STUDY MATERIAL FOR

BG 4th Semester

SUBJECT: BIOTECHNOLOGY

IMMUNOLOGY

IMMUNOLOGY

The word immunity has been derived from a Latin word "IMMUNIS" means 'Exempt from' and the term "Immunology" is used to study the defense mechanisms against infectious diseases.

Defence mechanisms fell into two Broad categories: specific and non-specific. These two categories/ grouping are inter-related/inter-dependent on each other.

The first line defence mechanisms include all non-specific factors while the second line of defence is under specific immune mechanism

Innate immunity (Latin; innatus: inborn)

This type of immunity is inherited to the organism from the parents and protects it from birth throughout life.

First line defence: this defence mechanism consists of four types of defensive barriers: physical (anatomic), physiologic, cellular (phagocytic), and cytokine (inflammatory) barriers.

Anatomic barriers

- Skin Mechanical barrier retards entry of microbes.
- Acidic environment (pH 3–5) retards growth of microbes.
- Mucous membranes Normal flora compete with microbes for attachment sites and nutrients.
- Mucus entraps foreign microorganisms.
- Cilia propel microorganisms out of body.

Physiologic barriers

- Temperature Normal body temperature inhibits growth of some pathogens.
- Fever response inhibits growth of some pathogens.
- Low pH Acidity of stomach contents kills most ingested microorganisms.
- Chemical mediators Lysozyme cleaves bacterial cell wall.
- Interferon induces antiviral state in uninfected cells.
- Complement lyses microorganisms or facilitates phagocytosis.
- Toll-like receptors recognize microbial molecules, signal cell to secrete immunostimulatory cytokines.
- Collectins disrupt cell wall of pathogen.

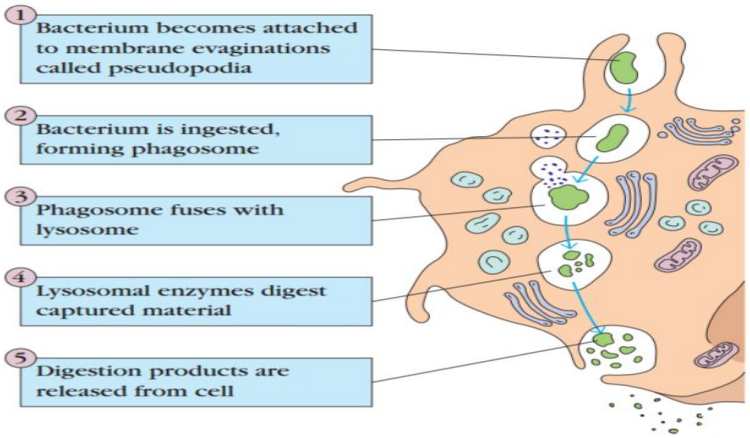
Phagocytic/endocytic / cellular barriers

Various cells internalize (endocytose) and break down foreign macromolecules.

Specialized cells (blood monocytes, neutrophils, tissue macrophages) internalize (phagocytose), kill, and digest whole microorganisms.

This important innate defense mechanism is the ingestion of extracellular particulate material by **phagocytosis.** Phagocytosis is one type of **endocytosis,** the general term for the uptake by a cell

of material from its environment. In phagocytosis, a cell's plasma membrane expands around the particulate material, which may include whole pathogenic microorganisms, to form large vesicles called **phagosomes** (Figure 1-5). Most phagocytosis is conducted by specialized cells, such as blood monocytes, neutrophils, and tissue macrophages (see Chapter 2). Most cell types are capable of other forms of endocytosis, such as *receptor-mediated endocytosis*, in which extracellular molecules are internalized after binding by specific cellular receptors, and *pinocytosis*, the process by which cells take up fluid from the surrounding medium along with any molecules contained in it.



Phagocytic mechanism

Inflammatory barriers

Tissue damage and infection induce leakage of vascular fluid, containing serum proteins with Antibacterial activity and influx of phagocytic cells into the affected area.

Tissue damage caused by a wound or by an invading pathogenic microorganism induces a complex sequence of events collectively known as the **inflammatory response.**

In the first century AD, the Roman physician Celsus described the "four cardinal signs of inflammation" as *rubor* (redness), *tumor* (swelling), *calor* (heat), and *dolor* (pain). In the second century AD, another physician, Galen, added a fifth sign: *functio laesa* (loss of function). The cardinal signs of inflammation reflect the three major events of an inflammatory response (Figure 1-4):

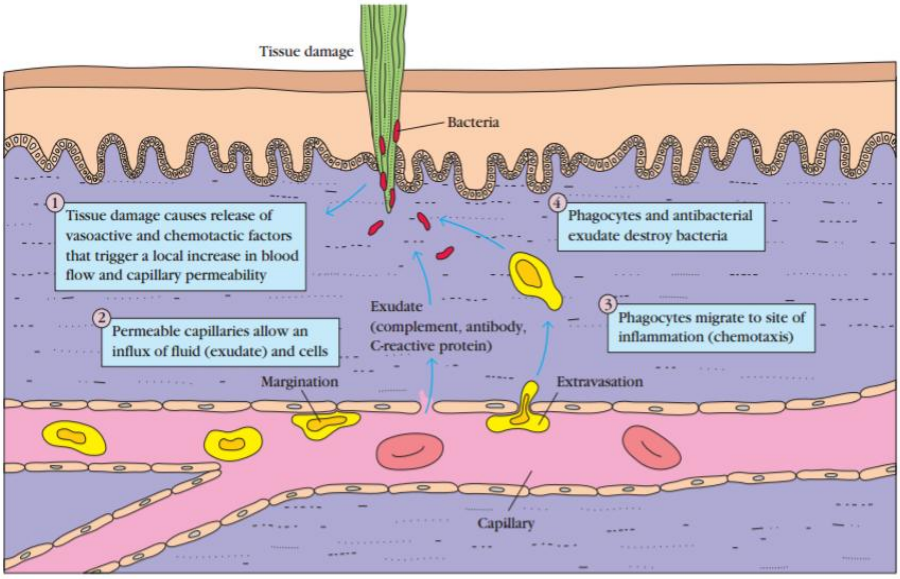


FIGURE 1-4 Major events in the inflammatory response. A bacterial infection causes tissue damage with release of various vasoactive and chemotactic factors. These factors induce increased blood flow to the area, increased capillary permeability, and an influx of white blood cells, including phagocytes and lymphocytes, from the blood into the tissues. The serum proteins contained in the exudate have antibacterial properties, and the phagocytes begin to engulf the bacteria, as illustrated in Figure 1-3.

1. *Vasodilation*—an increase in the diameter of blood vessels—of nearby capillaries occurs as the vessels that carry blood away from the affected area constrict, resulting in engorgement of the capillary network. The engorged capillaries are responsible for tissue redness (*erythema*) and an increase in tissue temperature.
2. An *increase in capillary permeability* facilitates an influx of fluid and cells from the engorged capillaries into the tissue. The fluid that accumulates **(exudate)** has much higher protein content than fluid normally released from the vasculature. Accumulation of exudate contributes to tissue swelling **(edema)**.
3. *Influx of phagocytes* from the capillaries into the tissues is facilitated by the increased permeability of the capillaries. The emigration of phagocytes is a multistep process that includes adherence of the cells to the endothelial wall of the blood vessels **(margination)**, followed by their emigration between the capillaryendothelial cells into the tissue **(diapedesis** or **extravasation)**, and, finally, their migration through the tissue to the site of the invasion **(chemotaxis)**. As phagocytic cells accumulate at the site and begin to phagocytose bacteria, they release lytic enzymes, which can damage nearby healthy cells. The accumulation of dead cells, digested material, and fluid forms a substance called pus.

ACQUIRED IMMUNITY / ADAPTIVE IMMUNITY/ SPECIFIC IMMUNITY

The resistance that an individual acquires during life is called acquired immunity. It defines the presence of functional immune system that is capable of recognizing and selectively eliminating specific foreign microorganisms and molecules (i.e., foreign antigens). Unlike innate immune responses, adaptive immune responses are not the same in all members of a species but are reactions to specific antigenic challenges.

Adaptive immunity displays four characteristic attributes:

1. _ Antigenic specificity
2. _ Diversity
3. _ Immunologic memory
4. _ Self/non-self-recognition

The **antigenic specificity** of the immune system permits it to distinguish subtle differences among antigens. Antibodies can distinguish between two protein molecules that differ in only a single amino acid. The immune system is capable of generating tremendous *diversity* in its recognition molecules, allowing it to recognize billions of unique structures on foreign antigens. Once the immune system has recognized and responded to an antigen, it exhibits *immunologic memory;* that is, a second encounter with the same antigen induces a heightened state of immune reactivity. Because of this attribute, the immune system can confer life-long immunity to many infectious agents after an initial encounter. Finally, the immune system normally responds only to foreign antigens, indicating that it is capable of *self/nonself recognition.* The ability of the immune system to distinguish self from nonself and respond only to nonself molecules is essential, for, as described below, the outcome of an inappropriate response to self-molecules can be fatal.

Adaptive immunity is not independent of innate immunity. The phagocytic cells crucial to nonspecific immune responses are intimately involved in activating the specific immune response. Conversely, various soluble factors produced by a specific immune response have been shown to augment the activity of these phagocytic cells. As an inflammatory response develops, for example, soluble mediators are produced that attract cells of the immune system. The immune response will, in turn, serve to regulate the intensity of the inflammatory response. Through the carefully regulated interplay of adaptive and innate immunity, the two systems work together to eliminate a foreign invader.

HUMORAL AND CELL MEDIATED IMMUNE RESPONSE

Immune responses can be divided into humoral and cell-mediated responses. Humoral immunity refers to immunity that can be conferred upon a non-immune individual by administration of serum antibodies from an immune individual. In contrast, cell-mediated immunity can be transferred only by administration of T cells from an immune individual.

The humoral branch of the immune system is at work in the interaction of B cells with antigen and their subsequent proliferation and differentiation into antibody-secreting plasma cells (Figure 1-7). Antibody functions as the effector of the humoral response by binding to antigen and

neutralizing it or facilitating its elimination. When an antigen is coated with antibody, it can be eliminated in several ways. For example, antibody can cross-link several antigens, forming clusters that are more readily ingested by phagocytic cells. Binding of antibody to antigen on a microorganism can also activate the complement system, resulting in lysis of the foreign organism. Antibody can also neutralize toxins or viral particles by coating them,which prevents them from binding to host cells.

Effector T cells generated in response to antigen are responsible for cell-mediated immunity (see Figure 1-7). Both activated TH cells and cytotoxic T lymphocytes (CTLs) serve as effector cells in cell-mediated immune reactions. Cytokines secreted by TH cells can activate various phagocytic cells, enabling them to phagocytose and kill microorganisms more effectively. This type of cell-mediated immune response is especially important in ridding the host of bacteria and protozoa contained by infected host cells. CTLs participate in cell-mediated immune reactions by killing altered self-cells; they play an important role in the killing of virus infected cells and tumor cells.

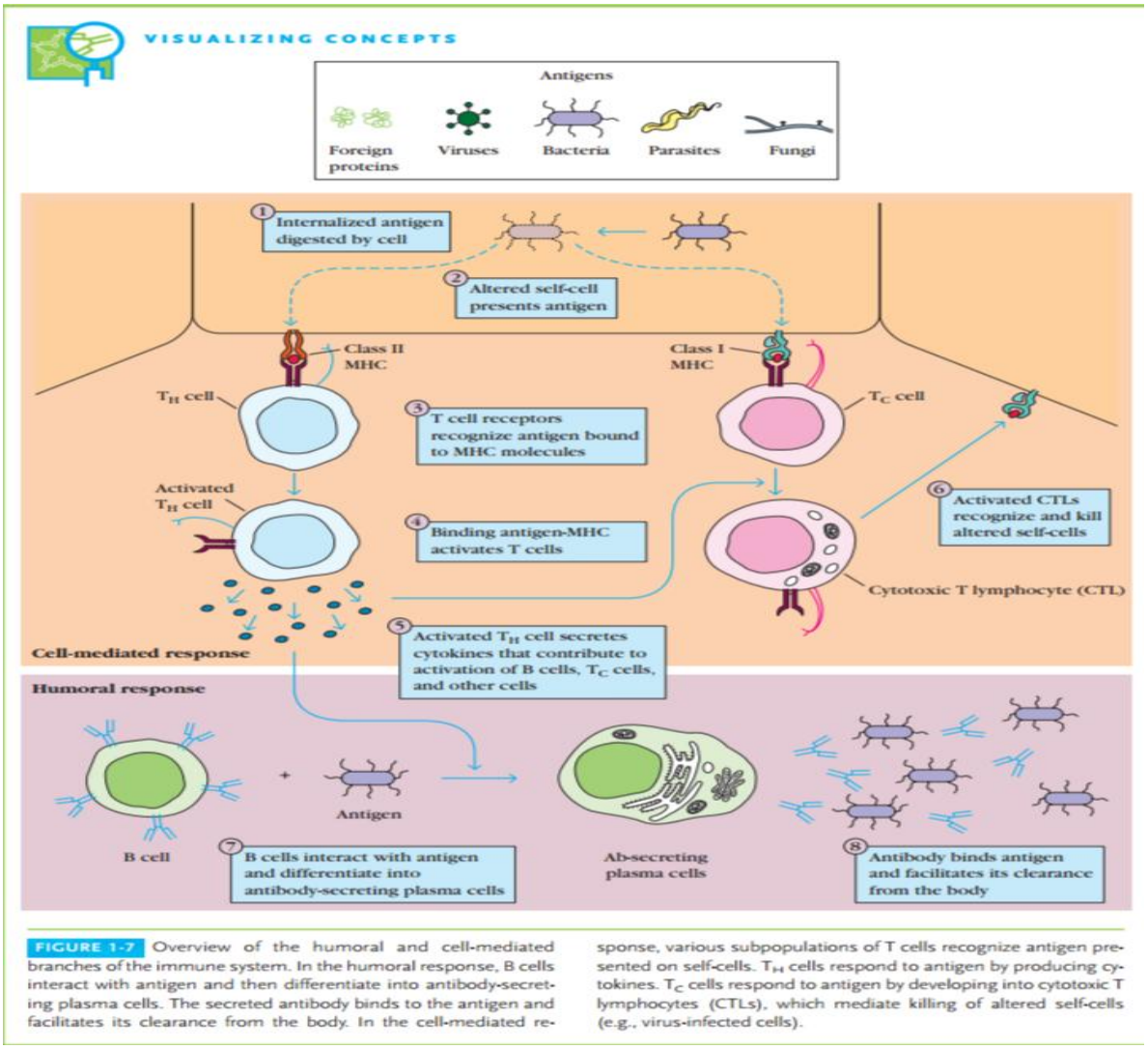


FIGURE 1-7 Overview of the humoral and cell-mediated branches of the immune system. In the humoral response, B cells interact with antigen and then differentiate into antibody-secreting plasma cells. The secreted antibody binds to the antigen and facilitates its clearance from the body. In the cell-mediated response, various subpopulations of T cells recognize antigen presented on self-cells. T_H cells respond to antigen by producing cytokines. T_C cells respond to antigen by developing into cytotoxic T lymphocytes (CTLs), which mediate killing of altered self-cells (e.g., virus-infected cells).

HUMORAL IMMUNE RESPONSE/ ANTIBODY MEDIATED IMMUNE RESPONSE

The HIR or AMIR defend body against free viruses, bacteria with polysaccharide capsule and toxins that enter the body. The word 'Humor' pertains to fluid and this AMIR consists of antibodies that circulate in the body fluids. It is regulated by B- CELLS that produce antibodies. Since B- Lymphocytes produce antibodies, therefore this immunity is called anitibody mediated or Humoral immunity. It is also called B- cell immunity. The B-cells give rise to two types of cells- plasma cells and memory cells

Plasma cells (Effector B- Cells): some of the activated B- cells enlarge divide and differentiate into a clone of plasma cells. Although plasma cells live for a few days, they secrete enormous amount of antibody during this period. A few days after exp[osure to an antigen, a plasma cells secretes hundreds of millions of antibodies daily and secretion occurs for about 4-5 days until the plasma cells dies.

Memory B Cells: some activated B-cells do not differentiate into plasma cells but rather remain as memory cells. They have a larger life span. The memory cells remain dormant until activated once again by a new quantity of the same antigen. They serve to dispose of the antigens in case of the infection of the same virus or bacterium occurs.

The antibodies bind to their antigen and activate the invading microorganisms or foreign molecules so that they are conveniently disposed by phagocytes. The immunoglobin fights antigen in 5 different ways:

1. Neutralization
2. Opsanization
3. Agglutination
4. Complement activation
5. Precipitation

CELL MEDIATED IMMUNE RESPONSE

The specific role of CMI is to protect body from viruses and bacteria. It also reacts against foreign tissue transplants. The cellular immune response is separate for each type of antigen that invades the body. This cellular immune response is given by T-Cells. These T- cells are similar morphologically but of **4 types B** functionally--- Killer or Cytotoxic cells, Helper T- Cells, suppressor T- Cells and Memory T- cells briefly written as T_K , T_H, T_S and T_M respectively.

Killer T-Cells:- These cells directly attack the foreign cells. They secrete a protein, **perforin** which punctures invaders cell membrane. Water and ions flow into the non-self-cells, which swells up and lyses.

Helper T-Cells:- These cells stimulate B-Cells to produce antibodies and also stimulate the Killer T-cells to destroy the non-self cells.

Suppressor T- Cells:- They are capable of suppressing the functions of immune system from attacking the body's own cells.

Memory T-Cells:- They are also called primed cells. They keep ready to mount a rapid and vigorous attack as soon as the same Pathogen attacks the body again.

LYMPHATIC SYTEM; ORGANIZATION AND STRUCTURE OF LYMPHOID ORGANS

Lymphatic System

As blood circulates under pressure, its fluid component **(plasma)** seeps through the thin wall of the capillaries into the surrounding tissue.Much of this fluid, called **interstitial fluid,** returns to the blood through the capillary membranes. The remainder of the interstitial fluid, now called **lymph,** flows from the spaces in connective tissue into a network of tiny open lymphatic capillaries and then into a series of progressively larger collecting vessels called **lymphatic vessels**The largest lymphatic vessel, the **thoracic duct,** empties into the left subclavian vein near the heart (see Figure 2-13). In this way, the lymphatic system captures fluid lost from the blood and returns it to the blood, thus ensuring steady-state levels of fluid within the circulatory system. The heart does not pump the lymph through the lymphatic system; instead the flow of lymph is achieved as the lymph vessels are squeezed by movements of the body's muscles. A series of one-way valves along the lymphatic vessels ensures that lymph flows only in one direction.

When a foreign antigen gains entrance to the tissues, it is picked up by the lymphatic system (which drains all the tissues of the body) and is carried to various organized lymphoid tissues such as lymph nodes, which trap the foreign antigen. As lymph passes from the tissues to lymphatic vessels, it becomes progressively enriched in lymphocytes.

Thus, the lymphatic system also serves as a means of transporting lymphocytes and antigen from the connective tissues to organized lymphoid tissues where the lymphocytes may interact with the trapped antigen and undergo activation.

Lymphoid Organs

A number of morphologically and functionally diverse organsand tissues have various functions in the development of immune responses. These can be distinguished by function as the **primary** and **secondary lymphoid organs** (Figure 2-13). The thymus and bone marrow are the primary (or central) lymphoid organs, where maturation of lymphocytes takes place. The lymph nodes, spleen, and various mucosal associated lymphoid tissues (MALT) such as gut-associated lymphoid tissue (GALT) are the secondary (or peripheral) lymphoid organs, which trap antigen and provide sites for mature lymphocytes to interact with that antigen. In addition, **tertiary lymphoid tissues,** which normally contain fewer lymphoid cells than secondary lymphoid organs, can import lymphoid cells during an inflammatory response.Most prominent of these are cutaneous-associated lymphoid tissues. Once mature lymphocytes have been generated in the primary lymphoid organs, they circulate in the blood and **lymphatic system,** a network of vessels that collect fluid that has escaped into the tissues from capillaries of the circulatory system and ultimately return it to the blood.

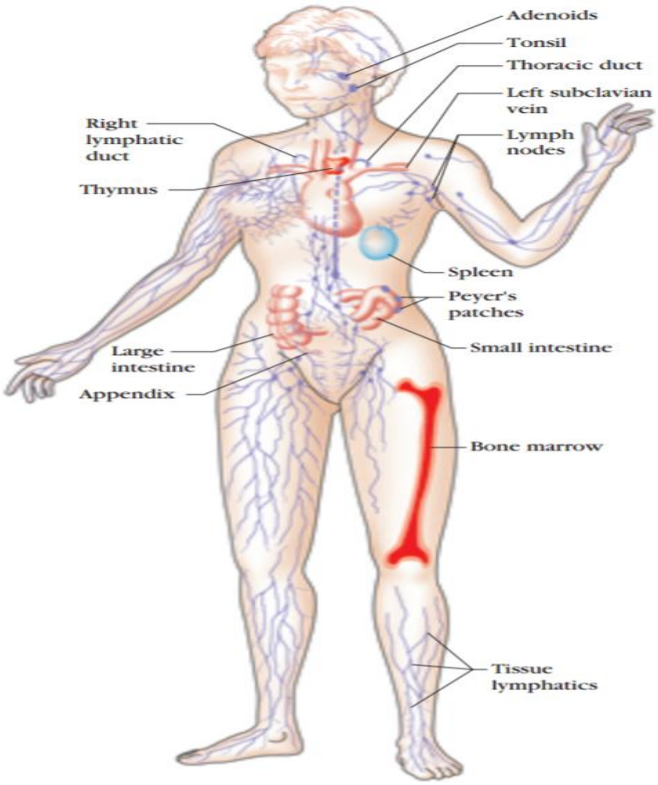


FIGURE 2-13 The human lymphoid system. The primary organs (bone marrow and thymus) are shown in red; secondary organs and tissues, in blue. These structurally and functionally diverse lymphoid organs and tissues are interconnected by the blood vessels (not shown) and lymphatic vessels (purple) through which lymphocytes circulate. Only one bone is shown, but all major bones contain marrow and thus are part of the lymphoid system. [*Adapted from H. Lodish et al., 1995,* Molecular Cell Biology, *3rd ed., Scientific American Books.*]

Primary Lymphoid Organs

Immature lymphocytes generated in hematopoiesis mature and become committed to a particular antigenic specificity within the primary lymphoid organs. Only after a lympho- cyte has matured within a primary lymphoid organ is the cell **immunocompetent** (capable of mounting an immune response).T cells arise in the **thymus,** and in many mammals—humans and mice for example—B cells originate in **bone marrow.**

THYMUS

The thymus is the site of T-cell development and maturation. It is a flat, bilobed organ situated above the heart. Each lobe is surrounded by a capsule and is divided into lobules, which are separated from each other by strands of connective tissue called trabeculae. Each lobule is organized into two compartments: the outer compartment, or *cortex,* is densely packed with immature T cells, called thymocytes, whereas the inner compartment, or *medulla,* is sparsely populated with thymocytes. Both the cortex and medulla of the thymus are crisscrossed by a

three-dimensional stromal-cell network composed of epithelial cells, dendritic cells, and macrophages, which make up the framework of the organ and contribute to the growth and maturation of thymocytes. Many of these stromal cells interact physically with the developing thymocytes (Figure 2-14). Some thymic epithelial cells in the outer cortex, called **nurse cells,** have long membrane extensions that surround as many as 50 thymocytes, forming large multicellular complexes. Other cortical epithelial cells have long interconnecting cytoplasmic extensions that form a network and have been shown to interact with numerous thymocytes as they traverse the cortex.

The function of the thymus is to generate and select a repertoire of T cells that will protect the body from infection. As thymocytes develop, an enormous diversity of T-cell receptors is generated by a random process (see Chapter 9) that produces some T cells with receptors capable of recognizing antigen-MHC complexes.However, most of the T-cell receptors produced by this random process are incapable of recognizing antigen-MHC complexes and a small portion react with combinations of self antigen-MHC complexes. The thymus induces the death of those T cells that cannot recognize antigen- MHC complexes and those that react with self-antigen–MHC and pose a danger of causing autoimmune disease. More than 95% of all thymocytes die by apoptosis in the thymus without ever reaching maturity.

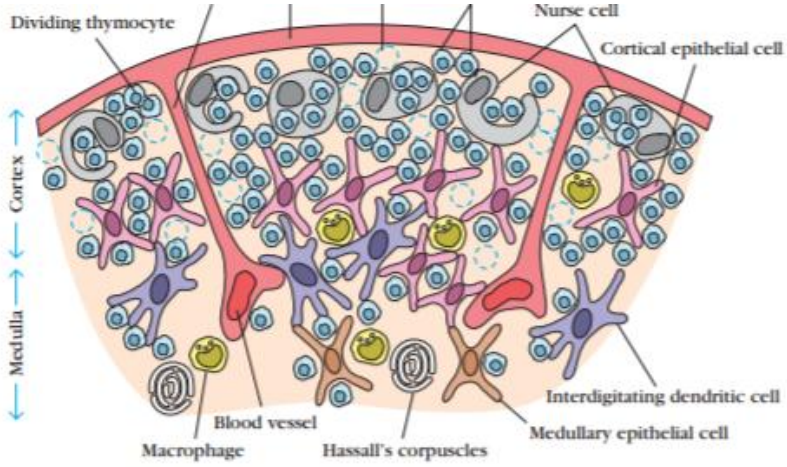


FIGURE 2-14 Diagrammatic cross section of a portion of the thymus, showing several lobules separated by connective tissue strands (trabeculae). The densely populated outer cortex is thought to contain many immature thymocytes (blue), which undergo rapid proliferation coupled with an enormous rate of cell death. Also present in the outer cortex are thymic nurse cells (gray), which are specialized epithelial cells with long membrane extensions that surround as many as 50 thymocytes. The medulla is sparsely populated and is thought to contain thymocytes that are more mature. During their stay within the thymus, thymocytes interact with various stromal cells, including cortical epithelial cells (light red), medullary epithelial cells (tan), interdigitating dendritic cells (purple), and macrophages (yellow). These cells produce thymic hormones and express high levels of class I and class II MHC molecules. Hassalls corpuscles, found in the medulla, contain concentric layers of degenerating epithelial cells. [*Adapted, with permission, from W. van Ewijk, 1991,* Annu. Rev. Immunol. **9**:591, *© 1991 by Annual Reviews.*]

BONE MARROW

In humans and mice, bone marrow is the site of B-cell origin and development. Arising from lymphoid progenitors, immature B cells proliferate and differentiate within the bone marrow, and stromal cells within the bone marrow interact directly with the B cells and secrete various cytokines that are required for development. Like thymic selection during Tcell maturation, a selection process within the bone marrow eliminates B cells with self-reactive antibody receptors. Bone marrow is not the site of B-cell development in all species. In birds, a lymphoid organ called the bursa of Fabricius, a lymphoid tissue associated with the gut, is the primary site of B-cell maturation. In mammals such as primates and rodents, there is no bursa and no single counterpart to it as a primary lymphoid organ. In cattle and sheep, the primary lymphoid tissue hosting the maturation, proliferation, and diversification of B cells early in gestation is the fetal spleen. Later in gestation, this function is assumed by a patch of tissue embedded in the wall of the intestine called the ileal Peyer's patch,which contains a large number (1010) B cells. The rabbit, too, uses gut-associated tissues such as the appendix as primary lymphoid tissue for important steps in the proliferation and diversification of B cells.

SECONDARY LYMPHOID ORGANS

Various types of organized lymphoid tissues are located along the vessels of the lymphatic system. Some lymphoid tissue in the lung and lamina propria of the intestinal wall consists of diffuse collections of lymphocytes and macrophages. Other lymphoid tissue is organized into structures called lymphoid follicles, which consist of aggregates of lymphoid and nonlymphoid cells surrounded by a network of draining lymphatic capillaries. Until it is activated by antigen, a lymphoid follicle—called a **primary follicle**—comprises a network of follicular dendritic cells and small resting B cells. After an antigenic challenge, a primary follicle becomes a larger **secondary follicle**—a ring of concentrically packed B lymphocytes surrounding a center (the **germinal center**) in which one finds a focus of proliferating B lymphocytes and an area that contains nondividing B cells, and some helper T cells interspersed with macrophages and follicular dendritic cells

Lymph nodes and the **spleen** are the most highly organized of the secondary lymphoid organs; they comprise not only lymphoid follicles, but additional distinct regions of Tcell and B-cell activity, and they are surrounded by a fibrous capsule. Less-organized lymphoid tissue, collectively called mucosal-associated lymphoid tissue (MALT), is found in various body sites. MALT includes Peyer's patches (in the small intestine), the tonsils, and the appendix, as well as numerous lymphoid follicles within the lamina propria of the intestines and in the mucous membranes lining the upper airways, bronchi, and genital tract.

LYMPH NODES

Lymph nodes are the sites where immune responses are mounted to antigens in lymph. They are encapsulated bean shaped structures containing a reticular network packed with lymphocytes, macrophages, and dendritic cells. Clustered at junctions of the lymphatic vessels, lymph nodes are the first organized lymphoid structure to encounter antigens that enter the tissue spaces. As

lymph percolates through a node, any particulate antigen that is brought in with the lymph will be trapped by the cellular network of phagocytic cells and dendritic cells (follicular and inter digitating). The overall architecture of a lymph node supports an ideal microenvironment for lymphocytes to effectively encounter and respond to trapped antigens.

Morphologically, a lymph node can be divided into three roughly concentric regions: the cortex, the paracortex, and the medulla, each of which supports a distinct microenvironment

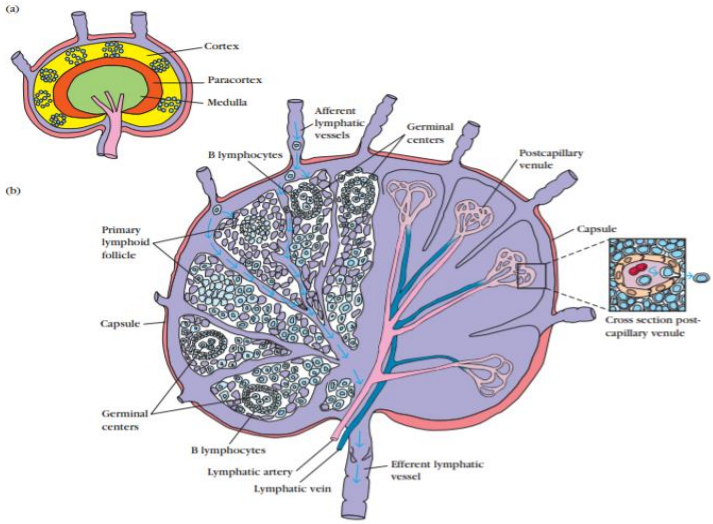
(Figure below). The outermost layer, the **cortex,** contains lymphocytes (mostly B cells), macrophages, and follicular dendritic cells arranged in primary follicles. After antigenic challenge, the primary follicles enlarge into secondary follicles, each containing a germinal center. In children with B-cell deficiencies, the cortex lacks primary follicles and germinal centers. Beneath the cortex is the **paracortex,** which is populated largely by T lymphocytes and also contains interdigitating dendritic cells thought to have migrated from tissues to the node. These interdigitating dendritic cells express high levels of class II MHC molecules,which are necessary for presenting antigen to TH cells. Lymph nodes taken from neonatally thymectomized mice have unusually few cells in the paracortical region; the paracortex is therefore sometimes referred to as a **thymus-dependent area** in contrast to the cortex, which is a **thymus-independent area.** The innermost layer of a lymph node, the **medulla,** is more sparsely populated with lymphoid-lineage cells; of those present, many are plasma cells actively secreting antibody molecules.

Functions of Lymph nodes

Lymph nodes must be looked upon as important structures of defence against the invasion of microorganisms entering the body via blood stream and lymphatic channels, lymph nodes, therefore, must be considered as second line of defence acting as filters and bearing the first onslaught of bacterial attack. The lymph in its course from the tissue spaces to the point where it is returned to the blood must be filtered through the lymph nodes.

Lymph nodes are found throughout the body but mainly in the neck, armpits, groin, tommy (abdomen).they are made of tissue that contain special cells that help fight against infection and disease such as cancer.

Lymphnodes vary in size. Some are as small as pinhead and others as big as the size of baked bean. The no. of lymph nodes in the body varies from person to person



SPLEEN

The spleen plays a major role in mounting immune responses to antigens in the blood stream. It is a large, ovoid secondary lymphoid organ situated high in the left abdominal cavity. While lymph nodes are specialized for trapping antigen from local tissues, the spleen specializes in filtering blood and trapping blood-borne antigens; thus, it can respond to systemic infections. Unlike the lymph nodes, the spleen is not supplied by lymphatic vessels. Instead, bloodborne antigens and lymphocytes are carried into the spleen through the splenic artery. Experiments with radioactively labeled lymphocytes show that more recirculating lymphocytes pass daily through the spleen than through all the lymph nodes combined.

The spleen is surrounded by a capsule that extends a number of projections (trabeculae) into the interior to form a compartmentalized structure. The compartments are of two types, the red pulp and white pulp, which are separated by a diffuse marginal zone (Figure 2-19). The splenic **red pulp** consists of a network of sinusoids populated by macrophages and numerous red blood cells (erythrocytes) and few lymphocytes; it is the site where old and defective red blood cells are destroyed and removed.Many of the macrophages within the red pulp contain engulfed red blood cells or iron pigments from degraded hemoglobin. The splenic **white pulp** surrounds the branches of the splenic artery, forming a **periarteriolar lymphoid sheath (PALS)** populated mainly by T lymphocytes. Primary lymphoid follicles are attached to thePALS. These follicles are rich in B cells and some of them contain germinal centers. The **marginal zone,** located peripheral to the PALS, is populated by lymphocytes and macrophages.

Blood-borne antigens and lymphocytes enter the spleen through the splenic artery, which empties into the marginal zone. In the marginal zone, antigen is trapped by interdigitating dendritic cells, which carry it to the PALS. Lymphocytes in the blood also enter sinuses in the marginal zone and migrate to the PALS.

The initial activation of B and T cells takes place in the Tcell- rich PALS. Here interdigitating dendritic cells capture antigen and present it combined with class II MHC molecules to TH cells. Once activated, these TH cells can then activate B cells. The activated B cells, together with some TH cells, then migrate to primary follicles in the marginal zone. Upon antigenic challenge, these primary follicles develop into characteristic secondary follicles containing germinal centers (like those in the lymph nodes), where rapidly dividing B cells (centroblasts) and plasma cells are surrounded by dense clusters of concentrically arranged lymphocytes.

The effects of splenectomy on the immune response depend on the age at which the spleen is removed. In children, splenectomy often leads to an increased incidence of bacterial sepsis caused primarily by *Streptococcus pneumoniae, Neisseria meningitidis*, and *Haemophilus influenzae.* Splenectomy in adults has less adverse effects, although it leads to some increase in blood-borne bacterial infections **(bacteremia).**

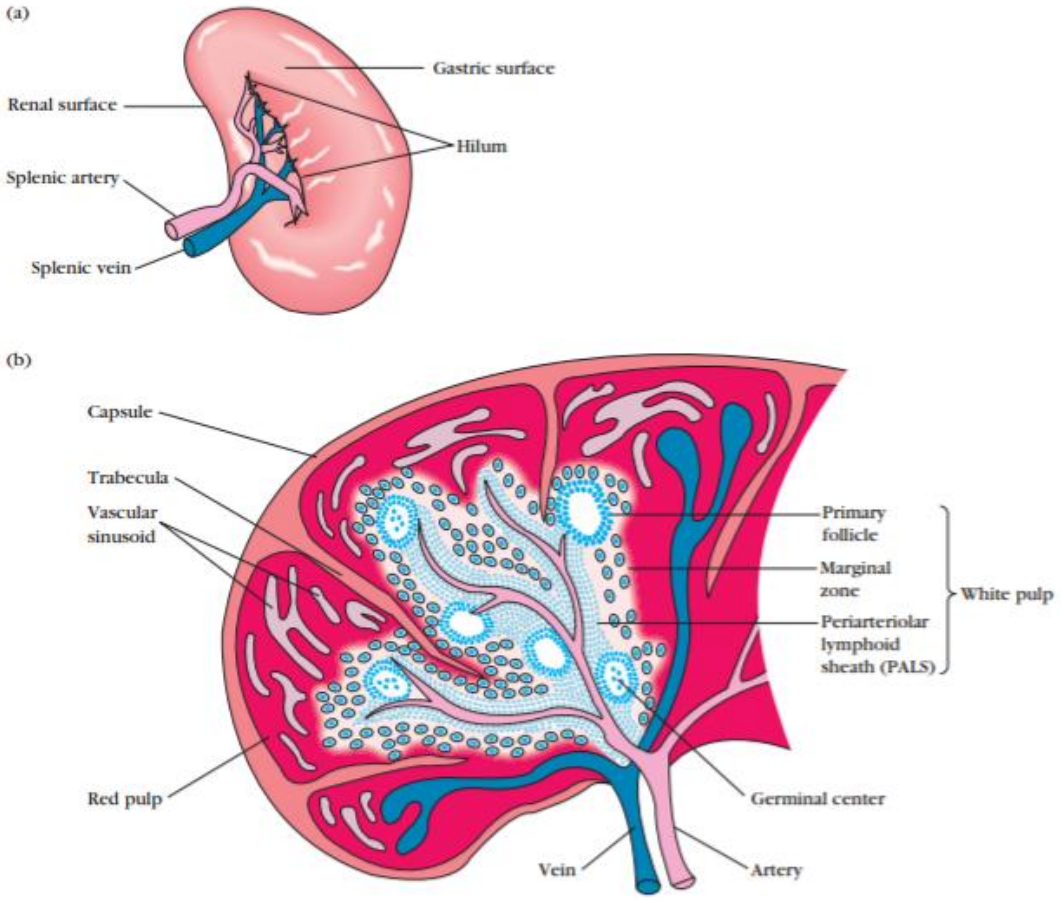


FIGURE 2-19 Structure of the spleen. (a) The spleen, which is about 5 inches long in adults, is the largest secondary lymphoid organ. It is specialized for trapping blood-borne antigens. (b) Diagrammatic cross section of the spleen. The splenic artery pierces the capsule and divides into progressively smaller arterioles, ending in vascular sinusoids that drain back into the splenic vein. The erythrocyte-filled red pulp surrounds the sinusoids. The white pulp forms a sleeve, the periarteriolar lymphoid sheath (PALS), around the arterioles; this sheath contains numerous T cells. Closely associated with the PALS is the marginal zone, an area rich in B cells that contains lymphoid follicles that can develop into secondary follicles containing germinal centers.

HEMATOPOIESIS AND DIFFERENTIATION

All blood cells arise from a type of cell called the **hematopoietic stem cell (HSC). Stem cells** are cells that can differentiate into other cell types; they are self-renewing—they maintain their population level by cell division. In humans, **hematopoiesis,** the formation and development of red and white blood cells, begins in the embryonic yolk sac during the first weeks of development. Here, yolk-sac stem cells differentiate into primitive erythroid cells that contain embryonic hemoglobin. In the third month of gestation, hematopoietic stem cells migrate from the yolk sac to the fetal liver and then to the spleen; these two organs have major roles in hematopoiesis from the third to the seventh months of gestation. After that, the differentiation of HSCs in the bone marrow becomes the major factor in hematopoiesis, and by birth there is little or no hematopoiesis in the liver and spleen.

It is remarkable that every functionally specialized, mature blood cell is derived from the same type of stem cell. In contrast to a *unipotent* cell, which differentiates into a single cell type, a hematopoietic stem cell is *multipotent,* or *pluripotent,* able to differentiate in various ways and thereby generate erythrocytes, granulocytes, monocytes, mast cells, lymphocytes, and megakaryocytes. These stem cells are few, normally fewer than one HSC per 5 _ 104 cells in the bone marrow.

The study of hematopoietic stem cells is difficult both because of their scarcity and because they are hard to grow in vitro. As a result, little is known about how their proliferation and differentiation are regulated. By virtue of their capacity for self-renewal, hematopoietic stem cells are maintained at stable levels throughout adult life; however, when there is an increased demand for hematopoiesis, HSCs display an enormous proliferative capacity. This can be demonstrated in mice whose hematopoietic systems have been completely destroyed by a lethal dose of x-rays (950 rads; one rad represents the absorption by an irradiated target of an amount of radiation corresponding to 100 ergs/gram of target). Such irradiated mice will die within 10 days unless they are infused with normal bone-marrow cells from a syngeneic (genetically identical) mouse. Although a normal mouse has 3 _ 108 bone-marrow cells, infusion of only 104–105 bone-marrow cells (i.e., 0.01%–0.1% of the normal amount) from a donor is sufficient to completely restore the hematopoietic system, which demonstrates the enormous proliferative and differentiative capacity of the stem cells.

Early in hematopoiesis, a multipotent stem cell differentiates along one of two pathways, giving rise to either a common **lymphoid progenitor cell** or a common **myeloid progenitor cell** (Figure 2-1). The types and amounts of growth factors in the microenvironment of a particular stem cell or progenitor cell control its differentiation. During the development of the lymphoid and myeloid lineages, stem cells differentiate into **progenitor cells,** which have lost the capacity for self-renewal and are committed to a particular cell lineage. Common lymphoid progenitor cells give rise to B, T, and NK (natural killer) cells and some dendritic cells .Myeloid stem cells generate progenitors of red blood cells (erythrocytes), many of the various white blood cells (neutrophils, eosinophils, basophils, monocytes, mast cells, dendritic cells), and platelets. Progenitor commitment depends on the acquisition of responsiveness to particular growth factors and **cytokines.** When the appropriate factors and cytokines are present, progenitor cells proliferate and differentiate into the corresponding cell type, either a mature erythrocyte, a particular type of leukocyte, or a platelet-generating cell (the megakaryocyte). Red and white blood cells pass into bone marrow channels, from which they enter the circulation.

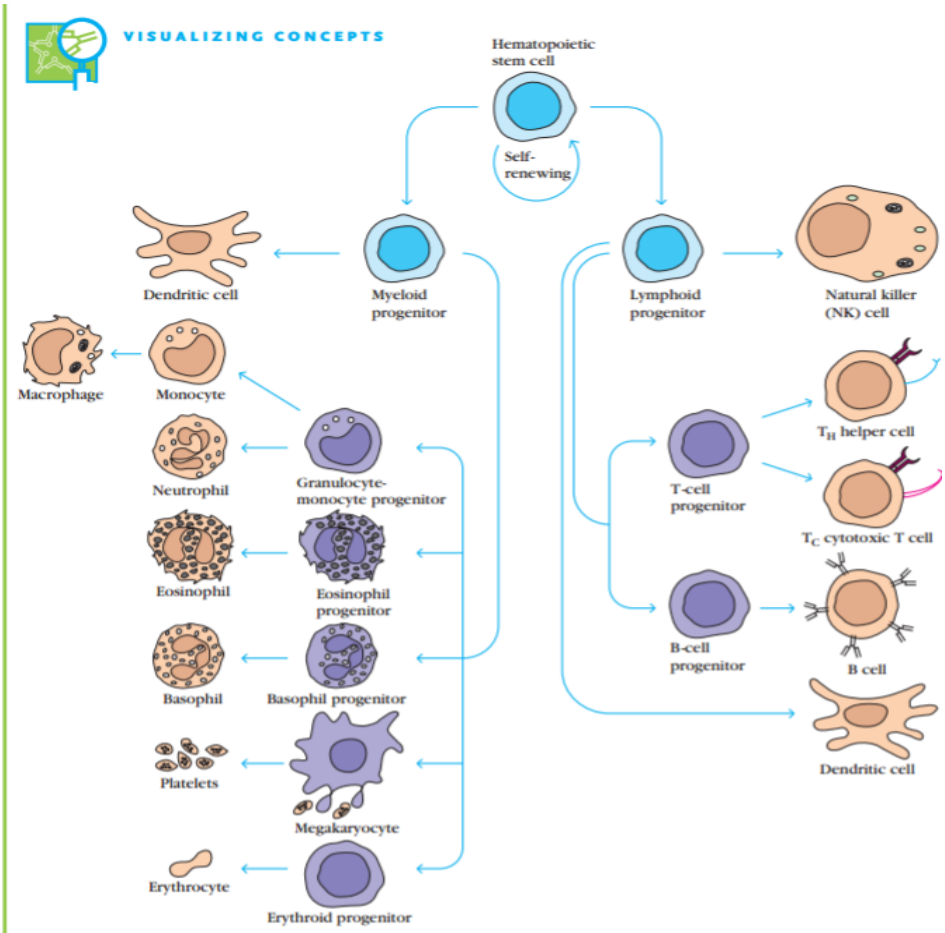


FIGURE 2-1 Hematopoiesis. Self-renewing hematopoietic stem cells give rise to lymphoid and myeloid progenitors. All lymphoid cells descend from lymphoid progenitor cells and all cells of the myeloid lineage arise from myeloid progenitors. Note that some dendritic cells come from lymphoid progenitors, others from myeloid precursors.

ROLE OF CYTOKINES

Cytokines

The development of an effective immune response involves lymphoid cells, inflammatory cells, and hematopoietic cells. The complex interactions among these cells are mediated by a group of proteins collectively designated **cytokines** to denote their role in cell-to-cell communication. Cytokines are low-molecularweight regulatory proteins or glycoproteins secreted by white blood cells and various other cells in the body in response to a number of stimuli. These proteins assist in regulating the development of immune effector cells, and some cytokines possess direct effector functions of their own.

Cytokines bind to specific receptors on the membrane of target cells, triggering signal-transduction pathways that ultimately alter gene expression in the target cells (Figure below). The susceptibility of the target cell to a particular cytokine is determined by the presence of specific membrane receptors. In general, the cytokines and their receptors exhibit very high

affinity for each other, with dissociation constants ranging from 10–10 to 10–12 M. Because their affinities are so high, cytokines can mediate biological effects at picomolar concentrations.

Cytokines Have Numerous Biological Functions

Although a variety of cells can secrete cytokines, the two principal producers are the TH cell and the macrophage. Cytokines released from these two cell types activate an entire network of interacting cells (Figure 12-5). Among the numerous physiologic responses that require cytokine involvement are development of cellular and humoral immune responses, induction of the inflammatory response, regulation of hematopoiesis, control of cellular proliferation and differentiation, and the healing of wounds. Although the immune response to a specific antigen may include the production of cytokines, it is important to remember that cytokines act in an antigen-nonspecific manner. That is, they affect whatever cells they encounter that bear appropriate receptors and are in a physiological state that allows them to respond.

Cytokines are involved in a staggeringly broad array of biological activities including innate immunity, adaptive immunity, inflammation, and hematopoiesis. Altogether, the total number of proteins with cytokine activity easily exceeds 100 and research continues to uncover new ones.

Below table summarizes the activities of some cytokines and places them into functional groups.

TABLE 12-1 Functional groups of selected cytokines[1]

Cytokine*	Secreted by**	Targets and effects
SOME CYTOKINES OF INNATE IMMUNITY		
Interleukin 1 (IL-1)	Monocytes, macrophages, endothelial cells, epithelial cells	Vasculature (inflammation); hypothalamus (fever); liver (induction of acute phase proteins)
Tumor Necrosis Factor-α (TNF-α)	Macrophages	Vasculature (inflammation); liver (induction of acute phase proteins); loss of muscle, body fat (cachexia); induction of death in many cell types; neutrophil activation
Interleukin 12 (IL-12)	Macrophages, dendritic cells	NK cells; influences adaptive immunity (promotes T_H1 subset)
Interleukin 6 (IL-6)	Macrophages, endothelial cells	Liver (induces acute phase proteins); influences adaptive immunity (proliferation and antibody secretion of B cell lineage)
Interferon α (IFN-α) (This is a family of molecules)	Macrophages	Induces an antiviral state in most nucleated cells; increases MHC class I expression; activates NK cells
Interferon β (IFN-β)	Fibroblasts	Induces an antiviral state in most nucleated cells; increases MHC class I expression; activates NK cells
SOME CYTOKINES OF ADAPTIVE IMMUNITY		
Interleukin 2 (IL-2)	T cells	T-cell proliferation; can promote AICD. NK cell activation and proliferation; B-cell proliferation
Interleukin 4 (IL-4)	T_H2 cells; mast cells	Promotes T_H2 differentiation; isotype switch to IgE
Interleukin 5 (IL-5)	T_H2 cells	Eosinophil activation and generation
Interleukin 25 (IL-25)	Unknown	Induces secretion of T_H2 cytokine profile
Transforming growth factor β (TGF-β)	T cells, macrophages, other cell types	Inhibits T-cell proliferation and effector functions; inhibits B-cell proliferation; promotes isotype switch to IgE; inhibits macrophages
Interferon γ (IFN-γ)	T_H1 cells; $CD8^+$ cells; NK cells	Activates macrophages; increases expression MHC class I and class II molecules; increases antigen presentation

[1]Many cytokines play roles in more than one functional category.

*Only the major cell types providing cytokines for the indicated activity are listed; other cell types may also have the capacity to synthesize the given cytokine.

**Also note that activated cells generally secrete greater amounts of cytokine than unactivated cells.

*It should be kept in mind that most of the listed functions have been identified from analysis of the effects of recombinant cytokines, often at nonphysiologic concentrations, added individually to in vitro systems. In vivo, however, cytokines rarely, if ever, act alone. Instead, a target cell is exposed to a milieu containing a mixture of cytokines, whose combined synergistic or antagonistic effects can have very different consequences. In addition, cytokines often induce the synthesis of other cytokines, resulting in cascades of activity.

TOLL LIKE RECEPTORS (TLRs)

TLRs constitute key components of innate immunity surveillance system. Mammalian TLRs are a family of 11 receptors that sense a broad range of microbial products. Each TLR is a single pass Trans- membrane receptor with an extracellular domain, containing multiple leucine- rich repeats and an intracellular signaling domain that is homologous to the cytoplasmic tail of TL-1 receptor. Stimulation of TLRs directs activation of NFκB (Nuclear transcription factor kappa B) and the production of pro-inflammatory cytokines. Vast arrays of microbial molecules have been shown to stimulate TLRs, including lipopolysaccharide by TLR4, bacterial flagelin by TLR 5, double stranded RNA by TLR 3 and bacterial DNA by TLR 9.

Mouse and human studies have revealed that TLR signaling plays a motor role in host defence. E.g. mice that are deficient in TLR4 are more susceptible to infection with a variety of Gram-Negative bacteria. Mutations and polymorphism in TLRs and TLR signaling molecules have revealed the importance of TLRs in human defence against pathogenic organisms. Humans with a genetic deficiency in IRAK-4, a proximal kinase required for TLR signaling show unresponsiveness to a wide range of TLR stimuli and patients are plagued by variety of childhood infections.

These Toll receptors were first described in the fruit-fly. They appear not to recognize and bind pathogens directly, but clearly are involved in signaling the appropriate response to different classes of pathogen. In the fruit-fly, the Toll receptor itself triggers the production of antifungal peptides in response to fungal infections, while a different member of the Toll family is involved in activating an antibacterial response. In mammals, a Toll-family protein, called **Toll-like receptor 4**, or **TLR-4**, signals the presence of LPS by associating with CD14, the macrophage receptor for LPS. TLR-4 is also involved in the immune response to at least one virus, respiratory syncytial virus, although in this case the nature of the stimulating ligand is not known. Another mammalian Toll-like receptor, TLR-2, signals the presence of a different set of microbial constituents, which include the proteoglycans of gram-positive bacteria, although how it recognizes these is not known. TLR-4 and TLR-2 induce similar but distinct signals, as shown by the distinct responses resulting from LPS signaling through TLR-4 and proteoglycan signaling through TLR-2. There are at least nine distinct proteins in this newly discovered family in mammals, and further functions of Toll-like receptors may soon be revealed as mice lacking one or other of these proteins are produced and analyzed.

The effects of bacterial lipopolysaccharide on macrophages are mediated by CD14 binding to Toll-likereceptor 4.

Bacterial LPS is a cell-wall component of gram-negative bacteria that has long been known for its ability to induce a dramatic systemic reaction known as septic shock. Perhaps because of this, the best-characterized proteins in innate immunity are the plasma protein **LPS-binding protein** (**LBP**), and the receptor protein CD14 which binds LBP-bound LPS. Both LBP and CD14 have leucine-rich repeat motifs. Although the structural details of the binding are not yet characterized, the LPS:LBP complex binds to CD14, which is either free in the plasma or bound to the cell surface by a phosphoinositol glycolipid tail (Fig. below). This binding triggers a cell response, but until recently the mechanism by which the signals were transduced across the cell membrane was unknown.

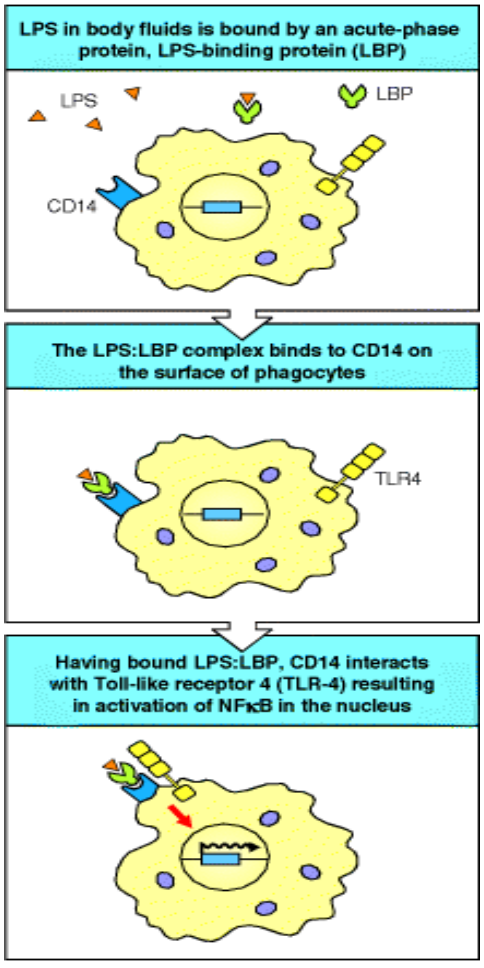


Fig.

Bacterial lipopolysaccharide signals through the Toll-like receptor TLR-4 to activate the
Transcription factor NFκB. TLR-4 is activated by the binding of bacterial lipopolysaccharide (LPS) through two other proteins. LPS is bound by the soluble LPS-binding protein, which then loads its bound LPS onto the phosphoinositol glycolipid-linked peripheral membrane protein CD14. This then triggers the membrane protein TLR- 4 to signal to the nucleus, activating the transcription factor NFκB, which in turn activates genes involved in defense against infection.

CELLS OF THE IMMUNE SYSTEM

The ability of the immune system to perform its function is dependent upon the no. of constituent cells. The principal cells of the immune system are : Lymphocytes, accessory cells and effector cells

LYMPHOCYTES

B LYMPHOCYTES

The B lymphocyte derived its letter designation from its site of maturation, in the ***bursa of Fabricius*** in birds; the name turned out to be apt, for *b*one marrow is its major site of maturation in a number of mammalian species, including humans and mice. Mature B cells are definitively distinguished from other lymphocytes by their synthesis and display of membrane-bound immunoglobulin (antibody) molecules, which serve as receptors for antigen. Each of the approximately $1.5x10^5$ molecules of antibody on the membrane of a single B cell has an identical binding site for antigen. Among the other molecules expressed on the membrane of mature B cells are the following:

- **B220** (a form of CD45) is frequently used as a marker for B cells and their precursors. However, unlike antibody, it is not expressed uniquely by B-lineage cells.
- **Class II MHC molecules** permit the B cell to function as an antigen-presenting cell (APC).
- **CR1** (CD35) and **CR2** (CD21) are receptors for certain complement products.
- **FcγRII** (CD32) is a receptor for IgG, a type of antibody.
- **B7-1** (CD80) and **B7-2** (CD86) are molecules that interact with CD28 and CTLA-4, important regulatory molecules on the surface of different types of T cells, including TH cells.
- **CD40** is a molecule that interacts with CD40 ligand on the surface of helper T cells. In most cases this interaction is critical for the survival of antigen stimulated B cells and for their development into antibody-secreting plasma cells or memory B cells.

Interaction between antigen and the membrane-bound antibody on a mature naive B cell, as well as interactions with T cells and macrophages, selectively induces the activation and differentiation of B-cell clones of corresponding specificity. In this process, the B cell divides repeatedly and differentiates over a 4- to 5-day period, generating a population of plasma cells and memory cells. Plasma cells, which have lower levels of membrane-bound antibody than B cells, synthesize and secrete antibody. All clonal progeny from a given B cell secrete antibody molecules with the same antigen-binding specificity. Plasma cells are terminally differentiated cells, and many die in 1 or 2 weeks.

B-cells are the only cell type of immune system that are specialized to secrete antibodies and therefore constitute the principal mediators of the humoral (i.e, antibody) immune response. B cells can be distinguished from other cell types by the presence of unique cell-surface markers.

T LYMPHOCYTES

T lymphocytes derive their name from their site of maturation in the thymus. Like B lymphocytes, these cells have membrane receptors for antigen. Although the antigen binding
T-cell receptor is structurally distinct from immunoglobulin, it does share some common structural features with the immunoglobulin molecule, most notably in the structure of its antigen-binding site. Unlike the membrane- bound antibody on B cells, though, the T-cell receptor (TCR) does not recognize free antigen. Instead the TCR recognizes only antigen that is bound to particular classes of self-molecules (antigen presenting cells). Most T cells recognize antigen only when it is bound to a self-molecule encoded by genes within the major histocompatibility complex (MHC). Thus, a fundamental difference between the humoral and cell-mediated branches of the immune system is that the B cell is capable of binding soluble antigen, whereas the T cell is restricted to binding antigen displayed on self-cells(antigen presenting cells). To be recognized by most T cells, this antigen must be displayed together with MHC molecules on the surface of antigen-presenting cells or on virus-infected cells, cancer cells, and grafts. The T-cell system has developed to eliminate these altered self-cells,which pose a threat to the normal functioning of the body.

Like B cells, T cells express distinctive membrane molecules. All T-cell subpopulations express the T-cell receptor, a complex of polypeptides that includes CD3; and most can be distinguished by the presence of one or the other of two membrane molecules, CD4 and CD8.

T cells that express the membrane glycoprotein molecule CD4 are restricted to recognizing antigen bound to class II MHC molecules, whereas T cells expressing CD8, a dimeric membrane glycoprotein, are restricted to recognition of antigen bound to class I MHC molecules. Thus the expression of CD4 versus CD8 corresponds to the MHC restriction of the T cell. In general, expression of CD4 and of CD8 also defines two major functional subpopulations of T lymphocytes. $CD4^+$ T cells generally function as T helper (T_H) cells and are class-II restricted; $CD8^+$ T cells generally function as T cytotoxic (T_C) cells and are class-I restricted. Thus the ratio of T_H to T_C cells in a sample can be approximated by assaying the number of $CD4^+$and $CD8^+$ T cells. This ratio is approximately 2:1 in normal human peripheral blood, but it may be significantly altered by immunodeficiency diseases, autoimmune diseases, and other disorders.

The classification of $CD4^+$ class II–restricted cells as T_H cells and $CD8^+$class I–restricted cells as T_C cells is not absolute. Some $CD4^+$ cells can act as killer cells. Also, some T_C cells have been shown to secrete a variety of cytokines and exert an effect on other cells comparable to that exerted by T_H cells. The distinction between T_H and T_C cells, then, is not always clear; there can be ambiguous functional activities. However, because these ambiguities are the exception and not the rule, the generalization of T helper (T_H) cells as being $CD4^+$ and class-II restricted and of T cytotoxic cells (T_C) as being $CD8^+$ and class-I restricted is assumed throughout this text, unless otherwise specified.

Another subpopulation of T lymphocytes—called **T suppressor (T_S) cells**—has been postulated. It is clear that some T cells help to suppress the humoral and the cell-mediated branches of the immune system, but the actual isolation and cloning of normal T_S cells is a matter of controversy and dispute among immunologists. For this reason, it is uncertain whether T_S cells do indeed constitute a separate functional subpopulation of T cells. Some immunologists believe that the suppression mediated by T cells observed in some systems is simply the consequence of activities of T_H or T_C subpopulations whose end results are suppressive.

TH1 AND TH2

T cells recognize and interact with antigen presented on class II MHC molecule on an antigen presenting cell. Following this interaction the T_H cells get activated and undergo extensive proliferation to give rise to many daughter cells of same antigen specific T_H cells called as effector cells. These T_H cells secrete specific cytokines which are responsible for activation of B-Cells, T_C cells and other cells of immune response. The type of immune response depends on the change in pattern of cytokines produced by T_H cells. The cytokine that activates only T cytotoxic cells ($T_{C)}$ and macrophages is called T_H1 response and the one which activates mainly B cells is called T_H2 response.

Effector T cells, fall into three functional classes that detect peptide antigens derived from different types of pathogen. Peptides from intracellular pathogens that multiply in the cytoplasm are carried to the cell surface by MHC class I molecules and presented to CD8 T cells. These differentiate into cytotoxic T cells that kill infected target cells. Peptide antigens from pathogens multiplying in intracellular vesicles, and those derived from ingested extracellular bacteria and toxins, are carried to the cell surface by MHC class II molecules and presented to CD4 T cells. These can differentiate into two types of effector T cell, called **TH1** and **TH2**. Pathogens that accumulate in large numbers inside macrophage and dendritic cell vesicles tend to stimulate the differentiation of TH1 cells, whereas extracellular antigens tend to stimulate the production of TH2 cells. TH1 cells activate the microbicidal properties of macrophages, and induce B cells to make IgG antibodies that are very effective at opsonizing extracellular pathogens for uptake by phagocytic cells. TH2 cells initiate the humoral immune response by activating naive antigen-specific B cells to produce IgM antibodies. These TH2 cells can subsequently stimulate the production of different isotypes, including IgA and IgE, as well as neutralizing and/or weakly opsonizing subtypes of IgG. Fig. 8.1 shows the involvement of the different effector T cells in the immune responses to different classes of pathogens.

	Cell-mediated immunity		Humoral immunity
Typical pathogens	Vaccinia virus Influenza virus Rabies virus *Listeria*	*Mycobacterium tuberculosis* *Mycobacterium leprae* *Leishmania donovani* *Pneumocystis carinii*	*Clostridium tetani* *Staphylococcus aureus* *Streptococcus pneumoniae* Polio virus *Pneumocystis carinii*
Location	Cytosol	Macrophage vesicles	Extracellular fluid
Effector T cell	Cytotoxic CD8 T cell	T_H1 cell	T_H2/T_H1 cell
Antigen recognition	Peptide:MHC class I on infected cell	Peptide:MHC class II on infected macrophage	Peptide:MHC class II on antigen-specific B cell
Effector action	Killing of infected cell	Activation of infected macrophages	Activation of specific B cell to make antibody

Figure 8.1. The role of effector T cells in cell-mediated and humoral immune responses to representative pathogens.

NATURAL KILLER CELLS

The natural killer cell was first described in 1976, when it was shown that the body contains a small population of large, granular lymphocytes that display cytotoxic activity against a wide range of tumor cells in the absence of any previous immunization with the tumor. NK cells were subsequently shown to play an important role in host defense both against tumor cells and against cells infected with some, though not all, viruses. These cells, which constitute 5%–10% of lymphocytes in human peripheral blood, do not express the membrane molecules and receptors that distinguish T- and B-cell lineages. Although NK cells do not have T-cell receptors or immunoglobulin incorporated in their plasma membranes, they can recognize potential target cells in two different ways. In some cases, an NK cell employs NK cell receptors to distinguish abnormalities, notably a reduction in the display of class I MHC molecules and the unusual profile of surface antigens displayed by some tumor cells and cells infected by some viruses. Another way in which NK cells recognize potential target cells depends upon the fact that some tumor cells and cells infected by certain viruses display antigens against which the immune system has made an antibody response, so that antitumor or antiviral antibodies are bound to their surfaces. Because NK cells express CD16, a membrane receptor for the carboxyl-terminal end of the IgG molecule, called the Fc region, they can attach to these antibodies and subsequently destroy the targeted cells. This is an example of a process known as **antibody-dependent cell mediated cytotoxicity (ADCC).**

Several observations suggest that NK cells play an important role in host defense against tumors. For example, in humans the **Chediak-Higashi syndrome**—an autosomal recessive disorder—is associated with impairment in neutrophils, macrophages, and NK cells and an increased incidence of lymphomas. Likewise, mice with an autosomal mutation called *beige* lack NK cells; these mutants are more susceptible than normal mice to tumor growth following injection with live tumor cells.

MONONUCLEAR PHAGOCYTES / MACROPHAGES AND PHAGOCYTOSIS

The mononuclear phagocytic system consists of **monocytes** circulating in the blood and **macrophages** in the tissues (Figure 2-8). During hematopoiesis in the bone marrow, granulocyte-monocyte progenitor cells differentiate into promonocytes, which leave the bone marrow and enter the blood, where they further differentiate into mature monocytes. Monocytes circulate in the bloodstream for about 8 h, during which they enlarge; they then migrate into the tissues and differentiate into specific tissue macrophages or, as discussed later, into dendritic cells.

Differentiation of a monocyte into a tissue macrophage involves a number of changes: The cell enlarges five- to tenfold; its intracellular organelles increase in both number and complexity; and it acquires increased phagocytic ability, produces higher levels of hydrolytic enzymes, and begins to secrete a variety of soluble factors. Macrophages are dispersed throughout the body. Some take up residence in particular tissues, becoming fixed macrophages, whereas others remain motile and are called free, or wandering, macrophages. Free macrophages travel by

amoeboid movement throughout the tissues. Macrophage-like cells serve different functions in different tissues and are named according to their tissue location:

- **Alveolar macrophages** in the lung
- **Histiocytes** in connective tissues
- **Kupffer cells** in the liver
- **Mesangial cells** in the kidney
- **Microglial cells** in the brain
- **Osteoclasts** in bone

Not all macrophages become fixed in specific tissues, however, with a large number of them retaining their motility and function as free or 'wandering' macrophages.

Macrophages are capable of actively ingesting and digesting exogenous antigens, such as whole microorganisms and insoluble particles, and endogenous matter, such as injured or dead host cells, cellular debris, and activated clotting factors. The process of phagocytosis involves the following series of steps:

1. In the first step in phagocytosis, macrophages are attracted by and move toward a variety of substances generated in an immune response; this process is called **chemotaxis.**
2. The next step in phagocytosis is adherence of the antigen to the macrophage cell membrane. Complex antigens, such as whole bacterial cells or viral particles, tend to adhere well and are readily phagocytosed; isolated proteins and encapsulated bacteria tend to adhere poorly and are less readily phagocytosed. Adherence induces membrane protrusions, called **pseudopodia,** to extend around the attached material.
3. Fusion of the pseudopodia encloses the material within a membrane-bounded structure called a **phagosome,**
4. The phagosome then enters the endocytic processing pathway. In this pathway, a phagosome moves toward the cell interior, where it fuses with a **lysosome** to form a **phagolysosome.** Lysosomes contain lysozyme and a variety of other hydrolytic enzymes that digest the ingested material.
5. The digested contents of the phagolysosome are then eliminated in a process called **exocytosis.**

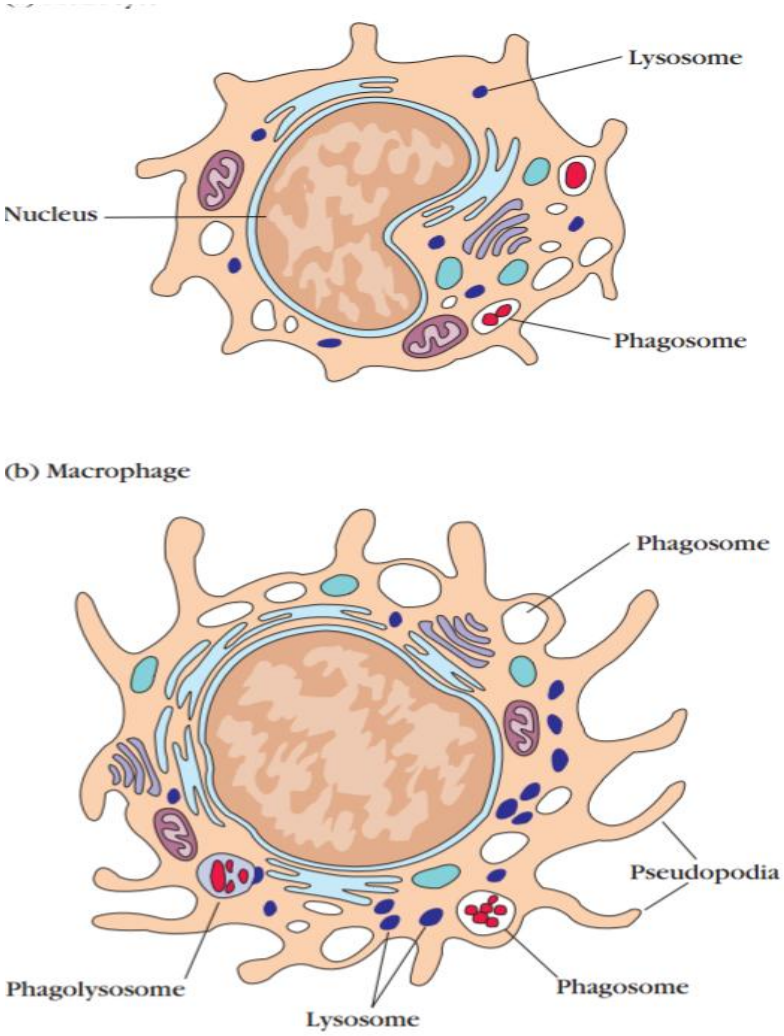


FIGURE 2-8 Typical morphology of a monocyte and a macrophage. Macrophages are five- to tenfold larger than monocytes and contain more organelles, especially lysosomes.

OPSONIZATION

The macrophage membrane has receptors for certain classes of antibody. If an antigen (e.g., a bacterium) is coated with the appropriate antibody, the complex of antigen and antibody binds to antibody receptors on the macrophage membrane more readily than antigen alone and phagocytosis is enhanced. In one study, for example, the rate of phagocytosis of an antigen was 4000-fold higher in the presence of specific antibody to the antigen than in its absence. Thus, antibody functions as an **opsonin,** a molecule that binds to both antigen and macrophage and enhances phagocytosis. The process by which particulate antigens are rendered more susceptible to phagocytosis is called **opsonization.**

NEUTROPHILS

Neutrophils are around 10-20 µm in diameter and are the most common polymorphonuclear granulocyte to be found in the blood, constituting 90% of all granulocytes Neutrophils are produced by hematopoiesis in the bone marrow. They are released into the peripheral blood and

circulate for 7–10 h before migrating into the tissues, where they have a life span of only a few days. In response to many types of infections, the bone marrow releases more than the usual number of neutrophils and these cells generally are the first to arrive at a site of inflammation. The resulting transient increase in the number of circulating neutrophils, called **leukocytosis,** is used medically as an indication of infection. Movement of circulating neutrophils into tissues, called **extravasation,** takes several steps: the cell first adheres to the vascular endothelium, then penetrates the gap between adjacent endothelial cells lining the vessel wall, and finally penetrates the vascular basement membrane, moving out into the tissue spaces.

A number of substances generated in an inflammatory reaction serve as **chemotactic factors** that promote accumulation of neutrophils at an inflammatory site. Among these chemotactic factors are some of the complement components, components of the blood-clotting system, and several cytokines secreted by activated TH cells and macrophages.

Like macrophages, neutrophils are active phagocytic cells. Phagocytosis by neutrophils is similar to that described for macrophages, except that the lytic enzymes and bactericidal substances in neutrophils are contained within primary and secondary granules. The larger, denser primary granules are a type of lysosome containing peroxidase, lysozyme, and various hydrolytic enzymes. The smaller secondary granules contain collagenase, lactoferrin, and lysozyme. Both primary and secondary granules fuse with phagosomes, whose contents are then digested and eliminated much as they are in macrophages. Neutrophils also employ both oxygen-dependent and oxygen-independent pathways to generate antimicrobial substances. Neutrophils are in fact much more likely than macrophages to kill ingested microorganisms.

EOSINOPHILS

Eosinophils constitute 2-5 % of white blood cells in people who do not have allergies. This figure is considered higher in individuals with Asthama, Eczema and / or hay fever. Eosinophils have a bilobed nucleus and their cytoplasm is filled with granules that bind the red dye, eosin, hence the name eosinophil. These granules contain several products including enzymes such as acid phosphatase, glucoronidase, catheprins, ribonuclease, histaminase, arylsulphatase and peroxidase. Eosinophils also produce a protein known as the ‘ major basic protein’ which is toxic. Eosinophils, like neutrophils, are motile phagocytic cells that can migrate from the blood into the tissue spaces. Their phagocytic role is significantly less important than that of neutrophils, and it is thought that they play a role in the defense against parasitic organisms. The secreted contents of eosinophilic granules may damage the parasite membrane.

BASOPHILS

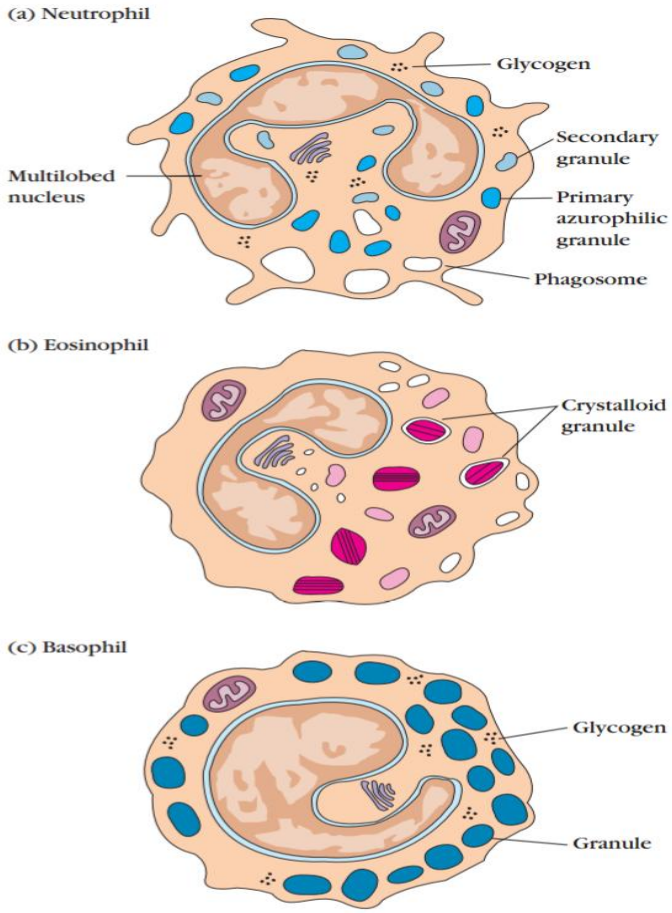
Basophils are non-phagocytic granulocytes that function by releasing pharmacologically active substances from their cytoplasmic granules. These substances play a major role in certain allergic responses. The basophil has a lobed nucleus and heavily granulated cytoplasm that stains with the basic dye methylene blue. Both neutrophils and eosinophils are phagocytic, whereas basophils are not.

Basophils are also present at the site of an inflammatory reaction. Basophils share a common stem-cell precursor with eosinophils; growth factors for basophils are very similar to those for eosinophils and include IL-3, IL-5, and GM-CSF. There is evidence for reciprocal control of the maturation of the stem-cell population into basophils or eosinophils. For example, transforming growth factor (TGF)-β in the presence of IL-3 suppresses eosinophil differentiation and enhances that of basophils. Basophils are normally present in very low numbers in the circulation and seem to have a similar role to eosinophils in defense against pathogens. Like eosinophils, they are recruited to the sites of allergic reactions.

MAST CELLS

Mast cells are very similar in structure and function to Basophils. Like Basophils, Mast cells also contain granules composed of heparin and histamine. Mast cells are found in Lymph nodes, spleen and bone marrow, around vessels, nerves, glands and throughout the skin. However it is extremely rare to found one in the blood. Mast-cell precursors, which are formed in the bone marrow by hematopoiesis, are released into the blood as undifferentiated cells; they do not differentiate until they leave the blood and enter the tissues. Mast cells can be found in a wide variety of tissues, including the skin, connective tissues of various organs, and mucosal epithelial tissue of the respiratory, genitourinary, and digestive tracts. Mast cells, together with blood basophils, play an important role in the development of allergies.

Like Basophils they have a very high affinity for IgE antibodies. The cell surface receptors for these antibodies are the triggers for the release of histamine and other products and this release results in anaphylactic and allergic reactions.



DENDRITIC CELLS

The **dendritic cell (DC)** acquired its name because it is covered with long membrane extensions that resemble the dendrites of nerve cells. The **DCs** belong to the family of antigen presenting cells (APCs), and thought to be the part of the innate immune system. They reside in almost all tissues in the body. DCs play a critical role in the initiation of the immune response. They serve as link between the adaptive and innate immune responses. acquired its name because it is covered with long membrane extensions that resemble the dendrites of nerve cells. There are many types of dendritic cells, although most mature dendritic cells have the same major function, the presentation of antigen to T_H cells. Four types of dendritic cells are known: Langerhans cells, interstitial dendritic cells, myeloid cells, and lymphoid dendritic cells. Each arises from hematopoietic stem cells via different pathways and in different locations.

Figure 2-11 shows that they descend through both the myeloid and lymphoid lineages. Despite their differences, they all constitutively express high levels of both class II MHC molecules and members of the co-stimulatory B7 family. For this reason, they are more potent antigen-presenting cells than macrophages and B cells, both of which need to be activated before they can function as antigen-presenting cells (APCs). Immature or precursor forms of each of these types of dendritic cells acquire antigen by phagocytosis or endocytosis; the antigen is processed, and mature dendritic cells present it to T_H cells. Following microbial invasion or during inflammation, mature and immature forms of Langerhans cells and interstitial dendritic cells migrate into draining lymph nodes, where they make the critical presentation of antigen to TH cells that is required for the initiation of responses by those key cells.

Another type of dendritic cell, the **follicular dendritic cell** (Figure 2-12), does not arise in bone marrow and has a different function from the antigen-presenting dendritic cells described above. Follicular dendritic cells do not express class II MHC molecules and therefore do not function as antigen presenting cells for TH-cell activation. These dendritic cells were named for their exclusive location in organized structures of the lymph node called lymph follicles, which are rich in B cells. Although they do not express class II molecules, follicular dendritic cells express high levels of membrane receptors for antibody,which allows the binding of antigen-antibody antibody complexes. The interaction of B cells with this bound antigen can have important effects on B cell responses.

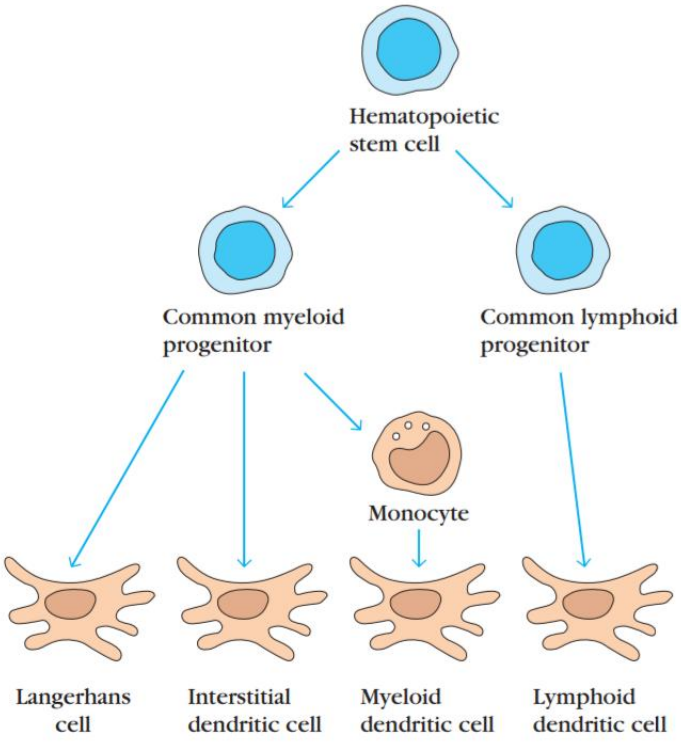
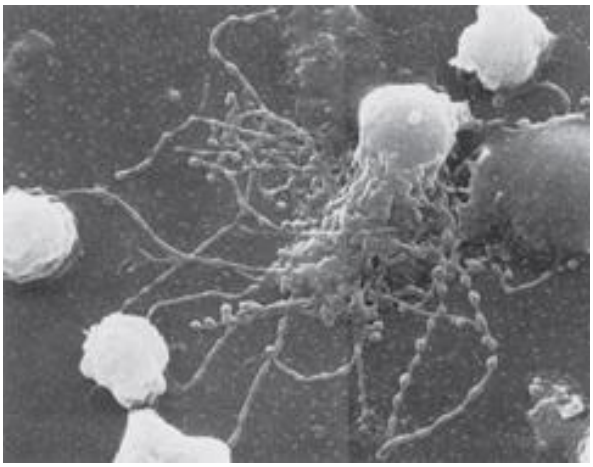


FIGURE 2-11 Dendritic cells arise from both the myeloid and lymphoid lineages. The myeloid pathway that gives rise to the monocyte/macrophage cell type also gives rise to dendritic cells. Some dendritic cells also arise from the lymphoid lineage. These considerations do not apply to follicular dendritic cells, which are not derived from bone marrow.



Scanning electron micrograph of follicular dendritic cells showing long, beaded dendrites. The beads are coated with antigen-antibody complexes. The dendrites emanate from the cell body.

LYMPHOKINE ACTIVATED KILLER (LAK) CELLS

Animal studies have shown that lymphocytes can be activated against tumor antigens in vitro by culturing them with x-irradiated tumor cells in the presence of IL-2 and added tumor antigens. These activated lymphocytes mediate more effective tumor destruction than untreated lymphocytes when they are re-injected into the original tumor-bearing animal.

It is difficult, however, to activate in vitro enough lymphocytes with antitumor specificity to be useful in cancer therapy. While sensitizing lymphocytes to tumor antigens by this method, S. Rosenberg discovered that, in the presence of high concentrations of cloned IL-2 but without the addition of tumor antigens, large numbers of activated lymphoid cells were generated that could kill fresh tumor cells but not normal cells. He called these cells **lymphokine-activated killer (LAK) cells.** In one study, for example, Rosenberg found that infusion of LAK cells plus recombinant IL-2 into tumor bearing animals mediated effective tumor-cell destruction. LAK-cell populations are typically >90% activated NK cells. However, small numbers of TCR-bearing cells are present in LAK populations and it is possible that these may also contribute to their tumoricidal activity.

Because large numbers of LAK cells can be generated in vitro and because these cells are active against a wide variety of tumors, their effectiveness in human tumor immunotherapy has been evaluated in several clinical trials. In these trials, peripheral-blood lymphocytes were removed from patients with various advanced metastatic cancers and were activated in vitro to generate LAK cells. In an early study, patients were then infused with their autologous LAK cells together with IL-2. In this trial, which involved 25 patients, cancer regression was seen in some patients. Subsequently, a more extensive trial with 222 patients resulted in complete regression in 16 patients. However, a number of undesirable side effects are associated with the high levels of IL-2 required for LAK cell activity. The most noteworthy is vascular leak syndrome, in which lymphoid cells and plasma emigrate from the peripheral blood into the tissues, leading to shock.
Tumors contain lymphocytes that have infiltrated the tumor and presumably are taking part in an antitumor response.

NATURE AND BIOLOGY OF ANTIGENS

An antigen is any foreign substance that can induce a specific immune response. We can also say as "The Substances that can be recognized by the immunoglobulin receptor of B cells, or by the T cell receptor when complexed with MHC, are called **antigens"**. The molecular properties of antigens and the way in which these properties ultimately contribute to immune activation are central to our understanding of the immune system. Typically antigens are characterized by two distinct but interrelated properties 'Immunogenicity and antigenicity'.
Immunogenicity and antigenicity are related but distinct immunologic properties that sometimes are confused.
Immunogenicity is the ability to induce a humoral and/or cell mediated immune response:

B cells + antigen ⟶ effector B cells + memory B cells

↓

(Plasma cells)

T cells + antigen ⟶ effector T cells + memory T cells

↓

(e.g., CTLs, THs)

Although a substance that induces a specific immune response is usually called an antigen, it is more appropriately called an **immunogen.**
Antigenicity is the ability to combine specifically with the final products of the above responses (i.e., antibodies and/or cell-surface receptors). Although all molecules that have the property of immunogenicity also have the property of antigenicity, the reverse is not true. Some small

molecules, called *haptens*, are antigenic but incapable, by themselves, of inducing a specific immune response. In other words, they lack immunogenicity.

The extent of immunogenicity of a given antigen determined, in part, by four properties of the: its foreignness, molecular size, chemical composition and complexity, and ability to be processed and presented with an MHC molecule on the surface of an antigen- presenting cell or altered self-cell. It is important to note that immune cells do not recognize or interact with an entire immunogen. Rather, lymphocytes recognize discrete sites on the macromolecule called epitopes or antigenic determinants. Epitopes are the immunologically active regions of an immunogen that bind to antigen-specific membrane receptors on lymphocytes or to secreted antibodies. Studies with small antigens have revealed that B and T cells recognize different epitopes on the same antigenic molecule. For example, when mice were immunized with glucagon, a small human hormone of 29 amino acids, antibody was elicited to epitopes in the amino-terminal portion, whereas the T cells responded only to epitopes in the carboxyl-terminal portion. The recognition of antigens by T cells and B cells is fundamentally different (Table 3-4). B cells recognize soluble antigen when it binds to their membrane-bound antibody.

Because B cells bind antigen that is free in solution, the epitopes they recognize tend to be highly accessible sites on the exposed surface of the immunogen.

Even if a macromolecule has the properties that contribute to immunogenicity, its ability to induce an immune response will depend on certain properties of the biological system that the antigen encounters. These properties include the genotype of the recipient, the dose and route of antigen administration, and the administration of substances, called adjuvants, that increase immune responses.

Adjuvants (from Latin *adjuvare*, to help) are substances that, when mixed with an antigen and injected with it, enhance the immunogenicity of that antigen. Adjuvants are often used to boost the immune response when an antigen has low immunogenicity or when only small amounts of an antigen are available. For example, the antibody response of mice to immunization with BSA can be increased fivefold or more if the BSA is administered with an adjuvant. Precisely how adjuvants augment the immune response is not entirely known, but they appear to exert one or more of the following effects:

- Antigen persistence is prolonged.
- Co-stimulatory signals are enhanced.
- Local inflammation is increased.
- The nonspecific proliferation of lymphocytes is stimulated.

STRUCTURE AND FUNCTION OF ANTIBODY

Antibodies are immunoglobulins (Igs) which are produced in the body in response to antigen or foreign bodies. Thus all antibodies are immunoglobulins but all immunoglobulins are not antibodies.

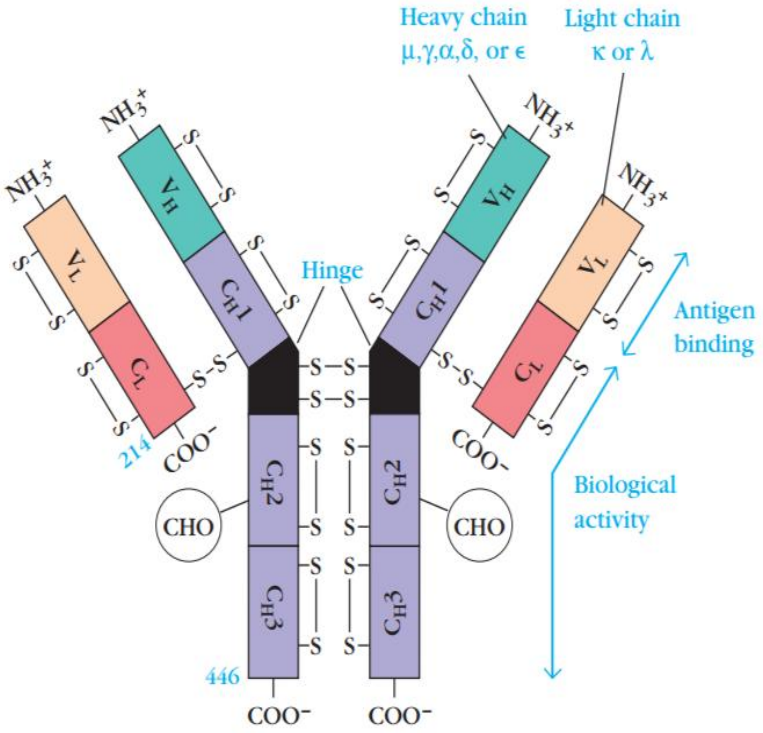
Location and formation

Antibodies are the antigen-binding proteins that are present on the B-cell membrane and secreted by plasma cells. Membrane-bound antibody confers antigenic specificity on B cells; antigen-specific proliferation of B-cell clones is elicited by the interaction of membrane antibody with antigen. Secreted antibodies circulate in the blood, where they serve as the effectors of humoral immunity by searching out and neutralizing antigens or marking them for elimination. All antibodies share structural features, bind to antigen, and participate in a limited number of effector functions.

The antibodies produced in response to a particular antigen are heterogeneous. Most antigens are complex and contain many different antigenic determinants, and the immune system usually responds by producing antibodies to several epitopes on the antigen. This response requires the recruitment of several clones of B cells. Their outputs are monoclonal antibodies, each of which specifically binds a single antigenic determinant. Together, these monoclonal antibodies make up the polyclonal and heterogeneous serum antibody response to an immunizing antigen.

Antibody structure

There are five main classes of antibodies, all of which share a common basic structure which was first hypothesized in 1962 by R. porter. The basic unit of each immunoglobulin molecule comprises four polypeptide chains- two identical long chains called heavy chains or H- chains whose molecular mass varies b/w 50 & 70 kDa and two identical short chains called light chains or L- chains of roughly 25 kDa. Each light chain is linked to a heavy chain by a covalent disulphide bond, and additional disulphide bonds link the two heavy chains to each other.these polypeptide chains that are linked together by disulphide bonds form a γ- shaped molecule. The amino terminal regions of both heavy and light chains contain highly variable amino acid sequences and are therefore termed as variable regions (VH and VL region respectively). Most of the differences present in b/w various antibodies fall within this region. The variable region of the antibody performs the antigen- binding function, whereas constant region performs the effector function of the Abs by interacting with other cells and the molecules of the immune system. Since each immunoglobulin molecule is made of two heavy and two light chains, there are two antigen -binding sites for each of these molecules.



Types/ classes of antibody

The various immunoglobulin isotypes and classes have been mentioned. Each class is distinguished by unique amino acid sequences in the heavy-chain constant region that confer class-specific structural and functional properties.

There are five main classes of immunoglobulins and can be differentiated by difference in both the general structure and conformation of their heavy and light chains. These are:

- IgG (Ig Gamma)
- IgM (Ig Mu)
- IgA (Ig Alpha)
- IgD (Ig Delta)
- IgE (Ig Epsilon)

Among the antibodies, IgG forms 80% of the antibodies in the body.

There are four human IgG subclasses, distinguished by differences in **γ** chain sequence and numbered according to their decreasing average serum concentrations: IgG1, IgG2, IgG3, and IgG4. The molecular properties and biological activities of the immunoglobulin classes are summarized in below Table

TABLE 4-2 Properties and biological activities* of classes and subclasses of human serum immunoglobulins

Property/Activity	IgG1	IgG2	IgG3	IgG4	IgA1	IgA2	IgM‡	IgE	IgD
Molecular weight†	150,000	150,000	150,000	150,000	150,000–600,000	150,000–600,000	900,000	190,000	150,000
Heavy-chain component	γ1	γ2	γ3	γ4	α1	α2	μ	ε	δ
Normal serum level (mg/ml)	9	3	1	0.5	3.0	0.5	1.5	0.0003	0.03
In vivo serum half life (days)	23	23	8	23	6	6	5	2.5	3
Activates classical complement pathway	+	+/−	++	−	−	−	+++	−	−
Crosses placenta	+	+/−	+	+	−	−	−	−	−
Present on membrane of mature B cells	−	−	−	−	−	−	+	−	+
Binds to Fc receptors of phagocytes	++	+/−	++	+	−	−	?	−	−
Mucosal transport	−	−	−	−	++	++	+	−	−
Induces mast-cell degranulation	−	−	−	−	−	−	−	+	−

*Activity levels indicated as follows: ++ = high; + = moderate; +/− = minimal; − = none; ? = questionable.

†IgG, IgE, and IgD always exist as monomers; IgA can exist as a monomer, dimer, trimer, or tetramer. Membrane-bound IgM is a monomer, but secreted IgM in serum is a pentamer.

‡IgM is the first isotype produced by the neonate and during a primary immune response.

CHARACTERISTICS AND FUNCTION OF IMMUNOGLOBULINS

Immunoglobulin G (IgG)

IgG, the most abundant class in serum, constitutes about 80% of the total serum immunoglobulin. The IgG molecule consists of two γ heavy chains and two κ or two λ light chains. It is found in the blood, lymph and intestine. It protects against bacteria and viruses by enhancing phagocytosis, neutralizing toxins and complement activation. It is the only class of antibody to cross the placenta from mother to fetus thereby conferring considerable immune protection in newborns.

There are four human IgG subclasses, distinguished by differences in γ-chain sequence and numbered according to their decreasing average serum concentrations: IgG1, IgG2, IgG3, and IgG4.

- IgG1, IgG3, and IgG4 readily cross the placenta and play an important role in protecting the developing fetus.
- IgG3 is the most effective complement activator, followed by IgG1; IgG2 is less efficient, and IgG4 is not able to activate complement at all.
- IgG1 and IgG3 bind with high affinity to Fc receptors on phagocytic cells and thus mediate opsonization. IgG4 has an intermediate affinity for Fc receptors, and IgG2 has an extremely low affinity.

Immunoglobulin M (IgM)

IgM accounts for 5%–10% of the total serum immunoglobulin, with an average serum concentration of 1.5 mg/ml. Monomeric IgM, with a molecular weight of 180,000, is expressed as membrane-bound antibody on B cells. IgM is secreted by plasma cells as a pentamer in which five monomer units are held together by disulfide bonds that link their carboxyl- terminal heavy chain domains ($C_\mu4/C_\mu4$) and their $C_\mu3/C_\mu3$ domains. The five monomer subunits are arranged with their Fc regions in the center of the pentamer and the ten antigen-binding sites on the periphery of the molecule.

Immunoglobulin A (IgA)

Although IgA constitutes only 10%–15% of the total immunoglobulin in serum, it is the predominant immunoglobulin class in external secretions such as breast milk, saliva, tears, and mucus of the bronchial, genitourinary, and digestive tracts. In serum, IgA exists primarily as a monomer, but polymeric forms (dimers, trimers, and some tetramers) are sometimes seen, all containing a J-chain polypeptide. The IgA of external secretions, called **secretory IgA,** consists of a dimer or tetramer, a J-chain polypeptide, and a polypeptide chain called **secretory component.**

It cannot cross the placental barrier. IgM is 500-1000 times more effective than IgG in opsonization, in bacterial action and in bacterial agglutination. But in neutralization of toxins and viruses, it is less effective than IgG. It helps in complement activation.

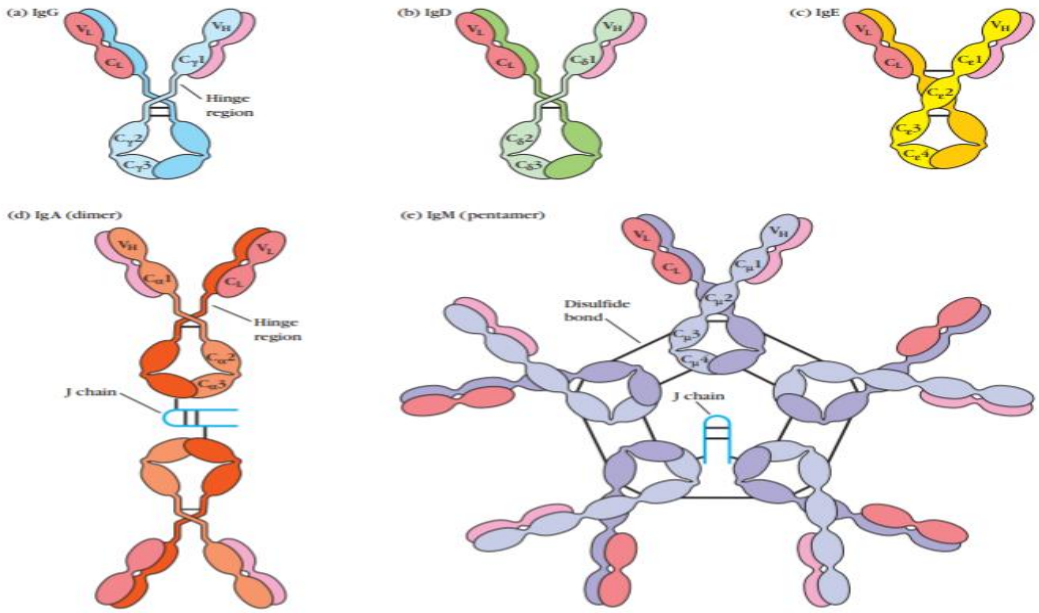
Immunoglobulin E (IgE)

The potent biological activity of IgE allowed it to be identified in serum despite its extremely low average serum concentration (0.3µg/ml). IgE antibodies mediate the immediate hypersensitivity reactions that are responsible for the symptoms of hay fever, asthma, hives, and anaphylactic shock. The presence of a serum component responsible for allergic reactions was first demonstrated in 1921 by K. Prausnitz and H. Kustner, who injected serum from an allergic person intra-dermally into a nonallergic individual. When the appropriate antigen was later injected at the same site, a wheal and flare reaction (analogous to hives) developed there. This reaction, called the **P-K reaction** (named for its originators, Prausnitz and Kustner), was the basis for the first biological assay for IgE activity.

Immunoglobulin D (IgD)

IgD was first discovered when a patient developed a multiple myeloma whose myeloma protein failed to react with anti-isotype antisera against the then-known isotypes: IgA, IgM, and IgG. When rabbits were immunized with this myeloma protein, the resulting antisera were used to

identify the same class of antibody at low levels in normal human serum. The new class, called IgD, has a serum concentration of 30µg/ml and constitutes about 0.2% of the total immunoglobulin in serum. IgD, together with IgM, is the major membrane bound immunoglobulin expressed by mature B cells, and its role in the physiology of B cells is under investigation. No biological effector function has been identified for IgD.



MONOCLONAL ANTIBODIES

Most antigens offer multiple epitopes and therefore induce proliferation and differentiation of a variety of B-cell clones, each derived from a B cell that recognizes a particular epitope. The resulting serum antibodies are heterogeneous, comprising a mixture of antibodies, each specific for one epitope. Such a **polyclonal antibody** response facilitates the localization, phagocytosis, and complement-mediated lysis of antigen; it thus has clear advantages for the organism in vivo. Unfortunately, the antibody heterogeneity that increases immune protection in vivo often reduces the efficacy of an antiserum for various in vitro uses. For most research, diagnostic, and therapeutic purposes, **monoclonal antibodies,** derived from a single clone and thus specific for a single epitope, are preferable.

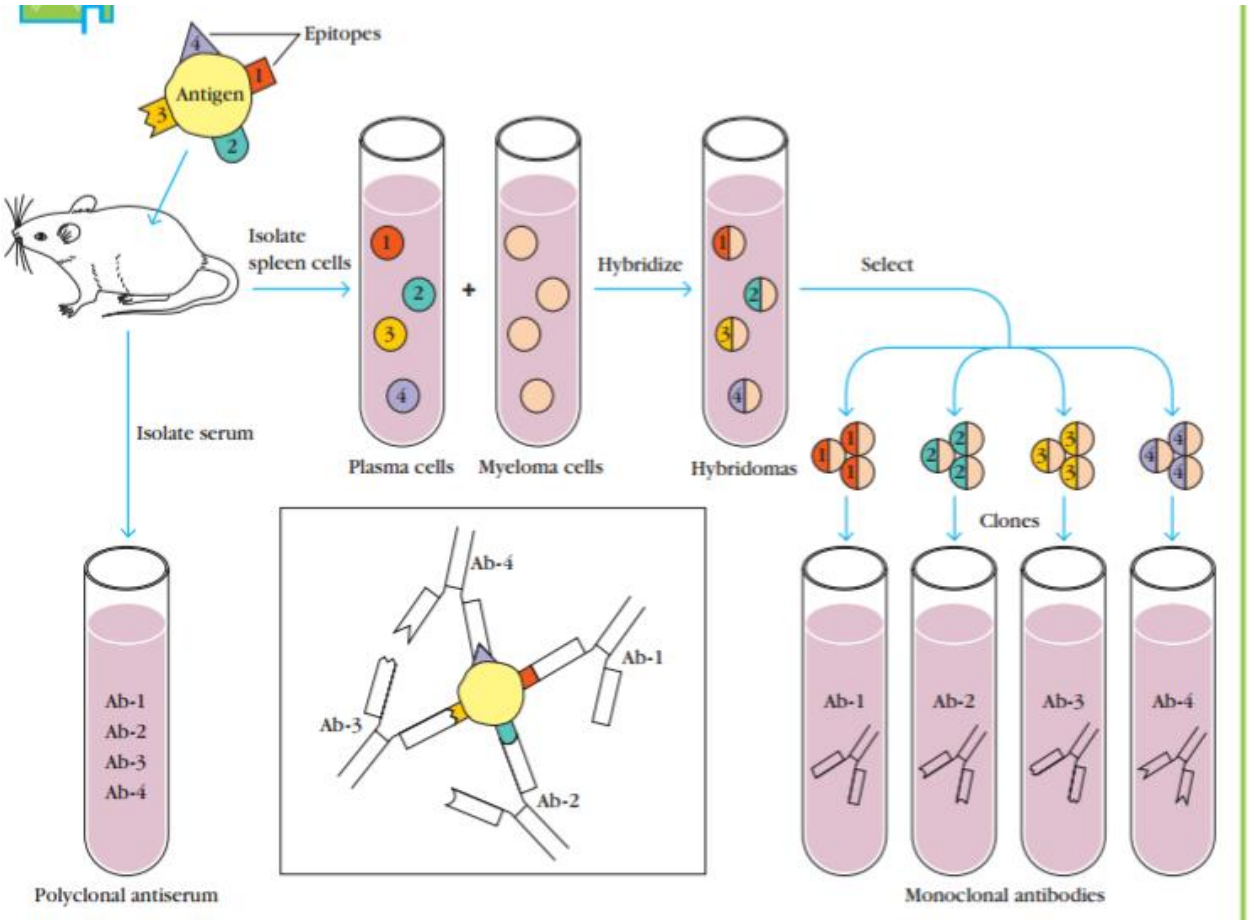
PRODUCTION

Direct biochemical purification of a monoclonal antibody from a polyclonal antibody preparation is not feasible. In 1975, Georges Köhler and Cesar Milstein devised a method for preparing monoclonal antibody, which quickly became one of immunology's key technologies. By fusing a normal activated, antibody-producing B cell with a myeloma cell (a cancerous plasma cell), they were able to generate a hybrid cell, called a **hybridoma that** possessed the immortal growth properties of the myeloma cell and secreted theantibody produced by the B cell. The resulting

clones of hybridoma cells, which secrete large quantities of monoclonal antibody, can be cultured indefinitely. The significance of the work by Köhler and Milstein was acknowledged when each was awarded a Nobel Prize.

Procedure

The conventional polyclonal antiserum produced in response to a complex antigen contains a mixture of monoclonal antibodies, each specific for one of the four epitopes shown on the antigen (fig.) In contrast, a monoclonal antibody, which is derived from a single plasma cell, is specific for one epitope on a complex antigen. The outline of the basic method for obtaining a monoclonal antibody is illustrated below if fig.



Clinical uses

Monoclonal antibodies are proving to be very useful as diagnostic, imaging, and therapeutic reagents in clinical medicine. Initially, monoclonal antibodies were used primarily as in vitro diagnostic reagents. Among the many monoclonal antibody diagnostic reagents now available are products for detecting pregnancy, diagnosing numerous pathogenic microorganisms, measuring the blood levels of various drugs, matching histocompatibility antigens, and detecting antigens shed by certain tumors.

Radiolabeled monoclonal antibodies can also be used in vivo for detecting or locating tumor antigens, permitting earlier diagnosis of some primary or metastatic tumors in patients. For example, monoclonal antibody to breast-cancercells is labeled with iodine-131 and introduced into the blood to detect the spread of a tumor to regional lymph nodes. This monoclonal imaging technique can reveal breast-cancer metastases that would be undetected by other, less sensitive scanning techniques.

Immunotoxins composed of tumor-specific monoclonal antibodies coupled to lethal toxins are potentially valuable therapeutic reagents. The toxins used in preparing immune-toxins include ricin, *Shigella* toxin, and diphtheria toxin, all of which inhibit protein synthesis. These toxins are so potent that a single molecule has been shown to kill a cell. Each of these toxins consists of two types of functionally distinct polypeptide components, an inhibitory (toxin) chain and one or more binding chains,which interact with receptors on cell surfaces; without the binding polypeptide(s) the toxin cannot get into cells and therefore is harmless.

ANTIGEN-ANTIBODY INTERACTION:

The antigen-antibody interaction is bimolecular association similar to an enzyme-substrate interaction, with an important distinction: it does not lead to an irreversible chemical alteration in either the antibody or the antigen. The association between an antibody and an antigen involves various non-covalent interactions between the antigenic determinant, or epitope, of the antigen and the variable-region (V_H/V_L) domain of the antibody molecule, particularly the hyper-variable regions, or complementarity-determining regions (CDRs).

The non-covalent interactions that form the basis of antigen-antibody (Ag-Ab) binding include hydrogen bonds, ionic bonds, hydrophobic interactions, and van der Waals interactions. Because these interactions are individually weak (compared with a covalent bond), a large number of such interactions are required to form a strong Ag-Ab interaction.

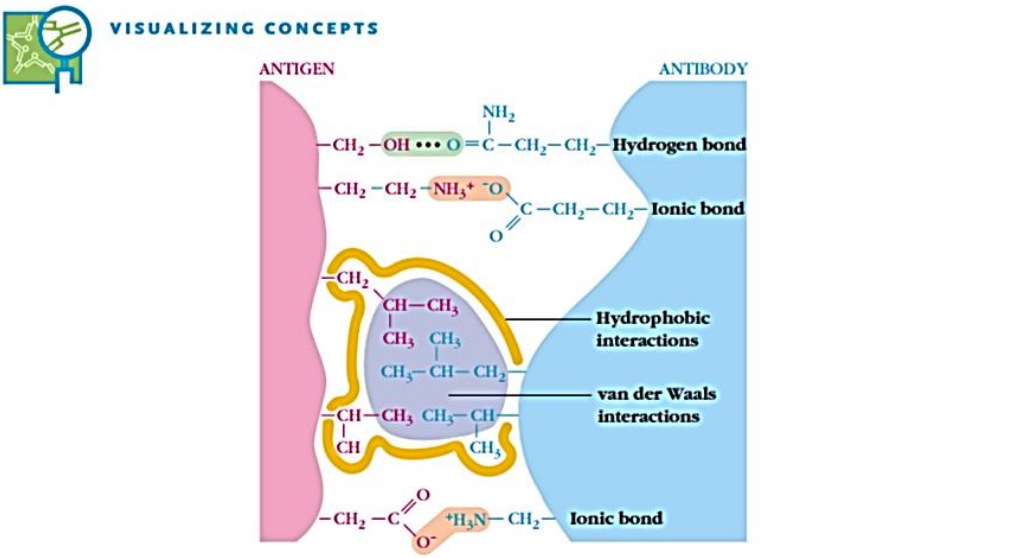


FIGURE 6-1 The interaction between an antibody and an antigen depends on four types of noncovalent forces: (1) hydrogen bonds, in which a hydrogen atom is shared between two electronegative atoms; (2) ionic bonds between oppositely charged residues; (3) hydrophobic interactions, in which water forces hydrophobic groups together; and (4) van der Waals interactions between the outer electron clouds of two or more atoms. In an aqueous environment, noncovalent interactions are extremely weak and depend upon close complementarity of the shapes of antibody and antigen.

The strength of Antigen-Antibody interaction is indicated by:

- Antibody Affinity
- Antibody Avidity
- Antibody cross reactivity

Antibody Affinity

The combined strength of the non-covalent interactions between a *single* antigen-binding site on an antibody and a *single* epitope is the **affinity** of the antibody for that epitope. Low-affinity antibodies bind antigen weakly and tend to dissociate readily, whereas high-affinity antibodies bind antigen more tightly and remain bound longer. The association between a binding site on an antibody (Ab) with a monovalent antigen (Ag) can be described by the equation:

$$Ag + Ab \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} Ag\text{-}Ab$$

Where k_1 is the forward (association) rate constant and $_{k\text{-}1}$ is the reverse (dissociation) rate constant. The ratio $k1/k_{-1}$ is the association constant K_a (i.e., $k1/k^{-}{}_1 = K$a), a measure of affinity. Because K_a is the equilibrium constant for the above reaction, it can be calculated from the ratio of the molar concentration of bound Ag-Ab complex to the molar concentrations of unbound antigen and antibody at equilibrium as follows:

$$K_a = \frac{[Ag\text{-}Ab]}{[Ab][Ag]}$$

Antibody Avidity

The affinity at one binding site does not always reflect the true strength of the antibody-antigen interaction. When complex antigens containing multiple, repeating antigenic determinants are mixed with antibodies containing multiple binding sites, the interaction of an antibody molecule with an antigen molecule at one site will increase the probability of reaction between those two molecules at a second site. The strength of such multiple interactions between a multivalent antibody and antigen is called the **avidity.** The avidity of an antibody is a better measure of its binding capacity within biological systems (e.g., the reaction of an antibody with antigenic determinants on a virus or bacterial cell) than the affinity of its individual binding sites. High avidity can compensate for low affinity. For example, secreted pentameric IgM often has a lower affinity than IgG, but the high avidity of IgM, resulting from its higher valence, enables it to bind antigen effectively.

Antibodies cross reactivity

Although Ag-Ab reactions are highly specific, in some cases antibody elicited by one antigen can cross-react with an unrelated antigen. Such **cross-reactivity** occurs if two different antigens share an identical or very similar epitope. In the latter case, the antibody's affinity for the cross-reacting epitope is usually less than that for the original epitope.

Cross-reactivity is often observed among polysaccharide antigens that contain similar oligosaccharide residues. The **ABO blood-group antigens,** for example, are glycoproteins expressed on red blood cells. Subtle differences in the terminal residues of the sugars attached to these surface proteins distinguish the A and B blood-group antigens. An individual lacking one or both of these antigens will have serum antibodies to the missing antigen(s). The antibodies are induced not by exposure to red blood cell antigens but by exposure to cross-reacting microbial antigens present on common intestinal bacteria. These microbial antigens induce the formation of antibodies in individuals lacking the similar blood-group antigens on their red blood cells. (In individuals possessing these antigens, complementary antibodies would be eliminated during the developmental stage in which antibodies that recognizes self-epitopes are weeded out.)

ANTIGEN PROCESSING AND PRESENTATION

Recognition of foreign protein antigens by a T cell requires that peptides derived from the antigen be displayed within the cleft of an MHC molecule on the membrane of a cell. The formation of these peptide-MHC complexes requires that a protein antigen be degraded into peptides by a sequence of events called **antigen processing.** The degraded peptides then associate with MHC molecules within the cell interior, and the peptide- MHC complexes are transported to the membrane, where they are displayed (**antigen presentation**).

Class I and class II MHC molecules associate with peptides that have been processed in different intracellular compartments. Class I MHC molecules bind peptides derived from **endogenous antigens** that have been processed within the cytoplasm of the cell called as **cytosolic pathway** (e.g., normal cellular proteins, tumor proteins, or viral and bacterial proteins produced within infected cells). Class II MHC molecules bind peptides derived from **exogenous antigens** that are internalized by phagocytosis or endocytosis and processed known as **endocytic pathway.**

Since all cells expressing either class I or class II MHC molecules can present peptides to T cells, strictly speaking they all could be designated as antigen-presenting cells. However, by convention, cells that display peptides associated with class I MHC molecules to $CD8^+$ T_C cells are referred to as ***target cells;*** cells that display peptides associated with class II MHC molecules to $CD4^+$ T_H cells are called **antigen-presenting cells (APCs).**

A variety of cells can function as antigen-presenting cells. Their distinguishing feature is their ability to express class II MHC molecules and to deliver a co-stimulatory signal. Three cell types are classified as *professional* antigen-presenting cells: dendritic cells, macrophages, and B lymphocytes. These cells differ from each other in their mechanisms of antigen uptake, in whether they constitutively express class II MHC molecules, and in their co-stimulatory activity.

- Dendritic cells are the most effective of the antigen-presenting cells. Because these cells constitutively express a high level of class II MHC molecules and co-stimulatory activity, they can activate naive TH cells.
- Macrophages must be activated by phagocytosis of particulate antigens before they express class II MHC molecules or the co-stimulatory B7 membrane molecule.
- B cells constitutively express class II MHC molecules but must be activated before they express the co-stimulatory B7 molecule.

Several other cell types, classified as ***nonprofessional* antigen-presenting cells**, can be induced to express class II MHC molecules or a co-stimulatory signal. Many of these cells function in antigen presentation only for short periods of time during a sustained inflammatory response. Because nearly all nucleated cells express class I MHC molecules, virtually any nucleated cell is able to function as a target cell presenting endogenous antigens to T_C cells. Most often, target cells are cells that have been infected by a virus or some other intracellular microorganism. However, altered self-cells such as cancer cells, aging body cells, or allogeneic cells from a graft can also serve as targets.

TWO DIFFERENT PATHWAYS

The immune system uses two different pathways to eliminate intracellular and extracellular antigens. Endogenous antigens (those generated within the cell) are processed in the ***cytosolic pathway*** and presented on the membrane with class I MHC molecules; exogenous antigens (those taken up by endocytosis) are processed in the ***endocytic pathway*** and presented on the membrane with class II MHC molecules

CYTOSOLIC PATHWAY

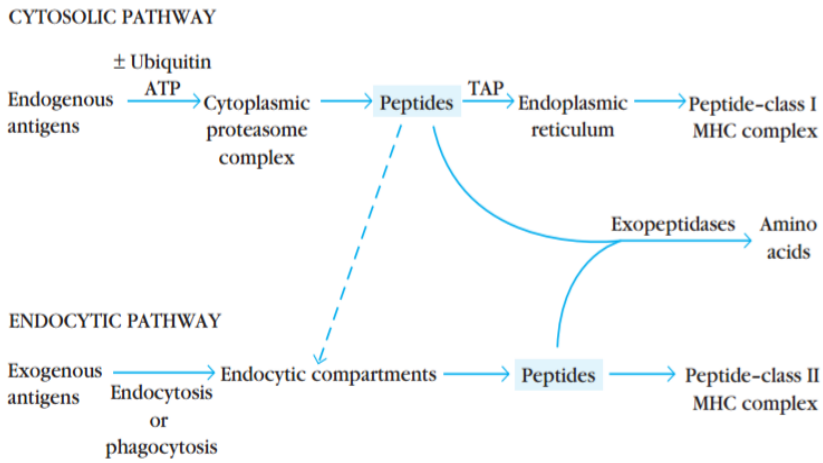


FIGURE 8-4 Overview of cytosolic and endocytic pathways for processing antigen. The proteasome complex contains enzymes that cleave peptide bonds, converting proteins into peptides. The antigenic peptides from proteasome cleavage and those from endocytic compartments associate with class I or class II MHC molecules, and the peptide-MHC complexes are then transported to the cell membrane. TAP (*t*ransporter of *a*ntigenic *p*eptides) transports the peptides to the endoplasmic reticulum. It should be noted that the ultimate fate of most peptides in the cell is neither of these pathways, but rather to be degraded completely into amino acids.

In eukaryotic cells, protein levels are carefully regulated. Every protein is subject to continuous turnover and is degraded at a rate that is generally expressed in terms of its half-life. Some proteins (e.g., transcription factors, cyclins, and key metabolic enzymes) have very short half-lives; denatured, misfolded, or otherwise abnormal proteins also are degraded rapidly. The pathway by which endogenous antigens are degraded for presentation with class I MHC molecules utilizes the same pathways involved in the normal turnover of intracellular proteins.

Intracellular proteins are degraded into short peptides by a cytosolic proteolytic system present in all cells. Those proteins targeted for proteolysis often have a small protein, called *ubiquitin,* attached to them (Figure 8-5a). Ubiquitin-protein conjugates can be degraded by a multifunctional protease complex called a **proteasome.** Each proteasome is a large (26S), cylindrical particle consisting of four rings of protein subunits with a central channel of diameter 10–50 Å. A proteasome can cleave peptide bonds between 2 or 3 different amino acid combinations in an ATP-dependent process (Figure 8-5b). Degradation of ubiquitin-protein complexes is thought to occur within the central hollow of the proteasome.

ENDOCYTIC PATHWAY

Figure below recapitulates the endogenous pathway discussed above and compares it with the separate exogenous pathway. Whether an antigenic peptide associates with class I or with class II molecules is dictated by the mode of entry into the cell, either exogenous or endogenous, and by the site of processing.

Antigen-presenting cells can internalize antigen by phagocytosis, endocytosis, or both. Macrophages internalize antigen by both processes, whereas most other APCs are not phagocytic or are poorly phagocytic and therefore internalize exogenous antigen only by endocytosis (either receptor-mediated endocytosis or pinocytosis). B cells, for example, internalize antigen very effectively by receptor-mediated endocytosis using antigen-specific membrane antibody as the receptor.

Once an antigen is internalized, it is degraded into peptides within compartments of the endocytic processing pathway. The endocytic pathway appears to involve three increasingly acidic compartments: early endosomes (pH 6.0–6.5); late endosomes or endolysosomes (pH 5.0–6.0); and lysosomes (pH 4.5–5.0). Internalized antigen moves from early to late endosomes and finally to lysosomes, encountering hydrolytic enzymes and a lower pH in each compartment. Lysosomes, for example, contain a unique collection of more than 40 acid-dependent hydrolases, including proteases, nucleases, glycosidases, lipases, phospholipases, and phosphatases. Within the compartments of the endocytic pathway, antigen is degraded into oligopeptides of about 13–18 residues, which bind to class II MHC molecules.

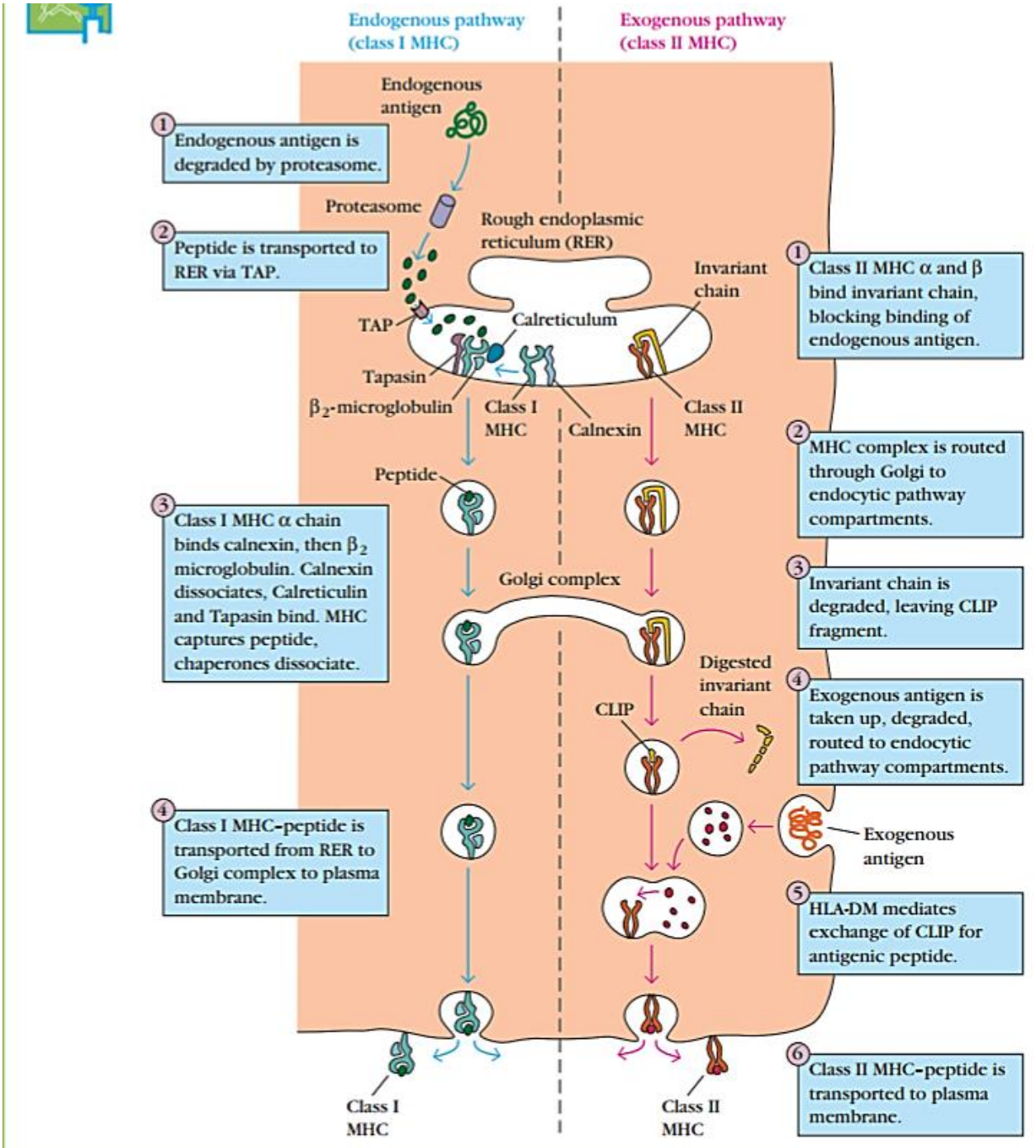


FIGURE 8-8 Separate antigen-presenting pathways are utilized for endogenous (green) and exogenous (red) antigens. The mode of antigen entry into cells and the site of antigen processing determine whether antigenic peptides associate with class molecules in the rough endoplasmic reticulum or with molecules in endocytic compartments.

FIG. ILLUSTRATING TWO DIFFERENT PATHWAYS OF ANTIGEN PROCESSING AND PRESENTATION

STRUCTURE AND FUNCTIONS OH MHC MOLECULES

Major Histocompatibility Complex

Every mammalian species studied to date possesses a tightly linked cluster of genes, the Major Histocompatibility Complex (MHC), whose products play important roles in intercellular recognition and in discrimination between self and non-self. The MHC participates in the development of both humoral and cell mediated immune responses. While antibodies may react with antigens alone, most T cells recognize antigen only when it is combined with an MHC molecule. Furthermore, because MHC molecules act as antigen-presenting structures, the particular set of MHC molecules expressed by an individual influences the collection of antigens to which that individual's T_H and T_C cells can respond. The concept that the rejection of foreign tissue is the result of an immune response to cell-surface molecules, now called **histocompatibility antigens,** originated from the work of Peter Gorer in the mid-1930s

The major histocompatibility complex is a collection of genes arrayed within a long continuous stretch of DNA on chromosome 6 in humans and on chromosome 17 in mice. The MHC is referred to as the **HLA complex** in humans and as the **H-2 complex** in mice. Although the arrangement of genes is somewhat different, in both cases the MHC genes are organized into regions encoding three classes of molecules

STRUCTURE

Class I MHC molecules encoded by the K and D regions in mice and by the A, B, and C loci in humans were the first discovered, and they are expressed in the widest range of cell types. These are referred to as *classical class I molecules.* Additional genes or groups of genes within the H-2 or HLA complexes also encode class I molecules; these genes are designated *nonclassical class I genes.* Expression of the non-classical gene products is limited to certain specific cell types. Although functions are not known for all of these gene products, some may have highly specialized roles in immunity. For example, the expression of the class I HLAG molecules on cyto-trophoblasts at the fetal-maternal interface has been implicated in protection of the fetus from being recognized as foreign (this may occur when paternal antigens begin to appear) and from being rejected by maternal T_C cells.

The two chains of the class II MHC molecules are encoded by the IA and IE regions in mice and by the DP, DQ, and DR regions in humans. The terminology is somewhat confusing, since the D region in mice encodes class I MHC molecules, whereas the D region (DR, DQ, DP) in humans refers to genes encoding class II MHC molecules! Fortunately, the designation D for the general chromosomal location encoding the human class II molecules is seldom used today; the sequence of the entire MHC region is available so the more imprecise reference to region is seldom necessary. As with the class I loci, additional class II molecules encoded within this region have specialized functions in the immune process.

The class I and class II MHC molecules have common structural features and both have roles in antigen processing. By contrast, the class III MHC region, which is flanked by the class I and II regions, encodes molecules that are critical to immune function but have little in common with class I or II molecules. Class III products include the complement components C4, C2, BF and inflammatory cytokines, including tumor necrosis factor (TNF) and heat-shock proteins.

FUNCTIONS

VISUALIZING CONCEPTS

Mouse H-2 complex

Complex	H-2						
MHC class	I	II		III		I	
Region	K	IA	IE	S		D	
Gene products	H-2K	IA αβ	IE αβ	C′ proteins	TNF-α TNF-β	H-2D	H-2L

Human HLA complex

Complex	HLA							
MHC class	II			III		I		
Region	DP	DQ	DR	C4, C2, BF		B	C	A
Gene products	DP αβ	DQ αβ	DR αβ	C′ proteins	TNF-α TNF-β	HLA-B	HLA-C	HLA-A

FIGURE 7-1 Simplified organization of the major histocompatibility complex (MHC) in the mouse and human. The MHC is referred to as the H-2 complex in mice and as the HLA complex in humans. In both species the MHC is organized into a number of regions encoding class I (pink), class II (blue), and class III (green) gene products. The class I and class II gene products shown in this figure are considered to be the classical MHC molecules. The class III gene products include complement (C′) proteins and the tumor necrosis factors (TNF-α and TNF-β).

Class I MHC genes encode glycoproteins expressed on the surface of nearly all nucleated cells; the major function of the class I gene products is presentation of peptide antigens to T_C cells.

Class II MHC genes encode glycoproteins expressed primarily on antigen-presenting cells (macrophages, dendritic cells, and B cells), where they present processed antigenic peptides to T_H cells.

Class III MHC genes encode, in addition to other products, various secreted proteins that have immune functions, including components of the complement system and molecules involved in inflammation.

THE COMPLEMENT SYSTEM

The complement system is the major effector of the humoral branch of the immune system. Research on complement began in the 1890s, when Jules Bordet at the Institut Pasteur in Paris showed that sheep antiserum to the bacterium *Vibrio cholera* caused lysis of the bacteria and that heating the antiserum destroyed its bacteriolytic activity. Surprisingly, the ability to lyse the bacteria was restored to the heated serum by adding fresh serum that contained no antibodies directed against the bacterium and was unable to kill the bacterium by itself. Bordet correctly reasoned that bacteriolytic activity requires two different substances: first, the specific antibacterial antibodies, which survive the heating process, and a second, heat-sensitive component responsible for the lytic activity. Bordet devised a simple test for the lytic activity, the easily detected lysis of antibody-coated red blood cells, called **hemolysis.** Paul Ehrlich in Berlin independently carried out similar experiments and coined the term *complement,* defining it as "the activity of blood serum that completes the action of antibody." In ensuing years, researchers discovered that the action of complement was the result of interactions of a large and complex group of proteins.

The Functions of Complement System

Research on complement now includes more than 30 soluble and cell-bound proteins. The biological activities of this system affect both innate and acquired immunity and reach far beyond the original observations of antibodymediated lysis of bacteria and red blood cells. Structural comparisons of the proteins involved in complement pathways place the origin of this system in primitive organisms possessing the most rudimentary innate immune systems.

By contrast, the realization that interaction of cellular receptors with complement proteins controls B-cell activities gives this system a role in the highly developed acquired immune system. Thus we have a system that straddles innate and acquired immunity, contributing to each in a variety of ways.

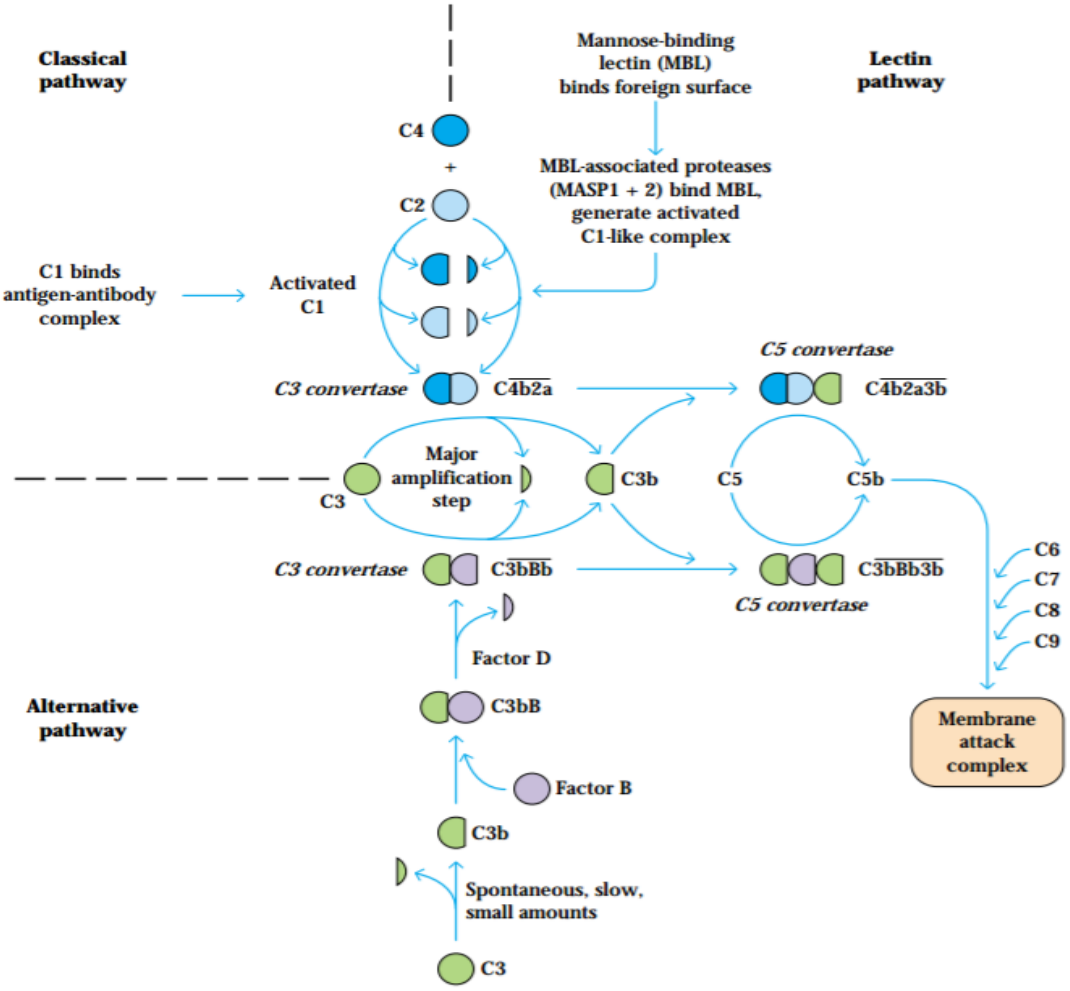
After initial activation, the various complement components interact, in a highly regulated cascade, to carry out a number of basic functions (Figure 13-1) including:

- Lysis of cells, bacteria, and viruses.
- Opsonization, which promotes phagocytosis ofparticulate antigens.
- Binding to specific complement receptors on cells of the immune system, triggering specific cell functions, inflammation, and secretion of immunoregulatory molecules.
- Immune clearance, which removes immune complexes from the circulation and deposits them in the spleen and liver.
-

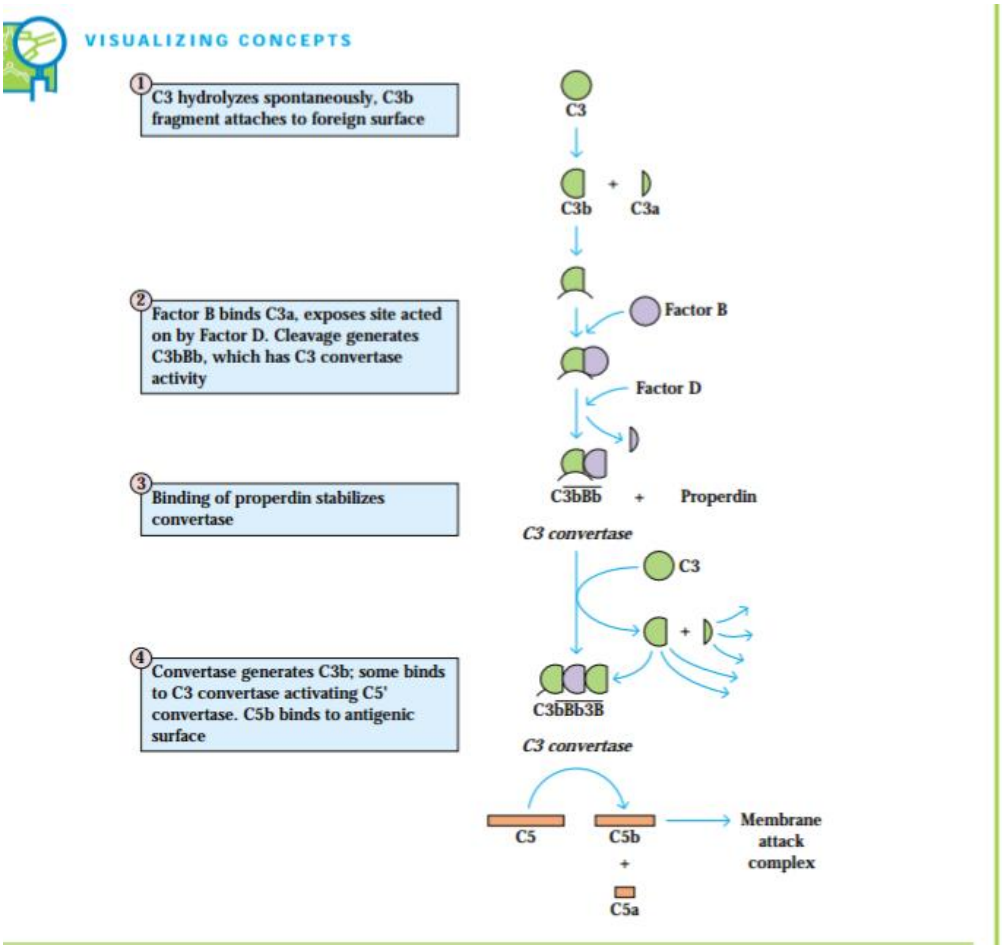
THE COMPLEMENT SYSTEM PATHWAYS

The early steps, culminating in formation of C5b, can occur by the **classical pathway,** the **alternative pathway,** or the **lectin pathway.** The final steps that lead to a membrane attack are the same in all pathways.

OUTLINE OF 3 DIFFERENT PATHWAYS



The classical pathway is initiated when C1 binds to antigen-antibody complexes. The alternative pathway is initiated by binding of spontaneously generated C3b to activating surfaces such as microbial cell walls. The lectin pathway is initiated by binding of the serum protein MBL to the surface of a pathogen. All three pathways generate C3 and C5 convertases and bound C5b, which is converted into a membrane- attack complex (MAC) by a common sequence of terminal reactions. Hydrolysis of C3 is the major amplification step in all pathways, generating large amounts of C3b, which forms part of C5 convertase. C3b also can diffuse away from the activating surface and bind to immune complexes or foreign cell surfaces, where it functions as an opsonin.



FORMATION OF INTERMEDIATE C5b

Regulation of the Complement System

Because many elements of the complement system are capable of attacking host cells as well as foreign cells and microorganisms, elaborate regulatory mechanisms have evolved to restrict complement activity to designated targets. A general mechanism of regulation in all complement pathways is the inclusion of highly labile components that undergo spontaneous inactivation if they are not stabilized by reaction with other components. In addition, a series of regulatory proteins can inactivate various complement components (Table below).

TABLE 13-2 Proteins that regulate the complement system

Protein	Type of protein	Pathway affected	Immunologic function
C1 inhibitor (C1Inh)	Soluble	Classical	Serine protease inhibitor: causes $C1r_2s_2$ to dissociate from C1q
C4b-binding protein (C4bBP)*	Soluble	Classical and lectin	Blocks formation of C3 convertase by binding C4b; cofactor for cleavage of C4b by factor I
Factor H*	Soluble	Alternative	Blocks formation of C3 convertase by binding C3b; cofactor for cleavage of C3b by factor I
Complement-receptor type 1 (CR1)*	Membrane bound	Classical, alternative, and lectin	Block formation of C3 convertase by binding C4b or C3b; cofactor for factor I-catalyzed cleavage of C4b or C3b C3bBb
Membrane-cofactor protein (MCP)*			
Decay-accelerating factor (DAE or CD55)*	Membrane bound	Classical, alternative, and lectin	Accelerates dissociation of $\overline{C4b2a}$ and $\overline{C3bBb}$ (classical and alternative C3 convertases)
Factor-I	Soluble	Classical, alternative, and lectin	Serine protease: cleaves C4b or C3b using C4bBP, CR1, factor H, DAE, or MCP as cofactor
S protein	Soluble	Terminal	Binds soluble C5b67 and prevents its insertion into cell membrane
Homologous restriction factor (HRF)	Membrane bound	Terminal	Bind to C5b678 on autologous cells, blocking binding of C9
Membrane inhibitor of reactive lysis (MIRL or CD59)*			
Anaphylatoxin inactivator	Soluble	Effector	Inactivates anaphylatoxin activity of C3a, C4a, and C5a by carboxypeptidase N removal of C-terminal Arg

*An RCA (regulator of complement activation) protein. In humans, all RCA proteins are encoded on chromosome 1 and contain short consensus repeats.

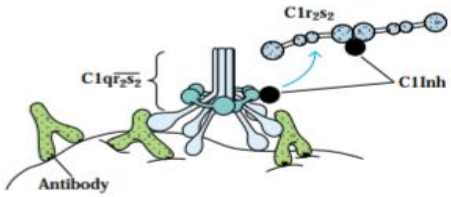
The reaction catalyzed by the C3 convertase enzymes of the classical, lectin, and alternative pathways is the major amplification step in complement activation, generating hundreds of molecules of C3b. The C3b generated by these enzymes has the potential to bind to nearby cells, mediating damage to the healthy cells by causing their opsonization by phagocytic cells bearing C3b receptors or by induction of the membrane attack complex. Damage to normal host cells is prevented because C3b undergoes spontaneous hydrolysis by the time it has diffused 40 nm away from the C4b2a or C3bBb convertase enzymes, so that it can no longer bind to its target site. The potential destruction of healthy host cells by C3b is further limited by a family of related proteins that regulate C3 convertase activity in the classical and alternative pathways. These regulatory proteins all contain repeating amino acid sequences (or motifs) of about 60 residues, termed *short consensus repeats* (SCRs). All these proteins are encoded at a single location on chromosome 1 in humans, known as the *regulators of complement activation* (RCA) gene cluster.

VISUALIZING CONCEPTS

Regulation of the Complement System

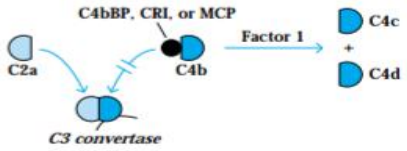
(a) Before assembly of convertase activity

1. C1 inhibitor (C1Iab) binds $C1r_2s_2$, causing dissociation from C1q



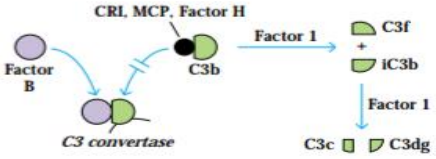
2. Association of C4b and C2a is blocked by binding C4b-binding protein (C4bBP), complement receptor type I, or membrane cofactor protein (MCP)

3. Inhibitor-bound C4b is cleaved by Factor 1



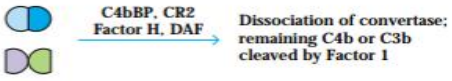
4. In alternative pathway, CR1, MCP, or Factor H prevent binding of C3b and Factor B

5. Inhibitor-bound C3b is cleaved by Factor 1



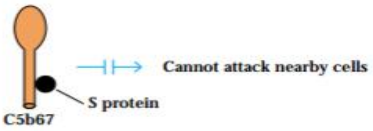
(b) After assembly of convertase

C3 convertases are dissociated by C4bBP, CR1, Factor H, and decay-accelerating Factor (DAF)



(c) Regulation at assembly of membrane-attack complex (MAC)

1. S protein prevents insertion of C5b67 MAC component into the membrane



2. Homologous restriction factor (HRF) and membrane inhibitor of reactive lysis (MIRL or CD59) bind $C8_1$, preventing assembly of poly-C9 and blocking formation of MAC

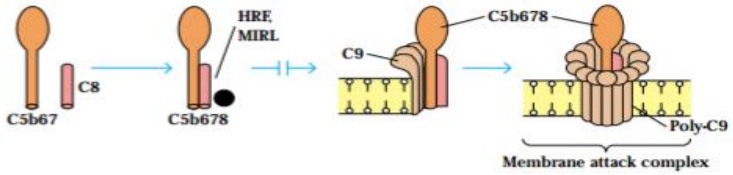


FIGURE 13-9 Regulation of the complement system by regulatory proteins (black).

IMMUNODIFFUSION

In this technique an antigen is allowed to diffuse in a semi-solid gel containing antibody. As the antigen diffuses away from the original site, i.e. the well containing antigen, its concentration gradually decreases and it forms a precipitation ring or zone where the optimal antigen and antibody concentration ratio is achieved. The area of ring or zone is proportional to the antigen concentration.

Two types of *immunodiffusion reactions* can be used to determine relative concentrations of antibodies or antigens, to compare antigens, or to determine the relative purity of an antigen preparation. They are **radial immunodiffusion** (the Mancini method) and **double immunodiffusion** (the Ouchterlony method); both are carried out in a semisolid medium such as agar. In radial immunodiffusion, an antigen sample is placed in a well and allowed to diffuse into agar containing a suitable dilution of an antiserum. As the antigen diffuses into the agar, the region of equivalence is established and a ring of precipitation, a precipitin ring, forms around the well (Figure, upper section). The area of the precipitin ring is proportional to the concentration of antigen. By comparing the area of the precipitin ring with a standard curve (obtained by measuring the precipitin areas of known concentrations of the antigen), the concentration of the antigen sample can be determined. In the Ouchterlony method, both antigen and antibody diffuse radially from wells toward each other, thereby establishing a concentration gradient. As equivalence is reached, a visible line of precipitation, a precipitin line, forms (Figure, lower section).

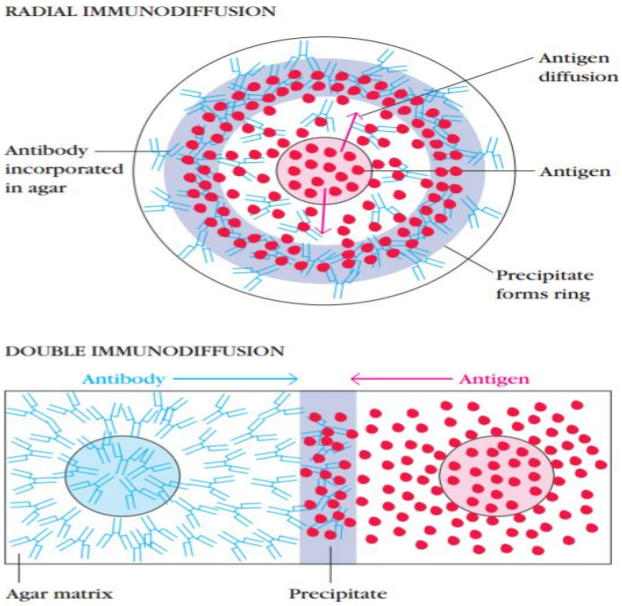


FIGURE 6-5 Diagrammatic representation of radial immunodiffusion (Mancini method) and double immunodiffusion (Ouchterlony method) in a gel. In both cases, large insoluble complexes form in the agar in the zone of equivalence, visible as lines of precipitation (purple regions). Only the antigen (red) diffuses in radial immunodiffusion, whereas both the antibody (blue) and antigen (red) diffuse in double immunodiffusion.

IMMUNOELECTROPHORESIS

Principle

Immunoelectrophoresis **(IEP)** is a two-step technique; first, a small sample of serum is applied in a well and electrophoresed through a support medium, usually agarose. After electrophoresis is completed, specific antisera are deposited in a trough cut into the agarose, parallel to the axis of the electrophoretic separation. The electrophoretically separated proteins and the antisera diffuse toward one another and, at the zone of antigen-antibody equivalence, a precipitin pattern in the form of an arc will appear.

Advantages and Limitations

The problems of poor sensitivity and long waiting time for the development and visualization of precipitin lines discussed above also apply to immunoelectrophoresis. In addition, the interpretation of the precipitin patterns requires an experienced interpreter.

Applications

Immunoelectrophoresis is particularly useful for analytical studies in patients suspected of plasma cell malignancies. Quantitative assays of immunoglobulins in these patients show a marked increase in one immunoglobulin class. IEP analysis can establish or disprove the monoclonal nature of the immunoglobulin increase by determining whether or not the quantitative increase is due to a homogeneous population, with a single light chain type. Monoclonal free light chains (Bence-Jones proteins) may also be present in the urine of these patients, and IEP of urine is the method classically used for their detection.

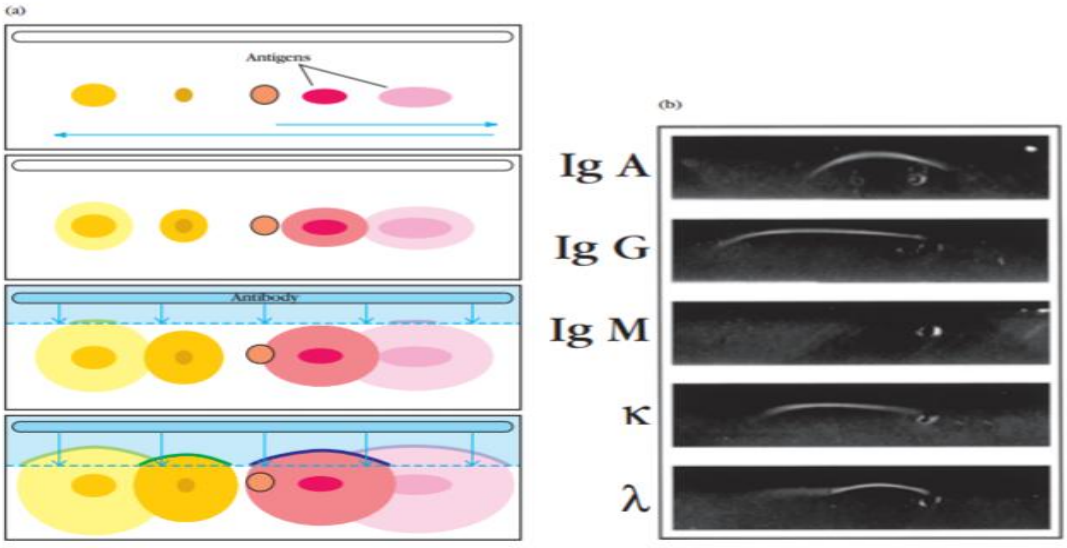


FIGURE 6-6 Immunoelectrophoresis of an antigen mixture. (a) An antigen preparation (orange) is first electrophoresed, which separates the component antigens on the basis of charge. Antiserum (blue) is then added to troughs on one or both sides of the separated antigens and allowed to diffuse; in time, lines of precipitation (colored arcs) form where specific antibody and antigen interact. (b) Immunoelectrophoretic patterns of human serum from a patient with myeloma. The patient produces a large amount of a monoclonal IgG (λ-light-chain-bearing) antibody. A sample of serum from the patient was placed in the well of the slide and electrophoresed. Then antiserum specific for the indicated antibody class or light chain type was placed in the top trough of each slide. At the concentrations of patient's serum used, only anti-IgG and anti-λ antibodies produced lines of precipitation. *[Part (b), Robert A. Kyle and Terry A. Katzman, Manual of Clinical Immunology, 1997, N. Rose, ed., ASM Press, Washington, D.C., p. 164.]*

RADIOIMMUNOASSAY (RIA)

One of the most sensitive techniques for detecting antigen or antibody is **radioimmunoassay (RIA).** The technique was first developed in 1960 by two endocrinologists, S. A. Berson and Rosalyn Yalow, to determine levels of insulin–anti-insulin complexes in diabetics. Yalow.

PRINCIPLE

The principle of RIA involves competitive binding of radiolabeled antigen and unlabeled antigen to a high-affinity antibody. The labeled antigen is mixed with antibody at a concentration that saturates the antigen-binding sites of the antibody. Then test samples of unlabeled antigen of unknown concentration are added in progressively larger amounts. The antibody does not distinguish labeled from unlabeled antigen, so the two kinds of antigen compete for available binding sites on the antibody. As the concentration of unlabeled antigen increases, more labeled antigen will be displaced from the binding sites. The decrease in the amount of radiolabeled antigen bound to specific antibody in the presence of the test sample is measured in order to determine the amount of antigen present in the test sample.

TYPES

1. The classical radioimmunoassay

The classical radioimmunoassay methods were based on the principle of **competitive binding:** unlabeled antigen competes with radiolabeled antigen for binding to antibody with the appropriate specificity. Thus, when mixtures of radiolabeled and unlabeled antigen are incubated with the corresponding antibody, the amount of free (not bound to antibody) radiolabeled antigen is directly proportional to the quantity of unlabeled antigen in the mixture.

a. Mixtures of known variable amounts of cold antigen and fixed amounts of labeled antigen and mixtures of samples with unknown concentrations of antigen with identical amounts of labeled antigen are prepared in the first step.

b. Identical amounts of antibody are added to the mixtures.

c. Antigen-antibody complexes are precipitated either by cross-linking with a second antibody or by means of the addition of reagents that promote the precipitation of antigen-antibody complexes.

d. Counting radioactivity in the precipitates allow the determination of the amount of radiolabeled antigen co-precipitated with the antibody.

e. A standard curve is constructed by plotting the percentage of antibody-bound radiolabeled antigen against known concentrations of a standardized unlabeled antigen (Fig. 14.12), and the concentrations of antigen in patient samples are extrapolated from that curve.

2. The solid-phase RIA

The separation of free and antibody-bound radiolabeled antigen becomes considerably more simple if the antibody is immobilized. This is the hallmark of **solid-phase RIA**.

a. Antibody is adsorbed or coupled to a solid surface (test tube wall, polystyrene beads, etc.).

b. Mixtures of labeled and unlabeled antigen and samples with added labeled antigen, prepared as described above, are added to the solid phase containing immobilized antibody.

c. Unbound reagents are washed off from the solid phase and radioactivity is counted to determine the amount of labeled antibody retained in each one of the different mixtures tested.
d. A calibration curve is constructed and unknown concentrations determined.

3. **Noncompetitive Solid-Phase RIA**

Noncompetitive Solid-Phase RIA for the detection of specific antibodies have also been described. The antigen is bound to solid phase, the antigencoated solid phase is exposed to a sample containing antibody, and a radiolabeled antihuman immunoglobulin is used to assay the antibody that becomes bound to the immobilized antigen.

APPLICATIONS

RIA have been used with extremely good results in the assay of many different hormones (insulin, aldosterone, human FSH, progesterone, testosterone, thyroxin, vasopressin, etc.), proteins (alpha-fetoprotein, carcinoembryonic antigen, IgE, hepatitis B surface antigen), nucleic acids (DNA), vitamins (vitamin B12), drugs (digoxin, LSD, barbiturate derivatives), enzymes (pepsin, trypsin), etc.

ADVANTAGES AND LIMITATIONS

The extremely high sensitivity of RIA is its major advantage, allowing the assay on the nanogram/milliliter range or even lower. Their main drawbacks lie in the cost of equipment and reagents, short shelf-life of radiolabeled compounds, and in the problems associated with the disposal of radioactive waste. In recent years, RIA have been virtually replaced by quantitative fluorescence assays or by enzymoimmunoassays.

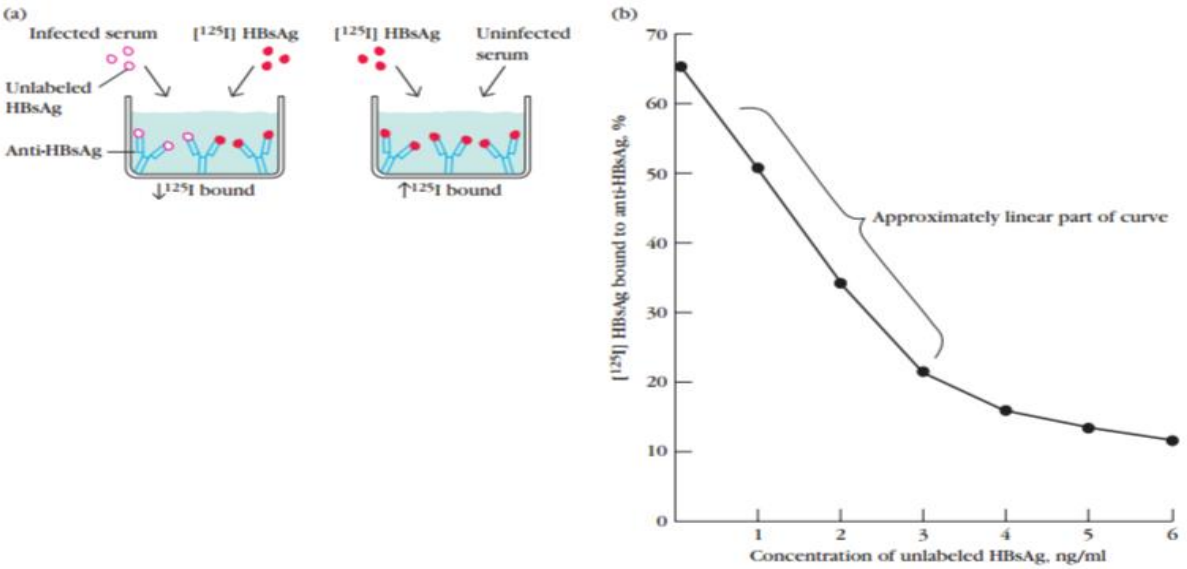
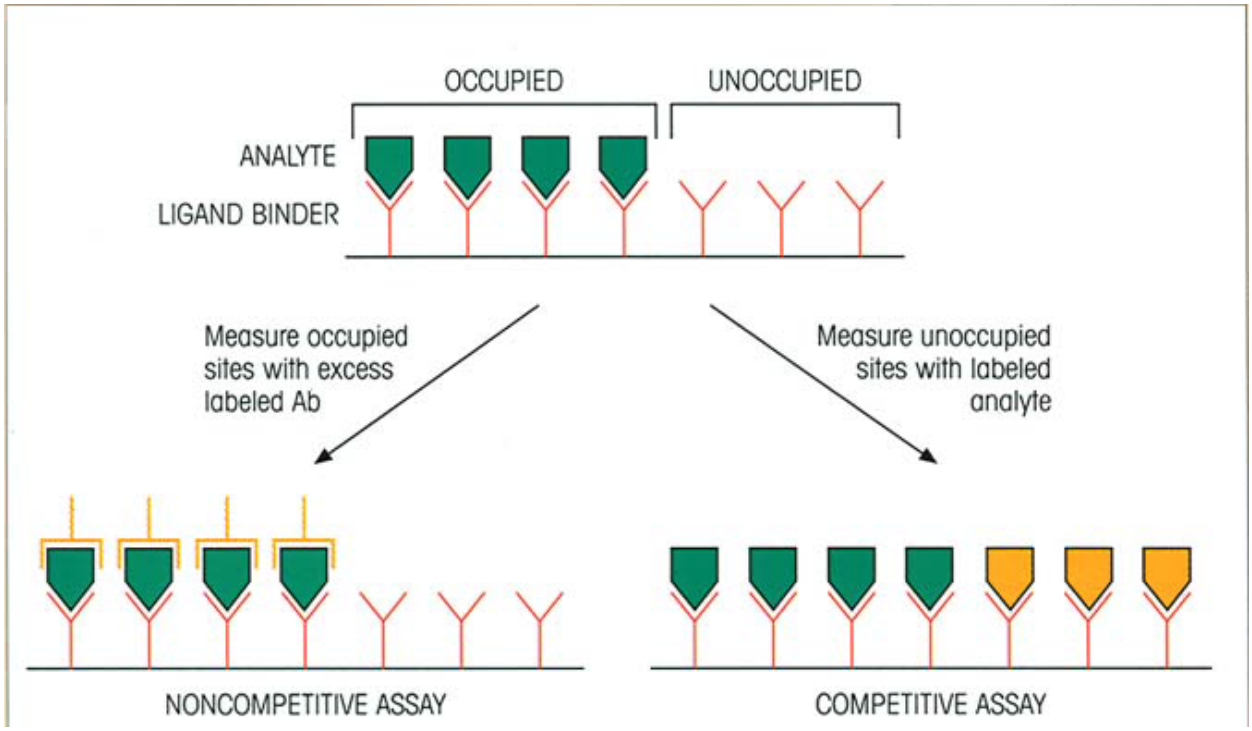


FIGURE 6-9 A solid-phase radioimmunoassay (RIA) to detect hepatitis B virus in blood samples. (a) Microtiter wells are coated with a constant amount of antibody specific for HBsAg, the surface antigen on hepatitis B virions. A serum sample and [^{125}I]HBsAg are then added. After incubation, the supernatant is removed and the radioactivity of the antigen-antibody complexes is measured. If the sample is infected, the amount of label bound will be less than in controls with uninfected serum. (b) A standard curve is obtained by adding increasing concentrations of unlabeled HBsAg to a fixed quantity of [^{125}I]HBsAg and specific antibody. From the plot of the percentage of labeled antigen bound versus the concentration of unlabeled antigen, the concentration of HBsAg in unknown serum samples can be determined by using the linear part of the curve.



RADIOIMMUNOASSAY

ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

Enzyme-linked immunosorbent assay, commonly known as **ELISA** (or EIA), is similar in principle to RIA but depends on an enzyme rather than a radioactive label. An enzyme conjugated with an antibody reacts with a colorless substrate to generate a colored reaction product. Such a substrate is called **a chromogenic substrate.** A number of enzymes have been employed for ELISA, including alkaline phosphatase, horseradish peroxidase, and β-galactosidase. These assays approach the sensitivity of RIAs and have the advantage of being safer and less costly.

TYPES

A number of variations of ELISA have been developed, allowing qualitative detection or quantitative measurement of either antigen or antibody. Each type of ELISA can be used qualitatively to detect the presence of antibody or antigen. Alternatively, a standard curve based on known concentrations of antibody or antigen is prepared, from which the unknown concentration of a sample can be determined.

1. INDIRECT ELISA

Antibody can be detected or quantitatively determined with an indirect ELISA (Figure 6-10a). Serum or some other sample containing primary antibody (Ab1) is added to an antigen- coated microtiter well and allowed to react with the antigen attached to the well. After any free Ab1 is washed away, the presence of antibody bound to the antigen is detected by adding an enzyme-conjugated secondary anti-isotype antibody (Ab_2), which binds to the primary antibody. Any free Ab_2 then is washed away, and a substrate for the enzyme is added. The amount of colored reaction product that forms is measured by specialized spectrophotometric plate readers, which can measure the absorbance of all of the wells of a 96-well plate in seconds.

Indirect ELISA is the method of choice to detect the presence of serum antibodies against human immunodeficiency virus (HIV), the causative agent of AIDS. In this assay, recombinant envelope and core proteins of HIV are adsorbed as solid-phase antigens to microtiter wells. Individuals infected with HIV will produce serum antibodies to epitopes on these viral proteins. Generally, serum antibodies to HIV can be detected by indirect ELISA within 6 weeks of infection.

2. SANDWICH ELISA

Antigen can be detected or measured by a sandwich ELISA. In this technique, the antibody (rather than the antigen) is immobilized on a microtiter well. A sample containing antigen is added and allowed to react with the immobilized antibody. After the well is washed, a second enzyme- linked antibody specific for a different epitope on the antigen is added and allowed to react with the bound antigen. After any free second antibody is removed by washing, substrate is added, and the colored reaction product is measured.

3. COMPETITIVE ELISA

Another variation for measuring amounts of antigen is competitive ELISA. In this technique, antibody is first incubated in solution with a sample containing antigen. The antigen-antibody

mixture is then added to an antigen coated microtiter well. The more antigen present in the sample, the less free antibody will be available to bind to the antigen-coated well. Addition of an enzyme-conjugated secondary antibody (Ab_2) specific for the isotype of the primary antibody can be used to determine the amount of primary antibody bound to the well as in an indirect ELISA. In the competitive assay, however, the higher the concentration of antigen in the original sample, the lower the absorbance.

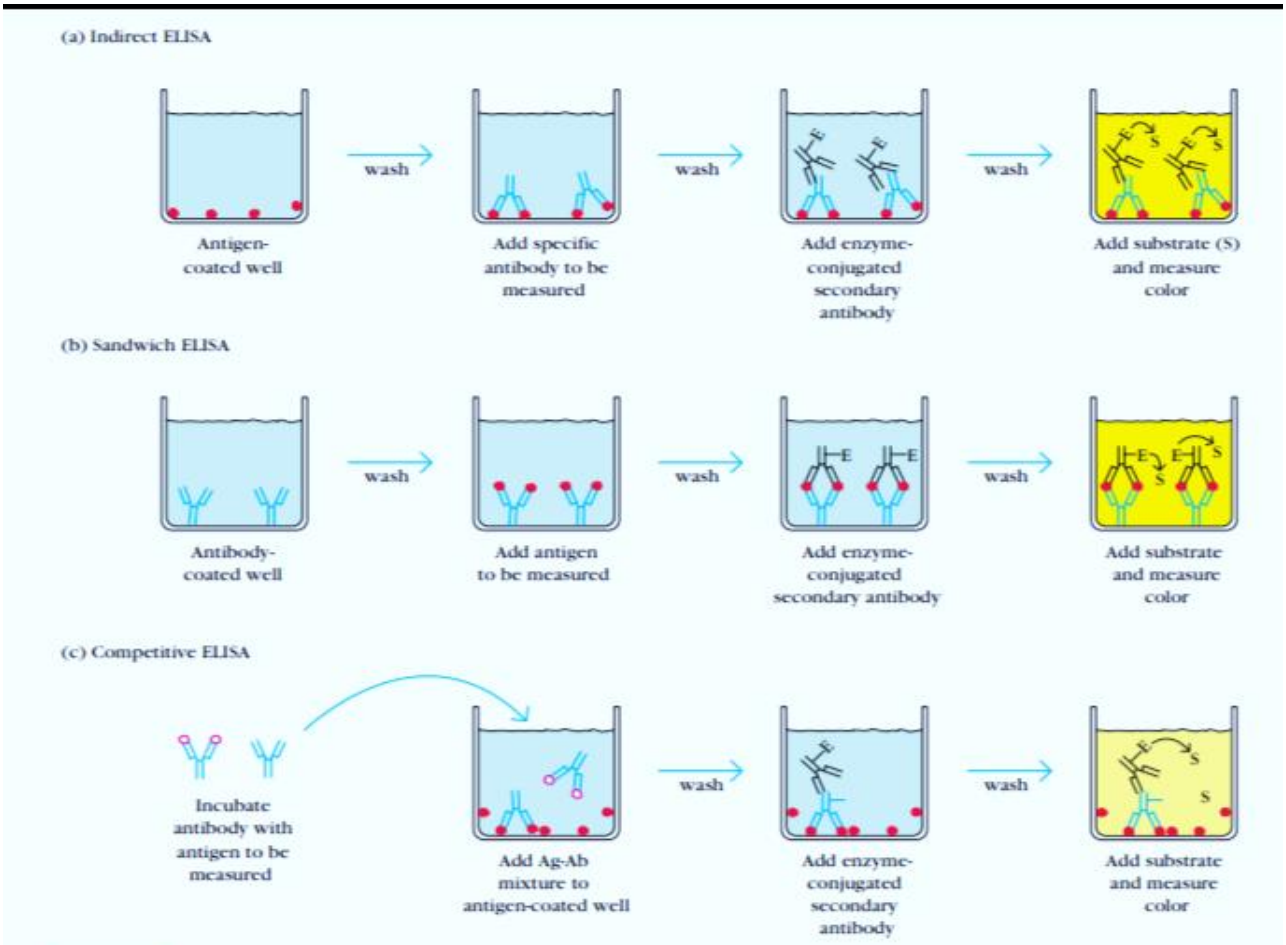


FIGURE 6-10 Variations in the enzyme-linked immunosorbent assay (ELISA) technique allow determination of antibody or antigen. Each assay can be used qualitatively, or quantitatively by comparison with standard curves prepared with known concentrations of antibody or antigen. Antibody can be determined with an indirect ELISA (a), whereas antigen can be determined with a sandwich ELISA (b) or competitive ELISA (c). In the competitive ELISA, which is an inhibition-type assay, the concentration of antigen is inversely proportional to the color produced.