Chapter 31 Host-Microbe Interactions: The Process of Infection

OUTLINE **Pathogenicity, Virulence, and Infection**

Microbial Adherence
Examples of Adherence of Pathogenic Bacteria • Examples of Adherence of Viruses

Penetration of Epithelial Cell Layers
Passive Penetration into the Body • Active Penetration into the Body

Events in Infection Following Penetration
Growth in Underlying Tissues • Infection of the Lymphatic System • Infection of the Blood

Microbial Virulence Factors
Antiphagocytic Factors • Exotoxins • Endotoxins • Other Virulence Factors

Infectious diseases occur as the result of interactions between **pathogenic** (disease-producing) microorganisms and the host. All infectious diseases begin at some surface of the host, whether it be the external surfaces such as the skin and conjunctiva or internal surfaces such as the mucous membranes of the respiratory tract, intestine, or urogenital tract. Many pathogens can selectively attach to particular host surfaces. In most infectious diseases the pathogenic microorganism penetrates the body surface and gains access to the internal tissues. In some kinds of infections the pathogen may remain **localized**, growing near its point of entry into the body. In other instances it may be transported to some other body site. Some pathogens may cause **generalized** infections, in which the microorganism becomes widely distributed and grows throughout the body. Some pathogens may be capable of growth within the cells of the host, causing severe disruption of normal physiological processes. In other infections the pathogen may grow extracellularly; here, damage to body cells usually occurs as the result of elaboration of poisonous substances **(toxins)** by the microorganism.

If a host is to recover from an infection, it must eradicate the pathogenic microorganisms. However, as a group, pathogenic microorganisms exhibit a vast array of weapons, termed **virulence factors**, that can combat the various defense mechanisms of the host; each species of pathogen possesses only one or a few of these factors. Thus, an infection represents a battle between the defenses mounted by the host and the particular armamentarium of virulence factors

produced by the pathogen. Often the infection proves lethal to the host; however, it is to the microbe's advantage if the battle is somewhat indecisive, i.e., if the disease the pathogen causes is not so severe as to kill the host. Killing the host would diminish the pathogen's chance of survival; consequently, host-microbe interactions that result in **chronic**, long-lasting infections are regarded as being more highly evolved than interactions that are **acute**, i.e., have a short and relatively severe course.

PATHOGENICITY, VIRULENCE, AND INFECTION

Pathogenicity is the capability of a microbial species to cause disease. However, various strains of a pathogenic species may differ with regard to their degree of pathogenicity, i.e., with regard to their virulence. For instance, some strains are highly virulent: only a few bacterial cells from a highly virulent strain are needed to cause disease in a host. Other strains may be less virulent, and larger numbers of cells of such strains are needed to cause the disease. Some strains may be avirulent, incapable of causing the disease even when large numbers of cells are inoculated into the host. Virulent strains of many pathogens, when repeatedly cultured on laboratory media or grown in vivo in hosts other than their normal hosts, may lose their virulence: such avirulent strains are called **attenuated** strains and are widely used as vaccines to elicit immunity to various diseases.

The virulence of a pathogen is usually measured by determining its LD_{50} dose for a particular type of laboratory animal. The LD_{50} dose is defined as that number of organisms which, when administered to a number of laboratory animals, will kill 50 percent of them. For example, an LD_{50} dose of 10 cells of strain X compared with 100,000 cells of strain Y would indicate that X is 10,000 times more virulent than Y. The LD_{50} dose can be determined more precisely than other endpoints such as the dose that kills 100 percent of the animals (LD_{100} dose, sometimes also termed **minimum lethal dose** or **MLD**) because the rate of change in mortality versus change in dose is greatest around the point of 50 percent mortality.

Infection represents the most intimate way in which a microorganism may cause disease: the host is invaded by the microorganisms which subsequently multiply in close association with the host's tissues. Most, but not all, microbially caused diseases are infections. An example of one that is not is a type of food poisoning called botulism, in which there is no invasion of the body by the causative microorganism; rather, the disease is contracted by ingesting the poison (toxin) in a food in which the bacterium *Clostridium botulinum* has previously grown.

In order to cause infectious disease a pathogen must accomplish the following:

1 It must enter the host.
2 It must metabolize and multiply on or in the host tissue.
3 It must resist host defenses (see Chaps. 32 and 33).
4 It must damage the host.

Each process is complex, and all four processes must be fulfilled to produce infectious disease. Some infections may result in only a very minor amount of damage to the host, so minor that there are no detectable clinical symptoms of

Table 31-1. Some Types of Infections

Term	Definition	Example
Acute	Has a short and relatively severe course	Streptococcal pharyngitis (sore throat caused by *Streptococcus pyogenes*)
Chronic	Has a long duration	Tuberculosis
Fulminating	Occurs suddenly and with severe intensity	Cerebrospinal meningitis caused by *Neisseria meningitidis*
Localized	Restricted to a limited area of the body	Urinary tract infection caused by *Escherichia coli*
Generalized	Affects many or all parts of the body	Blood infections, such as typhoid fever
Mixed, or polymicrobial	More than one kind of microorganism contributes to the infection	Gaseous gangrene, in which a combination of *Clostridium* species may occur
Primary	An initial localized infection that decreases resistance and thus paves the way for further invasion by the same microorganism or other microorganisms	Viral influenza
Secondary	Infection that is established after a primary infection has caused a decreased resistance	Pneumococcal pneumonia following viral influenza

the infection; such infections are called subclinical infections. Other infections vary in regard to severity, location, and the number of microbial species involved (see Table 31-1).

MICROBIAL ADHERENCE

Unless a pathogen is introduced directly into the tissues (as by a wound, injection by an arthropod, or other similar means), the first step in initiation of infection is usually adherence or attachment of the pathogen to some surface of the host. As indicated in Chap. 30, such surfaces represent hostile environments and the microorganism must compete with normal flora organisms for surface attachment. Moreover, the attachment is selective: various pathogens attach only to certain tissues. For most pathogens, the precise means of attachment are not yet understood, particularly for pathogenic fungi and protozoa.

Examples of Adherence of Pathogenic Bacteria

Neisseria gonorrhoeae, the causative agent of gonorrhea, adheres specifically to the epithelial cell layer of the human cervix, urethra, and conjunctiva by means of pili and thus avoids being washed away by the flow of mucus or tears. *Escherichia coli* strains that cause "scours," a diarrheal disease of newborn pigs, also possess pili that allow the bacteria to attach firmly to the mucosal lining of the small intestine. *Vibrio cholerae* adheres to the epithelial layer of the small intestine of humans (see Fig. 31-1); although the bacterial surface component responsible for the attachment is not yet certain, it may be a hemagglutinin (so named because it also permits attachment to erythrocytes in laboratory experiments). In another example, certain proteins located on the outer surface of the bacterial cell wall have been shown to be essential for the initiation of infection. For instance, *Streptococcus pyogenes*, the causative agent of streptococcal sore

Figure 31-1. Scanning electron micrograph of *Vibrio cholerae* attached to the microvilli of a brush border membrane isolated from the small intestine of a rabbit (X10,000). *(Courtesy of G. W. Jones, G. D. Abrams, and R. Freter, Infect Immun* **14**:*232-239, 1976.)*

throat, attaches specifically to the epithelial cells of the throat by means of cell-wall proteins called **M proteins.**

Examples of Adherence of Viruses

The surface of influenza virus particles is studded with hemagglutinin spikes that can cause attachment of the virus to specific mucoprotein receptors on the surface of host cells. Neuraminidase spikes on the virion surface also may possibly aid attachment by degrading the protective mucus layers of mucous membranes and allowing viral attachment to the underlying epithelial cells. Another example is a protein on the surface of poliovirus, which seems to be critical for attachment of the virus to lipid- and glycoprotein-containing receptors on host cells; the attachment is specific for cells of the intestinal tract and the central nervous system, and subsequent infection of the latter can lead to paralysis. In this regard, it is interesting that the attenuated strains of poliovirus used for vaccination against poliomyelitis (see Chap. 37) can attach to the gastrointestinal tract as the wild-type poliovirus does; however, because of mutation in the genes for the viral surface proteins, these attenuated strains have lost the ability to attach to cells of the central nervous system and thus do not cause the paralysis that is characteristic of poliomyelitis.

PENETRATION OF EPITHELIAL CELL LAYERS

Although penetration of the epithelial layer follows adherence in most infections, this is not always a prerequisite to infection. The microorganism may merely multiply on the epithelial surface and cause damage without penetration into the body. For example, *V. cholerae*, the causative agent of the severe diarrheal disease known as cholera, multiples on the epithelial layer of the small intestine where it produces a toxin that causes the loss of fluid from the epithelial cells and kills the cells.

Passive Penetration into the Body

It should be emphasized that penetration of body surfaces may be achieved not only **actively** (i.e., by the adherence and penetration mechanisms of the pathogen itself) but also **passively**, by mechanisms having nothing to do with the properties of the microorganism. Any mechanically caused breach in the body surfaces can introduce pathogens directly into the underlying tissues. Wounds or burns represent one passive mechanism. For example, soldiers wounded on the battlefield may develop gas gangrene if the wound becomes contaminated

by *Clostridium perfringens* present in soil and fecal matter. Burns often become infected by *Pseudomonas aeruginosa* or other aerobic or facultatively anaerobic bacteria from the surrounding environment. Another mode of passive penetration is by arthropods. For example, *Borrelia* species cause relapsing fever in humans when the spirochetes are introduced through the bite of a tick or a body louse.

Active Penetration into the Body

Some pathogenic microorganisms are capable of penetrating the epithelial layer to which they have become attached. For example, in bacillary dysentery, *Shigella* bacteria penetrate into and kill the epithelial cells of the colon, then spread to adjacent cells, which are in turn killed. The result is the formation of lesions (areas of damage) known as **ulcers**, i.e., areas on the intestinal wall which have disintegrating or necrotic (dead) tissue. In another example, the influenza virus penetrates the epithelial cells lining the nasopharynx, trachea, and bronchi. The virus then undergoes replication, and new viral progeny are subsequently liberated from the infected cell. The severity of influenza depends mainly on the degree of host cell destruction during viral multiplication.

After penetration through or between the epithelial cells, some pathogens may penetrate into the deeper tissues of the body and may even become widely disseminated throughout the body, particularly if the organism obtains access to the lymphatic system or the blood vascular system.

EVENTS IN INFECTION FOLLOWING PENETRATION

Whether the means of penetration of a body surface by a microorganism is passive or active, the microorganism multiplies, resists the defense mechanisms of the host, and begins to cause tissue damage.

Growth in Underlying Tissues

In some infections the microorganism may simply grow in the tissue in which it finds itself, causing a localized infection. An example is the type of infection caused by *Staphylococcus aureus*, where the characteristic lesion is an **abscess**, i.e., a walled-off cavity in the tissues containing the staphylococci, numerous white blood cells (that collectively form a pasty mass called pus), and dead, disintegrating tissue cells that have been killed by the toxins elaborated from the staphylococci.

In other infections the organism may not remain localized but may spread through the tissues. An example is the anaerobic bacterium *C. perfringens*, which causes the wound infection gas gangrene. Initiation of gas gangrene depends on the occurrence of anaerobic conditions in the wound, as occurs in crushed tissue or clotted blood. As *C. perfringens* begins to grow, the bacteria elaborate toxins that kill some of the surrounding healthy tissue. This dead tissue becomes anaerobic and can support the growth of more clostridia, which in turn elaborate more toxins that kill more tissue and allow the organisms to spread further. Another factor that contributes to the rapidity of tissue invasion is the production of large amounts of hydrogen gas by the bacteria; the pressure of this gas separates connective tissue sheaths from muscle tissue, forming a space that can rapidly be filled in by clostridia-containing fluid. By this means the clostridia can quickly invade the entire length of a muscle. Amputation of an affected limb is often the only way to stop the spread of *C. perfringens* to the rest of the body.

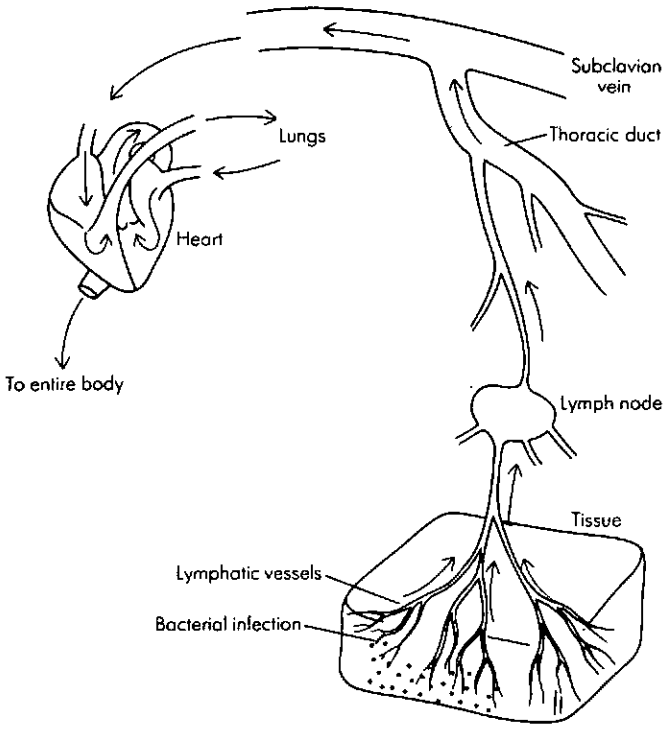


Figure 31-2. Schematic diagram illustrating how infection of the lymphatic system can lead to a bacteremia and generalized infection. Microorganisms infecting a tissue may gain access to thin-walled, finely branched lymphatic vessels which collect excess tissue fluid. The organisms may then pass via the lymphatic vessels to a regional lymph node. If the defenses of the lymph node are overwhelmed, the microorganisms may multiply and then pass to the thoracic duct, which empties lymph into the venous blood circulation via the subclavian vein. Thus the organisms reach the heart and, ultimately, are eventually distributed throughout the body.

Infection of the Lymphatic System

Many pathogenic microorganisms can spread from the initial site of infection to the lymphatic system. Body tissues are permeated by a network of blood capillaries which supply oxygen and nutrients to tissue cells. Capillaries have very thin walls, and some of the fluid portion of the blood leaks out into the tissues. Such fluid would cause tissues to become swollen unless it is returned to the blood vascular system. The function of the lymphatic vascular system is to collect this fluid by means of thin-walled lymphatic vessels (see Fig. 31-2) and return it to the blood vascular system. The fluid within the lymphatic vessels, called **lymph**, passes to **lymph nodes** (Fig. 31-2). These are ovoid structures ranging in size from one to several millimeters and are widely distributed throughout the body. Lymph enters a lymph node by any of several lymphatic vessels, passes through the lymph node along tortuous, winding channels, and emerges via a single efferent lymph vessel. From here it passes into larger and larger vessels (lymphatic trunks) and eventually reaches the main lymphatic ducts, which drain into the great veins of the blood vascular system (Fig. 31-2).

Microorganisms present in tissues may also be collected by lymphatic vessels and can infect them. For instance, in erysipelas, an inflammatory disease of the skin caused by *S. pyogenes*, the painful lesions are spread by invasion of the lymphatic system of the subepidermal tissue.

Pathogenic microorganisms may also be carried by lymphatic vessels to lymph nodes. The winding channel within a node is lined with cells called **macro-**

phages, which can engulf and destroy the bacteria that enter the lymph node (see Phagocytosis, Chap. 32). Thus the lymph node removes nearly all of the bacteria that enter it, and it may become inflamed, enlarged, and sore when infected; in this condition it is sometimes called a **bubo**. In bubonic plague, such swollen lymph nodes are particularly prominent (hence the name of the disease). The exudates (fluids) draining from such buboes are filled with plague bacilli.

Infection of the Blood

From the initial site of entry into the body by passive or active means, a pathogenic microorganism may be able to enter a blood capillary or venule and thereby gain direct access to the blood vascular system, causing a **bacteremia** (presence of bacteria in the blood). Once in the bloodstream the organism can be carried to various parts of the body and cause localized infections. For instance, *Neisseria meningitidis* present in the nasopharynx can reach the **meninges** (membranes that cover the brain and spinal cord) by means of a transient bacteremia, and can thereby cause a severe meningitis. By means of a bacteremia, spirochetes of the genus *Leptospira* can reach the kidneys, where they may eventually cause acute renal failure.

In other instances a pathogenic microorganism may gain access to the bloodstream directly by *first infecting the lymphatic system.* If the defenses of a lymph node are overwhelmed, the organisms may appear in the efferent lymph and ultimately reach the bloodstream. For example, in typhoid fever, *Salmonella typhi* organisms first attach to the epithelium of the small intestine; they penetrate this layer by passing through the epithelial cells, infect the **lamina propria mucosae** (the underlying connective tissue layer), enter the lymphatic system, and reach the mesenteric lymph nodes. Here, the organisms are not easily destroyed by the macrophages of the lymph node; they multiply and eventually begin to enter the blood circulation, causing a bacteremia. The bacteria eventually localize in various parts of the body, particularly in the macrophages of the liver and spleen, and they also reinfect various lymph nodes, since these are nourished by the blood.

In some infections, bacteria may actively multiply in the bloodstream and produce toxic products—a condition known as **septicemia**. Septicemic infections range from chronic to acute. One of the most severe is anthrax, a disease of animals and sometimes humans, in which the number of *Bacillus anthracis* organisms may often exceed the number of erythrocytes in the blood! Septicemic infections often begin as localized infections that later become generalized; for example, streptococcal pharyngitis, a staphylococcal abscess, and bubonic plague may all subsequently give rise to a septicemia.

MICROBIAL VIRULENCE FACTORS

Antiphagocytic Factors

The virulence of many pathogenic bacteria is influenced by the presence or absence of a nontoxic polysaccharide material composing the **capsules** surrounding the cells. Such capsules can prevent the engulfment and destruction of the bacteria by the phagocytic defense mechanisms of the body (see Chap. 32). The importance of capsules can be demonstrated with *Streptococcus pneumoniae*; capsulated cells are virulent, but mutant cells that can no longer make capsules are avirulent.

Other bacteria that produce capsular material with a direct bearing on virulence are *Haemophilus influenzae*, *Klebsiella pneumoniae*, *N. meningitidis*, and *B. anthracis*. The capsule of *B. anthracis* is unusual in that it is composed of a polypeptide rather than a polysaccharide.

Capsular polysaccharides can be isolated in pure form. For instance, when the capsular polysaccharides of pneumococci are injected into humans, they stimulate the production of antibodies that protect against the type of pneumococcus from which they were obtained. This is the basis of the vaccine that protects against pneumococcal pneumonia.

Some antiphagocytic factors made by pathogenic bacteria are not capsules, yet like capsules they are located on the outer surface of the bacterial cell wall. One example is the M protein of *S. pyogenes* (this protein is also responsible for adherence of the organisms to epithelial cells and has been mentioned previously in this chapter).

Exotoxins

Some microorganisms produce poisonous substances known as **toxins**. Their potency can be expressed in various ways: the most precise is in terms of the LD_{50} dose (the dose that kills 50 percent of test animals that are injected with the toxin), but MLD (minimum lethal dose or LD_{100} dose, the dose that kills 100 percent of test animals) is also frequently used. Toxins can be divided into two main categories, **exotoxins** and **endotoxins**. These are differentiated by the properties listed in Table 31-2.

Potency of Exotoxins

Exotoxins are toxic proteins that are secreted by living microorganisms. Some exotoxins have extraordinarily high potency, with only minute amounts being needed to kill animals. For example, *Clostridium botulinum* type A produces the most potent toxin known: 1 MLD for a mouse is 2.5×10^{-5} μg of the purified toxin, about 1 million times more toxic than strychnine. The toxin of *Clostridium tetani* is also highly potent, the MLD for a mouse being 4×10^{-5} μg. The high potency of these two toxins is attributable to their action on the mammalian nervous system. Most toxins affect other kinds of tissues and are less potent; for example, the MLD of diphtheria toxin for a guinea pig is 6×10^{-2} μg, and the MLD of the α toxin of *S. aureus* is 5 μg for a rabbit.

Table 31-2. Some Characteristics of Exotoxins and Endotoxins

Feature	Exotoxins	Endotoxins
Bacterial source	Secreted by living microorganisms	Released from cell walls of lysed Gram-negative bacteria
Chemical nature	Protein	Lipopolysaccharide
Heat tolerance	Inactivated easily by boiling; heat-labile	Will withstand autoclaving; heat-stable
Immunology	Can be converted to toxoids and readily neutralized by antitoxin	Cannot form toxoids; neutralization with antitoxin not possible or only with difficulty
Pharmacology	Each has a highly characteristic mechanism of action	All act similarly to cause their effects; action characterized by pyrogenicity, blood changes, and shock
Lethal dose	Small	Much larger

Fungi:

a *Histoplasma capsulatum*, the causative agent of histoplasmosis, showing macroconidia.

b *Sporotrichum schenkii*, the causative agent of sporotrichosis, as seen in a lacto-phenol blue stained preparation.

c *Candida albicans*, causative agent of thrush, monilia vaginitis, and other infections, showing chlamydospores.

d *C. albicans*, A Gram stain preparation of specimen from patient.

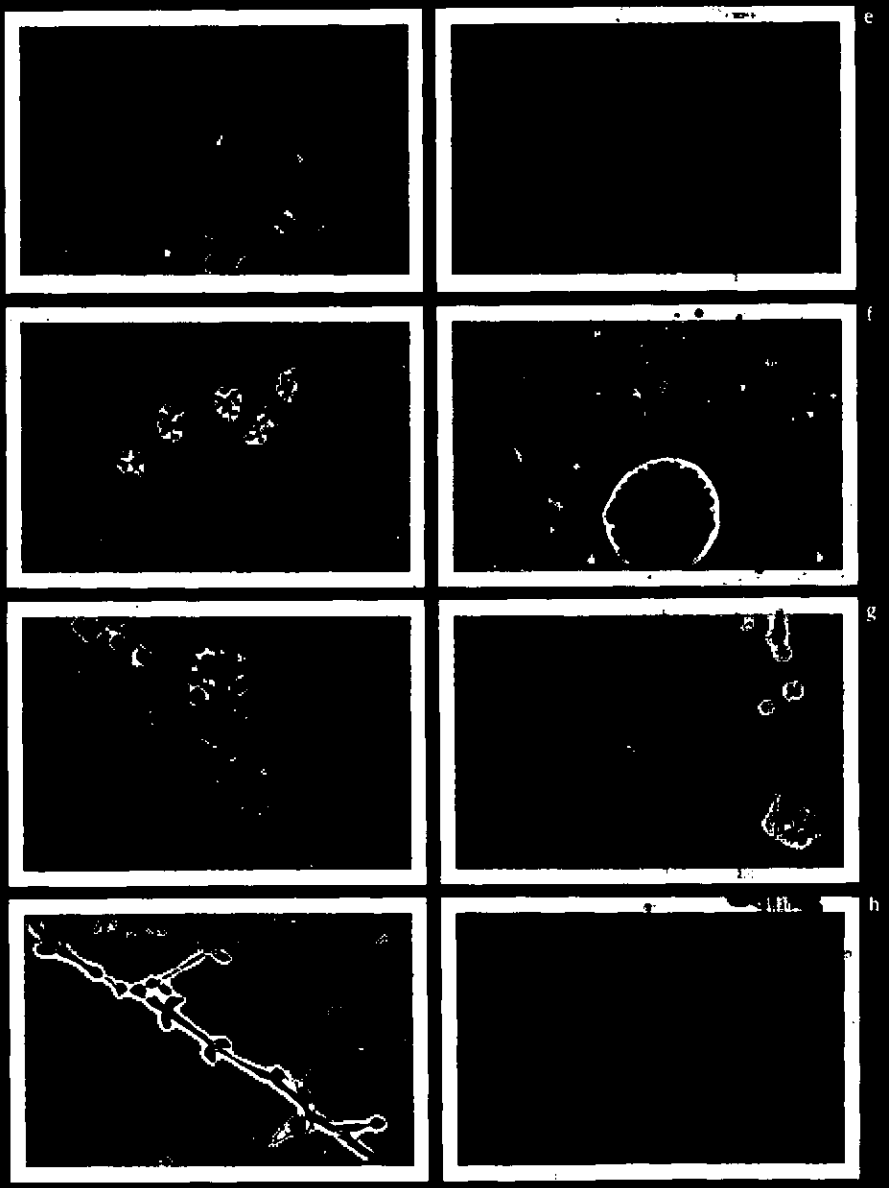
e *Coccidioides immitis*, causative agent of coccidioidomycosis, showing arthrospores.

f Spherules of *C. immitis* in a tissue section from a patient with coccidioidomycosis

g *Blastomyces dermatitidis*, causative agent of blastomycosis, showing yeast phase

h *B. dermatitidis*, mycelial phase with conidia.

(Courtesy of National Medical Audiovisual Center, Centers for Disease Control, Atlanta, Georgia.)



Virus infections of plants:

a Aster "Blue Boy" [*Callistephus chinensis* (L.) Nees.] systemically infected with tobacco rattle virus (aster ringspot strain).

b Leaf of *Gomphrena globosa* L. six days after inoculation with tobacco necrosis virus.

c Veinbandung symptom of grapevine fanleaf virus in *Vitis vinifera* var. Gewürztraminer.

d Tobacco leaf *Nicotiana tabacum* L. 19 days after infection with the apricot strain of tomato ringspot virus.

e Necrotic ringspots on a *Grammatophyllum scriptum* orchid leaf infected with a bacilliform virus. (Rhabdovirus)

f Flower of Cattleya orchid exhibiting necrosis resulting from Cymbidium mosaic virus infection.

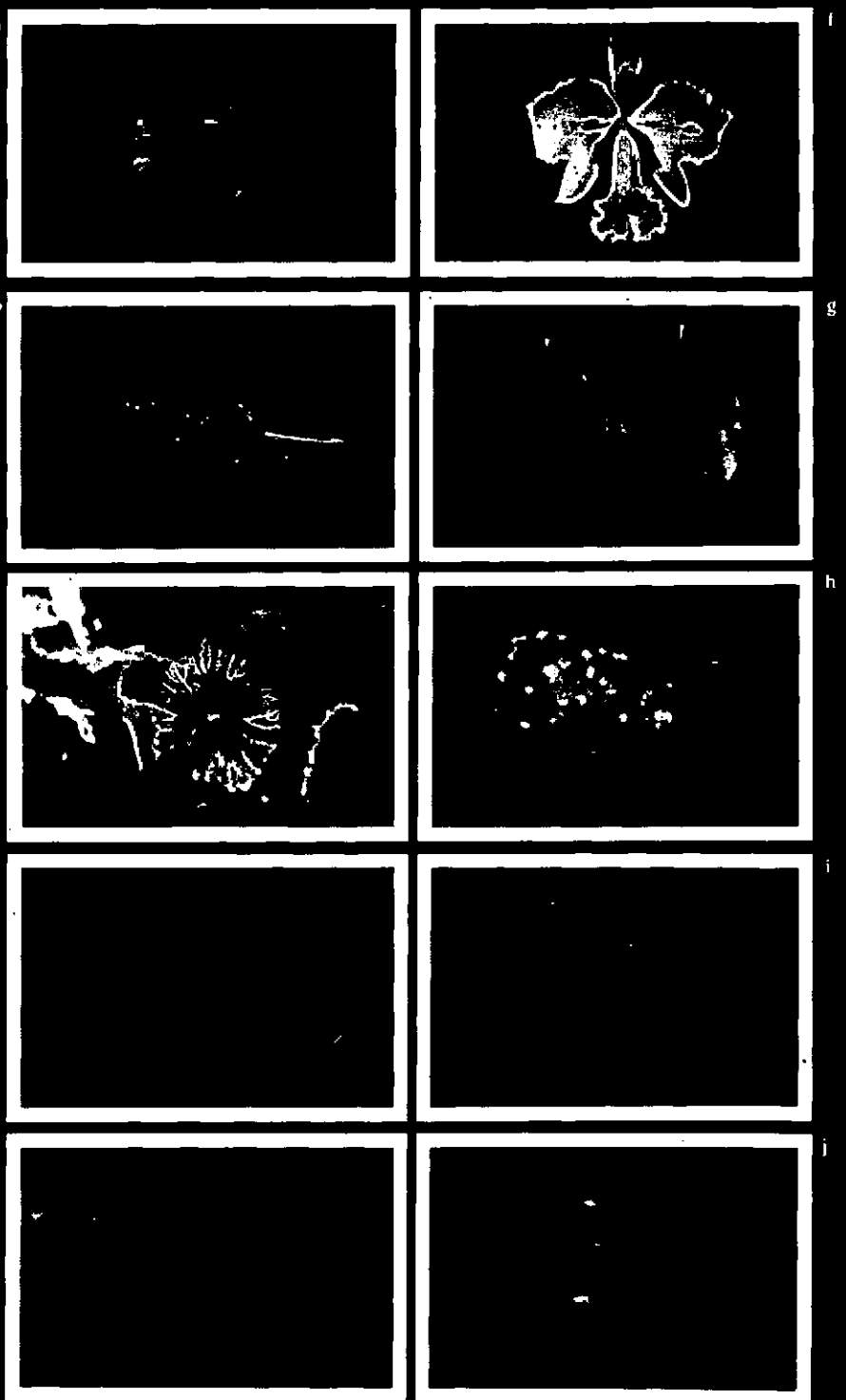
g Chlorotic ringspots on leaves of *Euonymus fortunei* (Turcz.) Hand.-Mazz. var. *vegetus* inoculated with the euonymus strain of tomato ringspot virus.

h Fruit symptoms (green) on summer crook-neck squash (*Cucurbita pepo* L.) infected with watermelon mosaic virus.

i Leaf of *Nicotiana glutinosa* L. 4 days after inoculation with tobacco mosaic virus.

j Flower of tulip (*Tulipa gesneriana* L.) var. Darwin exhibiting color break resulting from infection by color-breaking virus.

(Courtesy of Kenneth M. Corbett, University of Maryland.)



Not all exotoxins are lethal; some merely cause unpleasant effects. For example, many strains of *S. aureus* produce a toxin which, when ingested by a human in quantities of as little as 1 μg, gives rise to severe nausea and vomiting (staphylococcal food poisoning). Although this type of food poisoning is most unpleasant, few if any victims have died from it.

Classification of Exotoxins

Exotoxins are often divided into various categories based on the site of the damage that they cause or the kind of cells that are affected. For instance, botulinum and tetanus toxins affect nerve tissue and are termed **neurotoxins**. The toxin made by *V. cholerae* affects the intestinal tract and is termed an **enterotoxin** (from the Greek word *enteron*, meaning "intestine"). Diphtheria toxin kills several different kinds of cells and is thus termed a **cytotoxin**. Some cytotoxins may kill leukocytes and hence are known as **leukocidins**; some may cause the lysis of red blood cells and therefore are termed **hemolysins**.

Toxoids

Exotoxins are proteins. They lose their toxicity when treated with formaldehyde, although their antigenic properties are retained; in this form they are called **toxoids** and have the ability to stimulate the production of **antitoxins** (antibodies that react with toxins and neutralize them) in the body of the host animal. This is important in the protection of susceptible hosts from diseases caused by bacteria that produce exotoxins. For instance, toxoids are widely used as vaccines for immunization against tetanus and diphtheria.

Role of Bacteriophages and Plasmids

The ability of a bacterium to make a particular exotoxin (**toxigenicity**) may be due to a chromosomal gene. However, in some instances, such as diphtheria toxin or certain types of botulism toxin, toxigenicity can be conferred on a bacterium as the result of acquiring a temperate bacteriophage that carries the gene for the toxin. This process, called **lysogenic conversion**, has been described in Chap. 20. Bacteria can also become toxigenic by acquiring a plasmid that carries the gene for a toxin. For example, some strains of *E. coli* are able to cause diarrhea because they contain a plasmid whose DNA codes for an enterotoxin. Another plasmid-mediated toxin is the enterotoxin made by food-poisoning strains of *S. aureus*.

Mechanisms of Action of Exotoxins

It is becoming increasingly apparent that, just as adherence of microorganisms to tissue cells is an important first step in the process of infection, *an initial adherence of exotoxins to tissue cells* is an important first step in their toxic activity. The function of attachment is usually assumed by a particular region or subunit of the toxin. Other regions or subunits of the toxin may have enzymatic activity that causes damage to the tissue cell. The mechanisms of action of some exotoxins are well understood; in other instances they are completely unknown.

Diphtheria Toxin

This toxin consists of a single polypeptide chain which consists of two regions, A and B. By means of the B region the toxin becomes bound to the membrane of a tissue cell. The A region is then cleaved from the B region and enters the cell (Fig. 31-3). The A fragment has enzymatic activity and catalyzes a reaction that inactivates **elongation factor 2 (EF-2)**, a factor essential for the elongation

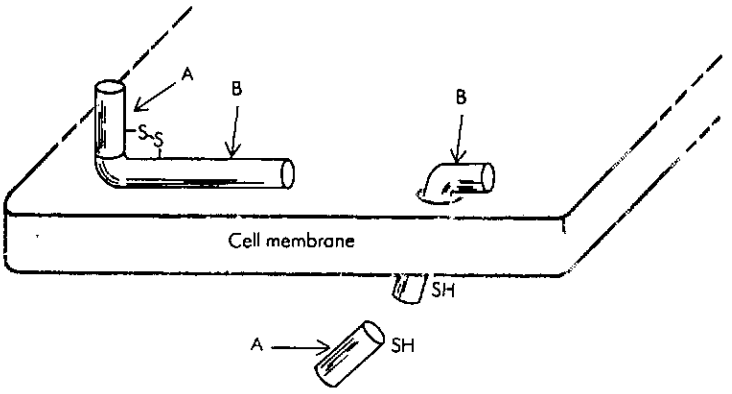


Figure 31-3. Hypothetical model showing certain features of diphtheria toxin. The toxin consists of a single polypeptide composed of two regions, A and B. A and B are linked not only by a peptide linkage but also by a disulfide (—S—S—) bridge. The B portion binds to the membrane of a tissue cell. The B portion then allows the A portion to penetrate the membrane. During this transport process the A fragment is liberated by cleavage of the peptide linkage and by reduction of the disulfide bridge. The A fragment then proceeds to inhibit protein synthesis within the host cell.

of growing polypeptide chains during protein synthesis by ribosomes. As a result, the tissue cell can no longer make proteins, and the cell eventually dies. Diphtheria antitoxin, i.e., antibodies formed against the toxin, can neutralize the toxicity of diphtheria toxin. More specifically, the antitoxin reacts with the B portion of the toxin, preventing the toxin from attaching to tissue cells. If attachment of the toxin to a host cell has already occurred, however, antitoxin cannot neutralize the toxin. Only toxin that has not yet attached can be neutralized by antitoxin. This is why prompt administration of antitoxin is required in cases of diphtheria: This principle applies to many other exotoxins as well.

Botulinum Toxin

In the normal operation of a voluntary muscle, a nerve impulse sent along the axon of a motor neuron reaches the **neuromuscular junction**, i.e. the junction between the axon and the muscle; secretion of acetylcholine by the end feet of the terminal twigs of the axon then initiates contraction of the muscle. In botulism, the toxin binds to the axon near the neuromuscular junction and prevents the secretion of acetylcholine; thus the muscle cannot contract. If this paralysis extends to the muscles of the chest and diaphragm, death by respiratory failure can result.

Tetanus Toxin

It is common to find that muscular movement involves cooperative action between a set of two opposing muscles. For example, in order to raise one's arm, the biceps muscle must contract and at the same time the opposing muscle, the triceps, must be allowed to stretch. As the triceps begins to stretch, however, stretch receptors in the muscle automatically send signals to the central nervous system that stimulate the firing of the motor neurons controlling the triceps. Thus it is necessary to render those neurons temporarily insensitive to stimulation. This is normally accomplished in the central nervous system by the action of a chemical called **inhibitory transmitter**, viz., the amino acid glycine. In tetanus, the toxin acts on the central nervous system to prevent the release of the glycine, and the result is that opposing sets of muscles often contract simultaneously and in uncontrollable fashion, giving rise to characteristic tetanic spasms that may be powerful enough to break bones and tear tissue. Death usually results from respiratory failure, i.e., an inability to control the muscles of the chest and diaphragm that are involved in breathing.

Cholera Toxin

The toxin consists of *one A subunit* and *five B subunits*. The B subunits are responsible for attachment of the toxin to the surface of the epithelial cells of the small intestine. The A subunit then penetrates the cell membrane and is cleaved to yield a fragment, A_1. This fragment causes an alteration of the regulatory protein that governs the activity of the enzyme **adenylate cyclase** in the cell. The modified regulatory protein becomes "turned on" permanently, causing adenylate cyclase to convert ATP to high levels of **cyclic AMP (cAMP)**:

$$\text{ATP} \rightarrow 3',5'\text{-cAMP} + \text{pyrophosphate}$$

The high cAMP level in turn causes a loss of water and electrolytes from the intestinal cells into the lumen of the intestine, i.e. diarrhea. The result of this sequence of events is dramatic: *as much as 10 to 12 liters of fluid may be excreted per day by a patient*. Ultimately the blood is the source of this fluid. Since bicarbonate ions are lost from the blood, the pH of the blood falls, which may lead to death from acidosis. Moreover, as the diarrhea proceeds, proteins and red cells become increasingly concentrated in the blood **(hemoconcentration)**, leading eventually to hypovolemic shock and circulatory collapse. The treatment of cholera is to replace the fluids and electrolytes intravenously in amounts equivalent to those being lost (Fig. 31-4).

Streptolysin O (SLO)* and Streptolysin S (SLS)

SLO is produced by *Streptococcus pyogenes* and is inactivated by oxygen (hence the "O" in its name). It has multiple activities based on its ability to damage cell membranes. For example, it is a hemolysin, causing β hemolysis around the colonies on blood-agar plates incubated anaerobically. In infections, however, this hemolytic action is much less important than its action as a leukocidin. The streptococci do not need to be ingested by a leukocyte in order to kill it: the soluble toxin penetrates the leukocyte from without and damages the membranes of cytoplasmic granules **(lysosomes)** within the cell. The powerful hydrolytic enzymes contained within the lysosomes are liberated into the cyto-

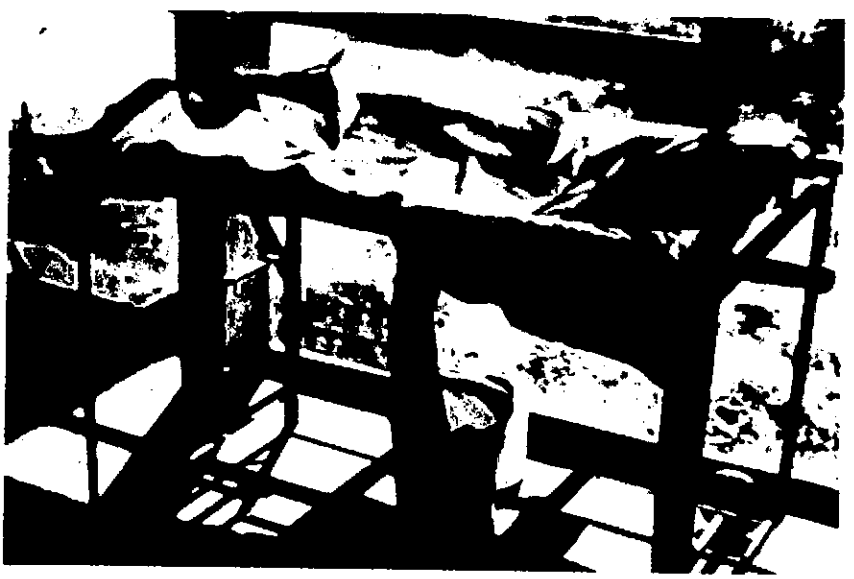


Figure 31-4. A cholera cot as used for the treatment of cholera patients in Bangladesh. It is a simple and vet effective device to collect diarrheal stools, while the patient is kept comfortable and clean. The loss of fluid can also be measured roughly using the collecting bucket so that the same fluid volume can be replaced. *(Courtesy of R. Oseasohn, McGill University.)*

plasm and proceed to destroy the phagocyte. When injected intravenously into rabbits, SLO also acts as a **cardiotoxin**, causing complete disruption of the cardiac pumping cycle within seconds; whether this cardiotoxic activity contributes to human infections is not known.

SLS is also produced by *S. pyogenes* and kills leukocytes in the same manner as SLO. However, SLS is not soluble but rather is bound to the bacterial cells; thus it kills leukocytes only after the bacteria have been engulfed. SLS is oxygen-stable (hence the "S" in the name). Thus, unlike SLO, SLS can cause β hemolysis on *aerobically incubated* blood-agar plates.

Other Exotoxins

Examples of several other exotoxins and their modes of action are indicated in Table 31-3.

Endotoxins

As discussed in Chap. 5, endotoxins are lipopolysaccharides located in the

Table 31-3. Additional Examples of Exotoxins

Toxin	Organism	Action	Mechanism
Clostridial α toxin	*Clostridium perfringens*	Hemolysin, cytotoxin	Phospholipase; hydrolyzes lecithin, thereby damaging the cytoplasmic membranes of blood and tissue cells
Staphylococcal α toxin	*Staphylococcus aureus*	Hemolysin, leukocidin, cytotoxin	Mechanism uncertain; may be due to disruption of hydrophobic regions of cytoplasmic membranes or conversion to an active protease
Staphylococcal enterotoxin	*S. aureus*	Enterotoxin, causing nausea and vomiting (staphylococcal food poisoning)	Mechanism unknown
Panton-Valentine factor	*S. aureus*	Leukocidin	Causes degranulation of leukocytes
LT toxin	*Escherichia coli*	Enterotoxin, causing diarrhea	Mechanism similar to that of cholera toxin (see text)
Exotoxin A	*Pseudomonas aeruginosa*	Cytotoxin	Mechanism similar to that of diphtheria toxin (see text)
Anthrax toxin	*Bacillus anthracis*	Possibly a neurotoxin	Mechanism unknown; the toxin probably acts on the central nervous system, causing respiratory failure; the toxin consists of three distinct proteins: EF, PA, and LF; no single one of these is lethal, but a combination of all three, or even just PA + LF, is lethal
Pertussis toxin (pertussigen)	*Bordetella pertussis*	Cytotoxin	Mechanism unknown; causes increased sensitivity to histamine, causes an increase in the number of leukocytes in the blood, and causes hypoglycemia (subnormal levels of glucose in the blood)
Erythrogenic toxin	*Streptococcus pyogenes*	Cytotoxin, causing red rash in scarlet fever	Damages small blood vessels by an unknown mechanism; also kills macrophages, is pyrogenic (fever-inducing), and enhances susceptibility to endotoxic shock

outer membrane of the cell wall of many Gram-negative bacteria. The presence of an endotoxin in the medium in which Gram-negative bacteria are grown is due to lysis of some of the cells, which may occur during late growth stages of the culture. In Table 31-2 the general properties of endotoxins are differentiated from those of exotoxins. It should be noted that much larger quantities of endotoxins are required to kill experimental animals. All endotoxins exhibit similar pharmacologic effects, which may be described as follows:

1 ***Pyrogenicity.*** This is the ability to cause a change in body temperature. In humans, endotoxins cause an *increase* in temperature (i.e., a fever response). The pyrogenic effect is indirect: the active chemical agent that causes the temperature change is an endogenous pyrogen that is released from the blood leukocytes under the influence of endotoxins. This pyrogen affects the hypothalamus of the brain, which regulates body temperature.
2 ***Blood changes.*** When administered to experimental animals, endotoxins first cause a temporary decrease, and later a marked increase, in the number of leukocytes in the blood. Endotoxins also damage blood platelets (thrombocytes), which release factors that may cause blood to clot within blood vessels (intravascular clotting). Moreover, endotoxins cause an increase in the permeability of blood capillaries, causing them to leak the fluid portion of the blood, and sometimes even whole blood (hemorrhage); these effects can cause serious changes in circulation and blood pressure.
3 ***Shock.*** When Gram-negative bacteria are present in the blood in large numbers, or when endotoxin is injected intravenously, severe shock may occur as evidenced by a decreased blood pressure, feeble rapid pulse, decreased respiration, and sometimes unconsciousness. A high dose can result in circulatory collapse and death.

A sensitive test has been devised for the presence of minute amounts of endotoxin in body fluids, as an aid to the early diagnosis and treatment of Gram-negative bacterial infections. The test is based on the ability of endotoxin to cause gelling of extracts of the amoebocytes (blood cells) of the horseshoe crab, *Limulus polyphemus*.

Other Virulence Factors

A number of factors produced by pathogenic bacteria or occurring as part of their cellular structure may contribute to virulence, although they are not toxins. They may enhance yirulence by aiding the spread of the pathogens through tissues, by enhancing abscess formation, by causing tissue damage, or by allowing pathogens to compete more effectively with a host for an essential nutrient. Some of these factors are described below.

Hyaluronidase

This enzyme is produced by the gas gangrene–causing organism *C. perfringens* and has been thought to enhance penetration of the host tissues by hydrolyzing hyaluronic acid, an essential intercellular "tissue cement." However, the failure of antihyaluronidase serum to inhibit the spread of *C. perfringens* through tissues suggests that the enzyme may play only a minor role.

Streptokinase

This substance is produced by the A, C, and G groups of the β-hemolytic streptococci. It converts blood plasminogen to plasmin, a protease that dissolves

the fibrin of blood clots. Streptokinase has long been thought to enhance the ability of streptococci to spread through tissue by dissolving the fibrin that tends to wall off areas of tissue damaged by infection; however, its role may be minor in view of the failure of antistreptokinase serum to inhibit streptococcal invasiveness.

Deoxyribonuclease (DNase)

This enzyme is produced by *S. pyogenes*, *S. aureus*, *C. perfringens*, and certain other pathogens. Its ability to destroy DNA would seem to indicate that it is a formidable cytotoxin; however, it cannot penetrate living tissue cells to gain access to the intracellular DNA. It may possibly contribute to the ability of some pathogens to spread through tissue by destroying the viscosity of DNA liberated from cells that have been damaged or killed by other means.

Coagulase

Staphylococcus aureus produces an enzyme commonly called coagulase which reacts with an activator in plasma to cause **clotting**, i.e., conversion of soluble **fibrinogen** to insoluble **fibrin**. The fibrin coats the cell walls of the cocci and has been thought to protect them against phagocytosis. Coagulase is also involved in the walling-off process of staphylococcal abscesses. However, mutants of *S. aureus* that cannot make coagulase have been found to retain their virulence, indicating that coagulase is probably not a major virulence factor.

Protein A

This protein is located on the cell wall of *S. aureus* and has the ability to bind antibodies, regardless of their specificity. The antibody molecules are distorted so that their binding site for complement (C) is exposed. (See Chap. 32 for a discussion of complement.) A subsequent series of reactions results in formation of a substance known as **C5a**. This substance, also called **anaphylatoxin**, causes release of the toxic compound, **histamine**, from certain body cells. The histamine causes a variety of damaging effects in tissues.

Hydrogen Peroxide and Ammonia

Bacteria of the genera *Mycoplasma* and *Ureaplasma* adhere firmly to epithelial tissue of the respiratory or urogenital tract, where they excrete toxic by-products of their metabolism, viz., H_2O_2 and NH_3. These compounds accumulate locally to high concentrations, thereby damaging the epithelial cells.

Microbial Iron Chelators

The ability of aerobic microbial pathogens to compete with a host for available iron has considerable bearing on microbial virulence. Aerobic or aerotolerant organisms are continually faced with the difficulty of obtaining enough iron for growth, i.e., for biosynthesis of iron-containing enzymes such as cytochromes and catalase. Most of the iron that is available for aerobic or aerotolerant organisms is present in the oxidized ferric form, which is extremely insoluble. (Organisms that can grow under anaerobic conditions have less difficulty in obtaining iron, since in the reduced environments in which anaerobic organisms occur iron is in its reduced, or ferrous form, which is very soluble.) Aerobic organisms have had to evolve ferric iron–binding compounds in order to solubilize and take up ferric iron. The iron-binding compounds formed by microorganisms are termed **siderophores**. These generally belong to two major classes, the phenolates and the hydroxamates. One well-known member of the phenolate group is **enterochelin**. For instance, cells of *E. coli* secrete entero-

chelin, which solubilizes polymeric ferric iron and forms complexes with the ferric ions; the ferric-enterochelin complex is then transported into the bacterial cells, where the complex is degraded and the iron reduced to the ferrous form.

The role of microbial siderophores in virulence is to compete with the host for available iron. The host, being aerobic, also possesses iron-binding compounds, proteins known as **lactoferrin** and **transferrin**, which can limit the amount of iron available to an invading pathogen (see Chap. 32). Much of the success of an infection appears to be due to the ability of the microbe to "steal" some of this iron, by means of its siderophores. In experimental infections of laboratory animals, strains of bacteria that do not secrete siderophores have markedly reduced virulence.

QUESTIONS

1 Define the following terms:

Pathogenic	Virulent
Avirulent	Attenuated
LD_{50}	MLD
Leukocidin	Primary infection
Secondary infection	Siderophore

2 List two components of microorganisms that are responsible for adherence to host tissue.

3 By what means may pathogenic microorganisms passively penetrate the body to initiate infection? How does passive penetration differ from active penetration?

4 Contrast the type of infection caused by *Staphylococcus aureus* with that caused by *Clostridium perfringens* in terms of the ability of the organisms to spread through tissues.

5 List the various means by which pathogenic microorganisms can become distributed within the human body.

6 Give two examples of bacteria that produce antiphagocytic factors.

7 What role do bacteriophages play in the toxigenicity of *Corynebacterium diphtheriae*? What role do plasmids play in the toxigenicity of diarrhea-causing strains of *Escherichia coli*?

8 Differentiate between exotoxins and endotoxins. Differentiate between neurotoxins and enterotoxins.

9 What accounts for the ability of the α toxin of *C. perfringens* not only to act as a hemolysin but also to destroy tissue cells other than erythrocytes?

10 In what exotoxins does a B region or B subunit play an important role in adherence of the toxin to a host cell?

11 Both the botulinum toxin and the tetanus toxin are neurotoxins; however, they act in entirely different ways. What differences occur between their mechanisms of action?

12 Explain how diphtheria antitoxin neutralizes the toxicity of diphtheria toxin.

13 Why might shock occur as a feature of bacteremias or septicemias caused by Gram-negative bacteria?

14 What sort of evidence suggests that hyaluronidase, coagulase, and streptokinase may not be as important with regard to virulence as once thought?

15 Why might a chronic infection represent a more highly evolved host-microbe relationship than an acute infection?

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Chapter 32 Natural Resistance and Nonspecific Defense Mechanisms

OUTLINE

While a pathogen uses all the means at its disposal to establish infection, the host's body has a number of defense mechanisms to prevent infection. The intricacies of the host-pathogen relationship are many and varied, and some aspects of the process of infection were discussed in Chap. 31.

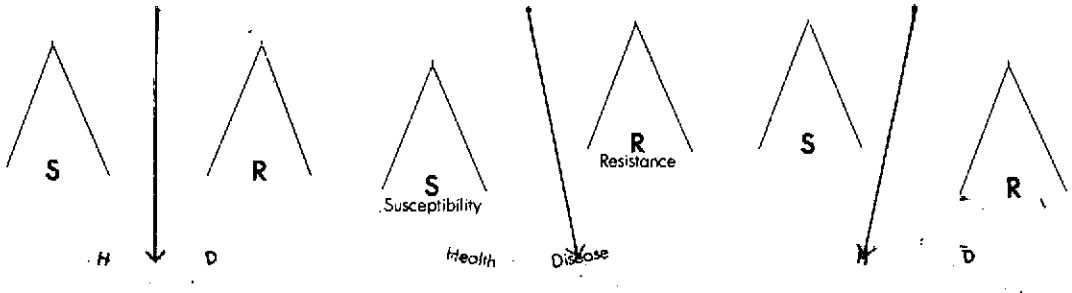
This chapter is concerned with **natural** resistance (which provides defense against infection by a number of inherent, mechanical, and chemical barriers) and **nonspecific** defense mechanisms (such as phagocytosis, the complement system, and interferon). Immunity constituting the specific aspects of the body's defense against infection is presented in Chap. 33.

NATURAL RESISTANCE

The metabolic and physiological requirements of a pathogen are important in determining the range of potentially susceptible hosts. Naturally resistant hosts either fail to provide some of the essential environmental factors required by the microorganisms for growth or have defense mechanisms to resist infection. In addition, the host's general health and state of nutrition, social and economic conditions, and other intangible factors (stress, depression, etc.) all play a part. However, these factors are so intertwined that it is difficult to evaluate their individual importance (see Fig. 32-1).

Species Resistance

Resistance to infection varies with the species of animal or plant, but the following discussion will be confined to humans and other animals. In general, species resistance has become physiologically more complex as the species of the animal kingdom have evolved. However, basic physiological characteristics of a species, such as its normal body temperature, can determine whether or



1 Microbial factors:
Virulence
Invasiveness
Portal of entry
Dosage
Microenvironment
Indigenous microbiota

2 Physiological factors:
Injury, constitutional disease
Physiological stress
Psychological stress
Misdirected tissue response, inflammation, histamine release

3 Cellular factors:
Phagocytosis by macrophages and polymorphonuclear leukocytes
Lymphocytes

4 Humoral factors:
Antibodies
Complement
Interferon

5 Constitutional factors:
Nutrition
Age
Tissue tone
Hormonal balance
Vascular condition
Lysozyme
Race

6 Socioeconomic factors:
Housing
Hygiene
Occupational hazards
Environment

Figure 32-1. Host-parasite relationship in health and disease: the balance between susceptibility and resistence.

not a microorganism can be pathogenic. For this reason, many diseases of mammals do not affect fish or reptiles, and vice versa. Frogs, being cold-blooded, are resistant to anthrax unless they are warmed to 37°C. Conversely, if the body temperature of chickens is lowered from 39°C to 37°C, they become susceptible to anthrax. Most mammals are resistant to *Mycobacterium avium*, and the type of mycobacterium found in cold-blooded reptiles does not produce characteristic infections in either mammals or fowls. Although generally host-specific, the human and bovine species of the tubercle bacillus will cross-infect between humans and cattle as well as other animals having about the same body temperature.

Metabolic, physiological, and anatomical differences between species affect the ability of a pathogen to cause infection. Herbivorous animals are often resistant to diseases of carnivores and vice versa for a variety of reasons. Intestinal anatomy differs greatly between carnivores and ruminants, which have multiple stomachs and different intestinal microbial flora and digestive juices. Diseases of the skin, to which humans are quite susceptible, are often resisted by animals because they have more hair and thicker hides.

We can conclude that the characteristics of species resistance are (1) the inability of an organism to cause disease in the resistant species under natural conditions, (2) the production in resistant species of a localized or transient infection by experimental inoculation as opposed to a generalized or progressive disease in naturally susceptible species and, (3) the induction of experimental disease in resistant species only by massive doses of the microorganisms, often by an unnatural route or under unnatural conditions.

Racial Resistance

Various animal as well as plant breeds show marked differences in their resistance to certain infectious diseases. A well-known example is that Brahman cattle are resistant to the protozoan parasite responsible for tick fever in other breeds of cattle. That this type of resistance is an inherited racial characteristic is indicated by the fact that crossbreeding the highly susceptible Texas longhorn with Brahman cattle produces a resistant variety. In humans, evidence of racial resistance is explained on the basis of selectivity and survival in most cases.

The presence of a pathogen in isolated races results in a gradual selection for resistant members as the susceptible members die of progressive infection. The introduction of new pathogens, such as the tubercle bacillus by resistant Europeans to an isolated population such as the American Indians (who had not previously developed a resistance to the organism), resulted in epidemics which decimated the population. Similarly, blacks show a relatively high resistance to tropical diseases such as malaria and yellow fever, and Orientals exhibit a reduced susceptibility to syphilis. In addition to physiological factors, racial customs play an important role in racial resistance. Hygienic practices, food taboos, and domestic and migratory habits all affect the opportunity for exposure to infectious disease-causing agents.

Individual Resistance

Some people appear to experience fewer or less severe infections than others, even though they apparently have the same racial background and opportunity for exposure. Such individual resistance is due to a combination of both natural and adaptive resistance factors. Age is important, as young individuals are susceptible to "children's diseases" such as measles and chicken pox prior to the acquisition of immunity, which follows both overt and inapparent infections. Conversely, the aged are susceptible to diseases such as pneumonia, presumably as a result of a decline of immune functions with age. Certain individuals have genetic defects which result in selective or general immunodeficiencies (i.e., an inability to develop immunity to pathogens), which greatly increase the susceptibility to disease. Other factors include nutrition, personal hygiene, and the individual's sex. The nature of the workplace and its hazards, the opportunity for contacts with infected individuals, and the individual's hormonal and endocrine balance all affect the selectivity and frequency of certain diseases.

External Defense Mechanisms

External defense mechanisms are another factor in natural resistance. These are largely mechanical, but chemical barriers are also involved. Mechanical barriers, together with host secretions, constitute the body's first line of defense against invading microorganisms.

Mechanical barriers include the unbroken skin and mucous membranes, which are generally impervious to infectious agents. It is possible for microorganisms to enter through hair follicles, openings of sweat glands, or abrasions, but skin and mucous membranes form a generally effective barrier. However, certain fungi will readily produce skin infections when the skin becomes moist and soft, e.g., fungi that cause athlete's foot. Most bacteria are inhibited by lactic acid and fatty acids in sweat and sebaceous glands and by the low pH which these chemicals bring about. Mucous secretions of the respiratory tract, the digestive tract, the urogenital tract, and other tissues form a protective covering of mucous membranes and collect and hold many microorganisms until they

can be disposed of or lose their infectivity. **Peristalsis** (the progressive and rhythmic contraction of the intestines) traps microorganisms in mucus and other material present in the intestines and expels them from the body. Small hairlike appendages, or **cilia**, of epithelial cells lining many of the body cavities and orifices sweep bacteria away from susceptible surfaces. Coughing, sneezing, shedding tears, perspiring, and salivating provide mechanical flushing or clearing that removes microorganisms.

In addition to the mechanical action of mucus, saliva, and tears in removing bacteria, some of these secretions contain chemical substances that inhibit or even destroy microorganisms. An example is **lysozyme**, an enzyme found in many body fluids and secretions, which has an effective antimicrobial action due to its ability to lyse certain Gram-positive bacteria by hydrolyzing peptidoglycan. Other enzymes and hormones may produce chemical, physiological, or mechanical effects that reduce susceptibility to infection. The acidity or alkalinity of some body fluids has a deleterious effect on many microorganisms and helps to prevent potential pathogens from entering the deeper tissues of the body.

Another protein with known antimicrobial activity is **lactoferrin**. It is a red, iron-containing protein found in milk (both bovine and human) as well as in most of the secretions that bathe human mucosal surfaces, including bronchial mucus, saliva, nasal discharges, tears, hepatic bile, pancreatic juice, seminal fluid, and urine. It is also an important component of the specific granules of polymorphonuclear leukocytes. Its serum counterpart is **transferrin**. Both have similar molecular weights (about 78,000 daltons) and metal binding sites. These proteins chelate or tie up available iron in the environment, thus limiting the availability of this essential metal nutrient to invading microorganisms.

INTERNAL DEFENSE MECHANISMS

The second line of defense consists of the body's internal mechanisms, which can be mobilized against invading microorganisms. These mechanisms can be nonspecific in their action (e.g., phagocytosis) or specifically directed against the pathogen (e.g., antibodies and sensitized cells). These two types are called **nonspecific defense mechanisms** and **specific acquired immunity**, respectively. Specific acquired immunity is a result of an infection or artificial immunization and is directed to the specific causative organism; it is discussed in Chap. 33. During infection both mechanisms act together to rid the body of the invading microorganisms. This interrelationship, and the interrelationships between the defense mechanisms discussed above, are illustrated in Fig. 32-2.

In the remainder of this chapter the nonspecific defense mechanisms, or cellular defense factors, will be discussed. At this point it is sufficient to define an **antibody** as a globular serum protein which can specifically combine with an organism, such as a bacterium, parasite, or virus.

NONSPECIFIC DEFENSE MECHANISMS

The body can mobilize many factors which act nonspecifically to the invasion of foreign organisms. These include complement, phagocytic cells, naturally cytotoxic lymphocytes, and interferon, among others.

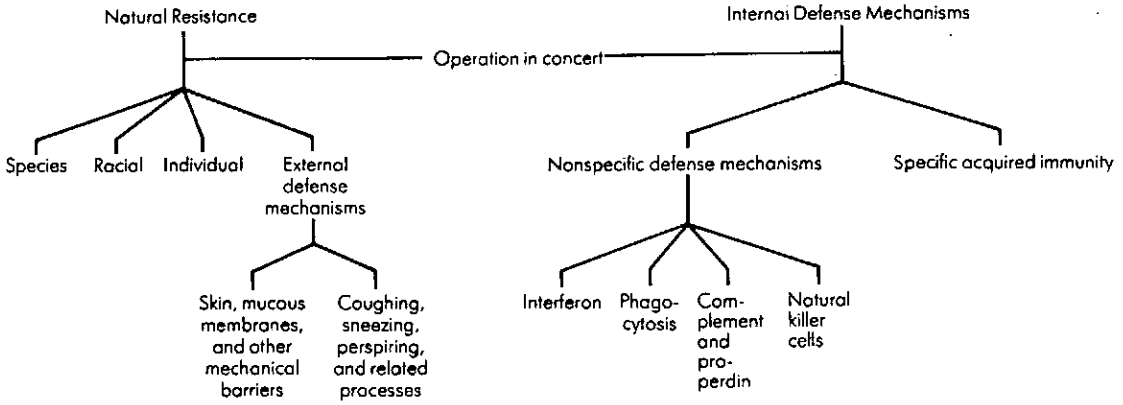


Figure 32-2. Interrelationships between defense mechanisms.

Table 32-1. Functional Activities of the Host Complement System in Host Defense against Infection

Activity	Complement Components or Fragments
Lysis of viruses, virus-infected cells, tumor cells, mycoplasma, protozoa, and bacteria	C1–C9
Endotoxin inactivation	C1–C5
Virus neutralization	C1, C4, C2, C3
Anaphylatoxin release (capillary dilatation)	C3a, C4a, C5a
Opsonization; enhancement of cell-mediated cytotoxicity; stimulation of production of B-cell lymphokines	C3b
Enhanced induction of antibody formation	C3b, C3d
Chemotaxis of neutrophils, monocytes, eosinophils	C5a
Stimulation of macrophage adherence and spreading	Bb

Complement System

The serum of higher animals contains a group of eleven proteins referred to collectively as complement because its action complements that of certain antibody-mediated reactions. Complement plays an essential role in resistance against infection and is the principal mediator of the inflammatory response. Upon activation of the first protein of the group of eleven, there occurs a sequential cascade in which active molecules are generated from inactive precursors. Certain of the different proteins activated along the cascade may function as mediators of a particular response as well as activators of the next step. Some of the functional activities of complement are shown in Table 32-1. In general, complement is capable of attacking and killing invading cells only after antibody binds to the cell membrane, thus initiating the complement attachment or fixation; this is illustrated in Fig. 32-3.

There are several characteristics of the complement system. It has a recognition unit to respond to the antibody molecules that have identified an invading cell. It has receptor sites to combine with the surface of the foreign cell when it is activated. Its activity must be limited in time to minimize damage to the

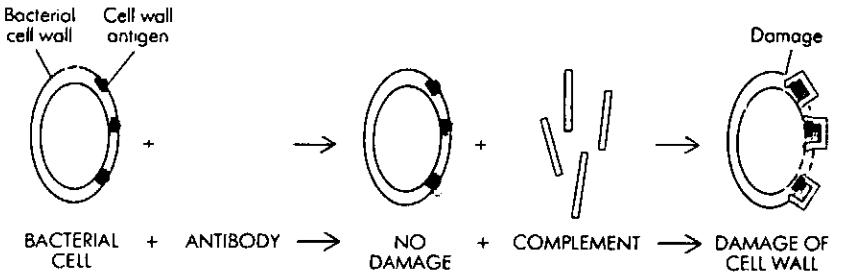


Figure 32-3. Mechanism of complement action: the bacterial-killing effects of antibody and complement. *(Courtesy of A. J. Vander, J. H. Sherman, and D. S. Luciano, Human Physiology, The Mechanisms of Body Function, McGraw-Hill, New York, 1970.)*

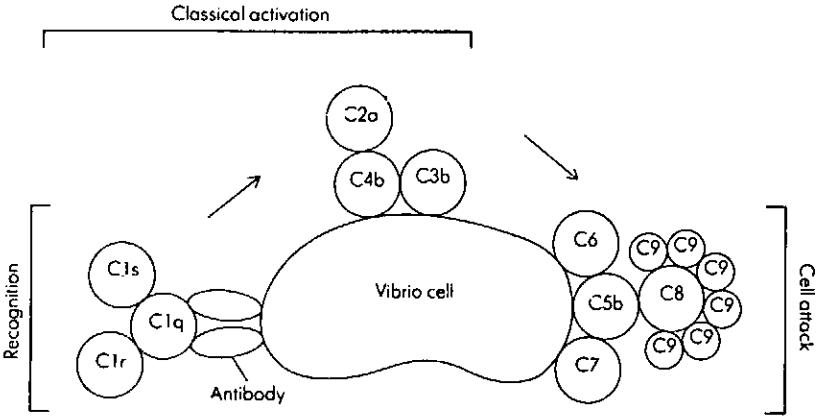


Figure 32-4. Model of complement assembly on the membrane of a bacterial cell in the sequence of events in complement recognition, activation, and cell attack. *(After a diagram suggested by Dr. Russell Siboo, McGill University.)*

host's own cells. This limitation is brought about partly by the spontaneous decay of activated complement and partly by interference from destructive enzymes and inhibitors.

As stated before, complement is composed of eleven components. These components are named according to the following rules. Each component has been assigned a number in the order of its discovery, and that number is preceded by the capital letter C. Unfortunately, the first four components do not interact in the order of their discovery, but rather in the sequence C1, C4, C2, and C3. But the remaining components do react in the appropriate numerical order: C5, C6, C7, C8, and C9. C1 has three subcomponents, C1q, C1r, and C1s. Fragments of components resulting from cleavage by other components acting as enzymes are assigned lowercase letters a, b, c, d, or e, for example, C3a and C3b. The mechanisms of complement action in bacterial lysis shown in Fig. 32-3 are represented in greater detail in Fig. 32-4 as a cascade of events in complement *recognition* and *activation*, culminating in *cell attack*. This is the *classical* or antibody-dependent pathway requiring activation by antibody, C1, C4, C2, and C3.

The activation of complement may also occur by an alternate pathway sometimes called the **properdin** pathway. Properdin refers to one of the factors in blood serum that, acting together with complement, is a mechanism for the activation of a natural defense when sufficient quantities of specific antibody are unavailable for immune activation by the classical pathway. Components of

the alternate pathway have been assigned the letters B, D, and P (properdin). They activate the C3 component of complement directly. That is, the alternate pathway does not use antibody, C1, C4, or C2, which are the early reactants of the classical pathway. Since C3 is a component of both the classical and the properdin pathway, it is apparent that these two pathways of complement activation have much in common, differing only in the initial activation events. The term *complement system* refers generally to both pathways, which interact and are dependent upon each other for their full activity. Among the substances that can activate the alternate pathway are lipopolysaccharides of Gram-negative bacteria, bacterial capsules, teichoic acids of Gram-positive cell walls, inulin, dextran, fungal cell walls, and aggregated globulins high in carbohydrate content.

Since the complement system consists of so many distinct proteins (as well as the many active fragments split during their enzymatic activation), the overall system is very complex, and no further attempt will be made in this chapter to identify the specific roles of individual complement proteins (except as noted in Table 32-1).

Phagocytosis

The importance of phagocytosis in protecting the body from infection was first recognized by the Russian zoologist Elie Metchnikoff in 1883. He called the amoeboid particulate-eating cells *phagocytes* (from *phagein*, "to eat," and *kytos*,

Table 32-2. Cell Types Associated with Host Resistance to Infection

Cell Type	Location	Derivation	Description and Function
1 Leukocytes	Peripheral blood	Bone marrow stem cells	Classified according to structure and affinity for dyes
(a) Polymorphonuclear granulocytes			Lobar nuclei and abundant cytoplasmic granules
(1) Neutrophils			No dye preference; phagocytic
(2) Eosinophils			Stain with eosin, an acid dye; phagocytic
(3) Basophils			Stain with basic stains; bind IgE and produce histamine
(b) Lymphocytes		Lymphoid organs, bone marrow stem cells	Smaller than monocytes; large nucleus; scanty cytoplasm; form T and B cells (see Chap. 33); nonphagocytic
(c) Monocytes		Bone marrow stem cells	Larger than granulocytes; single horseshoe-shaped or oval nucleus; few cytoplasmic granules; phagocytic
2 Plasma cells	Lymphoid organs (lymph nodes, spleen, thymus)	From B lymphocytes	Produce antibodies; eccentric nucleus; nonphagocytic
3 Macrophages	Other tissues	Transformed from monocytes	Numerous cytoplasmic granules; phagocytic
(a) Wandering			
(1) Alveolar	Lung		
(2) Peritoneal	Abdomen		
(b) Fixed			
(1) Histiocytes	Connective tissue		
(2) Kupffer cells	Liver		

"hollow vessel"). He believed that cellular activity, in destroying bacteria and other microorganisms, was the primary defense mechanism of the body. Even though this concept was well entrenched in the minds of people since that time, phagocytes were neglected by research workers from the 1920s on. Little was added to our understanding of phagocytosis after the initial pioneer studies. In recent years, however, there has been a resurgence of interest in phagocytosis as a central resistance process.

It is now known that cellular activity responsible for phagocytosis and other aspects of nonspecific and specific resistance is carried out by a diverse group of blood cells (Table 32-2). In higher organisms, there are two principal classes of phagocytic cells. These are the *polymorphonuclear granulocytes*, or *polymorphs* for short (mainly *neutrophils*), so called because their nuclei come in many shapes, and the *macrophages* (mononuclear phagocytes), large phagocytic cells derived from monocytes. (See Fig. 32-5.)

The polymorphs constitute the front line of internal defense for the host. They are produced in the bone marrow and are discharged into the blood in vast numbers. The 50 to 100 billion polymorphs that are present in normal human blood carry out their functions after leaving the circulation and entering sites of inflammation in tissues. They live for a few days only, and about 10^{11} polymorphs disappear from the blood daily; however, they are replaced by new ones from the bone marrow. Polymorphs contain numerous enzymes and antimicrobial substances for the killing and degradation of bacteria. These substances are contained in membrane-bound organelles called lysosomes.

Macrophages are formed from circulating precursor *monocytes* (blood cells with horseshoe-shaped nuclei; see Fig. 32-6) which also arise from the bone marrow. As soon as the monocytes leave the circulation and begin to carry out phagocytosis, they are called macrophages. Unlike the polymorphs, macrophages are long-lived and can persist in tissues for weeks or months. They are

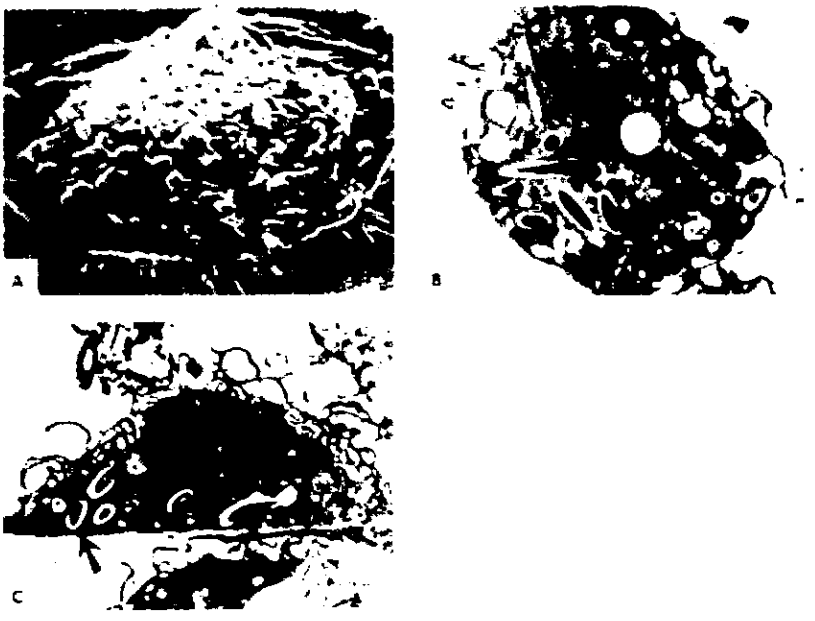


Figure 32-5. (A) Scanning electron micrograph of a peritoneal macrophage from a mouse fixed during locomotion (X2,700). (B, C). Thin sections of cultured mouse peritoneal macrophage showing phagocytosed cells of *Listeria monocytogenes* (a bacterium) indicated by arrows. (B) Horizontal section (X4,000). (C) Vertical section (X3,800). *(Courtesy of P. Gill, McGill University.)*

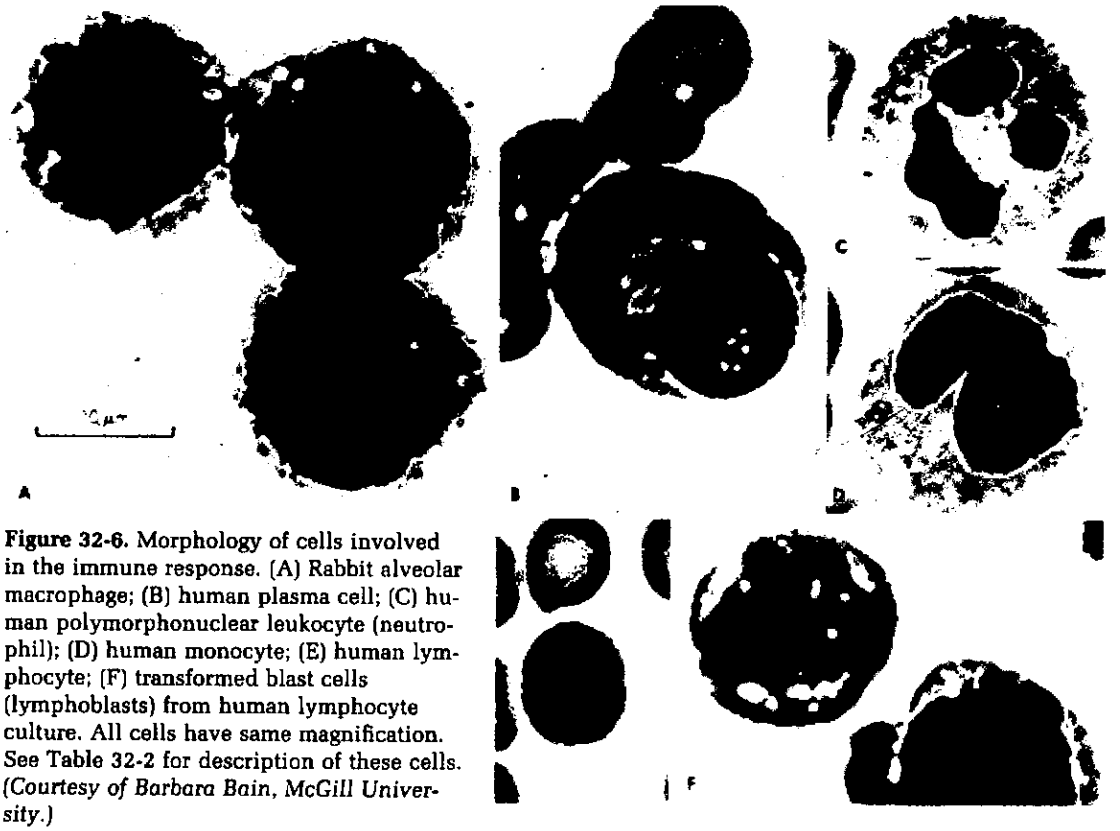


Figure 32-6. Morphology of cells involved in the immune response. (A) Rabbit alveolar macrophage; (B) human plasma cell; (C) human polymorphonuclear leukocyte (neutrophil); (D) human monocyte; (E) human lymphocyte; (F) transformed blast cells (lymphoblasts) from human lymphocyte culture. All cells have same magnification. See Table 32-2 for description of these cells. *(Courtesy of Barbara Bain, McGill University.)*

widely distributed throughout the body, but they are not as numerous as polymorphs (about one-twentieth the concentration). Under certain conditions, macrophages can synthesize DNA and multiply. When they differentiate in connective tissue, they are called *histiocytes*, in the liver, *Kupffer cells*, and in the lung, *alveolar macrophages*.

Two types of mature macrophages are recognized: (1) those wandering in tissues and body spaces (e.g., alveolar and peritoneal macrophages) and (2) those fixed to vascular endothelium (e.g., Kupffer cells and fixed macrophages of the spleen and lymph nodes).

Macrophages, then, are strategically placed throughout the body to combat invading microorganisms. Macrophages also have lysosomes with bactericidal substances. (See Fig. 32-6 for the morphology of the polymorphs and macrophages.)

Mechanism of Phagocytosis

The process of phagocytosis requires a preliminary attachment of the microbe to the phagocytic cell surface. Electrostatic forces are necessary for initial attachment, since divalent cations such as Ca^{2+} and Mg^{2+} are required. The firm attachment is facilitated by serum substances called opsonins. Opsonins are antibodies that allow microbes to be more easily ingested by the phagocyte.

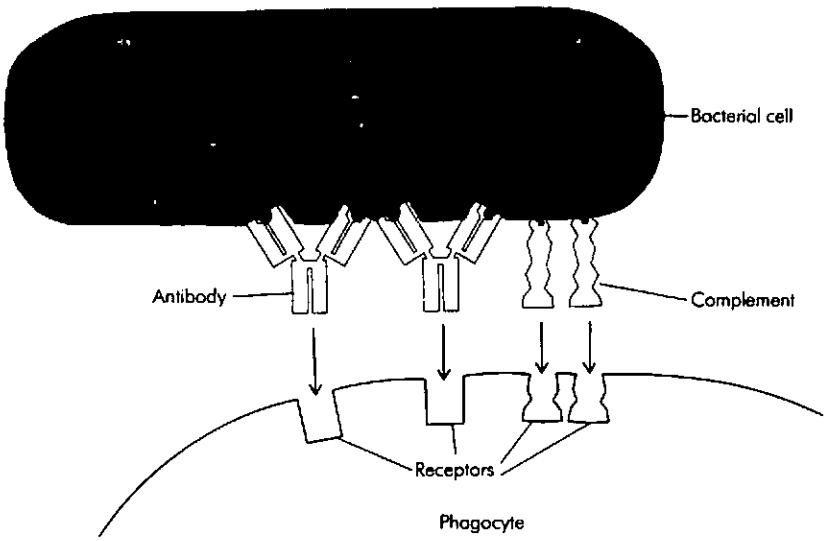


Figure 32-7. Attachment of bacterial cell antigen to surface of phagocyte. The phagocyte has receptors on its surface both for the antibody and for the complement. Phagocytosis occurs more readily with this kind of binding.

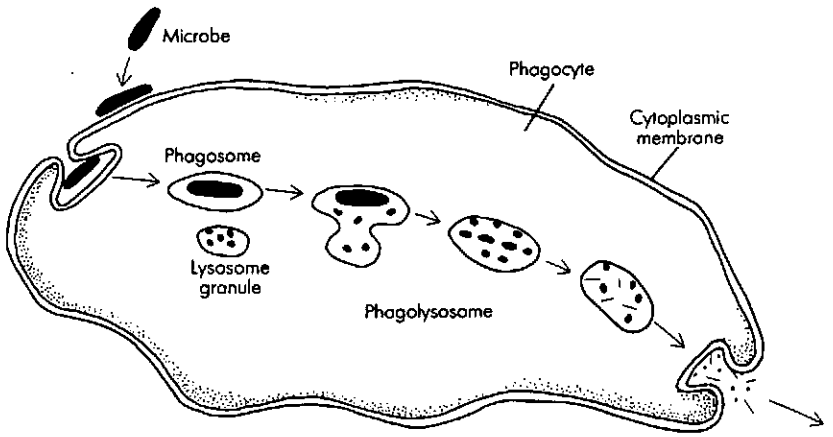


Figure 32-8. Phagocytosis of a microbial cell.

Phagocytic cells have a special receptor affinity for antibody attached to microorganisms. If complement is also bound to the microbial surface, there are complement receptors on the phagocytic cell which provide additional attachment forces. (See Fig. 32-7.)

In phagocytosis, the phagocyte extends small pseudopods around the microbe after adherence. These pseudopods fuse and form a vacuole by means of invagination of the phagocyte plasma membrane engulfing or surrounding the bacterium. The vacuole is now called a phagosome. Subsequent events depend on the activity of the lysosomal granules. These move toward the phagosome, fuse with its membrane to form a phagolysosome, and discharge their contents

(hydrolytic enzymes) into the vacuole, thus initiating the intracellular killing and digestion of the microbe. Within the phagolysosome, most microbes are killed in minutes, although the complete degradation may take a few hours. The process of phagocytosis is shown schematically in Fig. 32-8.

The major mechanism of bacterial killing within phagocytic cells is by the so-called "respiratory burst." This activity is initiated when a particle such as a bacterium binds to a polymorph surface or when a macrophage ingests a particle: the phagocytic cell increases its oxygen consumption—thus the term respiratory burst. This is brought about by an enhanced activity of the hexose monophosphate shunt pathway of metabolism (see Chap. 10). This pathway generates large quantities of $NADPH_2$ (reduced nicotinamide adenine dinucleotide phosphate). When the $NADPH_2$ is oxidized back to NADP, highly toxic oxygen metabolites are generated. These include singlet oxygen, superoxide, hydrogen peroxide, hydroxyl radicals, chloramines, and aldehydes. All of these are effective in killing invading bacteria. The major enzymes involved in the generation of these oxygen metabolites are superoxide dismutase and myeloperoxidase (in neutrophils) or catalase (in macrophages). Their specific activity is shown in Fig. 32-9.

In addition, the lysosome granules of phagocytic cells contain many potent hydrolytic enzymes. These granules migrate through the cytoplasm and fuse with the phagosome containing the invading microbe (Fig. 32-8). The enzymes, now present in the phagolysosome, proceed to destroy the ingested bacteria. Over 60 different enzymes have been found within lysosomes. These include lysozyme, hyaluronidase, and other enzymes that act on carbohydrates; lipases; ribonuclease and deoxyribonuclease; collagenases, elastases, and other enzymes that act on proteins and peptides; and the enzymes of the respiratory burst, namely, myeloperoxidase, superoxide dismutase, and catalase.

The combined actions of the lysosomal enzymes and of the respiratory burst are usually sufficient to destroy all invading microorganisms. However, microbes vary in their response to phagocytic activity. Gram-positive bacteria are rapidly

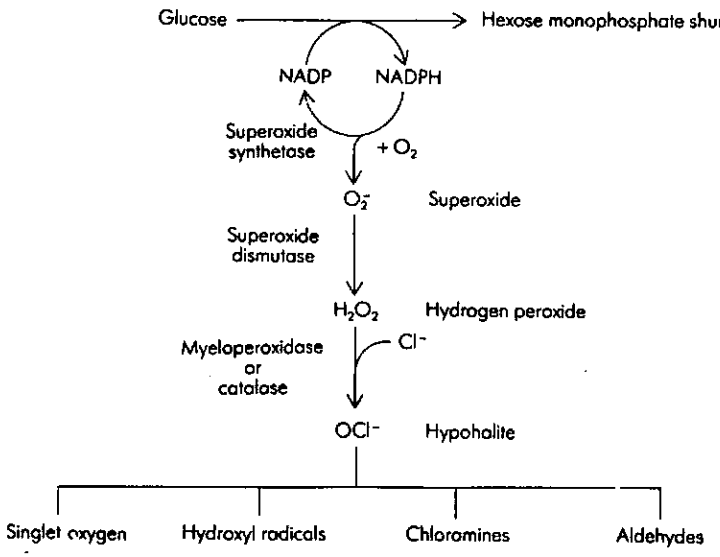


Figure 32-9. The respiratory burst that produces bactericidal products.

Macrophages Have Many Functions

In addition to maintaining antimicrobial defenses, macrophages play a very important role in the body's day-to-day functioning. Macrophages are involved in the breakdown of coagulated blood, in wound healing, in several pathways of immediate and delayed allergic reactions, and, very importantly, in the digestion of dead cells and tissues. For example, each day, 300 billion senescent red blood cells are removed from the circulation by macrophages in the liver and spleen. In the course of a year, a person's macrophages ingest and digest about 5 lb of hemoglobin.

Macrophages can also be activated to kill cells without ingesting them. Activation is by protein factors called *lymphokines* produced by antigen-stimulated thymus-dependent lymphocytes (T cells; see Chap. 33). Activated (also called angry) macrophages have the capacity to destroy tumor cells, possibly by the secretion of large amounts of hydrogen peroxide and other toxic forms of oxygen.

Macrophages also secrete many molecules into their surrounding environment, affecting the activity of other cells. For instance, one of the macrophage products acts strongly to attract circulating polymorphs and monocytes to the lungs in response to infections and tumors.

destroyed. Gram-negative bacteria are somewhat more persistent because their cell wall is relatively resistant to digestion. But some bacteria like *Mycobacterium tuberculosis* are so resistant to phagocytic action that they may literally multiply within the phagocytes.

Natural Killer Cells

Recently, a subpopulation of lymphocytes has been discovered which are referred to as **null cells** because they do not possess certain surface antigens. Morphologically, null cells are about the size of large lymphocytes (12 to 15 μm in diameter) but have a larger cytoplasm-to-nucleus ratio. Null cells generally possess surface receptors for immunoglobulin; reports are conflicting as to whether they have complement receptors. The best-defined null cells are known as **natural killer (NK) cells**. They can kill cellular targets without prior exposure (immunization) to antigens of the target cells. Thus they can directly kill tumor cells and virally infected cells without ingesting them. NK activity is potentially the first line of immune surveillance against neoplastic cells and virus-infected cells. However, little is known about how they recognize and destroy their targets. Figure 32-10 shows a NK cell–target cell interaction.

Interferon

Interferon was discovered in 1957 by Alick Isaacs and Jean Lindenmann of the

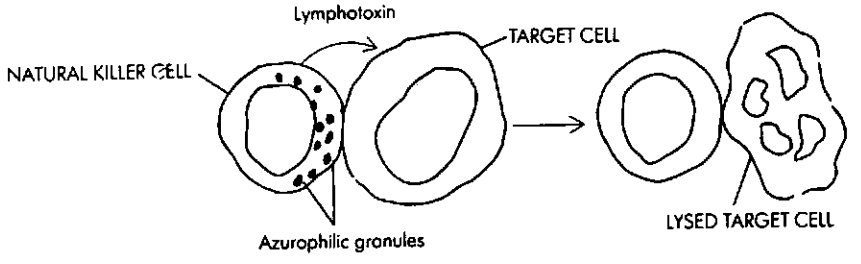


Figure 32-10. Extracellular cytotoxicity by natural killer cells. *(After a drawing suggested by Malcolm Baines, McGill University.)*

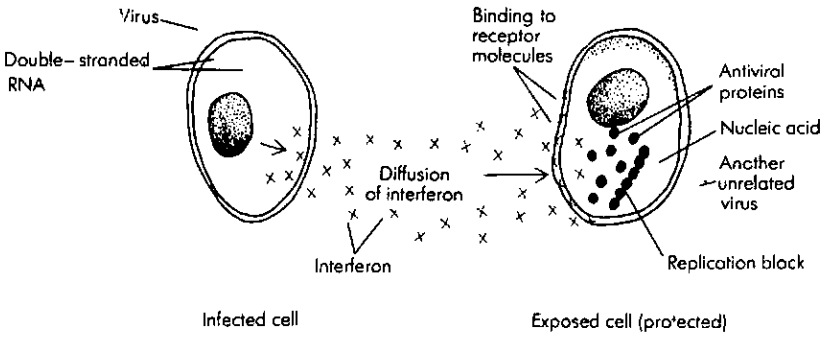


Figure 32-11. Mechanism of interferon induction and action.

National Institute for Medical Research in London while working on the mechanisms of viral interference (resistance of an animal or cell infected with one virus to superinfection with a second unrelated virus). Interferon is still virtually the only natural substance with the proven ability to inhibit intracellular viral replication, although results from clinical trials of its therapeutic use are ambiguous. Interferons are small proteins produced by normal eucaryotic cells in response to viral infection or double-stranded RNAs (viral or synthetic). Interferons lack virus specificity since they do not react directly with the virion but instead induce the host cell to synthesize a nonspecific antiviral protein (Fig. 32-11). Conversely, interferons were found to be species-specific with respect to the cells that produced them and so would induce little or no resistance in cells from other species. Thus human interferon was most effective in protecting human cells and poorly protective for mouse or chicken cells. However, recent work with purified interferon shows that its antiviral activity is not strictly species-specific. Though purified interferons are stable at low pH and fairly heat-resistant, they are relatively unstable at physiological pH in tissue fluids. Interferon is secreted by cells only in minute amounts and is extremely difficult to purify. For these reasons interferon has not been particularly useful clinically, although recent advances in molecular genetics have permitted the production and synthesis of interferons in large quantities for experimental use and clinical trials. Specifically, recombinant DNA carrying a gene for human α interferon has been inserted into the bacterium *Escherichia coli* (see Chap. 29 on genetic engineering). Such recombinant *E. coli* cells are grown in large quantities (e.g., in 400-liter fermentation tanks), centrifuged out, and broken open to release intracellular material, including interferon. The cellular debris is separated by centrifugation. Nucleic acids and other viscous materials are removed, and the proteins in the extract are passed through *monoclonal-antibody* columns to yield purified interferon.

Our interest in interferon, in the context of the present chapter, is in its antiviral effect in preventing cell infection. But this antiviral effect is merely one facet of interferon activity. There are other aspects which should interest the microbiologist, e.g., interferon's effect on cell membrane–related events, its inhibition of cell proliferation, and its involvement as a primary regulator of the immune system. Interferon is also capable of promoting the natural cytotoxic activity of natural killer cells, which implies that it has a role in immunologic

surveillance against malignancies. However, the primary protective role of interferons is against naturally acquired viral infections because interferons are produced locally and more promptly than specific antibodies.

Inflammatory Response

The final consideration in our discussion of defense mechanisms is how the nonspecific factors discussed above combine in what is termed the *inflammatory response* to combat an invasion by pathogens. If the microbe is able to activate and fix complement by the alternate pathway, the chemotactic complement-derived factors released attract leukocytes to the site, and anaphylatoxin also causes the degranulation of tissue basophils called mast cells. These in turn release histamine and serotonin, which cause constriction of smooth muscles (e.g., in bronchioles and blood vessels) and increased capillary permeability, which promotes the passage of plasma and leukocytes into the affected tissue. The leukocytes pass through the junction between the capillary endothelial cells in response to the chemotactic influence of the complement cleavage fragments. Migration continues until the phagocytes encounter complement fixed to the microbial surface, which causes adherence and facilitates engulfment.

If antibodies specific for the microbe are present, they opsonize it and also increase complement fixation. This greatly enhances leukocyte adherence and promotes phagocytosis. The plasma also contains other microbicidal substances which may inhibit replication and growth of pathogens and modulate the subsequent immune response.

The symptoms of the inflammatory response are local swelling, erythema (reddening), and local and systemic heat. The local swelling is due in part to the accumulation of large numbers of phagocytic cells at the site of infection. The increase in temperature and the erythema are due to the increased blood flow to the local site, enzymatic activity, and the release of bacterial endotoxin. The inflammatory response is usually acute and resolves spontaneously. During long-term infections the inflammatory response may become chronic. The inflammatory response is the most important factor in resisting infection by pyogenic (pus-forming) bacteria.

QUESTIONS

1 What is meant by a naturally resistant host?
2 Discuss species resistance and give several specific examples.
3 Discuss some specific factors governing individual resistance to disease.
4 Describe two chemical factors that form part of the external defense mechanisms.
5 Discuss two functional activities of the host complement system in host defense against infection.
6 Why is complement considered a resistance factor?
7 Explain the terminology for the components of complement.
8 Compare the initial complement activation events of the classical pathway with those of the alternate pathway.
9 Compare and contrast the two principal classes of phagocytic leukocytes in higher organisms.
10 Describe the mechanism of phagocytosis as well as the known mechanism for killing phagocytosed bacteria.

11 What are natural killer cells? What role do they play in the defense of the body?
12 Why is interferon effective as a resistance factor against virus infections?
13 Why is interferon sometimes used in cancer therapy?
14 Explain how nonspecific factors of immunity elicit the inflammatory response.
15 What factors cause the symptoms of the inflammatory response?

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Chapter 33 Basic and Theoretical Aspects of the Immune Response

OUTLINE

Immunology is a rapidly developing field of study concerned with the discovery of the mechanisms of the immune system, the development of vaccines, and the regulatory procedures for manipulating the immune response. The type of immunity most commonly encountered is naturally acquired active immunity to pathogens (see Fig. 33-1). However, diverse pathological effects have also been shown to arise from immune responses to nontoxic and noninfectious antigens (macromolecules that will induce the formation of immunoglobulins or sensitized cells that react specifically with the antigens). Thus the immune response is directly responsible for allergies, autoimmune diseases (e.g., rheumatoid arthritis), and graft rejection. Also, the failure of various aspects of the immune system can result in the development of malignancies and death due to overwhelming infections (e.g., acquired immunodeficiency syndrome, better known as AIDS, which will be discussed later in this chapter).

The immune system is composed of a single integrated cellular system producing effector products of two types: serum antibodies that constitute part of the humoral immunity and sensitized cells called lymphocytes that constitute cell-mediated immunity. While antibodies are effective in opsonizing bacteria and neutralizing toxins and viruses, lymphocytes are important in eliminating intracellular parasites and viruses and rejecting tumors and transplants. Thus the immune response, in eliciting reactive antibodies and cells in response to antigens, not only forms the principal means of defense in vertebrates against

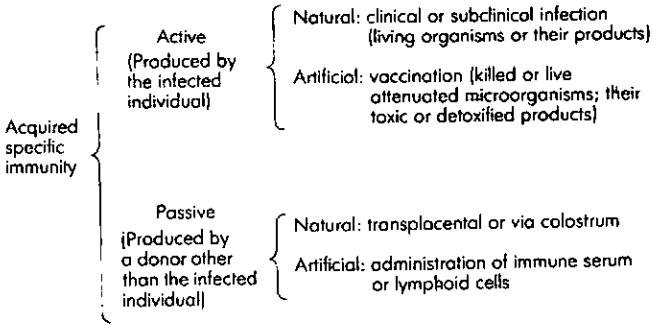

Figure 33-1. The various types of acquired specific immunity.

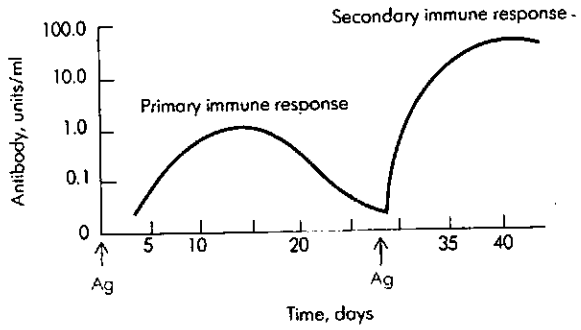

Figure 33-2. Antibody production due to administration of antigen (Ag). Note that the secondary immune response is faster and stronger than the primary immune response because of immunologic memory.

infection by pathogenic microorganisms and larger parasites, but also acts as a surveillance mechanism against the transformation of host cells into cancer cells.

THE IMMUNE RESPONSE

As mentioned above, immune responses are processes in which animals form *specifically reactive* proteins (antibodies) and cells in response to a great variety of *foreign* organic macromolecules and molecules. The generalized immune response has four primary characteristics: discrimination, specificity, anamnesis, and transferability by living cells. The first primary characteristic refers to the ability of the immune system to *discriminate* between "self" and "nonself," and therefore it responds only to materials which are foreign to the host. Second, the response is highly *specific* for the inducing material or antigen to which the immune antibodies or cells will react in greatest strength. The third characteristic refers to the ability to elicit a larger specific response more quickly when induced by a second exposure to the same foreign antigen. This is called immunologic memory or the *anamnestic* response (Fig. 33-2). Finally, *active* immunity is only transferable from one inbred animal to another by the immune cells or lymphocytes and not by serum. Whereas immune serum is capable of temporarily transferring *passive* immunity, transfer of active immunity requires the long-term regenerative ability of living cells.

Some nonspecific materials, such as mineral oil and alum, have the ability to prolong and intensify the immune response to a specific antigen when they are

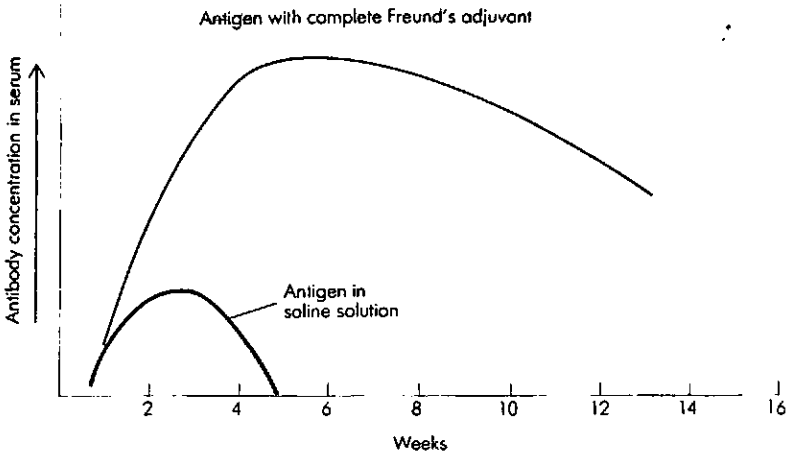

Figure 33-3. Effect of an adjuvant on the antibody response.

injected together with the antigen. Such materials are called adjuvants because they help the immune response (Fig. 33-3).

Antigens

An antigen is any substance which, when introduced into the vertebrate host, stimulates the production of antibodies and reacts with preformed antibodies, if they are already present. This demonstrates the two properties of antigens: specificity, or their ability to react specifically with immune antibodies or cells, and immunogenicity, or the capacity to stimulate an immune response. The terms *antigenicity* and *immunogenicity* differ in that the former refers to the "foreignness" of a substance or its specific reactivity while the latter refers to the ability of a substance to stimulate an immune response.

The parts of an antigen responsible for its particular properties are separable into the low-molecular-weight antigenic determinants or haptens normally linked to the larger supporting portion of the molecule, which may be referred to as the carrier. The hapten is the specificity-determining portion which is capable of reacting with a specific antibody but is incapable of inciting an immune response by itself. For this reason haptens are often referred to as incomplete antigens. High molecular-weight antigens which possess both immunogenicity and specificity are called complete antigens.

Antigens are generally proteins or polysaccharides with molecular weights greater than 10,000 daltons. They may be nucleoproteins, lipoproteins, glycoproteins from any biological source, or synthetic polypeptides or polysaccharides. Antigenic determinants or haptens generally have a molecular weight of less than 1000 daltons. Many naturally occurring substances may act as antigens: bacteria, viruses, and other microorganisms; foreign proteins such as pollens, egg white, and metabolic products of microorganisms; and living cells from different animal species.

Antigens stimulate the production of antibodies that may have prophylactic or therapeutic properties directed against the specific organism that expresses

the antigen. The antigenicity of a substance or organism is not, however, related to its ability to produce disease or damage tissue. Antigenicity can be measured only in terms of the antibody response it elicits on the basis of its foreignness to the host's immune system.

Sources of Antigens

When an immune response is elicited in an animal to the multitude of antigens in the tissues of a different species, a variety of antibody specificities are produced. Some antibodies recognize all tissues of the donor species and therefore can identify *species-specific* antigens. Other antibodies recognize the *organ-specific* antigens of the immunizing tissue. Such antibodies are useful in forensic medicine and for product identification in the food industry.

A type of tissue-specific antigen which is present in one individual of a species but not in another is called an alloantigen or isoantigen. Human sera contain *isoantibodies* against the red blood cell isoantigens of some other persons (Table 33-1). For example, individuals of blood group O contain both anti-A and anti-B isoantibodies in their sera. Individuals of blood group A have only anti-B antibodies, while group B individuals possess anti-A antibodies. It follows that blood group AB individuals have no anti-A and anti-B antibodies in their sera. The designations A, B, AB, and O describe the antigenic phenotype of a person's red blood cells. These antigens are inherited according to a simple Mendelian system involving three allelic genes called A, B, and O. Gene A controls the formation of A antigen, gene B controls the formation of B antigen, while gene O is not expressed (see Table 33-1). Thus a person with AB genes is heterozygous, having inherited an A gene from one parent and a B gene from the other. A person with O gene must be homozygous for O, while persons in A and B groups must be either homozygous (AA or BB) or heterozygous (AO or BO).

There are other isoantigens on human red blood cells. Individuals of blood group O actually produce an antigen called H antigen, which is a precursor of the A and B antigens. The H gene is situated at a different locus from the ABO locus on the chromosomes. The H gene controls the production of the H substance that, in the presence of A and B genes, is converted to A, B, or AB antigens. In addition to the A, B, and H antigens, two other blood group specificities also belong to this group but are inherited at a different locus. These are Lewis a (Le^a) and Lewis b (Le^b) antigens. The three loci, ABO, H, and Le, all code for glycosyl transferases that determine the structure of the blood group substances.

In 1940, Landsteiner and Weiner demonstrated that antibodies produced in rabbits against rhesus monkey erythrocytes agglutinated the red blood cells of 85 percent of a human population. The antibodies were specific for a new

Table 33-1. Human Major Blood Types: The ABO Blood Group System

Blood Group (Isoantigen Present)	Isoantigen on Red Blood Cells	Genotype	Isoantibodies Present
A	A	AO, AA	Anti-B
B	B	BO, BB	Anti-A
AB	AB	AB	No anti-A or anti-B
O	None	OO	Anti-A and anti-B

antigen which came to be called the rhesus (Rh) antigen. Individuals possessing this antigen were called Rh positive. The remaining 15 percent who did not carry this antigen were called Rh negative. Natural antibodies against the Rh antigens are not found. Since then the Rh system has been shown to be highly complex, and approximately 30 different antigenic types have been identified. (Rh incompatibility resulting in hemolytic disease of the newborn is discussed in the next chapter.)

Humans also possess a large number of other blood group systems, e.g., the Kidd group, the Duffy group, and the MNS system. Thus the antigenic mosaic on the red blood cells of humans is extremely complex.

Microbial Antigens

The antigens with which we are primarily concerned are those of microbial origin. The exotoxins produced by *Corynebacterium diphtheriae, Clostridium tetani*, and certain other microorganisms are potent antigens that stimulate the production of antitoxins (toxin-specific antibodies) in the body of the host. Most pathogenic bacteria, viruses, and rickettsias are good antigens which stimulate the production of antibodies capable of protecting against infection and which are useful in diagnostic tests. Fungi, however, are not strongly antigenic. Heat or chemical treatment destroys the viability of most microorganisms without necessarily decreasing or altering their antigenicity.

Bacterial cells may contain one antigenic component in the cell wall, another in the body of the cell, and others in the flagella and capsules. The antigen in the capsule of the pneumococcus gives the organism its type specificity. Antigens at the surface of cells of *Salmonella* are somatic antigens called the O antigens. They are used by bacteriologists in classifying related species into serological groups, whereas the H antigens from the flagella of *Salmonella* detect serological differences of species in a group (Fig. 33-4).

Vaccines

Vaccines, which are suspensions of killed, living, or attenuated (having weakened virulence) cultures of microorganisms, are used as antigens to produce immunity against infection due to the particular microorganism. Typhoid fever vaccine consists of killed cells of *Salmonella typhi*; the Salk poliomyelitis vaccine is composed of killed poliomyelitis virus, but the oral type, such as the Sabin vaccine, contains attenuated live virus. Another example of a vaccine made from an attenuated culture is that used for rabies. It is made by treating the rabies virus chemically so that it cannot produce infection but still can stimulate the body to make antibodies. The yellow fever virus is attenuated by growing it in embryonated eggs, thus somehow reducing the virulence of the virus for humans. The vaccine used for immunization against smallpox is different from either of these, since a living culture of a virus, vaccinia, closely

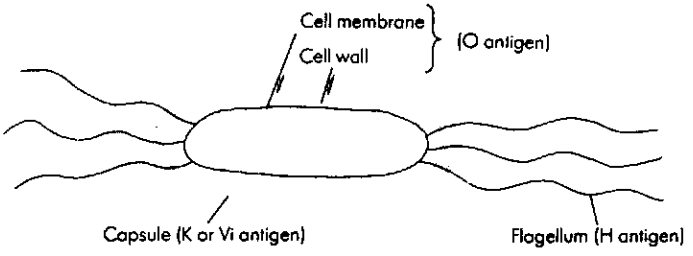

Figure 33-4. External antigens of Gram-negative bacilli such as the salmonellas.

related to variola virus, which is not highly infectious for humans, produces antibodies that protect people from infection with the human pathogen. Hepatitis B vaccine consists of highly purified, formalin-inactivated antigens obtained from the plasma of persistently infected carriers (since the virus has not yet been grown in cell cultures).

Toxoids

Toxoids are made from extracellular toxins (exotoxins) by destroying the poisonous portion with heat, ultraviolet light, or chemicals without altering their antigenic specificity and often enhancing their immunogenicity. Toxoids made from toxin-producing microorganisms such as *C. diphtheriae* and *Cl. tetani* are used to immunize against the harmful effects of diphtheria and tetanus, respectively.

Heterophile Antigens

Substances that stimulate the production of antibodies capable of reacting with tissues of a wide variety of unrelated animals or plants are called heterophile antigens. Heterophile antigen was first demonstrated in 1911 by Forssman, who showed that when emulsions of tissues, including brain, liver, kidney, and adrenals (but not blood or serum), from guinea pigs are injected into rabbits, they stimulate the rabbits to produce hemolysin capable (in the presence of complement) of lysing the red blood cells of sheep. This particular reaction is known as the Forssman reaction. Many other heterophile systems have since been demonstrated. Some pathogenic bacteria contain heterophile antigen, but it is rarely found in saprophytes. Heterophile antigen is found in the tissues of many animals, including horses, cats, dogs, fish, and fowl. A Forssman type of heterophile antigen is of diagnostic importance in mononucleosis, which results in the production of an antibody which agglutinates animal red blood cells as detected in the so-called Paul Bunnell test. Some malignant (cancerous) tissues express Forssman antigens as a consequence of neoplastic transformation.

Antibodies

Specific acquired immunity against infection is primarily a property of a group of serum glycoproteins (proteins with attached carbohydrate) called antibodies. These antibodies have been produced by a subpopulation of white blood cells in the immune system called lymphocytes. These are small round cells, 6 to 7 μm in diameter, with a high nuclear-to-cytoplasmic ratio in their resting stage, and they are capable of expanding greatly in volume and activity in response to an antigen. This process is called lymphocyte activation.

Antibodies are termed immunoglobulins (abbreviated Ig) since they are globular proteins with immune function, the bulk of which generally separate in the gamma region on electrophoresis (Fig. 33-5). All antibody molecules have two basic functions: (1) antigen binding and (2) participation in effector functions depending upon the physical properties of the antibody. These properties can be separated by cleavage of the antibody molecule with a proteolytic enzyme, papain. This produces two antigen-binding fragments (named Fab, or antigen-binding fragment) and a single crystallizable fragment (termed Fc, or crystallizable fragment) which possesses the physical properties of the molecule (Fig. 33-6). Only the Fc fragment has been shown to fix complement and bind to Fc receptors, and the Fab does not interact.

Subsequent biochemical analysis demonstrated that every monomeric anti-

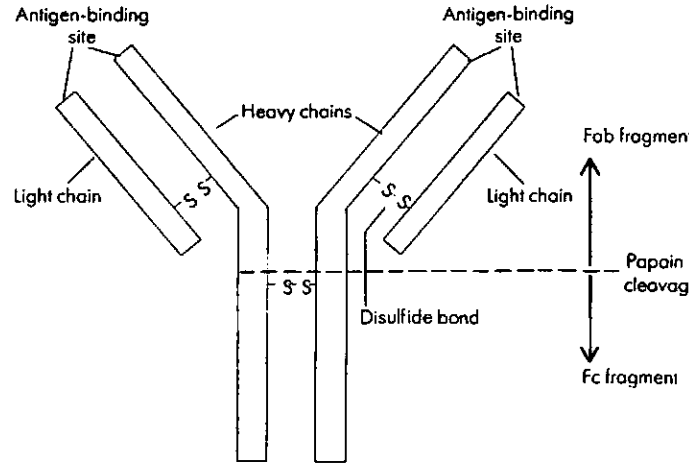

Figure 33-6. (Right) Monomer of the immunoglobulin molecule with two heavy and two light polypeptide chains held together by interchain disulfide bonds. Shaded areas indicate the variable regions or domains.

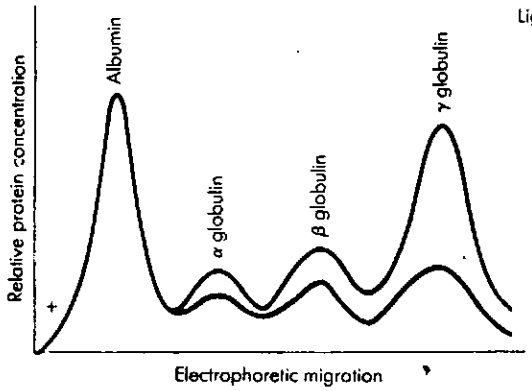

Figure 33-5. Electrophoretic profile of rabbit serum containing antibodies to egg albumin before (shaded zone included) and after absorption with the same antigen (egg albumin). Note the decrease in the globulin peaks, especially that of gamma globulin, demonstrating that the anti-egg albumin antibodies were globulin proteins.

body molecule consisted of two pairs of polypeptide chains linked to each other by disulfide bonds. The larger pair is composed of two heavy chains, each chain having a molecular weight of 50,000 daltons. The smaller pair is composed of two light chains, each with a molecular weight of 25,000 daltons. (See Fig. 33-6.)

Amino acid analysis of the antibody molecule revealed that the antigen-binding site is flanked by a variable amino acid sequence portion of the chains (Fig. 33-6) while the remainder has a relatively constant amino acid sequence. Thus the amino acid sequence of the constant regions determines the common biological and physical properties of the immunoglobulin, and the variable region determines its individual specificity.

In actual fact, there are five separate classes of immunoglobulins: immunoglobulin G (IgG), immunoglobulin M (IgM), immunoglobulin A (IgA), immunoglobulin D (IgD), and immunoglobulin E (IgE). (See Table 33-2.) Their re-

Table 33-2. Some Characteristics of the Different Classes of Human Immunoglobulins

Immunoglobulin	Heavy-Chain Designation	Molecular Weight	Physical State	J Chain	Subclasses
IgG	γ (gamma)	150,000	Monomer	–	4
IgM	μ (mu)	900,000	Pentamer	+	1
IgA	α (alpha)	160,000 (serum)	Monomers	–	2
		(160,000)n (serum)	Polymer	+	
		390,000 (secretions)	Dimer[1]	+	
IgD	δ (delta)	180,000	Monomer	–	1
IgE	ε (epsilon)	185,000	Monomer	–	1

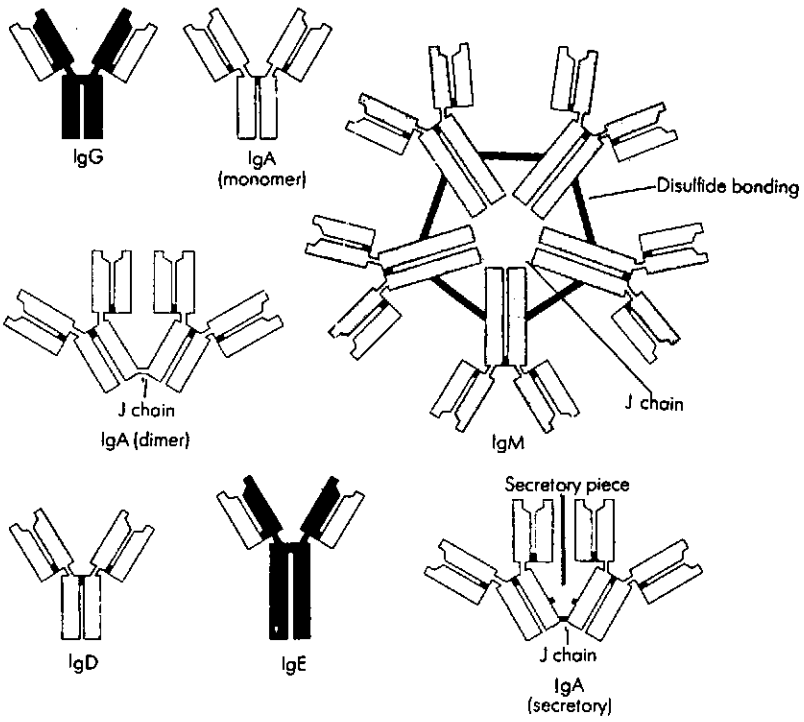

Figure 33-7. Structures of the different classes of immunoglobulins. IgG, IgD, and IgE each consists of a monomer of two light and two heavy polypeptide chains. IgM is a large molecule having five monomers in a star formation joined with a J (joining) polypeptide chain. IgA has three forms. When it appears in the serum, it may consist of one, two, or three monomers (not shown); when it is found in such body fluids as saliva, tears, and nasal secretions, it contains two monomers joined by a special component known as the "secretory piece ' (this dimer also has a J chain). The exact location of the J chain in the IgM and IgA molecules is uncertain.

spective heavy chains are designated by the Greek letters gamma (γ), mu (μ), alpha (α), delta (δ), and epsilon (ϵ). Some of these immunoglobulins are polymers of the basic unit (Fig. 33-6), and some have subclasses. Each immunoglobulin is linked with one of two types of light chains called kappa (κ) or lambda (λ). A primary rule for antibodies is that each basic unit consists of two identical heavy chains and two identical light chains. For example, an IgG antibody could have the formula of either $\gamma_2\lambda_2$ or $\gamma_2\kappa_2$, where γ refers to the type of heavy chain and λ and κ refer to light-chain types. Each class of immunoglobulin has its own characteristic structure and special function (Tables 33-2 and 33-3). Figure 33-7 shows the structures of the different classes of immunoglobulins.

IgG. The most common antibody in serum is IgG, which accounts for 80 percent of the total 10 to 20 mg of immunoglobulin present in 1 ml of serum. IgG can be subdivided on the basis of antigenic differences into four subclasses designated as IgG_1, IgG_2, IgG_3, and IgG_4; they constitute 70, 19, 8, and 3 percent, respectively, of the IgG class. As seen in Table 33-3, IgG with a molecular weight of 150,000 daltons is the only immunoglobulin capable of passing from the mother to the fetus via the placenta, and so it is the major source of passive immunoglobulin protection for the fetus and newborn of many higher vertebrate species, including humans. Monomeric IgG antibody is said to have a valence of 2 since it has two antigen-combining sites per molecule.

Table 33-3. Biological Properties of Different Classes of Human Immunoglobulins

Immunoglobulin	Site Found	Complement Fixation	Crosses Placenta	Functions
IgG	Internal body fluids, particularly extravascular	+	+	Major line of defense against infection during the first few weeks of a baby's life; neutralizes bacterial toxins; binds to microorganisms to enhance their phagocytosis and lysis
IgM	Largely confined to bloodstream	+ + +	–	Efficient agglutinating and cytolytic agent; effective first line of defense in cases of bacteremia (bacteria in blood)
IgA	Serum, external body secretions	–	–	Protects mucosal surfaces from invasion by pathogenic microbes
IgD	Serum, on lymphocyte surface of newborn	–	–	Regulator for the synthesis of other immunoglobulins; fetal antigen receptor
IgE	Serum	–	–	Responsible for severe acute and occasionally fatal allergic reactions; combats parasitic infections

IgM. The largest immunoglobulin with a molecular weight of 900,000 daltons is the pentameric macroglobulin IgM. The five monomers of IgM are held together by disulfide bonds and a single J protein (mol wt 15,000 daltons), and though the structural valence is 10, the observable valence is only 5, i.e., only five antigen molecules can be bound, possibly because of steric hindrence. Because of its large size, IgM does not cross the placenta, but monomeric IgM molecules coupled to a secretory protein usually associated with IgA have been found in mucous secretions. IgM is the first antibody produced in a primary immune response and constitutes 6 percent of the total immunoglobulin pool.

IgA. The protection of respiratory and gastrointestinal mucous membranes and external body secretions such as tears, saliva, seminal fluid, urine, and colostrum is accomplished by IgA, which constitutes 13 percent of the total serum immunoglobulin. Though IgA can exist as monomers, dimers, and polymers in serum, it exists as a dimer in secretions containing a single J chain and a secretory protein with a molecular weight of 60,000 daltons. Whereas the light, heavy, and J chains are produced by an antibody-producing lymphocyte, the secretory piece is added by epithelial cells during secretion. The function of the secretory piece is unknown, but it may be involved in the transport of IgA to an external environment, and it may protect the secreted IgA from proteolytic digestion by extracellular proteases present in gastrointestinal and other secretions. Secretory IgA in colostrum provides passive immune protection to the gut of the newborn against microorganisms which might cause gastroenteritis. In respiratory mucus, IgA may act as a first line of defense against bacteria and viruses and may also neutralize allergens.

IgD. The rare classes of immunoglobulins IgD and IgE were identified as a result of their excess production in patients with a cancer of the antibody-forming cells, called a myeloma. IgD has a molecular weight of 180,000 daltons and constitutes 1 percent of the total serum immunoglobulins. It appears to act as a primary receptor for specific antigen on the surface of fetal lymphocytes destined

to become antibody-forming cells in adult life. As such it also may determine to some extent what the immune system will accept as being "self" or respond to as being "foreign" by allowing the removal of specifically autoreactive cells prior to their maturation.

IgE. Even though they make up only 0.002 percent of total serum immunoglobulins, IgE proteins are responsible for some of the most severe immunologic reactions called **hypersensitivity** or **allergic** reactions. IgE is called a **homocytotropic** antibody since it can bind to IgE-specific Fc receptors on **autologous** (one's own) tissue basophils called **mast cells.** When antigen, such as a pollen allergen or parasite, combines with the IgE on a mast cell, the cytoplasmic basophil granules are activated, releasing chemical mediators (e.g., histamine and serotonin). In the respiratory tract these mediators cause the symptoms of allergy: sneezing, wheezing, and excess mucous secretion; in the skin, these antigens cause a typical wheal-and-flare reaction in sensitized individuals. In particularly severe sensitivities, the allergic reaction may produce fatal systemic **anaphylaxis** caused by excessive smooth muscle contraction in, for example, the bronchioles of the lungs.

Even though IgE is noted for its harmful properties, it may have protective functions. The release of histamine may result in the destruction of parasites. This hypothesis arose because it was observed that individuals in tropical areas (where parasites are more prevalent than in northern regions) may have 20 times more IgE antibodies than those who live in colder climates. This, of course, is of little comfort to northerners who suffer the miseries of summer allergies.

Functional Names of Antibodies

Antibodies react against specific microorganisms, their toxic products, and other compounds. They can be used in the treatment of infection caused by the specific micoorganisms, and, more importantly, they prevent infection and disease caused by these agents. Antibodies may be designated by names that describe their reaction in vitro or in vivo when they are allowed to act on certain types of antigens: (1) antitoxins, (2) agglutinins, (3) precipitins, (4) lysins, (5) complement-fixing antibodies, and (6) opsonins. Any antibody may be multifunctional and therefore can be called by more than one of the above terms.

These antibodies are all produced as a result of antigenic stimuli. They are humoral antibodies and can be differentiated as follows:

1 Antitoxins neutralize toxins.
2 Agglutinins cause clumping of the bacterial cells for which they are specific.
3 Precipitins cause precipitation or flocculation of extracts of bacterial cells or other soluble antigens.
4 Lysins cause lysis, or breakdown, of bacterial or other cells that are specifically sensitive to their action.
5 Complement-fixing antibodies participate in the complement-fixation reactions described in Chap. 34.
6 Opsonins render microorganisms more susceptible to ingestion by phagocytes.

Monoclonal Antibodies

As mentioned previously, myeloma is a cancer of the antibody-forming' cells (lymphocytes). Because it is derived from a single neoplasic (aberrantly growing) cell, it consists of a huge clone of identical lymphocytes. The antibody produced

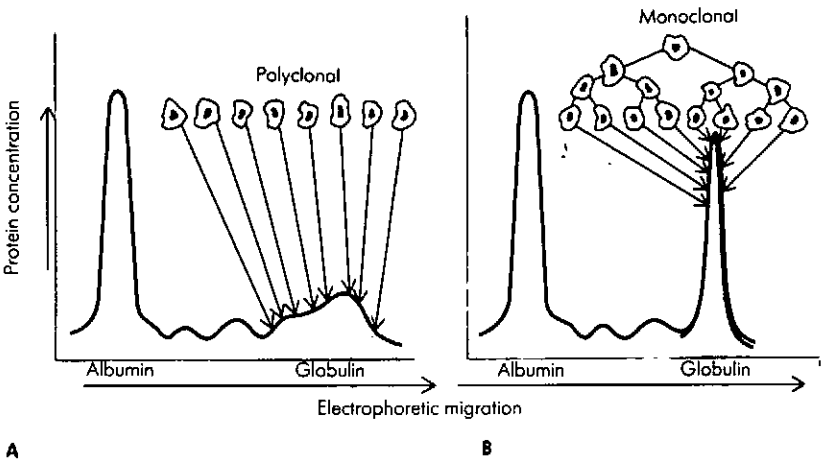

Figure 33-8. Electrophoretic distribution of antibodies in serum. (A) Heterogeneous electrophoretic distribution of antibodies typical of a polyclonal response. (Serum from an animal or human producing antibodies to a protein or other macromolecule contains a complex mixture of antibodies.) The heterogeneity has been shown to be due to the many clones of plasma cells, each of which produces a different antibody molecule. (B) Electrophoretic pattern shows the production of homogeneous, or monoclonal, antibody, such as from a myeloma patient. Myelomas (tumors of the antibody-forming cells) produce a single antibody.

by these lymphocytes is homogeneous because it is all derived from the single homogeneous clone of cells and is therefore called a **monoclonal antibody** or M protein, which forms a single peak on electrophoretic separation of the serum (Fig. 33-8). Unfortunately, the antibody produced by a myeloma tumor is generally to an unknown antigen.

A recent development in cell biology has allowed the fusion of different cells to form a single "hybrid" cell which expresses properties of both "parental" cell lines. The fusing agent can be a defective virus (Sendai virus that characteristically causes cell fusion) or various chemicals (such as polyethylene glycol). If one uses as the fusion partners an immune lymphocyte producing a specific antibody in limited quantity and a myeloma cell, one is able to select fused cells called **hybridomas** (see Fig. 33-9). In these cells the myeloma provides properties of unrestricted proliferation and production of vast quantities of monoclonal antibody and the immune lymphocyte provides the information for the specificity of the antibody, that is, an antibody with known antigen-binding characteristics. Such hybridoma-derived monoclonal antibodies are becoming increasingly important in diagnostic and therapeutic medicine. For example, in cancer therapy, monoclonal antibodies can be used directly to attack and destroy tumor cells. They can be labeled with radioactive isotopes to locate tumors and to deliver specifically lethal doses of radiation to inaccessible tumors. They can also be used to deliver anticancer drugs to tumor cells in a similar manner.

Microbiologists already have generated monoclonal antibodies that allow new differentiations between strains of rabies viruses as well as between strains of influenza viruses. There is no doubt that the diagnosis and epidemiology of many viral, rickettsial, parasitic, and bacterial diseases will be greatly facilitated by the availability of monoclonal antibodies. Such antibodies have been produced against the bacterial pathogens *Streptococcus pneumoniae, Mycobacterium leprae, Treponema pallidum, Neisseria gonorrhoeae, N. meningitidis, Pseudomonas aeruginosa,* and *Haemophilus influenzae.*

The most successful use of monoclonal antibodies so far is in diagnostic kits

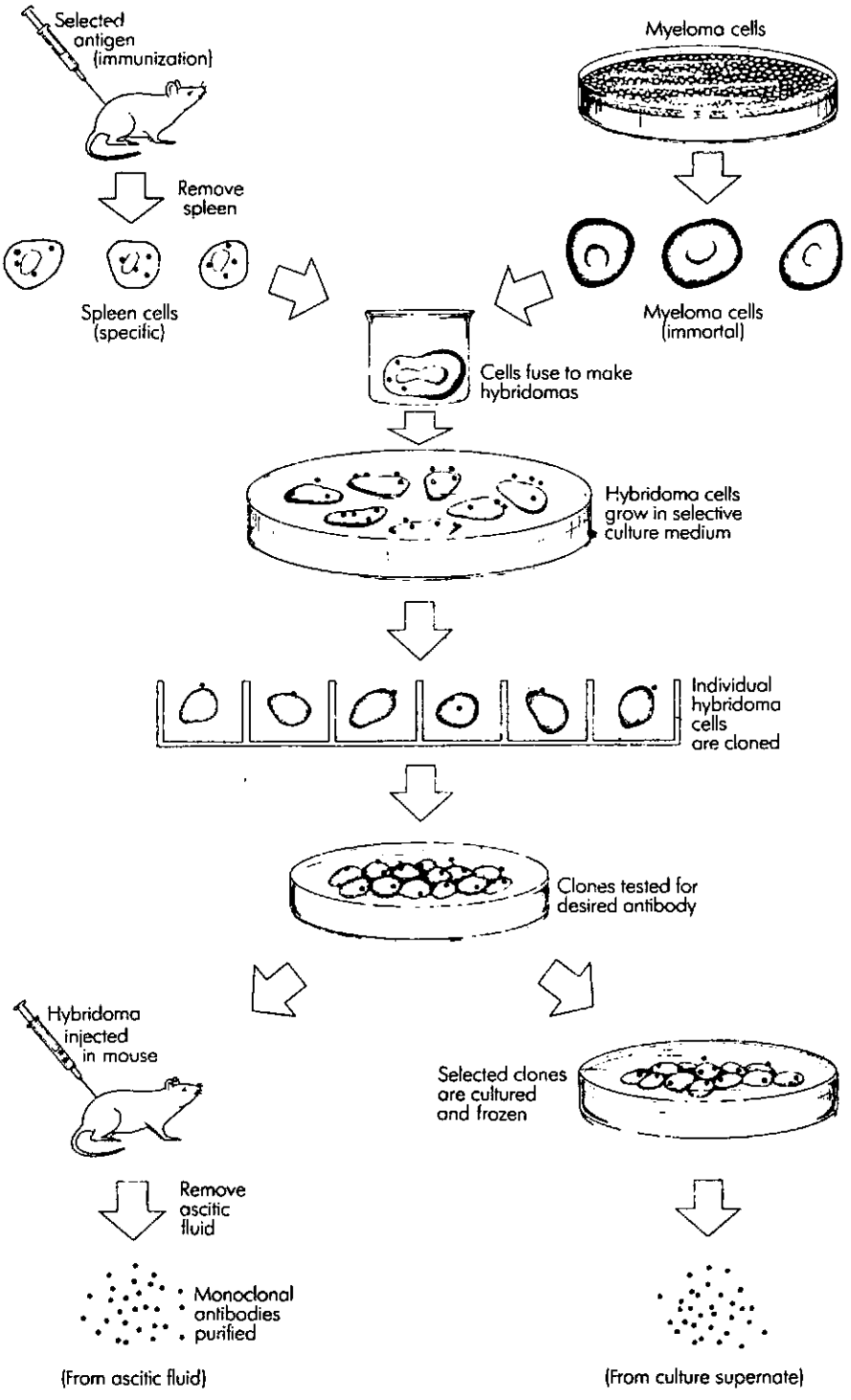

Figure 33-9. How monoclonal antibodies are made. The selected antigen is injected into a mouse. A few days later, the spleen is removed from the mouse. The antibody-synthesizing cells from the spleen are then mixed with fast-growing mouse cancer cells, called myelomas, and a chemical "glue," polyethylene glycol. The result is a hybridoma, a fusion of an antibody-making cell and a cancer cell. The hybridomas are separated, cloned, and tested to select those that make the desired monoclonal antibodies. The chosen hybridomas are injected into the peritoneal cavity of mice, where they induce the accu mulation of 2 to 10 ml of ascitic fluid containing large amounts of antibody. Or the hybridoma cells may be cultured in the laboratory. (They may be stored frozen until they are needed to generate additional supplies of monclonal antibody.) The final step is to separate the pure monoclonal antibodies from the hybridomas by differential centrifugation. *(Erwin F. Lessel, illustrator.)*

Development of the Hybridoma Technique

The hybridoma technique was developed by Georges Köhler and Cesar Milstein in 1975 at the Medical Research Council laboratories in Cambridge, England. Although the technique was never patented, its commercial applications were recognized immediately. In 1982 alone, more than three dozen independent ventures in monoclonal antibodies were initiated, and at least 20 large chemical and pharmaceutical companies are developing monoclonal antibodies for a wide variety of uses. One projection puts the world market value of hybridoma antibodies at a half billion dollars annually by the late 1980s.

For the discovery of the principle that led to production of monoclonal antibodies, Köhler and Milstein were awarded a Nobel prize in medicine in 1984.

used to test specimens of blood, urine, or tissues for blood type, viral disease, and other factors. For example, in 1981, the Food and Drug Administration approved four such kits for use in diagnosing allergies, prostate cancer, pregnancy, and anemia.

Cell-Mediated Immunity

Whereas antibodies in the serum and extracellular fluid form part of the humoral immune system, the living lymphocytes, particularly those derived from the thymus (T lymphocytes), are responsible for cell-mediated immunity. This includes immunity to tubercle bacilli, viruses, fungi, and many other intracellular parasites, the rejection of foreign tissue grafts, a delayed type of hypersensitivity, and resistance to some cancers. In all these cell-mediated immune reactions, specific lymphocytes recognize and bind the relevant antigen or cell and transform into cytotoxic T lymphocytes. The activated T lymphocytes also proliferate and release soluble mediators called lymphokines which recruit and activate other host cells. One of the lymphokines is called migration inhibition factor (MIF), which is instrumental in the inflammatory response since it prevents the migration of lymphocytes and phagocytes away from the focus of the immune response. Interferon is another example of a lymphocyte-produced lymphokine.

THE IMMUNE SYSTEM

Anatomy and Development

The lymphocyte is the basic cell responsible for both humoral and cellular immunity. This cell in its resting stage is small (6 μm in diameter), with a high nuclear-to-cytoplasmic ratio, indicative of its lack of activity.

A pool of recirculating lymphocytes passes from the blood into the lymph nodes, spleen, and other tissues and back to the blood by the major lymphatic channels such as the thoracic duct. Lymphocytes are found in high concentrations in the lymph nodes and spleen and at the sites where they are manufactured and processed: the bone marrow and the thymus (Fig. 33-10).

The bone marrow hemopoietic stem cells are the ultimate origin of erythrocytes and all leukocytes including the lymphocytes. Many lymphocytes pass through the thymus where they become processed by the hormonal microenvironment prior to release. These lymphocytes are now called thymus-derived lymphocytes, T lymphocytes, or T cells. (See Fig. 33-11.) The majority of the

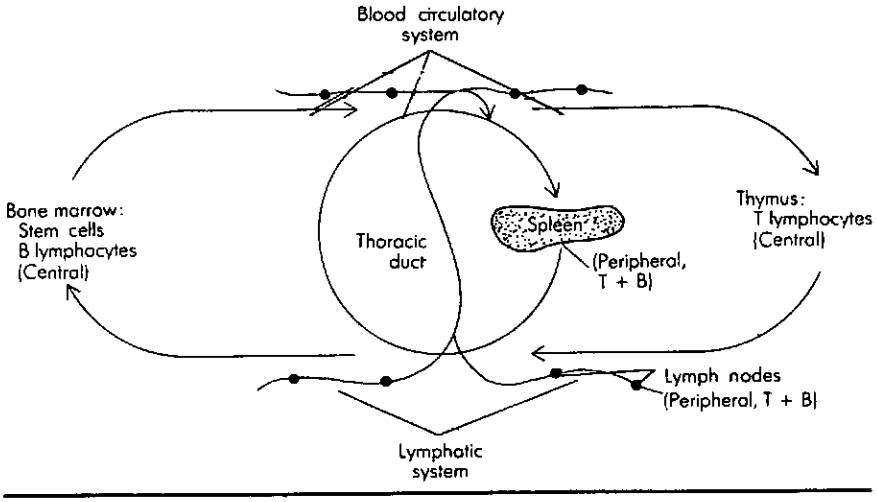

Figure 33-10. Basic anatomy of the immune system. Immune responses occur in the peripheral lymphoid organs, such as the spleen and lymph nodes. The central lymphoid organs (thymus and bone marrow) are the sources of the lymphocyte subpopulations (T and B cells) which seed to the peripheral organs. *(After an original drawing by Malcolm Baines, McGill University.)*

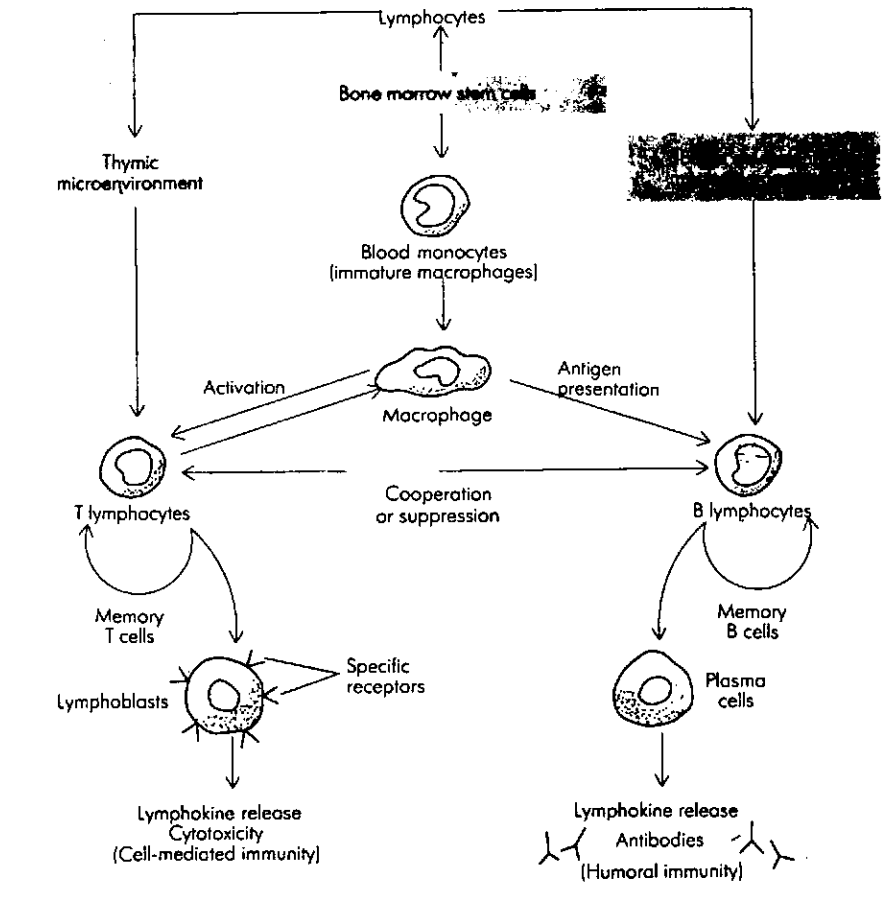

Figure 33-11. The generation of humoral and cell-mediated immune responses. *(After an original drawing by Malcolm Baines, McGill University.)*

bone marrow–derived lymphocytes which do not enter or become processed by the thymus are called B cells. Studies of the chicken have revealed the existence of a central lymphoid organ called the **bursa of Fabricius** responsible for inducing mature B-lymphocyte function just as the thymus induces T-cell function. It is thought that the site in which human B cells mature (i.e., the bursal equivalent) is the gut-associated lymphoid tissues (GALT), such as the Peyer's patches in the intestines, and the spleen and bone marrow. Though both populations of lymphocytes are morphologically identical, the B cells are destined to synthesize specific humoral antibody and the T cells become primarily responsible for cellular immunity. Lymphocytes which do not express either B- or T-cell markers or functions are called, appropriately, **null lymphocytes.**

T and B Lymphocytes

The interrelationships between the T and B cells are very complex in that each can augment or regulate the activity of the other (Fig. 33-11). In addition, specific antigen must be presented to the lymphocytes in the proper format by macrophages.

The B cells have on their surface membrane the immunoglobulin receptors representative of the specific antibody which they are capable of producing. One B cell is destined to synthesize only one specificity of antibody, although it may be able to synthesize more than one class of antibody. Thus during an immune response the first or early antibody produced is IgM, and after a few days the predominant antibody synthesized is IgG, even though it may express the same specificity. The B cell must receive a "signal" from an interacting cell (T cell and/or macrophage) in order to be stimulated to proliferate and, eventually, differentiate into the plasma cell form (see Figs. 32-6 and 33-11). Plasma cells are the ultimate antibody-secreting cells; they are also end-stage cells which die after they have served their function. A mature plasma cell can produce immunoglobulins of a unique class and subclass, as well as a unique type of heavy and light chain. Synthesis proceeds at a rate of some 300 molecules per second for its life span of a few days to weeks.

After the immune response wanes, the surviving lymphocytes return to their resting stage and the serum antibodies, mostly IgG, gradually disappear, with a half-life of about 25 days. However, the anamnestic response (Fig. 33-2) suggests the presence of an additional type of differentiated cell (a specific B lymphocyte) that arises following initial exposure to an antigen. It is extremely long-lived, exists in rather low numbers, and is capable of greatly accelerated proliferation into effector cells upon restimulation (boosting) with antigen. This cell is called a **memory cell** since it is the basis of immunologic memory (Fig. 33-11).

Macrophages have also been shown to produce several lymphokines that serve many of the same functions as those produced by T cells (see below). This release of lymphokines probably serves as a regulatory mechanism to activate or inactivate either T cells, B cells, or null cells.

T cells perform a wide variety of functions. These functions can generally be classified into three types: (1) helper function, (2) suppressor function, and (3) killer function. The helper function is to recognize an antigen and then facilitate a B-cell response to it through the release of lymphokines and interleukins. The suppressor function of T cells is the recognition of antigen followed by the release of factors which result in suppression of B-cell or other T-cell responses.

Table 33-4. Some Known Immunodeficiency Diseases

Immunodeficiency	Cellular Defect	Result
Agammaglobulinemia (Bruton's disease)	B lymphocytes low or absent	Low or absent antibodies; increase in pyogenic infections
Thymic aplasia (DiGeorge's syndrome)	T lymphocytes absent	Low or absent cell-mediated immunity; increase in viral infections; inability to reject grafts; increase in cancers
Phagocytic dysfunction (Chediak-Higashi syndrome)	Phagocytic leukocyte activity	Decreased intracellular killing of microbes; increase in pyogenic infections; decrease in natural killer cell functions; increase in cancers
Complement defects	C3 production	Increase in pyogenic infections

The killer function is cytotoxicity: the ability to cause the death of foreign cells, such as cells from a genetically different donor, parasite cells, single-celled pathogens, and tumor cells. Each of these three types of T-cell functions can be executed either by direct cell-to-cell contact with the interacting cell or by the effect of lymphokines, which were discussed previously in relation to cell-mediated immunity.

Immunodeficiency Diseases

Defects in one or more components of the immune system can lead to **immunodeficiency diseases** caused by impaired or absent immunity of one type or another. Table 33-4 summarizes a few of the genetic immunodeficiencies currently known. A new immunodeficiency disease of unknown cause and high virulence has come to our attention since late 1978. It is called acquired immunodeficiency syndrome or AIDS. As of April, 1984, physicians and health departments in the United States and Puerto Rico had reported a total of 4,087 cases of the disease. Of these patients, 1,758 (43 percent) are known to have died. About a third of the patients contracted a rare form of cancer called Kaposi's sarcoma. The other major disease manifestation was infection by any of several opportunistic pathogens. But by far the most common life-threatening opportunistic infection in AIDS patients was due to the protozoan *Pneumocystis carinii*, which causes a severe pneumonia.

The underlying problem is a defective immune system, which leaves AIDS patients unable to resist infections and malignancies. Most investigators think that AIDS is caused by an infectious agent, possibly a new virus or a new varient of an existing virus. Recently, virologists at the National Institutes of Health in Bethesda, Maryland, have obtained evidence that the etiologic agent is a member of the retrovirus family, HTLV-III (see Chap. 37). The causative virus is possibly carried by sperm or blood.

Most of the patients are homosexual men and bisexual men (71 percent of cases), intravenous drug users (some 17 percent), and persons born in Haiti and now living in the United States (5 percent of cases). Only 6 percent of the known cases are women.

A defective cellular immunity has been implicated as the cause of AIDS. The patients have low lymphocyte counts, often half or less than half of the normal lower limit of about 1500 lymphocytes per milliliter of blood. The T cells are both low in number and abnormal in composition. Apparently the B lymphocytes (and therefore humoral immunity) are not much affected. However, some

recent reports indicate a defect in B-cell function as well; that is, the B cells are not able to respond to a variety of stimuli, presumably because of a lack of helper T cells. In addition to T-cell abnormality, AIDS patients may have a reduced population of natural killer cells, which are important in cancer cell surveillance.

The study of AIDS is providing immunologists with information about the normal workings of the immune system and how its breakdown can result in disease, including cancer.

THEORETICAL CONCEPTS

There are several basic questions concerning the functioning of the immune response which are still only partly understood. They deal with the mechanism of the generation of antibody diversity and how the immune response actually operates at the cellular level.

Generation of Antibody Diversity

It has been estimated that a single individual is capable of producing in excess of a million different specificities of antibody in a normal lifetime. If all this information were stored in every cell, this would occupy an excessive amount of its DNA "memory." Recent studies of the genetics of immunoglobulin inheritance and the biochemistry of antibody specificity have produced a more realistic explanation for the generation of antibody diversity.

This explanation is based on the fact that in the genomes of higher organisms the genes occur in pieces, rather than as continuous stretches of DNA. It is the assembly of the individual genes by combining separate segments of DNA that accounts for much of the great diversity of antibodies. Let us examine this situation in a little more detail.

Each light chain of an antibody molecule consists of about 220 amino acid residues. It consists of two regions of approximately equal length: the variable region, which differs from one chain to the next, and the constant region, which is the same for all light chains of the same class. In a similar manner, heavy chains of antibodies (each containing 440 amino acid residues) have a variable region consisting of 110 amino acids; the rest of the molecule constitutes the constant region. It will be recalled that the variable regions of both the heavy and light chains form the antigen-binding site (see Fig. 33-6).

Two gene segments are needed to produce the variable region of the light chain. One segment (designated V) codes for the first 95 or so amino acids of the variable region. A second segment (designated J because its product *joins* the constant and variable regions) codes for the remaining 15 amino acids. A third segment (designated C) codes for the constant region.

The existence of a separate J-region gene segment increases the amount of diversity that can be programmed into the variable region. For example, the mouse genome is estimated to carry about 300 V gene segments and 4 J gene segments. They can combine to produce variable regions for light chains of the κ class. The κ chains constitute about 95 percent of all the light chains of mice and about 60 percent of those of humans; the remainder belong to the λ class. Simple V-J joining can thus generate about 1200 different κ variable regions. This light-chain gene assembly is shown in Fig. 33-12.

Knowledge about the rearrangement of the genes coding for antibody heavy

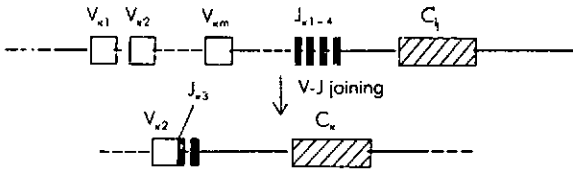

Figure 33-12. Light chain gene assembly. The upper diagram shows the arrangement of κ chain gene segments in the DNA of germ-line cells. During V-J joining, any of the variable gene segments ($V_{\kappa 1}$ through $V_{\kappa m}$) can be connected to any of the joining gene segments ($J_{\kappa 1-4}$), as shown in the lower diagram for the $V_{\kappa 2}$–$J_{\kappa 3}$ combination. The variable region gene thus assembled remains separate from the constant region gene (C_κ) until this whole stretch of DNA is copied into RNA transcripts. Then the left end of the variable-region gene is spliced to the right end of the constant-region gene, and the resulting messenger RNA is translated to yield a κ light chain. *(From J. L. Marx, "Antibodies: Getting Their Genes Together," Science, 212:1015–1017, 1981. By permission of the American Association for the Advancement of Science.)*

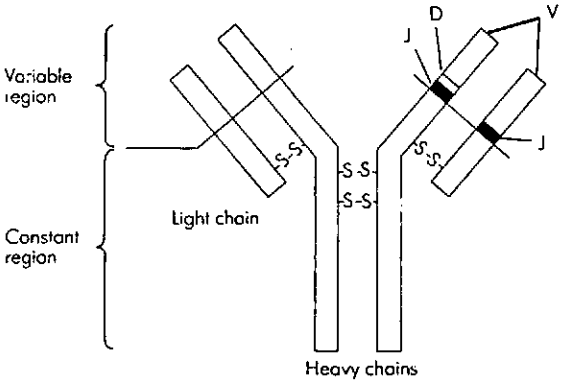

Figure 33-13. Regional structure of the antibody molecule in gene assembly. V, D, and J refer to the variable, diversity, and joining regions of the appropriate chains.

chains has been slower in coming, probably because these proteins are larger than the light chains. However, present evidence shows that heavy-chain genes are also assembled together from separate DNA pieces. Three pieces must be joined together for a complete heavy-chain variable region (unlike the two pieces for the light-chain variable region). A third piece of DNA is inserted between the V and J segments and is designated D for diversity (because it codes for amino acids in one of the most variable sections of the heavy-chain variable region; see Fig. 33-13). Like the V-J joining of light chains, V-D-J joining occurs after an undifferentiated immune cell begins to develop into an antibody-synthesizing cell. The heavy-chain gene assembly is shown in Fig. 33-14. As may be seen, one assembled variable region can be attached to any of five different constant regions. The type of constant region determines the class to which an antibody belongs. For the five classes of immunoglobulin (Ig) M, D, G, A, and E, the corresponding constant-region genes are designated Cμ, Cδ, Cγ, Cα, and Cε, respectively.

The sequence in which the different antibody classes are expressed follows a

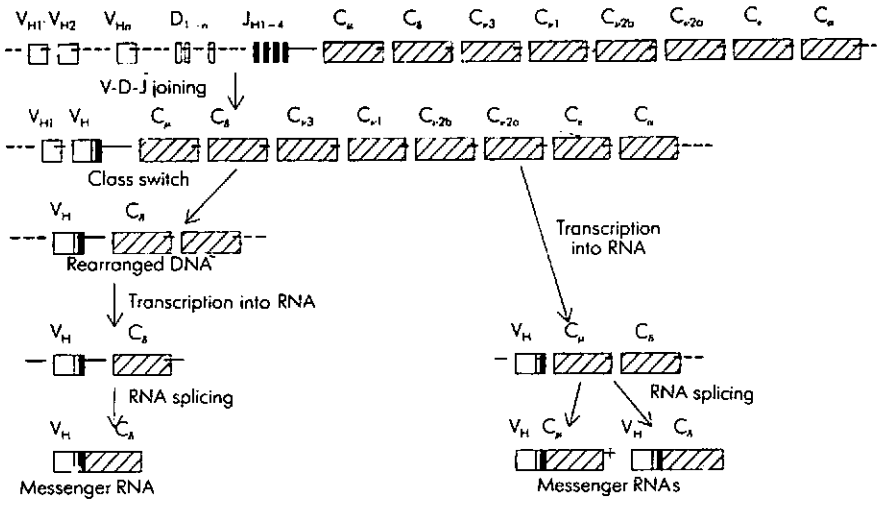

Figure 33-14. Heavy-chain gene assembly. The upper diagram shows the arrangement in germ-line cells of the gene segments for antibody heavy chains. The assembly of a complete variable region gene (V_H) reguires the joining of three gene segments: one of those designated V_{H1} through V_{Hn}, one D, and one J. After V-D-J joining, transcription of the rearranged DNA into RNA, followed by differential splicing of the transcript, allows the simultaneous production of messenger RNAs for two different classes of heavy chain (shown in the right-hand branch of the diagram). The messengers direct the synthesis of either an IgM or IgD heavy chain. The shift to the final class of heavy chain that will be produced by the cell involves a further gene rearrangement (left-hand branch). In this case, the cell will make an IgD heavy chain and the Cμ gene segment has been deleted to bring the completed variable gene nearer to the Cδ gene segment. If the cell were going to make an IgA heavy chain, gene segments Cμ through Cε would have to be deleted. The rearranged DNA is transcribed and the RNA copy spliced to form the final messenger. *(From J. L. Marx, "Antibodies: Getting Their Genes Together," Science, 212:1015–1017, 1981. By permission of the American Association for the Advancement of Science.)*

set pattern: the cell first makes IgM, then it may make both an IgM and an IgD at the same time, and finally it becomes committed to producing a single class of heavy chain, which may be any of the five. This necessitates that the completed variable-region gene be switched from one type of constant-region gene to another at the appropriate time during development. That is, as B cells develop, they can replace the Cμ gene with any of the other C genes. This kind of change is termed **class switching** and is effected either at the level of RNA or by gene rearrangement.

As seen in Fig. 33-14, the assorted gene segments that must be rearranged to produce a complete heavy chain are arranged on the chromosome in order of their appearance, with the V, D, and J gene segments followed by the Cμ, Cδ, Cγ, Cε, and Cα genes. During the early stages of development, before the antibody-producing cell becomes committed to producing a single type of heavy

chain, processing of RNA transcripts is the most important factor in determining the type of chain to be made. For example, after V-D-J joining, a single RNA transcript, which extends from before the beginning of the completed variable-region gene to beyond the end of the Cδ gene, is made. When the noncoding portions of the transcript are excised, the variable-region copy can be attached to either a Cμ or a Cδ gene copy, giving the cell the ability to make an IgM and an IgD at the same time.

In the later stages of development, when the cell becomes committed to production of a particular class of heavy chain to produce, the constant-region genes between the assembled V-D-J region and the desired C gene are apparently removed. This suggests that gene rearrangement is one means of generating the almost limitless diversity of antibodies.

Theories on Antibody Diversity

Historically, three major competing theories were formulated to explain this antibody diversity:

1 The germ-line theory postulated that there was a separate variable gene for every possible antibody chain.
2 The somatic mutation theory held that there need be only one or a few such genes; diversity was brought about by mutations of these genes.
3 The minigene theory held that the genes were assembled from the shuffling of small gene segments in varying combinations.

Present evidence indicates that each of these theories contained some elements of truth. There are at least a few hundred different V gene segments for both light and heavy chains (germ-line theory). Their diversity can be further increased by V-J or V-D-J joining (minigene theory). Mutations also contribute to antibody diversity because there is more variation in the protein structures of the variable regions than can be explained by the genes (somatic mutation theory).

Cooperation and Regulation

As previously stated, many B cells require T-cell help to produce an antibody response to the majority of antigens (viruses, parasites, bacteria, allergens, and transplantation antigens). These antigens are termed **thymus-dependent** antigens and serve to illustrate how T cells cooperate with B cells by providing a second stimulus to trigger antibody synthesis by B cells. The first stimulus is of course specific antigen. A few antigens are directly *mitogenic* (stimulate cell division) for B cells and do not require helper T cells (T_H), and they are therefore called **thymus-independent** antigens (e.g., cell-wall lipopolysaccharides of bacteria).

Once an immune response has been initiated, the primary regulator is also specific antigen. Once it has been masked or covered by the specific antibody and removed and degraded by macrophages, the primary stimulus has been eliminated and the immune response stops. In addition, a group of regulatory T cells, called suppressor T cells (T_S), actively turns off the immune response by directly inhibiting the T_H cells. These types of controls are necessary to prevent the generation of uncontrolled immune responses to foreign and autologous (self) substances. The occurrence of *autoimmune diseases*, such as rheumatoid arthritis and hemolytic anemia, serves as evidence that the normal immunologic *tolerance* of self can break down.

HYPERSENSITIVITY

The result of an immune response is that the individual has an increased state of reactivity to the specific antigen or pathogen. Situations in which host immune responses contribute to tissue injury are collectively referred to as **hypersensitivity states**. Hypersensitivity reactions can be conveniently divided into four major types as advocated by two British immunologists, Philip Gel and Robin Coombs.

Type I: Immediate Hypersensitivity Reactions

Type I hypersensitivity is mediated by IgE antibodies which have bound to the Fc receptors of circulating basophils and tissue mast cells, thereby sensitizing them. When antigen contacts the sensitized cells, an *immediate* (within 1 to 10 min) skin reaction occurs leading to intense local inflammation typified by diffuse infiltrations by polymorphonuclear leukocytes, which results in a soft, swollen, red skin reaction. For this reason it is thought to be important in effecting resistance to pathogens.

The chemical basis for this reaction is the release by the sensitized basophils and mast cells of a number of preformed substances (histamine, serotonin, heparin, and chemotactic factors) and unstored (synthesized de novo) mediators (e.g., slow reacting substance of anaphylaxis, leukotactic activity substance, and platelet-activating factors). Besides an immediate skin reaction, the biological effects of these substances or mediators account for a more generalized clinical picture because certain target organs (smooth muscle, vessel walls, etc.) are affected. So-called **anaphylactic reactions** are due to this type of hypersensitivity reaction and are characterized by severe symptoms, including irritation, rash, swelling, wheezing, shock, and occasionally death. Allergic reactions to insect bites (by mosquitoes or wasps) are also due to this type of hypersensitivity reaction.

Type II: Antibody-Dependent Cytotoxic Hypersensitivity Reactions

Type II hypersensitivity is mediated by IgM and IgG antibodies reacting with cellular or particulate antigens. This **antibody-dependent cytotoxic** hypersensitivity results in complement-mediated cytolysis, antibody-dependent cell-mediated cytotoxicity (ADCC), or the opsonization and increased phagocytosis of particles sensitized by antibody or fixed complement. The latter process is particularly effective against bacteria and free viruses in a systemic infection and provides protection against infectious agents. Unfortunately, type II hypersensitivity can also damage the host because of the circulating antibodies. Thus it also is responsible for clinical transfusion reactions (mismatching), erythroblastosis fetalis (Rh isoimmune reactions), and some autoimmune reactions (occurring secondarily to certain infections and diseases). Extrinsic antigens, e.g., penicillin, may bind to red cell surfaces, where they act like haptens and cause the formation of antibodies. These antibodies are capable of fixing complement and lysing the red blood cells (drug-induced hemolytic anemias).

Type II hypersensitivity reactions differ in three ways from type I reactions:

1 Antibody combines with antigenic determinants on the cell surface through its Fab portion before any Fc interactions occur.
2 Interaction of antibody with the target cell directly results in cell death.
3 IgE is not involved.

Type III: Immune-Complex-Mediated Hypersensitivity Reactions

Type III hypersensitivity reactions are also mediated by IgG and IgM antibodies which react with soluble antigens. This causes the formation of circulating immune complexes (IC) which can cause widespread inflammatory responses called serum sickness (IC formed intravascularly and deposited at distant sites) or intense local inflammatory responses in the peripheral tissues (IC formed in situ) called an Arthus reaction after its original investigator. Bacterial toxins and free viral proteins are presumably the intended targets, but this type of hypersensitivity can cause severe organ dysfunction such as kidney failure, rheumatoid arthritis, and types of toxic shock syndrome.

Type IV: Cell-Mediated Hypersensitivity Reactions

Type IV hypersensitivity is mediated by sensitized T lymphocytes which can cause direct target cell–mediated lysis and the release of soluble lymphokines. Since these cells arrive at a skin site containing antigen 24 to 72 h after infection, producing a typical hard swelling, this type of reaction is called a delayed reaction. This contrasts with the 4- to 24-h time course of the Arthus reaction. (Types I, II, and III hypersensitivity reactions have historically been described as immediate hypersensitivity reactions. In addition, these reactions are dependent on the activity of antibody for their pathological effects.) Upon histological examination the delayed reaction site is densely infiltrated by mononuclear cells (lymphocytes, monocytes). The cytotoxic T lymphocytes are important in killing cells infected with intracellular parasites such as viruses, bacteria, and trypanosomes as well as fungi and perhaps cancer cells. On the other hand, T-cell-mediated hypersensitivity is responsible for accelerated graft reactions and allergic skin reactions caused by contact with chemical or metal allergens (e.g., nickel) termed contact sensitivity. The cutaneous delayed-type tuberculin reaction (sensitivity test for tuberculosis using partially purified protein derivative of *Mycobacterium tuberculosis*) is the prototype for cell-mediated immunity. Thus nonsensitized individuals (with no prior exposure to the organism) give no reaction.

QUESTIONS

1 What are the two types of effector products of the immune system? Describe the overall division of labor between the two types of effectors with respect to pathogens and transformed (cancer) cells.
2 Explain the four primary characteristics of a generalized immune response.
3 Explain by means of diagrams the significance of (*a*) anamnestic response, (*b*) use of adjuvants.
4 Identify the following terms with respect to antigens: (*a*) specificity, (*b*) immunogenicity, (*c*) antigenicity, (*d*) haptens, (*e*) carrier, (*f*) complete antigen.
5 Distinguish between the following pairs of items related to antigens:
   (*a*) Toxins and toxoids
   (*b*) H and O antigens of *Salmonella*
   (*c*) Salk vaccine and Sabin vaccine for poliomyelitis
6 Why are antibodies termed *immunoglobulins*? How do they behave in an electrophoretic field?
7 Provide an account of the general structure of an immunoglobulin molecule giving reference to: variable region, constant region, Fab, and Fc.

8 Compare and contrast the structure and biological functions of IgM and IgE.
9 Write a brief account of the general properties of IgG.
10 Identify the special functional names of antibodies when they are allowed to act on certain types of antigens.
11 What is a monoclonal antibody, and how is it produced?
12 What are the useful properties of hybridomas? How are they developed?
13 Compare and contrast cell-mediated immunity and humoral immunity with respect to their activity.
14 Compare the derivation of the B cells and T cells.
15 Briefly describe the B cells and their production of antibodies.
16 What is the basis of immunologic memory?
17 Describe the three types of functions ascribed to T lymphocytes.
18 What kind of disease is AIDS? Do we know its etiology?
19 Explain the means by which an individual generates the great diversity of antibodies produced in a normal lifetime.
20 What were the theories formulated over the years to explain the immense repertoire of antibodies produced during an individual's lifetime? Are they still sound? Explain.
21 Describe briefly cooperation and regulation between T and B cells.
22 List the four major types of hypersensitivity reactions.
23 Which type of hypersensitivity reaction causes (*a*) anaphylactic shock, (*b*) opsonization, (*c*) transfusion reactions, (*d*) allergy to penicillin, (*e*) toxic shock syndrome, (*f*) tuberculin reaction?

REFERENCES

The references cited in Chap. 32 are also applicable to this chapter.

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Chapter 34 Assays and Applications of the Immune Response

OUTLINE

As discussed in the previous chapter, the induction of a specific immune response usually results in the production of specific effector cells (cell-mediated immunity) and antibodies (humoral immunity). The detection and quantitation of a specific immune response to a pathogen usually indicates that the patient has had an infection at some time within the past year, and is now immune and no longer at risk. If two blood specimens are taken at 1- to 2-week intervals during a current or recent infection, a rising quantity of antibody in the serum from the first to the second bleeding (called a rising antibody titer), specific for the pathogen, indicates that the pathogen is, in all probability, the causative or etiologic agent. The total lack of any immune antibodies or of a positive reaction to a test dose of the specific antigen indicates a susceptible individual who should be immunized. The above shows the usefulness of immunoassays, and further applications of them will be discussed in this chapter.

Generally, unknown pathogens are identified using antibodies of known specificity, and serodiagnosis is made by reacting patient sera with antigens from known pathogens. When antibodies combine specifically with antigens, usually an immune complex is formed which can be seen or detected using an appropriate technique.

This chapter gives an overall view of assays and applications encountered in the diagnosis of infectious disease and is not intended to be a step-by-step methods manual. For such information, the student should refer to the *Manual of Clinical Immunology* (cited in References).

MEASUREMENT OF HUMORAL ANTIBODIES

Since antibodies alone cannot be seen by the naked eye, we must detect them by what they do in the presence of the specific antigen or pathogen. Many of the in vitro tests depend upon the formation of a visible reaction endpoint by the cross-linking of antigen and antibody to form a large complex.

The Lattice Hypothesis

The most favored model to explain antigen-antibody interactions to form large visible complexes is the lattice hypothesis (Fig. 34-1). In chemical terms, antibody (Ab) usually has a very high binding affinity for the specific antigen (Ag) and can be expressed by the equation shown at the top of the next page.

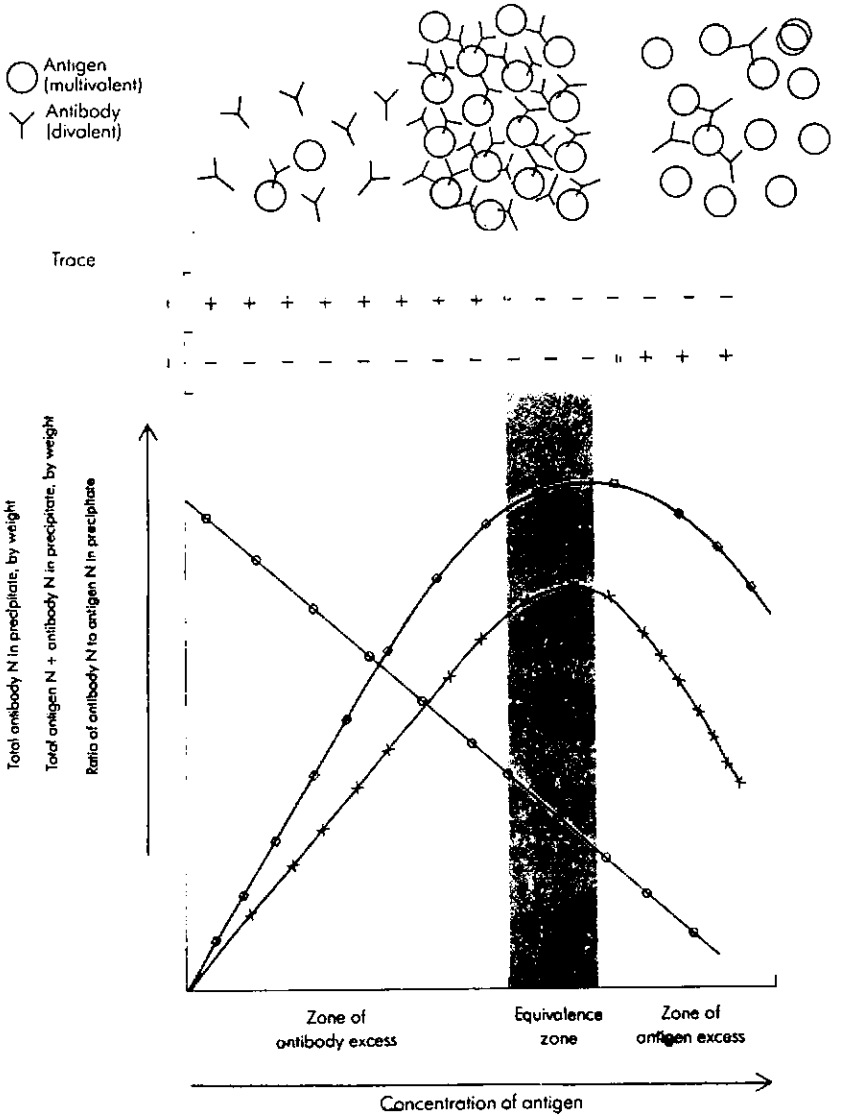

Figure 34-1. Graph and schematic illustration of the events of the precipitin reaction. In this reaction, increasing concentrations of antigen are added to a series of serological tubes containing a constant concentration of antibody (antiserum). The concentration of antigen and antibody present in any tube is determined by assaying for nitrogen (N), a component of both the antigen and antibody. I and II refer to tests for excess antibody and excess antigen, respectively, in the fluid remaining after removal of the precipitate (+ = positive; tr = trace; − = negative).

$$_{v1}(Ag) + {}_{v2}(Ab) \rightleftharpoons Ag_{v1}{:}Ab_{v2}$$

where v_1 = valence of antibody and v_2 = valence of antigen.

The equilibrium constant K is therefore

$$K = \frac{[Ag{:}Ab]}{[Ag][Ab]}$$

where the brackets indicate the actual concentrations of the reactants and product in moles per liter. As can be seen, the amount of complex formed increases with the K value. In order for the combination of antigen and antibody to form a visible complex their respective valences must be greater than 1. We already know from Chap. 33 that antibodies are bivalent even in their monomeric form (e.g., IgG). However, if the antigen is a monovalent hapten, then two moles of hapten would combine with one mole of antibody forming soluble complexes with the formula $Ag_2{:}Ab_1$, which would be too small to see. If the antigen were polyvalent (has many antigenic determinants), the antibody now has the opportunity of cross-linking molecules of antigen to form lattices. If these become large enough, then they precipitate out of solution and flocculate or agglutinate particles to form a visible reaction (Fig. 34-1).

To form the greatest amount of precipitate or agglutinate in an immune reaction it is necessary that antigen and antibody be present in optimal or equivalence amounts such that there is just enough antigen to saturate the antibody-combining sites. This is called the **equivalence zone** and can be used to quantitate antibody or antigen. An excess of antigen or antibody will result in the formation of smaller, and probably invisible, soluble immune complexes due to a lack of extensive lattice formation (Fig. 34-1). Some serological tests based on antigen-antibody reactions are shown in Fig. 34-2.

Precipitin Tests

In the **precipitin test** a reaction takes place between a soluble antigen and a solution of its homologous antibody. The reaction is manifested by the formation of a visible precipitate at the interface of the reactants but may be inhibited by an excess of either antigen or antibody. The most useful precipitin tests therefore provide for diffusion of the reactants until optimum concentration is reached (see Fig. 34-1). This equivalence zone represents the concentrations of antigen and antibody where complete precipitation occurs. Culture filtrates or animal sera contain a number of different antigens. If they are used for immunization, they give rise to a correspondingly large number of different antibodies. Unless special precaution is taken to remove all but one precipitin from the solution, several layers of precipitate are formed—one for each precipitin system present. The formation of several precipitate layers is not necessarily a disadvantage since these are used to identify and distinguish between different antigens and antibodies in a solution. Factors which influence precipitin tests are electrolytes, pH, temperature, and time.

Precipitin tests are not limited to the laboratory diagnosis of bacterial infections of humans and other animals by means of specific antigens but are also useful in many other ways, such as (1) serologic screening tests for syphilis where a nonspecific antigen not related to the spirochete is usually used; (2) serological identification of various pathogens; (3) identification of blood or

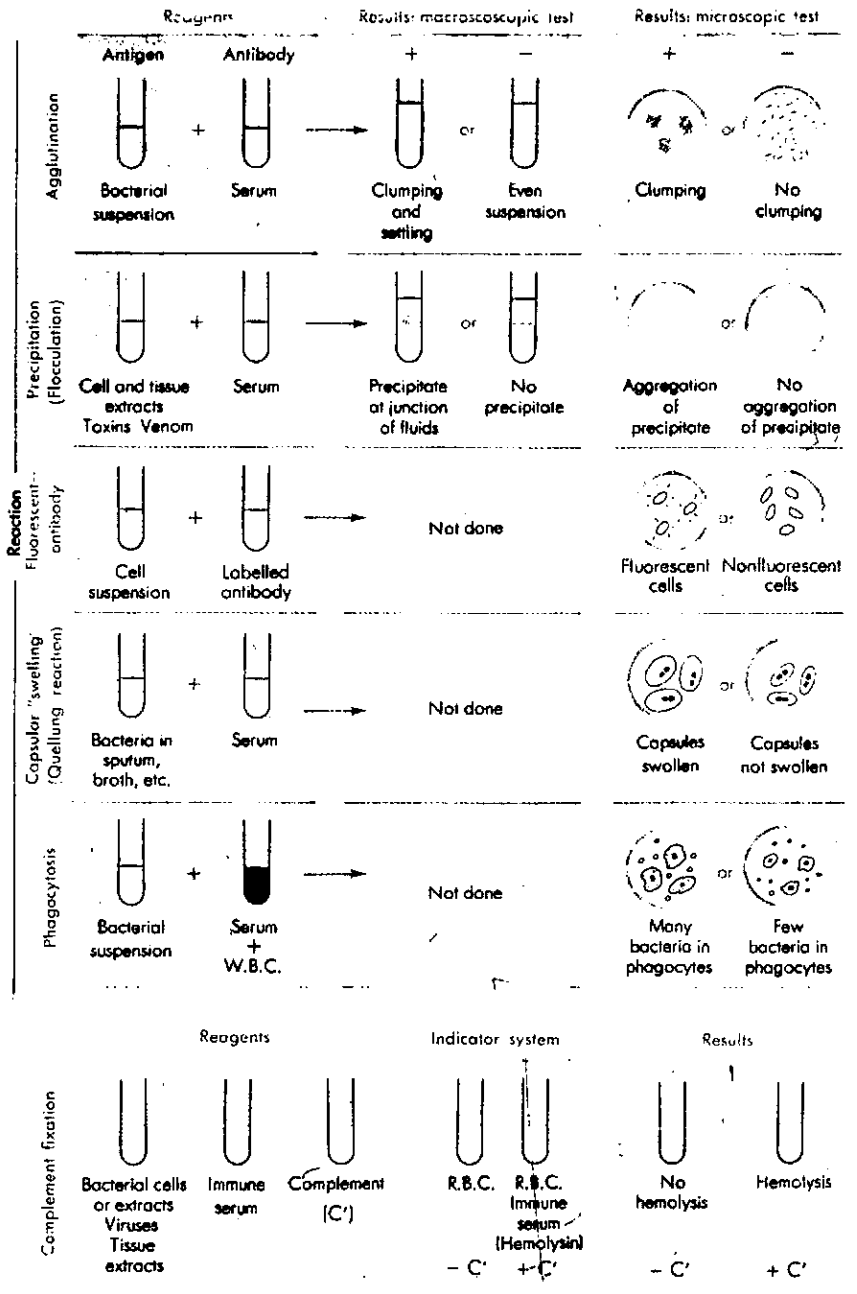

Figure 34-2. Some serological reactions.

seminal fluid in stains on clothing, weapons, or other exhibits for medicolegal purposes; (4) postmortem diagnosis of anthrax from tissue of a dead or decomposed animal by the Ascoli test; (5) determination of the kind of animal a

mosquito has recently fed on (this information helps entomologists and epidemiologists to prevent the spread of arthropod-borne diseases); and (6) detection of the adulteration of foods.

Antigen for precipitin tests is prepared by making an extract from bacterial cells, tissues, or other suitable material. For example, in the Ascoli test for anthrax, a small amount of infected spleen is boiled in physiological salt solution. The test consists in layering a milliliter or so of the extract over a similar amount of antiserum prepared by injecting a rabbit with killed anthrax bacilli.

Precipitating antigens for streptococcus typing and diagnosis of certain other bacterial infections are made from 24- to 48-h cultures that are extracted to yield a clear solution of antigenic precipitable material.

One- and Two-Dimensional Immunodiffusion

A tube precipitin test can be performed in a fluid phase. This is called a **ring test** because the precipitate forms a ring or white disk at the interface between the clear solution of antigen and antibody (Fig. 34-3A). For greater accuracy,

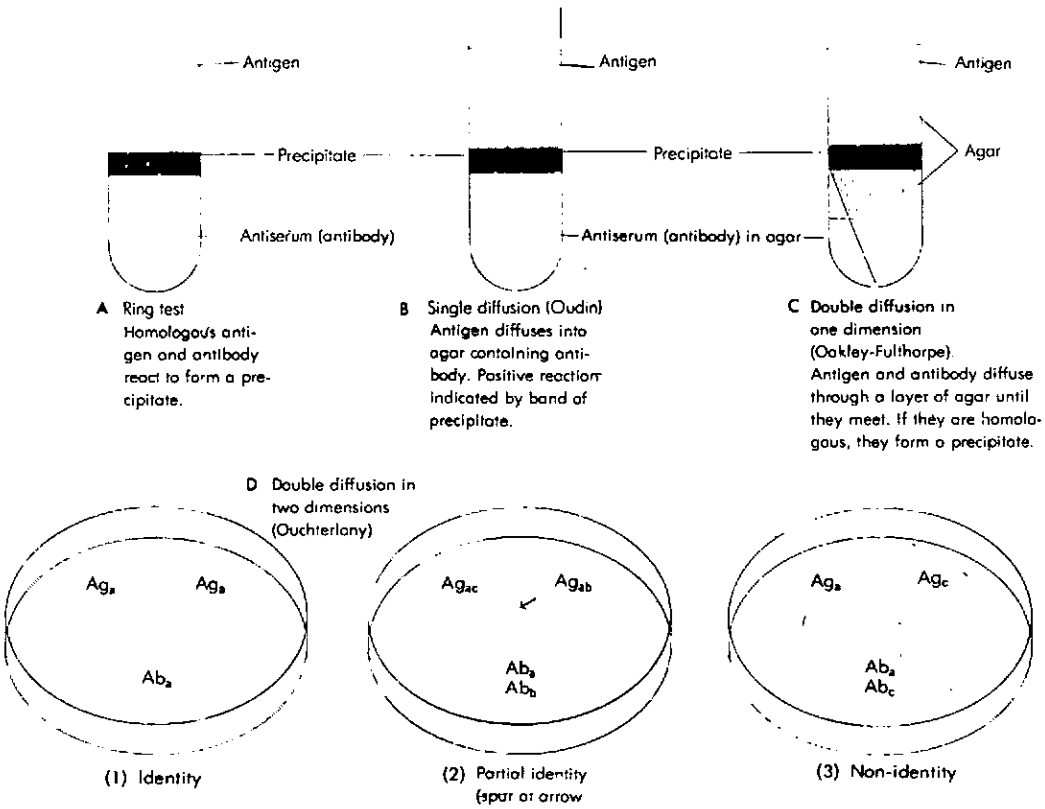

Figure 34-3. Qualitative precipitin tests (Ag = antigen; Ab = antibody).

Antigen and antibody are placed in separate wells cut into agar in a Petri dish. Homologous reactants produce a line of precipitate where they meet in the agar. Like antigens produce bands which meet exactly as in (1). Unlike antigens produce bands which cross as in (2) and (3).

ease of handling, and preservation of the tests the antibody can be contained in agar gel and the antigen allowed to diffuse into it. This is called a **single-diffusion** method. The method of Oudin directly layers antigen over antibody in agar (Fig. 34-3B), and the Oakley-Fulthorpe method separates these two reagents with simple agar (Fig. 34-3C). This results in the diffusion of both reagents into the agar and the formation of precipitin ring(s) with greater accuracy. This method is described as a **double-diffusion** precipitin test.

The simple method for double diffusion in two dimensions developed by Ouchterlony allows the comparison of various antigens and antisera for specific identities. The antigen and antibody diffuse from wells cut in a layer of agar to form a precipitate at the equivalence zone point. By cutting the wells in the agar in two-dimensional patterns, several antigens can be compared. A fusion of immune precipitates of an antibody preparation with antigens from two separate wells indicates identity of the two antigens, whereas the crossing of precipitates shows a lack of identity (Fig. 34-3D).

Single Radial Immunodiffusion in Agar

Whereas the former precipitin tests described are qualitative, antigens can also be quantitated. The Mancini method allows antigen to diffuse from a well into a layer of agar which contains specific antibodies. Thus, a ring of precipitate forms, the diameter of which is proportional to the antigen concentration. This is a simple but sensitive method for quantitating antigens to concentrations of 1 μg/ml (Fig. 34-4).

Immunoelectrophoresis

Electrophoresis is an electrochemical process in which colloidal (suspended) particles or macromolecules with a net electric charge migrate in a solution or agar gel under the influence of an electric current. A characteristic of living cells in suspension and biological compounds (such as protein antigens) in solution or in a gel is that in an electric field they travel to the positive or negative electrode, depending on the charge on the substances. Positively charged substances travel to the cathode, while negatively charged ones go to the anode; this movement is called **electrophoretic mobility.** When electrophoresis is applied to the study of antigen-antibody reactions, it is called **immunoelectrophoresis.**

When a fluid containing protein antigens is placed in a well in a thin layer of buffered agar, and an electric current is applied, antigens will be distributed in separate spots along a line passing through the well and parallel to the direction of current flow (Fig. 34-5A). The spots are analogous to antigen wells in an Ouchterlony plate. When the current is shut off, diffusion will begin from each of these spots. By placing antiserum in a trench cut in the agar parallel to the electrophoretic distribution of the antigens, the precipitin reaction can be used to demonstrate the nature of the diffusing molecules. In this case, a broad band of antibody diffuses toward the antigens from the linear antibody trench, while the antigens diffuse as expanding disks. This results in a complex pattern

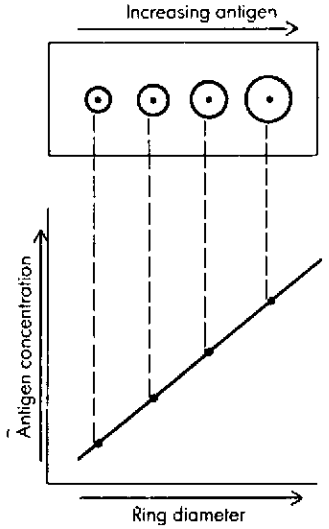

Figure 34-4. Single radial immunodiffusion. The rings, initially faint, become heavier as diffusion nears completion. At equilibrium, the diameter of each ring is directly proportional to the concentration of monospecific antibodies.

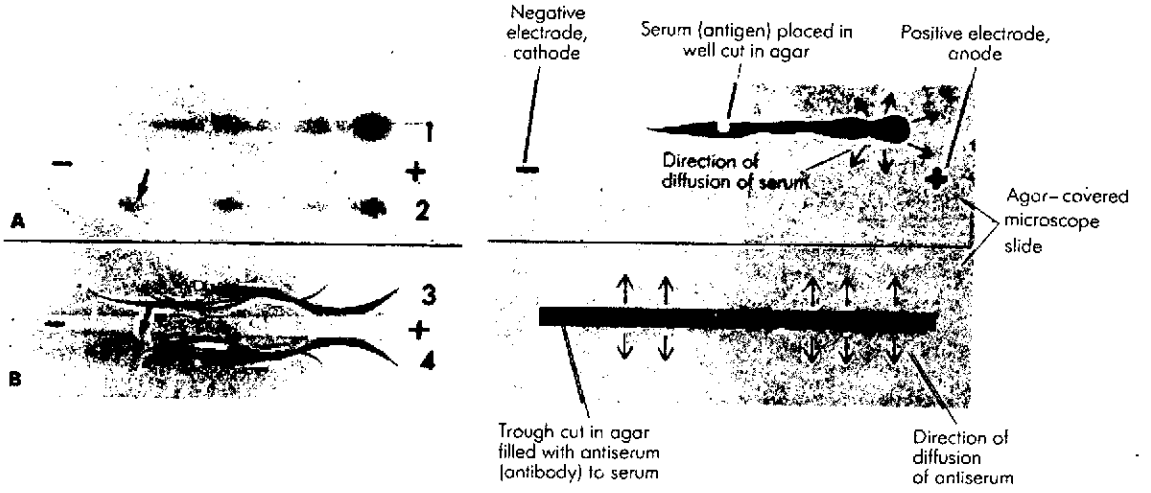

Figure 34-5. Left: Agar-gel electrophoresis (A) and immunoelectrophoresis (B) of sera from normal mice and from mice bearing tumors. 1. Normal mouse serum. 2. Tumor mouse serum. 3. Normal mouse serum. 4. Tumor mouse serum. The precipitin bands were developed with antimouse serum prepared from rabbits inoculated with mouse serum. Those bands corresponding to the tumor protein are indicated with arrows. *(Courtesy of R. A. Murgita, McGill University.)* Right: Explanatory sketches for photographs (A) and (B).

of arc-shaped zones of precipitate (Fig. 34-5B). When the antigen molecules are from human serum, there may be 20 or more such zones, each of which represents at least one distinct precipitin system. Precipitin specificity provides a far more critical indicator of the identity of protein molecules than the physical characteristics which control the movement of the molecules in an electrophoretic field.

Rocket Immunoelectrophoresis

A combination of the immunoelectrophoresis assay and the Mancini assay involves the electrophoretic migration of antigen from wells into an agar gel which contains specific antiserum. This results in the formation of a rocket-shaped precipitate. The height of each "rocket" is proportional to the concentration of antigen in the well. The sensitivity of this assay allows the measurement of antigens in concentrations as low as 0.5 μg/ml. (See Fig. 34-6).

Precipitin Test for Streptococcus Grouping and Typing

Differentiation of the streptococci into serological groups and according to types within some of the groups is accomplished best by a precipitin test. It is an example of how a serological reaction is used for the identification of microorganisms. The serological groups A, B, C, D, E, F, G, H, and K to O of streptococci have been identified on the basis of the carbohydrate antigen known as C substance. Types are determined by the presence of M substance, which is also on the cell wall of the streptococci.

Streptococcus-precipitating antiserum is obtained by immunizing rabbits with

heat-killed suspensions of the bacteria or extracts containing both group-specific C and type-specific M substances.

The test is performed by layering a small amount of type-specific antiserum and antigen in a capillary tube (ring test). If the test is positive, a cloudy ring or disk appears at the junction of serum and antigen in a few minutes. In practice, the group is determined, and if it is group A, the antigen is tested against type-specific antisera for type determination.

Agglutination Tests

The agglutination reaction is relatively easy to perform, is simple to interpret, and is the serological method of choice when a suitable cellular antigen can be prepared. Among the human diseases for which the agglutination test is of diagnostic value are typhoid fever, salmonellosis, brucellosis, tularemia, typhus fever, and Rocky Mountain spotted fever. Agglutination tests are useful in the diagnosis of many animal diseases such as brucellosis in cattle and goats, glanders in horses, swine erysipelas, and pullorum disease of chickens.

Not only can the diagnosis of certain infectious diseases be confirmed in the laboratory by agglutination of known antigens by the patient's serum, but unknown cultures of bacteria or other microorganisms can be identified by the capacity of a known antiserum to agglutinate the suspension of unknown cells.

The **Widal test** was devised specifically to aid in diagnosis of typhoid fever by agglutinating typhoid bacilli with patients' serum, but the term is sometimes loosely applied to other agglutination tests using heat-killed cultures of organisms other than *Salmonella typhi*.

Another agglutination test used in laboratory diagnosis is the **Weil-Felix reaction**. This test is based on the fact that several of the rickettsias have a common antigen with strains of *Proteus* spp.; therefore serum from patients with rickettsial infections agglutinates suspensions of the *Proteus* organisms. The strains of *Proteus* used most commonly are *Proteus* OX-19, OX-2, and OX-K. The Weil-Felix reaction is differential for certain rickettsial diseases because of the selective agglutination of these strains.

Agglutination tests are classified as **macroscopic** when the test is carried out in small test tubes referred to as agglutination tubes and **microscopic** when antigen and antiserum are mixed on a slide and examined under a microscope (see Fig. 34-2). Measuring the agglutination of a particulate antigen by its specific antibody (direct agglutination) is the simplest way to estimate the quantity of that antibody in serum. Very small amounts of antibody can be detected by this method, since only a small number of antibody molecules is necessary for the formation of the antigen-antibody lattice. The agglutination reaction has been extended to include a wide variety of antigens by attaching soluble antigens to the surface of inert particles, such as bentonite, latex, or red blood cells. The role of these particles, once coated, is a passive one; i.e., they react as if they themselves possessed the antigenic specificity of the coating antigen.

Red blood cells (erythrocytes) have been found to be extremely convenient carriers of antigen. When specific antibody is added to antigen-coated red blood cells, antibody bridges are formed between neighboring erythrocytes, and large aggregates of erythrocytes are produced which are visible to the naked eye. This agglutination is designated **passive** or **indirect hemagglutination.**

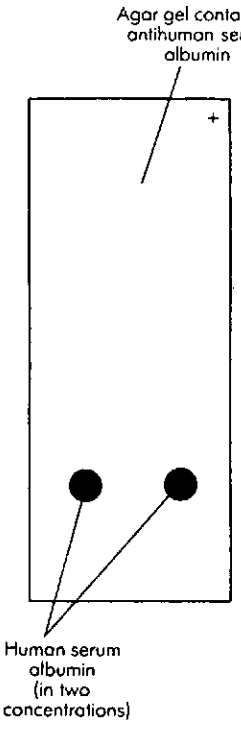

Figure 34-6. Rocket immunoelectrophoresis of human serum albumin into agar containing anti-human serum albumin. At equilibrium, the height of the rocket is proportional to the antigen concentration.

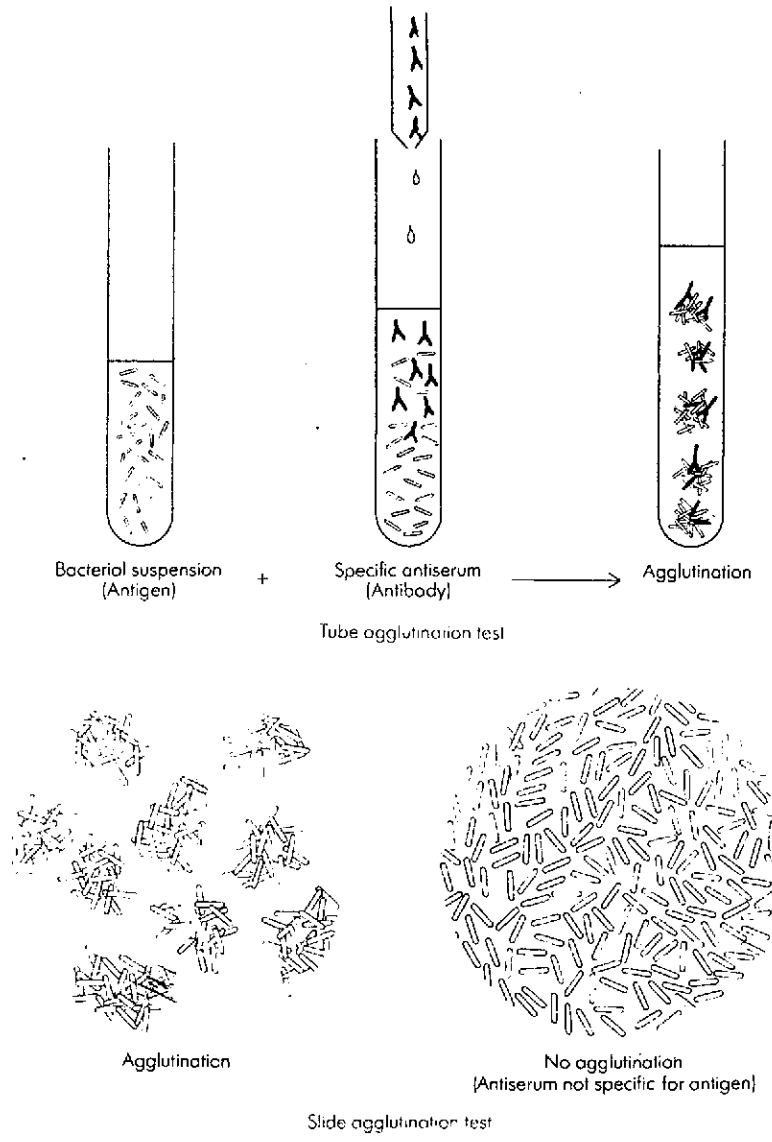

Figure 34-7. Agglutination occurs when bacterial cell antigens are exposed to homologous antibodies. (Note that antibody size is not depicted to scale.)

Tube Agglutination

The most widely used agglutination test is the macroscopic tube agglutination test (Fig. 34-7). By this method not only can the presence of specific agglutinins in serum be detected, but their approximate concentration can be determined because the antiserum can be diluted serially in a set of test tubes and the antigen (suspension of bacterial cells) mixed with it.

Antigens for agglutination tests are made by preparing a homogeneous suspension of the organisms taken from a young culture in physiological saline

(0.85% NaCl solution). The salt used for dilutions provides an electrolyte without which agglutination will not occur even though the antibodies are specific for the antigen used. A concentrated suspension of the organisms can be stored and diluted for use as required. A standardized suspension of antigen for use in the macroscopic tube agglutination test is made by diluting the cell suspension with physiological salt solution.

The serum is diluted serially with physiological salt solution in several small test tubes. To each tube is added a constant amount of bacterial cell suspension. A control tube contains no serum.

After incubation, bacterial cells (antigen) in the control tubes should not be clumped. If they are, the results of agglutination in other tubes are not reliable. Agglutination in tubes with small amounts of serum indicates a higher titer, i.e., high concentration of antibody in the serum.

Macroscopic Slide Agglutination Test

This is a quick and convenient method for determining the presence of agglutinating antibodies. A drop of a dense suspension of the organisms (antigen) in saline is placed on a clean glass slide. One to three drops of the patient's serum are mixed with the antigen by gentle rotation. Positive agglutination, observable with the unaided eye, occurs in 3 to 5 min.

The test can be made semiquantitative by using exact amounts of antigen and antibody. Graduated amounts of antibody are placed in separate spots on a slide. If a constant amount of antigen is added to each, a range of dilutions results. Positive and negative controls must be set up on separate slides. Since this is a screening test, doubtful or positive reactions should be confirmed.

Microscopic Slide Agglutination Test

For the microscopic agglutination test, serial dilutions of serum in physiological saline are prepared, one loopful of each dilution is placed on a cover glass, and to each a loopful of antigen, which may be a young, living broth culture of bacteria, is added. For the control, one loopful of salt solution (containing no

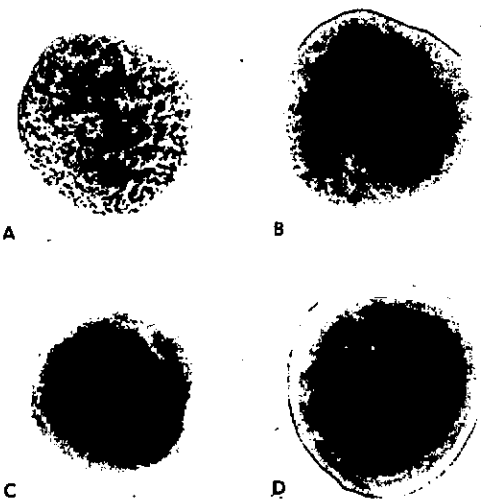

Figure 34-8. Agglutination tests performed with whole blood are particularly useful in testing for certain animal diseases in the field. Agglutination of *Salmonella pullorum* antigen with a drop of whole blood taken from chickens gives prompt reactions and makes it possible to remove infected birds from the flock without delay. (A) Strongly positive, (B) positive, (C) weakly positive, and (D) negative reactions. *(Courtesy of U.S. Department of Agriculture.)*

serum) and a loopful of antigen are used. Each cover glass is placed over a concave slide ringed with petrolatum to prevent drying, incubated for 1 h, and then examined with the high-dry objective of the microscope for clumping (Fig. 34-7). This method can also be used for rapid identification of unknown cultures of bacteria using known antisera.

Whole-Blood Agglutination Tests

Agglutination tests with whole blood are used in pullorum-disease eradication programs and for the diagnosis of swine erysipelas. The test is similar to the rapid serum test except that whole blood is used rather than serum.

The test for pullorum disease is made by mixing a loopful of freshly drawn blood with a drop of the antigen on a glass plate with a white background. Positive serum from infected birds causes clumping within 2 min (Fig. 34-8). It can be carried out quickly and simply in the field.

A positive test with *Erysipelothrix rhusiopathiae* antigen may indicate either infection or contact with the causative organisms of swine erysipelas.

Agglutinin-Adsorption Tests

Related organisms such as those of the genus *Rickettsia* and the organisms in the genus *Salmonella* or the genus *Shigella* may contain common antigens that cause several species or strains to agglutinate with antiserum prepared from a specific species or variety of the group. To illustrate, suppose that antibodies are produced against a given organism X, and by analysis we know that X antigen is composed of fractions A, B, C, and D. The antiserum would then contain corresponding antibodies *a*, *b*, *c*, and *d* and would agglutinate X antigen at high titer. If, however, organism Y contains antigen fractions A, F, G, and H, because of the common antigen A, it, too, would be agglutinated by antiserum specific for organism X but usually at a lower titer than X, since only fraction A is common. This cross agglutination can be eliminated by adsorbing out *a* antibodies using Y antigen, i.e., by incubating Y antigen (organism) with the X antiserum. After removal of the Y cells the antiserum would no longer agglutinate organism Y since *a* antibodies had been adsorbed out, but it would still agglutinate X cells. Unless the phenomenon of cross agglutination is understood, this reaction leads to misinterpretation of results of routine agglutination tests. Proof of cross agglutination can be obtained by agglutinin-adsorption tests, and the results of these tests are valuable aids in the identification of bacteria. By the agglutinin-adsorption test, the antigenic pattern of closely related organisms such as the salmonellas can be determined.

Hemagglutination Test

Certain viruses have the ability to agglutinate red blood cells from certain species of animals, notably chicken, guinea pig, or human type O red blood cells. This is due to an interaction in which the virus is adsorbed on the erythrocytes and then the blood cell–virus aggregates agglutinate (the viral attachment protein cross-links erythrocytes). Because this aggregation dissociates spontaneously, the test must be read in 1 h or less. The amount or intensity of the agglutination depends on the concentration and infective titer of the virus. The procedure consists in making serial dilutions of the virus antigen; then the red blood cell suspension is added to each tube, and the tubes are incubated for 1 h. Agglutination is indicated by an even coating of red blood cells in the bottom of a tube.

In a negative reaction (no agglutination) the red cells merely settle to form a compact button in the bottom of the tubes.

The exact technique for conducting a hemagglutination test varies with the virus, i.e., antigen. This test is useful for serological diagnosis of many viruses including the influenza virus, mumps, and the ECHO viruses. Vaccinia and variola viruses, those of the coxsackie and encephalitides groups, and several viral pathogens of domestic animals also agglutinate erythrocytes.

Hemagglutination Inhibition Test

During the course of many infectious diseases the etiologic agent stimulates the body to produce antibodies. This forms the basis for the use of serological tests used for diagnosis. For example, if two or more serum specimens are taken from the patient at intervals of 10 to 14 days during his or her illness and a serological test reveals an increasing antibody titer, this is an indication that the infective agent is serologically related to—or is the same as—the antigen used in the test. In the case of the influenza or other viruses which cause hemagglutination, an increase in the antibody which inhibits this agglutination (neutralizes the viral hemagglutin) indicates the presence of the virus of the type of antigen employed.

The hemagglutination inhibition test can also be used to identify virus strains isolated from a patient. This requires rather complex techniques for the preparation of specimens and reagents and for excluding false reactions.

Complement-Fixation Tests

Complement-fixation tests are based on the presence of complement-fixing antibodies in serum (see Fig. 34-2). These antibodies are produced in an animal body stimulated by bacterial antigens. In the presence of the antibodies, complement causes lysis of the specific bacterial cells. The purpose of the complement-fixation test is to determine whether specific bacterial antibodies are present in serum. In the actual test, two systems are involved. One is the bacteriolytic or complement-fixing system in which serum, bacterial suspension (or other antigen), and complement are mixed. If the antigen and antibody in the serum are capable of union, the complement is said to be fixed. The second system is simply an indicator system in the test. Rabbit antibodies against sheep red blood cells are added together with sheep red blood cells. If complement is available, the red blood cells are lysed. If, however, the complement is fixed by being used in the reaction between the bacteriolytic antibody and antigen, no hemolysis will occur. Therefore, a hemolytic reaction indicates a *negative* test. Obviously, all reactants in the complement-fixation test must be accurately adjusted.

The Wassermann Test for Syphilis

The complement-fixation test is widely used in the laboratory diagnosis of many infectious diseases, including those of bacterial, viral, protozoan, and fungal etiology. It is also used for the identification of many microorganisms. One of the best-known applications of the complement-fixation test is the Wassermann test for syphilis, which differs from many other serological reactions in that the antigen is not prepared from the causative organism of the disease but is made by extracting beef-heart powder with ether and alcohol. This extract contains a complex phospholipid called cardiolipin. Cholesterol and lecithin are added to the extract to increase the sensitivity and specificity of the antigen. Since the

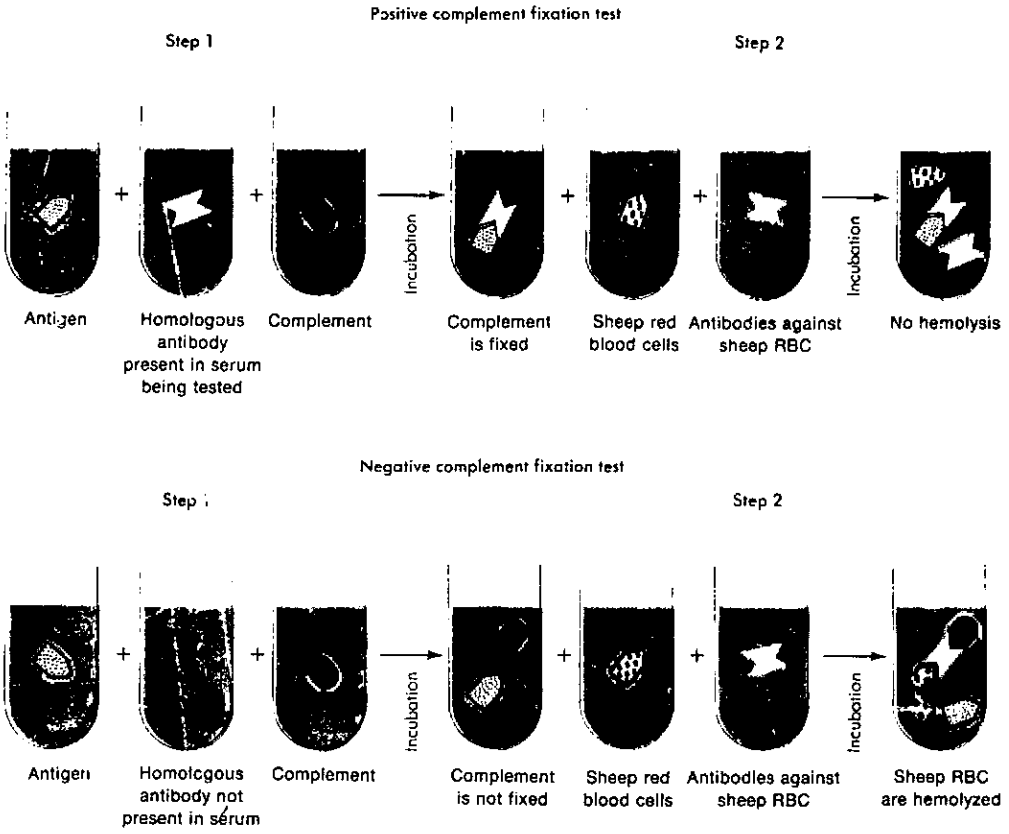

Figure 34-9. The complement-fixation test is based on the fact that if complement, which is found in blood, is "fixed" by reacting with antigen and its homologous antibody, lysis of sheep red blood cells does not occur even though antibodies against the sheep red blood cells are present.

material is not capable of inducing antibody formation in an animal, it is not a true antigen; but because it combines with syphilitic "antibodies" in the complement-fixation test, it is referred to as a nonspecific antigen.

The principle for all complement-fixation tests is shown in Fig. 34-9.

The Wassermann and other tests for the serodiagnosis of syphilis which employ nonspecific cardiolipin antigens were in use for many years before it was possible to prepare an antigen from the specific organism. Since techniques for cultivating virulent *Treponema pallidum* in vivo have been developed, several new tests have been introduced. One is the ***Treponema pallidum*** **complement-fixation (TPCF) test**, in which the antigen is an extract of the virulent treponemes. This antigen can be standardized and stored for several months. The principle is the same as that of other complement-fixation tests, but the actual techniques differ somewhat.

Radioimmunoassays (RIAs)

The sensitivity with which antigens can be detected has been increased by the use of radiolabeled (radioactive-labeled) antibodies or pure antigens. The method is also rapid and precise and has an enormous diversity of applications. The direct method employs labeled antibody which can be precipitated with

the antigen in immune complexes. In the Farr test, by labeling the antigen, the amount of radioactivity in an antigen-antibody precipitate measures the antigen-binding capacity of the immune sera. A further development often used in quantitating hormone levels is the inhibition assay. In this type of radioimmunoassay the clinical specimen containing an unknown quantity of hormone is reacted with a known quantity of antibody. Labeled pure hormone is then added to the reaction, and its binding is inhibited in direct proportion to the antibody sites occupied by the unknown hormone. In these tests the isotope used to label the proteins is usually ^{125}I, and the final measurement of the radioactive label is made using a solid (gamma) scintillation counter. RIAs are used to detect human chorionic gonadotropin in the serum of women as a test for pregnancy.

Enzyme-Linked Immunosorbent Assays (ELISA)

It is now possible to couple enzymes to antibodies or antigens for use in immunoassays. With the increasing concern over environmental contamination with radioisotopes, all the above radioimmunoassays can be performed using enzyme-labeled reagents. In addition to being as sensitive as radioimmunoassays (antigens and antibodies detectable at levels of about 10^{-10} g/ml or 1 part in 10 billion), enzyme immunoassays are less expensive, safer, and just as reliable and accurate as radioimmunoassays.

The principle of ELISA is based on these two observations:

1 Antibodies and some antigens can attach to polystyrene plastic plates (or other solid-phase supports) and still maintain their full immunologic capabilities.
2 Antigens and antibodies can be bonded to enzymes, and the resulting complexes are still fully functional both immunologically and enzymatically.

It is the enzyme activity which is the measure of the quantity of antigen or antibody present in the test sample. Enzymes used in ELISA include β-galactosidase, glucose oxidase, peroxidase, and alkaline phosphatase.

There are two methods of enzyme immunoassay that have significant clinical value: the double-antibody-sandwich procedure for the detection and measurement of antigen and the indirect-microplate-ELISA procedure for the detection and measurement of antibody.

Double-Antibody-Sandwich Procedure

In this technique (Fig. 34-10A), the wells or depressions in a polystyrene plate receive antiserum. The antibodies in the antiserum adhere to the surface of each well. The test antigen is added, and if the antigen is homologous, it attaches to the antibody immobilized on the well surface. Enzyme-labeled specific antibody is then added; it will bind to the antigen already fixed by the first antibody. This results in an antibody (with enzyme)-antigen-antibody "sandwich." Finally, the enzyme substrate is introduced for reaction with the enzyme. The rate of enzyme action is directly proportional to the quantity of enzyme-labeled antibody present, and that, in turn, is proportional to the amount of test antigen. Enzyme activity may be followed by a color change (brought about by substrate hydrolysis) which can be inspected visually or measured by means of a colorimeter (an instrument used to analyze color changes in a solution). This method has been used to assay hepatitis B antigen with great success.

Indirect ELISA Procedure

The initial step involves the coating of polystyrene wells with antigen by passive adsorption. (See Fig. 34-10B.) Test antiserum is added and allowed to incubate.

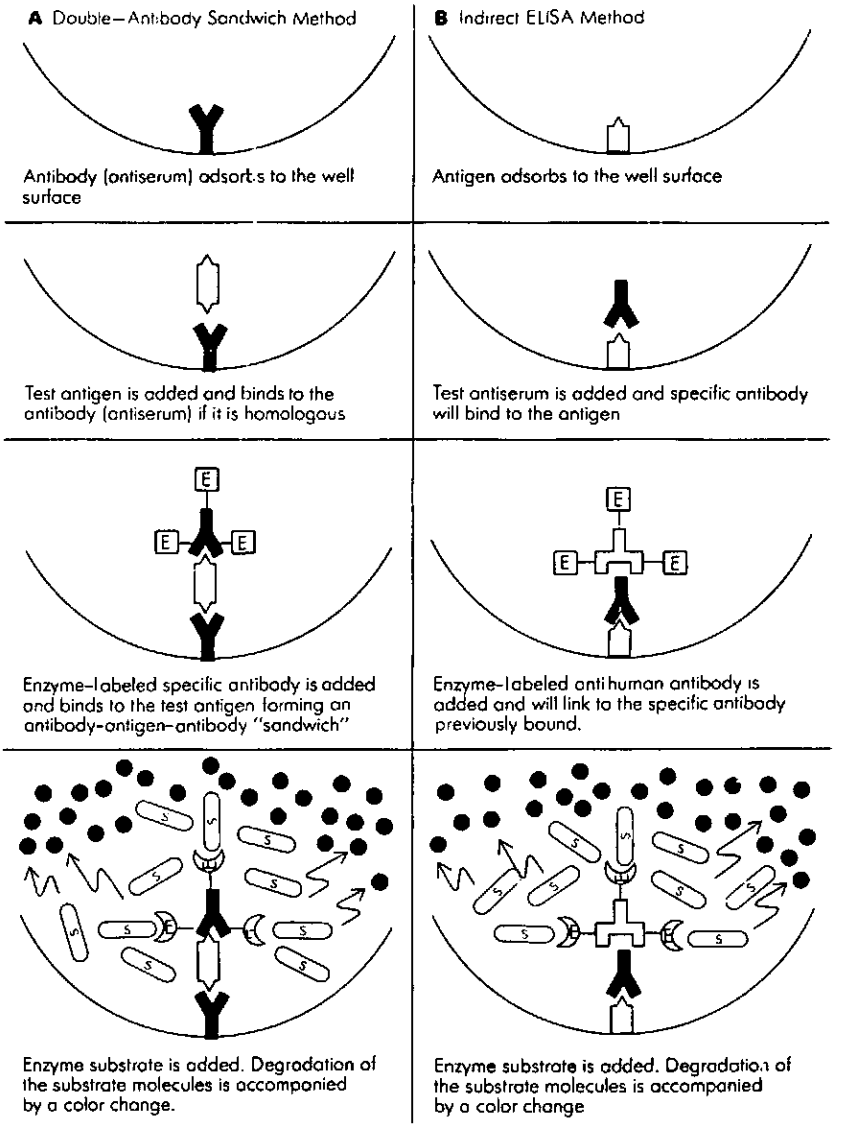

Figure 34-10. Enzyme-immunoassay techniques. (A) Double-antibody-sandwich method for the detection and the measurement of antigen. (B) Indirect ELISA method for the detection and measurement of antibody. (Note that the necessary washing after each step is not indicated.)

If the antibodies in the antiserum are homologous they will bind to the immobilized antigen. Enzyme-labeled antihuman antibodies (antiglobulins or antiantibodies) are added to the system which will link to the antibody-antigen complexes formed in the previous step. Finally, as in the double-antibody-sandwich method above, the enzyme substrate is added and the rate of its degradation (hydrolysis) is associated with a color change proportional to the concentration of antibody present in the test sample. This color change can be monitored visually or by a colorimeter.

This method has been used for the immunodiagnosis of many infectious

pathogens such as viruses, parasites, and fungi. For example, one antiserum sample from a pregnant woman can be screened simultaneously for infections of rubella virus (the agent for German measles, which causes congenital malformations and fetal death), type 2 herpesvirus (causes severe congenital nervous system malformations and small heads), and other infections that can affect the fetus.

Special Serological Tests

Fluorescent-Antibody Technique

The fluorescent-antibody technique is a rapid procedure for the identification of an unknown infectious agent. The technique is based on the behavior of certain dyes which fluoresce (glow) when exposed to certain wavelengths of light. Examples of such dyes are fluorescein isothiocyanate and rhodamine isothiocyanate. Antibodies can be conjugated, or tagged, with these dyes and are then termed **labeled** or **fluorescent antibodies.**

If a mixed culture or specimen is placed on a slide, combined with serum containing fluorescent antibodies, and examined by fluorescence microscopy, only those organisms (antigens) that reacted with the specific labeled antibodies will be visible (Fig. 34-11). Thus only a few organisms need to be present to be observed. This is the direct method, in which the fluorescent dye is conjugated with the antibody specific for the antigen.

In the indirect method the initially applied antibody is not labeled. Instead, a second labeled antibody against the globulin of the animal species used for the preparation of the initial specific antibody is applied. This binds the fluorescent label to the specific antibody that has already reacted with antigen in the smear (see Fig. 34-12).

Neufeld Quellung Reaction

Pneumococci can be differentiated into at least 80 serological types on the basis of the polysaccharides in their capsular material. The great majority of clinical cases in adults are caused by types 1 to 8, whereas types 14 and 19 occur frequently in children, along with types 1, 4, 5, and 7. When specific antiserum was used in the treatment of pneumococcus pneumonia (before antibiotics were available), it was necessary to determine the *type* of *Streptococcus pneumoniae* causing the infection. Agglutination and precipitin reactions could be used, but a more convenient method of typing is based on the swelling of the capsules in the presence of type-specific antiserum (see Fig. 34-2). This reaction was described by Neufeld, who gave it the name *quellung*, from the German meaning

Figure 34-11. Fluorescent-antibody technique is used to detect microorganisms. A vaginal smear from a patient infected with *Neisseria gonorrhoeae*, the etiologic agent of gonorrhea, was treated with gonococcus antibody tagged with fluorescein dye. (A) Slide preparation viewed with dark-field microscopy. Note the presence of several kinds of microorganisms. (B) The same field viewed by fluorescence microscopy. Note the presence only of gonococci, which are paired, oval cells. *(Courtesy of W. E. Deacon, Bull WHO, 24:349–355, 1961.)*

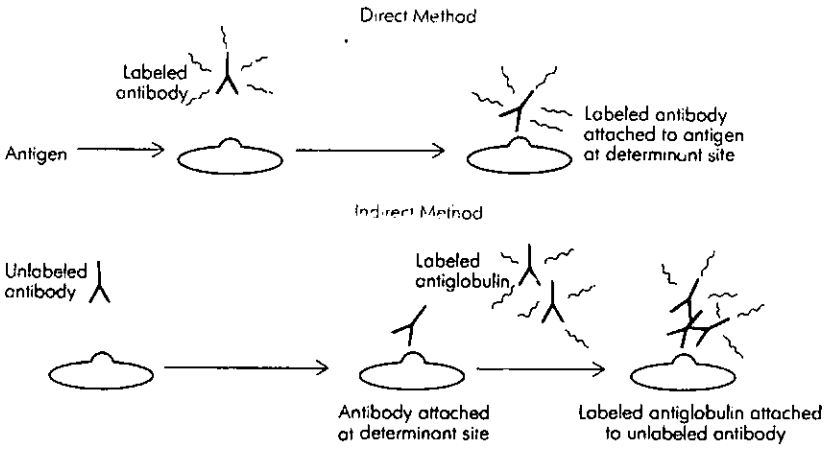

Figure 34-12. Fluorescent-antibody technique—direct and indirect methods. The indirect method is more sensitive as two or more labeled antiglobulin molecules can be attached to each antibody bound to its antigen.

"to swell." This is now considered to be a misnomer because actual swelling takes place only in the presence of complement, whereas in this test the appearance of swelling is due to a combination of the specific antibody with the capsular polysaccharide, which increases the refractive index of the capsular material.

The test can be made on pneumococci in sputum, from cultures, from peritoneal exudate of mice inoculated with sputum, or from other material containing the organisms. Loopfuls of material containing pneumococci are placed on microscope slides and mixed with specific antipneumococcal serums (made by inoculating rabbits with specific types of the organisms) containing a small amount of methylene blue. The dye stains the body of the cell and the surrounding fluid, leaving the capsules clear (Fig. 34-13). A positive test is indicated by a very prominent capsule.

Figure 34-13. Quellung reaction causes the capsules of the pneumococcus to appear enlarged when mixed with type-specific antiserum. This photomicrograph illustrates the appearance of *Streptococcus pneumoniae*, type 3, in the presence of its specific antibody. (*Courtesy of Robert C. Austrian, J Exp Med*, **99**:21–34, 1953.)

Detection of Heterophile Antibodies

When emulsions of organs from guinea pigs or certain other animals are injected into rabbits, antibodies against the red blood cells of sheep are produced in the rabbit. The antigenic material in this case is called a **heterophile antigen**, or **Forssman antigen**, for the discoverer of the phenomenon. The antibodies produced by injection of sheep cells differ from the heterophile antibody produced as a result of heterophile antigen stimulation.

The test used for the detection of heterophile antibodies in human infectious mononucleosis is based on the agglutination of sheep red blood cells by the serum of the patient.

Virus-Neutralizing Antibodies

Neutralizing antibodies indicate the presence of certain viruses. This test is especially useful in virus infections, but it can also be used to determine antibody titers to other organisms.

Virus obtained from the patient is grown in tissue culture (the brain cells of mice or in some other appropriate tissue), extracted, and exposed to patient's serum and to normal rabbit serum for 2 h. A small amount of each of these mixtures is inoculated into mice. Subsequently, the effectiveness of the patient's serum in neutralizing the virus is determined by the degree of protection afforded the test mice as compared with the lack of protection afforded the mice given normal serum. Neutralizing antibodies can also be detected by tissue-culture or egg-culture techniques.

MEASUREMENT OF CELL-MEDIATED IMMUNITY

The tuberculin, brucellergin, and some other skin tests (Table 34-1) are based on the detection of a delayed type IV hypersensitivity response typical of T-cell-mediated immunity. In contact with specific antigen, the T cells release various substances (see Chap. 33) that may cause tissue necrosis. Delayed-type reactions are apparent only after 18 h and may persist for days. Similar tests can be performed in vitro using these microbial antigens to stimulate the patients' lymphocytes to divide. A positive stimulation indicates the presence of immune T lymphocytes. Similarly, the T lymphocytes of patients who have

Table 34-1. Some Intradermal Tests Based on Cell-Mediated Immunity

Test	Etiologic Agent	Disease
Tuberculin	Bacterium: *Mycobacterium tuberculosis*	Tuberculosis—pulmonary disease with necrotic tubercles in lungs; may spread to other parts of body
Lepromin	Bacterium: *Mycobacterium leprae*	Leprosy—chronic disease that affects peripheral nerves as well as face, hands, and feet, to cause disfiguring
Brucellergin	Bacterium: *Brucella* spp.	Brucellosis—undulant (fluctuating) fever in humans
Blastomycin	Fungus: *Blastomyces dermatitidis*	Blastomycosis—lesions develop in lungs, skin, and bones
Histoplasmin	Fungus: *Histoplasma capsulatum*	Histoplasmosis—lesions in lung and lymph nodes, but may spread to other organs, including the liver and spleen
Leishmanin	Protozoan: *Leishmania* spp.	Leishmaniasis—any disease caused by these parasitic flagellates, e.g., kala azar, a visceral disease with enlarged liver and spleen

rejected an organ graft can be stimulated to divide by the foreign lymphocytes of the organ donor in a mixed lymphocyte culture (MLC). The cell-mediated immune response in these cultures can be detected either by counting the dividing cells or by allowing them to incorporate radioactive thymidine into their DNA and measuring newly synthesized DNA in a liquid scintillation counter (for counting radioactivity).

The effector cells of the cell-mediated immune system have the ability to kill foreign cells, virally infected cells, and tumor cells within the human body. Cytolytic T lymphocytes (CTL) can be induced in a mixed lymphocyte culture and can lyse radioisotope-labeled cells in vitro; the amount of label released from the target cells is counted in a solid scintillation counter. Thus cytolytic activity is directly proportional to the number of target cells lysed and the amount of label released. This type of cytotoxicity assay is used to quantitate specific CTL-mediated immunity as well as antibody-dependent cellular cytotoxicity (ADCC) and the nonspecific activity of natural killer lymphocytes.

OTHER DIAGNOSTIC APPLICATIONS OF IMMUNOASSAYS

At this point in our discussion, examples have been given of the use of immunoassays to classify microorganisms and identify specific pathogens responsible for disease. But it should be remembered that there are many other uses for immunoassays in biology and medicine.

Classification of Cellular Antigens

One of the most common uses of immunoassays is tissue cross matching. With respect to the ABO blood typing system (see Chapter 33), specific antisera are used in cross-match hemagglutination tests to type the donor and recipient cells for compatibility. Similarly, tissue type (histocompatibility) antigens coded for by the major histocompatibility complex (MHC), a group of genes determining cellular membrane antigens, can be typed using specific antisera as well as the mixed lymphocyte culture reaction. Such tissue typing of the MHC antigens is essential to the successful transplantation of organs and tissue grafts.

Tests to Evaluate Virulence

Antifibrinolysin Test

Most group A, some group C and G, and a few strains of groups B and F hemolytic streptococci produce an extracellular heat-stable protein that has the ability to dissolve fibrin clots. This substance is known as **fibrinolysin** or **streptokinase**. This enzyme has some specificity for fibrin from certain animal species. For example, the fibrinolysin produced by *Streptococcus pyogenes* will dissolve fibrin from humans and bovines but not from rabbits. The ability of streptococcus cultures to produce fibrinolysin (streptokinase) can be determined by mixing oxalated human plasma coagulated with calcium chloride with a broth culture of the streptococci. The time required for the clot to be liquefied is determined.

Antifibrinolysin, or **antistreptokinase**, is the antibody produced by the host stimulated antigenically with fibrinolysin, or streptokinase. Presence of this antibody can be determined by comparing the time required for different sera to dissolve a plasma clot prepared as described above.

When normal blood is used, the plasma clot will be completely dissolved within 1 h. Longer lysing time indicates the presence of the antibody, and the time required for complete lysis of the clot is used as an index of antifibrinolysin (antistreptokinase) concentration.

Table 34-2. Intracutaneous Tests

Name of Test	Material	Administration
Schick test (for determining susceptibility to diphtheria): indicates lack of immunity if positive	Diluted diphtheria toxin; control is heated toxin	0.1 ml intradermally; same amount of control on opposite arm
Dick test (for determining susceptibility to scarlet fever): indicates lack of immunity if positive	Diluted erythrogenic toxin; no control needed	0.1 ml intradermally
Schultz-Charlton test (for diagnosis of scarlet fever)	Antiscarlatinal serum or convalescent serum	0.1 to 0.2 ml injected intradermally into an erythematous area
Frei test (for determining sensitivity to the causative agent of lymphogranuloma venereum)	Chick-embryo culture of *Chlamydia trachomatis*	0.1 ml intradermally; same amount of control on opposite arm
Tuberculin test (for determining allergy to the tubercle bacillus); indicates hypersensitivity	Either PPD (purified protein derivative) or old tuberculin (OT)	0.1 ml intradermally. If PPD is desired, use first dilution (0.002 mg/ml); if OT, use 1:10,000
Ducrey test (for determining sensitivity to *Haemophilus ducreyi*)	A suspension of the specific organisms	0.1 ml intradermally; no control necessary
Brucellergin test (for demonstrating allergy to *Brucella*): indicates hypersensitivity	An extract of brucellas	0.1 ml intradermally; no control necessary
Trichinella test (for determining sensitivity to trichinella protein)	An extract of the worms	0.01 ml intradermally with saline control
Echinococcus test (for determining sensitivity to echinococcus protein)	An extract prepared from hydatid fluid	0.01 ml intradermally with saline control

SOURCE: *Laboratory Guide for Bacteriology*, U.S. Naval Medical School, Bethesda, Md., 1946.

Test for Antistreptolysin O

Many strains of group A streptococci produce a hemolytic, oxygen-labile factor called **streptolysin O.** The specific antibody produced by the body upon stimulation by this antigen is called **antistreptolysin O.** The titer of this antibody is of diagnostic value in streptococcal infections which may lead to rheumatic fever, glomerulonephritis, and other serious complications. Early diagnosis is important, so that treatment to prevent permanent injury to the heart or kidneys can be started. However, a single high antistreptolysin O titer is not significant since it may represent only a "normal" base line for that individual. Antistreptolysin O determinations at 4- to 6-week intervals are highly significant if there

Recording	Remarks
Read at end of 48 h and record as positive or negative; a positive reaction shows edema and usually scaling for 7 days.	The control rules out sensitivity to bacterial protein; in general, a false reaction comes on earlier, fades faster, and leaves less pigmentation than a positive Schick; antibody (IgG) neutralizes toxin
Read between 18 and 24 h; positive test is an erythema over 10 mm in diameter; record as positive or negative	The Dick test reflects only presence or absence of antitoxin to the erythrogenic toxin; it has no significance as a measure of immunity to other streptococcal infections; antibody (IgG) neutralizes toxin
Positive reaction (for scarlet fever) is indicated by a definite and permanent blanching of the surrounding scarlatinal rash in 5 to 6 h	This reaction is due to neutralization of the toxin produced by streptococci that cause scarlet fever; it is useful in making di agnosis in doubtful cases; antibody (IgG) neutralizes toxin
In 48–72 h for first reading, with subsequent check on fourth and seventh days; record presence of a papule greater than 6 mm and without significant reaction of control as positive	This test is supposedly reliable in 90% of cases; a positive reaction may reflect past disease rather than present infection; about 3 weeks must elapse between development of infection and appearance of a positive test; detects cell-mediated immunity
Read in 36–48 h; record amount of redness and of swelling by diameter in millimeters	When results are not clear-cut, it is sometimes advisable to use a dilution of greater strength and repeat the test; detects cell-mediated immunity
A positive test appears in 48 h and is manifested as an area of induration in excess of 7 mm; record as positive or negative	Too little is known of this test for didactic appraisal; an attempt to isolate the organism from local lesion or lymph node should be made; detects cell-mediated immunity
In 24–48 h, inspect for erythema or induration; an area of edema of over 20 mm is recorded as positive	About 15% of people show positive skin test, so that the significance of the test in relation to symptoms must be carefully weighed; detects cell-mediated immunity
A positive reaction usually appears as a wheal within 20 min	There is evidence that some individuals may display a delayed type of reaction, and tests immediately negative should be checked after 24 h; this test is believed to have about 95% specificity; immediate sensitivity (IgE) to trichinella protein
A positive reaction is indicated by a wheal within 20 min	Some individuals may show a delayed reaction, and all negative tests should be checked at the end of 24 h; immediate sensitivity to echinococcus protein

is a consistent drop or increase in titer, because they indicate recovery or increased inflammation, respectively. The test is based on the ability of the antibody to prevent hemolysis in tubes containing patient's serum, standard buffer, streptolysin O, a suspension of red blood corpuscles, and saline solution.

Intracutaneous Diagnostic Tests

The intradermal injection of a small quantity of a test material is often used to diagnose an infectious disease or determine the susceptibility of a patient (Table 34-2). These tests can be used (1) to detect the presence or absence of an antibody in the patient which reacts with antigenic material from the pathogen (e.g.,

Schick test); (2) to detect microbial antigens in the diseased patient (e.g., Schultz-Charlton test); and (3) to detect cell-mediated immunity to microbial antigens (see Table 34-1).

In Vivo Tests

Animal tests sometimes provide conclusive data on the mechanism of microbial pathogenicity and may aid in the diagnosis of disease. If a human pathogen produces detectable disease in a laboratory animal, it is possible to measure the protective efficiency of antibodies in the patient's serum. The patient's serum is injected into the test animals to provide *passive protection*. The animals are then challenged with the pathogen, and the increase in the numbers of the microorganism required to establish infection is determined. If no increase is seen, then the patient lacks specific antibody. The pathogens can also be injected into previously vaccinated animals to quantitate the immunogenicity of the vaccine and the protective value of the vaccine.

IMMUNOTHERAPY

In the preceding chapters vaccines have been mentioned many times, and this emphasis underlies their importance in safeguarding our health. Vaccines are either killed or attenuated pathogens which, when injected, elicit an immune response which is protective against the original pathogen. In addition, toxins can be inactivated and still retain their antigenic specificity as an immunogenic toxoid. New vaccines are being developed even today.

Once used more often than it is now, passive immunization still has its uses. Now largely confined to the use of hyperimmune human antisera to avoid problems with serum sickness, passive immunization is also used in combatting some infections (e.g., rabies and hepatitis) and in the prevention of erythroblastosis fetalis, the hemolytic disease of the newborn. This disease occurs frequently in newborns of Rh-negative mothers and Rh-positive fathers and more frequently in babies whose mothers have had multiple pregnancies. The disease occurs because Rh-negative women can become immunized to their babies' Rh-positive antigen during or following pregnancy. The IgG antibody to the Rh-positive antigen can cross the placenta and cause the lysis of the babies' red blood cells, causing a potentially fatal anemia. Passive immunization of the mother with anti-Rh antibodies before and after the birth of a Rh-positive child prevents her from developing a primary antibody response to the Rh factor since these anti-Rh antibodies destroy Rh-positive red blood cells in the mother. This forestalls the development of an Rh-incompatibility problem in a subsequent pregnancy.

Specifically labeled antibodies, especially monoclonal antibodies, are being tested as in vivo tracers for diagnosing and treating certain cancers. The possibility that therapeutic drugs can be coupled with specific antitumor antibodies may finally provide medicine with a "magic bullet" that has been searched for since the days of Paul Ehrlich, who discovered a chemical compound for the treatment of syphilis.

QUESTIONS

1 What is the significance of a rising antibody titer in a patient?
2 Describe the lattice hypothesis for the combination of antigen and antibody.

3 What is meant by the equivalence zone in a quantitative precipitin test?
4 List several ways in which precipitin tests can be useful.
5 Describe the Ouchterlony method of precipitin testing and explain how the results are interpreted.
6 Describe the technique of radial immunodiffusion and explain why it has advantage(s) over other methods of precipitin testing.
7 What is immunoelectrophoresis? Why is one type of immunoelectrophoretic test called rocket immunoelectrophoresis?
8 How is the precipitin test performed for the differentiation of streptococci?
9 How do the Widal test and the Weil-Felix test differ in purpose and procedure?
10 What are agglutinin-adsorption tests? How do they aid in the identification of bacteria?
11 Describe the hemagglutination test. What is its importance?
12 Describe the principle of the complement-fixation test.
13 Explain the following tests briefly: (*a*) RIA, (*b*) ELISA.
14 Compare the direct fluorescent-antibody technique with the indirect one.
15 What constitutes a positive test in the Neufeld quellung reaction? What is the basis for the positive reaction?
16 Describe some in vitro tests for cell-mediated immunity.
17 Indicate the purpose of the Schick test and the tuberculin test. What kind of immunity is involved in each case?
18 What causes erythroblastosis fetalis? How can this disease be prevented?

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