A wide variety of organisms and their associated molecules pose a constant threat to the human body. **The human immune system is the defensive mechanisms that identify and neutralize these threats**. The human immune system is able to distinguish "**non-self**" organisms and molecules from "**self**," that which is part of the body .

Threats may enter the body from :

1- **the outside** (e.g. infectious organisms or toxic agents) .

2- or may arise from potentially harmful changes occurring within the body (e. g . , the malignant transformation of a previously normal cell into a cancer cell) .

Fortunately, the immune system consists of three layers of defense :

1- The first line of defense is provided by a set of mechanical (e. g . , skin) , chemical (e . g . , acidic environment of stomach) , and biologic (e. g . , commensal microbes) barriers that protect the body.

2- the innate immune system .

3- adaptive immune system .

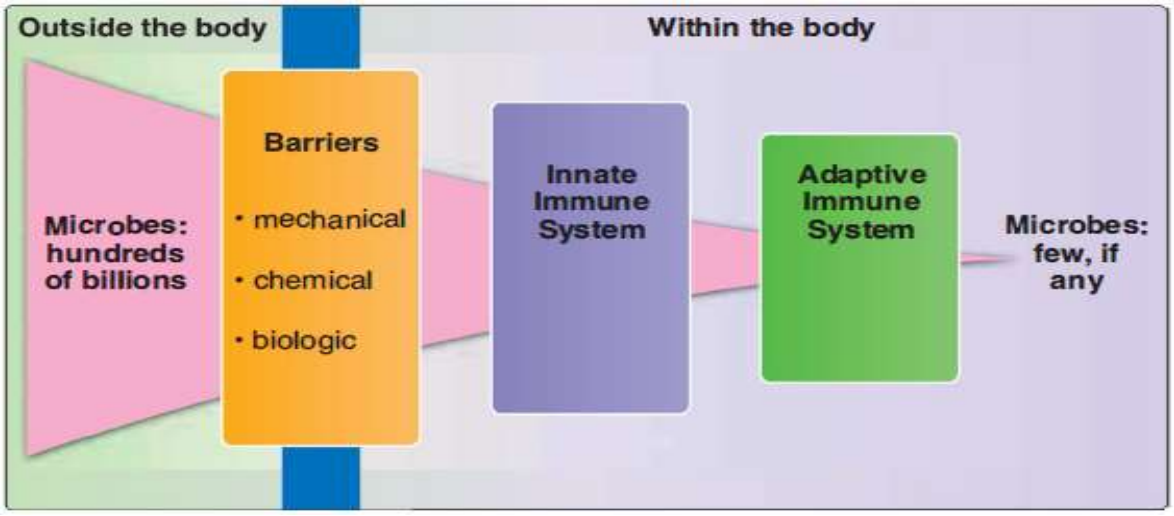


Figure: Protection from and response to microbial invasion . Initial protection is provided by a set of barriers. When breached, invading

microbes trigger the innate immune system and, if necessary, the adaptive immune system .

Innate Immunity: Nonspecific Defenses of the Host

Innate immunity		adaptive immunity
First line of defense	Second line of defense	Third line of defense
1- Intact skin. 2-Mucous membranes and their secretions. 3- Normal microbiota.	1-Phagocytes, such as neutrophils, dendritic cells and macrophages. 2- Inflammation. 3- Fever. 4-Antimicrobial substances	1-Specialized lymphocytes T cells and B cells. 2- Antibodies.

- Innate immunity involves defenses against **any pathogen** (**nonspecific)**. regardless of species.
- adaptive immunity involves defenses against a **specific pathogen**.

CHARACTERISTICS	INNATE IMMUNITY (NON-SPECIFIC)	ACQUIRED IMMUNITY (SPECIFIC)
DEFINITION	The resistance to infection that an individual possess by virtue of genetic and constitutional makeup; i.e., by birth.	The resistance that an individual acquires in response to exposure to a foreign substance during their lifetime.
TIME TAKEN TO DEVELOP	Hours (rapid)	Days (slow)
USED AGAINST	For microbes	For microbes and non-microbial antigens
MEMORY	None; immune response does not improve with repeated exposure.	Long-term memory; acquired immune response improves with repeated exposure to pathogen.
COMPONENTS:		
1. PHYSICAL & CHEMICAL BARRIER	- Skin, - Mucosal epithelia, - Antimicrobial chemicals.	- Lymphocytes in epithelia - Antibodies secreted at epithelial surfaces.
2. BLOOD & TISSUE ANTIMICROBIAL SUBSTANCES	- Complement - Leukins from leukocytes, - Plakins from platelets - Lactic acid found in muscle tissue, - Lactoperoxidase in milk, and - Interferons (antiviral)	- Antibodies
3. CELLS	- Phagocytes (macrophages and neutrophils) - Natural killer cells	- Lymphocytes

The Concept of Immunity

-**Innate immunity** refers to defenses that are present at birth. They are always present and available to provide rapid responses to protect us against disease.

- **Innate immunity** does not involve specific recognition of a microbe. Further, innate immunity does not have a memory response, that is, a more rapid and stronger immune reaction to the same microbe at a later date.

-**Adaptive immunity** is based on a specific response to a specific microbe once a microbe has breached the innate immunity defenses. It adapts or adjusts to handle a particular microbe.

-Unlike innate immunity, adaptive immunity is **slower** to respond, but it does have a **memory component**.

First line of defense

Physical Factors

1- The intact skin

is the human body's largest organ in terms of surface area and weight and is an extremely important component of the first line of defense. It consists of two distinct portions: the **dermis** and the **epidermis**. The top layer of epidermal cells is dead and contains a protective protein called **keratin**. The periodic shedding of the top layer helps remove microbes at the surface. In addition, the dryness of the skin is a major factor in inhibiting microbial growth on the skin.

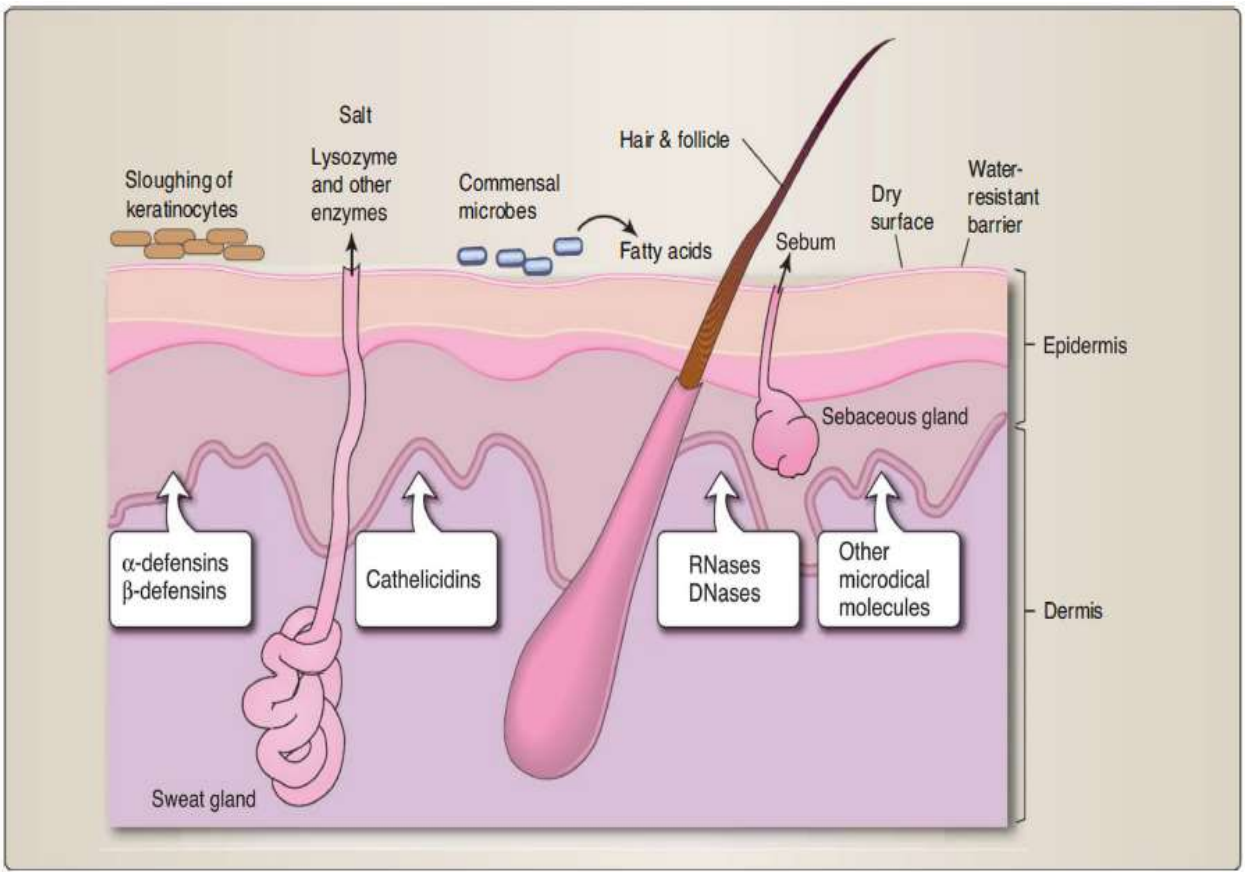


Figure: Skin contains various defense mechanisms. The epidermis provides a dry, watertight barrier continually sloughing dead cells

(keratinocytes). Dermal glands bathe the epidermis with microcidal molecules as well as with sebum and sweat producing an acidic pH and deposit salt on the surface of the skin. The dermis contains additional defense molecules and phagocytic molecules (e.g. neutrophils, macrophages) that attack invaders. Commensal microbes secrete fatty acids that inhibit colonization by other microbes.

2- Mucous membranes

line the entire gastrointestinal, respiratory, and genitourinary tracts. The epithelial layer of a mucous membrane secretes a fluid called mucus, a slightly viscous (thick) glycoprotein produced by **goblet cells** of a mucous membrane. Among other functions, mucus prevents the tracts from drying out.

Saliva: produced by the **salivary glands**, helps dilute the numbers of microorganisms and wash them from both the surface of the teeth and the mucous membrane of the mouth.

The mucous membrane of the nose also has mucus-coated hairs that filter inhaled air and trap microorganisms, dust, and pollutants. The cells of the mucous membrane of the lower respiratory tract are covered with **cilia**.

The cleansing of the **urethra** by the flow of urine is another physical factor that prevents microbial colonization in the genitourinary tract.

Vaginal secretions likewise move microorganisms out of the female body.

Peristalsis, defecation, and vomiting also expel microbes.

In response to microbial toxins, the muscles of the gastrointestinal tract contract vigorously, resulting in vomiting and/or diarrhea, which may also rid the body of microbes.

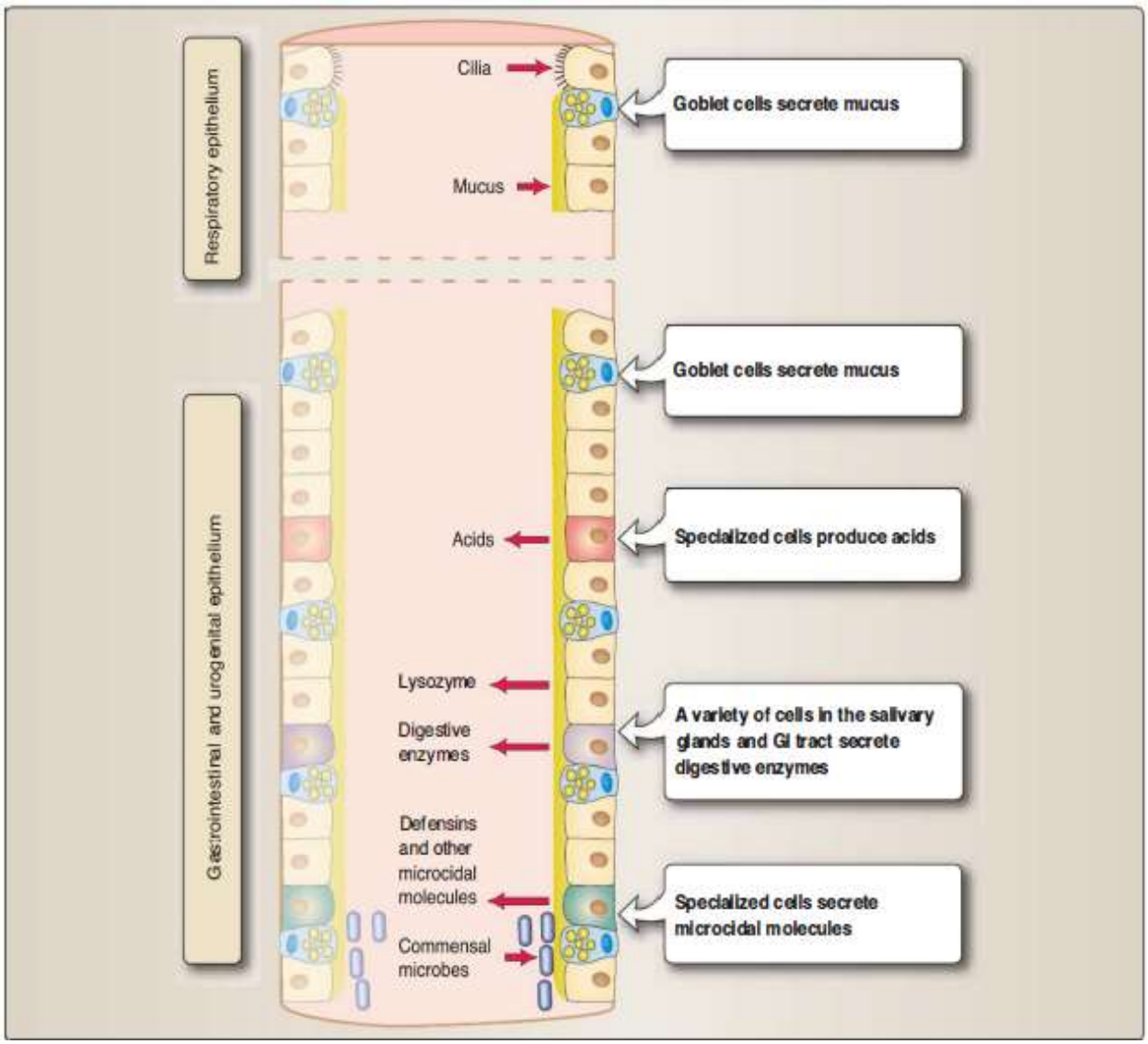


Figure : Defense mechanisms of the mucous membranes. Mucus entraps microbes and particulate matter (which, in the respiratory tract, is swept out by cilia) . Protective commensal microbes are present, and numerous microcidal molecules, enzymes, and acids are produced .

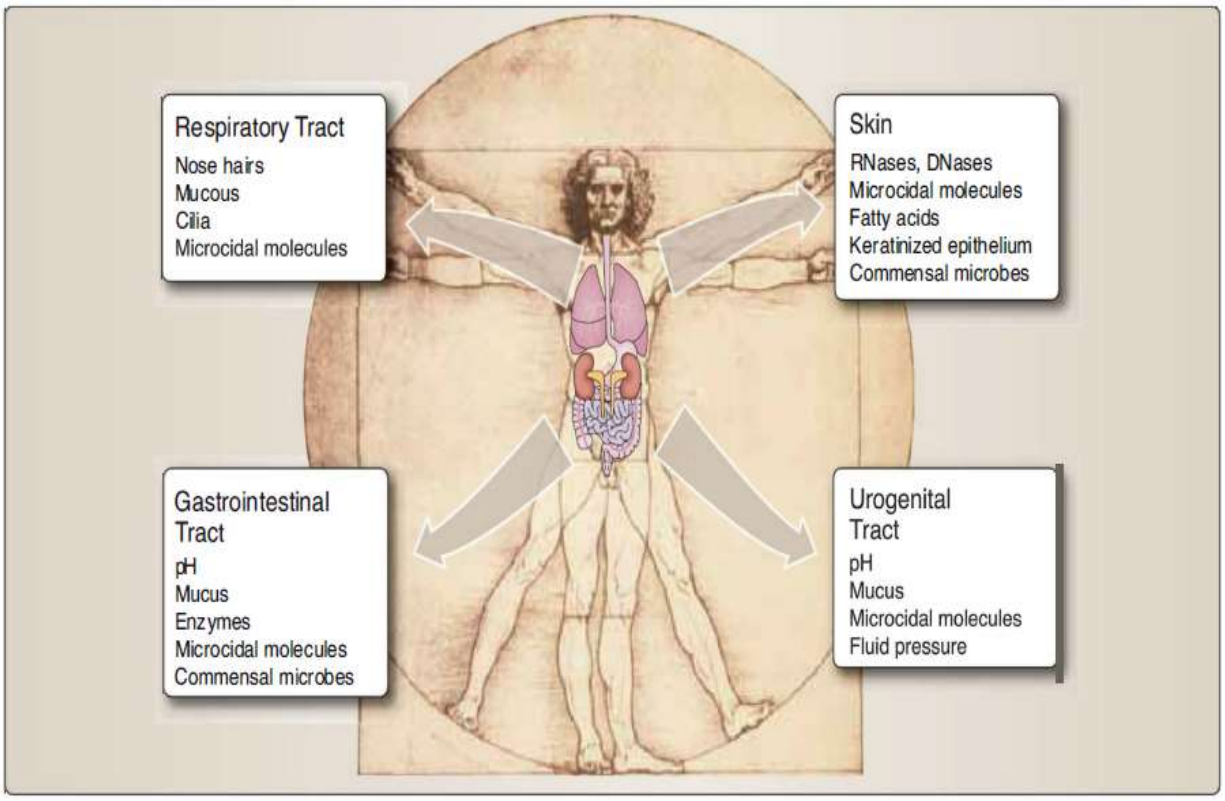


Figure: Protective barriers of the body. The barriers of the body represent the first line of defense and prevent or retard the entry cells and molecules into the body.

Chemical Factors

1- Skin : **Sebaceous** (oil) glands of the skin produce an oily substance called **sebum** that prevents hair from drying and becoming brittle.

One of the components of sebum is unsaturated fatty acids, which inhibit the growth of certain pathogenic bacteria and fungi. The **low pH of the skin**, between pH 3 and 5, is caused in part by the secretion of fatty acids and lactic acid. The **skin's acidity** probably discourages the growth of many other microorganisms.

The skin is protected in part by several **antimicrobial peptides** secreted by various cell types found within the skin. Among these are **α-defensins**, **β-defensins**, and **cathelicidin**. All are able to inhibit microbial growth by direct action on the microbes, perhaps by damaging the microbial membranes and causing lysis. They can also act as **chemoattractants** for cells of the innate immune system and facilitate the ingestion and destruction of microbes by phagocytes.

Also present in the skin are molecules that act on the RNA and DNA of a wide range of microbes such as **RNases** and **DNases** .

Sweat glands of the skin produce **perspiration**, which helps maintain body temperature, eliminate certain wastes, and flush microorganisms from the surface of the skin. Perspiration also contains **lysozyme**, an enzyme capable of breaking down cell walls of gram-positive bacteria and, to a lesser extent, gram-negative bacteria. Specifically, lysozyme breaks chemical bonds on peptidoglycan, which destroys the cell walls. Lysozyme is also found in **tears**, **saliva**, **nasal secretions, tissue fluids, and urine,** where it exhibits antimicrobial activity.

2- Respiratory tract: To protect the mucosal surfaces of the lungs, some cells of the respiratory epithelium secrete microcidal molecules such as **β-defensins**. These and other molecules in the respiratory tract can attach to microbes and make them more susceptible to ingestion and destruction by phagocytic cells.

Saliva also contains an antibody (**immunoglobulin A**) that prevents attachment of microbes so that they cannot penetrate mucous membranes.

3- Gastrointestinal tract : Gastric juice is produced by the glands of the stomach. It is a mixture of hydrochloric acid, enzymes, and mucus. The very high acidity of gastric juice (pH 1.2-3.0) is sufficient to destroy bacteria and most bacterial toxins, except those of *Clostridium botulinum* and *Staphylococcus aureus*. However, many enteric pathogens are protected by food particles and can enter the intestines via the gastrointestinal tract. In contrast, the bacterium *Helicobacter pylori* neutralizes stomach acid, thereby allowing the bacterium to grow in the stomach.

4- **Vaginal secretions** play a role in antibacterial activity in two ways. Glycogen produced by vaginal epithelial cells is broken down into lactic acid by *Lactobacillus acidophilils.* This creates an **acid pH (3-5)** that inhibits microbes. **Urine**, in addition to containing lysozyme, has an acid pH (average 6) that inhibits microbes. Also, urine contains urea and other metabolic by-products, such as uric acid, hippuric acid, which inhibit microbes.

5- Lacrimal secretions: Lacrimal glands are small almond-shaped structures, located above the outer corner of the eye, that produce **tears**. As part of protecting the eyes, the secretions of lacrimal glands contain **lysozyme**.

Normal Microbiota

several relationships between normal microbiota and host cells.

Some of these relationships help prevent the overgrowth of pathogens and thus may be considered components of innate immunity.

- Antagonism, the normal microbiota prevent pathogens from colonizing the host by competing with them for nutrients, by producing substances that are harmful to the pathogens, and by altering conditions that affect the survival of

the pathogens, such as pH and oxygen availability. The presence of normal microbiota in the vagina, for example, alters pH, thus preventing overpopulation by *Candida albicans*, a pathogenic yeast that causes vaginitis. In the large intestine, *E. coli* bacteria produce **bacteriocins** that inhibit the growth of *Salmonella* and *Shigella*.

- Commensalism is one organism benefits while the other is unaffected. Most microbes that are part of the commensal microbiota are found on the skin and in the gastrointestinal tract. The majority of such microbes are bacteria that have highly specialized attachment mechanisms and precise environmental requirements for survival. Normally, such microbes are harmless, but they may cause disease if their environmental conditions change. These opportunistic pathogens include *E. coli, Staphylococcus aureus S. epidermids, Enterococcus faecalis,* and oral streptococci.

Question

Q/ Describe the role of the skin and mucous membranes in innate immunity.

Q/ Differentiate physical from chemical factors. and list five examples of each.

Q/ Describe the role of normal microbiota in innate Immunity.

Q/ Identify one physical factor and one chemical factor that prevent Q/ microbes from entering the body through skin and mucous membranes.

Q/ Identify one physical factor and one chemical factor that prevent microbes from entering or colonizing the body through the eyes, digestive tract and respiratory tract.

Q/ Distinguish microbial antagonism from commensalism.

Q/ A 30-year-old female developed vaginal candidiasis (a fungal infection) after receiving antibiotic therapy for a sinus infection . One possible explanation for the fungal infection is antibiotic-induced reduction in vaginal

A. lysozyme secretion.

B. mucus secretion.

C. normal commensal bacteria.

D. pH .

E. RNases and DNases.

Lecture 2:

Antigens and Receptors

Immune responses are initiated by the interaction between a **ligand** and a **receptor** protein on the cell's surface of a soluble receptor. These interactions trigger the activation **white blood cells**. The complementary shapes of the ligand and its receptor are critical. The effectiveness of interaction often increases with the affinity or strength of interaction between ligand and receptor (Fig. 1). Receptors may be :

1- displayed on cell surfaces (e. g . , cell-surface receptors)

2- or may be soluble molecules (e. g . , secreted products of leukocytes) . Ligands may be expressed by :

1- cells as cell-surface molecules (e. g . , on microbes)

2- or as soluble molecules (e.g . , the secreted products of cells).

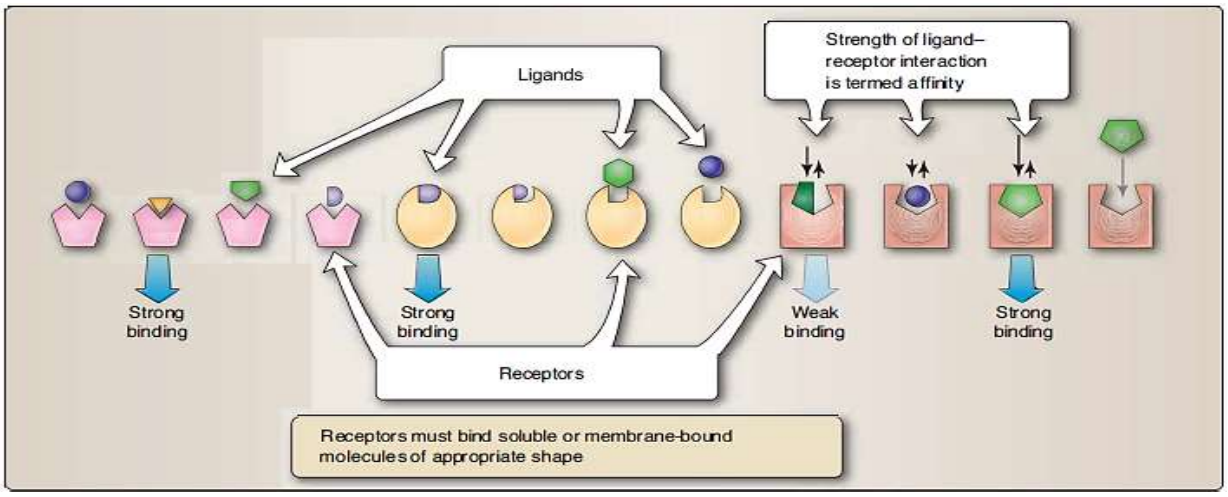


Figure 1: Receptor-ligand interactions. Receptors bind molecules or ligands that may be either soluble or bound to membranes. If the binding is sufficient, the receptor is able to provide a signal to the cell .

Several factors influence the binding of a ligand to a cell-surface receptor: A) The **shape** and B) **charge** affect binding affinity, the collective affinities where multiple receptors may be involved (**avidity**) , C) the **intracellular signals** that are triggered , and D) the presence of other receptors that may also influence the action in question . The context in which cells receive signals can influence whether they respond to those signals (Fig. 2) . Cells must often correlate information from multiple activated receptors, some providing **positive signals** and others providing **negative signals**, to determine what action they will ultimately take.

Antigens

Classically, an antigen is defined as an organism, a molecule, or part of a molecule that is recognized by the immune system . Antigens may be simple or complex, protein, carbohydrate, or synthetic in origin.

A. Epitopes : The basic recognition unit

Antigen receptors recognize discrete regions of molecules called epitopes, the smallest part of an antigen that is "seen" by immune cell receptors (Fig. 3). Different lymphocytes, each with a unique set of receptors, may recognize different epitopes on the same antigen. Depending on the nature of the immune responses they trigger, antigens/epitopes are divided into three broad functional types: **immunogens**, **haptens**, and **tolerogens**.

Immunogens : an immunogen refers to a molecule that is capable of eliciting an immune response by an organism's immune system, whereas an antigen refers to a molecule that is capable of binding to the product of that immune response. All immunogens are antigens, but all antigens may not be immunogens

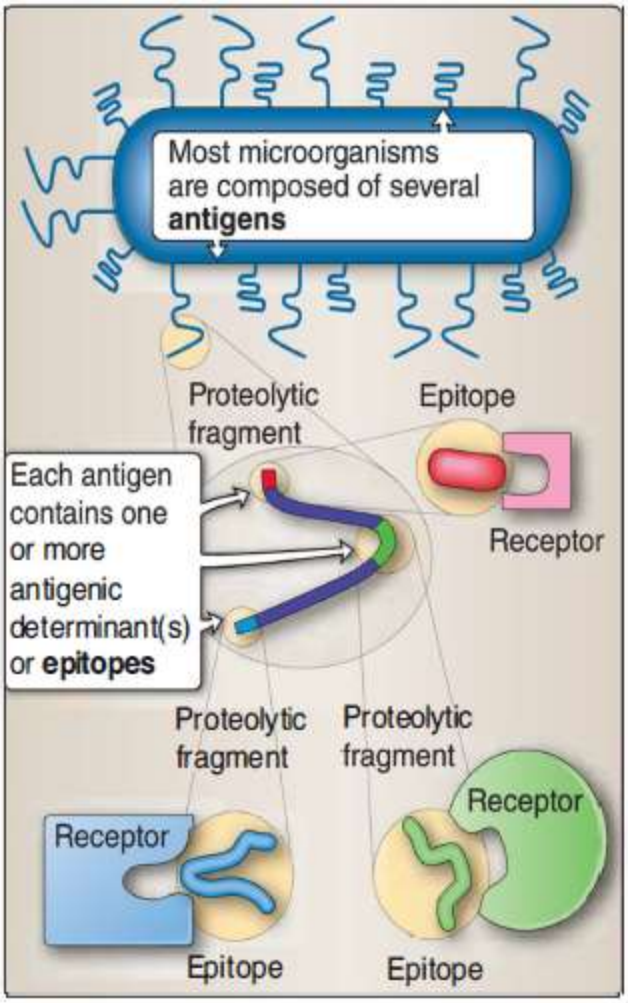


Figure.3 : Epitopes and antigens: degrees of complexity. Complex antigens may contain large numbers of different epitopes.

Haptens

- Haptens are small molecules that are usually of non-biologic origin.
- Haptens are antigens and can bind to immune receptors but cannot by themselves induce a specific immune response and hence are not immunogenic
- when a hapten is chemically bound to an immunogen (also called a carrier) , immune responses may be generated against both the hapten and the epitopes on the immunogen.

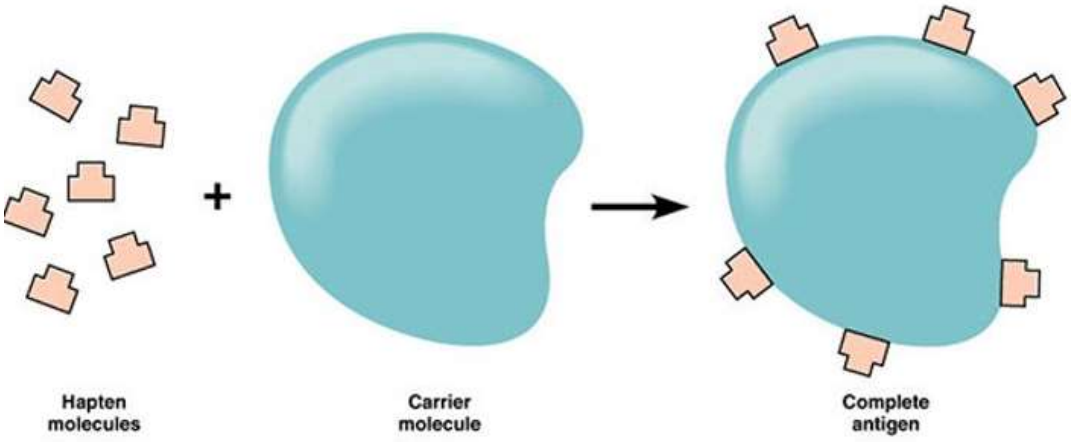


Figure 4: Haptens

Tolerogens

- A foreign antigen that suppresses immune response, or produces immune tolerance.
- Instead of inducing the immune system to be active, the tolerogen binds to the antigen receptor of the lymphocytes in order to suppress it.

B. immunogenicity

Although there are no firm rules for predicting whether a substance is an immunogen prior to exposure to the immune system , there are several guidelines:

• **Size:** Proteins greater than 10 kDa are usually more immunogenic.

• **Complexity:** Complex proteins with numerous, diverse epitopes are more likely to induce an immune response than are simple peptides that contain only one or a few epitopes.

• **Conformation and accessibility**: Epitopes must be "seen by" and be accessible to the immune system .

• **Chemical properties:** A protein immunogen has to be enzymatically cleavable by phagocytes. For example, L-amino acid-containing polypeptides are generally good immunogens, whereas D-amino acid-containing polypeptides are poor immunogens because proteolytic enzymes are able to cleave only the L-forms of amino acids.

Many carbohydrates, steroids, and lipids tend to be poor immunegens. Amino acids and haptens are, by themselves, not immunogenic .

Receptors of immune systems

A. Preformed receptors

The initial defense to an infectious agent comes from elements of the innate immune system that contain **preformed receptors** that allow a quick response. This response confers some protection whereas the adaptive immune system prepares respond.

1- Pattern recognition receptors (PRRs) :

- Receptors of the innate immune system recognize broad structural motifs that found on microbes.
- pattern recognition receptors are present in soluble forms (e.g ., complement proteins) or on host cell surfaces.
- They recognize pathogen-associated molecular patterns (PAM Ps), which include combinations of sugars, some proteins, lipids, and nucleic acids broadly associated with microbes (Fig. 5). PRR binding

to PAMPs triggers various forms of inflammation intended to destroy the pathogens.

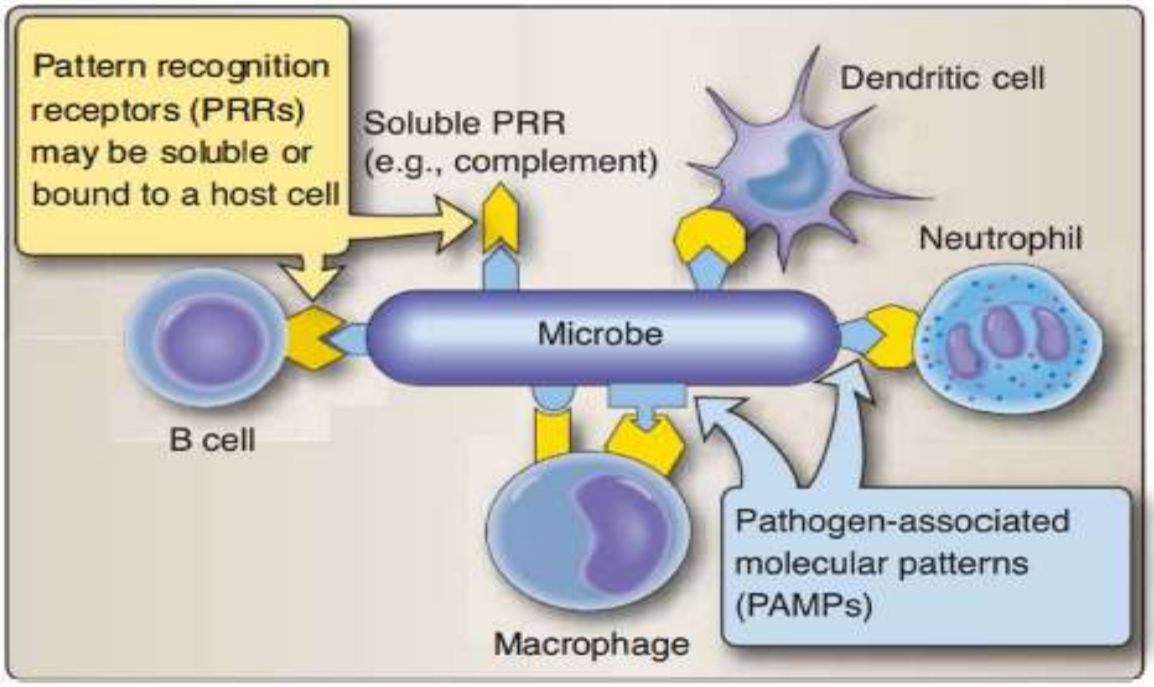


Figure 5: Pattern recognition receptors

2- Toll - like receptors :

- In humans, PRRs also include toll - like receptors (TLRs) that are present on various host cells.
- When triggered by binding to a PAMP on an infectious organism, TLRs mediate the generation of defensive responses that include the secretion of cytokines (immune chemicals secreted by immune cells) to promote inflammation , and the attraction of macrophages, neutrophils, natural killer (N K) cells, and dendritic cells to the site of infection.

3- Killer activation receptors :

- NK cells are part of the lymphocyte that bear receptors that are able to detect alterations in host cells that have been infected by viruses.
- Killer activation receptors (KARs) on NK cells allow them to recognize the presence of stress-related molecules (called MICA and MICB molecules in humans) expressed by host cells that are unhealthy.
- Binding of MICA or MICB molecules by the NK cell's KARs induces the NK cell to attach and destroy the targeted (e.g. , infected) host cell (Fig. 6).

4- Killer inhibition receptors:

- The killer inhibition receptors (KI Rs) , is used by NK cells to monitor major histocompatibility complex (MHC) class I molecules normally displayed on the cell surfaces of all nucleated cells in the body (Fig. 6).
- Many processes, including some cancers and some types of viral infection, decrease the number of MHC class I molecules displayed on the surface of the affected cell .
- Via KIRs , if NK cells determine that the level of MHC class I is subnormal , they kill the target cell . If they determine that normal levels are present, the killing process is terminated and the target cell is released unharmed.

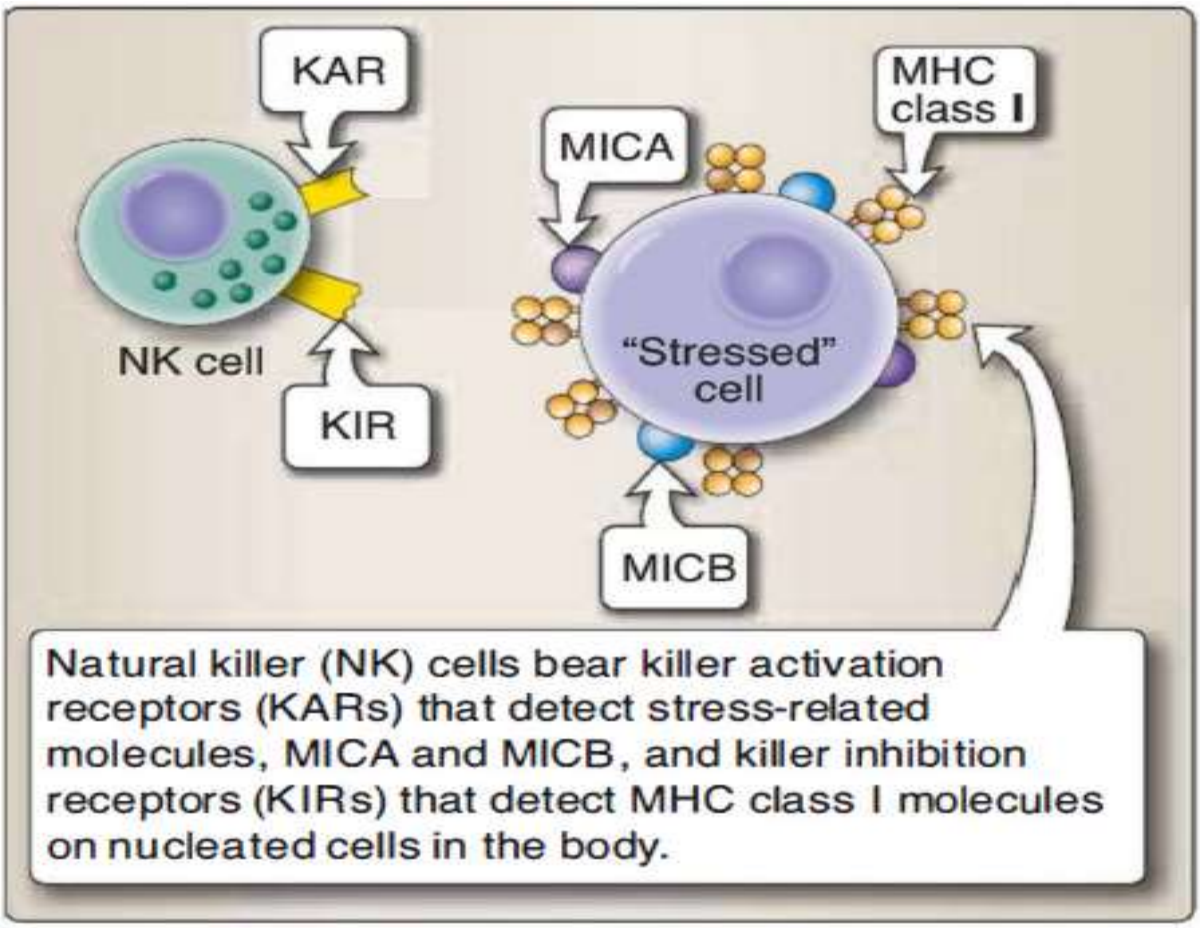


Figure 6. Killer-cell activation receptors (KARs) and killer-cell inhibition receptors (KI Rs).

5. Complement receptors:

- The complement system is a complex set of soluble molecules that generate various reactions that attract immune cells to the site of infection and lead to destruction of microbes.
- Cell-surface bound complement receptors on **phagocytic cells** and **B cells** recognize these bound complement fragments and facilitate the binding, ingestion, and internal degradation of the tagged microbes (Fig. 7).

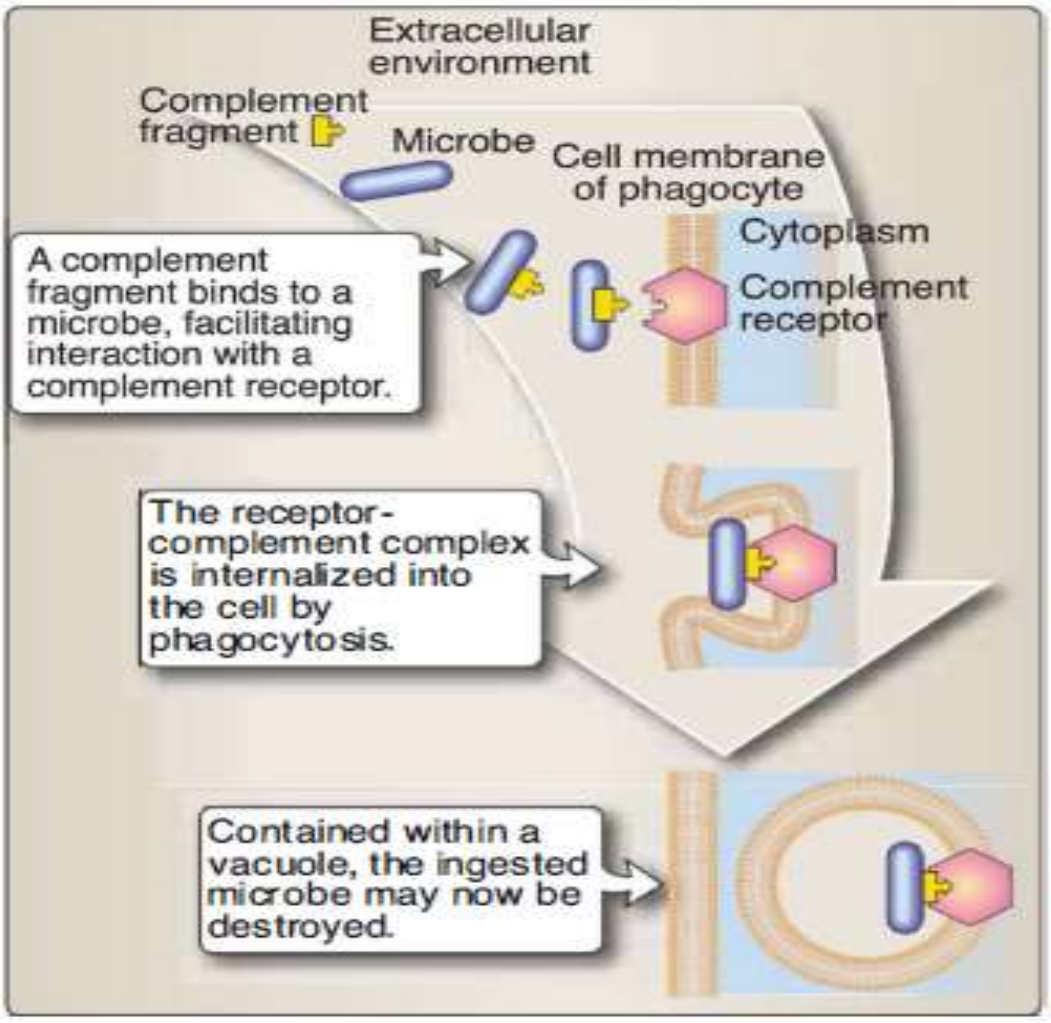


Figure 7: Complement receptors. Binding by complement receptors on phagocytic cells facilitates binding, ingestion, and destruction of microbes.

6 . Fc receptors:

- Immunoglobulins are classified as **lgA** (immunoglobulin A) , **lgD**, **lgE**, **lgG**, and **lgM** based on their structure.
- Epitope binding by lgA, lgG, or lgM antibodies triggers a conformational change **Fc portion** of the antibody.
- Fe receptors (FcRs) are expressed on the surfaces of phagocytic cells (Fig. 8 recognize epitope-engaged antibodies (recognizable by the altered conformation of the Fe region), which leads to the phagocytosis of the epitope-antibody-FcR complex.

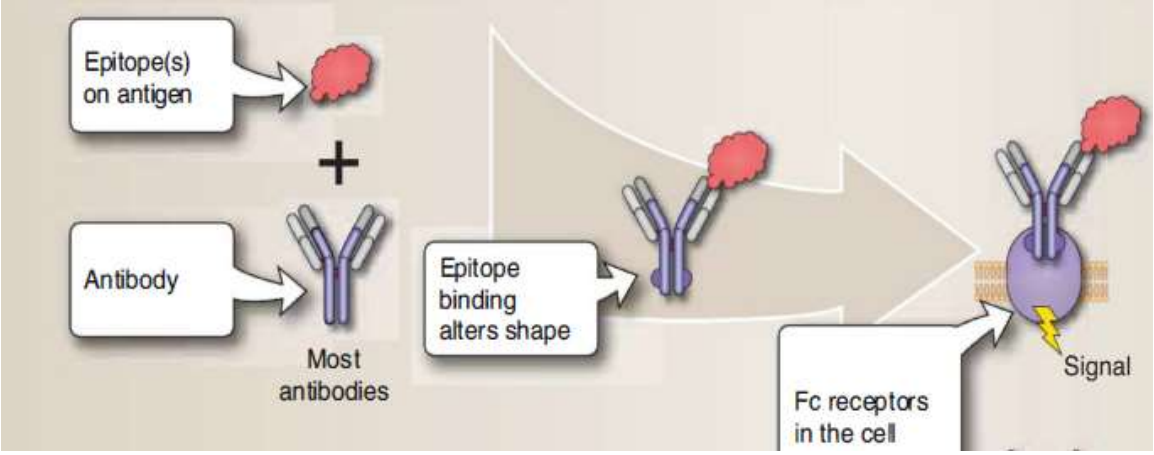


Figure 8 : Fc receptors. Like complement receptors, Fc receptors permit phagocytes to identify and ingest microbes and molecules that antibodies have previously "tagged" for destruction.

B. Somatically generated receptors

The preformed receptors of the innate immune system (e.g. , PRRs, TLRs, and complement) are encoded in the **germline** and passed on intact from one generation to the next. In contrast, the specialized receptors of B cells and T cells of the adaptive immune system are regenerated anew in the lymphocytes of each individual through **random somatic chromosomal rearrangements** and **mutations**. The result is a vast array of receptors specific for precise molecular details found in unique epitopes that may be encountered in the future.

1. B-cell receptors :

- B-cell receptors (BCRs) are cell-surface bound monomeric immunoglobulin associated with disulfide-linked heterodimers called **lgα and lgβ** (Fig. 9).
- When a BCR binds an epitope, the specialized cytoplasmic tails of lgα and lgβ initiate an intracellular signaling cascade that may lead to **B-cell activation**.

- In addition, some activated B cells terminally differentiate into **plasma cells**, which secrete immunoglobulins that have the same epitope-binding specificity as their BCR.

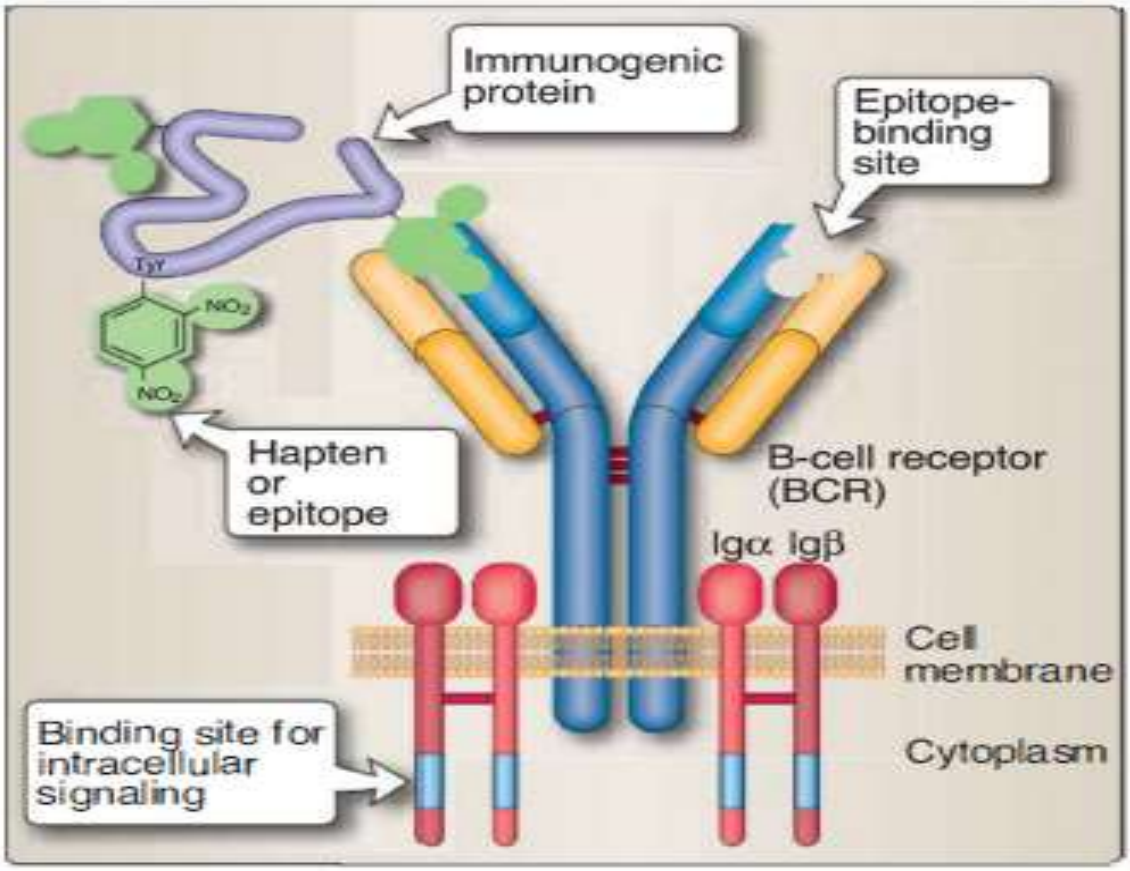


Figure 9: Immunoglobulins serve as B-cell receptors (BCRs) . B cells bear receptors that are composed of two identical large (heavy) chains and two identical smaller (light) chains. Molecules such as lgα and lgβ are associated with BCRs and help provide a signal to the cell when the BCR binds an epitope.

2. T-cell receptors :

- Structurally similar to immunoglobulin molecules.
- T-cell receptors (TC Rs) are heterodimers, consisting of either an a αβ or γδ chain pair (Figure. 10) .

- TCRs are always membrane bound and recognize fragments of antigen as peptides bound to MHC molecules.

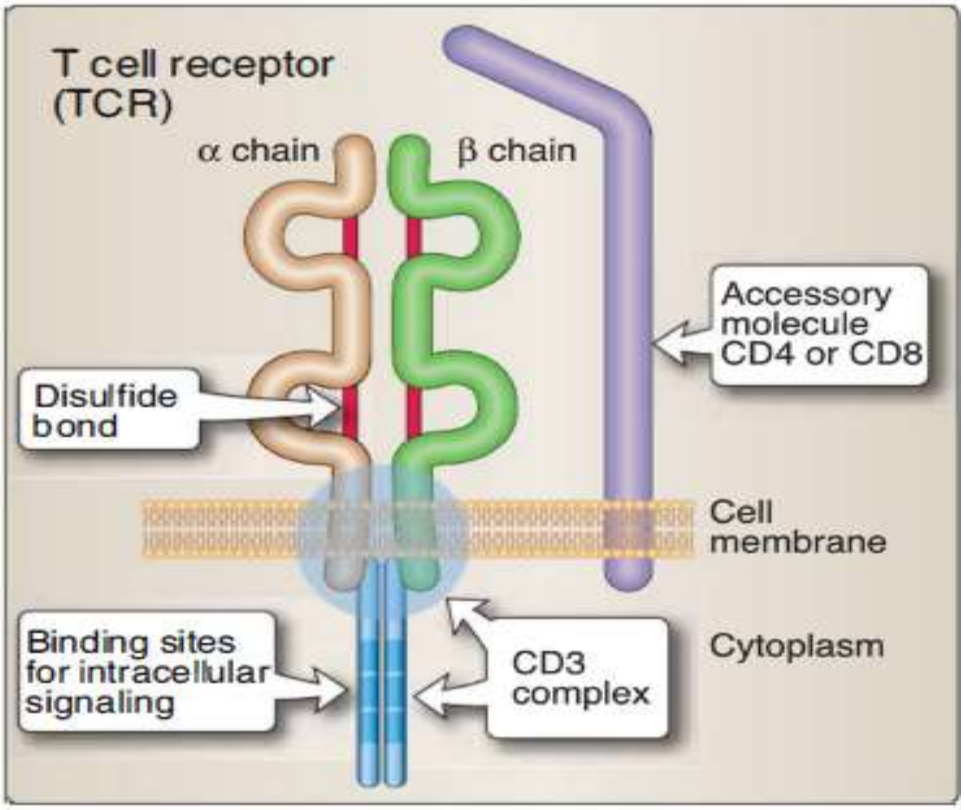


Figure 10. a αβ T-cell receptors (TCRs) . T cells bear receptors that are composed of two chains, either an a αβ combination (shown) or γδ combination. The **CD3** complex is associated with the TCR and facilitates cell signaling .

Lec 3

Cells of the Innate Immune System

White blood cells or leukocytes serve as guards and defenders against infection by patrolling the tissues and organs of the body. They move around

the body via the lymphatic and blood circulatory systems and can leave and reenter the circulation to move through body tissues.

Leukocytes are classified by: **A) morphology**, including the number of lobes that their nuclei possess **B)** the **presence or absence** of microscopically visible **granules** in their cytoplasm (Fig. 1).

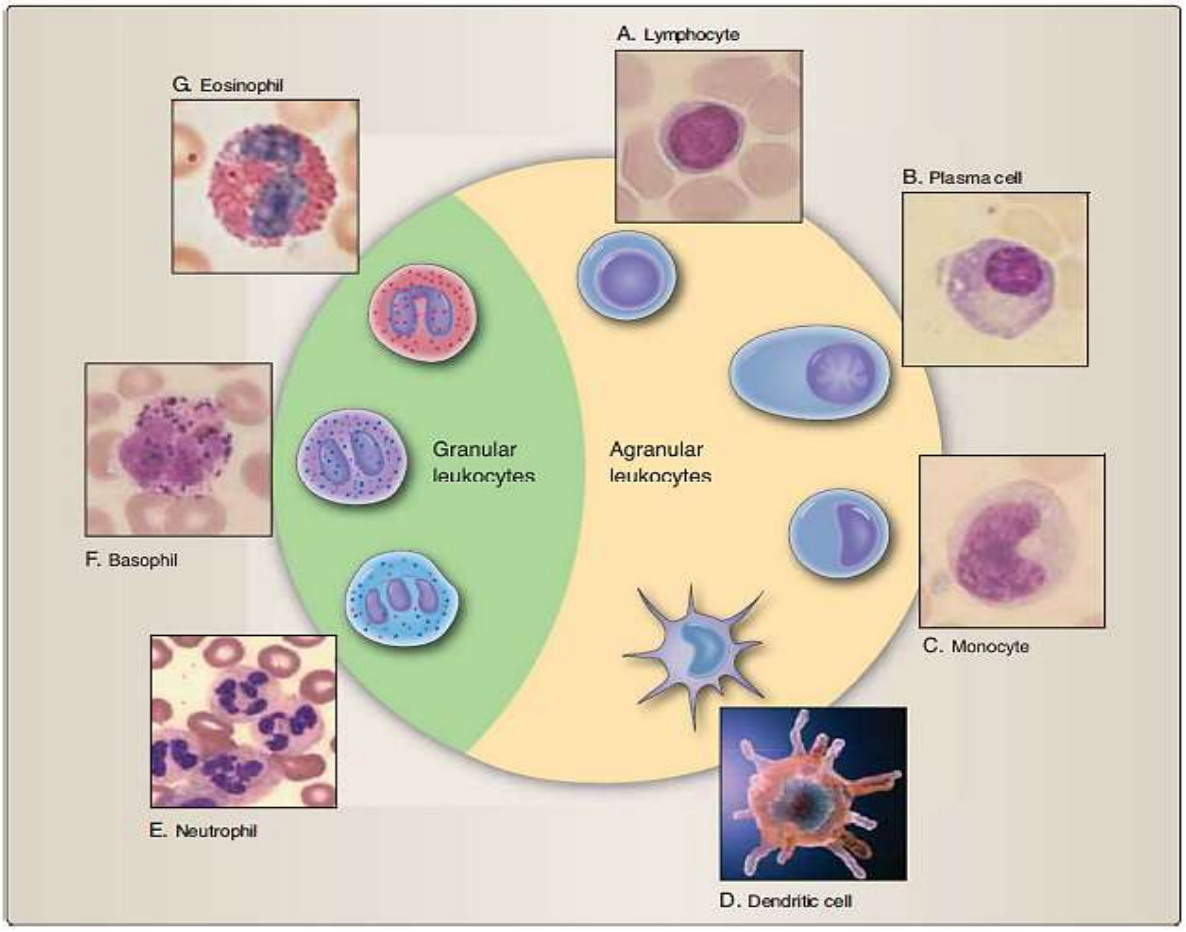


Figure1: Types of leukocytes.

All blood-borne cells ultimately derive from **pluripotent hematopoietic stem cells**. They are called **pluripotent** because each stem cell has the capacity to produce all **leukocytes** as well as **red blood cells** and **platelets**.

Agranular Leukocytes

White blood cells that have multilobed nuclei and contain cytoplasmic granules are known as **granulocytes** . Others with a single, un-lobed nucleus and cytoplasm that contains few or no granules are known as **agranular leukocytes**. Agranular leukocytes derive from lymphoid or myeloid lineage precursors and account for approximately **35% to 38%** of the leukocytes in circulation.

A. Lymphoid lineage cells

Cells that differentiate along one of several of the lymphocytic pathways are known as **lymphocytes** and comprised : (see Fig. 2)

1) B lymphocytes (B cell) reside in the **bone marrow** and are able to synthesize immunoglobulin molecules. **B cells** can further differentiated into **plasma cells** , are the only cells that are capable of immunoglobulin synthesis.

2) T lymphocytes (T cell) of bone marrow origin migrate to, differentiate, and are vetted with in the environment of the **thymus** .

3) Natural killer (N K) cell : These large, nonphagocytic, granular lymphocytes are named for their ability to kill abnormal (e. g., infected or malignant) host cells. They account for **5 % to 10%** of all lymphocytes in the circulation.

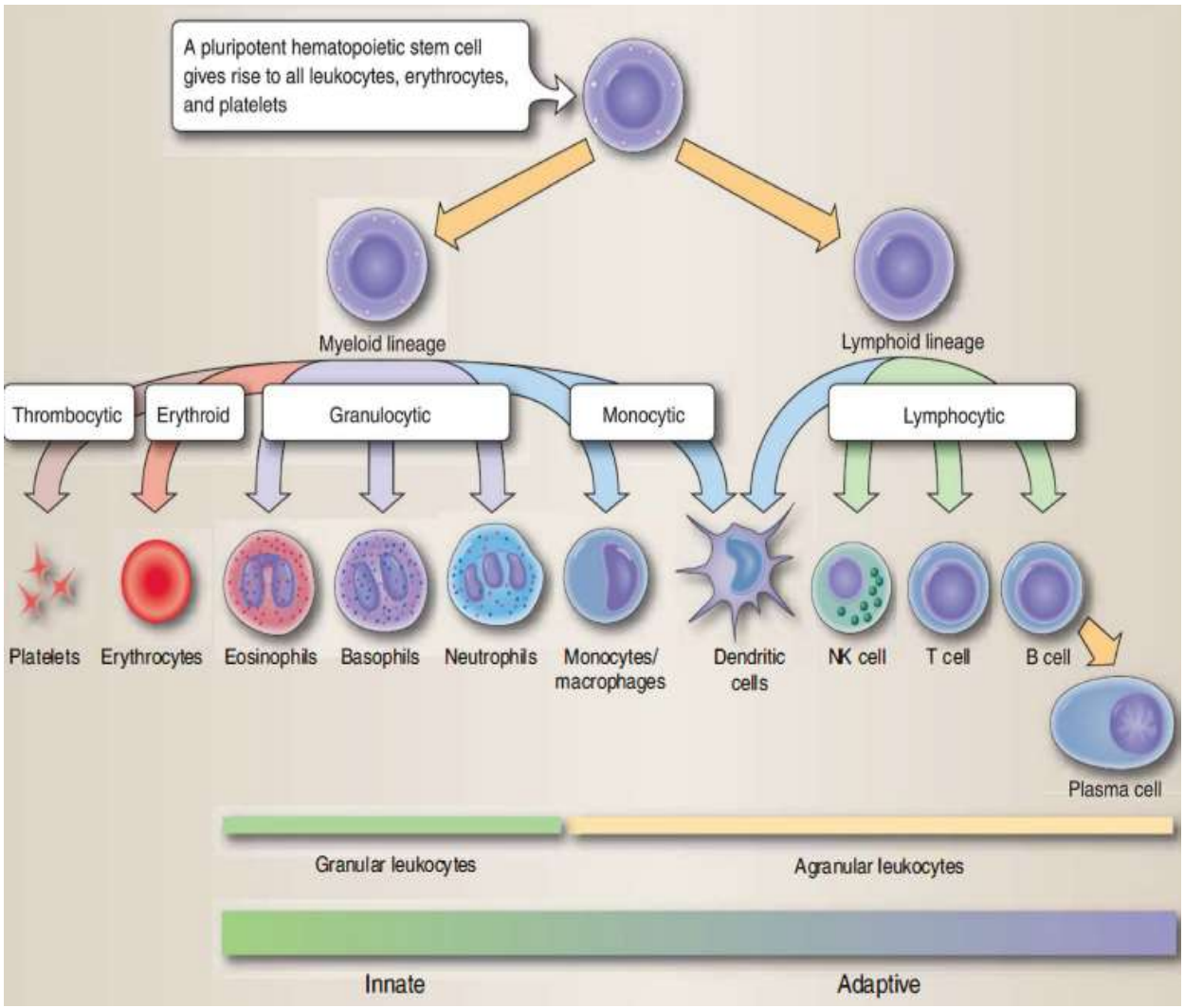


Figure 2: Hematopoietic lineages.

B. Monocytic lineage cells

Mononuclear cells that differentiate from **myeloid precursors** are known as monocytes in the circulation or macrophages once they leave the circulation and enter the tissues. Another group of phagocytic cells with both **myeloid** and **lymphoid** origins is collectively known as **dendritic cells** .

1. Monocytes and macrophages : Monocytes are large mononuclear cells and account for approximately 5% to 7% of the leukocytes in the peripheral blood (Fig. 4.4). Monocytes spend 1 to 2 days in the circulation, then enter

tissues throughout the body, where they reside for up to several months as **macrophages**. Both monocytes and macrophages actively sample their environment by phagocytosis and serve as scavengers to remove cellular debris. Ingested materials are enzymatically degraded.

2. Dendritic cells: Found throughout the body but predominantly in portals of microbial entry (e. g . , skin, lung, gastrointestinal tract), these cells are named for their **branchlike cytoplasmic projections** . dendritic cells engulf cells and particles in their environment by phagocytosis .

Granular Leukocytes

Leukocytes that contain **cytoplasmic granules** are known as **granulocytes**. These cells have multilobed nuclei and cytoplasmic granules that contain amines (stained by basic dyes) , basic proteins (stained with acidophilic or eosinophilic dyes) , or both (neutral staining) .

A. Neutrophils

- Comprising approximately **60%** of the peripheral blood leukocytes.

- They are also called **polymorphonuclear** (PMN) cells because of their of variable number nuclear segments (two to five) .

- A half-life of approximately 7 hours, more than 100 billion neutrophils enter the circulation daily in normal adults.

- Neutrophils are very effective at killing **bacteria**. An increase in the number of peripheral blood neutrophils is often an indication of acute infection.

- They require about **2 weeks** to mature from **metamyelocytes** through intermediate stages and become **mature-segmented neutrophils**.

B. Basophils and mast cells

- The acidic cytoplasmic granules of basophils contain vasoactive amines (e.g . , histamine) that cause smooth muscle contraction and are readily stained with "base-loving" dyes.

- These bilobed cells are found in low numbers in the peripheral blood (0% to 1 %) or in their tissue resident form, known as **mast cells**. Both basophils and mast cells are important in **allergic reactions** of the adaptive immune response.

C. Eosinophils

- So named because of their "**eosin-loving**" granules (eosin is a dye used in histology) .

- eosinophils are bilobed granulocytes with cytoplasmic granules that contain basic proteins.

- Although they comprise **0% to 5%** of the peripheral blood leukocytes, eosinophils are active participants in innate and adaptive immune responses to **parasitic helminth** (worm) infections.

Lec 4

Innate Immune Function

If microbes should penetrate the body's first line of defense (the mechanical, chemical , and biological barriers) the innate immune system provides the second line of defense (the first immunologic line of defense) against infection.

Innate immune responses include :

1. the rapid destruction of an infectious organism

2. activation of phagocytic cells, and

3. the localized protective response known as **inflammation**.

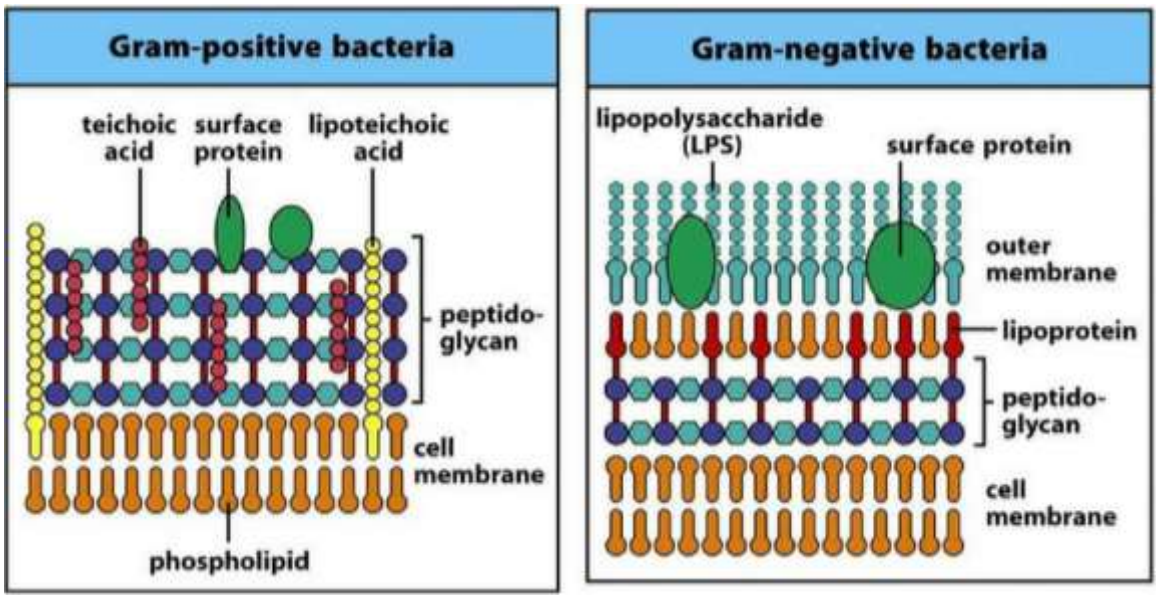
I. Recognition

The innate immune system uses a limited number of **pattern recognition receptors (PRRs)** to recognize **pathogen-associated molecular patterns (PAMPs)** . the genes encoding **PRRs** are encoded within the genome and require no additional modification. Because the host does not produce PAMPs, the innate immune system is able to distinguish between self and non-self.

Pathogen-associated molecular patterns (PAMPs)

PAMPs may be **sugars**, **proteins**, **lipids**, **nucleic acids**, or combinations of these types of molecules. Two common bacterial products that contain PAMPs are **lipopolysaccharide** and **peptidoglycan** . Bacterial **lipopolysaccharide (LPS)** is a major component of the outer cell membrane of gram-negative bacteria. Cell-surface molecules on monocytes, macrophages, dendritic cells, mast cells, and intestinal epithelial cells bear **toll-like receptor 4** (TLR4) that bind **LPS**.

Peptidoglycans are major components of the cell walls of gram-positive bacteria and are recognized by **TLR2 receptors** on host phagocytic cells .



ll. Soluble Defense Mechanisms

In addition to the actions of whole cells, the innate immune system employs **soluble molecules** as weapons for : **1) protection from viral infection**, **2) lytic destruction of microbes**, or **3) increasing the susceptibility of microbes to ingestion by phagocytic cells**.

A. Type I interferons

Type I interferons (IFNs) are produced by 1) a subset of dendritic cells (**IFN-α**) , 2) by fibroblasts (**IFN-β**) , 3) and by other cells in response to viral infection (Fig. 2). IFN-α and IFN - β are rapidly produced, within 5 minutes, by cells when **viral PAMPs** interact with certain PRRs.

Secreted **type I IFNs** induce both virally infected and non-infected cells to activate numerous antiviral defenses including **RNA-dependent protein kinase (PKR)** and **apoptotic (programmed cell death)** pathways.

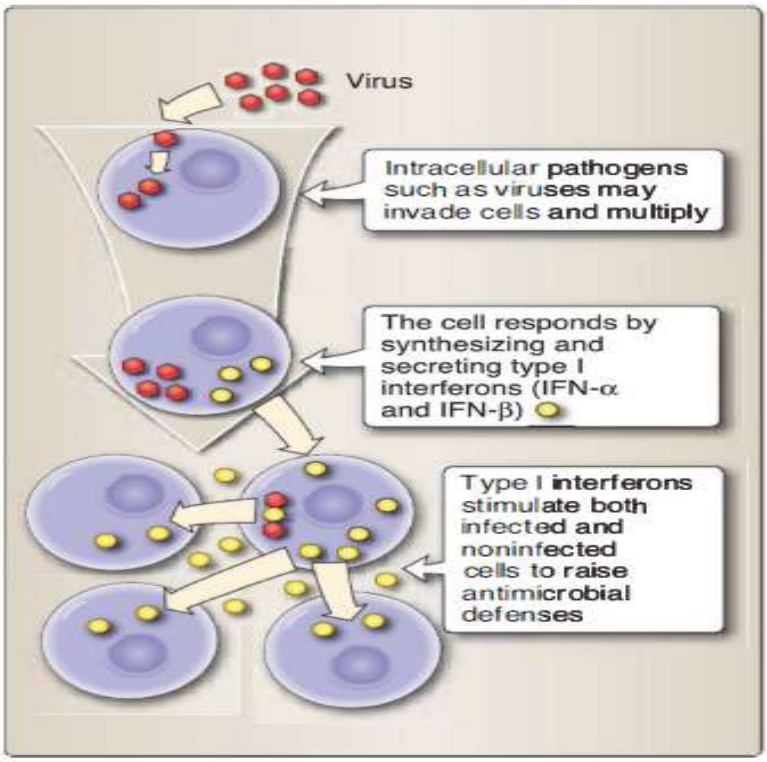


Figure 2. Type 1 interferon response to intracellular microbial invasion. Some cells respond to infection by producing and secreting type I interferons that signal adjacent cells to activate their antimicrobial defenses.

B. Microcidal molecules

Various cells, including **epithelial cells**, **neutrophils**, and **macrophages**, in the **skin** and **mucous membranes** secrete peptides called **defensins**. These peptides form channels in the cell membranes of bacteria, which cause eventually bacterial death. Other molecules with microcidal functions include **cathelicidin** , **lysozyme**, **DNases** and **RNases**, and others.

C. Complement

- The complement system is a defensive **system consisting of over 30 proteins** produced by the **liver** and found circulating in **blood serum** and within **tissues** throughout the body. The complement system is so-named because it "complements" the cells of the immune system in destroying microbes.

- Complement is a collective term for a system of enzymes and proteins that function in both the innate and adaptive branches of the immune system as soluble means of protection against pathogens that evade cellular contact.

- In the innate immune system , complement can be activated in two ways: via the **alternative pathway**, in which antigen is recognized by particular characteristics of its surface, or via the **mannan-binding lectin** (MBL) pathway. Complement can also be activated in the adaptive immune system via the **classical pathway** that begins with antigen-antibody complexes (Fig. 3).

- Regardless of the pathway of activation, **functions of complement** include : 1- lysis of bacteria, cells, and viruses

2- promotion of phagocytosis (opsonization)

3- triggering of inflammation and secretion of immunoregulatory molecules

4- clearance of immune complexes from circulation .

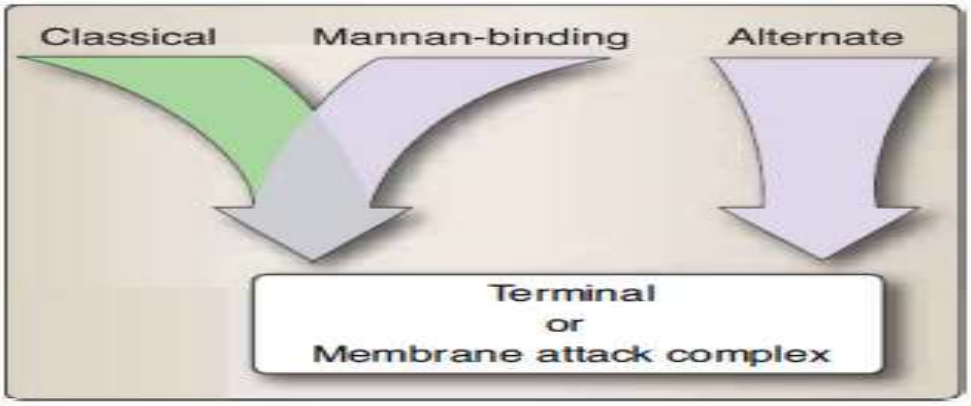


Figure 3. Three complement pathways lead to formation of the membrane attack complex.

1 . The alternative pathway is initiated by cell-surface components that are recognized as foreign to the host, such as **LPS** (Fig. 4). Various enzymes (e.g . , kallikrein , plasmin , elastase) cleave **C3** (the most abundant serum complement component) into several smaller fragments. One of these, the continuously present, short-lived, and unstable **C3b fragment**, is the major opsonin of the complement system and readily attaches to receptors on cell surfaces .

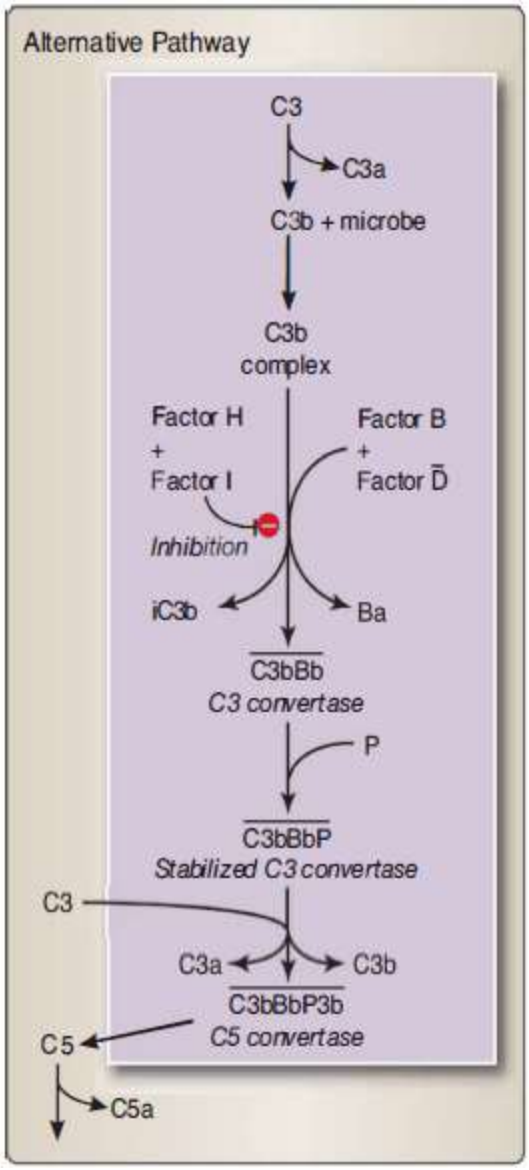


Figure 4 . Alternative pathway of complement activation. Beginning with the binding of C3b to a microbial surface, this pathway results in an amplified production of C3b and formation of a C5 convertase.

1 . C3b binds Factor B .

2. Factor B in the complex is cleaved by Factor D to produce **C3bBb**, an unstable C3 convertase.

3. Alternatively, C3bBb binds properly in (Factor P) to produce

stabilized C3 convertase, **C3bBbP**.

4. Additional C3b fragments join the complex to make **C3bBbP3b**, also known as **C5 convertase**. C5 convertase cleaves **C5** into C5a and C5b.

5. C5b inserts into the cell membrane and is the necessary step leading to formation of the membrane attack complex (MAC) and cell lysis.

2**. Mannan-binding lectin pathway:**

Lectins are proteins that bind to specific **carbohydrates**. This pathway is activated by binding of mannan-binding lectin (MBL) to mannose-containing residues of glycoproteins on certain microbes (e.g . , *Listeria* spp. , *Salmonella* spp. , *Candida albicans*) . MBL is an acute phase protein, one of a series of serum proteins whose levels can rise rapidly in response to infection, inflammation, or other forms of stress. MBL, once bound to appropriate mannose-containing residues, can interact with **MBL-activated serine protease** (MASP) . Activation of MASP leads to subsequent activation of components C2, C4, and C3 (Fig. 5) .

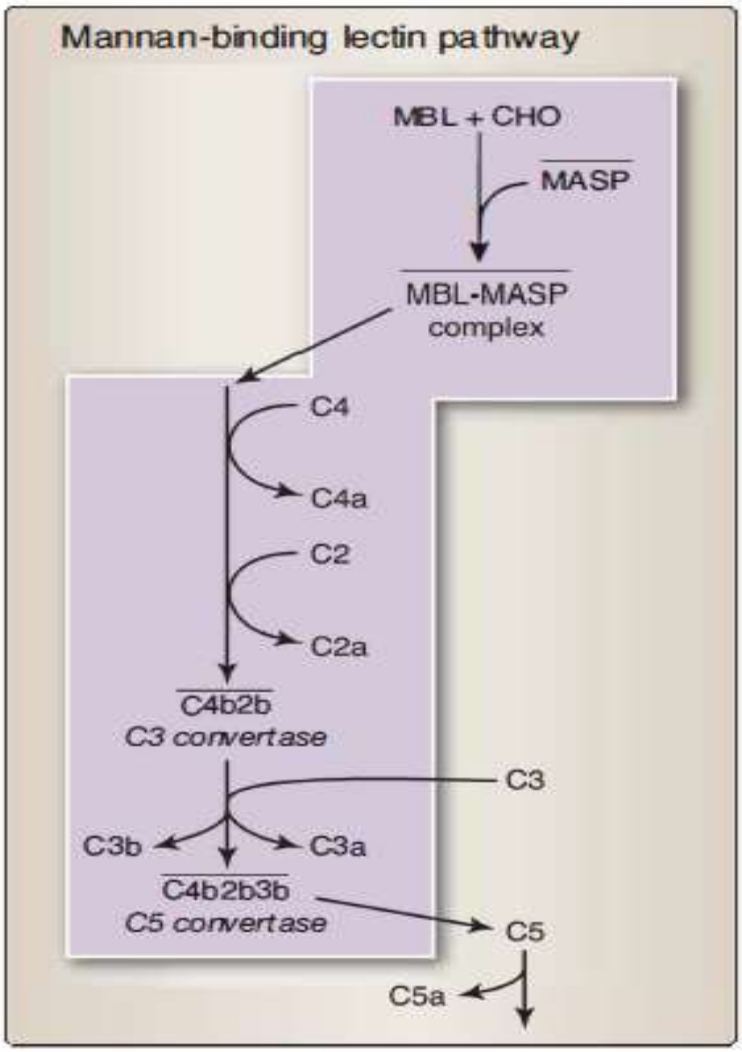


Figure 5. Mannan-binding lectin (MBL) pathway of complement activation. The lectin binding pathway is initiated by the binding of certain glycoproteins commonly found on microbial surfaces, and results in the formation of a C3 convertase (that acts to produce C3b) and a C5 convertase (that can lead to MAC formation).

Classical pathway of complement

Interaction of **antibody** with **antigen** initiates the classical pathway of complement activation . This biochemical cascade of enzymes and protein fragments facilitates destruction of microbes by :

- the membrane attack complex (MAC)

- by increased opsonization through C3b binding of microbial surfaces and

- by the production of anaphylotoxins C3a, C5a, and C4a. The cascade begins with the activation of component C1 .

1. Activation of C1

Binding of lgM or lgG antibody to antigen causes a conformational change in the Fc region of the immunoglobulin molecule. This conformational change enables binding of the first component of the classic pathway, **C1q** . Upon binding to antibody, **C1q** undergoes a conformational change that leads to the sequential binding and activation of the serine proteases **C1r** and **C1s**. The **C1qrs** complex has enzymatic activity for both C4 and C2 (Fig.6)

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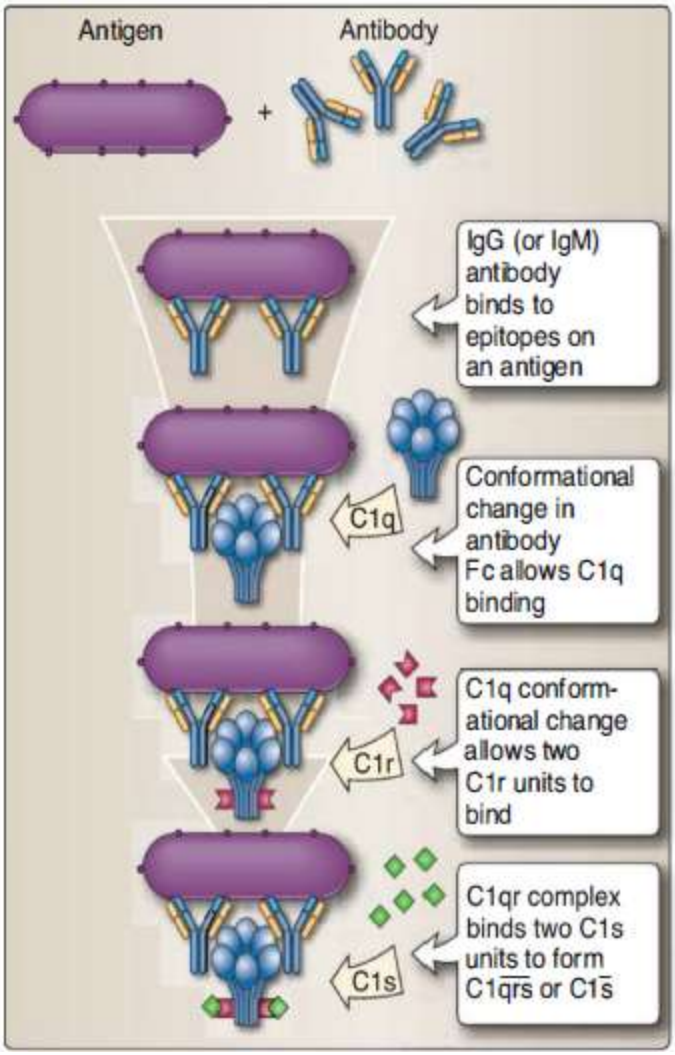


Figure 6. Activation of complement component C1 . Activation of C1 involves serial binding and activation of its three subunits (C1q , C1 r, C1s) .

2. Production of C3 convertase

Activation of **C1qrs** leads to the rapid cleavage and activation of components **C4, C2, and C3**. In fact, both the classical and mannan-binding lectin (MBL) pathways of complement activation are identical in the cleavage and activation of C4, C2, and C3 .

3. Production of C5 convertase

The binding of **C4b2b** to **C3b** leads to the formation of the **C4b2b3b** complex. This complex, a **C5 convertase** initiates the construction of the membrane attack complex on the microbial surface . Thus, as in the case of the alternative and MBL pathways, production of **C5 convertase** by the classical pathway leads to the development and insertion of a structure that is capable of damaging the cell surfaces.

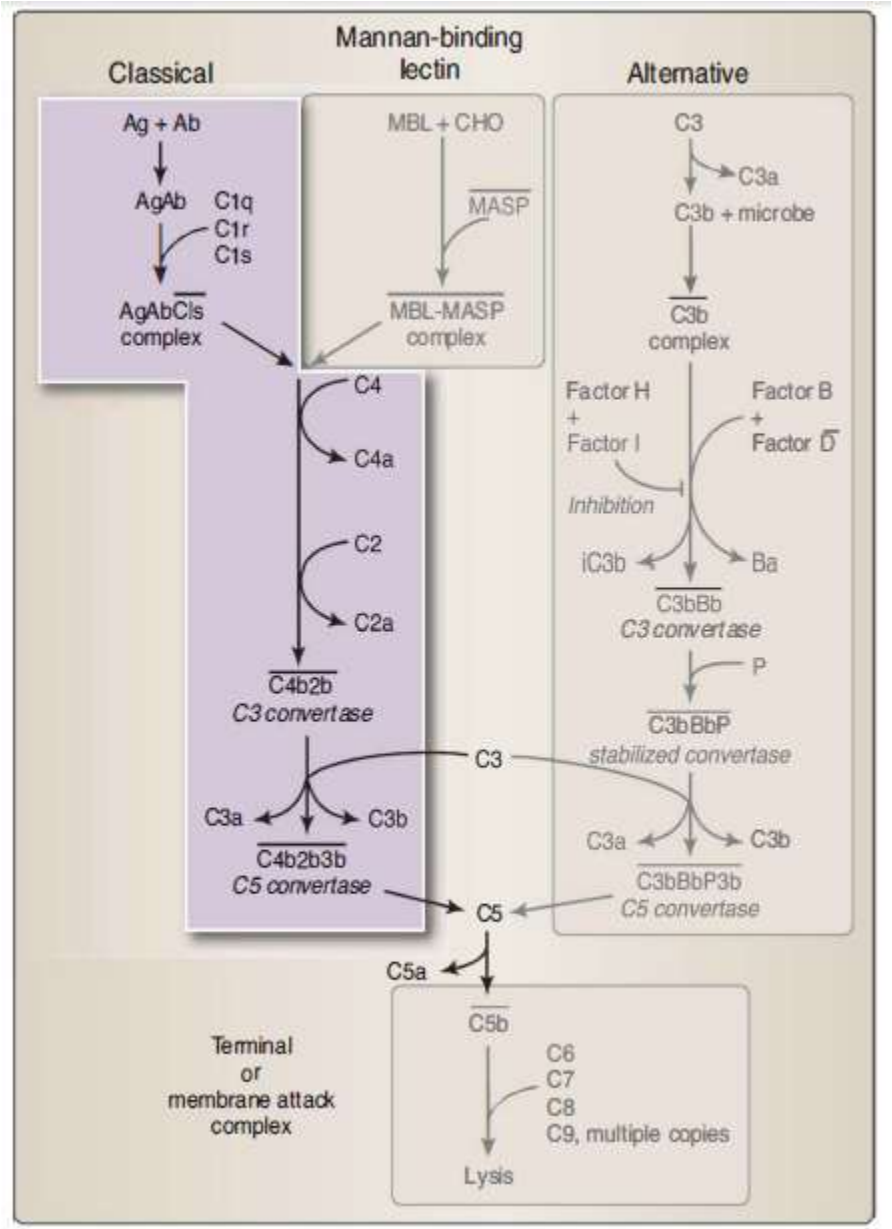


Figure 6.5. Classical pathway of complement activation. The classical pathway is initiated by the binding of antibody (usually lgG or lgM) to an antigen and then to the C1 complement component. The pathway produces a **C3 convertase** (responsible for the cleavage of C3 into its component parts: C3a, C3b, etc.) and a **C5 convertase** (that can lead to MAC formation).

Anaphylotoxins: The small fragments (C3a, C4a, C5a) generated by the cleavage of C3 and C5 in the alternative pathway and of C3, C4, and C5 in the MBL pathway act as anaphylotoxins.

Anaphylotoxins attract and activate different types of leukocytes . They draw additional cells to the site of infection to help eliminate the microbes. C5a has the most potent effect, followed by C3a and C4a.

D. Cytokines and chemokines

Cytokines are secreted by leukocytes and other cells and are involved in innate, adaptive immunity, and inflammation . Cytokines are involved in a wide array of biologic activities ranging from chemotaxis to activation of specific cells to induction of broad physiologic changes.

Chemokines are a subgroup of cytokines of low molecular weight and particular structural patterns that are involved in the chemotaxis (chemical-induced migration) of leukocytes.

Lecture 5

IV. Cellular Defense Mechanisms

In addition to soluble means of defense, the innate immune system employs cellular mechanisms to combat infection. Receptors that recognize ligands from pathogens trigger inflammation and destruction of microbes by

phagocytes. In addition, NK cells detect and destroy host cells that have been infected, injured, or transformed.

A. Phagocytosis

Phagocytosis is the **engulfment** and **degradation** of microbes and other particulate substance by cells such as **macrophages**, **dendritic cells**, **neutrophils**, and even **B lymphocytes** (prior to their activation). These cells are part of the body's "cleansing" mechanism. They not only defend the body by ingesting microbes, but also remove cellular debris and particulate substance that arise from normal physiologic functions.

Some macrophages, called **fixed macrophages**, are resident in certain tissues and organs of the body. Fixed macrophages are found in the liver (**Kupffer's cells**), lungs (**alveolar macrophages**), nervous system (**microglial cells**).

Phagocytosis involves cell-surface receptors associated with specialized regions of the plasma membrane called **clathrin-coated pits**. Dendritic cells use an additional mechanism to sample large amounts of soluble molecules, a process known as **macropinocytosis**. This process does not involve clathrin. Instead , plasma membrane "ruffles" or projections fold back on the membrane to engulf extracellular fluids in large intracellular vesicles.

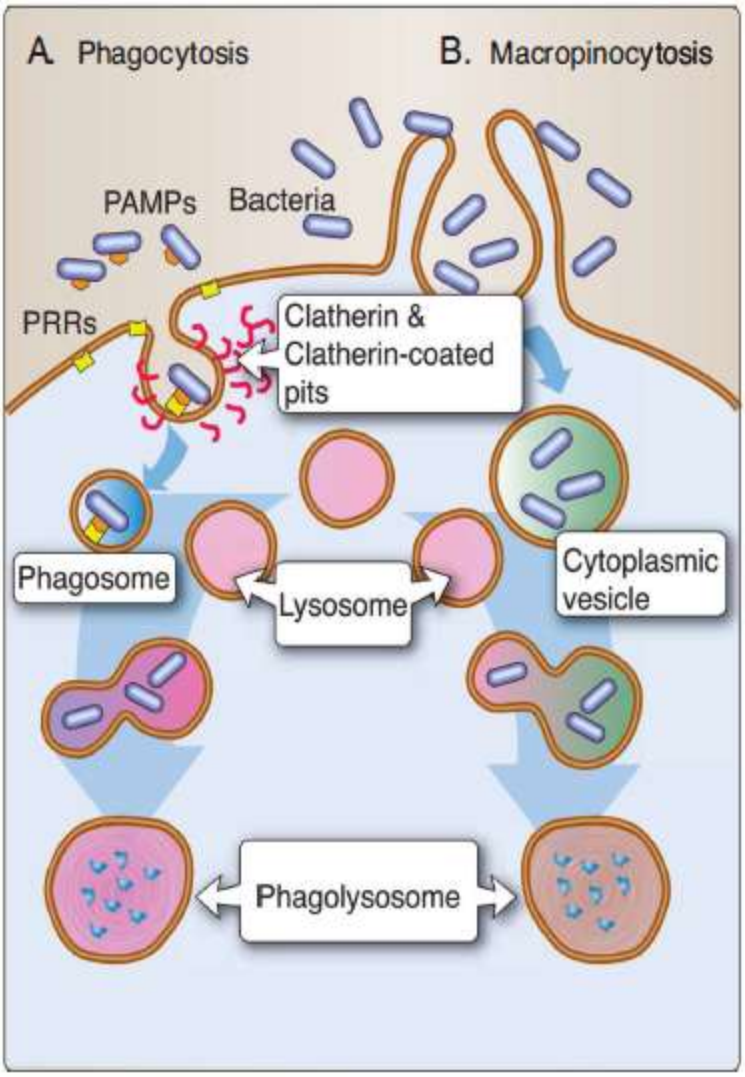


Figure : Phagocytosis, phagosome, and phagolysosome formation. **A.** In phagocytosis, molecules and particles are captured and ingested by receptors associated with membrane regions called clathrin-coated pits. **B.** In macropinocytosis, protrusions of the plasma membrane capture extracellular fluids whose contents are subsequently ingested. In both cases, the ingested material is degraded in phagolysosomes.

The Mechanism of Phagocytosis

phagocytosis will divide into four main phases:

1- Chemotaxis

Chemotaxis is the chemical attraction of phagocytes to microorganisms. Among the chemotactic chemicals that attract phagocytes are **microbial products**, **components of white blood cells** , **damaged tissue cells**, **cytokines** released by other white blood cells, and **peptides derived from complement.**

2- Adherence

Adherence is the attachment of the phagocyte's plasma membrane to the surface of the microorganism or other foreign material. Adherence is facilitated by the attachment of pathogen-associated molecular patterns (PAMPs) of microbes to receptors, such as Toll –like receptors (TLRs), on the surface of phagocytes. The binding of PAMPs to TLRs not only initiates phagocytosis, but also induces the phagocyte to release specific cytokines that recruit additional phagocytes.

Microorganisms can be more readily phagocytized if they are first coated with certain serum proteins that promote attachment of the microorganisms to the phagocyte. This coating process is called **opsonization**. The proteins that act as **opsonins** include some components of the complement system and antibody molecules.

3-Ingestion

During this process, the plasma membrane of the phagocyte extends projections called **pseudopods** that engulf the microorganism. Once the microorganism is surrounded, the pseudopods meet and fuse, surrounding the microorganism with a sac called a **phagosome**, or **phagocytic vesicle**.

The membrane of a phagosome has enzymes that pump protons (**H+**) into the phagosome, reducing the pH to about 4. At this pH, hydrolytic enzymes arc activated.

4- Digestion

In this phase of phagocytosis, the phagosome pinches off from the plasma membrane and enters the cytoplasm. Within the cytoplasm, it contacts **Lysosomes** that contain digestive enzymes and bactericidal substance on contact, the phagosome and lysosome membranes fuse to form a single larger structure called a **phagolysosome**.

Intracellular killing:

Intracellular killing is done by two mechanisms:

- **Oxygen dependent mechanisms**

Oxygen is converted to the following products which kill the microbe: Lysosomes also contain enzymes that can produce **toxic oxygen** products such as **superoxide radical (O^{2-})** , hydrogen peroxide (H_2O_2), nitric oxide (NO), singlet oxygen, and hydroxyl radical (OH•)

- **Non oxygen dependent mechanisms**

The microbe is killed by many hydrolytic and proteolytic enzymes such as lysozyme which hydrolyzed peptidoglycan in bacterial cell walls. A variety of other enzymes, such as lipases, proteases, ribonuclease, and deoxy ribonuclease, hydrolyze other macromolecular components of microorganisms.

B. Natural Killer Cell Responses

NK cells detect aberrant host cells and target them for destruction . NK cells possess **killer activation receptors** (KAR) that recognize stress-associated binding occurs, the NK cell will kill the target host cell . Sufficient binding by the KI Rs will override the KAR kill signal, and the host cell will be allowed to survive . molecules, including **MICA** and **MICB** in humans, which appear on the surface of infected and transformed host cells. Binding of KAR to MICA and MICB generates a kill signal. Before proceeding to kill the targeted cells, however, NK cells use killer inhibition receptors (KIR) to assess MHC I molecules on the target cell surface. Some viruses and malignant events often depress expression of these molecules. If insufficient levels of KIR-MHCI

Microbial Evasion of Phagocytosis

The ability of a pathogen to cause disease is related to its ability to **evade phagocytosis**. Some bacteria have structures that inhibit adherence, such as the **M protein** and **capsules**.

- **M protein** of *Streptococcus pyogenes* inhibits the attachment of phagocytes to their surfaces and makes adherence more difficult.

- Organisms with large **capsules** include *Streptococcus pneumoniae* and *Haemophilus influenzae* type b. Heavily encapsulated microorganisms like these can be phagocytized only if the phagocyte traps the microorganism against a rough surface, such as a **blood vessel**, **blood dot**, or **connective tissue fiber**, from which the microbe cannot slide away.

- Other microbes may be ingested but not killed. For example, *Staphylococcus* produces **leukocidins** (pore-forming toxins) that may kill phagocytes by causing the release of the phagocyte's own lysosomal enzymes into its cytoplasm.

- *Listeria monocytogenes*, *Shigella* (the causative agent of shigellosis), and *Rickettsia* (the causative agent of Rocky Mountain spotted fever and typhus) have the ability to escape from a phagosome before it fuses with a lysosome.

- Biofilms also play a role in evading phagocytes. Bacteria that are part of biofilms are much more resistant to phagocytosis because the phagocytes cannot detach bacteria from the biofilm prior to phagocytosis.

V. Inflammation

Damage to the body's tissues triggers a local defensive response called inflammation, another component of the second line of defense. The damage can be caused by microbial infection, physical agents (such as heat, radiant energy, electricity, or sharp objects), or chemical agents (acids, bases, and gases). Inflammation is usually characterized by five signs and symptoms: **redness**, **pain**, **heat**, **swelling, and loss of functions**.

Acute inflammation If the cause of an inflammation is removed in a relatively short period of time.

Chronic inflammation. If the cause of an inflammation is difficult or impossible to remove, the inflammatory response is longer lasting but less intense. such as tuberculosis, caused by *M. tuberculosis.*

functions of inflammation:

(1) to destroy the injurious agent, if possible, and to remove it and its by-products from the body.

(2) if destruction is not possible, to limit the effects on the body by confining or walling off the injurious agent and its by-products.

(3) to repair or replace tissue damaged by the injurious agent or its by-products.

During the early stages of inflammation, microbial structures, such as flagellin, lipopolysaccharides (LPS), and bacterial DNA stimulate the macrophages to produce cytokines, such as **tumor necrosis factor alpha** (α-TNF). In response to α-TNF in the blood, the liver synthesizes a group of proteins called **acute phase proteins;** other acute-phase proteins are present in the blood in an inactive form and are converted to an active form during inflammation. **Acute-phase proteins** induce both local and systemic responses and include proteins such as **C- reactive protein**, **mannose-binding lectin** and several specialized proteins such as **fibrinogen** for blood clotting and **kinins** for vasodilation.

<u>Inflammation can be divide into three stages:</u>

1-Vasodilation and Increased Permeability of Blood Vessels

 The increase in permeability, which permits fluid to move from the blood into tissue spaces, is responsible for the **edema.**

 The release of histamine, kinins, prostaglandins and Leukotrienes causes vasodilation and increased permeability of blood vessels.

 Blood clots can form around an abscess to prevent dissemination of the infection.

2-Phagocyte Migration and Phagocytosis

 Phagocytes have the ability to stick to the lining of the blood vessels (margination).

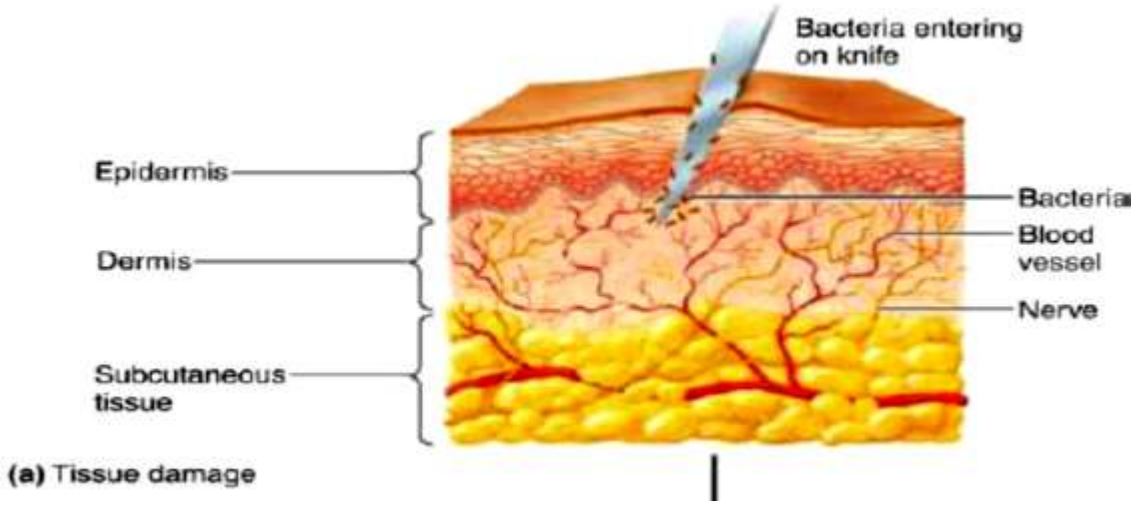
 They also have the ability to squeeze through blood vessels (diapedesis).

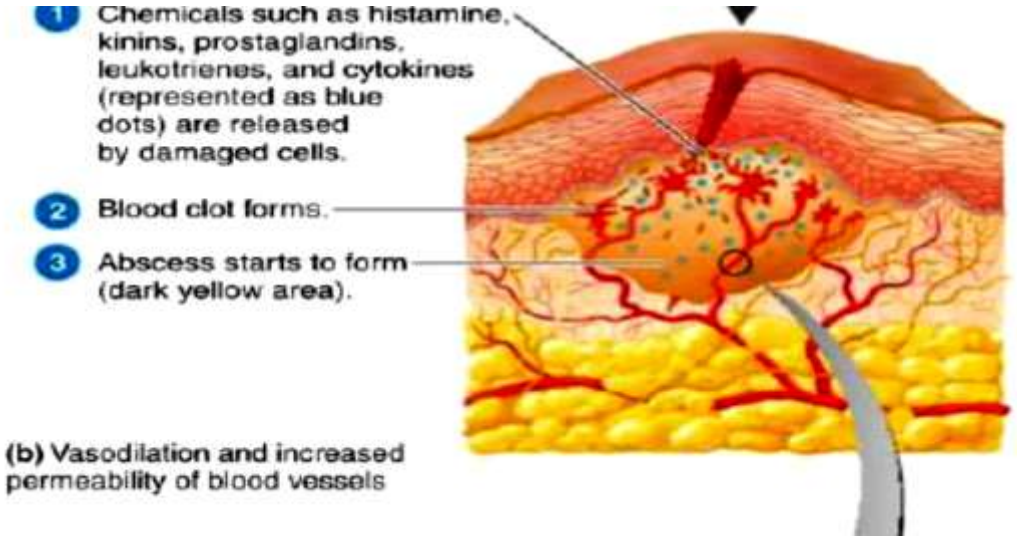
 Pus is the accumulation of damaged tissue and dead microbes, granulocytes, and macrophages.

3-Tissue Repair

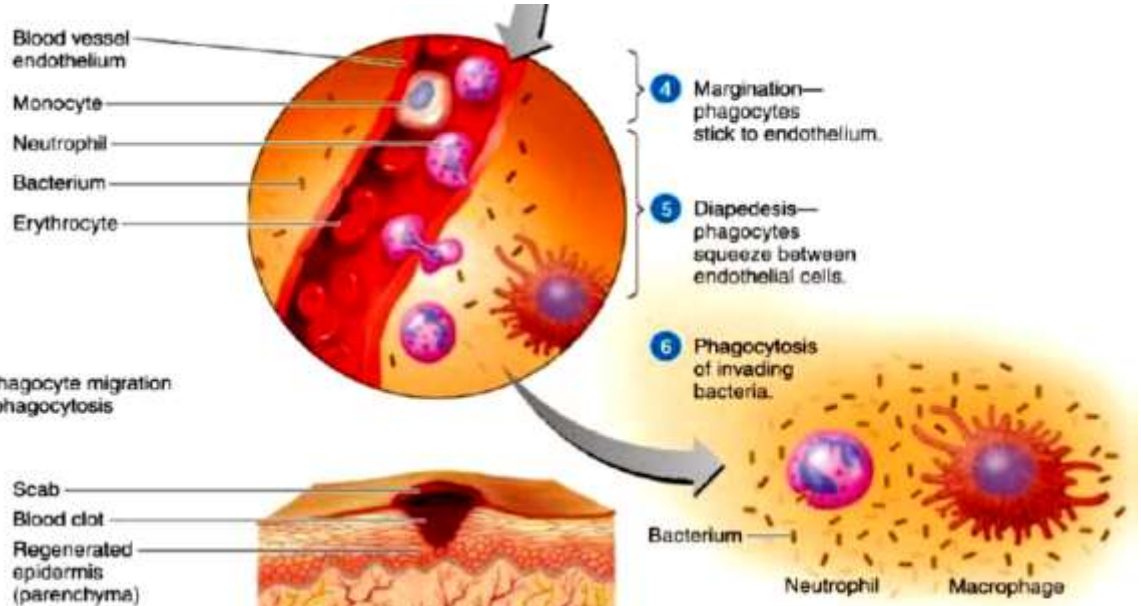
 A tissue is repaired when the stroma (supporting tissue) or parenchyma (functioning tissue) produces new cells.

 Stromal repair by fibroblasts produces scar tissue.

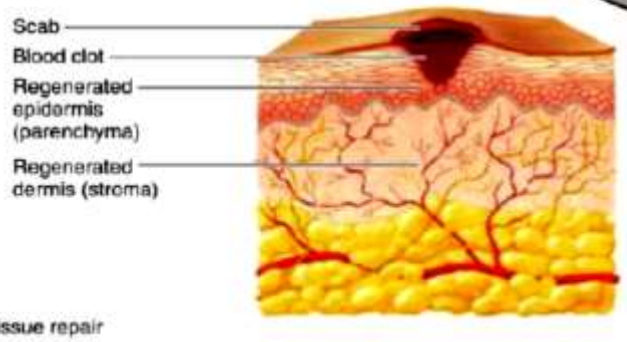




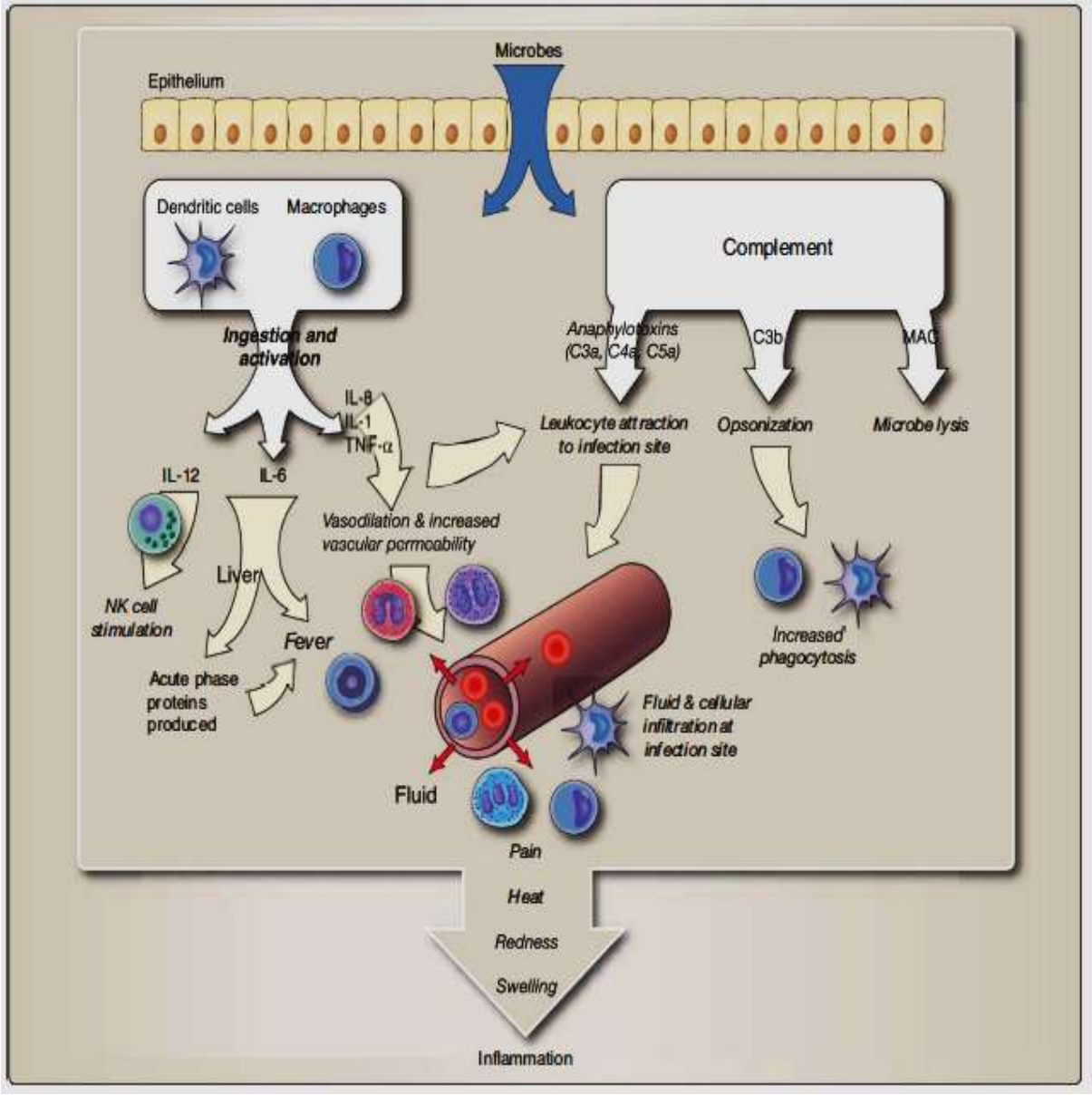
(b) Vasodilation and increased permeability of blood vessels



(c) Phagocyte migration and phagocytosis



(d) Tissue repair



Some Questions

1- What purposes does inflammation serve?

2- What causes the redness, swelling, and pain associated with inflammation?

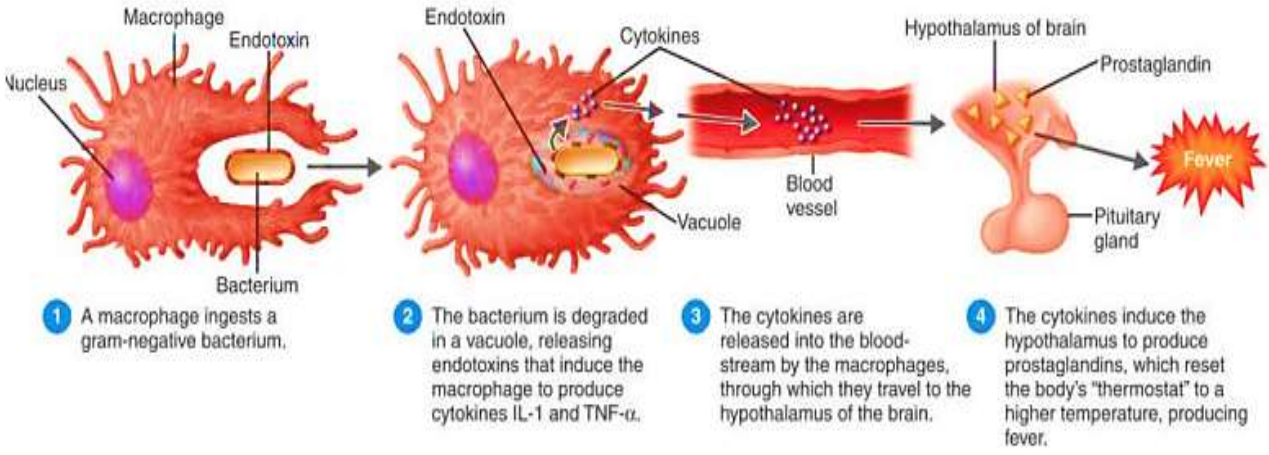
3- What is margination?

Lecture 6

Fever

Inflammation is a local response of the body to injury. There are also systemic, or overall, responses; one of the most important is fever, an abnormally high body temperature, a third component of the second line of defense. The most frequent cause of fever is infection from bacteria (and their toxins) or viruses.

Body temperature is controlled by a part of the brain called the hypothalamus. The hypothalamus is sometimes called the body's thermostat, and it is normally set at 37°C. It is believed that certain substances affect the hypothalamus by setting it at a higher temperature. Recall that when phagocytes ingest gram-negative bacteria, the lipopolysaccharides (LPS) of the cell wall (endotoxins) are released, causing the phagocytes to release the cytokines interleukin- I (formerly called endogenous pyrogen), along with TNF- α. These cytokines cause the hypothalamus to release prostaglandins that reset the hypothalamic thermostat at a higher temperature, thereby causing fever.



Assume that the body is invaded by pathogens and that the thermostat setting is increased to 39°C. To adjust to the new thermostat setting, the body responds by constricting blood vessels, increasing the rate of metabolism, and shivering, all of which raise body temperature. Even though body temperature is climbing higher than normal, the skin remains cold, and shivering occurs. This condition, called a chill, is a definite sign that body temperature is rising. When body temperature reaches the setting of the thermostat, the chill disappears. The body will continue to maintain its temperature at 39"C until the cytokines are eliminated. The thermostat is then reset to 37°C. As the infection subsides, heat-losing mechanisms such as vasodilation and sweating go into operation. The skin becomes warm, and the person begins to sweat. This phase of the fever, called the crisis, indicates that body temperature is falling. Interleukin-I helps step up the production of T cells. High body temperature intensifies the effect of antiviral interferons and increases production of **transferrins** that decrease the iron available to microbes. Also, because the high temperature speeds up the body's reactions, it may help body tissues repair themselves more quickly. Among the complications of fever are tachycardia (rapid heart rate), which may compromise older persons with cardiopulmonary disease; increased metabolic rate, which may produce acidosis; dehydration; electrolyte imbalances; seizures in young children; and delirium and coma. As a rule, death results if body temperature rises above 44 to 46°C.

Interferons

Interferons (IFNs) are a class of similar antiviral proteins produced by certain animal cells, such as lymphocytes and macrophages, after viral stimulation.

One of the principal functions of interferons is to interfere with viral multiplication.

Human interferons are of three principal types:

- alpha interferon (IFN -α),

- beta interferon (IFN - β),

- gamma interferon (IFN-γ).

In the human body, interferons are produced by **fibroblasts** in connective tissue and by **lymphocytes** and other **leukocytes**. Each of the three types of interferons produced by these cells can have a slightly different effect on the body.

All interferons are small proteins, with molecular weights between 15,000 and 30,000. They are quite stable at low pH and are fairly resistant to heat.

Gamma interferon is produced by **lymphocytes**; it induces neutrophils and macrophages to kill bacteria. IFN- γ causes macrophages to produce **nitric oxide** that appears to kill bacteria as well as tumor cells by inhibiting ATP production.

Both IFN- α and IFN- β are produced by **virus-infected host cells** only in very small quantities and diffuse to uninfected neighboring cells. They react with plasma or nuclear membrane receptors, inducing the uninfected cells to manufacture mRNA for the synthesis of **antiviral proteins** (AVPs). These proteins are enzymes that disrupt various stages of viral multiplication.

- interferons are effective for only short periods

- they do not remain stable for long periods of time in the body.

- And when injected, interferons have side effects, such as nausea, fatigue, headache, vomiting, weight loss, and fever.

- High concentrations of interferons are toxic to the heart, liver, kidneys, and red bone marrow.

- They typically play a major role in infections that are acute and short term, such as colds and influenza.

- Another problem is that they have no effect on viral multiplication in cells already infected.

- Also, some viruses, such as adenoviruses (which cause respiratory infections), have resistance mechanisms that inhibit AVPs.

- Further, some viruses, such as the hepatitis B virus, do not induce the production of sufficient amounts of interferon in host cells following viral stimulation.

Cytokines and chemokines

Cytokines are secreted by leukocytes and other cells and are involved in innate immunity, adaptive immunity, and inflammation (Table 1). Cytokines act in an antigen-nonspecific manner and are involved in a wide array of biologic activities ranging from chemotaxis to activation of specific cells to induction of broad physiologic changes.

Chemokines are a subgroup of cytokines of low molecular weight and particular structural patterns that are involved in the chemotaxis (chemical-induced migration) of leukocytes.

Table 1: cytokines and Chemokines Produced by activated phagocytes

Cytokine/Chemokine	Acts on	Actions
Interleukin-1 (IL-1)	Vascular endothelium	Increased permeability of vascular endothelium Stimulates production of IL-6
Interleukin-6 (IL-6)	Liver	Production of acute phase proteins (e.g., C-reactive protein); elevated temperature (fever)
Interleukin-8 (IL-8), a chemokine	Vascular endothelium	Activation of vascular endothelium Attraction/activation of neutrophils
Interleukin-12 (IL-12)	NK cells	Activates NK cells Influences lymphocyte differentiation
Tumor necrosis factor-α (TNF-α)	Vascular endothelium	Increased permeability of vascular endothelium Activation of vascular endothelium

Lecture 7

Adaptive Immunity: Specific Defenses of the Host

The clonal nature of the adaptive immune system permitted the emergence of a third feature that enabled the immune system to alter its responses to molecules (whether free or cell-bound) that it encountered on multiple occasions.

This ability to modify its activity on the basis of previous exposure is the basis of immunologic memory.

The combination of receptors **generated by DNA rearrangement**, and **immunologic memory** allows the adaptive immune system to function in ways that the innate system cannot. However, the innate and adaptive immune systems also interact constantly. The innate system is required to "**ignite**" the adaptive immune system. The adaptive immune system, in tum, can identify an extremely broad range of targets (e.g., a specific part of a specific molecule on a specific infectious organism) and then direct and focus the destructive activities of the innate system on those targets.

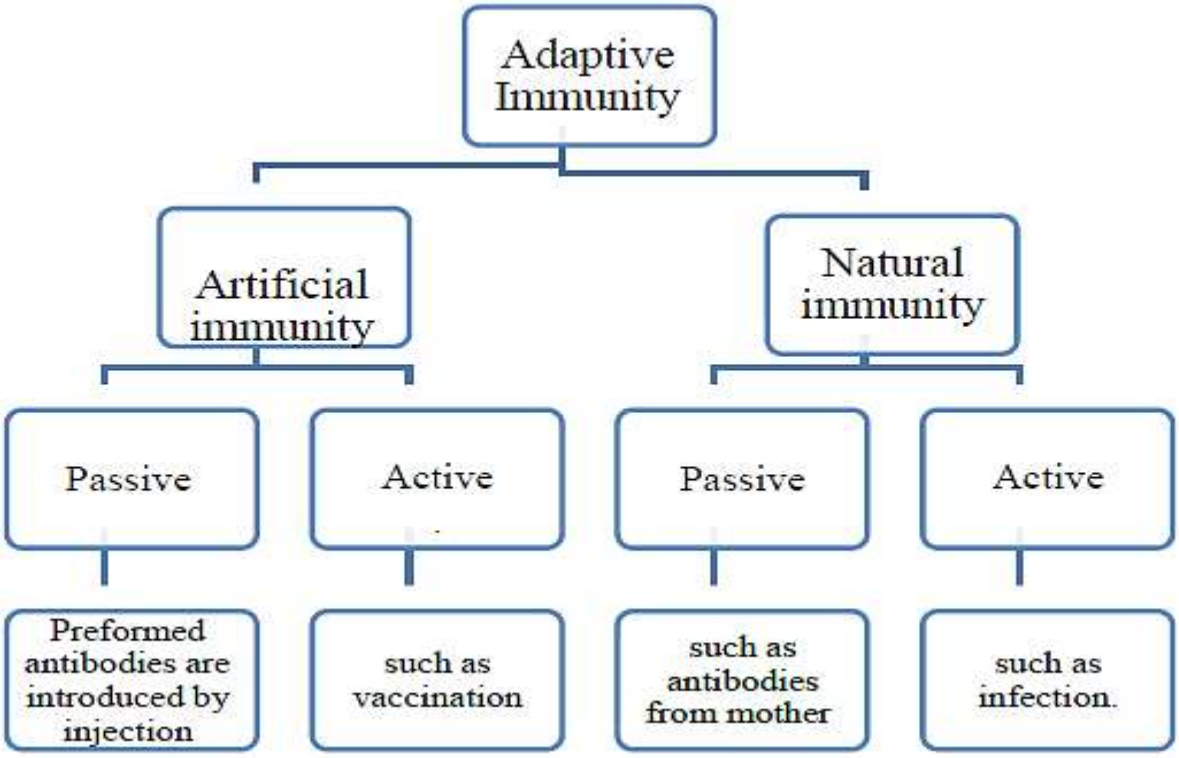
❖ **Characters :**

- Highly specific for the invading antigen.
- Can differentiate between self and non self-antigens. The response occurs only to non-self-antigen.
- Diversity: responds to millions of different antigens.
- Immunological memory due to presence of memory cells.

❖ **Mechanisms of acquired immunity:**

- **Humoral immunity:**
  - ***Mediated by antibodies secreted from B lymphocytes.***
- **Cell mediated immunity:**
  - ***Mediated by T lymphocytes, NK cells and macrophages.***

❖ **Types of acquired immunity:**



The Nature of Antibodies

Antibodies are globulin proteins therefore; we have come to use the term immunoglobulins (Ig) for antibodies. Globulin proteins arc relatively soluble. Antibodies are made in response to an antigen and can recognize and bind to the antigen. Each antibody has at least two identical sites that bind to epitopes. These sites are known as antigen-binding sites.

Antibody Structure

Because a bivalent antibody has the simplest molecular structure, it is called a monomer. A typical antibody monomer has four protein chains: two identical light chains and two identical heavy chain. The chains are joined by

disulfide links and other bonds to form a Y-shaped molecule. The Y-shaped molecule is flexible and can assume a T shape (notice the hinge region in).

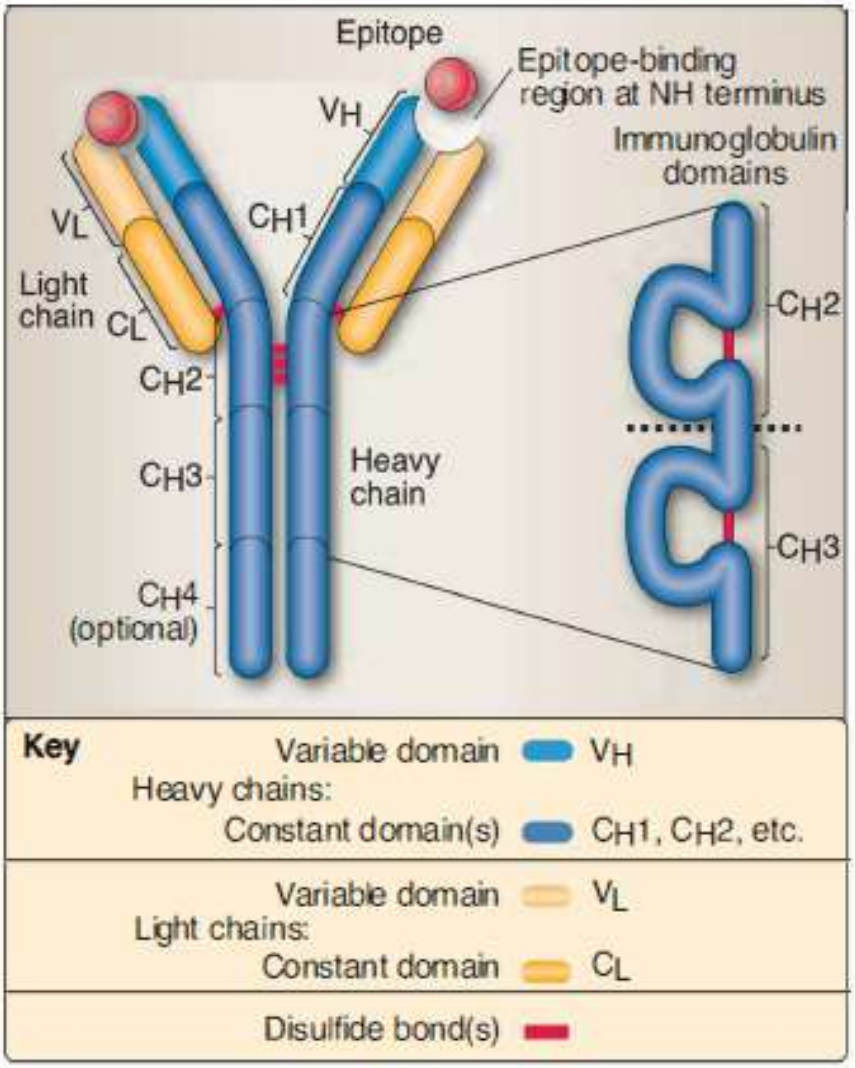


Fig 1: Immunoglobulin monomer. An immunoglobulin monomer contains two identical light (L) chains and two identical heavy (H) chains connected by disulfide bonds. Each chain contains a variable domain and one or more constant domains.

1. Light chains: An immunoglobulin monomer contains two identical **Kappa** (*k*) or two identical **lambda** (ʎ) A light chains but never one of each. Light or L chains contain a variable (V_L) domain and a constant (C_L) domain. Variable regions (in both heavy and light chains) are so named for their

variation in amino acid sequences between immunoglobulins synthesized by different B cells.

2. Heavy chains: Heavy chains contain one variable (V_H) and three or four constant (C_H) domains. Heavy (H) chain variable domains (V_H) are extremely diverse, and constant domains (C_H) display a relatively limited variability for members of an isotype.

3. Antigen-binding sites: A light chain variable domain and a heavy chain variable domain together form a pocket that constitutes the **antigen (epitope)-binding region** of the immunoglobulin molecule. Because an immunoglobulin monomer contains two identical light chains and two identical heavy chains, the two binding sites found in each monomeric immunoglobulin are also identical. The variability in the amino acid sequences of the V_L and V_H domains, together with the random pairing of light and heavy chain that occurs from one B cell to another, creates a pool of binding sites capable of recognizing a very large number of different epitopes.

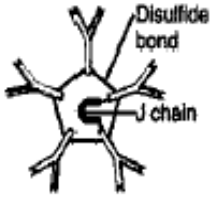
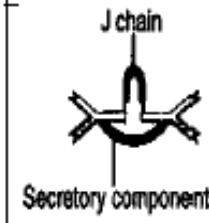
4. Immunoglobulin landmarks of two proteases: Immunoglobulin molecules can be enzymatically cleaved into discrete fragments by either pepsin or papain (Fig. 6.4). Disulfide bonds join the heavy chains at or near a proline-rich hinge region, which confers flexibility on the immunoglobulin molecule.

The fragments of immunoglobulin are as follows:

- **Fab or antigen (epitope)-binding fragment**, produced by papain cleavage of the immunoglobulin molecule. Two Fab fragments are produced by papain cleavage of an immunoglobulin monomer; each fragment has an epitope binding site.

- **Fc or constant (crystallizable) fragment** is produced by cleavage of the immunoglobulin molecule with papain. It is responsible for many biologic activities that occur following engagement of an epitope.

Immunoglobulin Classes

Characteristics	IgG	IgM	IgA	IgD	IgE
Structure					
Percentage of Total Serum	80%	5- 10%	10- 15%'	0.2%	0.002%
Location	Blood. lymph. intestine	Blood, lymph, B cell surface (as monomer)	Secretions (tears. saliva. mucus. intestine. milk). blood. lymph	B cell surface. blood, lymph	Bound to mast and basophil cells throughout body. blood
Half-life in Serum	23 days	5 days	6 days	3 days	2 days
Complement Fixation	yes	yes	No	No	No
placental Transfer	yes	No	No	No	No