the eyes is the **lacrimal apparatus**, a group of structures that manufactures and drains away tears. Normally**, the tears** are spread over the surface of the eyeball by blinking and evaporate or pass into the nose as fast as they are produced. This continuous washing action helps to keep microorganisms from settling on the surface of the eye. If an irritating substance or large numbers of microorganisms come in contact with the eye, the lacrimal glands start to secrete heavily, and the tears accumulate more rapidly than they can be carried away. This excessive production is a protective mechanism because the excess tears dilute and wash away the irritating substance or microorganisms.

In a cleansing action very similar to that of tears, **saliva** is produced by the salivary glands and washes microorganisms from both the surface of the teeth and the mucous membrane of the mouth. This mechanism helps prevent colonization by microbes.

Even though urine can support bacterial growth in the **bladder, the acidic pH of urine** and voiding serve as effective defensive mechanisms against most uropathogens. Urinary tract infections are also generally less common in males than females because of the longer urethra in males. Urinary stasis caused by reflux, prostatic hypertrophy, or calculi facilitates growth of organisms in the retained urine and subsequent infections.

10.2 NONSPECIFIC HUMORAL DEFENSE MECHANISMS

Numerous enzymes, proteins, and other factors contribute to a host’s nonspecific immunity. Some are humoral defenses and others, to be discussed later, are cellular defenses.

10.2.1 Inflammatory Response

Acute inflammation represents an early defense mechanism to contain an infection and prevent its spread from the initial focus. When microbes multiply in host tissues, two principal defensive mechanisms mounted against them are antibodies and leukocytes. The three major events in acute inflammation are;

(1) dilatation of capillaries to increase blood flow,

(2) changes in the microvasculature structure leading to escape of plasma proteins and leukocytes from the circulation, and

(3) leukocyte emigration from the capillaries and accumulation at the site of injury.

Widening of interendothelial cell junctions of venules or injury of endothelial cells facilitates escape of plasma proteins from the vessels. Neutrophils, attached to the endothelium through adhesion molecules, escape the microvasculature and are attracted to sites of injury by chemotactic agents. This is followed by phagocytosis of the microorganisms that may lead to their intracellular destruction. Activated leukocytes may produce toxic metabolites and proteases that injure endothelium and tissues when they are released. Activation of the third component of complement (C3) is also a critical step in inflammation.

Multiple chemical mediators of inflammation derived from either plasma or cells have been described. Mediators in plasma, such as complement, are present as precursors that require activation for them to become biologically active. Mediators derived from cells are present as precursors in intracellular granules, such as histamine in mast cells. Following activation, these substances are secreted. Other mediators such as **prostaglandins** may be synthesized following stimulation. These mediators are quickly activated by enzymes or other substances such as antioxidants. A chemical mediator may also cause a target cell to release a secondary mediator with a similar or opposing action.

Besides **histamine**, other preformed chemical media tors in cells include **serotonin** and **lysosomal enzymes**. Those that are newly synthesized include prostaglandins, leukotrienes, platelet-activating factors, cytokines, and nitric oxide. Chemical mediators in plasma include **complement fragments C3a and C5a and the C5b-9 sequence**. Three plasma-derived factors, including **kinins**, complement, and clotting factors, are involved in inflammation. **Bradykinin** is produced by activation of the kinin system. It induces arteriolar dilatation and increased venule permeability through contraction of endothelial cells and extravascular smooth muscle contraction. Activation of bradykinin precursors involves activated factor XII (Hageman factor) generated by its contact with injured tissues.

During clotting, **fibrinopeptides** produced during the conversion of fibrinogen to fibrin increase vascular permeability and are chemotactic for leukocytes. The fibrinolytic system participates in inflammation through the kinin system.

Products produced during arachidonic acid metabolism also affect inflammation. These include **prostaglandins and leukotrienes**, which can mediate essentially every aspect of acute inflammation. In inflammation, they act locally on the endothelium, participate in systemic acute phase reactions, and affect fibroblasts.

They promote surface expression of adhesion molecules on the endothelial surface, which leads to increased adherence of leukocytes and increased thrombogenicity of the endothelium. **They are pyrogenic** (induce fever) during acute inflammation. **TNF** promotes neutrophil aggregation and activation and the escape of proteolytic enzymes from mesenchymal cells, promoting tissue injury. **IL-8** has powerful chemoattractant qualities and activates PMN. Oxygen-derived metabolites from leukocytes may contribute to inflammation. They may injure endothelial cells, causing increased vascular permeability, and injury to other cells.

Local clinical manifestation are: RUBOR, CALOR, DOLOR, TUMOR.

10.2.2 Cytokines

Cells responding to invading microorganisms may produce a variety of **peptides** called cytokines that are **able to modulate the immune system**. Cytokines produced by macrophages and monocytes are known as **monokines**, and those produced by lymphocytes are known as **lymphokines**. Cytokines are not antigen specific but play an important function in modifying the immune response. Examples of cytokines are interleukin 1 (IL-1) and **tumor necrosis factor (TNF)** produced by macrophages, and **interleukin 2 (IL-2), interferons**, and colony-stimulating factors synthesized by T lymphocytes.

Cytokines with the greatest role in inflammation are **IL-1, TNF, and IL-8.** IL-1 and TNF both are produced by activated macrophages, although other types of cells may also produce IL-1. Cytokine secretion is promoted by endotoxin, immune complexes, toxins, physical injury, and inflammation.

Cytokines that act on the cells producing them are said to have an **autocrine action**, whereas those that affect neighboring cells are said to have a **paracrine effect.** When they act like any other hormone (i.e., on cells distant from their site of synthesis), they have an endocrine effect. In inflammation, they act locally on the endothelium, participate in systemic acute phase reactions, and affect fibroblasts. **IL-8 has powerful chemoattractant qualities** and activates neutrophils.

Oxygen-derived metabolites from leukocytes may contribute to inflammation. They may injure endothelial cells, causing increased vascular permeability, inactivation of antiproteases, and injury to other cells. The effect of oxygen-derived free radicals in inflammation depends on their synthesis and inactivation by host cells.

10.2.3 Acute Phase Response

The acute phase response is a **nonspecific reaction** by an individual stimulated by infection, inflammation, tissue injury, and infrequently neoplasm; it is mediated by IL-1, I IL-6, tumor necrosis factor, prostaglandin (PGE_1), and interferons. Serum proteins elevated in the circulation during the acute phase response include complement, coagulation proteins, transport proteins, protease inhibitors, and adherence proteins that activate complement, promote phagocytosis, and stimulate migration of leukocytes. These acute phase reactants are: α - 1 antitrypsin, α - 1 glycoprotein, amyloid A and P, antithrombin III, C - reactive protein, C1 esterase inhibitor, C3 complement, ceruloplasmin, fibrinogen, haptoglobin, orosomucoid, plasminogen, and transferrin.

C-reactive protein (CRP) is produced by IL-1 stimulation of the liver. Within 24 to 48 hours of the onset of acute inflammation, the CRP concentration increases a thousandfold (thus elevated levels are a nonspecific indicator of inflammation). CRP complexes with polysaccharides of numerous bacteria and fungi; it activates the alternate complement pathway, which facilitates removal of these organisms from the body through increased phagocytosis.

Interleukin-1, tumor necrosis factor K, and α-interferon released during the acute phase response act on the hypothalamus to induce fever.

10.2.4 Complement

The complement system consists of **approximately 20 proteins** that are present in normal human (and other animal) serum. They are termed: C1, C2,…..C9. Any activation splits the unit (eg: C5 into C5a and C5b fragments). The term "complement" refers to the ability of these proteins to complement, ie, **augment, the effects of other components of the immune system**, eg, antibody. Complement is an **important component of our innate host defenses**.

There are **three main effects** of complement:

(1) **lysis of cells** such as bacteria, allografts, and tumor cells;

(2) generation of **mediators that participate in inflammation** and attract neutrophils;

(3) **opsonization**, ie, enhancement of phagocytosis.

Complement proteins are synthesized mainly by the liver. Complement is heat-labile; ie, it is inactivated by heating serum at 56°C for 30 minutes. Immunoglobulins are not inactivated at this temperature.

ACTIVATION

Several complement components are proenzymes, which must be cleaved to form active enzymes. Activation of the complement system can be initiated either by antigen-antibody complexes or by a variety of nonimmunologic molecules, eg, endotoxin. Sequential activation of complement components occurs via one of two pathways: **the classic pathway and the alternative pathway**.

Of the two pathways, **the alternative** one is more important the first time we are infected by a microorganism, since the antibody required to trigger the classic pathway is not present. Both pathways lead to the **production of C3b**, the central molecule of the complement cascade. C3b has two important functions:

(1) It combines with other complement components to **generate C5 convertase**, the enzyme that leads to the production of the membrane attack complex, and

(2) it **opsonizes bacteria** because phagocytes have receptors for C3b on their surface.

Classical Pathway

The first complement component, designated C1 consists of a complex of three separate proteins designated Clq, C1r, and C1s. One molecule each of Clq and C1s with two molecules of C1r compose the Cl complex or recognition unit. Clq facilitates binding of the recognition unit to cell surface antigen-antibody complexes. Activation of the classical complement cascade requires linkage of Clq to two IgG antibodies through their Fc regions. In contrast, one pentameric IgM molecule attached to a cell surface may interact with Clq to initiate the classical pathway. Binding of Clq activates C1r (referred to, now as C1r*) and in turn C1s (C1s*). C1s* then splits C4 (to C4a and C4b) and C2 (to C2a and C2b). The ability of a single recognition unit to split numerous C2 and C4 molecules represents an amplification mechanism in the complement cascade. The union of C4b and C2a produces C4b2a, which is known as C3 convertase. This complex binds to the cell membrane and splits C3 into C3a and C3b fragments. The ability to split multiple C3 molecules is another amplification mechanism. The interaction of C3b with C4b2a bound to the cell membrane produces a complete activation unit, C4b3b2a, which is termed C5 convertase. This activation unit splits C5 into C5a and C5b fragments and represents yet another amplification step.

The terminal stage of the classical pathway involves creation of the membrane attack complex, which is also called the lytic unit. C5b binds to the cell membrane, followed by the successive interaction of single molecules of C6, C7, C8, and C9 with the membrane-bound C5b. Formation of a membrane attack complex leads to a cell membrane lesion that permits loss of potassium and ingress of sodium and water, leading to hypotonic lysis of cells.

Not all C3b produced in the classical complement activation unites with C4b2a to produce C5 convertase. Some of it binds directly to the cell membrane and makes it more attractive for phagocytic cells such as neutrophils and macrophages, which have receptors for C3b. Complement fragments C3a and C5a also serve as powerful anaphylatoxins that stimulate mast cells to release histamine, which enhances vascular permeability and smooth muscle contraction. C5a also acts as an attractant for neutrophils and macrophages that release hydrolytic enzymes and stimulate platelet aggregation, leading to microthrombosis, blood stasis, edema, and local tissue injury and destruction.

Alternate Pathway

Endotoxin, human IgA, microbial polysaccharides, and other factors may activate complement by an alternative pathway. This pathway does not depend on antibody activation and does not involve the early complement components (C1, C2, and C4). The initial activation of the alternate pathway is mediated by properdin factor B binding to C3b and then with properdin factor D, which splits factor B in the complex to yield the Bb active fragment that remains linked to C3b (activation unit). Inactive Ba is split off from this complex, which leads to C3 activation and then continuation of the complement cascade in a manner analogous to the classical pathway. Activation of the late components results in opsonic activity, chemotaxis of leukocytes, enhanced permeability of organisms, and cytolysis.

Membrane Attack Complex

Five terminal complement proteins (C5-C9) associate into a membrane attack complex (MAC) on target cell membranes to mediate injury. Initiation of MAC assembly begins with C5 cleavage into C5a and C5b fragments (**the common pathway**). A $(C5b,6,7,8)_1(C9)_n$ complex then either forms on natural membranes or, in their absence, combines with plasma inhibitors such as lipoproteins, antithrombin III, and S protein.

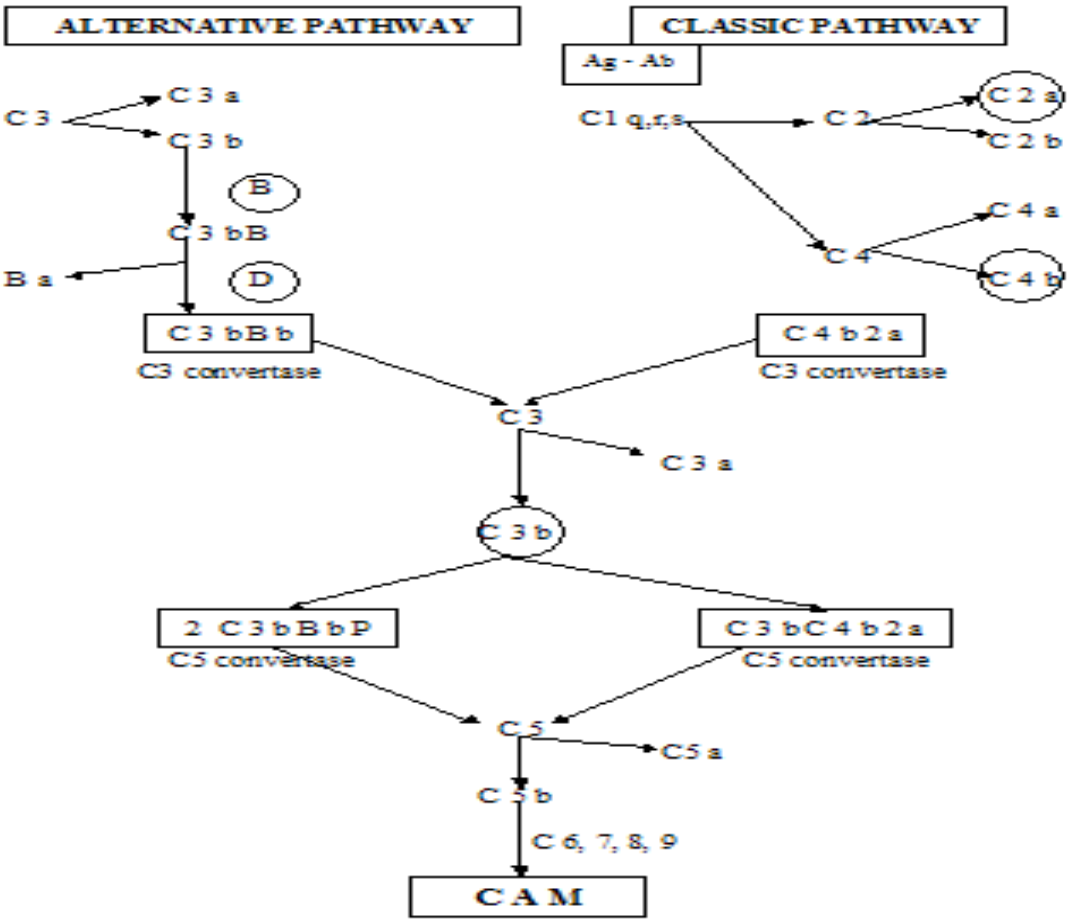

Figure : Complement activation pathways

There is also a third activation pathway: **Lectinic pathway** (Ali/ Krueger)

It is similar with the clasical pathway (C4 și C2), but without the presence of imunoglobulines. It binds to the mannose residues, on the surface of microorganisms (like *Salmonella, Listeria, Neisseria, Cryptococcus neoformans,* HIV-1 , VRS). MBL (Mannose binding lectin) binds 2 proteases, MASP – I și MASP II (Manan binding lectin Associated Serine Protease), which activate C4 and C2 which will form C4bC2a – lectinic pathway convertase.

REGULATION OF THE COMPLEMENT SYSTEM

The first regulatory step in the classic pathway is at the level of the antibody itself. The complement-binding site on the heavy chain of IgM and IgG is unavailable to the C1 component of complement if antigen is not bound to these antibodies. This means that complement is not activated by IgM and IgG, despite being present in the blood at all times. However, when antigen binds to its specific antibody, a conformational shift occurs and the C1 component can bind and initiate the cascade.

BIOLOGIC EFFECTS

Opsonization Cells, antigen-antibody complexes, and viruses are **phagocytized much better in the presence of C3b**. This is due to the presence of C3b receptors on the surface of many phagocytes.

Chemotaxis C5a and the C567 complex attract neutrophils. They migrate especially well toward C5a. C5a also enhances the adhesiveness of neutrophils to the endothelium.

Anaphylatoxin C3a, C4a, and C5a cause degranulation of mast cells with release of mediators, eg, histamine, leading to increased vascular permeability and smooth muscle contraction, especially contraction of the bronchioles leading to bronchospasm. C5a is, by far, the most potent of these anaphylatoxins. Anaphylaxis caused by these complement components is less common than anaphylaxis caused by type I (IgE-mediated) hypersensitivity (see Chapter 65).

Cytolysis - Insertion of the C5b6789 complex into the cell membrane leads to killing or lysis of many types of cells including erythrocytes, bacteria, and tumor cells. Cytolysis is not an enzymatic process; rather, it appears that insertion of the complex results in disruption of the membrane and the entry of water and electrolytes into the cell.

Enhancement of Antibody Production - The binding of C3b to its receptors on the surface of activated B cells greatly enhances antibody production compared with that by B cells that are activated by antigen alone. The clinical importance of this is that patients who are deficient in C3b produce significantly less antibody than do those with normal amounts of C3b. The low concentration of both antibody and C3b significantly impairs host defenses, resulting in multiple, severe pyogenic infections.

CLINICAL ASPECTS

(1) **Inherited (or acquired) deficiency of some complement components**, especially **C5-C8**, are associated with defective MAC activity. This action increases the susceptibility to disseminated *Neisseria* infections. A deficiency of C3 leads to severe, recurrent pyogenic sinus and respiratory tract infections.
(2) Inherited deficiencies of **Clq, C1r, C1s, C4, or C2** components are associated with defects in activation of the classical pathway that lead to increased susceptibility to **pyogenic (pus-producing) infections.**
(3) A **deficiency of C3** leads to a defect in activation of both the classical and alternative pathways, which also results in an **increased incidence of pyogenic infections.** Such individuals also have defective opsonization and phagocytosis.
(4) **Defects of the properdin factors** impair activation of the alternative pathway, which also results in an increased **susceptibility to pyogenic infections**.
(5) **In transfusion mismatches**, eg, when type A blood is /given by mistake to a person who is type B, antibody to the A antigen in the recipient binds to A antigen on the donor red cells, complement is activated, and large amounts of anaphylatoxins and membrane attack complexes are generated. The anaphylatoxins cause shock, and the membrane attack complexes cause red cell hemolysis.
(6) **Immune complexes bind complement**, and thus complement levels are low in **immune complex diseases**, eg, acute glomerulonephritis and systemic lupus erythematosus. Binding (activating) complement attracts polymorphonuclear leukocytes, which release enzymes that damage tissue.
(7) Inherited deficiency of C1 esterase inhibitor results in angioedema. When the amount of inhibitor is reduced, an overproduction of esterase occurs. This leads to an increase in anaphylatoxins, which cause capillary permeability and edema.

10.2.5 Opsonins and Opsonization

An opsonin is a substance that adheres to the surface of a microorganism and makes it more attractive to a phagocytic cell. Opsonins **enhance phagocytosis** of microbes, constituting a cornerstone of defense against infection. Both **nonimmune and immune substances** may serve as opsonins. **C3b**, produced during complement activation, covalently binds to microbes.

Fibronectin, it is a glycoprotein of relatively high molecular weight found on cells and in plasma. It may serve as an opsonin, as well as function as an adhesion molecule in cellular interactions. Fibronectin may also react with complement components.

Specific opsonines are represented by antibodies, part of the acquired imunity.

10.2.6 Interferons

These low molecular weight glycoproteins are characterized as **alpha, beta, and gamma**. **Alpha and beta interferon are activated by viral infections** and induce antiviral proteins that prevent viral mRNA translation. The activity of interferon is cell specific and not virus specific (e.g., chicken interferon will not protect humans). Interferons act at the cell surface through specific receptors with a common receptor for alpha and beta interferon. Interferon can also activate NK cells and macrophages.

Interferon-γ modulates the immune response.

Interferon can also activate NK cells and macrophages

10.3 NONSPECIFIC CELLULAR DEFENSE MECHANISMS

10.3.1 Phagocytic Cells

Phagocytosis is an important clearance mechanism for the removal and disposition of microbes or damaged cells. Macrophages, monocytes, eosinophils, and polymorphonuclear (PMN) leukocytes are phagocytic cells. In special circumstances other cells such as fibroblasts may show phagocytic properties; these cells are called facultative phagocytes. **PMNs are the first cells to arrive** at the infected focus. The cells contain both primary or azurophilic granules and secondary or specific granules. Azurophilic granules serve as reservoirs for myeloperoxidase and also for other lysosomal hydrolases such as β-glucuronidase, elastase, and cathepsin G. Specific granules serve as reservoirs for lysozyme and lactoferrin. These digestive and hydrolytic enzymes are delivered to phagosomes to aid in the breakdown of ingested material. Frequently, the PMNs die after ingesting and destroying the invading microorganisms – a property commonly observed with pyogenic bacteria (e.g., *Staphylococcus*, *Streptococcus*, *Neisseria*).

Mononuclear Phagocytes

These include **monocytes in the blood and macrophages in the tissues**. These cells have cell surface receptors for Fc gamma and C3b. They are also able to phagocytize microorganisms coated with opsonins and kill many but not all of them. Some microorganisms (including but not limited to mycobacteria, *Listeria*, *Brucella*, *Cryptococcus*, *Toxoplasma*) survive and multiply within macrophages. In this case the cell may serve as a protective reservoir or transport system to help spread the organisms throughout the body. However, in the presence of cell-mediated immunity, the macrophages are activated and can kill the intracellular pathogens.

10.3.2 Phagocytosis

PMNs, eosinophils, and macrophages have an important role in defending the host against microbial infection. PMNs and occasionally eosinophils appear first in response to acute inflammation, followed later by macrophages. Chemotactic factors (e.g., formyl-methionyl-leucyl-phenylalanine [f-met-leu-phe]) are released by actively multiplying microbes. These chemotactic factors are powerful attractants for phagocytic cells, which have specific membrane receptors for the factors.

Phagocytosis of microbes involves several steps: **attachment, internalization, and digestion**. After attachment, the particle is engulfed within a membrane fragment and a phagocytic vacuole is formed. This vacuole fuses with the primary lysosomes to form the phagolysosome, in which the lysosomal enzymes are discharged and the enclosed material is digested. Remnants of indigestible material can be recognized subsequently as residual bodies.

Phagocytic dysfunction may be due to either extrinsic or intrinsic defects. Consequences of phagocytic dysfunction include **increased susceptibility to bacterial infections but not to viral or protozoal infections**. Selected phagocytic function disorders may be associated with **severe fungal infections**. The severity of bacterial infections associated with phagocytic dysfunction may range **from mild skin infections to fatal systemic infections**.

Chemotaxis

The locomotion of cells may be stimulated by the presence of certain substances in their environment. Chemotactic factors are released by actively multiplying microbes. They are powerful attractants for phagocytic cells, which have specific membrane receptors for the factors

Oxygen-Dependent Killing

Phagocytic killing of microbes is either oxygen dependent or oxygen independent. Oxygen-dependent killing is activated by a powerful oxidative burst that culminates in the formation of hydrogen peroxide and other antimicrobial substances.

Oxygen-Independent Killing

Following adherence of opsonized microbes to the neutrophil plasma membrane and engulfment, cationic proteins (e.g., cathepsin G) from azurophil granules and lysozyme and lactoferrin from specific granules are discharged into the phagosomes. These proteins kill gram-negative bacteria by interrupting their cell membrane integrity but are far less effective against gram-positive bacteria (which are killed principally through the oxygen-dependent mechanism).

Cationic protein deficiency may be associated with chronic skin infections or abscesses.

10.3.3 Natural Killer Cells and Antibody-Dependent Cytotoxic Cells

Although natural killer (NK) cells are not phagocytic, **they can attack and destroy certain virus-infected cells.** They constitute an important part of the natural immune system, **do not require prior contact with antigen,** and **are not MHC restricted** by the major histocompatibility complex (MHC) antigens. On contact with the virus-infected cell, NK cells produce perforin that leads to the formation of pores in the infected cell membrane and subsequent osmotic lysis. Interferon enhances NK cell activity.

11. ACQUIRED IMMUNITY

Humans are confronted with a host of microorganisms that have the potential to induce serious or fatal infections. Yet nature has provided appropriate molecules, cells, and receptors that can protect against these microbes. Many of these defenses are general or nonspecific and do not require previous exposure to the offending pathogen (or closely related organism). These important mechanisms constitute the innate or constitutive defense system.

Another important defense system is **acquired immunity, which can develop after previous contact with the organism through infection** (overt or subclinical) **or by deliberate immunization** with a vaccine prepared from the etiological agent.

Naturally acquired immunity describes the protection provided by previous exposure to a pathogenic microorganism or antigenically related organism. In contrast, **artificially acquired immunity** develops as a result of immunization with vaccines - either with attenuated organisms or with killed organisms or subunit components. Toxoids provide excellent immunity against the effects of microorganisms such as *Corynebacterium diphtheriae* and *Clostridium tetani* that produce powerful exotoxins.

Active immunization with appropriate booster injections leads to the development of IgG, which provides immunity of long duration. Acquired immunity depends on antibodies and T cells.

Passive immunity involves the transfer of resistance against an infectious disease agent from an immune individual to a previously susceptible recipient. **Natural passive immunity** describes the transfer of IgG antibodies across the placenta from mother to child. IgA secretory antibodies may also be passively transferred from mother to child in breast milk. **Artificially acquired passive immunity** describes the transfer of immunoglobulins from an immune individual to a nonimmune, susceptible recipient. Passive immunity of this type is more often used for prophylaxis than for therapy. It provides immediate protection of the recipient for relatively short periods (few weeks). Human sera are preferred for passive immunization to avoid serum sickness induced by foreign serum proteins. Adoptive immunization refers to the transfer of specifically immune lymphoid cells from one individual to another, such as occurs in bone marrow transplantation.

11.1 ANTIGENS

Definition: antigens are molecules (infectious agent, whether a bacterium, fungus, virus, or parasite) that react with antibodies, and are capable of inducing an immune response.

After exposure to an antigen, immunological tolerance or antibody synthesis and/or cell-mediated immunity may result. To stimulate an immunogenic response, a substance usually needs to be foreign (some autoantigens are exceptions). Antigens usually have a molecular weight of at least 10,000 d and are either proteins or polysaccharides. Nevertheless, immunogenicity depends on the genetic capacity of the host to respond and on the immunogenic properties of the antigen.

Classification

A complete antigen is one that both properties:

- induces an immune response (antibodies or T lymphocytes) -immunogenity
- and also reacts with the products- antigenicity.

An incomplete antigen or hapten is unable to induce an immune response alone but will react with the products. Haptens can be rendered immunogenic (capable of stimulating an immune response) by covalently linking them to a carrier molecule such as a foreign protein. In addition, haptens often have highly reactive chemical groupings that permit them to couple with a substance such as a tissue protein.

A hapten is a molecule that is not immunogenic by itself, but can react with specific antibody. Haptens are usually small molecules, but some high-molecular-weight nucleic acids are haptens as well. Many drugs, e.g., penicillins, are haptens and the catechol in the plant oil that causes poison oak and poison ivy is a hapten.

Haptens are not immunogenic, because they cannot activate helper T cells. The failure of haptens to activate is due to their inability to bind to MHC proteins; Furthermore, haptens are univalent and therefore cannot activate B cells by themselves. Although haptens cannot stimulate a primary or secondary response by themselves, they can do so when covalently bound to a "carrier" protein. In this process, the hapten interacts with an IgM receptor on the B cell and the hapten-carrier protein complex is internalized. A peptide of the carrier protein is presented in association with class II MHC protein to the helper T cells. The activated helper T cell then produces interleukins, which stimulate the B cells to produce antibody to the hapten.

Epitopes

The specific parts of antigen molecules that elicit immune reactivity are known as antigenic determinants or epitopes. The epitope reacts with the specific antigen binding site in the variable region of an antibody molecule known as a paratope. The excellent fit between epitope and paratope is based on their three-dimensional interaction and noncovalent union. An epitope may also react with a T cell receptor (TCR) for which it is specific.

A single antigen molecule may have several different epitopes. Antigenic determinants may be either conformational or linear. **A conformational epitope** is produced by spatial juxtaposition during folding of amino acid residues from different segments of the linear amino acid sequence. Conformational determinants are usually associated with natural rather than denatured proteins. They are recognised by B cells

A linear epitope is one produced by adjacent amino acid residues in the proteins. They usually interact with antibody only after denaturation and are not customarily in the native configuration. They are recognised by T cells.

Antigens may be classified as either **T-cell dependent (TD) or T-cell independent (TI).**

The TD antigens are much more complex than the TI antigens, are usually proteins, stimulate all five classes of Ig, elicit an anamnestic or memory response, and are present in most pathogenic microorganisms. By contrast, the simpler TI antigens are often polysaccharides or lipopolysaccharides, elicit an IgM response only, and fail to stimulate an anamnestic response. An antigen has two or more epitopes per molecule. Epitopes consist of approximately six amino acids or six monosaccharides.

Epitopes that stimulate a greater antibody response than others are referred to as immunodominant epitopes. Antigens must be degradable by phagocytes to be able to stimulate an immune response. Ag processing includes enzymatic digestion to prepare soluble macromolecules. Substances that cannot be digested cannot function as Ag.

The features of molecules that determine immunogenicity are as follows.

1. **Foreignness**: In general, molecules recognized as "self are not immunogenic; i.e., we are tolerant to those self-molecules. To be immunogenic, molecules must be recognized as "nonself," i.e., foreign.
2. **Molecular Size**: The most potent immunogens are proteins with high molecular weights, i.e., above 100,000. Generally, molecules with molecular weight below 10,000 are weakly immunogenic, and very small ones, e.g., an amino acid, are nonimmunogenic. Certain small molecules, e.g., haptens, become immunogenic only when linked to a carrier protein.
3. **Chemical-Structural Complexity**: A certain amount of chemical complexity is required; e.g., amino acid homopolymers are less immunogenic than heteropolymers containing two or three different amino acids.
4. **Antigenic Determinants** (Epitopes): Epitopes are small chemical groups on the antigen molecule that can elicit and react with antibody. An antigen can have one or more determinants. Most antigens have many determinants; i.e., they are multivalent. In general, a determinant is roughly 5 amino acids or sugars in size. The overall three-dimensional structure is the main criterion of antigenic specificity.
5. **Dosage, Route, and Timing of Antigen Administration**: These also affect immunogenicity. In addition, the genetic constitution of the host (HLA genes) determines whether a molecule is immunogenic. Different strains of the same species of animal may respond differently to the same antigen.

6. **Factors in host response to antigens** are represented by: response to a given Ag is heterogeneous-ediated by several factors, host age (very young or old - less response), port of entry (mucosa, blood, lymph), genetic capability, general health, length of time in the body (role of adjuvants) and dose, how administered (IM, subcutaneous, IV).

Adjuvants enhance the immune response to an immunogen. They are chemically unrelated to the immunogen and may act by nonspecifically stimulating the immunoreactive cells or by releasing the immunogen slowly. Some human vaccines contain adjuvants such as aluminum hydroxide or lipids.

Major Histocompatibility Complex MHC

The success of tissue and organ transplants depends on the donor's and recipient's human leukocyte antigens (HLA) encoded by the HLA genes. These proteins are alloantigens; i.e., they differ among members of the same species. The genes for the HLA proteins are clustered in the major histocompatibility complex (MHC). These genes are very diverse (polymorphic). They actively communicate through specialized surface molecules, including:

- the class I MHC molecules and
- class II MHC molecules.

The class I molecules consist of one three-domain MHC polypeptide chain and a β2-microglobulin component, are important for presenting endogenous antigens to T lymphocytes, and are present on all nucleated cells.

Class II MHC molecules consist of an alpha and a beta MHC polypeptide chain, are primarily responsible for presentation of exogenous antigens, and are present only on macrophages, B lymphocytes, and a few other cell types (like:dendritic cells of the spleen, and Langerhans cells of the skin).

BIOLOGIC IMPORTANCE OF MHC:

MHC genes and proteins are important in two medical contexts:

- one is that many autoimmune diseases occur in people who carry certain MHC genes, and the other is that
- the success of organ transplants is, in large part, determined by the compatibility of the MHC genes of the donor and recipient.

The ability of T cells to recognize antigen is dependent on association of the antigen with either class I or class II proteins.

For example, **cytotoxic T cells respond to antigen in association with class I MHC proteins**. Thus, a cytotoxic T cell that kills a virus-infected cell will not kill a cell infected with the same virus if the cell does not also express the appropriate class I proteins.

Helper T cells recognize class II proteins. Helper-cell activity depends in general on both the recognition of the antigen on antigen-presenting cells and the presence on these cells of “self” class II MHC proteins. This requirement to recognize antigen in association with a “self” MHC protein is called MHC restriction. T cells recognize antigens only when the antigens are presented on the surface of cells (in association with either class I or II MHC proteins), whereas B cells do not have that requirement and can recognize soluble antigens in plasma with their surface monomer IgM acting as the antigen receptor.

MICROBIAL ANTIGENS

Are particulate-bounded to the microbial cell (bacteria, viruses, fungi):

-released in the medium after bacterial lysis (endotoxines)

-soluble-released in the medium (enzimes, exotoxines)

The most important in bacterial pathogenity are the pathogenity antigens:

-capsular antigens (that allow them to adhere to the surface of human cells, enhancing their ability to cause disease). These adherence mechanisms are essential for organisms that attach to mucous membranes; For example capsula in *Klebsiella* spp., *Streptococcus pneumoniae*, the glycocalyx of *Staphylococcus epidermidis* and certain viridans streptococci allows the organisms to adhere strongly to the endothelium.

-cell wall antigens (endotoxines in Gram negative rods)

-exoenzimes(coagulase, hemolisines,)

-exotoxines (*C.diphteriae, C.tetaniae*, etc.)

SUPERANTIGENS

A superantigen (like *S.aureus* exotoxines) is a substance such as a bacterial toxin that is capable of stimulating multiple T lymphocytes, leading to the release of relatively large quantities of cytokines. Superantigens are TD antigens that do not require phagocytic processing.

Instead of fitting into the T-cell receptor (TCR) internal groove where a typical processed peptide antigen fits, superantigens bind to the external region of the TCR.

Superantigens react with multiple TCR molecules whose peripheral structure is similar. Thus they stimulate multiple T cells that increase a protective T and B cell antibody response.

This enhanced response to antigens such as toxins produced by staphylococci and streptococci is an important protective mechanism in the infected individual.

11.2 ORIGIN OF IMMUNE CELLS

The capability to respond to immunologic stimuli rests mainly with lymphoyd cells. During embryonic development, blood cell precursors originate mainly in the fetal liver and yolk sac. In postnatal life, the stem cells reside in the bone marrow. Stem cells differentiate into cells of the erythroid, myeloid, or lymphoid series. The latter envolve into two main lymphocyte populations: T cells and B cells. The ratio of T cells to B cells is approximately 3:1.

I. CENTRAL LYMPHOID TISSUES

Here instruction is done without the presence of antigens:

- bone marrow- for B cells proliferation
- tymus-for T cells proliferation

II. PERIPHERICAL LYMPHOID TISSUES

Here instruction is done in the presence of antigens:

- lymphoid nodes
- spleen
- tonsils
- MALT(Mucosal associate lymphoid tissues), like GALT (gastro-enteral associate lymphoid tissue) and BALTbroncho-alveolar associate lymphoid tissues).

11.2.1 Encapsulated lymph nodes

Contains reticular cells and their fibers organized into sinuses. These act as a filter for lymph draining the body tissues and possibly bearing foreign antigens which enters the subcapsular sinus by the afferent vessels and diffuses pass the lymphocytes in the cortex to reach the macrophages of the medullary sinuses and thence the efferent lymphatics.

11.2.2 Spleen

On a fresh section of spleen, the lymphoid tissue forming the white pulp is seen as circular or elongated gray areas within the erythrocyte-filled red pulp which consists of splenic cords lined with macrophages and venous sinusoids. As in the lymph node, T- and B-cell areas are segregated. The spleen is a very effective blood filter removing of red and white cells and responding actively to blood-borne antigens, the more so if particulate. Plasmablasts and mature plasma cells are present in the marginal zone extending into the red pulp.

11.2.3 Mucosal – associated lymphoid tissue (MALT)

The respiratory, alimentary and genitourinary tracts are guarded immunologically by subepithelial accumulations of lymphoid tissue. These may occur as diffuse collections of lymphocytes, plasma cells and phagocytes throughout the lung and the lamina propria of the intestinal wall or follicles. In man, the latter includes the lingual, palatine and pharyngeal tonsils, the small intestinal Peyer's patches and the appendix.

It is generally agreed that this MALT forms a separate interconnected secretory system within which cells committed to IgA or IgE synthesis may circulate.

In the gut, antigen enters the Peyer's patches across specialized epithelial cells and stimulates the antigen-sensitive lymphocytes. After activation these drain into the lymph and after a journey through the mesenteric lymph nodes and the thoracic duct, they pass from the bloodstream into the lamina propria where they become IgA-forming cells which protect the bowel with antibodies.

Significance of Mucosal Immunity

Mucosal surfaces represent a vast surface area vulnerable to colonization and invasion by microorganisms. The total amount of secretory IgA exported on mucosal surfaces, exceeds production of circulating IgG. Antigens on mucosal surfaces are separated from mucosal immune tissue by the epithelial barrier. To elicit a mucosal immune response, antigens must be transported across the epithelium before they can be processed and presented to cells of immune system. MALT have an important role in protection from microbial colonization (adherence), prevention of environmental sensitization. May also have regulatory influence on systemic immunity and may block allergic sensitization.

11.2.4 Lymphocyte traffic

This traffic of lymphocytes between the tissues, the bloodstream and the lymph glands enables antigen-sensitive cells to seek the antigen and to be recruited to sites at which a response is occurring, while the dissemination of memory cells and their progeny enables a more widespread response to be organized throughout the lymphoid system.

The handling of antigen: where does antigen go when it enters the body?

If it penetrates the tissues it will tend to finish up in the draining lymph nodes. Antigens which are encountered in the upper respiratory tract or intestine are trapped by local mucosal-assodated lymphoid tissue. Antigens in the blood provoke a reaction in the spleen. Macrophages in the liver will filter blood-borne antigens and degrade them without producing an immune response since they are not strategically placed with respect to lymphoid tissue.

11.3 CELLS OF IMMUNITY

To properly understand the process of antigen presentation, it is necessary to understand the types of lymphocytes and MHC molecules with which an antigen must interact. All lymphocytes develop from hematopoietic stem cells in the bone marrow. Precursor cells destined to follow the T-lymphocyte lineage migrate from the bone marrow to the thymus, whereas the B-lymphocyte precursors remain in the bone marrow.

The immune response consists of two limbs:

- **a T-cell limb responsible for cell-mediated immunity** and
- **a B-cell or humoral limb concerned with antibody production**.

A successful immune response against many microorganisms often involves stimulation of both T and B cells and involves a cooperative interaction between these two cell populations. T and B lymphocytes do not function in isolation. They actively communicate through specialized surface molecules, including the **class I and class II MHC molecules**.

11.3.1 Antigen Presenting Cells

Antigen presenting cells **(APC) include macrophages, Langerhans' cells, and dendritic reticulum cells.** These cells present antigen at the cell surface to immunoreactive lymphocytes (e.g., CD4+ helper/inducer T cells). Other antigen presenting cells that serve mainly as passive antigen transporters include B lymphocytes, endothelial cells, keratinocytes, and Kupffer cells.

Antigen presentation describes the expression of antigen molecules on the surface of a macrophage or other APC

(1) when, in association with **MHC class II molecules**, the antigen is presented to CD4+ T-helper lymphocytes or

(2) when, in association with cell surface **MHC class I molecules**, the antigen is presented to CD8+ cytotoxic T lymphocytes.

Protein antigens are ingested by mononuclear phagocytes, split into 8 to 10 amino acid residues, and then linked to cell surface MHC class II molecules. For appropriate presentation, peptides must bind securely to the MHC class II molecules. Those peptides that do not bind or bind only weakly are not presented and fail to elicit an immune response.

Following interaction of the peptide and CD4 helper T-lymphocyte receptor,

-the CD4 cell is activated,

-interleukin-2 (IL-2) is released, and IL-2 receptors are expressed on the CD4 lymphocyte surface

-the IL-2 produced by the activated cell stimulates its own receptors, as well as those of mononuclear phagocytes, increasing their killing activity. IL-2 also stimulates B cells to synthesize antibodies.

-whereas B cells may recognize a protein antigen in its native state, T lymphocytes recognize the oligopeptides that result from antigen processing.

11.3.2 Lymphocytes

Lymphocytes are classified as B cells, T cells, or null cells (i.e., natural killer [NK] cells, large granular lymphocytes [LGL]).

B cells release immunoglobulins into the circulation following antigenic stimulation of the cells. In contrast, the **T cells** have specific receptors that are retained on the cell surface and facilitate participation of the lymphocyte in cell-mediated immunity. When the T cell is stimulated, it produces powerful immunoregulatory chemicals (lymphokines) that affect B cell function.

In general, **lymphocytes constitute approximately 30% of human leukocytes**, with the NK cells and LGLs responsible for only about 5% of the lymphocyte population. In contrast with B cells that mature in the bone marrow, the T-cell populations migrate to the thymus where during maturation some surface markers are lost and others gained.

11.3.2.1 T Lymphocytes

Functionally, the CD4+ cell population is predominantly:

- **helper/inducer T cells (LTh) -** after antigen interaction they stimulate B cells and have as a marker CD4+
- **suppressor T Lymphocytes (LTS)** are responsible for suppressor cell activity (clonal proliferation) of T and B cells, they regulate the intensity of imun rsponse. Like cytotoxic T cells, T-suppressor cells are MHC class I restricted.
- **cytotoxic T Lymphocytes (LTC)** are stimulated by those antigens which are expresed on surface of the host cell (viruses, intracelular bacteria, tumoral cells)
- **CD8+ population is suppressor(LTS)/cytotoxic (LTC) cells**
- **helper T cells delay Lymphocytes (LTDhCD4 +)** also mediate delayed-type hyper-sensitivity. The cells secrete lymphokines (e.g., macro-phage chemotaxin, migration inhibitory factor [MIF], macrophage activating factor [MAF]) that produce histological changes in the tissues

After maturation of T and B cells, they will migrate to the peripheral lymphoid tissues such as lymph nodes and spleen. **T cells may be activated** by the interaction of antigen, in the context of MHC, with the T-cell receptor.

CD4 + lymphocytes also mediate delayed-type hyper-sensitivity. The cells secrete lymphokines (e.g., macrophage chemotaxin, migration inhibitory factor [MIF], macrophage activating factor [MAF]) that produce histological changes in the tissues (described in the section on hypersensitivity disorders).

11.3.2.2 B Lymphocytes

The activation of B cells by T-helpers through the TCR recognition of MHC-linked antigenic peptide, leads to up regulation of the surface receptor for IL4. Local release of this cytokine from the T - helper then drives powerful clonal proliferation and expansion of the activated B cell population. IL2 also contributes to this process.

Under the influence of **IL4 alone, the expanded clones can differentiate and mature into IgE synthesizing cells. IgM plasma cells emerge under the tutelage of IL4 plus 5, and IgG producers result from the combined influence of IL4, 5 and 6 with a probable contribution from IFN gama.**

11.4 ANTIBODIES

Antibodies are globulin proteins (immunoglobulins) that react specifically with the antigen that stimulated their production. They make up about 20% of the protein in blood plasma. Blood contains three types of globulins, alpha, beta, and gamma, based on their electrophoretic migration rate. Antibodies are gamma globulins. **There are five classes of antibodies: IgG, IgM, IgA, IgD, and IgE.**

Antibodies that arise in an animal in response to typical antigens are heterogeneous, because they are formed by several different clones of plasma cells; i.e., they are **polyclonal**. Antibodies that arise from a single clone of cells, e.g., in a plasma cell tumor (myeloma), are homogeneous; i.e., they are **monoclonal.**

11.4.1 Immunoglobulin structure

Immunoglobulins are glycoproteins made up **of light (L) and heavy (H) polypeptide chains**. The terms "light" and "heavy" refer to molecular weight; light chains have a molecular weight of about 25,000, whereas heavy chains have a molecular weight of 50,000-70,000. The simplest antibodymolecule has a **Y shape** and consists of four polypeptide chains: two H chains and two L chains. The four chains are linked by **disulfide bonds**. An individual antibody molecule always consists of **identical H chains and identical L chains**. This is primarily the result of two phenomena: allelic exclusion and regulation within the B cell, which ensure the synthesis of either kappa (κ) or lambda (λ) L chains but not both.

L and H chains are subdivided into **variable and constant regions**. The regions are composed of three-dimensionally folded, repeating segments called **domains**. An **L chain consists of one variable (VL) and one constant (CL) domain**. **Most H chains consist of one variable (VH) and three constant (CH) domains**. (IgG and IgA have three CH domains, whereas IgM and IgE have four.) Each domain is approximately 110 amino acids long. The **variable regions are responsible for antigen – binding, whereas the constant regions are responsible for various biologic functions**, e.g., complement activation and binding to cell surface receptors.

The variable regions of both L and H chains have three **extremely variable** ("hypervariable") amino acid sequences at the amino-terminal end that form the antigen-binding site. Only 5-10 amino acids in each hypervariable region form the antigen-binding site. Antigen-antibody binding involves electrostatic and van der Waals' forces and hydrogen and hydrophobic bonds rather than covalent bonds. The remarkable specificity of antibodies is due to these hypervariable regions.

L chains belong to one of two types, **κ (kappa) or λ (lambda),** on the basis of amino acid differences in their constant regions. Both types occur in all classes of immunoglobulins (IgG, IgM, etc), but any one immunoglobulin molecule contains only one type of L chain. **The amino-terminal portion of each L chain participates in the antigen-binding site. H chains** are distinct for each of the five immunoglobulin classes and are **designated γ, α, μ, ε, and δ.** The amino-terminal portion of each H chain participates in the antigen-binding site; the **carboxy terminal forms the Fc fragment, which has the biologic activities** described above.

If an antibody molecule is **treated with a proteolytic enzyme** such as papain, peptide bonds in the "hinge" region are broken, producing two identical Fab fragments, which carry the antigen-binding sites, and one Fc fragment, which is involved in placental transfer, complement fixation, attachment site for various cells, and other biologic activities.

11.4.2 Immunoglobulin classes

IgG

Each IgG molecule consists of two L chains and two H chains linked by disulfide bonds (molecular formula H2L2). Because it has two identical antigen-binding sites, it is said to be divalent. There are four subclasses, **IgGl-IgG4**, based on antigenic differences in the H chains and on the number and location of disulfide bonds. IgG1 makes up most (65%) of the total IgG. IgG2 antibody is directed against polysaccharide antigens and is an important host defense against encapsulated bacteria.

IgG is the predominant antibody in the secondary response and constitutes an important **defense against bacteria and viruses**. IgG is **the only antibody to cross the placenta**; only its Fc portion binds to receptors on the surface of placental cells. It is therefore **the most abundant immunoglobulin in newborns**. IgG is one of the two immunoglobulins that can **activate complement**; IgM is the other.

IgG is the immunoglobulin that **opsonizes**. It can opsonize, i.e., **enhance phagocytosis**, because there are receptors for the y H chain on the surface of phagocytes. IgM does not opsonize directly, because there are no receptors on the phagocyte surface for the γH chain. However, IgM activates complement, and the resulting C3b can opsonize because there are binding sites for C3b on the surface of phagocytes.

IgA

IgA is the main immunoglobulin in secretions such as colostrum, saliva, tears, and respiratory, intestinal, and genital tract secretions. It prevents attachment of microorganisms, e.g., bacteria and viruses, to mucous membranes. **Each secretory IgA molecule consists of two H2L2** units plus one molecule each of **J (joining) chain and secretory component**. The secretory component is a polypeptide synthesized by epithelial cells that provides for IgA passage to the mucosal surface. It also protects IgA from being degraded in the intestinal tract. In serum, some IgA exists as monomeric H2L2.

IgM

IgM is the main immunoglobulin **produced early in the primary response**. It is present as a monomer on the surface of virtually all B cells, where it functions as an antigen-binding receptor. In **serum, it is a pentamer composed of 5 H2L2 units plus one molecule of J (joining) chain**. Because the pentamer has 10 antigen-binding sites, it is **the most efficient immunoglobulin in agglutination**, **complement fixation (activation), and other antibody reactions** and is important in defense against bacteria and viruses. It can be produced by the fetus in certain infections. It has **the highest avidity of the immunoglobulins**; its interaction with antigen can involve all 10 of its binding sites.

IgD

This immunoglobulin has no known antibody function but may function as an antigen receptor; it is present on the surface of many B lymphocytes. It is present in small amounts in serum.

IgE

IgE is medically important for two reasons:

(1) it mediates immediate (anaphylactic) hypersensitivity, and

(2) it participates in host defenses against certain parasites, e.g., helminths (worms).

The **Fc region of IgE binds to the surface of mast cells and basophils.** Bound IgE serves as a receptor for antigen (allergen), and this antigen-antibody complex triggers allergic responses of the immediate (anaphylactic) type through the release of mediators. Although IgE is present in trace amounts in normal serum (approximately 0.004%), **persons with allergic reactivity have greatly increased amounts**, and IgE may appear in external secretions. IgE **does not fix complement and does not cross the placenta.**

IgE is the **main host defense against certain important helminth** (worm) infections, such as *Strongyloides, Trichinella, Ascaris*, and the hookworms. The serum IgE level is usually increased in these infections. Because worms are too large to be ingested by phagocytes, they are killed by **eosinophils** that

release worm-destroying enzymes. IgE specific for worm proteins binds to receptors on eosinophils, triggering the **antibody-dependent cellular cytotoxicity (ADCC) response.**

11.5 Antigen-Antibody Reactions in the Laboratory

Reactions of antigens and antibodies are highly specific. An antigen will react only with antibodies elicited by itself or by a closely related antigen. Because of the great specificity, reactions between antigens and antibodies are suitable for identifying one by using the other. This is the basis of serologic reactions.

However, cross-reactions between related antigens can occur, and these can limit the usefulness of the test. The results of many immunologic tests are expressed as a **titer, which is defined as the highest dilution of the specimen, e.g., serum, that gives a positive reaction in the test.**

11.5.1 Types of diagnostic tests

Many types of diagnostic tests are performed in the immunology laboratory. Most of these tests can be designed **to determine the presence of either antigen or antibody.** To do this, one of the components, either antigen or antibody, is known and the other is unknown. For example, with a known antigen such as influenza virus, a test can determine whether antibody to the virus is present in the patient's serum. Alternatively, with a known antibody, such as antibody to herpes simplex virus, a test can determine whether viral antigens are present in cells taken from the patient's lesions.

Agglutination In this test, the antigen is particulate (e.g., bacteria and red blood cells) or is an inert particle (latex beads) coated with an antigen. Antibody, because it is divalent or multivalent, cross-links the antigenically multivalent particles and forms a latticework, and clumping (agglutination) can be seen. This reaction can be done in a small cup or tube or with a drop on a slide. One very commonly used agglutination test is the test that determines a person's ABO blood group.

Precipitation (Precipitin) In this test, the antigen is in solution. The antibody cross-links antigen molecules in variable proportions, and aggregates (precipitates) form. In the zone of equivalence, optimal proportions of antigen and antibody combine; the maximal amount of precipitates forms, and the supernatant contains neither an excess of antibody nor an excess of antigen. In the zone of antibody excess, there is too much antibody for efficient lattice formation, and precipitation is less than maximal In the zone of antigen excess, all antibody has combined but precipitation is reduced because many antigen-antibody complexes are too small to precipitate; i.e., they are "soluble". Precipitin tests can be done in solution or in semisolid medium (agar).

A. Precipitation in Solution: This reaction can be made quantitative; i.e., antigen or antibody, can be measured in terms of micrograms of nitrogen present. It is used primarily in research.

B. Precipitation in Agar: This is done as either single or double diffusion. It can also be done in the presence of an electric field.

C. Precipitation in Agar With an Electric Field:

Radioimmunoassay (RIA) This method is used for the quantitation of antigens or haptens that can be radioactively labeled. It is based on the competition for specific antibody between the labeled (known) and the unlabeled (unknown) concentration of material. The complexes that form between the antigen and antibody can then be separated and the amount of radioactivity measured. The more unlabeled antigen is present, the less radioactivity there is in the complex. The concentration of the unknown (unlabeled) antigen or hapten is determined by comparison with the effect of standards. RIA is a highly sensitive method and is commonly used to assay hormones or drugs in serum. The radioallergosorbent test (RAST) is a specialized RIA that is used to measure the amount of serum IgE antibody which reacts with a known allergen (antigen).

Enzyme-Linked Immunosorbent Assay (ELISA) This method can be used for the quantitation of either antigens or antibodies in patient specimens. It is based on covalently linking an enzyme to a known antigen or antibody, reacting the enzyme-linked material with the patient's specimen, and then assaying for

enzyme activity by adding the substrate of the enzyme. The method is nearly as sensitive as RIA yet requires no special equipment or radioactive labels.

For measurement of antibody, known antigens are fixed to a surface (e.g., the bottom of small wells on a plastic plate), incubated with dilutions of the patient's serum, washed, and then re-incubated with antibody to human IgG labeled with an enzyme, e.g., horseradish peroxidase. Enzyme activity is measured by adding the substrate for the enzyme and estimating the color reaction in a spectrophotometer. The amount of antibody bound is proportional to the enzyme activity. The titer of antibody in the patient's serum is the highest dilution of serum that gives a positive color reaction.

Immunofluorescence (Fluorescent Antibody) Fluorescent dyes, e.g., fluorescein and rhodamine, can be covalently attached to antibody molecules and made visible by ultraviolet (UV) light in the fluorescence microscope. Such "labeled" antibody can be used to identify antigens, e.g., on the surface of bacteria (such as streptococci and treponemes), in cells in histologic section, or in other specimens. The immunofluorescence reaction is direct when known labeled antibody interacts directly with unknown antigen and indirect when a two-stage process is used (e.g., known antigen is attached to a slide, the patient's serum [unlabeled] is added, and the preparation is washed; if the patient's serum contains antibody against the antigen, it will remain fixed to it on the slide and can be detected on addition of a fluorescent dye-labeled antibody to human IgG and examination by UV microscopy). The indirect test is often more sensitive than direct immunofluorescence, because more labeled antibody adheres per antigenic site. Furthermore, the labeled antiglobulin becomes a "universal reagent"; i.e., it is independent of the nature of the antigen used because the antibody to IgG is reactive with all human IgG.

Complement Fixation The complement system consists of 20 or more plasma proteins that interact with one another and with cell membranes. Each protein component must be activated sequentially under appropriate conditions for the reaction to progress. Antigen-antibody complexes are among the activators, and the complement fixation test can be used to identify one of them if the other is known.

The reaction consists of the following two steps:

(1) Antigen and antibody (one known and the other unknown; e.g., use a known antigen and determine whether a patient's serum contains antibodies to that antigen) are mixed, and a measured amount of complement (usually from guinea pig) is added. If the antigen and antibody match, they will combine and take up ("fix") the complement.

(2) An indicator system, consisting of "sensitized" red blood cells (i.e., red blood cells plus anti-red blood cell antibody), is added. If the antibody matched the antigen in the first step, complement was fixed and less (or none) is available to attach to the sensitized red blood cells. The red blood cells remain unhemolyzed; i.e., the test is positive, because the patient's serum had antibodies to that antigen. If the antibody did not match the antigen in the first step, complement is free to attach to the sensitized red blood cells and they are lysed; i.e., the test is negative.

Complement must be carefully standardized, and the patient's serum must be heated to 56 °C for 30 minutes to inactivate any human complement activity. The antigen must be quantitated. The result is expressed as the highest dilution of serum that gives positive results. Controls to determine whether antigen or antibody alone fixes complement are needed to make the test results valid. If antigen or antibody alone fixes complement, it is said to be anticomplementary.

Neutralization Tests These use the ability of antibodies to block the effect of toxins or the infectivity of viruses. They can be used in cell culture (e.g., inhibition of cytopathic effect and plaque-reduction assays) or in host animals (e.g., mouse protection tests).

Immune Complexes Immune complexes in tissue can be stained with fluorescent complement. Immune complexes in serum can be detected by binding to Clq or by attachment to certain (e.g., Raji lymphoblastoid) cells in culture.

Hemagglutination Tests Many viruses clump red blood cells from one species or another (active hemagglutination). This can be inhibited by antibody specifically directed against the virus (hemagglutination inhibition) and can be used to measure the titer of such antibody. Red blood cells also can absorb many antigens and, when mixed with matching antibodies, will clump (this is known as passive hemagglutination, because the red cells are passive carriers of the antigen).

Western Blot This test is typically used to determine whether a positive result in a screening immunologic test is a true-positive or a false-positive. For example, patients who are positive in the screening ELISA for HIV infection or for Lyme disease should have a Western blot test performed. In this test, the HIV proteins are separated electrophoretically in a gel, resulting in discrete bands of viral protein. These proteins are then transferred from the gel, i.e., blotted, onto filter paper, and the person's serum is added. If antibodies are present, they bind to the viral proteins (primarily gp41 and p24) and can be detected by adding antibody to human IgG labelled with either radioactivity or an enzyme such as horseradish peroxidase, which produces a visible color change.