The Latin term immunis, meaning "exempt," is the source of the English word immunity, meaning the state of protection from infectious disease. Perhaps the earliest written reference to the phenomenon of immunity can be traced back to Thucydides, the great historian of the Peloponnesian War. In describing a plague in Athens, he wrote in 430 BC that only those who had recovered from the plague could nurse the sick because they would not contract the disease a second time .Although early societies recognized the phenomenon of immunity, almost two thousand years passed before the concept was successfully converted into medically effective practice.

The first recorded attempts to induce immunity deliberately were performed by the Chinese and Turks in the fifteenth century. Various reports suggest that the dried crusts derived from smallpox pustules were either inhaled into the nostrils or inserted into small cuts in the skin (a technique called variolation). In 1718, Lady Mary Wortley Montagu, the wife of the British ambassador to Constantinople, observed the positive effects of variolation on the native population and had the technique performed on her own children. The method was significantly improved by the English physician Edward Jenner, in 1798. Intrigued by the fact that milkmaids who had contracted the mild disease cowpox were subsequently immune to smallpox, which is a disfiguring and often fatal disease, Jenner reasoned that introducing fluid from a cowpox pustule into people (i.e., inoculating them) might protect them from smallpox. To test this idea, he inoculated an eight-year-old boy with fluid from a cowpox pustule and later intentionally infected the child with smallpox. As predicted, the child did not develop smallpox. Jenner's technique of inoculating with cowpox to protect against smallpox spread quickly throughout Europe. However,for many reasons, including a lack of obvious disease targets and knowledge of their causes, it was nearly a hundred

years before this technique was applied to other diseases. As so often happens in science, serendipity in combination with astute observation led to the next major advance in immunology, the induction of immunity to cholera. Louis Pasteur had succeeded in growing the bacterium thought to cause fowl cholera in culture and then had shown that chickens injected with the cultured bacterium developed cholera.After returning from a summer vacation, he injected some chickens with an old culture. The chickens became ill, but, to Pasteur's surprise, they recovered. Pasteur then grew a fresh culture of the bacterium with the intention of injecting it into some fresh chickens. But, as the story goes, his supply of chickens was limited, and therefore he used the previously injected chickens.Again to his surprise, the chickens were completely protected from the disease. Pasteur hypothesized and proved that aging had weakened the virulence of the pathogen and that such an attenuated strain might be administered to protect against the disease. He called this attenuated strain a **vaccine** (from the Latin *vacca*, meaning "cow"), in honor of Jenner's work with cowpox inoculation. Pasteur extended these findings to other diseases, demonstrating that it was possible to **attenuate,** or weaken, a pathogen and administer the attenuated strain as a vaccine. In a now classic experiment at Pouilly-le-Fort in 1881, Pasteur first vaccinated one group of sheep with heat-attenuated anthrax bacillus (*Bacillus anthracis*); he then challenged the vaccinated sheep and some unvaccinated sheep with a virulent culture of the bacillus.All the vaccinated sheep lived, and all the unvaccinated animals died. These experiments marked the beginnings of the discipline of immunology. In 1885, Pasteur administered

his first vaccine to a human, a young boy who had been bitten repeatedly by a rabid dog . The boy, Joseph Meister, was inoculated with a series of attenuated rabies virus preparations. He lived and later became a custodian at the Pasteur Institute.

I - Anatomy of the Immune System

Immune (Lymphatic) System - The immune or lymphatic system consists of a complex network of specialized cells and organs designed to protect and defend the body against attacks by "foreign" invaders such as bacteria and viruses .

The lymphatic system is a component of the immune system that is responsible for the development and circulation of immune cells, specifically lymphocytes. Immune cells are produced in bone marrow. Certain types of lymphocytes migrate from bone marrow to lymphatic organs, such as the spleen and thymus, to mature into fully functioning lymphocytes. Lymphatic structures filter blood and lymph of microorganisms, cellular debris, and waste.

Lymph is an alkaline (pH > 7.0) fluid that is usually clear, transparent, and colorless. It flows in the lymphatic vessels and bathes tissues and organs in its protective covering. There are no RBCs in lymph and it has a lower protein content than blood. Like blood, it is slightly heavier than water (density = 1.019 ± .003).

The lymph flows from the interstitial fluid through lymphatic vessels up to either the thoracic duct or right lymph duct, which terminate in the subclavian veins, where lymph is mixed into the blood. (The right lymph duct drains the right sides of the thorax, neck, and head, whereas the thoracic duct drains the rest of the body.) Lymph carries lipids and lipid-soluble vitamins absorbed from the gastrointestinal (GI) tract. Since there is no active pump in the lymph system, there is no back-pressure produced. The lymphatic vessels, like veins, have one-way valves that prevent backflow. Additionally, along these vessels there are small bean-shaped lymph nodes that serve as filters of the lymphatic fluid. It is in the lymph nodes where antigen is usually presented to the immune system.

The human lymphoid system has the following:

· **primary organs (central)**: bone marrow (in the hollow center of bones) and the thymus gland (located behind the breastbone above the heart), and· **secondary organs** at or near possible portals of entry for pathogens: adenoids, tonsils, spleen (located at the upper left of the abdomen), lymph nodes (along the lymphatic vessels with concentrations in the neck, armpits, abdomen, and groin), Peyer's patches (within the intestines), and the appendix.

1. BONE MARROW

The primary point of production of the cells of the immune system, bone marrow is a substance found inside the bones primarily in the hips and thighs. Bone marrow is made up of white blood cells, red blood cells and platelets. It is the site of“B” cell development and maturation.

2. THYMUS

It 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 (Figure2.14). 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 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.

Aging is accompanied by a decline in thymic function.This decline may play some role in the decline in immune function during aging in humans and mice. The thymus reaches its maximal size at puberty and then atrophies, with a significant decrease in both cortical and medullary cells and an increase in the total fat content of the organ.Whereas the average weight of the thymus is 70 g in infants, its age-dependent involution leaves an organ with an average weight of only 3 g in the elderly (Figure).

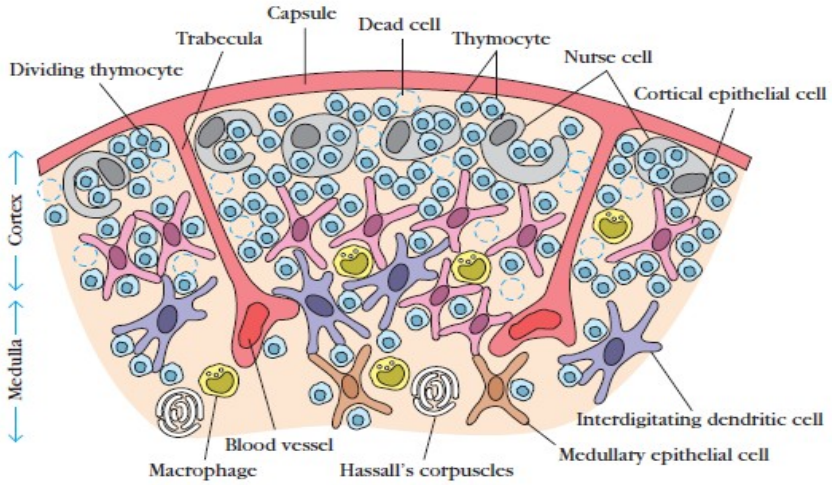

FIGURE 2-14 Diagrammatic cross section of a portion of the thymus, showing several lobules separated by connective tissue strands (trabeculae). The densely populated outer cortex is thought to contain many immature thymocytes (blue), which undergo rapid proliferation coupled with an enormous rate of 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.*]

3- LYMPH NODES

Lymph nodes are part of the lymphatic system that can be found widely distributed throughout the entire body. They are responsible for trapping foreign particles and filtering pathogens found within the body.

a) Structure: A fibrous capsule extends from outside the lymph node to the inner substance which includes the cortex and medulla to make up the lymph node.

b) Cortex: B cells arranged as follicles make up the outer cortex and the inner cortex is made up of t-cells.

c) Medulla: the medullary cords are made up of plasma, macrophages and B cells. The medullary sinuses separate the medullary cords and contain histiocytes and reticular cells. The large blood vessels, sinuses and medullary cords make up the medulla.

d) Passage of lymph: lymphatic circulation begins in the nodes and passes through the marginal sinus into the cortical sinuses. The passage of lymph continues until the lymph reaches the medullary sinuses and then exits the efferent lymphatic.

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4. SPLEEN

Located in the upper left abdominal section, the spleen is structured similar to an oversize lymph node and works as a blood filter.

a) Structure: made up of two distinct parts known as the red pulp and the white pulp, the spleen filters foreign bodies out of the blood keeping the person healthy.

b) Red pulp: this is where the filtration of red blood cells takes place removing damaged cells from the body.

c) White pulp: responsible for immune response, white pulp includes T cells and B cells which fight antigens in the blood stream for improved health.

5. MUCOSA-ASSOCIATED LYMPHOID TISSUE (MALT)

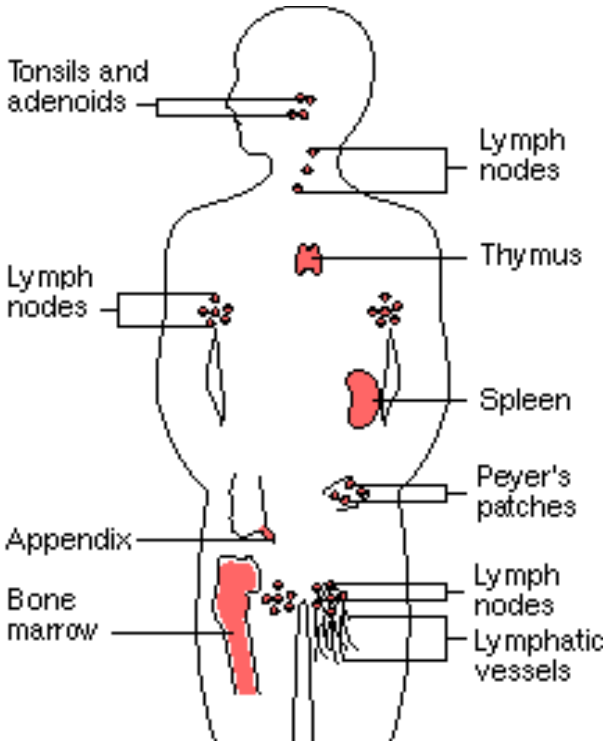
A diffusion system made up of small amounts of lymphoid tissue located in the body's mucosal linings, the mucosa-associated lymphoid tissue is the largest part of lymphatic tissue. The MALT protects the body from various antigens and has a differential naming structure which refers to various locations of the tissue within the body such as:

- Gut Associated Lymphoid Tissue
- Nasal Associated Lymphoid Tissue
- Bronchial or Tracheal-Associated Lymphoid Tissue

6. LYMPHOCYTE RECIRCULATION

The cycle in which lymphocytes circulate throughout the body, in both lymphoid and non-lymphoid tissues, to remove antigens from the body and keep the person free from disease, viruses and bacteria.

Figure - Lymphatic System



Types of Immunity

The immune system is composed of two major subdivisions, the innate or nonspecific immune system and the adaptive or specific immune system (Figure 1). The innate immune system is our first line of defense against invading organisms while the adaptive immune system acts as a second line of defense and also affords protection against re-exposure to the same pathogen. Each of the major subdivisions of the immune system has both cellular and humoral components by which they carry out their protective function (Figure 1). In addition, the innate immune system also has anatomical features that function as barriers to infection. Although these two arms of the immune system have distinct functions, there is interplay between these systems (i.e, components of the innate immune system influence the adaptive immune system and vice versa.

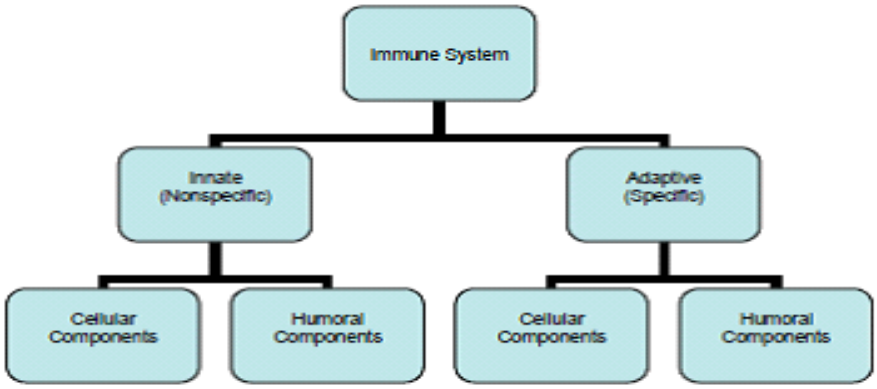


Figure 1. Overview of the Immune System

Although the innate and adaptive immune systems both function to protect against invading organisms, they differ in a number of ways. The adaptive immune system requires some time to react

to an invading organism, whereas the innate immune system includes defenses that, for the most part, are constitutively present and ready to be mobilized upon infection. Second, the adaptive immune system is antigen specific and reacts only with the organism that induced the response. In contrast, the innate system is not antigen specific and reacts equally well to a variety of organisms. Finally, the adaptive immune system demonstrates immunological memory. It "remembers" that it has encountered an invading organism and reacts more rapidly on subsequent exposure to the same organism. In contrast, the innate immune system does not demonstrate immunological memory.

All cells of the immune system have their origin in the bone marrow and they include myeloid (neutrophils, basophils, eosinpophils, macrophages and dendritic cells) and lymphoid (B lymphocyte, T lymphocyte and Natural Killer) cells (Figure 2), which differentiate along distinct pathways (Figure 2.1). The myeloid progenitor (stem) cell in the bone marrow gives rise to erythrocytes, platelets, neutrophils, monocytes/macrophages and dendritic cells whereas the lymphoid progenitor (stem) cell gives rise to the NK, T cells and B cells. For T cell development the precursor T cells must migrate to the thymus where they undergo differentiation into two distinct types of T cells, the CD4+ T helper cell and the CD8+ pre-cytotoxic T cell. Two types of T helper cells are produced in the thymus the TH1 cells, which help the CD8+ pre-cytotoxic cells to differentiate into cytotoxic T cells, and TH2 cells, which help B cells, differentiate into plasma cells, which secrete antibodies.

The main function of the immune system is self/non-self discrimination. This ability to distinguish between self and non-self is necessary to protect the organism from invading pathogens and to eliminate modified or altered cells (*e.g.* malignant cells). Since pathogens may replicate intracellularly (viruses and some bacteria and parasites) or extracellularly (most bacteria, fungi and parasites), different components of the immune system have evolved to protect against these different types of pathogens. It is important to remember that infection with an organism does not necessarily mean diseases, since the immune system in most cases will be able to eliminate the infection before disease occurs. Disease occurs only when the bolus of infection is high, when the virulence of the invading organism is great or when immunity is compromised. Although the immune system, for the most part, has beneficial effects, there can be detrimental effects as well. During inflammation, which is the response to an invading organism, there may be local discomfort and collateral damage to healthy tissue as a result of the toxic products produced by the immune response. In addition, in some cases the immune response can be directed toward self tissues resulting in autoimmune disease.

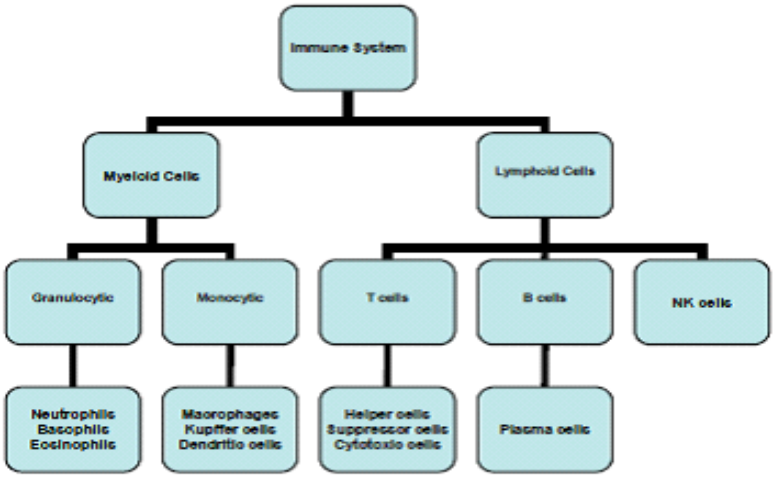


Figure 2. Cells of the Immune System

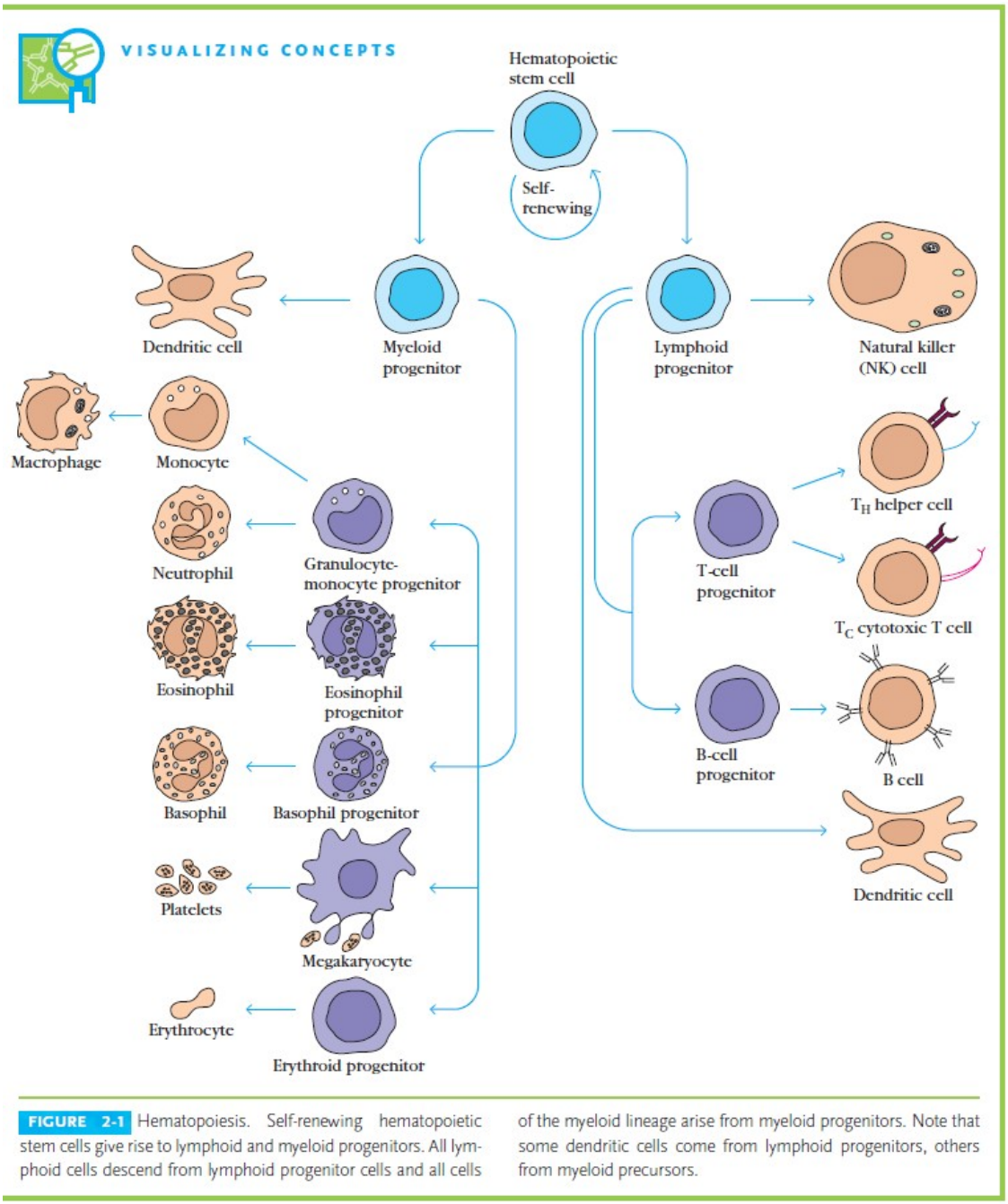


FIGURE 2-1 Hematopoiesis. Self-renewing hematopoietic stem cells give rise to lymphoid and myeloid progenitors. All lymphoid cells descend from lymphoid progenitor cells and all cells of the myeloid lineage arise from myeloid progenitors. Note that some dendritic cells come from lymphoid progenitors,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

II. INNATE HOST DEFENSES(non-specific) Innate immunity is the resistance that an individual possesses by birth. Innate immunity may be classified as (a) individual

immunity, (b) racial immunity, and (c) species immunity.

A. Anatomical barriers to infections

1. Mechanical or Physcical factors

The epithelial surfaces form a physical barrier that is very impermeable to most infectious agents. Thus, the skin acts as our first line of defense against invading organisms. The desquamation of skin epithelium also helps remove bacteria and other infectious agents that have adhered to the epithelial surfaces. Movement due to cilia or peristalsis helps to keep air passages and the gastrointestinal tract free from microorganisms. The flushing action of tears and saliva helps prevent infection of the eyes and mouth. The trapping affect of mucus that lines the respiratory and gastrointestinal tract helps protect the lungs and digestive systems from infection.

2. Chemical factors

Fatty acids in sweat inhibit the growth of bacteria. Lysozyme and phospholipase found in tears, saliva and nasal secretions can breakdown the cell wall of bacteria and destabilize bacterial membranes. The low pH of sweat and gastric secretions prevents growth of bacteria. Defensins (low molecular weight proteins) found in the lung and gastrointestinal tract have antimicrobial activity. Surfactants in the lung act as opsonins (substances that promote phagocytosis of particles by phagocytic cells).

3. Biological factors

The normal flora of the skin and in the gastrointestinal tract can prevent the colonization of pathogenic bacteria by secreting toxic substances or by competing with pathogenic bacteria for nutrients or attachment to cell surfaces.

B. Humoral barriers to infection

The anatomical barriers are very effective in preventing colonization of tissues by microorganisms. However, when there is damage to tissues the anatomical barriers are breeched and infection is occurs. Once infectious agents have penetrated tissues, another innate defense mechanism comes into play, namely acute inflammation. Humoral factors play an important role in inflammation, which is characterized by edema and the recruitment of phagocytic cells. These humoral factors are found in serum or they are formed at the site of infection.

1. **Complement system** – The complement system is the major humoral nonspecific defense mechanism . Once activated complement can lead to increased vascular permeability, recruitment of phagocytic cells, and lysis and opsonization of bacteria.

2. **Coagulation system** – Depending on the severity of the tissue injury, the coagulation system may or may not be activated. Some products of the coagulation system can contribute to the nonspecific defenses because of their ability to increase vascular permeability and act as chemotactic agents for phagocytic cells. In addition, some of the products of the coagulation system are directly antimicrobial. For example, β-lysin, a protein produced by platelets during coagulation can lyse many Gram + bacteria by acting as a cationic detergent.

3**. Lactoferrin and transferrin** – By binding iron, an essential nutrient for bacteria, these proteins limit bacterial growth.

4. **Interferons** – Interferons are proteins that can limit virus replication in cells. Interferons are secreted by an infected cell, diffuse to neighboring cells, where they stimulate the production of other proteins that inhibit synthesis of viral coat protein and viral replication. The defense is not virus specific.

5. **Lysozyme** – Lysozyme breaks down the cell wall of bacteria.

6**. Interleukin-1** – Il-1 induces fever and the production of acute phase proteins, some of which are antimicrobial because they can opsonize bacteria.

1. **interleukin 1** - (IL1) Discovered first, it stimulates cell division in T-cells when the proper combination of receptor proteins are linked between itself and the antigen presenting cell (APC) usually one of the macrophages.

interleukin 2 - (IL2) Released by Helper T-cells when stimulated by interleukin 1. This actually stimulates the T-cells to grow and divide and release a messenger - interferon gamma

interleukin 12 - appears to pack a double punch against tumors - shuts off new blood vessel growth and recruits natural killer cells (II,1B,3)

interleukin 16 - made by the CD8 lymphocyte - this lymphokine attacks CD4 (T-helper) lymphocytes (the cells infected by the aids virus. It attaches to the surface of the CD4 cell, sending a message to its nucleus to stop HIV replication.

6. **Histamin**e - speeds release of other agents both chemical and cellular and initiates the inflammatory response. Histamines (which are blocked by antihistamines) initiate the redness and swelling associated with inflammation and infection. Histamine is released by injured basophils and mast cells that are found in connective tissue. Histamine triggers local vasodilation and makes the capillaries leakier. Prostaglandins which are also released promote blood flow to the injury.

C. Cellular barriers to infection

Part of the inflammatory response is the recruitment of PMN eosinophiles and macrophages to sites of infection. These cells are the main line of defense in the nonspecific immune system.

1. Neutrophils – Polymorphonuclear cells (PMNs) are recruited to the site of infection where they phagocytose invading organisms and kill them intracellularly. In addition, PMNs contribute to collateral tissue damage that occurs during inflammation.

2. Macrophages – Tissue macrophages and newly recruited monocytes, which differentiate into macrophages, also function in phagocytosis and intracellular killing of microorganisms. In addition, macrophages are capable of extracellular killing of infected or altered self target cells. Furthermore, macrophages contribute to tissue repair and act as antigen presenting cells, which are required for the induction of specific immune responses.

3. Natural killer (NK) and lymphokine activated killer (LAK) cells – NK and LAK cells can nonspecifically kill virus infected and tumor cells. These cells are not part of the inflammatory response but they are important in nonspecific immunity to viral infections and tumor surveillance.

4. Eosinophils – Eosinophils have proteins in granules that are effective in killing certain parasites.

III. Adaptive specific host defences Adaptive immunity is also called acquired immunity, since the potency of immune response is acquired by experience only. Differences between innate and acquired immunity are summarized in Table 11-1.

A. Specific Chemical Defenses (Humoral responses - antibodies)

When a virgin B-cell encounters an appropriately displayed antigen and a secondary signal (usually found on a Helper T-cell) it is prompted to divide, giving rise to a population of effectors called plasma cells.

1. Specific antibodies secreted by plasma (B-cells) aid phagocytes. The circulating antibodies defend mainly against toxins, free bacteria, and viruses present in body fluids (humors). B-cells originate in the red bone marrow and in the fetal liver. Virgin B-cells mature to become plasma cells or memory B-cells by presenting a processed antigen to a corresponding helper T-cell which releases interleukin 1. When activated B-cells form an unmistakably extensive rough endoplasmic reticulum used to manufacture up to 2000 identical antibody proteins per second for the 4-5 day life span of these cells.

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2. Antibodies are large proteins composed of four polypeptides joined in the shape of a Y. Two chains are small and two large. All four polypeptides have constant regions that are the same for every antibody (of a class) and variable regions tailored to a specific foreign particle (antigen) Special proteins secreted by B-cells in response to and capable of combining with foreign substances are called antibodies. Also called immunoglobins (Igs). Every antibody has at least two identical sites that can reversibly bind to its antigen (epitope).

B. Specific Cellular (Tertiary) Defenses

Lymphocytes of the cell-mediated system defend against bacteria and viruses inside the host's cells, and against fungi, protozoans, and worms T-Cells . Some stem cells originating in the red bone marrow, migrate to and mature in the thymus gland to become virgin T-cells each with its own unique T-cell Receptor protein. When a Virgin T-cell encounters an antigen that it recognizes plus the appropriate secondary signal it divides to give rise to a population of effector cells.

Each T-cell is equipped with antigen-specific receptor molecules (Similar to, but not exactly antibodies) that enable it to recognize just one type of antigen fragment attached to an MHC molecule. If a T-cell finds a matching antigen on a presenting cell and if that presenting cell offers the appropriate signals, the T-lymphocyte responds in two major ways. One is to enlarge and repeatedly divide, thereby increasing the number of cells that react to the antigen. The other is to secrete lymphokines (cytokines such as interleukin), proteins that directly inhibit the pathogen or that recruit other cells to join in the immune response.

1. Helper T-cells which activate B-cells (and therefore antibody production). Helper T-cells recognize Class II MHC molecules, which are only found on macrophages and B-cells.

2. Cytotoxic T-cells that kill virus-infected cells. Cytotoxic T-cells only recognize Class I MHC molecules cradling a specific antigen. These cells are stimulated to reproduce by specific Helper T-

cells.Cytotoxic T-cells by recognizing specific antigens in association with class I MHC molecules, can bind to any cell of the body infected by that particular antigenic invader. If docking is successful the cytotoxic T-cell releases a protein called perforin which creates lesions in the infected cell's membrane leading to the cells lysis - spilling out the invader and other chemicals which quickly attract other lymphocytes and macrophages.

3. Suppresser T-cells which somehow suppresses the positive feedback characteristic of the immune response.

4. Memory T-cells act as a "reserve army"

TABLE 11-1 Differences between innate and acquired immunity

Feature	Innate immunity	Acquired immunity
Definition	The resistance to infection that an individual possesses by virtue of genetic and constitutional makeup	The resistance that an individual acquires during life
Types	Nonspecific and specific	Active and passive
Time taken to develop	Hours	Days
Specificity	For structures shared by groups of related microbes	For antigens of microbes and for nonmicrobial antigens
Memory	None; repeated exposure brings response like primary response	Yes; secondary response much faster than primary response
Components		
Physical and chemical barriers	Skin, mucosal epithelia, and antimicrobial chemicals	Lymphocytes in epithelia and antibodies secreted at epithelial surfaces
Blood and tissue antimicrobial substances	Complement; leukins from leukocytes, plakins from platelets, lactic acid found in muscle tissue, lactoperoxidase in milk, and interferons (antiviral)	Antibodies
Cells	Phagocytes (macrophages and neutrophils) and natural killer cells	Lymphocytes

PHAGOCYTOSIS AND INTRACELLULAR KILLING

A. Phagocytic cells

1. Neutrophiles/Polymorphonuclear cells (PMNs) - PMNs are motile phagocytic cells that have lobed nuclei. They can be identified by their characteristic nucleus or by an antigen present on the cell surface called CD66. They contain two kinds of granules the contents of which are involved in the antimicrobial properties of these cells. The primary or azurophilic granules, which are abundant in young newly formed PMNs, contain cationic proteins and defensins that can kill bacteria, proteolytic enzymes like elastase, and cathepsin G to breakdown proteins, lysozyme to break down bacterial cell walls, and characteristically, myeloperoxidase, which is involved in the generation of bacteriocidal compounds. The second type of granule found in more mature PMNs is the secondary or specific granule. These contain lysozyme, NADPH oxidase components, which are involved in

the generation of toxic oxygen products, and characteristically lactoferrin, an iron chelating protein and B12-binding protein.

2. Monocytes/Macrophages - Macrophages are phagocytic cells that have a characteristic kidney-shaped nucleus. They can be identified morphologically or by the presence of the CD14 cell surface marker. Unlike PMNs they do not contain granules but they have numerous lysosomes which have contents similar to the PNM granules.

Phagocytic cells have a variety of receptors on their cell membranes through which infectious agents bind to the cells. These include**:**

1. Fc receptors – Bacteria with IgG antibody on their surface have the Fc region exposed and this part of the Ig molecule can bind to the receptor on phagocytes. Binding to the Fc receptor requires prior interaction of the antibody with an antigen. Binding of IgG-coated bacteria to Fc receptors results in enhanced phagocytosis and activation of **the metabolic activity of phagocytes (respiratory burst).**

2. Complement receptors – Phagocytic cells have a receptor for the 3rd component of complement, C3b. Binding of C3b-coated bacteria to this receptor also results in enhanced phagocytosis and stimulation of the respiratory burst.

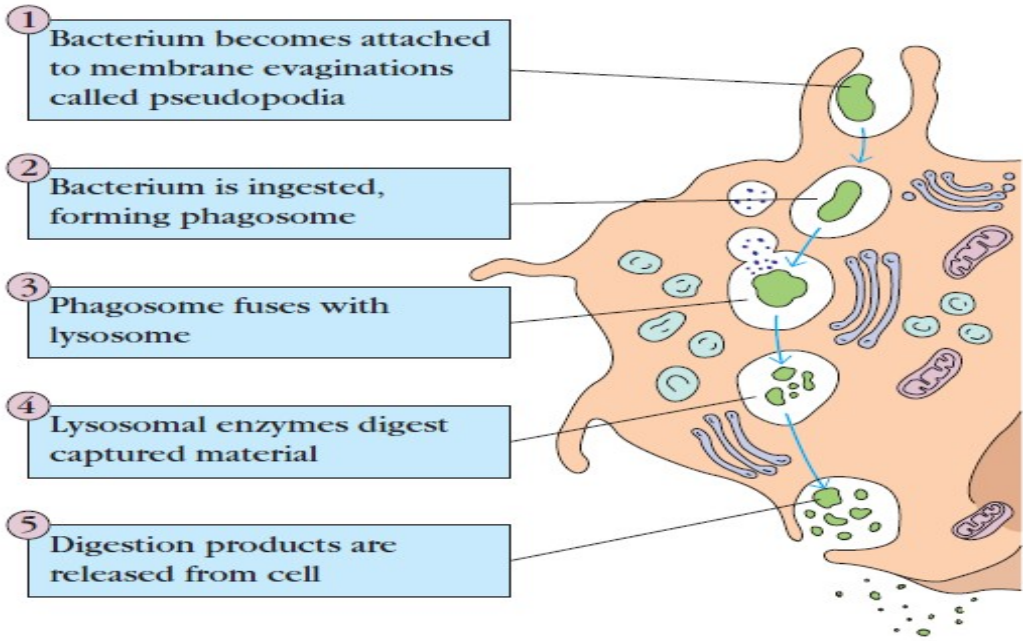
3. Scavenger receptors – Scavenger receptors bind a wide variety of polyanions on bacterial surfaces resulting in phagocytosis of bacteria.

4. Toll-like receptors – Phagocytes have a variety of Toll-like receptors (Pattern Recognition Receptors or PRRs) which recognize broad molecular patterns called PAMPs (pathogen associated molecular patterns) on infectious agents. Binding of infectious agents via Toll-like receptors results in phagocytosis and the release of inflammatory cytokines (IL-1, TNF-α and IL-6) by the phagocytes.

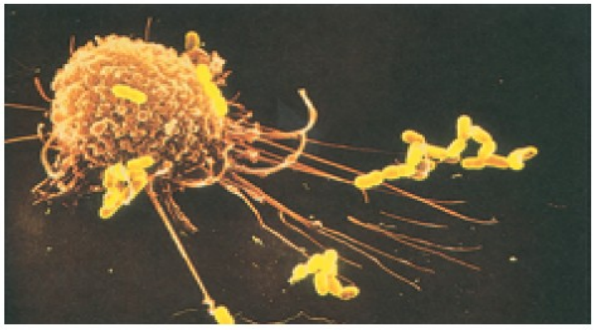
Macrophages are capable of ingesting and digesting exogenous antigens, such as whole microorganisms and insoluble particles, and endogenous matter, such as injured or dead host cells, cellular debris, and activated clotting factors. In the

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. 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 (Figure 2-9a). Fusion of the pseudopodia encloses the material within a membrane-bounded structure called a **phagosome,** which then enters the endocytic processing pathway (Figure 2-9b). 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. The digested contents of the phagolysosome are then eliminated in a process called **exocytosis** (see Figure 2-9b). 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.**



(a)



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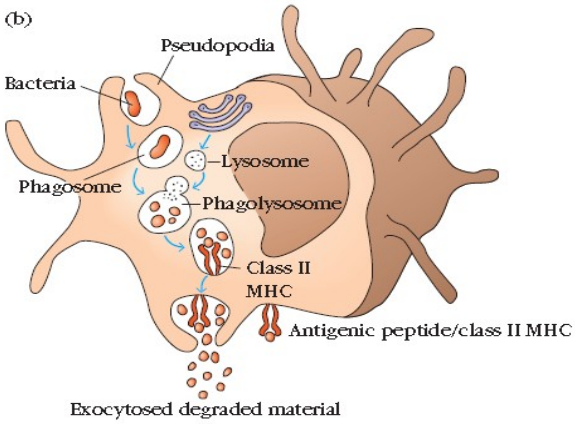


FIGURE 2-9 Macrophages can ingest and degrade particulate antigens, including bacteria. (a) Scanning electron micrograph of a macrophage. Note the long pseudopodia extending toward and making contact with bacterial cells, an early step in phagocytosis. (b) Phagocytosis and processing of exogenous antigen by macrophages. Most of the products resulting from digestion of ingested material are exocytosed, but some peptide products may interact with class II MHC molecules, forming complexes that move to the cell surface, where they are presented to T_H cells. [*Photograph by L. Nilsson, © Boehringer Ingelheim International GmbH.*]

ANTIMICROBIAL AND CYTOTOXIC ACTIVITIES

A number of antimicrobial and cytotoxic substances produced by activated macrophages can destroy phagocytosed microorganisms (Table 2-6).Many of the mediators of cytotoxicity listed in Table 2-6 are reactive forms of oxygen.

OXYGEN-DEPENDENT KILLING MECHANISMS

Activated phagocytes produce a number of reactive oxygen intermediates (ROIs) and reactive nitrogen intermediates that have potent antimicrobial activity. During phagocytosis, a metabolic process known as the respiratory burst occurs in activated macrophages. This process results in the activation of a membrane-bound oxidase that catalyzes the reduction of oxygen to superoxide anion, a reactive oxygen intermediate that is extremely toxic to ingested microorganisms. The superoxide anion also generates other powerful oxidizing agents, including hydroxyl radicals and hydrogen peroxide. As the lysosome fuses with the phagosome, the activity of myeloperoxidase produces hypochlorite from hydrogen per- oxide and chloride ions. Hypochlorite, the active agent of household bleach, is toxic to ingested microbes. When macrophages are activated with bacterial cell-wall components such as lipopolysaccharide (LPS) or, in the case of mycobacteria, muramyl dipeptide (MDP), together with a T-cell–derived cytokine (IFN-_), they begin to express high levels of nitric oxide synthetase (NOS), an enzyme that oxidizesL-arginine to yield L-citrulline and nitric oxide (NO), a gas:

L-arginine _ O2 _NADPH □→ NO _ L-citrulline _ NADP

Nitric oxide has potent antimicrobial activity; it also can combine with the superoxide anion to yield even more potent antimicrobial substances. Recent evidence suggests that much of the antimicrobial activity of macrophages against bacteria, fungi, parasitic worms, and protozoa is due to nitric oxide and substances derived from it .

OXYGEN-INDEPENDENT KILLING MECHANISMS Activated macrophages also synthesize lysozyme and various hydrolytic enzymes whose degradative activities do not require oxygen. In addition, activated macrophages produce a group of antimicrobial and cytotoxic peptides, commonly known as defensins.

TABLE 2-6 Mediators of antimicrobial and cytotoxic activity of macrophages and neutrophils

Oxygen-dependent killing	Oxygen-independent killing
Reactive oxygen intermediates	Defensins
$O^{\bullet}{}_2^-$ (superoxide anion)	Tumor necrosis factor α
$OH^{\bullet}$ (hydroxyl radicals)	(macrophage only)
H_2O_2 (hydrogen peroxide)	Lysozyme
ClO^- (hypochlorite anion)	Hydrolytic enzymes
Reactive nitrogen intermediates	
NO (nitric oxide)	
NO_2 (nitrogen dioxide)	
HNO_2 (nitrous acid)	
Others	
NH_2CL (monochloramine)	

3rd year : Pathological analysis / Lec. 4- Complement system

The term complement refers to the ability of a system of some nonspecific proteins in normal human serum to complement, i.e., augment the effects of other components of immune system, such as antibody. The complement system, which is an important component of the human innate host defense system, consists of approximately 20 proteins that are present in normal human serum(inactive state). When the complement components are activated, serial, rapid cascad events occur. Historically, the term complement (C) was used to a heat-labile serum component able to lyse bacteria and its activity is destroyed (or inactivated) by heating serum at 56 C^{o} for 30 min. Complement glycoproteins are synthesized by liver cells (hepatocytes) and macrophages and many other cell (e.g. gut epithelial cells). All normal individuals have complement components in their blood. The synthetic rates for the complement glycoproteins increase when complement is activated and consumed.

Complement activation takes place through any of the following three pathways:

1. The classical pathway
2. The alternative pathway
3. The lectin pathway

Of these, alternative and lectin pathways are important in the innate immunity of the host. These two are also more important when the human host is infected by a microorganism for the first time, because the antibody required to trigger the classical pathway is not present. All the three activation pathways lead to activation of C3, resulting in the production of C3b. Hence, C3b is considered as the central molecule in the activation of the complement cascade.

Classical Pathway:

C1activation C1, a multi-subunit protein containing three different proteins (C1q, C1r and C1s), binds to the Fc region of IgG and IgM antibody molecules that have interacted with antigen (it does not bind to free Ab) , binding requires calcium and magnesium ions. The binding of C1q results in the activation of C1r which in turn activates C1s. The result is the formation of an activated "C1qrs", which is an enzyme that cleaves C4 into two fragments C4a and C4b.

- **C4 and C2 activation** (generation of C3 convertase). The C4b fragment will stay (usually binds to the binds to the membrane of bacteria) and the C4a fragment is released. Activated "C1qrs" also cleaves C2 into C2a and C2b. C2a binds to the membrane in association with C4b, and C2b is released. The resulting C4bC2a complex is a C3 convertase (acts as enzyme), which cleaves C3 into C3a and C3b.

C3 activation (generation of C5 convertase):

C3b binds to the membrane in association with C4b and C2a, and C3a is released (which acts as anaphylaxis protein and a chemotactic factor). The resulting C4bC2aC3b is a C5 convertase. The generation of C5 convertase is the end of the classical pathway. Many products of the classical pathway have biological activities that support the host defenses:

Biological Activity of classical pathway products	
Biological Activity	**Component**
Prokinin; have role in kinin system, causes edema	C2b
Anaphylotoxin; can activate basophils and mast cells to degranulate resulting in increased vascular permeability and contraction of smooth muscle cells, which may lead to **anaphylaxis**	C3a
Opsonin; induces phagocytosis by binding to complement receptors. Activation of phagocytic cells	C3b
Anaphylotoxin (weaker than C3a)	C4a
Opsonin; induces phagocytosis by binding to complement receptors	C4b

B. Lectin Pathway

The lectin pathway is very similar to the classical pathway. It starts with the binding of mannose-binding lectin (MBL) to bacterial surfaces with mannose-containing polysaccharides (Mannans). Many serial events occur resulting C4bC2aC3b formation, which is the C5 convertase. The generation of C5 convertase is the end of the lectin pathway.

C.Alternative Pathway

Activation of this pathway starts spontaneously and C3 will be cleaved by the help of Factor B, Factor D, properdin and Mg^{+2} ions. Cleavage of C3will release C3a (which acts as anaphylaxis protein) and C3b. when C3b is formed, Factor B will bind to it and will be cleaved by Factor D. The resulting C3bBb complex is a C5 convertase and this is the end of the alternative pathway. generation of C3b is essential for the activation of the alternative pathway.

The component C3b can be generated:

-During normal C3 turnover in blood

-In the presence of bacterial proteases

During classical pathway activation (for this reason activation of the classical pathway is always associated with activation of the alternative pathway which generating more activated C3).

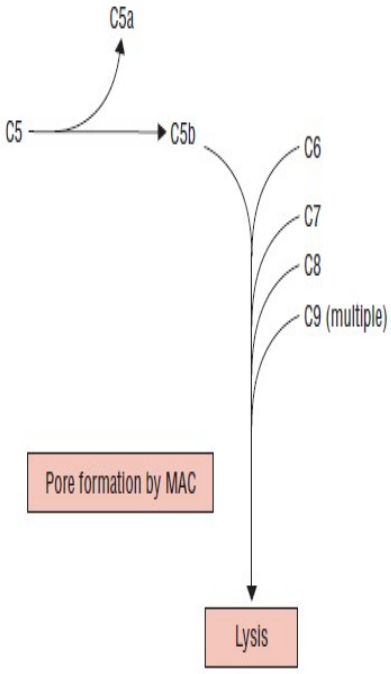
The alternative pathway of complement activation is important especially during the early phase of an infection, when the concentrations of specific antibody are very low and classical pathway activation is limited. Also, in the presence of large numbers of bacteria, during low specific Ab concentration and many bacteria escape from distraction.

The alternative pathway can be activated by many Gram-negative, some Gram-positive bacteria, viruses and parasites, and results of lysing of these organisms. The alternative pathway provides the non-specific resistance against infection without the participation of antibodies and hence provides a first line of defense against a number of infectious agents.

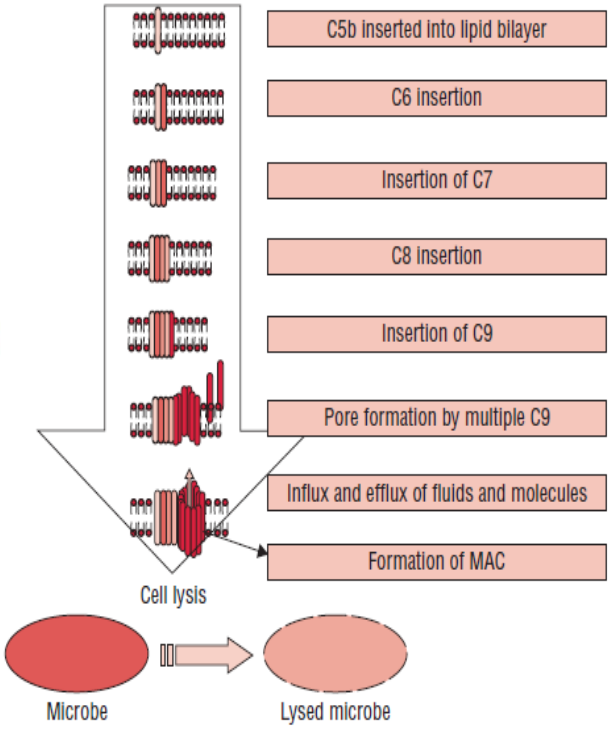
D. Membrane attack complex Formation:

Lytic pathway is the end of all the complement system pathways, C5 convertase from all pathways (classical, lectin or alternative) cleaves C5 into C5a and C5b. C5a has a special role and the C5b rapidly associates (bind) with C6 and C7 and inserts into the membrane. Then C8 binds, followed by several molecules of C9. The C9 molecules form a pore in the membrane and lysis occurs due to physical damage to the membrane. The complex of C5bC6C7C8C9 is the membrane attack complex (MAC).

C5a formed in the lytic pathway has many biological activities. It is the most active anaphylotoxin. It is a chemotactic factor for neutrophils and stimulates the respiratory burst in them and it stimulates inflammatory cytokine production by macrophages.



15-2. Formation of membrane attack complex.



IG. 15-3. Action of membrane attack complex.

TABLE 15-1 Comparison of classical, alternative, and lectin pathways

Classical pathway	Alternative pathway	Lectin pathway
Chain of events in which components react in specific sequence following activation of C1	Activation of C3 without prior participation of C1,4,2	Activated by binding of mannose-binding lectin to mannose residues on surface of microorganisms
Requires binding of C1 to antigen–antibody complex	Activators are bacterial endotoxins, IgA and IgD, cobra venom factor, and nephritic factor	No role for antibodies; similar to alternate pathway
Cannot be considered as a component of innate immune mechanism	It is a component of the innate immune mechanism	Can be considered as a component of innate immune mechanism

Function of the complement system:

The complement system takes part in both specific and non-specific resistance and generates a number of products of biological and immunological importance. The functions of the complement system are:

- Binding and neutralizing foreign substances that activate it.

-Induce the ingestion of complement-coated substances by phagocytic cells (help in the opsonization process when C3b and C4b linked with the surface of microorganisms and attach to Complement receptor on phagocytic cells then induce phagocytosis).

-Activation of many cells including polymorphonuclear cells (PMNs) and macrophages.

-Have roles in regulation of antibody responses.

-Clearance of immune complexes and apoptotic cells.

- Have roles in inflammation and tissue damage.

- Some components (C3a, C4a and C5a), have role in Anaphylaxis (a dangerous case of type I hypersensitivity), hence they are called anaphylotoxins.

- Some complement componants acts as chemotactic facters e.g. C5a and MAC.

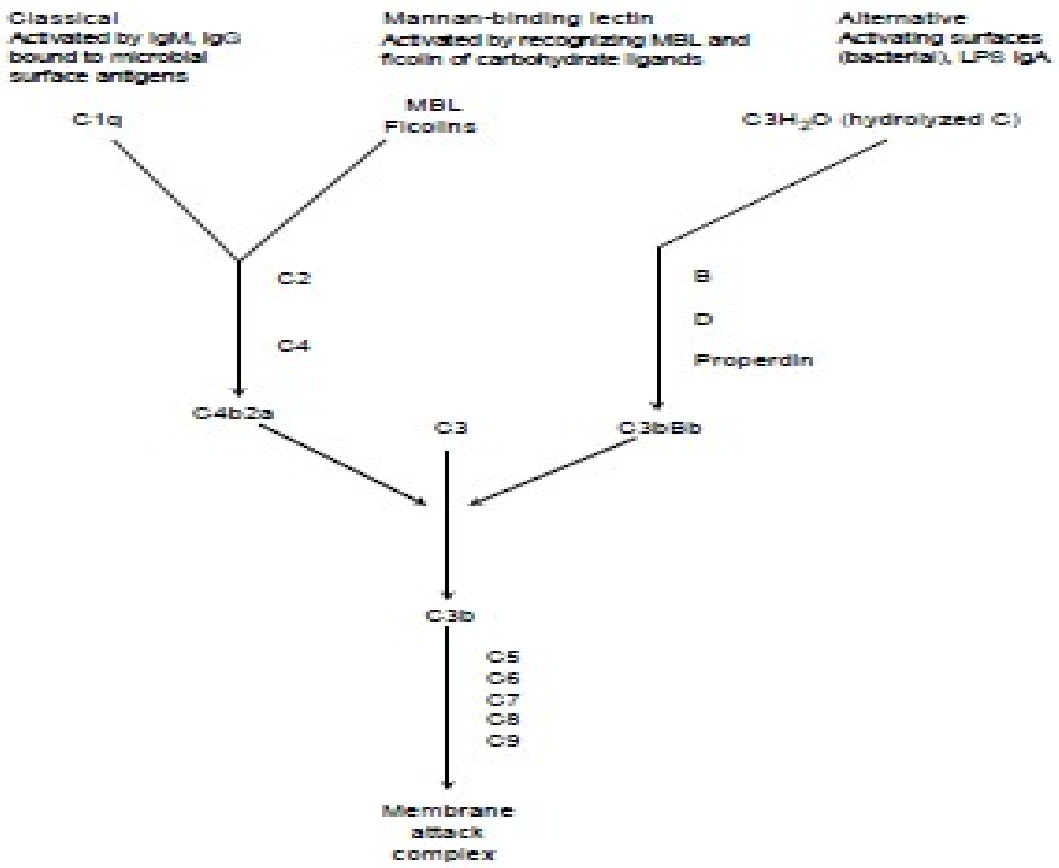


FIGURE 1.1 The three pathways of complement activation. (Modified from Seeleen, M., Roos, A., Daha, M.R., *J. Nephrol.*, 18, 642–653.)

Complement deficiencies and diseases

Mechanism	Disease	Component
Opsonization of immune complexes help keep them soluble, deficiency results in increased precipitation in tissues and inflammation	SLE	C1, C2, C4
Not enough opsonization of bacteria	Susceptibility to pyogenic (pus-forming) bacterial infections	Factors B or D
Lack of opsonization and inability to utilize the membrane attack pathway	Susceptibility to bacterial infections	C3
Inability to attack the outer membrane of Gram-negative bacteria	Susceptibility to Gram-negative infections	C5, C6, C7 C8, and C9

Lec.4 part-2 : Inflammatory Response

Tissue damage caused by a wound or by an invading pathogenic microorganism induces a complex sequence of events collectively known as the **inflammatory response**, a molecular component of a microbe, such as LPS, may trigger an inflammatory response via interaction with cell surface receptors. The end result of inflammation may be the marshalling of a specific immune response to the invasion or clearance of the invader by components of the innate immune system. Many of the classic features of the inflammatory response were described as early as 1600 BC, in Egyptian papyrus writings. 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):

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 a 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. The events in the inflammatory response are initiated by a complex series of events involving a variety of chemical mediators whose interactions are only partly understood. Some of these mediators are derived from invading microorganisms, some are released from damaged cells in response to tissue injury, some are generated by several plasma enzyme systems, and some are products of various white blood cells participating in the inflammatory response. Among the chemical mediators released in response to tissue damage are various serum proteins called **acute-phase proteins.** The concentrations of these proteins increase dramatically in tissue-damaging infections. C-reactive protein is a major acute-phase protein produced by the liver in response to tissue damage. Its name derives from its patternrecognition activity: C-reactive protein binds to the C-polysaccharide cell-wall component found on a variety of bacteria and fungi. This binding activates the complement system, resulting in increased clearance of the pathogen either by complement-mediated lysis or by a complementmediated increase in phagocytosis. One of the principal mediators of the inflammatory

response is **histamine,** a chemical released by a variety of cells in response to tissue injury. Histamine binds to receptors on nearby capillaries and venules, causing vasodilation and increased permeability. Another important group of inflammatory mediators, small peptides called **kinins,** are normally present in blood plasma in an inactive form. Tissue injury activates these peptides, which then cause vasodilation and in-creased permeability of capillaries. A particular kinin, called bradykinin, also stimulates pain receptors in the skin. This effect probably serves a protective role, because pain normally causes an individual to protect the injured area. Vasodilation and the increase in capillary permeability in an injured tissue also enable enzymes of the blood-clotting system to enter the tissue. These enzymes activate an enzyme cascade that results in the deposition of insoluble strands of **fibrin,** which is the main component of a blood clot. The fibrin strands wall off the injured area from the rest of the body and serve to prevent the spread of infection . Once the inflammatory response has subsided and most of the debris has been cleared away by phagocytic cells, tissue repair and regeneration of new tissue begins. Capillaries grow into the fibrin of a blood clot. New connective tissue cells, called fibroblasts, replace the fibrin as the clot dissolves. As fibroblasts and capillaries accumulate, scar tissue forms.

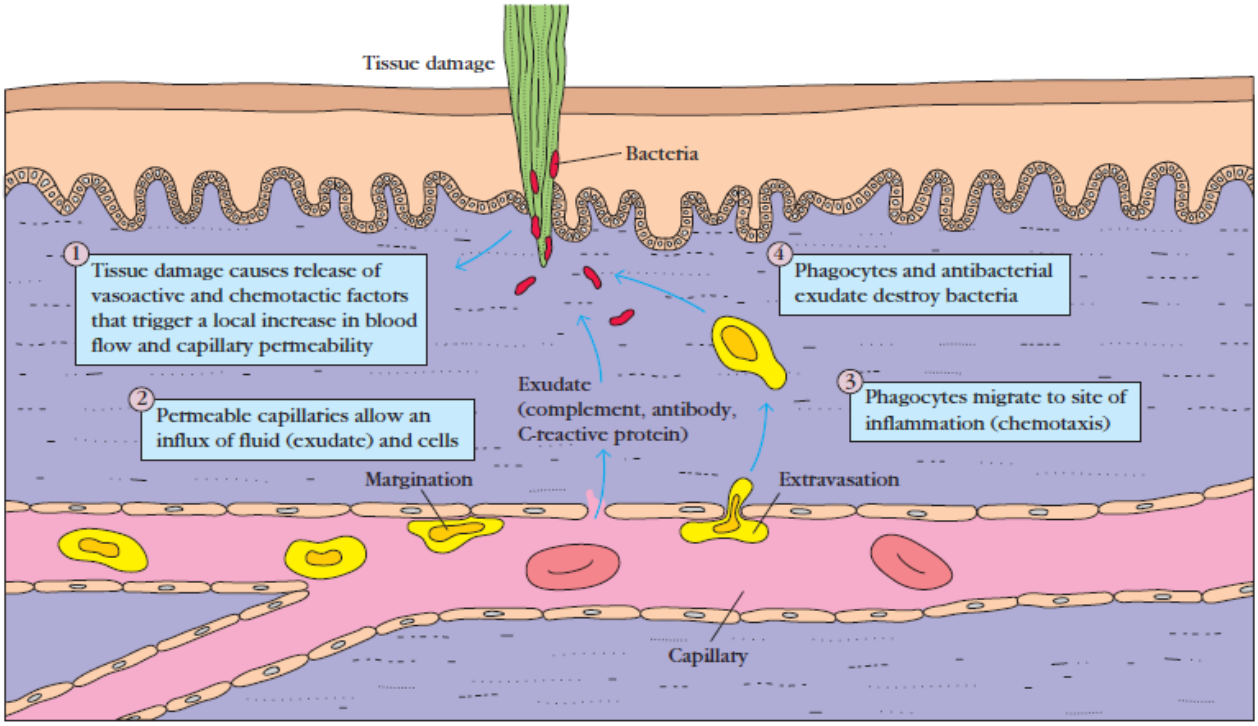


FIGURE 1-4 Major events in the inflammatory response. A bacterial infection causes tissue damage with release of various vasoactive and chemotactic factors. These factors induce increased blood flow to the area, increased capillary permeability, and an influx of white blood cells, including phagocytes 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.

3rd year : Pathological analysis : Lecture (5) : Antigens

Antigen is any substance capable of provoking the immune system of an animal or a person to respond by generating an immune reaction, specifically directed at the inducing substances and not

at other unrelated substances So it is any agent capable of binding specifically to components of immune response such as lymphocytes and antibodies.

Immunogen is any agent capable of inducing an immune response. Although all molecules that have the property of immunogenicity also have the property of antigenicity**, but not all antigens need to be immunogens**. This difference become obvious in the case of low molecular weight compounds, a group of substances includes many antibiotics and drugs.

Haptens are low molecular weight compounds that can combine with antibody but cannot initiate an immune response unless it is coupled to a larger carrier molecule. For example, nickel is a substance of small molecular weight which is incapable of provoking an immune response in its own right. **Nickel allergy**, however, is a common cause of contact dermatitis. This results when **nickel** combines with protein in the patient's skin. The nickel-protein complex is recognized as foreign and an immune response is mounted.

Requirements for immunogenicity

A substance must possess the following three characteristics to be immunogen.

1- Foreignness

2- High molecular weight

3- Chemical complexity.

Parameter	Increase immunogenicity	Decreased immunogenicity
Size	Large	Small (MW<2500)
Composition	Complex	Simple
Similarity to self protein	Multiple differences	Few differences
Interaction with host MHC	Effective	Ineffective

Table 1: Intrinsic properties of proteins that affect immunogenicity.

Foreignness

The immune system of an individual can normally distinguish between body components, i.e. 'self' and foreign substances, 'non-self'. Normally, the body is tolerant to its own components, and does not initiate an immune response against these.

High molecular weight

Small molecules such as amino acids or monosaccharides are usually not antigenic. As a rule, molecules with a molecular weight of less than 10,000 have no or only weal antigenicity. However, as mentioned above, if coupled to a suitable carrier moleculesuch as a protein, low molecular weight substance (haptens) can exhibit antigenicity.

Chemical complexity

The configuration and complexity of the molecule are important. Linear polypeptides and globular proteins are both capable of inducing an immune response. Antibody that is formed to these different

structures is highly specific and when the conformation of an antigen is changed the antibody induced by the original form no longer combines with it. For example, it is possible for an individual to produce and immune response to raw egg antigens, but when the egg is boiled the antigenic configuration is changed and no immune response is mounted. The need for complexity means that molecules containing a repeating unit of only one amino acid are generally poor antigens, even if the molecule is large.

Further requirements for antigenicity : In addition to the above characteristics several other factors play roles in the determining whether a substance is immunogenic. These include the susceptibility of the substance to enzymatic degradation and the genetic make up of the host.

Parameter	Increased immunogenicity	Decreased immunogenicity
Dose	Intermediate	High or low
Route	Subcutaneous > intraperitoneal > intravenous or intragastric	
Form	Particulate	Soluble
	Denatured	Native
Adjuvants	Slow release	Rapid release
	Bacteria	No bacteria

Table 2: Factors that influence the adapted immuno response to an antigen.

To activate T cell, the substance must be susceptible to partial enzymatic degradation that takes place during antigen processing and presentation by antigen presenting cells such as macrophage.

Genetic Constitution of the Host Two members of the same species of animals

may respond differently to the same antigen, because of a different composition of immune response genes. The **method of administration** and the **dose** are also important. The immune response to a substance can be enhanced by administering an **adjuvant** together with the antigen, while a state of immunological unresponsiveness can result if a very high or low doses of certain antigens are administered .

Antigenic determinants (epitopes)

Despite the fact that potent antigens are relatively large molecules, only limited parts of the molecule are involved in the binding to antibodies. These parts are called antigenic determinants or epitopes. A molecule must have at least two antigenic determinants in order to stimulate antibody production. **Epitopes recognized** by B cells and T cells .

B cells with their membrane-bound antibody, which serve as epitope receptors, recognize and bind **free antigen in solution.** Thus, the epitopes on the antigen must be on the 'outside' of the molecule, accessible for interaction with the receptor. On the other hand, the interaction of epitope with the T-cell receptor requires prior 'processing' of the antigen, and the association of an area of the processed antigen with MHC molecules present on the surface of the antigen-presenting cell. Generally such 'processed' epitopes are internal denatured proteins. Polysaccharides contain solely

B-cell recognizable epitopes, Figure 7. With respect to their epitopes, antigens may have the characteristics shown schematically in Figure 7.

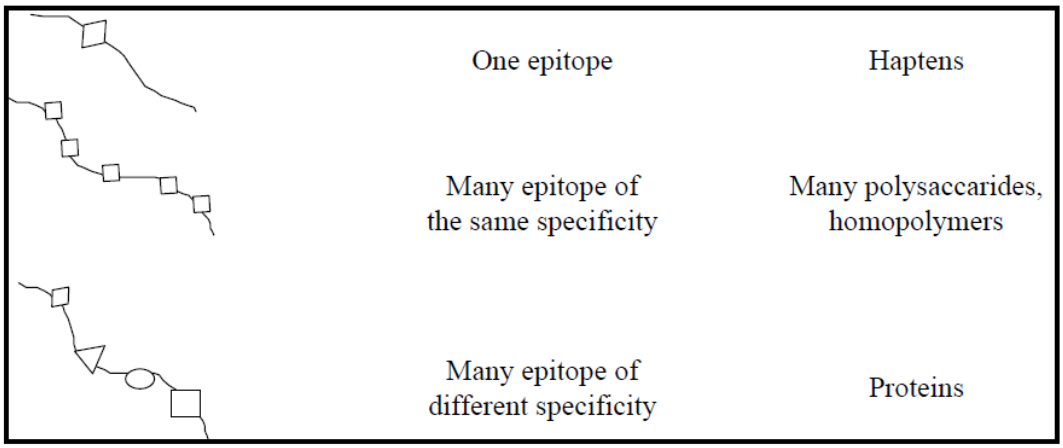


Figure 7: Representation of some possible antigenic structures.

Thus, they may consist of a single epitope (hapten) or have varying numbers of the same epitope on the same molecule (polysaccharides). The most common antigens (proteins) have varying numbers of different epitopes on the same molecule.

Major classes of antigens

The following major chemical families may be antigenic:

• **Carbohydrates (polysaccharides)**: polysaccharides are potentially, but not always, immunogenic. Antibodies, can be induced against many kinds of polysaccharide molecules, such as components of microorganisms. An excellent example of antigenicity of polysaccharides is the immune response associated with the ABO blood groups, which are polysaccharide on the surface of the red blood cells.

Lipids: lipids are rarely immunogenic, but an immune response to lipids may be induced if the lipids are conjugated to protein carriers.

• **Nucleic acids**: nucleic acids are poor immunogens by themselves, but they become immunogenic when they are conjugated to protein carriers.

• **Proteins**: virtually all proteins are immunogenic. Thus, the most common immune responses are those to proteins. Furthermore, the greater the degree of complexity of the protein, the more vigorous will be the immune response to that protein. In general, proteins are multi-determinant antigens.

Immunological adjuvants

An adjuvant is a substance that, when mixed with an immunogen, enhances the immune response against the immunogen.

Adjuvant name	Composition	Mechanism of action
Incomplete Freund's adjuvants	Oil in water emulsion	Delayed release of antigen; enhanced uptake by macrophage
Complete Freund's adjuvants	Oil in water emulsion with dead mycobacteria	Delayed release of antigen; enhanced uptake and induction of co-stimulators in macrophages

Table 3: Commonly used adjuvants.

It is important to distinguish between a carrier for a hapten and an adjuvant. **A hapten** will become immunogenic when conjugated covalently to a carrier; it will not become immunogenic if mixed with an adjuvant. Thus, an adjuvant enhances the immune response to immunogens but does not confer immunogenicity to haptens. The most widely used adjuvant in humans is alum precipitate, a suspension of aluminum hydroxide on which the antigen is absorbed. This adjuvant causes aggregation of a soluble antigen and allows continuous slow release of antigen. In addition, it has a slight irritant effect that enhances the ingestion and processing of an antigen by macrophages which present the antigen to T cells, leading to T-cell activation.

Major Histocompatibility MHC: They are genes **encoding** for proteins found on the surface of all human and animals cells. They have an important role in giving the identity of the cell and in the communication with the immune system cells and regulation of immune response:

MHC class I: found on the surface of all nucleated cells, have role in tissues and organs transplantation.

MHC class II: found on the surface of all immune cells especially APC.

MHC class III: found on the surface of certain cells for some complement components receptors.

Processing and Presentation of Antigens

For recognition of a foreign protein antigen by a T cell it is degraded into antigenic peptides by sequence of events within the cell. The degraded peptides then form complexes with class I or class II MHC molecules in different compartments within the cytoplasm and are then transported to the surface membrane of the cell where they are displayed. This is called antigen processing and **presentation**.

1. Exogenous antigen is produced outside the host such as in bacteria and is internalized by antigen presenting cells by endocytosis or phagocytosis. Antigen presenting cells (APC) are macrophages, dendritic cells, Langerhans cells and B cells. Within APC the antigen is degraded into peptides which is complexed with class II MHC molecule and is displayed qn the cell surface, and is recognized by CD4 T cell receptor (TCR). Processing occurs in endocytic pathway (class II MHC pathway).

2. Endogenous antigen is produced within the cell itself (e.g. virus-infected cell) of the host. The virus is degraded into peptide within the cell cytosole. The peptide is complexed with class I MHC molecule and is displayed on the cell surface, and is recognized by CD8 T cell receptor (TCR). Processing occurs in cytosolic pathway (class I MHC pathway) .

3rd year : Immunology : Lecture (6)

Antibodies are globulin proteins (immunoglobulins) that are synthesized in serum and tissue fluids, which react specifically with the antigen that stimulated their production. Three types of globulins are present in the blood: alpha, beta, and gamma. The antibodies are the gamma globulins. Antibodies are one of the major plasma proteins, and against infection often referred to as "first line of defense". The most important function of antibodies is to confer protection against microbial pathogens. Antibodies confer protection in the following ways:

1. They prevent attachment of microbes to mucosal surfaces of the host.
2. They reduce virulence of microbes by neutralizing toxins and viruses.
3. They facilitate phagocytosis by opsonization of microbes.
4. They activate complement, leading to complement-mediated activities against microbes.

Immunoglobulins are proteins of animal origin, endowed with known antibody activity and for certain other proteins related to them by chemical structure. That means the Ig include, besides antibody globulin, the abnormal proteins found in myeloma, macroglobulinemia, cryoglobulinemia, etc. While Ig satisfies the structural and chemical concept, the antibody provides biological and functional concept. All antibodies are Ig, but all Ig are not antibodies. Immunoglobulins constitute 20% to 25% of the serum protein. Based on the physicochemical, antigenic differences and the types of heavy chain Igs are classified into five types. All Igs are made up of light (molecular weight 25,000) and heavy polypeptide chains (molecular weight 50,000). Light (L) chains are of one of the two, kappa (K) or lambda (l). Both types can occur in all classes of Ig (IgG, IgM, IgA, IgE and IgD), but any one Ig contains only one type of L chain. Both the L chain of one Ig molecule cannot have both kappa and lambda chain. The amino-terminal portion of each L chain contains a part of antigen-binding site. Heavy (H) chains are distinct for each of the five Ig classes and are designated γ (gammammunoglobulins μ (mu), α (alpha), δ (delta) and e (epsilon).

The amino-terminal portion of each H chain participates in the antigen-binding site. The carboxy-terminal portion forms the fraction crystallizable (Fc) fragment, which has various biologic activities (complement activation, macrophage fixation, reactivity with rheumatoid factor and binding to cell-surface receptors). An individual antibody molecule consists of two H chains and L chains, covalently linked by disulfide bonds. Both the H chains and L chains are identical. Proteolytic cleavage of IgG by Porter, Edelman and their colleagues led to a better understanding of the detailed structure of the Ig molecule. Pepsin treatment produces a dimeric F(ab)2 fragment. Papain treatment produces monovalent antigen-binding fragment (Fab) and Fc fragments. The F(ab)2 and Fab fragments bind antigen, but lack a functional Fc region (Fig. 5.2). Light and heavy chains are subdivided into variable regions and constant regions. A L chain consists of one variable domain (VL) and one constant domain (CL). Most H chains consist of one variable domain (VH) and three or more constant domains (CH). Each domain is approximately 110 amino acids long. Variable regions are for antigen-binding and the constant regions are responsible for other biologic functions. In the variable regions of both L and H chains, there are three extremely variable (hypervariable) amino acid sequences that form the antigen-binding site. The hypervariable region (HVR) form the complementary region of the antigenic determinant and therefore, known as complementary determining regions (CDRs) (Fig. 5.3). Classes of Immunoglobulin .There are five classes of immunoglobulins, according to their properties (Table 5.1).

They are:

1. Immunoglobulin G (IgG).
2. Immunoglobulin A (IgA).
3. Immunoglobulin M (IgM).
4. Immunoglobulin D (IgD).
5. Immunoglobulin E (IgE).

Immunoglobulin G

Immunoglobulin G is the main class of immunoglobulin in serum. It exists as a molecule of molecular weight 146 to 160 kDa (7S) in serum and is abundant component of the secondary humoral response. This class of Ig is not only found in the bloodstream, but also in extravascular spaces. It contains less carbohydrate than other Igs. It has a half-life of approximately 23 days. It is also transported across the placenta and is therefore, responsible for passive immunity in the fetus and neonate. Passively administered IgG, suppresses the homologous antibody synthesis by a feed back mechanism. This process is utilized in the immunization of women by the administration of anti-RhD IgG during delivery. There are four subclasses of IgG isotypes in man (IgG1, IgG2, IgG3 and IgG4), each one is distinguished by a minor variation in the amino acid sequences in the C-region and by the numbers and location of disulfide bridges. The four subclasses are distributed in human serum, IgG1 (65%), IgG2 (23%), IgG3 (8%) and IgG4 (4%) (Fig. 5.4). Immunoglobulin G participates in most immunological reactions such as complement fixation (IgG1 and IgG3), precipitation, neutralization of toxins and viruses. IgG1 and IgG3 are capable of interacting with the Fc receptors on macrophages and therefore, acting as efficient opsonins

Immunoglobulin A

Immunoglobulin A is the second most abundant class of immunoglobulin constitute about 10% to 13% of all serum immunoglobulins. The normal serum level is 0.6 to 4.2 mg per mL. It has a half-life of 6 to 8 days. IgA is found in two forms in the body—in serum, where it occurs principally as monomer (160 kDa, 7S) and on secretory surfaces, where it exists as a dimeric molecule (385 kDa, 11S). The dimeric form is known as secretory IgA (sIgA) (Fig. 5.5) and is found in association of J chain and with secretory component; the latter is involved in the transport of IgA to the secretory surfaces. Secretory component is non-covalently associated with the IgA molecules in the sIgA complex. sIgA is the main Ig in the secretions such as milk, saliva and tears and in the secretions of respiratory, intestinal and genital tracts. It protects mucous membranes from attack by bacteria and viruses. There are two subclasses of IgA; IgA1 and IgA2 distinguished by their distribution and arrangement of disulfide bonds. IgA is the predominant form found in serum, where as IgA1 and IgA2 isotypes are present in roughly equal amounts in IgA. Immunoglobulin A is the component of the secondary humoral response. The principal antigens that elicit an IgA response are microorganisms in the gut or on the airways. IgA cannot cross the placental barrier, but however, sIgA can be passed to the neonate through milk. IgA does not fix complement, but can activate the alternative complement pathway. It promotes phagocytosis and intracellular killing of microorganisms.

Immunoglobulin M

Immunoglobulin M constitutes 5% to 8% of serum Ig with a normal level of 0.5 to 2 mg per mL. It has a half-life of about 5 days. It is a heavy molecule (19S; molecular weight 900,000 to 1,000,000, hence called the millionaire molecule). It has a pentameric structure comprising five identical four chain units (Fig. 5.6), i.e. it has 10 identical binding sites. The 'mu' heavy chains has five domains, VH plus 4 C regions (Cμ1, Cμ2, Cμ3, Cμ4) and lacks a hinge region. The pentameric structure is stabilized by disulfide bonding . between adjacent Cμ3 domains and by the presence of Joinez (J) chain. Though theoretically 10 antigen-binding sites are there, only five antigen-binding sites react with antigen probably due to steric hindrance. Immunoglobulin M is the principal component of primary immune response. Because of its large size (970 kDa, 19S), it is located mainly in the bloodstream. As it is not transported across the placenta, the presence of IgM in the fetus indicates intrauterine infection and its detection is useful to the diagnosis of congenital infections such as syphilis, rubella, human immunodeficiency virus (HIV) infection and toxoplasmosis. IgM antibodies are relatively short lived, disappears earlier than IgG. Hence, their demonstration in serum indicates recent infection. Treatment of serum with0.12 M 2-mercaptoethanol selectively destroys IgM without affecting IgG antibodies. The isohemagglutinins (anti-A, anti-B) and many other natural antibodies to microorganisms are IgM. Antibodies to typhoid O antigen (endotoxin) and Wassermann reaction (WR) antibodies in syphilis are also of this class. It is efficient in both opsonization and complement fixation.

Immunoglobulin D

Immunoglobulin D structurally resembles IgG. The concentration is about 0.03 mg per mL of serum. It has a half-life of about 3 days. IgD acts as an antigen receptor, when present on the surface of certain B lymphocyte. Two subclasses, IgD1 and IgD2 have been described (Fig. 5.7).

Immunoglobulin E

Immunoglobulin E is 8S molecule (molecular weight is about 190,000) with a half-life of 2 days. Normal serum contains only traces. It exhibits unique properties such as heat lability and affinity towards surface of mast cells. The Fc region of IgE binds to the receptor for the antigen on the surface of mast cell and basophil. The resulting antigen-antibody complex triggers immediate (type 1) hypersensitivity reaction by releasing the mediators. Serum IgE increased in anaphylactic reaction and helminthic infection. IgE is also known as reagin (Fig. 5.8).

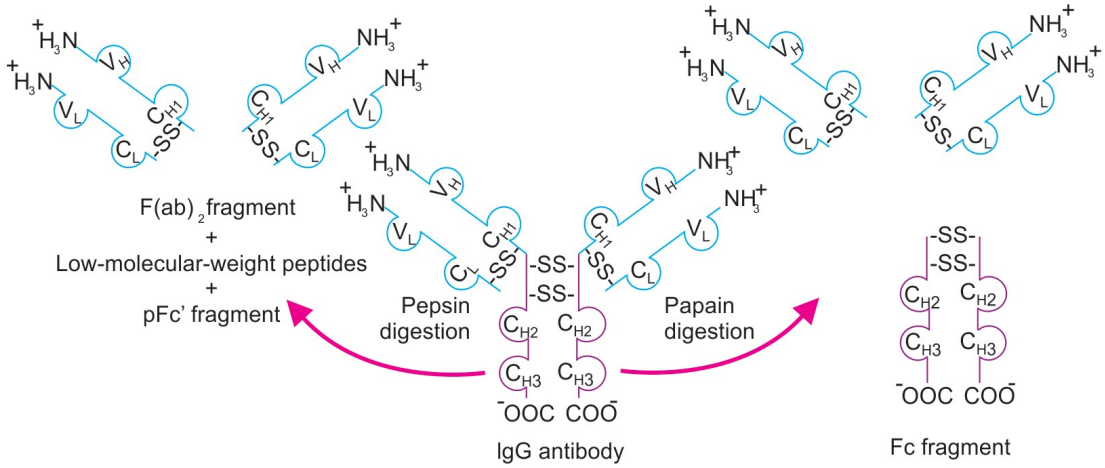


Fig. 5.2: Proteolytic digestion of immunoglobulin G (IgG). Pepsin treatment produces a dimeric F(ab)2 fragment. Papain treatment produces monovalent Fab fragments and an Fc fragment. The F(ab)2 and the Fab fragments bind antigen, but lack a functional Fc region.

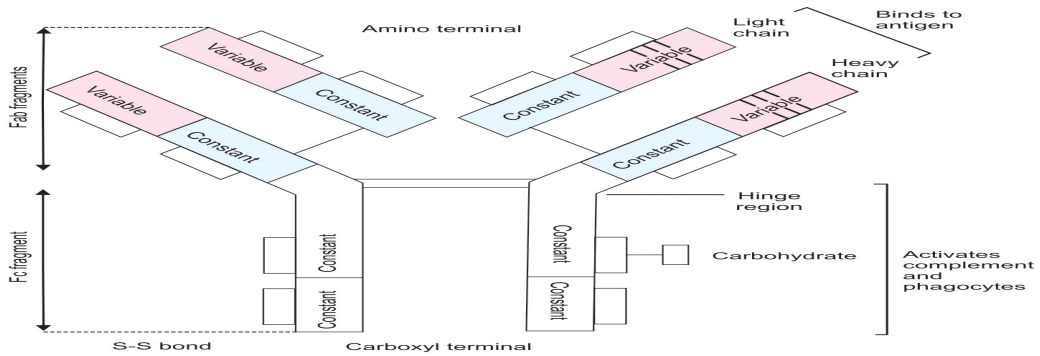


Fig. 5.3: Schematic representation of an IgG molecule, indicating the location of the constant and the

variable regions on the light and heavy chain

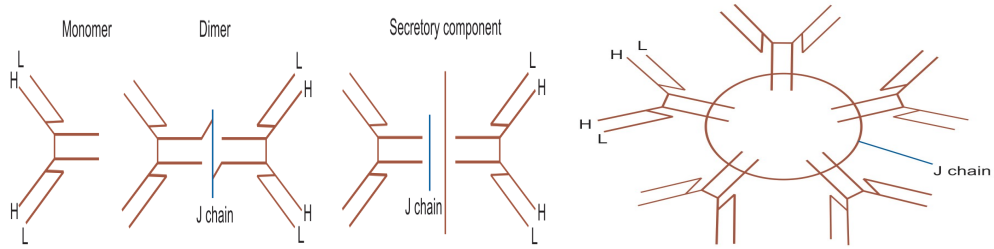


Fig. 5.5: Structure of IgA (both monomer and dimer) Fig. 5.6: Structure of human IgM (pentamer)

Antibody production follows a characteristic pattern after an initial antigenic challenge.

The pattern consists of:

1. A lag phase or latent phase, which lasts for 1 week in human beings during which no antibody is detected. During this period, activation of Th and B cells is taking place.

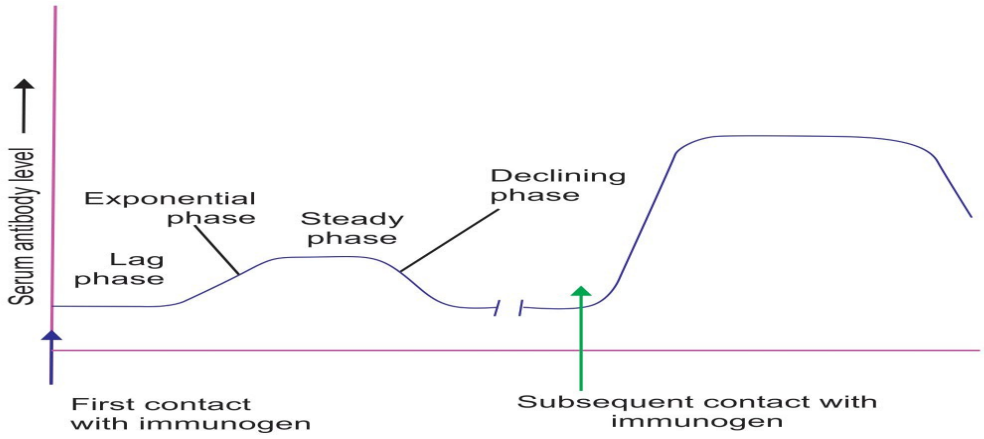
2. A log phase or exponential phase, where This phase results the increasing number of plasma cells.

3. A plateau or a steady state, where there is equilibrium between antibody synthesis and catabolism.

4. Phase of decline in which the antibody titer diminishes, which indicates that new plasma cells are no longer produced and the existing plasma cells are dying or ceasing antibody production.

Lec. 7 : Primary and secondar y response : The antibody response to an initial antigenic stimulus differs qualitatively and quantitatively from the response to the subsequent stimuli with the same antigen (Fig. 9.12). An individual's first encounter with a particular immunogen leads to a relatively slow, sluggish short-lived response designated as primary response. There is a longer lag phase and the titer of antibody is low, which does not persist longer and the antibodies formed are predominantly IgM in nature. In contrast, when the same individual encounters with the same immunogen subsequently, the response is prompt, powerful and prolonged, where there is no lag phase or shorter lag phase, antibody titer is high and persists for longer period and the predominant antibody formed is IgG . This is known as secondary or anamnestic reaction. The large numbers of

antigen-specific memory T and B cells, generated during the primary response are responsible for the rapid kinetics and the greater intensity and duration of secondary responses.



(HUMORAL IMMUNITY)

Humoral immune response (Fig. 9.11) involves the production of large quantity of antibodies following B-cell activation, on antigenic challenge. The activation of B cells need the help of T cells in case of T dependent antigens and may not require the help of T cells in case of TI antigens. The TI antigens fall into two groups such as TI-1 antigens and TI-2 antigens. TI-1 antigens, in high concentration, induce activation of many B cells, both specific and non-specific. Because they activate many B cells, they are known as polyclonal B-cell activators. Many polyclonal activators (LPS) also activate macrophage to produce IL-1 and TNF-α, which augment immune responses. In contrast, TI-2 antigens are not polyclonal activators nor do they activate macrophage. Usually, these are highly repetitive polymeric antigens, such as polysaccharide from bacterial cell walls or polymeric protein, such as bacterial flagella. B-cell activating properties in TI-2 antigen may be due to cross-linking of numerous BCR molecules inducing intracellular signaling reactions.

Humoral immune response plays important role in the primary defense against extracellular bacterial pathogens. Antibodies that coat bacteria or other particulate antigens can function as opsonin to promote phagocytosis. Antibodies specific for bacterial toxins or for the venom of the insects or snakes, bind these antigenic proteins and in many cases directly inactivate them by steric effects. In addition, the toxin-antitoxin complex may ultimately be phagocytosed by macrophages and other phagocytic cells. Certain types of antibodies, coated over the surface of the bacteria, can activate complement pathway leading to complement-mediated lysis. There is another mechanism, how antibodies play a role in causing cytolysis of bacteria and multicellular parasites, is by antibody-dependant cell-mediated cytotoxicity (ADCC). IgG binds Fc receptors on the surface of natural killer (NK) cells and certain other cell types and enables them to carry out a form of antigen-specific killing by cytotoxin. Antibodies also bring about defense against viruses that infect through the respiratory tract and intestinal tract. Antibodies specific for protein on the surface of a virus may block the adsorption site on the target cell, thus preventing the entry of the organism. Besides

elimination of foreign substances (antigens), humoral immunity participates in the mechanism of pathogenesis of immediate hypersensitivity reaction and autoimmune diseases.

Antigen crosslinks mIg, generating signal ①, which leads to increased expression of class II MHC and costimulatory B7. Antigen-antibody complexes are internalized by receptor-mediated endocytosis and degraded to peptices, some of which are bound by class II MHC and presented on the membrane as peptide-MHC complexes.

T_h cell recognizes antigen-class II MHC on B-cell membrane. This plus costimulatory signal activates T_h cell.

T_h cell begins to express CD40L. Interaction of CD40 and CD40L provides signal②. B7-CD28 interactions provide costimulation to the T_h cell.

B cell begins to express receptors for various cytokines. Binding of cytokines released from T_h cell in a directed fashion sends signals that support the progression of the B cell to DNA synthesis and to differentiation.

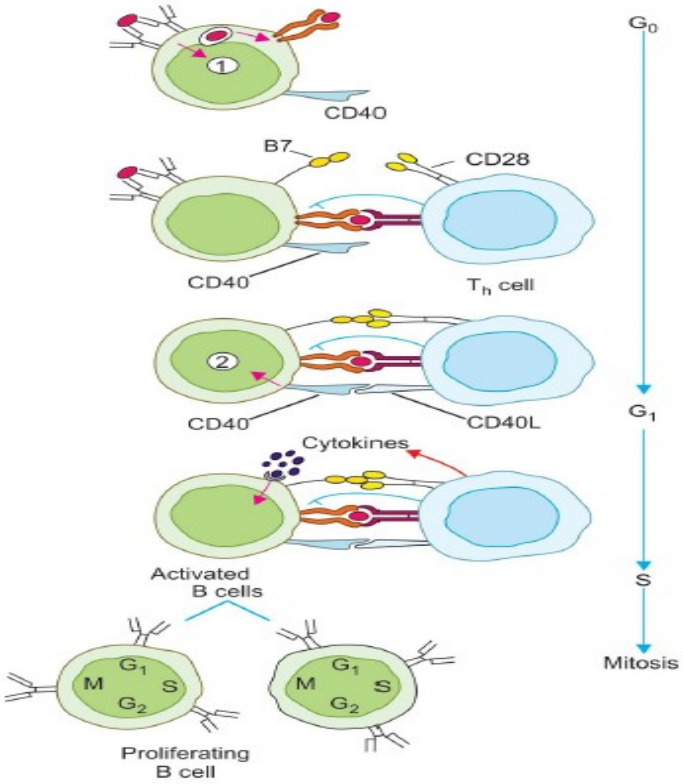


Fig. 9.9: Sequence of events in B cell activation by a thymus-dependent antigen. The cell cycle phase of the interacting B cell is indicated on the right (MHC, major histocompatibility complex; DNA, deoxyribonucleic acid).

TABLE 11-2 Differences between cell-mediated and humoral immunity

Cell-mediated immunity	Humoral immunity
Immune response mediated by cells	Immune response mediated by antibodies
Protects against fungi, viruses, and facultative intracellular bacterial pathogens	Protects against extracellular bacterial pathogens and viruses infecting respiratory or intestinal tract; and prevents recurrence of viral infections
Mediates delayed (type IV) hypersensitivity	Mediates immediate (types I, II, and III) hypersensitivity
Only T-cell-dependent antigens lead to cell-mediated immunity	B cells directly bind soluble antigens resulting in production of antibodies
Both CD4+ and CD8+ T cells are involved	Only T_H cells are involved
Provides immunological surveillance and immunity against cancer	No major role in immunological surveillance
Participates in rejection of homografts and graft-versus-host reaction	May be involved in early graft rejection due to preformed antibodies

Lec . 8& 9 : LYMPHOCYTES

The most fundamental distinction is the division of these cells into two major lineages known as T (thymus derived) cells and B (bone marrow derived) cells. The relative proportions of T and B cells vary in tissue to tissue, but in peripheral blood they constitute 75% and 15% respectively. The remaining 10 percent are a special class of granular lymphocytes known as natural killer (NK) cells. 'T' and 'B' lineage cells, both arise from a subset of hemopoietic stem cells in the bone marrow or fetal liver that become committed to the lymphoid path of development (Fig8.3). There are evidences that both T and B cells, as well as NK cells are the descendants of a common committed marrow progenitor cell called lymphoid stem cells. The growth of early lymphoid progenitors require at least two cytokines, called interleukin-7 (IL-7) and stem cell factor (SCF), found in both the thymic

and marrow microenvironments. The progeny of these putative stem cells follow divergent pathways to mature into either T or B cell. Human B lymphocytes develop exclusively in bone marrow. T cells, on the other hand, develop from the mature precursor that leave the marrow and travel through the bloodstream to the thymus, where they proliferate and differentiate into mature T lymphocytes under the influence of a number of thymic hormones. Mature lymphocytes that come out of thymus and bone marrow are in a resting state (mitotically inactive). Dispersed into the bloodstream these, so called naive (virgin), lymphocytes migrate efficiently into various secondary lymphoid organs such as spleen, lymph nodes, tonsils, etc. The function of the secondary organs is to maximize encounters between lymphocytes and foreign substances. And, it is from these sites most immune responses are carried out. Most virgin lymphocytes have an inherently short lifespan and are programmed to die, within few days after leaving the marrow or thymus. However, if such a cell receives signals that indicate the presence of foreign substance or pathogen, it may respond through a phenomenon known as activation. Such, activated or committed or sensitized cell undergoes successive cell division to form memory lymphocytes (Fig. 8.4), which can survive for years and the

effector lymphocytes, which survive only for few days, but carry out specific defense activities against the foreign invader.

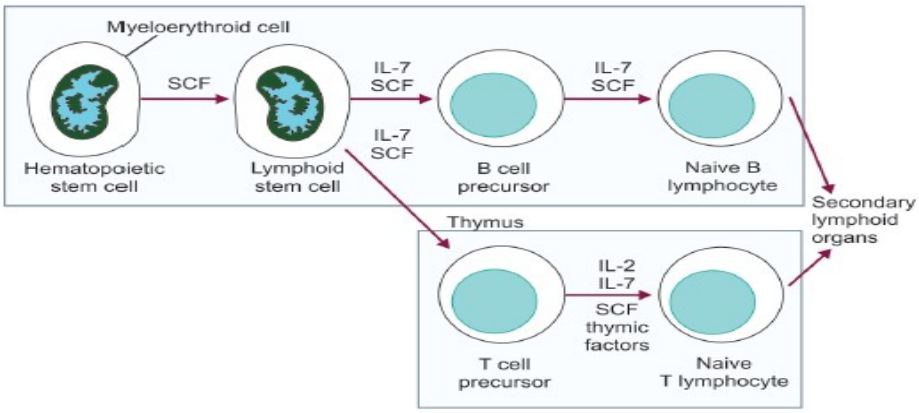


Fig. 8.3: Schematic overview of lymphocyte development (lymphopoiesis). In this simplified diagram, most intermediated stages are omitted. The characteristics of cells that migrate to the thymus are unknown. A few of the regulatory molecules needed for proliferation at particular stages of development are indicated (SCF, stem cell factor; IL, interleukin).

B Cells

B lymphocyte precursors, pro-B cells, develop in the fetal liver during embryonic life and in the bone marrow afterwards. The important feature of the cell, is its ability to synthesize proteins called immunoglobulin (Ig). Each Ig molecule binds specifically and with high affinity with its own molecular ligand known as antigen. A virgin (resting) B cell is one-stage of B cell, which has not had contact with antigen. Each resting lymphocyte may express good number of membrane Ig on its surface. On contact with its appropriate antigen, the mature B cell undergoes clonal proliferation. Some activated B cells become long-living memory cells responsible for the recall phenomenon seen in subsequent contact with the same antigen. The majority of the activated B cells are transformed into plasma cells (Fig. 8.5).

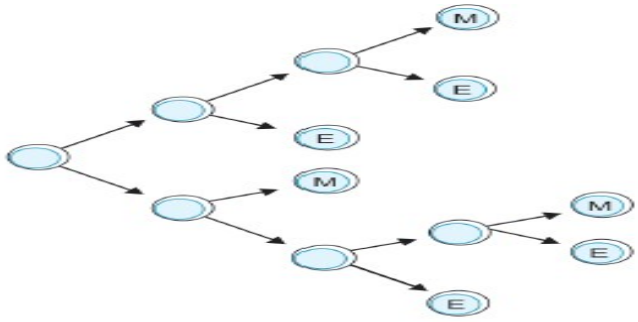


Fig. 8.4: Lymphocyte activation. This leads to both cell division and differentiation. At each cell division, individual cells can cease dividing and differentiate into memory (M) or effector (E) cells. In this example, a single activated lymphocyte gives rise to four effector memory cells after four cycles of division.

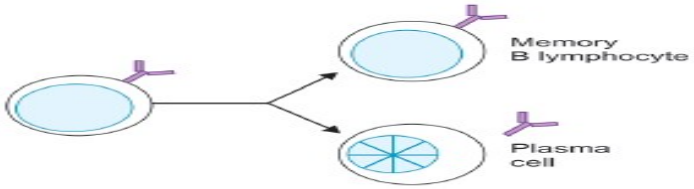


Fig. 8.5: The progeny of an activated B lymphocyte can differentiate into either memory B lymphocytes or antibody-secreting plasma cells

Surface Markers of B Cell at Different : Stages of Development

Various surface markers identify the developmental B lineage cells such as pro-B and pre- B cells. At the pro-B stage, the cells do not display the heavy or light chains of antibody, on the other hand, they do express CD45R, which is a form of the protein tyrosine phosphatase found on leukocytes and the signal transduction molecules Igα/Igβ, which are found in association with the membrane forms of antibody in later stages of B cell development (Fig. 8.6). In addition pro-B cells express CD19 (part of B cells coreceptor), CD43 (leukosialin), CD24 [heat stable antigen (HSA)] and C-kit (receptor for a growth promoting ligand present in the stromal cells). While developing from pro-B to pre-B cells, they cease to express some receptors (c-kit and CD43) and begin to express some other receptors (CD25, α-chain of IL-2 receptors and pre-BCR). After rearrangement of light chain, the surface Ig have both heavy and light chains, lose the [B cell receptor (BCR)] pre-BCR and CD25 and then convert to immature B cell.

B Cell Subtypes

There are two subsets of B cells. They are B-1 cells and B-2 cells, which constitute 5% and 95% respectively. B-1 cells are so named, because they are first to develop embryologically that dominate the pleural and peritoneal cavities. They appear during fetal life, express surface IgM, but little or no IgD and are marked by CD5. In contrast the conventional or B-2 cells arise during and after the neonatal period and continuously replaced from the bone marrow and are widely distributed throughout the lymphoid organs and tissues. Each B cell is specific, that is, it produces Ig of one specificity that recognizes only one epitope. The B-1 population of cells responds poorly to protein antigens, but much better to carbohydrates. The antibodies produced by high proportion of B-1 cells are of low affinity.

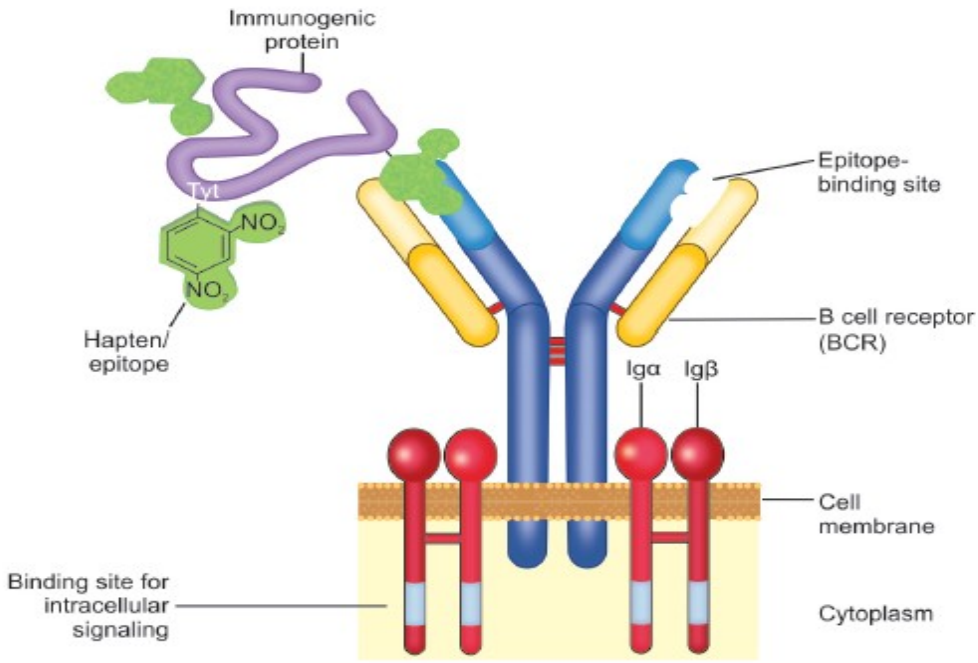


Fig. 8.6: Immunoglobulins serve as BCRs. B cells bear receptors that are composed of two identical large (heavy) chains and two identical smaller (light) chains. Molecules such as Igα and Igβ are associated with BCRs and help provide a signal to the cell when the BCR binds an epitope.

Plasma Cells

Plasma cells are the effector cells of the B lineage, are uniquely specialized to secrete large amount of Ig proteins to the surrounding milieu. Secreted immunoglobulins retain their ability to recognize and bind their specific ligands and are often referred to as antibodies. Binding of the antibodies to their specific ligands have a variety of effects that are beneficial to host cells. Plasma cells are oval or egg-shaped and have abundant cytoplasm and eccentrically placed round nuclei. Clumps of dark staining chromatin are often distributed around the inner aspect of the nuclear membrane in plasma cell, giving a cart-wheel or clock face appearance under light microscope. The cytoplasm contains abundant rough endoplasmic reticulum and a well-developed Golgi apparatus. Igs are not present in the surface of plasma cell, but produced in large amount in cytoplasm and are then secreted into the extracellular space. Plasma cells have relatively short span of life and are terminally differentiated. The main functions of the 'B' lineage cells are involvement in the following:

1. Humoral acquired immunity.

2. Antigen processing and presenting.

3. Production of an array of cytokines and other factors that influence the growth and activity of other immunologically important cells.

T Cells ('T' Lineage Cells)

A lymphocyte that has been educated by the thymus becomes an immunologically competent cell (ICC). Such cells are qualified to take up immunological response, when approximately stimulated by an antigen. The ICC subserves the function of recognition of antigen, storage of immunological memory and immune response. T lymphocytes do not express Igs, but instead, detect the presence of foreign antigen by way of surface protein called T cell receptors (TCR). These receptors are membrane proteins made up of a pair of polypeptide known as α and β chains or γ and δ chains. Large majority of the cells have α and β chains. TCR are closely related to immunoglobulins in evolution and share with them a number of structural and functional properties, including the ability to detect specific small molecular ligands called antigens. Unlike Igs however, TCR proteins are never secreted and therefore they are incapable of striking their targets at a long distance. Instead they extend their protective effects, either through direct contact or by influencing the activity of other immune cells. Together with macrophages, T cells are the primary cell type involved in a category of immune response called cellmediated immunity. Unlike B cells, T cells can detect foreign substances only in specific context. In particular, T lymphocyte will recognize a foreign protein only if it is first cleaved into small peptides and which are displayed on the surface of a second host cell called antigen-presenting cells (APCs), which constitute macrophages, dendritic cells, B cells and other cell types. Presentation depends in part, on MHC protein on the surface of APC, which is attached non-covalently to the cleaved peptides for display. It is the combination of cleaved peptide and MHC molecule that can attract T cells with appropriate receptors.

Surface Molecules (Proteins) on T Lymphocytes

Mature functional T lymphocytes expresses, a number of characteristic surface proteins in addition to TCR. These molecules have been identified by means of monoclonal antibodies. These markers reflect stage of differentiation and functional properties of the cell. Many of these proteins are referred by the initials of 'clusters of differentiation' (i.e. CD) followed by a unique identifying number like CD1, CD2, CD3, CD4, etc. Over 80 CD markers have been identified. Some of the CD markers with their cell association and other surface molecules are given (Table 8.1). Almost all the mature T cells found in the secondary lymph nodes and peripheral blood are CD2+, CD3+, that is, both the cells express CD2 and CD3 molecules on their surface. There are distinct subpopulations of cells with very different immunological functions. These subpopulations of cells express their own surface molecules and are referred to as T cell subsets (Table 8.2). The two most important major T cell subsets are (CD4+, CD8–) and (CD4–, CD8+), which constitute 70% and 25% respectively. Most T lymphocytes that express CD8 proteins are cytotoxic, i.e. they are able to kill cells that have foreign macromolecule ontheir surfaces. CD8 cells have virucidal effect. T lymphocytes that express CD4 protein are not cytotoxic. They are known as helper T cells (Th), which promote proliferation and maturation and immunologic function of other cell types by the help of specific lymphokines. The two minor subsets of T cells are (CD4–, CD8–) and (CD4+, CD8+),

which constitute 4% and 1% respectively. The double negative subsets (CD4–, CD8–) of T lymphocytes, instead of α and β polypeptide have γ and δ chains of polypeptide. The function of double-positive T cells (CD4+, CD8+) is unknown. Some distinguishing features between T cell, B cell and macrophage are summarized in Table 8.3. The correlation of CD4+ or CD8+ with helper (Th) or cytotoxic (TC) function is strong, but not absolute. A few CD8+ cells have helper activity and also CD4+ cells have cytotoxic activity. Expression of CD4 and CD8 actually correlates most closely with the type of MHC protein that a T cell can recognize.

T Cell Maturation

T cell precursors migrate to the thymus, after being developed in yolk sac, fetal liver and bone marrow, during the embryonic life and following birth. CD7+ pro T cells are the earliest identifiable cells of 'T' lineage, which acquire CD2 on entering the thymus. Then synthesis of CD3 occurs in the cytoplasm and they become pro T cells. TCR synthesis also takes place. TCR occurs as two pairs of glycoprotein chain either αβ or γδ. Pre T cells differentiate into two lineages, expressing either αβ or γδ TCR. Contact with self-antigens within the thymus leads, to the destruction of immature T cells carrying the corresponding TCR. Thus self-tolerance or elimination of T cells capable of reacting with the self-antigens take place in the thymus. The uncommitted T cells are also converted to committed T cells, against foreign antigens in thymus only. T cells also develop MHC restriction so that CD8+ cells respond, to foreign antigen presented along with MHC I molecule and CD4+ cells presented with MHC II molecule. Immature T cells in the thymus exhibit CD7, CD2, CD3, CD1, CD4 and CD8 besides TCR. On functional maturity, they lose CD1 and differentiate into two major subsets and (CD4+ CD8– and CD4– CD8+) and two minor subsets. Mature CD4+ CD8– cell serve helper/inducer function and CD4– CD8+ cells serve as suppressor/cytotoxic function. The minor subsets such as CD4+ CD8+ and CD4– CD8– also present in circulation (Fig. 8.7).

Null Cells

There are some lymphocytes, which lack the features of T cells and B cells. They are known as null cells (large granular lymphocytes), which constitute 5% to 10% of all lymphocytes. The null cells or large granular lymphocytes, may be NK cells, antibodydependent cellular cytotoxicity (ADCC) cells and lymphokine-activated killer (LAK cells). Natural killer cells possess spontaneous cytotoxicity towards various target cells. The ability of NK cells to kill tumor cells has been, a focus of research interest and therapeutic trials, but there is little evidence that NK cells normally protect against the development of tumor, instead the most important role for NK cell appears to be against infection by intracellular agents such as virus, bacteria and parasite. The cytotoxicity is not antibody dependant or MHC restricted like cytotoxic T lymphocytes. For their action they also do not require sensitization by antigenic contact. Unlike T cells, NK cells do not express cell surface CDR/CD3 complex. They also lack CD4 surface molecule. About half of human NK cells express CD8 molecule. But it is not required for natural killing. Most NK cells express CD16 (a receptor for Fc portion of IgG) and CD56 [a variant of neural cell adhesion molecule, (NCAM)]. These receptors are not required for natural killing. They are only required for identification of NK cells, which are generally CD16+, CD56+, CD3–where as T cells are CD3+, CD16–, CD56–.NK cells bind to the glycoprotein receptor of the target cells and release several cytolytic factors. One of them is perforin, which resemble complement component C9 that causes transmembrane pores through which cytotoxic factors such as tumor necrosis factor-β (TNF-β), serine esterase, lymphotoxins enter the cell and destroy it by apoptosis (programed cell death). NK cells are augmented by γ-interferon.

In addition to receptors mentioned earlier, recent findings suggest that the NK cells bear another set of receptors called killer activation receptors (KARs) and killer inhibition receptors (KIRs) that allow them to recognize host cells (Fig. 3.4). When the KAR is engaged by binding to its carbohydrate ligands, on target cells, 'the kill signal' to the NK cell is activated, causing destruction to the target cells. However, if the KIR is engaged by binding of ligands on the surface of the target

cell, then the NK cell receive ‘do not kill signal’ and target cell survives. A unique subset of T cells have been found out, which shares the functional properties and the receptors of NK cells, developed in thymus and express a rearranged TCR with extremely limited repertoire. This is known as NKT cell. Unlike conventional T cells, NKT cells respond to lipids, glycolipids or hydrophilic peptides, presented by specialized non-classical MHC I molecule, CD1d and secrete large amount of cytokines specially IL-4.

Table 8.1 Some important surface molecules on T lymphocytes	
Marker	**Major function or significance**
T cell receptor	Antigen binding
CD3 complex	Single transduction from T cell receptor; lineage-specific marker
CD2, CD5, CD7	Lineage-specific markers
CD4	Subset-specific marker (mainly on helper cells); interaction with class II MHC* proteins
CD8	Subset-specific marker (mainly on cytotoxic cells); interaction with class I MHC proteins
CD28	Activation-specific marker; receives B7-mediated costimulation from APC†
CD40 ligand (CD40L)	Activation-specific marker; delivers contact-mediated help to B cells
IL‡-2 receptor class II MHC proteins transferrin receptor CD25, CD29, CD54, CD69	Other activation-specific markers
IL-1 receptor IL-6 receptor TNF§-α receptor	Other cytokine receptors
Fc receptors	Immunoglobulin binding
LFA‖-1, ICAM¶-1	Cell-cell adhesion molecules

*MHC, major histocompatibility complex; †APC, antigen-presenting cell; ‡IL, interleukin; §TNF, tumor necrosis factor; ‖LFA, leukocyte functional antigen; ¶ICAM, intercellular adhesion molecule.

Table 8.2 Major T cell subsets found in blood and peripheral tissues			
Surface phenotype	**Predominant function**	**Proportion of total blood T lymphocytes**	**T cell receptor type**
$CD4^+ CD8^-$	Helper	70%	α/β
$CD4^- CD8^+$	Cytotoxic	25%	α/β, rarely γ/δ
$CD4^- CD8^-$	Cytotoxic	4%	γ/δ
$CD4^+ CD8^+$	–	1%	α/β

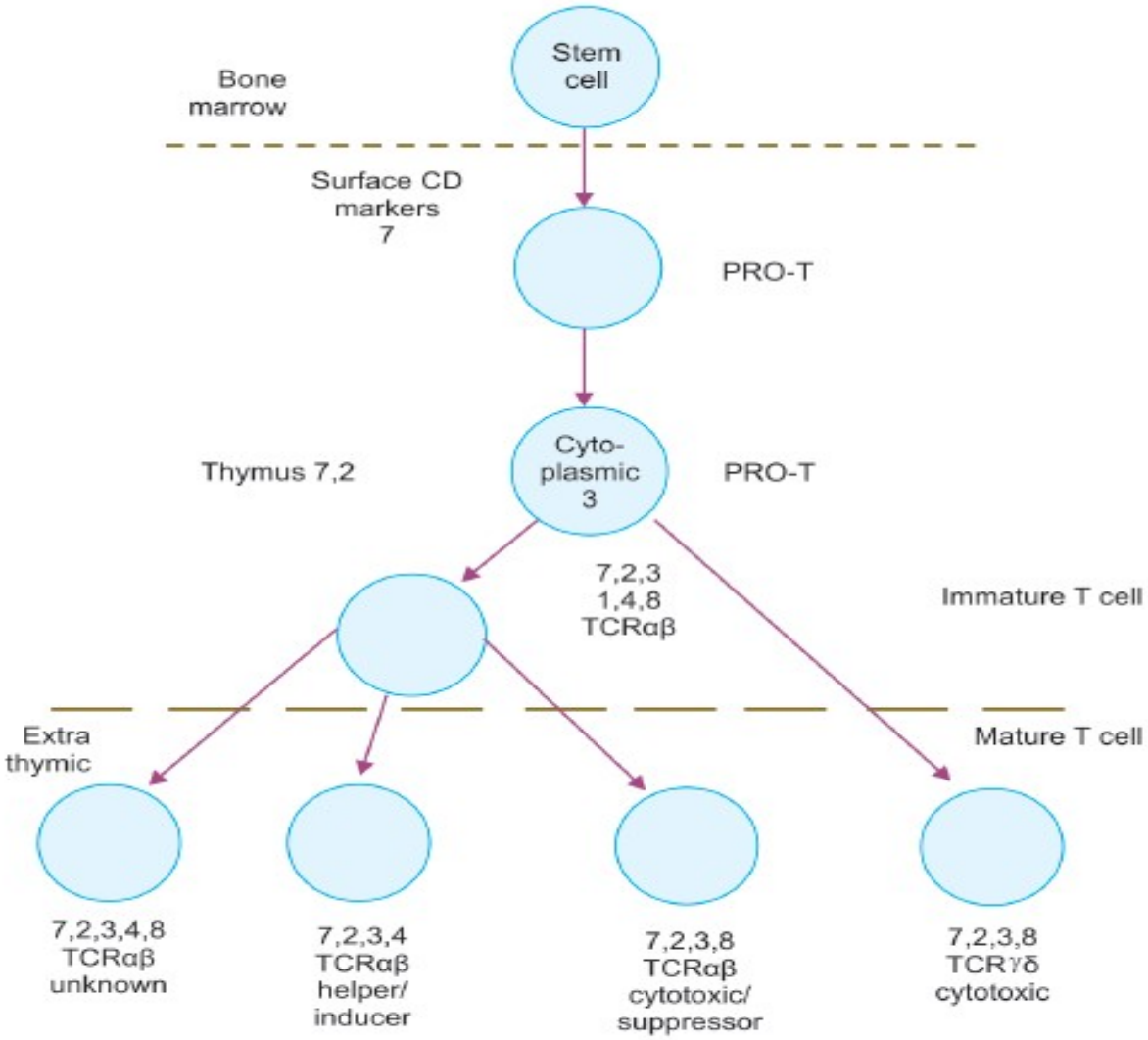


Fig. 8.7: T cell maturation