DR. UMAYAL RAMANATHAN COLLEGE FOR WOMEN,
KARAIKUDI – 3
ACCREDITED WITH B+ GRADE BY NAAC
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RUN BY DR.ALAGAPPA CHETTIAR EDUCATIONAL TRUST

URCW

DEPARTMENT OF BIOTECHNOLOGY

PRINCIPLES OF IMMUNOLOGY

7BBT5C1

DR.J.CHITRA

V	Principles of Immunology	7BBT1C1	CO1	Facilitate to identify the cellular and molecular basis of immune responsiveness.
			CO2	Understand the roles of the immune system in both maintaining health and contributing to disease.
			CO3	Learn about immunological response and how it is triggered and regulated.
			CO4	Demonstrate a capacity for problem-solving about immune responsiveness.
			CO5	Transfer knowledge of immunology into clinical decision-making through case studies presented in class.

III YEAR – V SEMESTER

COURSE CODE: 7BBT5C1

CORE COURSE - IX – PRINCIPLES OF IMMUNOLOGY (7BBT5C1)

Unit - I

Scope of immunology. Haematopoiesis. Cell and organs of immune responses and their functions. Basic mechanisms of innate, adaptive, humoral and cell mediated immunity.

Unit - II

Antigens: Immunogenicity Vs antigenicity. Properties of immunogen, haptens, epitopes and adjuvants. Antibodies: Structure and function.

Unit - III

Antigen – Antibody interactions: Avidity and affinity. Basic principles and applications of precipitation reactions (radial immuno diffusion, double immuno diffusion and immuno electrophoresis), agglutination reactions, RIA, ELISA and Western blotting

Unit - IV

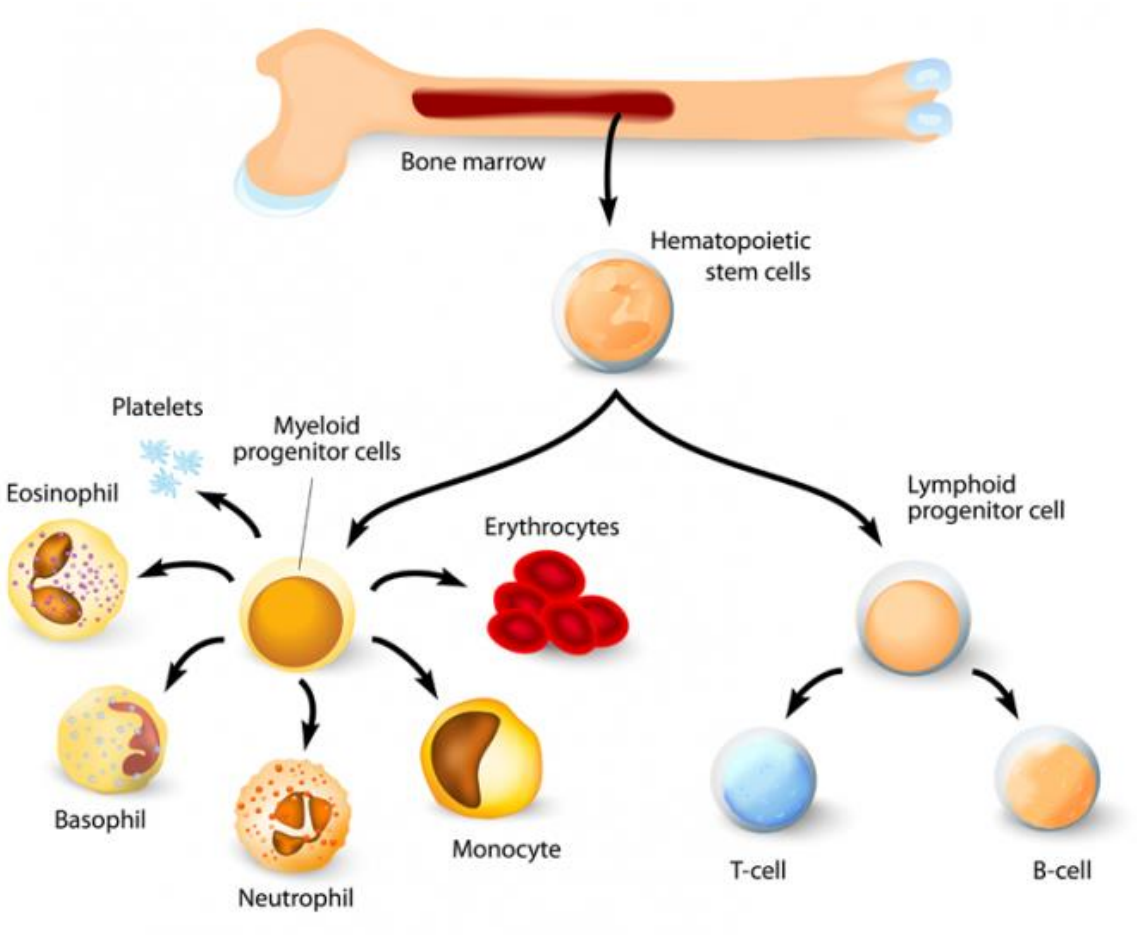
Antigen presentation: Class I and II MHC molecules, cytosolic and endocytic pathways. Complement pathways – classical and alternative

Unit - V

Vaccines: Active and Passive immunization. Hypersensitivity reactions and its types

Unit I

Mind Map



Scope of Immunology

Immunology is an emerging branch of medical science that deals with studies related to different aspects of the immune system like the cells, structure, function, response against antigens, and disorders.

- The immune system consists of a collection of cells, chemicals, processes, and mechanisms that function to protect the body from foreign antigens, such as microbes (organisms such as bacteria, fungi, and parasites), viruses, cancer cells, and toxins.
- Beyond, the structural and chemical barriers which protect us from infection, the immune system consists of two lines of defense; innate immunity and adaptive immunity.
- The innate immunity represents the first line of defense against the antigen, and it is an antigen-independent defense mechanism used by the host immediately or within hours of encountering an antigen.
- On the other hand, adaptive immunity is antigen-dependent and antigen-specific and, therefore, includes a lag time between exposure to the antigen and maximal response.

- Studies conducted in immunology are related to the measurement of physiological functioning of the immune system in both healthy and disease conditions, malfunctioning of the system in the case of disorders, and physical, chemical, and physiological characteristics of the cells of the immune system.
- Immunology is fast becoming an important branch of clinical medicine as it has a close relationship with organ transplantation, oncology, virology, bacteriology, and even dermatology.
- Immunology is focused on certain organs of the body like the bone marrow and the lymphatic system and the white blood cells found in the blood.
- These cells or organs producing these cells are responsible, directly or indirectly, for the defense mechanism of the body against a pathogenic agent or other antigens.
- These cells keep on circulating throughout the body via blood or lymph so that they can detect antigens entering the body from different sources.
- An important aspect of immunology is immunotherapy, where components of the immune system or antigens are used to treat a disease or disorder as a form of treatment.

I. Haematopoiesis

Haemopoiesis or haematopoiesis is the of process formation of new blood cellular components. It has been estimated that in an adult human, approximately 1011–1012 new blood cells are produced daily in order to maintain steady state levels in the peripheral circulation. The mother cells from which the progeny daughter blood cells are generated are known as haematopoietic stem cells. In an embryo yolk sac is the main site of haemopoiesis whereas in human the basic sites where haemopoiesis occurs are the bone marrow (femur and tibia in infants; pelvis, cranium, vertebrae, and sternum of adults), liver, spleen and lymph nodes (Table 1). In other vertebrates haemapoiesis occurs in loose stroma of connective tissue of the gut, spleen, kidney or ovaries.

Sites of Haemopoiesis in humans

Stage	Sites
Fetus	0–2 months (yolk sac) 2–7 months (liver, spleen) 5–9 months (bone marrow)
Infants	Bone marrow
Adults	Vertebrae, ribs, sternum, skull, sacrum and pelvis, proximal ends of femur

The process of haemopoiesis Pluripotent stem cells with the capability of self-renewal, in the bone marrow known as the haemopoiesis mother cell give rise to the separate blood cell lineages. This haemopoietic stem cell is rare, perhaps 1 in every 20 million nucleated cells in bone marrow. Figure 1 illustrates the bone marrow pluripotent stem cell and the cell lines that arise from it. Cell differentiation occurs from a committed progenitor haemopoietic stem cell and one stem cell is capable of producing about 106 mature blood cells after 20 cell divisions. The process leads to division of stem cells and commitment of each cell to differentiate into one of the different blood progenitor cells. The cell lineage chosen by the progenitor cells is a matter both chance and on the external stimuli received by progenitor cells. Internal transcription factors like PU.1 commits cells to the myeloid lineage whereas GATA-1 leads to erythropoietic and megakaryocytic differentiation. The proliferation and differentiation of haemopoietic progenitor cells and the function of mature blood cells is in turn regulated by glycoprotein hormones like Granulocyte colony stimulating factor or G-CSF. The growth factors may cause cell proliferation but can also stimulate differentiation, maturation, prevent apoptosis and affect the function of mature cells. The other growth factors that act at various levels of haemopoiesis are interleukin (IL-1 and IL-3); macrophage colony-stimulating factor; stem cell factor; and tumour necrosis factor.

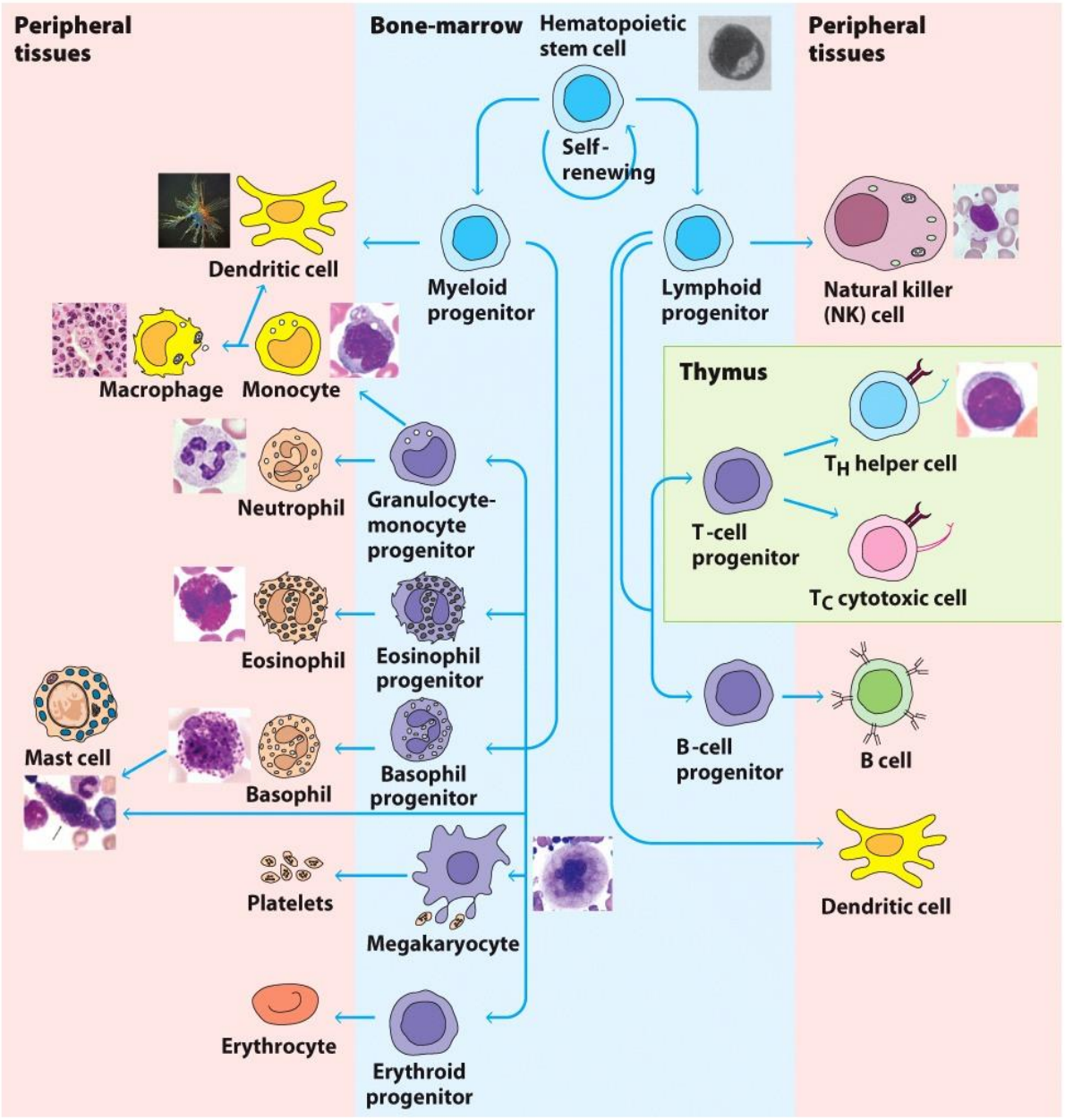


Figure 2-1
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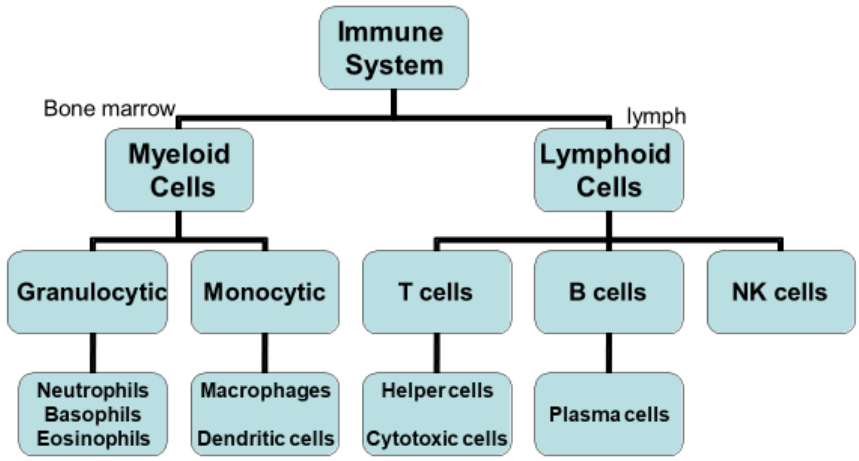
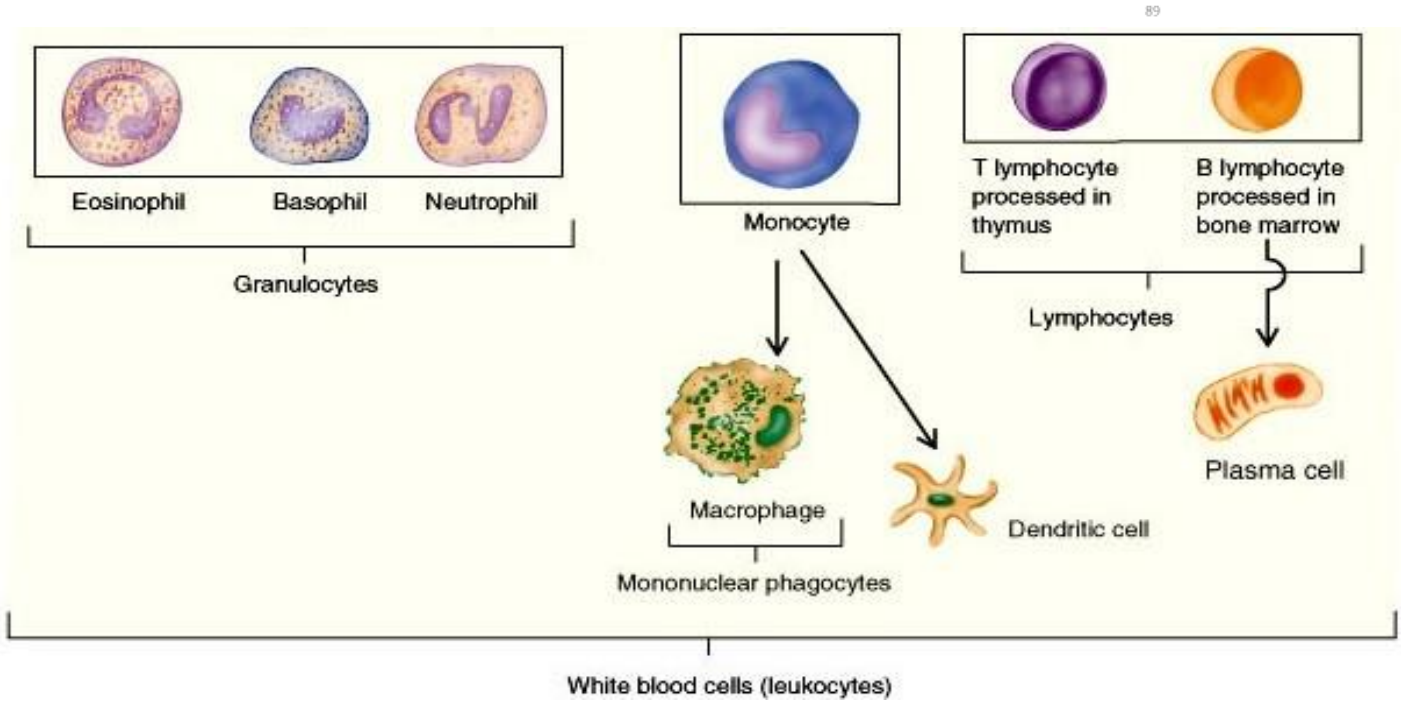
Growth factors in haemopoiesis

Acts On	Growth factor type
stromal cells	IL-1, TNF
pluripotential stem cells	SCF, Flt-L
multipotential progenitor cells	IL-3, GM-CSF, IL-6, G-CSF, Thrombopoietin
committed progenitor cells	G-CSF, M-CSF, IL-5, Thrombopoietin

II. Cell of immune responses and their functions

Cells of the Immune System





The cells that serve specialized roles in innate and adaptive immune responses are phagocytes, dendritic cells, antigen-specific lymphocytes, and various other leukocytes that function to eliminate antigens. Although most of these cells are found in the blood, their responses to microbes usually occur in lymphoid and other tissues.

1. Phagocytes

Phagocytes, including neutrophils and macrophages, are cells whose primary function is to ingest and destroy microbes and get rid of damaged tissues. The functional responses of phagocytes in host defense consist of sequential steps: recruitment of the cells to the sites of infection, recognition of and activation by microbes, ingestion of the microbes by the process of phagocytosis, and destruction of ingested microbes.

2. Neutrophils

Neutrophils, also called **polymorphonuclear leukocytes**, are the most abundant population of circulating white blood cells and mediate the earliest phases of inflammatory reactions. Neutrophils circulate as spherical cells about 12 to 15 μm in diameter with numerous membranous projections. The cytoplasm contains granules of two types. **Specific granules** filled with enzymes such as lysozyme, collagenase, and elastase. These granules do not stain strongly with either basic or acidic dyes (hematoxylin and eosin, respectively), which distinguishes neutrophil granules from those of two other types of circulating granulocytes, called basophils and eosinophils. The remainder of the granules of neutrophils, called **azurophilic granules**, are lysosomes that contain enzymes and other microbicidal substances, including defensins and cathelicidins.

Neutrophils are a type of white blood cell with multi-lobed nuclei and stainable cytoplasmic granules.

- These are the most abundant granulocytes, occupying about 40-60% of the total number of white blood cells in the blood.
- Neutrophils, like all other blood cells, are formed from the stem cells in the bone marrow.
- After differentiation in the bone marrow, neutrophils are released into the peripheral blood and circulate for 7 to 10 hours before migrating into the tissues, where they have a life span of only a few days.
- These are highly motile, allowing them to move in and out of the cells and tissue during infection quickly.
- The neutrophils are divided into two groups; neutrophil-killers and neutrophil-cagers.

- Neutrophils are at the front lines of attack during an immune response and are considered part of the innate immune system.

Neutrophil Structure

- Neutrophils are mostly circular ranging in size from 12-15 µm (in humans, the average size is 8 µm).
- Their shape changes into amoeboid once they are activated so that they can extend their pseudopodia to attack invaders.
- These are the smallest of all granulocytes with a characteristic multi-lobed nucleus with 3-5 lobes joined by a slender strand of genetic material.
- The nucleolus is present in young neutrophils but is lost as the neutrophil matures.
- The cytoplasm of the neutrophils has a large number of purple-colored granules, termed azurophilic or primary granules that have microbicidal activity.
- Additionally, secondary granules are also found int eh cytoplasm that contains lysozyme, collagenase, and other enzymes.
- Other cytoplasmic organelles like mitochondria, Golgi complex appear sparingly, and the endoplasmic reticulum is entirely absent.

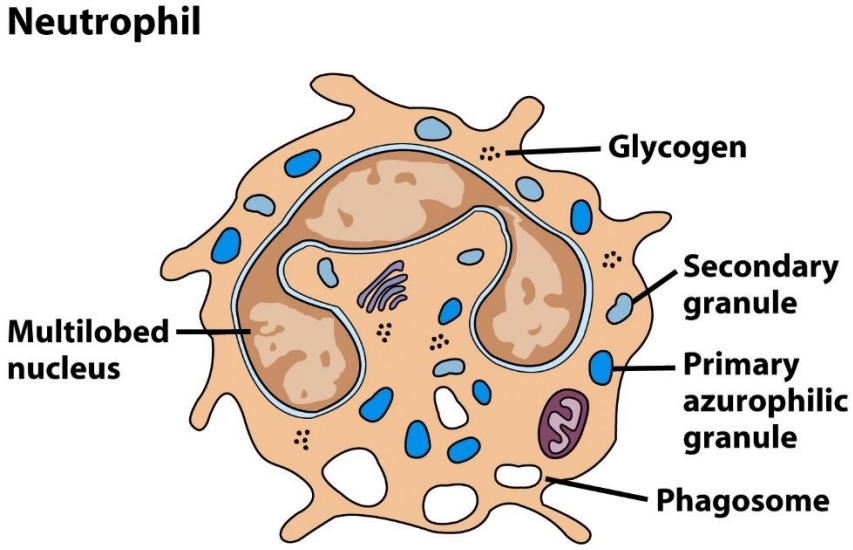


Figure 2-9a
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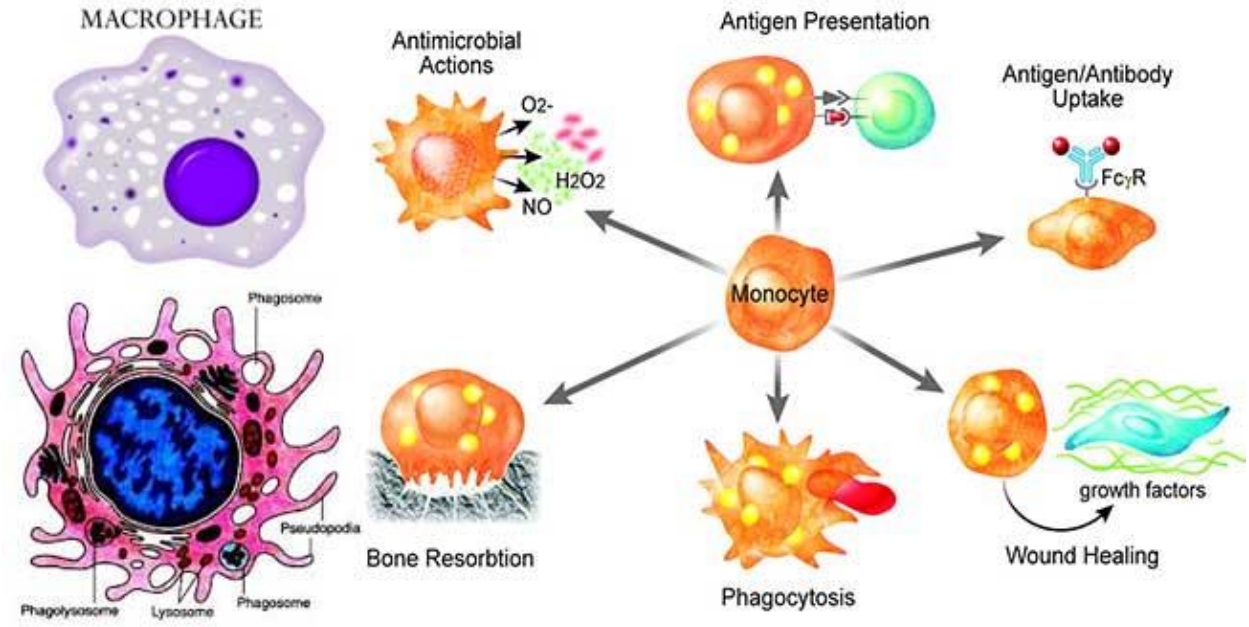
Neutrophils Functions

- Neutrophils are the most abundant granulocytes that makeup about 40% of white blood cells and 60% of the immune cells in the blood.
- Neutrophils are the first responders to infection, and they phagocytose bacteria into phagosomes before hydrolyzing and destroying them.

- These cells also secrete a range of proteins that have antimicrobial effects as well as tissue remodeling potential.
- Neutrophils have a short lifespan and thus destroy themselves during the degradation of foreign invaders. New neutrophils are then produced continuously in the bone marrow.
- The neutrophils of another subpopulation, cager neutrophils, perform a transport function of delivering foreign particles to the target site for the action of killer neutrophils.

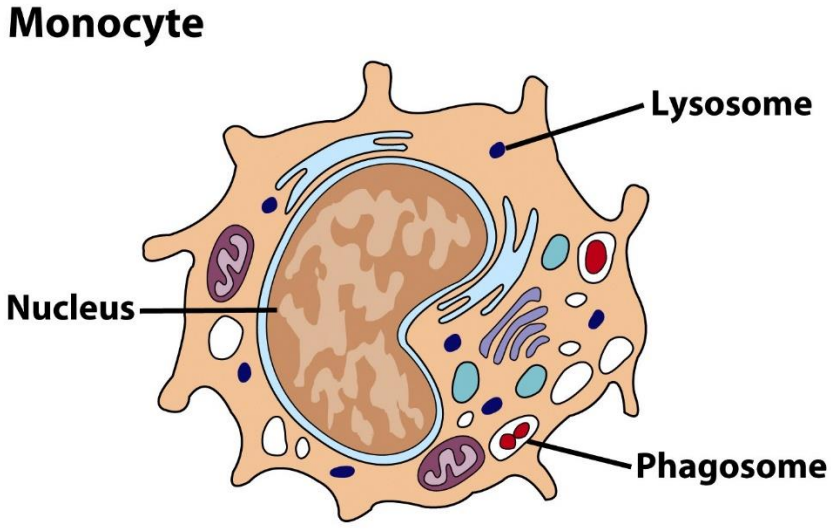
3. Mononuclear phagocyte



The mononuclear phagocyte system **includes circulating cells called monocytes and tissue resident cells called macrophages.** Monocytes are 10 to 15 μm in diameter, and they have bean-shaped nuclei and finely granular cytoplasm containing lysosomes, phagocytic vacuoles, and cytoskeletal filaments. Monocytes that migrate into tissues in response to infection can differentiate into specific tissue macrophages. Some macrophages are long-term residents in tissues and play an important role in regulating their repair and regeneration.

Other macrophages participate in the innate immune response and undergo a number of key changes when they are stimulated by encounters with pathogens or tissue damage. These are referred to as inflammatory macrophages and play a dual role in the immune system as effective phagocytes that can contribute to the clearance of pathogens from a tissue, as well as antigen-presenting cells that can activate T lymphocytes. Osteoclasts in the bone, microglial cells in the central nervous system, and alveolar macrophages in the lung are tissue-specific examples of macrophages with these properties.

The mononuclear phagocytic system consists of monocytes circulating in the blood and macrophages in the tissues. The monocyte is considered a leukocyte in transit through the blood, which becomes a macrophage when fixed in a tissue. Monocytes and macrophages as well as granulocytes are able to ingest particulate matter (microorganisms, cells, inert particles) and are said to have phagocytic functions. The phagocytic activity is greater in macrophages, particularly after activation by soluble mediators released during immune responses, than in monocytes.



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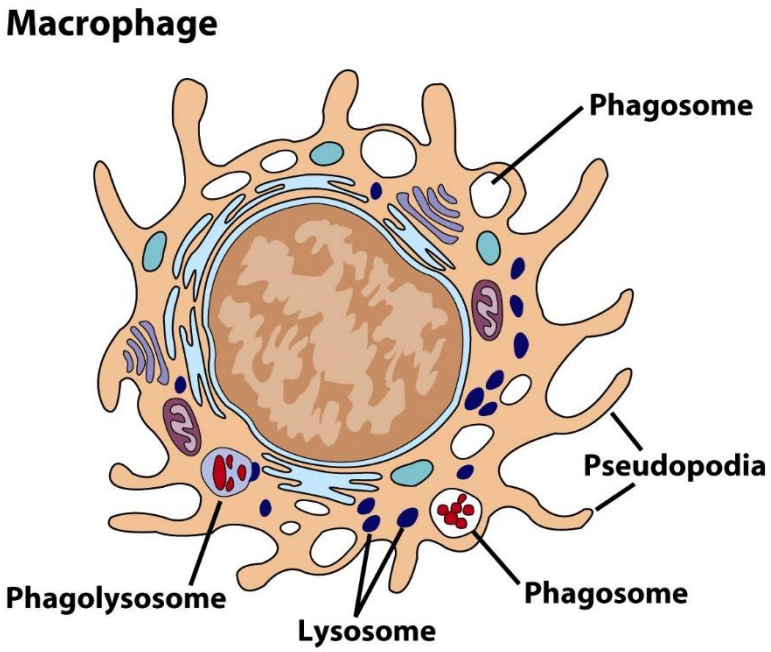

Figure 2-7b
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Differentiation of monocyte into a tissue macrophage involves a number of changes as follows:

1. The cell enlarges 5–10 folds.
2. Its intracellular organelles increase in number and complexity.
3. It acquires increased phagocytic ability.
4. It produces higher levels of hydrolytic enzymes.
5. It begins to secrete a variety of soluble factors.

Examples

1. Alveolar macrophages in the lung.
2. Histiocytes in connective tissues.
3. Kupffer cells in the liver
4. Mesangial cells in the kidney.
5. Microglial cells in the brain.
6. Osteoclasts in the bone.

For their participation in the immune reaction, the macrophages need to be stimulated and reach an "**activated state**." Macrophages can be activated by various cytokines, components of the bacterial cell wall, and mediators of the inflammatory response. Gamma interferon produced by helper T cells is a potent activator of macrophages and is secreted by various cells in response to appropriate stimuli. Bacterial lipopolysaccharides (endotoxin), bacterial peptidoglycan, and bacterial DNA are the substances that also activate macrophages.

Activated macrophages are more potent than normal macrophages in many ways, such as having greater phagocytic ability and increased ability to kill ingested microbes. They are better APCs, and they activate T-cell response in a more effective manner. By secreting various cytotoxic proteins, they help in eliminating a broad range of pathogens, including virus-infected cells, tumor cells, and intracellular bacteria.

Functions of macrophages

Tissue macrophages perform several important functions in innate and adaptive immunity.

Phagocytosis

A major function of macrophages in host defense is to ingest and kill microbes. The mechanisms of killing include the enzymatic generation of reactive oxygen and nitrogen

species that are toxic to microbes, and proteolytic digestion. Phagocytosis of bacteria, viruses, and other foreign particles is the most important function of macrophages. The macrophages on their cell surfaces have Fc receptors that interact with Fc component of the IgG, thereby facilitating ingestion of the opsonized organisms.

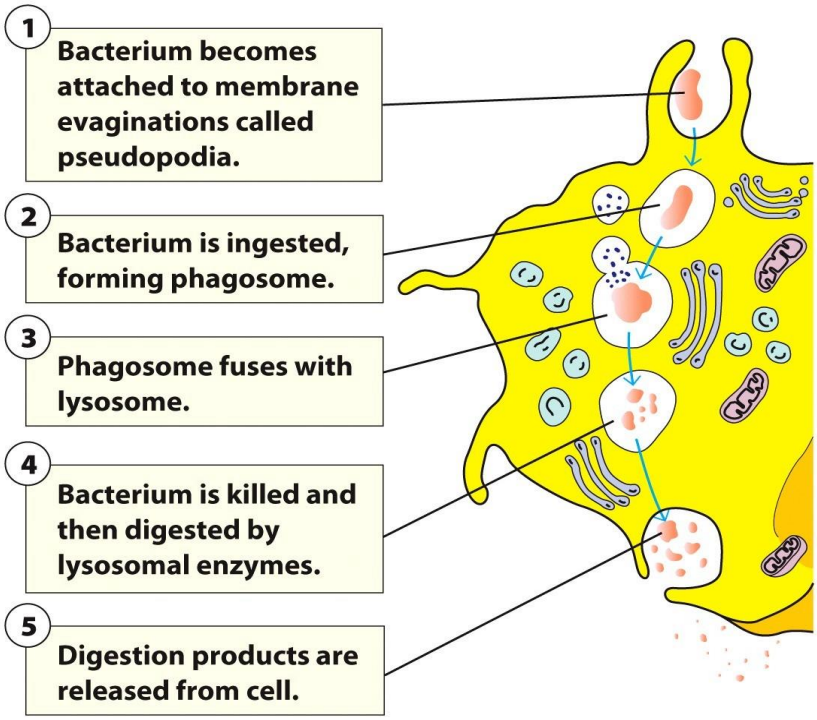

Figure 5-5b
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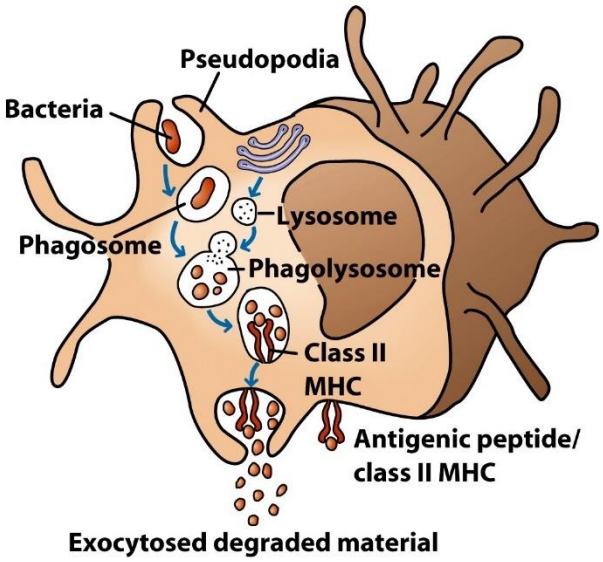
They also have receptors for C3b, another important opsonin. After ingestion, the phagosome containing the microbe fuses with a lysosome. The microbe within the phagolysosome is killed by reactive oxygen, reactive nitrogen compounds, and lysosomal enzymes.

Antigen presentation

Macrophages serve as APCs that display antigens and activate T lymphocytes. This function is important in the effector phase of T cell–mediated immune responses. After ingestion and degradation of foreign materials, the fragments of antigen are presented on the macrophage cell surface in conjunction with class II MHC proteins for interaction with the TCR of $CD4^+$ helper T cells. Degradation of the foreign protein is stopped following the association of antigen with the class II MHC proteins in the cytoplasm. This is followed by transportation of the complex to the cell surface by transporter proteins.



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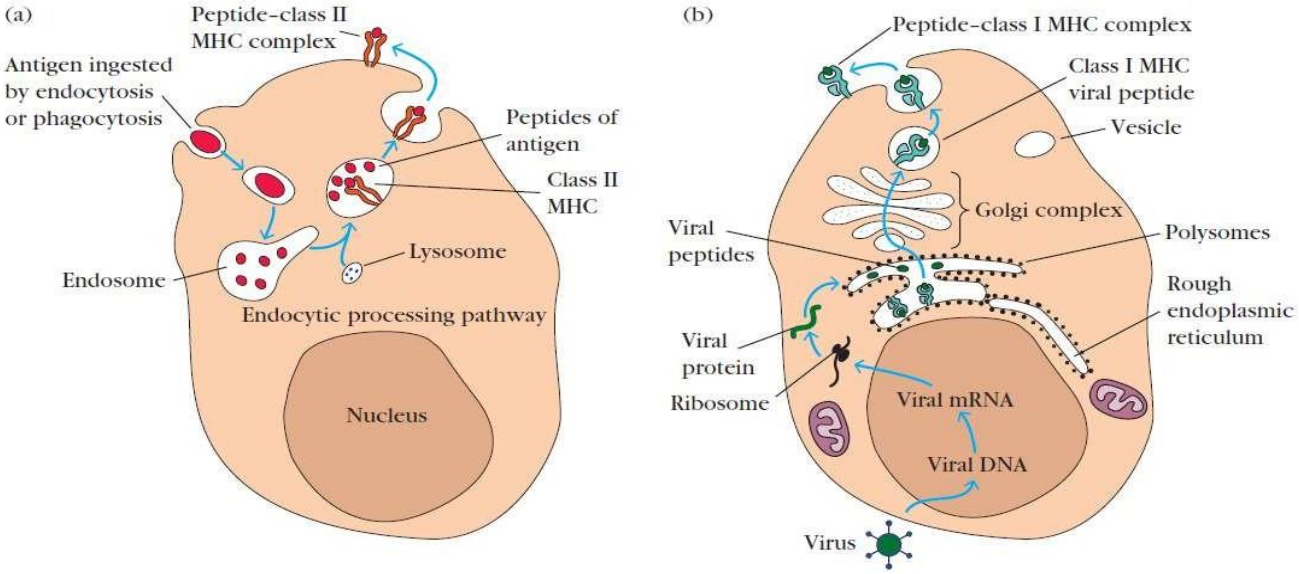


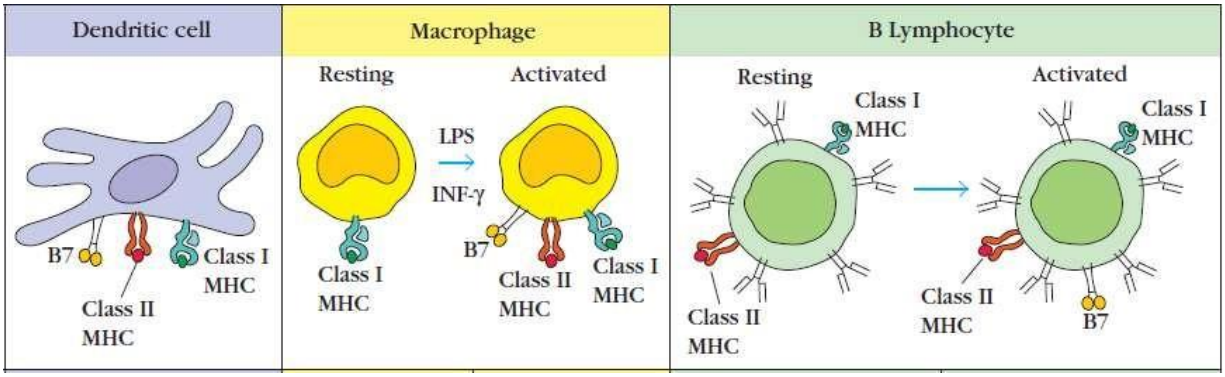
Cytokine production

Macrophages produce several cytokines including the IL-1, TNF, and IL8. IL-1 plays an important role in the activation of helper T cells, while TNF plays as important mediator in inflammatory reactions. IL-8 attracts neutrophils and T cells to the site of infection. Activated macrophages secrete several different cytokines that act on endothelial cells lining blood vessels to enhance the recruitment of more monocytes and other leukocytes from the blood into sites of infections, thereby amplifying the protective response against the microbes.

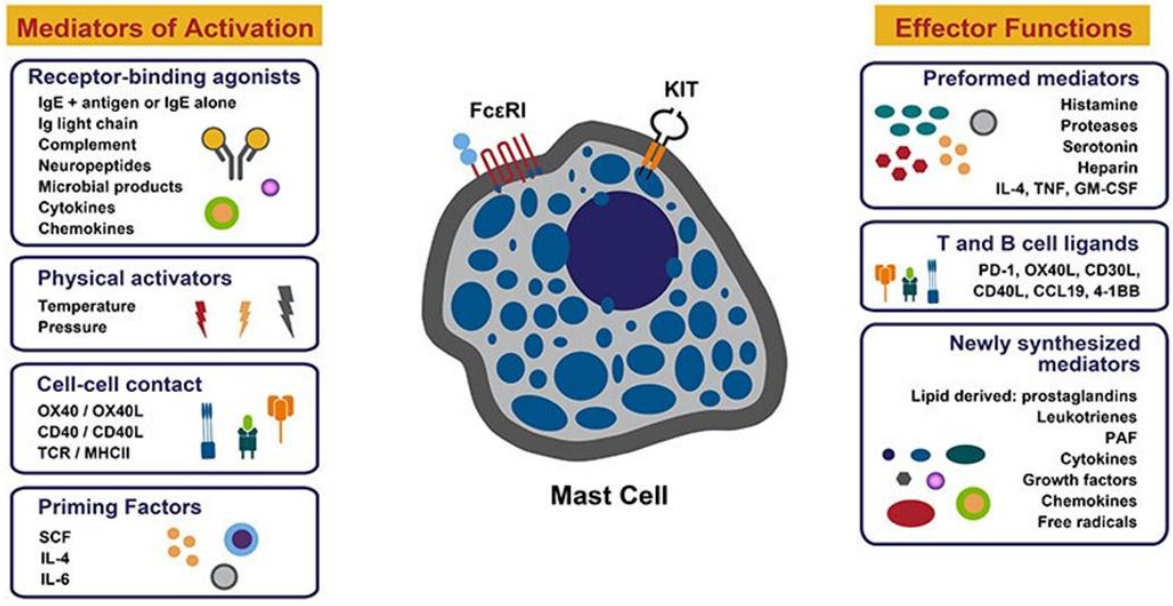
In addition to ingesting microbes, macrophages also ingest dead host cells, including cells that die in tissues because of trauma or interrupted blood supply and neutrophils that accumulate at

sites of infection. This is part of the cleaning up process after infection or sterile tissue injury. Macrophages also recognize and engulf apoptotic cells before the dead cells can release their contents and induce inflammatory responses. Throughout the life of an individual, unwanted cells die by apoptosis as part of many physiologic processes and the dead cells are eliminated by macrophages.

Macrophages promote the repair of damaged tissues by stimulating new blood vessel growth **(angiogenesis)** and synthesis of collagen-rich extracellular matrix (**fibrosis**). These functions are mediated by cytokines secreted by the macrophages that act on various tissue cells.



signaling events are induced that lead to release of the cytoplasmic granule contents into the extracellular space. The released granule contents, including histamine, promote changes in the blood vessels that cause inflammation. Mast cells function as sentinels in tissues, where they recognize microbial products and respond by producing cytokines and other mediators that induce inflammation.



5. Basophils:

Basophils are blood granulocytes with many structural and functional similarities to mast cells. Although they are normally not present in tissues, basophils may be recruited to some inflammatory sites. Like mast cells, basophils express IgE receptors, bind IgE, and can be triggered by antigen binding to the IgE. Because basophil numbers are low in tissues, their importance in host defense and allergic reactions is uncertain.

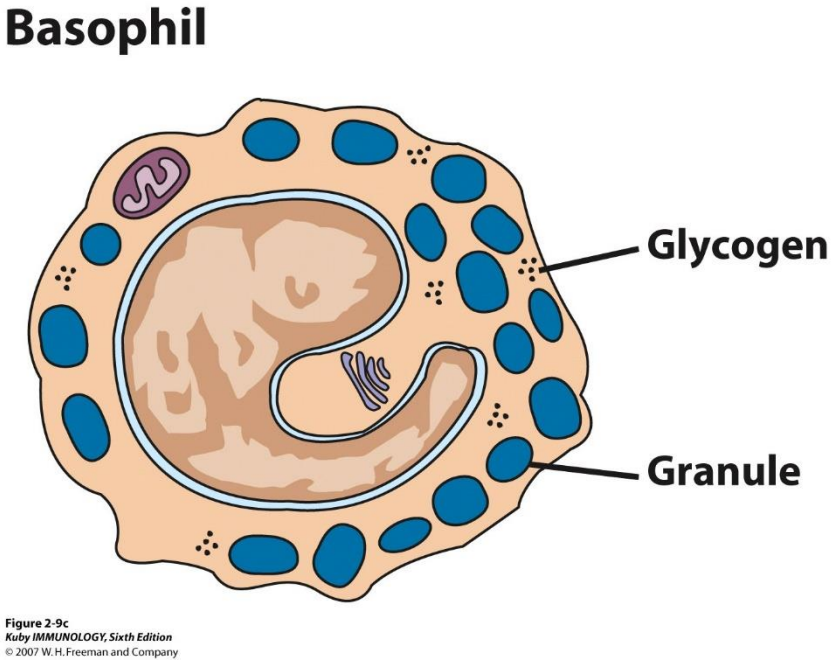


Figure 2-9c
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6. Eosinophils

Eosinophils are blood granulocytes that express cytoplasmic granules containing enzymes that are harmful to the cell walls of parasites but can also damage host tissues. Some eosinophils are normally present in peripheral tissues, especially in mucosal linings of the respiratory, gastrointestinal, and genitourinary tracts, and their numbers can increase by recruitment from the blood in the setting of inflammation.

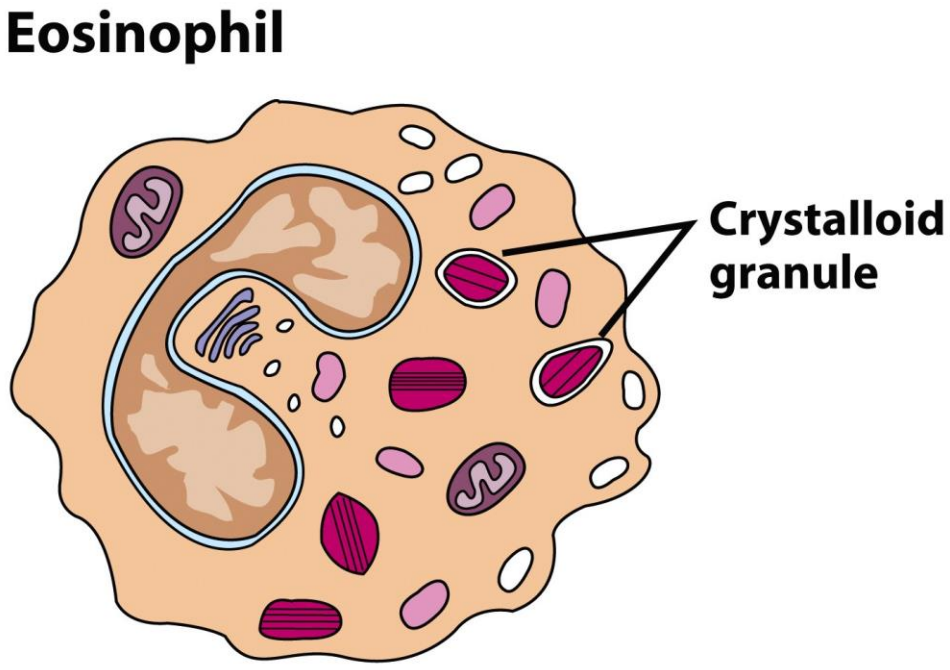

Figure 2-9b
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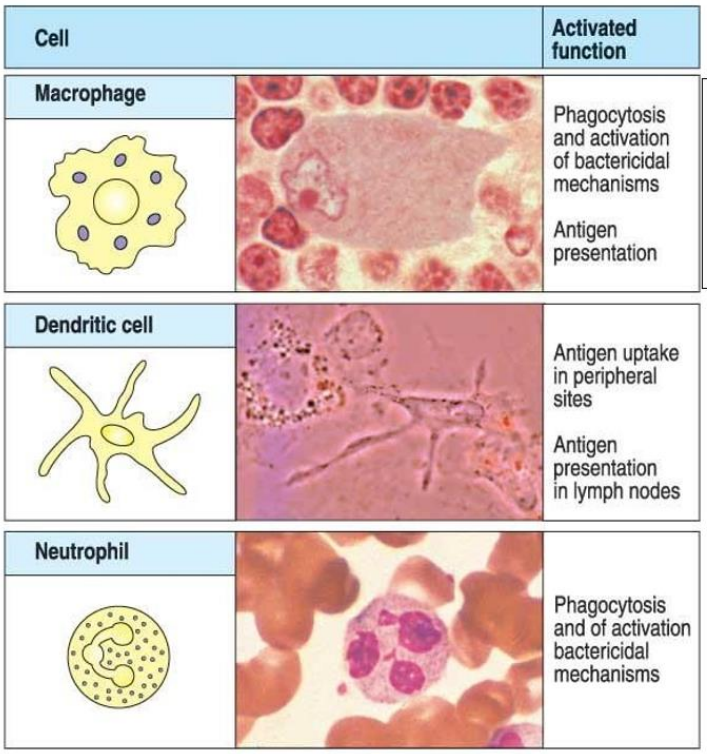
7. Antigen-presenting cells (APCs)

Antigen-presenting cells (APCs) are cells that capture microbial and other antigens, display them to lymphocytes, and provide signals that stimulate the proliferation and differentiation of the lymphocytes. The major type of APC that is involved in initiating T cell responses is the **dendritic cell. Macrophages and B cells** present antigens to T lymphocytes in cell mediated and humoral immune responses. are cells that capture microbial and other antigens, display them to lymphocytes, and provide signals that stimulate the proliferation and differentiation of the lymphocytes. The major type of APC that is involved in initiating T cell responses is the **dendritic cell. Macrophages and B cells** present antigens to T lymphocytes in cell mediated and humoral immune responses.

8. Dendritic cells

Dendritic cells are the most important APCs for activating naive T cells, and they play major roles in innate responses to infections and in linking innate and adaptive immune responses. They have long membranous projections and phagocytic capabilities and are widely distributed in lymphoid tissues, mucosal epithelium, and organ parenchyma. Most dendritic cells are part of the myeloid lineage of hematopoietic cells and arise from a precursor that can also differentiate into monocytes but not granulocytes.

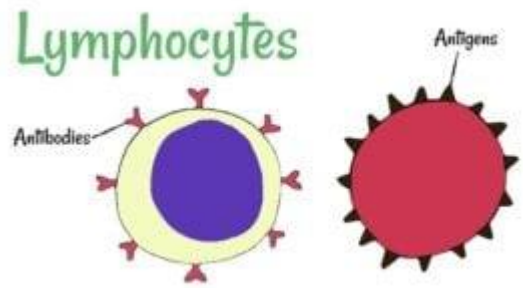


Big in size
Fix or moving
Professional APC
Secrete hydrolytic Enz.
Innate immunity

Professional APC
Present to T_H cells

Most abundant WBCs
Migrate to infection sites
kill the invader

9. Lymphocytes



Lymphocytes are the cells that specifically recognize and respond to foreign antigens and are mediators of humoral and cellular immunity. Lymphocytes, the unique cells of adaptive immunity, are the only cells in the body that express clonally distributed antigen receptors, each specific for a different antigenic determinant. Each clone of T and B lymphocytes expresses antigen receptors with a single specificity, which is different from the specificities of the receptors in all other clones. Thus, the antigen receptors of these lymphocytes are clonally distributed. Genes encoding the antigen receptors of lymphocytes are formed by recombination of DNA segments during the maturation of these cells. Lymphocytes are the principal cell in the adaptive immune response. They represent 20% to 40% of circulating white blood cells and 99% of cells in the lymph. The total number of lymphocytes in a healthy adult

is about 5×10^{11}. Of these, about 2% are in the blood, 4% in the skin, 10% in the bone marrow, 15% in the mucosal lymphoid tissues of the gastrointestinal and respiratory tracts, and 65% in lymphoid organs (mainly the spleen and lymph nodes).

Types/ Subsets of Lymphocytes

Lymphocytes consist of distinct subsets that are different in their functions and protein products. Lymphocytes can be broadly subdivided into three major populations on the basis of functional and phenotypic differences: B lymphocytes (B cells), T lymphocytes (T cells), and natural killer (NK) cells. In humans, approximately a trillion (10^{12}) lymphocytes circulate continuously through the blood and lymph and migrate into the tissue spaces and lymphoid organs.

Morphologically, all lymphocytes are similar, and their appearance does not reflect their heterogeneity or their diverse functions. **B lymphocytes**, the cells that produce antibodies, arise and maturation occur in the bone marrow. Thus, B lymphocytes now refer to bone marrow–derived lymphocytes. **T lymphocytes**, the mediators of cellular immunity, arise in the bone marrow, and migrate to and mature in the thymus; T lymphocytes refer to thymus-derived lymphocytes.

Subsets of B and T lymphocytes

They exist with distinct phenotypic and functional characteristics. The major subsets of B cells are follicular B cells, marginal zone B cells, and B-1 cells, each of which is found in distinct anatomic locations within lymphoid tissues. The two major T cell subsets are CD4+ helper T lymphocytes and CD8+ CTLs, which express antigen receptors called αβ T cell receptors (TCRs), and function as the mediators of cellular immunity. CD4+ regulatory T cells are a third subset of T cells expressing αβ receptors; their function is to inhibit immune responses. T lymphocytes (T cells) derive their letter designation from their site of maturation in the thymus. T-cell receptors only recognize processed pieces of antigen (typically peptides) bound to cell membrane proteins called major histocompatibility complex (MHC) molecules. They become activated, proliferate, and differentiate into an effector cell called a cytotoxic T lymphocyte (CTL). The CTL has a vital function inmonitoring the cells of the body and eliminating any cells that display foreign antigen complexed with class I MHC,

Natural killer (NK) cells Natural killer (NK) cells are lymphoid cells that are closely related to B and T cells. However, they do not express antigen specific receptors. NK cells constitute 5% to 10% of lymphocytes in human peripheral blood. They are efficient cell killers and attack a variety of abnormal cells, including some tumor cells and some cells infected with virus.

III. Organs of Immune system

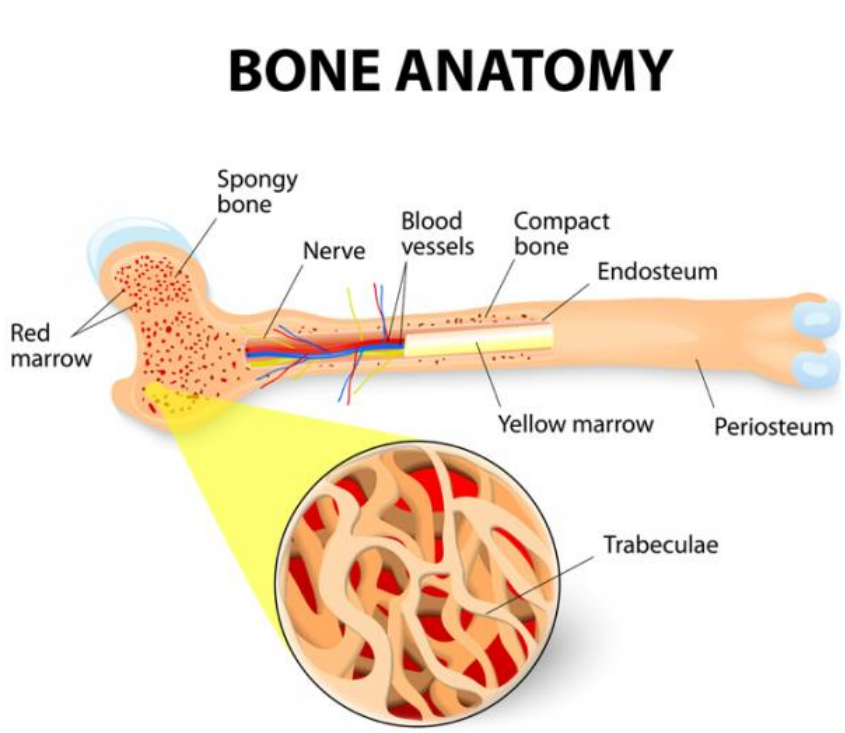
The immune system consists of many different organs and tissues that are found throughout the body. These organs can be classified functionally into two main groups:

Primary Lymphoid Organs- which provide appropriate microenvironments for the development and maturation of lymphocytes. This includes *Thymus, Bone Marrow, Fetal Liver*

Secondary Lymphoid Organs- which trap antigen from defined tissues or vascular spaces and are sites where mature lymphocytes can interact effectively with that antigen. This includes Lymph nodes, Spleen, Mucosa Associated Lymph tissue (MALT), Tonsils, Peyers patches, lamina propria (largest amount of lymphs), appendix collectively known Gut associated lymhoid tissue (GALT) and Bronchial associated lymphoid tissue (BALT)

a) Bone Marrow- Types, Structure and Functions

- **Bone Marrow** is the soft, highly vascular and flexible connective tissue within bone cavities which serve as the primary site of new blood cell production or hematopoiesis.
- In adult humans, bone marrow is primarily located in the ribs, vertebrae, sternum, and bones of the pelvis.
- The majority of the cell types involved in the immune system is produced from a common hemopoietic stem cell (HSC).
- HSC are found in the fetal liver, fetal spleen and neonate and adult bone marrow.
- Bone marrow is the primary source of pluripotent stem cells that give rise to all hemopoietic cells (blood cells) including lymphocytes.
- As a part of the lymphatic system, it is the major organ for B cell maturation and gives rise to the precursor cells of the thymic lymphocytes.
- The thymus and the bone marrow are primary lymphoid organs as T and B cells must first undergo maturation in these organs/tissues before migrating to the secondary lymphoid tissues, such as the spleen, lymph nodes and mucosa associated lymphoid tissues (MALT).
- Staring from the last months of fetal development when bone marrow becomes the dominant site of hemopoiesis (blood cell formation), the great majority of cells involved in mammalian immunity are derived from precursors in the bone marrow.



Types of Bone Marrow

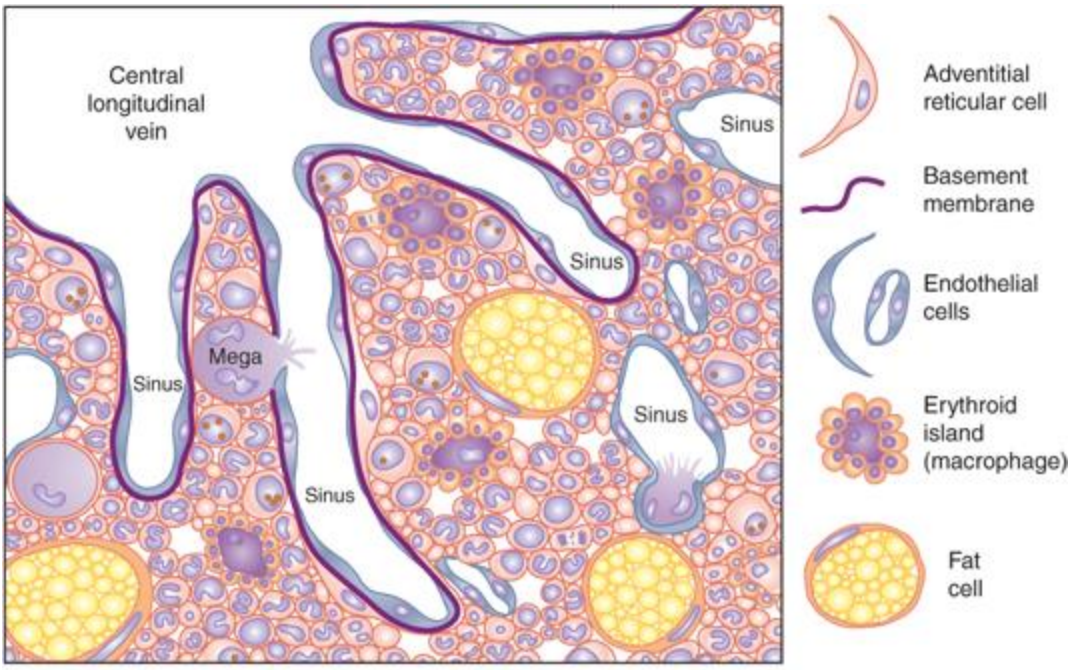
There are two categories of bone marrow tissue: **red marrow** and **yellow marrow**. Most of the bone marrow during birth to early adolescence is red marrow while the red marrow is replaced with yellow with age.

In adults, red marrow is confined mostly to skeletal system bones that serve to produce blood cells and help remove old cells from circulation. They contain hematopoietic stem cells that produce two other types of stem cells: **myeloid stem cells** and **lymphoid stem cells**. These cells develop into red blood cells, white blood cells, or platelets.

Yellow marrow found in spongy bones and in the shaft of long bones, is non-vascular and consists primarily of fat cells. It is composed of hematopoietic tissue that has become inactive.

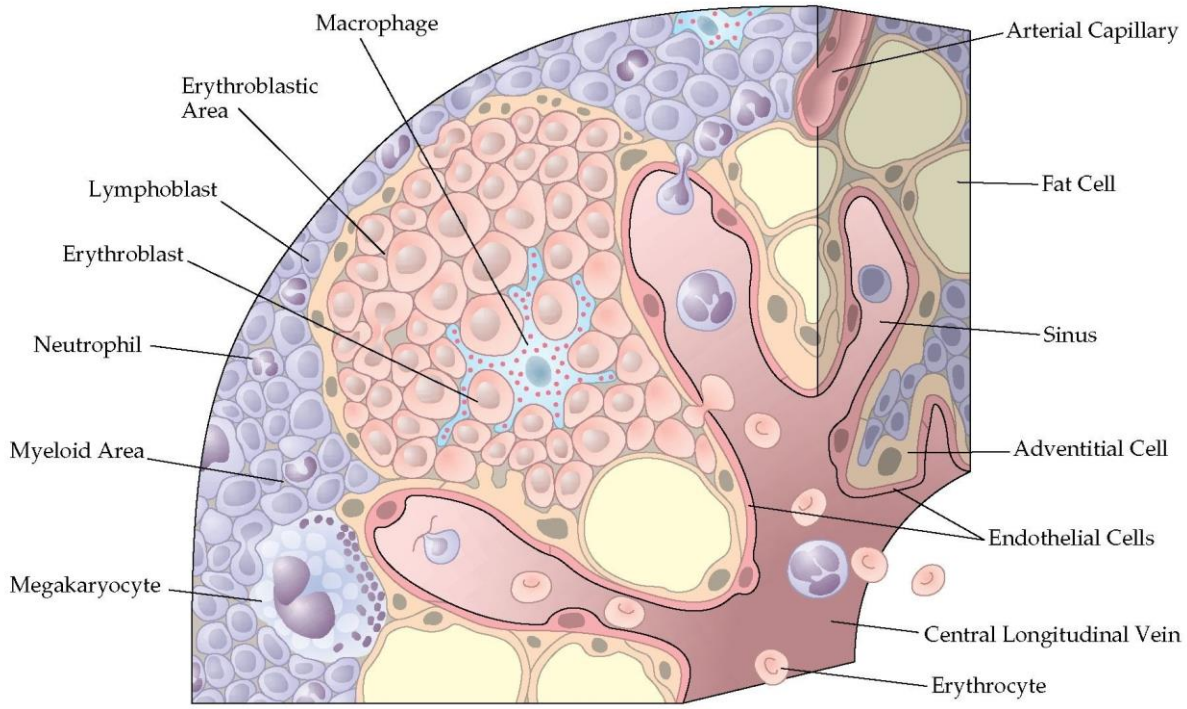
Structure of Bone Marrow

The structure of bone marrow constitutes of hematopoietic tissue islands and adipose cells surrounded by vascular sinuses interspersed within a meshwork of trabecular bone.



Source: H. Franklin Bunn, Jon C. Aster: Pathophysiology of Blood Disorders
www.accessmedicine.com

- The bone marrow is composed of both cellular and non-cellular components and structurally be divided into vascular and non-vascular regions.
- The non-vascular section of bone marrow is composed of hemopoietic cells of various lineages and maturity, packed between fat cells, thin bands of bony tissue (trabeculae), collagen fibers, fibroblasts and dendritic cells. This is where hematopoiesis takes place.
- The vascular section contains blood vessels that supply the bone with nutrients and transport blood stem cells and formed mature blood cells away into circulation.
- Ultrastructural studies show hemopoietic cells cluster around the vascular sinuses where they mature, before they eventually are discharged into the blood.
- Lymphocytes are found surrounding the small radial arteries, whereas most immature myeloid precursors are found deep in the parenchyma.



Functions of Bone Marrow

- The bone marrow gives rise to all of the lymphoid cells that migrate to the thymus and mature into T cells, as well as to the major population of conventional B cells.
- B cells mature in the bone marrow and undergo selection for non-self before making their way to the peripheral lymphoid tissues.
- Since the bone marrow constitutes of the hemopoietic cells derived from multipotential stem cells, they not only give rise to all of the lymphoid cells found in the lymphoid tissue, but also to all of the cells found in the blood.
- Platelets, which are crucial for the blood clotting process, are formed from bone marrow just like other blood cells.
- Yellow marrow is actively involved in lipid storage.

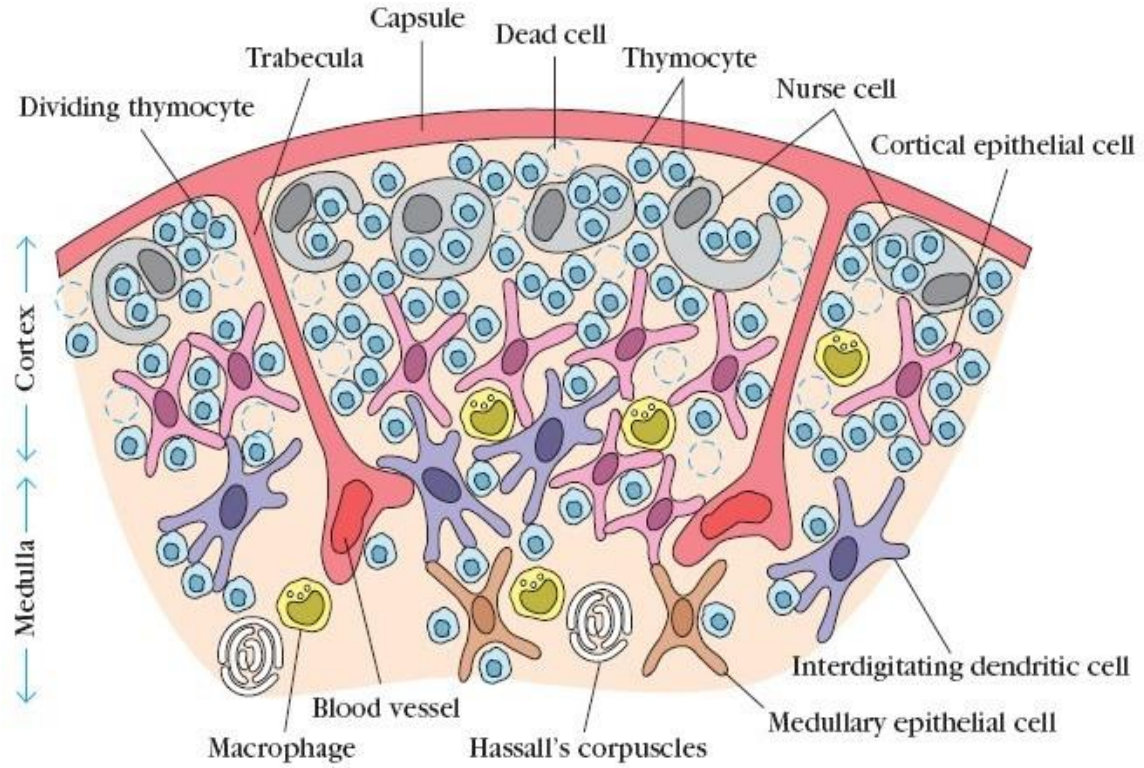
b) Thymus- Structure and Functions

- The thymus is a lymphocyte-rich, bilobed, encapsulated organ located behind the sternum, above and in front of the heart.
- The activity of the thymus is maximal in the fetus and in early childhood and then undergoes atrophy at puberty although never totally disappearing.
- The thymus is derived from the third and fourth pharyngeal pouches during embryonic life and attracts (with chemoattractive molecules) circulating T cell precursors derived from hemopoietic stem cells (HSC) in the bone marrow.

- It is essential for the maturation of T cells and the development of cell-mediated immunity thus referred to as primary lymphoid organ.
- In fact, the term ‘T cell’ means thymus-derived cell and is used to describe mature T cells.
- It is composed of cortical and medullary epithelial cells, stromal cells, interdigitating cells and macrophages.
- These cells are important in the differentiation of the immigrating T cell precursors and their ‘education’ (positive and negative selection) prior to their migration into the secondary lymphoid tissues.

Structure of Thymus

- It is a pink, flattened, asymmetrical structure lying between sternum and pericardium in anterior mediastinum.
- It is large in infants weighing upto 70 g while atrophied in adult to about 3g.
- The thymus consists of two lobes joined by aerolar tissues.
- The two thymic lobes are surrounded by a thin connective tissue capsule.
- Fibrous extensions of capsule around the thymus called trabeculae or septa divide thymus into lobules.



- Each lobule has:

1. An Outer Cortex

- Dark-staining outer part packed with lymphocytes, compartmentalized by elongated epithelial cells.
- It consists of immature T -cells.
- The process of proliferation and selection occurs mainly here.

2. An Inner Medulla

- Lighter central zone with fewer lymphocytes but more epithelial reticular cells.
- It consists of mature T-cells.
- It is predominantly the epithelial part, to which cortical lymphocytes migrate before export via venules and lymphatics.
- Also, the final stages of selection may occur at the cortico-medullary junction.
- Medulla also consists of thymic corpuscles alternatively called Hassall's corpuscles which are oval structures consisting of round whorls of flattened epithelial cells. They are believed to be aged and degenerated cells.

Functions of Thymus

1. T cell maturation and development

Immature T cell precursors travel from the bone marrow to the thymus (to become thymocytes) where they generate antigen specificity, undergo thymic education, and then migrate to the peripheral lymphoid tissues as mature T cells.

The main functions **of the thymus** as a primary lymphoid organ **are to:**

(a) To produce sufficient numbers (millions) of different T cells each expressing unique T cell receptors (generate diversity) such that in every individual there are at least some cells potentially specific for each foreign antigen in the environment.

(b) To select T cells for survival in such a way that the chance for an auto-immune response is minimized.

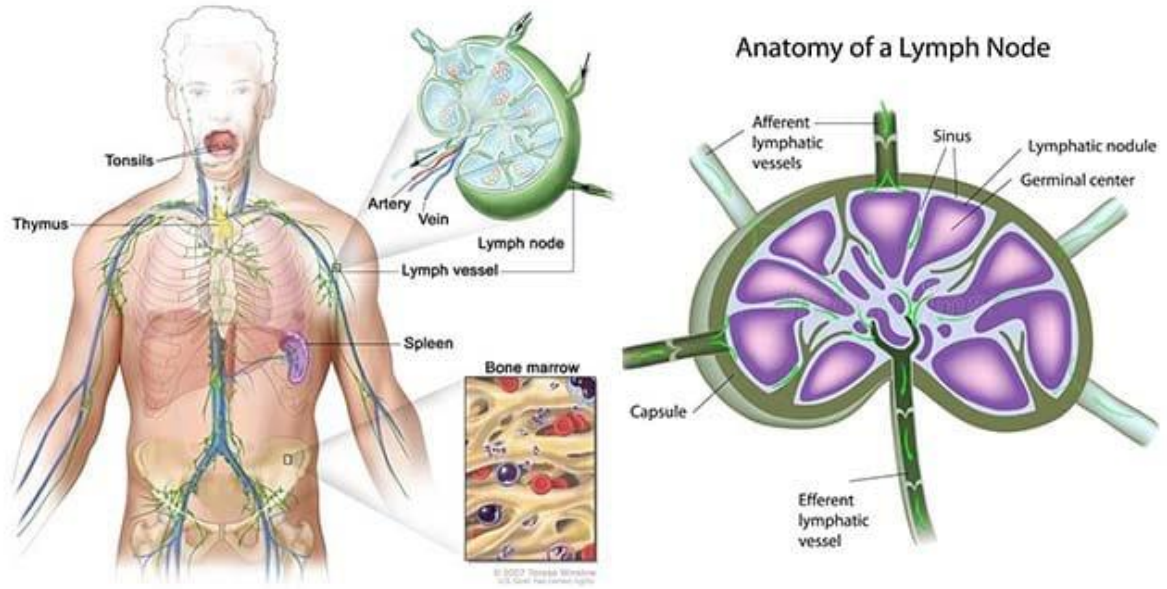
The thymus has an interactive role with the endocrine system. Thymic epithelial cells produce the hormones thymosin and thymopoietin and in concert with cytokines (such as IL-7) are probably important for the development and maturation of thymocytes into mature T cells.

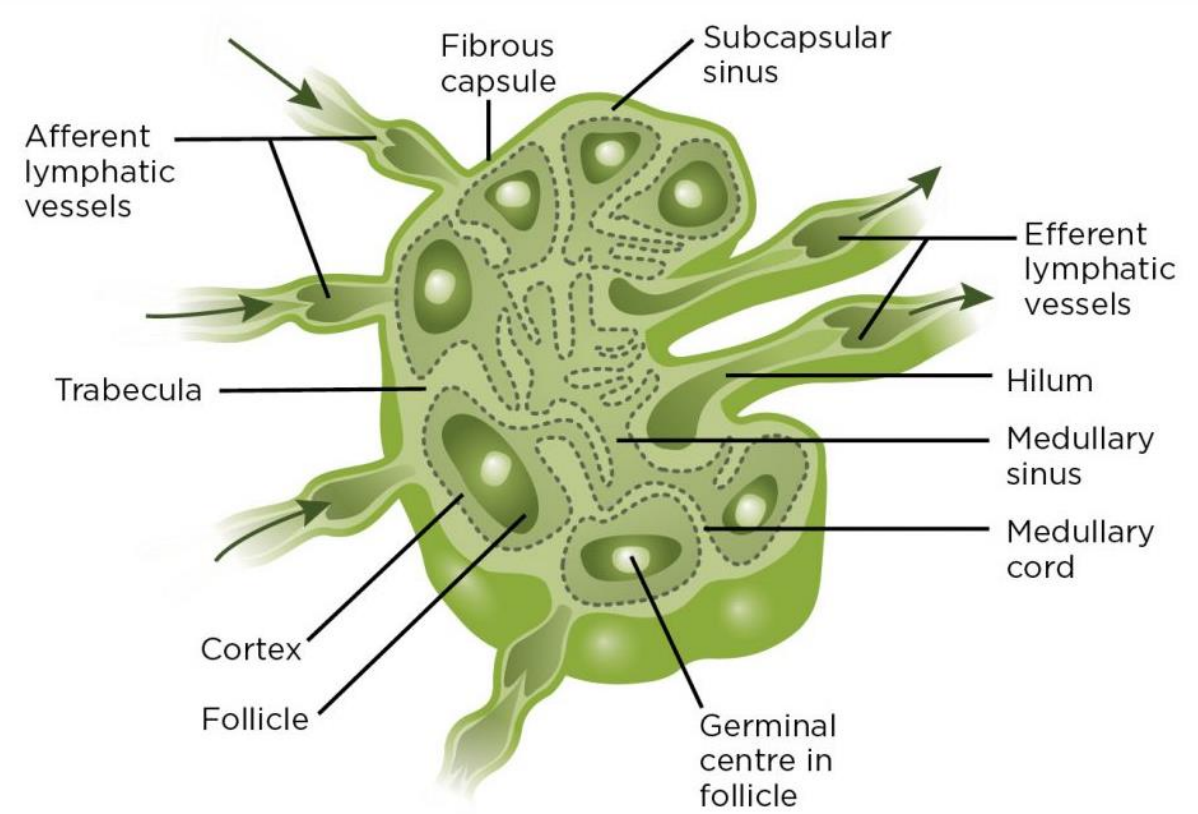
c) Lymph Nodes- Structure and Functions

- **Lymph nodes** are small solid structures placed at varying points along the lymphatic system such as the groin, armpit and mesentery.
- They contain both T and B lymphocytes as well as accessory cells and are primarily responsible for mounting immune responses against foreign antigens entering the tissues.
- Lymph nodes are situated at strategic positions throughout the body and serve to filter the lymph.

Structure of Lymph Nodes

- They range in size from 2 to 10 mm, are spherical in shape and are encapsulated.
- Lymph node is surrounded by a fibrous capsule which dips down into the node substance forming partition or trabeculae.
- The node is made by reticular and lymphatic tissues containing mainly lymphocytes and macrophages.
- Beneath the capsule is the subcapsular sinus, the cortex, a paracortical region and a medulla.
- The cortex contains many follicles and on antigenic stimulation becomes enlarged with germinal centers.
- The follicles are comprised mainly of B cells and follicular dendritic cells.
- The paracortical (thymus-dependent) region contains large numbers of T cells interspersed with interdigitating cells.
- Each lymph node has 4-5 afferent vessels that bring lymph to the node while only one efferent vessel draining lymph away from the node.
- It also has a concave surface called the hilum where an artery enters, a vein and the efferent lymph vessel leave.
- Depending upon the position, the lymph nodes may be superficial or deep lymph nodes. Groups of lymph nodes are present in the neck, collarbone, under the arms (armpit), and groin.





Lymph Circulation in Lymph Nodes

- Lymph arriving from the tissues or from a preceding lymph node in the chain, passes via the afferent lymphatics into the subcapsular sinus and then into the cortex, around the follicles, into the paracortical area and then into the medulla.

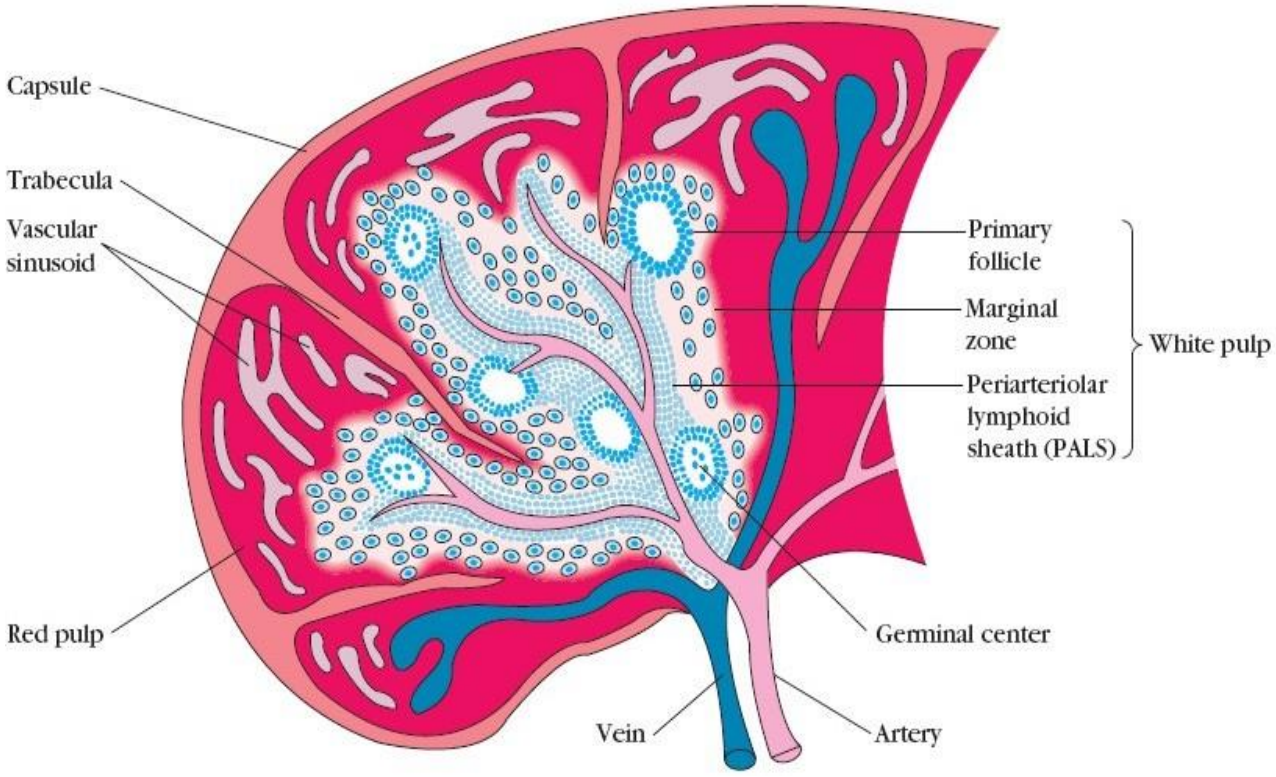
- Lymph in the medullary sinuses then drains into efferent lymphatics and hence through larger lymphatic vessels back into the bloodstream.

Functions of Lymph Nodes

- The primary role of the lymph node is to filter the lymph and then produce an immune response against trapped microbes/antigens.
- Filtering of the lymph helps in removal of particles not normally found in the serum.
- The lymphoide tissues in the nodes break down materials which have been filtered off such as microorganisms, tumor cells and cells damaged by inflammation.
- Lymphocyte develops from the reticular and lymphoid tissue in the nodes.
- Antibodies and antitoxins are also formed by the cells of lymph nodes.

d) Spleen

Spleen- Structure and Functions



The spleen is a large, encapsulated, bean-shaped organ that is situated on the left side of the body below the diaphragm. The spleen contains T and B lymphocytes as well as many phagocytes and is a major component of the mononuclear phagocyte system. Although the structure resembles that of the large lymph nodes, the spleen differs from a lymph node in having no lymphatic drainage, and also in containing large numbers of red cells.

Structure of Spleen

- It is a dark purple-coloured organ, which lies in the left hypochondriae region of the abdomen, between the fundus of the stomach and the diaphragm.
- It varies in size and weight during the lifetime of an individual but in an adult is usually about 12 cm long, 8cm broad and 3-4 cm thick weighing about 200gm.
- The spleen has diaphragmatic and visceral surfaces. The diaphragmatic surface is in contact with the inner surface of the diaphragm.
- The spleen has an outer coat of peritoneum which is firmly adherent to the internal fibro-elastic coat or splenic capsule that dips into the organ, forming trabeculae.
- he spleen has a spongy interior called splenic pulp. The splenic pulps are of two kinds:

1. **White Pulp:**

- It consists of periarteriolar sheaths of lymphatic tissue with enlargements called splenic lymphatic follicles containing rounded masses of lymphocytes.
- These follicles are center of lymphocytes production called primary lymphoid follicles, composed mainly of follicular dendritic cells (FDC) and B cells.
- They are visible to the naked eye in freshly cut surface of the spleen as whitish dots against the dark red background of red pulp.
- The white pulp forms ‘islands’ within a meshwork of reticular fibers containing red blood cells, macrophages and plasma cells (red pulp).

2. **Red Pulp:**

- It consists of numerous sinusoids containing blood, separated by a network of perivascular tissue which is referred to as the splenic cords.
- The splenic cords contain numerous microphages abd are the site of intense phagocytes activity.
- They also contain numerous lymphocytes, which are derived from the white pulp.

Functions of Spleen

1. The main immunological function of the spleen is to filter the blood by trapping bloodborne microbes and producing an immune response to them. It is particularly important for B cell responses to polysaccharide antigens.
2. The spleen is formed partly by lymphatic tissue which produces T lymphocytes and B lymphocytes.
3. Due to the presence of lymphoid reticulo-endothelial tissue, the spleen is involved in producing antibodies and antitoxin.
4. In the foetus, the spleen acts as an important haemopoitic organ.
5. It also removes damaged red blood cells and immune complexes.
6. It can act as an erythropoietic organ which acts as a reservoir of erythrocytes or a reservoir for blood.
7. Those individuals who have had their spleens removed (splenectomized) have a greater susceptibility to infection with encapsulated bacteria, and are at increased risk of severe malarial infections, which indicates its major importance in immunity.

E) Mucosa Associated Lymphoid Tissues (MALT)

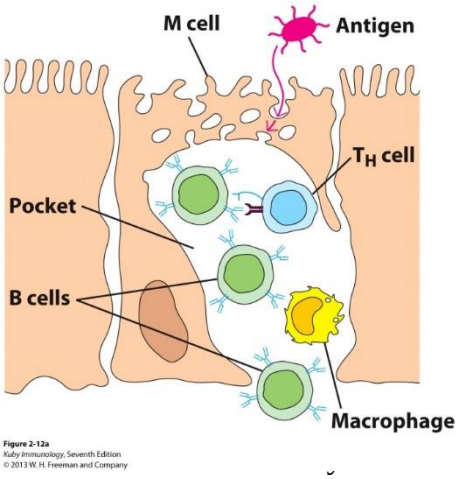


Figure 2-12a
Kuby Immunology, Seventh Edition
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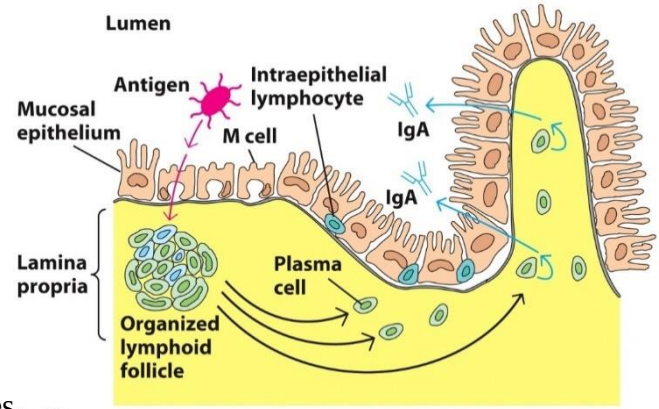

Figure 2-12b
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obes

- Hence, the majority (>50%) of lymphoid tissue in the human body is located within the lining of the respiratory, digestive and genitourinary tracts.
- Small concentrations of lymphoid tissue are also found in thyroid, breast, lung, salivary glands, eye, and skin.
- These lymphoid tissues collectively are thus referred to as mucosa associated lymphoid tissues (MALT). Examples include tonsils, the Peyer patches within the small intestine, and the vermiform appendix.

- Depending upon their location, they can be sub-divided into Nasal-associated lymphoid tissue (NALT), Gut-associated lymphoid tissue (GALT), Bronchus- associated Lymphoid Tissue (BALT) and lymphoid tissue associated with the genitourinary system.

Structure of MALT

All mucosal lymphoid tissues although present at various sites, contain the same basic compartments-follicles, interfollicular regions, subepithelial dome regions, and follicle-associated epithelium.

1. **Nasal-associated lymphoid tissue (NALT)**

- It includes immune cells underlying the throat and nasal passages and especially the tonsils.
- Their structure is similar to that of lymph nodes but they are not encapsulated and are without lymphatics.
- It consists of follicles composed mainly of B cells surrounded by T cells and the germinal center.
- Within the follicle is the site of antigen-dependent B cell proliferation.
- Antigens and foreign particles are trapped within the deep crypts of their lympho-epithelium from where they are transported to the lymphoid follicles.

2. **Gut-associated lymphoid tissue (GALT)**

- It is composed of lymphoid complexes that consist of specialized epithelium, antigen-presenting cells and intraepithelial lymphocytes.
- These structures occur strategically at specific areas in the digestive tract for example **Peyer's patches** in the terminal ileum.
- The specialized epithelial cells are called as **M cells** which are intimately associated with antigen presenting cells (APCs) which together carry out antigen sampling in the gut. M cells sample antigen from the lumen and deliver it to the lymphoid tissue through APCs.

3. **Bronchus- associated lymphoid tissue (BALT)**

- The lymphoid tissue associated with the bronchus (BALT) is structurally similar to Peyer's patches and other lymphoid tissues of the gut.
- It is composed mainly of aggregates of lymphocytes organized into follicles that are found in all lobes of the lung and along the main bronchi.
- The majority of lymphocytes in the follicles are B cells.

- Antigen sampling is carried out by epithelial cells lining the surface of the mucosa and by way of M cells which transport antigens to underlying APCs and lymphocytes.

Functions of MALT

- The mucosa-associated lymphoid tissue initiates immune responses to specific antigens encountered along all mucosal surfaces.
- Diffuse lymphoid tissue along all mucosal surfaces are the sites of IgA transport across the mucosal epithelium.
- The primary role of GALT is to protect the body against microbes entering the body via the intestinal tract.
- M cells take up foreign molecules and pass them to underlying APCs, which present them in the context of class I and class II MHC molecules to T cells. The helper T cells help to activate B cells and both T and B cells can migrate to other parts of the GI tract (including salivary glands) and other MALT sites, e.g. lactating mammary glands and respiratory and genitourinary tracts, and protect these surfaces from invasion by the same microbes.
- The location of nasal and bronchus associated lymphoid tissues in the airway suggest that they are directly involved in handling airborne microbes.
- MALT tissues comprise the mucosal immune system which can function independently of the systemic immune system and are, therefore, an important aspect of immunity.

IV. Basic mechanisms of innate, adaptive, humoral and cell mediated immunity

I. Innate Immunity

- The innate immune system provides the first line of host defense against microbes, before adaptive immune responses have had sufficient time to develop. The mechanisms of innate immunity exist before exposure to microbes.
- The cellular components of the innate immune system include **epithelial barriers** and **leukocytes** (neutrophils, macrophages, NK cells, lymphocytes with invariant antigen receptors, and mast cells).
- The innate immune system uses cell-associated pattern recognition receptors, present on plasma and endosomal membranes and in the cytosol, to recognize structures called **pathogen-associated molecular patterns (PAMPs)**, which are shared by microbes, are not present on mammalian cells, and are often essential for survival of

the microbes, thus limiting the capacity of microbes to evade detection by mutating or losing expression of these molecules.

- In addition, these receptors recognize molecules made by the host but whose expression or location indicates cellular damage; these are called **DAMPs (Damage-associated molecular pattern molecules).**
- **Toll-like receptors (TLRs),** present on the cell surface and in endosomes, are the most important family of pattern recognition receptors, recognizing a wide variety of ligands, including bacterial cell wall components and microbial nucleic acids.
- Cytosolic pattern recognition receptors exist that recognize microbial molecules.
- These receptors include the **RIG-I-like receptors (RLRs)**, which recognize viral RNA, CDSs which recognize microbial DNA, and **NOD-like receptors (NLRs)**, which recognize bacterial cell wall constituents and also serve as recognition components of many inflammasomes.
- Pattern recognition receptors, including TLRs, NLRs, and RLRs, signal to activate the transcription factors NF-κB and AP-1, which stimulate expression of cytokines, costimulators, and other molecules involved in inflammation, and the IRF transcription factors, which stimulate expression of the antiviral type I interferon genes.
- The inflammasome, a specialized caspase-1 containing enzyme complex that forms in response to a wide variety of PAMPs and DAMPs, includes recognition structures, which are often NLR family proteins, an adaptor, and the enzyme caspase-1, the main function of which is to produce active forms of the inflammatory cytokines IL-1 and IL-18.
- Soluble pattern recognition and effector molecules are found in the plasma, including pentraxins (e.g., CRP), collectins (e.g., MBL), and ficolins.
- These molecules bind microbial ligands and enhance clearance by complement-dependent and complement-independent mechanisms.
- Innate lymphoid cells are cells with lymphocyte morphology and functions similar to T lymphocytes, but do not express clonally distributed T cell antigen receptors.
- Three helper subsets of ILCs secrete the same cytokines as Th1, Th2, and Th17 helper T cells.

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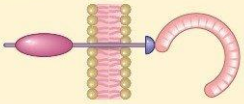
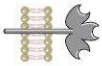
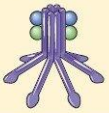
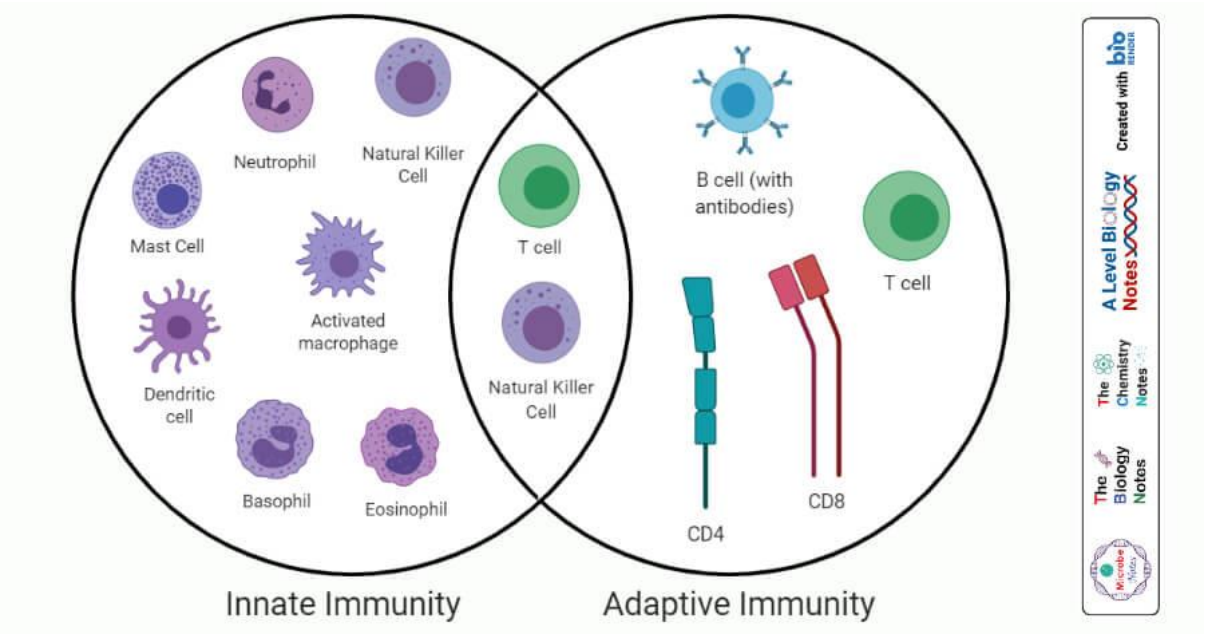
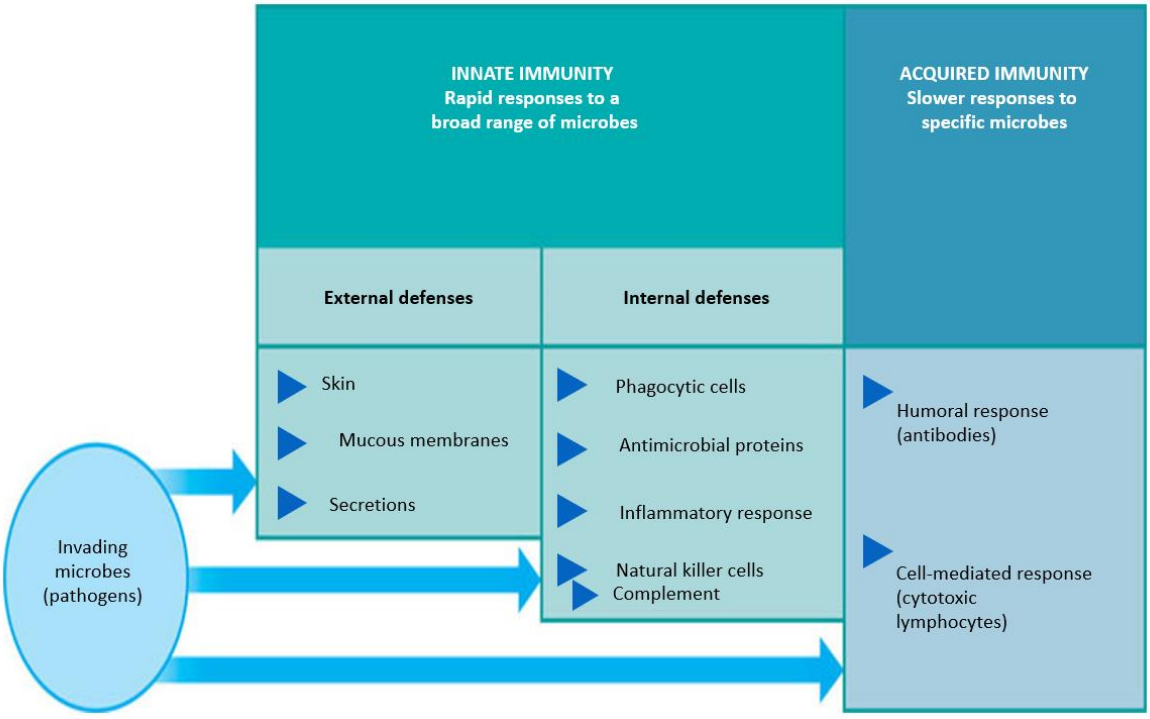
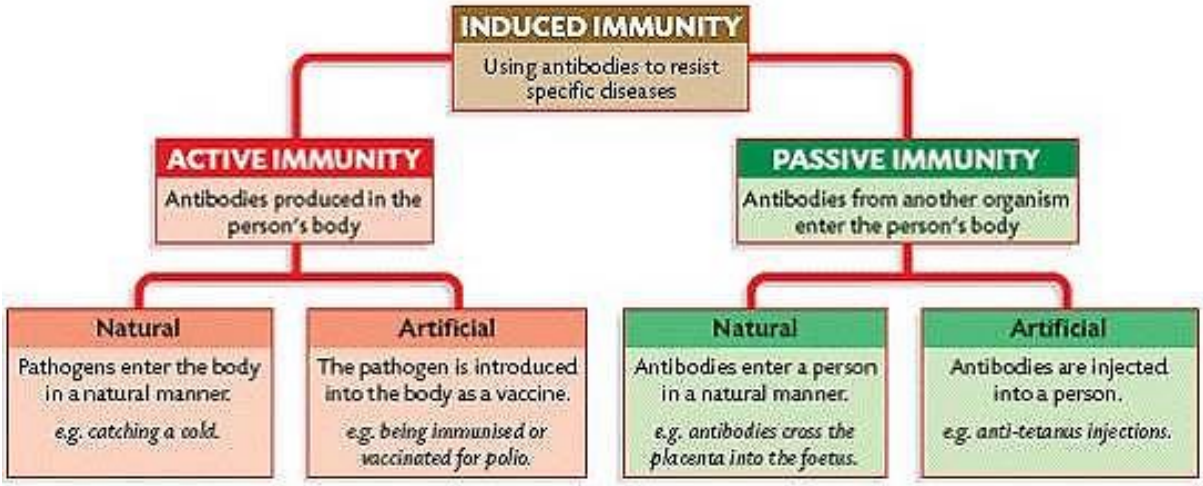
Pattern Recognition Receptors	Location	Specific Examples	Ligands (PAMPs or DAMPs)
Cell-Associated			
TLRs 	Plasma membrane and endosomal membranes of DCs, phagocytes, B cells, endothelial cells, and many other cell types	TLRs 1–9	Various microbial molecules including bacterial LPS and peptidoglycans; viral nucleic acids
NLRs 	Cytosol of phagocytes, epithelial cells, and other cells	NOD1/2 NLRP family (inflammasomes)	Bacterial cell wall peptidoglycans Intracellular crystals (urate, silica); changes in cytosolic ATP and ion concentrations; lysosomal damage
RLRs 	Cytosol of phagocytes and other cells	RIG-1, MDA-5	Viral RNA
CDSs 	Cytosol of many cell types	AIM2; STING-associated CDSs	Bacterial and viral DNA
CLRs 	Plasma membranes of phagocytes	Mannose receptor DC-sign Dectin-1, Dectin-2	Microbial surface carbohydrates with terminal mannose and fructose Glucans present in fungal and bacterial cell walls
Scavenger receptors 	Plasma membranes of phagocytes	CD36	Microbial diacylglycerides
N-Formyl met-leu-phe receptors 	Plasma membranes of phagocytes	FPR and FPRL1	Peptides containing *N*-formylmethionyl residues
Soluble			
Pentraxins 	Plasma	C-reactive protein	Microbial phosphorylcholine and phosphatidylethanolamine
Collectins 	Plasma Alveoli	Mannose-binding lectin Surfactant proteins SP-A and SP-D	Carbohydrates with terminal mannose and fructose Various microbial structures
Ficolins 	Plasma	Ficolin	*N*-acetylglucosamine and lipoteichoic acid components of the cell walls of gram-positive bacteria
Complement 	Plasma	Various complement proteins	Microbial surfaces


Neutrophil
Natural Killer Cell
Mast Cell
Activated macrophage
Dendritic cell
Basophil
Eosinophil
T cell
Natural Killer Cell
B cell (with antibodies)
T cell
CD4
CD8
Innate Immunity
Adaptive Immunity
Created with bio
A Level Biology Notes
The Chemistry Notes
The Biology Notes


INNATE IMMUNITY
Rapid responses to a broad range of microbes
ACQUIRED IMMUNITY
Slower responses to specific microbes
External defenses
Internal defenses
Skin
Mucous membranes
Secretions
Phagocytic cells
Antimicrobial proteins
Inflammatory response
Natural killer cells
Complement
Humoral response (antibodies)
Cell-mediated response (cytotoxic lymphocytes)
Invading microbes (pathogens)


INDUCED IMMUNITY
Using antibodies to resist specific diseases
ACTIVE IMMUNITY
Antibodies produced in the person's body
PASSIVE IMMUNITY
Antibodies from another organism enter the person's body
Natural
Pathogens enter the body in a natural manner.
e.g. catching a cold.
Artificial
The pathogen is introduced into the body as a vaccine.
e.g. being immunised or vaccinated for polio.
Natural
Antibodies enter a person in a natural manner.
e.g. antibodies cross the placenta into the foetus.
Artificial
Antibodies are injected into a person.
e.g. anti-tetanus injections.

S.N	Characteristics	Innate Immunity	Adaptive Immunity
1.	Synonyms	Nonspecific, natural immunity	Specific, acquired immunity
2.	Definition	The defense mechanisms that are non-antigen specific and immediately come into play on the antigen's appearance in the body.	The defense mechanism that is not always present but involve antigen-specific immune response.
3.	Order of defense	It is the first line of defense of immune system.	It is the action against pathogens that are able to evade or overcome innate immune defenses.
4.	State at birth	Presence since birth.	Acquired during lifetime.
5.	Presence	Always present in the body itself.	Developed only upon exposure to antigens.
6.	Inducible	No	Yes
7.	Cells involved	Physical epithelial barriers, Phagocytic leukocyte, Dendritic cells, Natural killer (NK) cell, Mast cells etc.	Killer CD8+ T-cells, Helper CD4+ T-cells, B-cells, Antigen presenting cells etc.
8.	Molecules involved	Cytokines, Complements ,Interferon, Acute phase proteins.	Antibodies Cytokines
9.	Fights against	Fights any foreign invader and thus is non-specific.	Ability to fight a specific infection.
10.	Receptors involved	Uses receptors that recognize conserved pathogen-associated molecular patterns (PAMPs)	Uses recombined B- and T-cell receptors that recognize specific antigens on pathogens

		such as LPS, flagellin, nucleic acids.	
11.	Effector function	Constitutive effective functions encoded in the germline (inflammation, phagocytosis)	Inducible effector functions (proliferation, activation, maturation, differentiation)
12.	Response time	Occurs rapidly from minutes to hours.	Occurs over days to weeks.
13.	Immunological memory	Does not confer memory	Confer immunological memory
14.	Directed against	Innate immunity is directed towards types of molecules.	It is directed towards specific epitopes.
15.	Subsequent exposure	The immune response does not alter on repeated exposure.	Immune response improves with each successive exposure.
16.	Types of immune response	Types of adaptive immune responses: Inflammation, Complement mediated killing, Phagocytosis etc.	Two types of adaptive immune responses: humoral immunity, mediated by antibodies produced by B lymphocytes, and cell-mediated immunity, mediated by T lymphocytes.
17.	Changeability	May vary between individuals but does not change over course of an individual lifetime	Immunity is generated by recombination of V, D, and J regions and further hypervariation thus may change.
18.	Diversity	Limited	Diverse

19.	Potency	Limited and Lower potency	Higher potency
20.	Inheritance	Inherited from parents	Not inherited from parents
21.	Time span	Once activated against a specific type of antigen, the immunity remains throughout the life.	The span of developed immunity can be lifelong or short.
22.	Complexity	Innate immune response is less complex.	More complex than the innate immune response.
23.	Anatomic and physiological barriers	Skin, Mucous membranes, Temp, pH, chemicals, etc.	Lymph nodes, spleen, mucosal associated lymphoid tissue.
24.	Allergy or Hypersensitivity reaction	None	Immediate and Delay hypersensitivity
25.	Complement system activation	Alternative and lectin pathways	Classical pathway
26.	Found in	Found in nearly all forms of life.	Found only in jawed vertebrates.
27.	Factors causing immune evasion	Caused by pathogenic virulence factor. Often involves disabling the conserved pattern recognition used by innate system	Caused by mutation of the recognized antigen.

28.	Functions	a) Recruiting immune cells to sites of infection b) Activation of the complement cascade to identify antigens c) Identification and removal of foreign substances present in organs, tissues, blood and lymph. d) Activation of the adaptive immune system through antigen presentation. e) Acting as a physical and chemical barrier to infectious agents.	a) Recognition of specific "non-self" antigens during the process of antigen presentation. b) Generation of responses that are tailored to maximally eliminate specific pathogens or pathogen-infected cells. c) Development of immunological memory, through memory B cells and memory T cells.
29.	Examples	White blood cells fighting bacteria, causing redness and swelling during a cut.	Administration of Chickenpox vaccination such that an individual do not develop chickenpox as adaptive immunity forms immunological memory.

V. Cell mediated and Humoral Mediated Immunity

The immune system distinguishes two groups of foreign substances. One group consists of antigens that are freely circulating in the body. These include molecules, viruses, and foreign cells. A second group consists of self-cells that display aberrant MHC proteins. Aberrant MHC proteins can originate from antigens that have been engulfed and broken down (exogenous antigens) or from virus-infected and tumor cells that are actively synthesizing foreign proteins (endogenous antigens). Depending on the kind of foreign invasion, two different immune responses occur:

The humoral response (or antibody-mediated response) involves B cells that recognize antigens or pathogens that are circulating in the lymph or blood ("humor" is a medieval term for body fluid). The response follows this chain of events:

1. Antigens bind to B cells.
2. Interleukins or helper T cells costimulate B cells. In most cases, both an antigen and a costimulator are required to activate a B cell and initiate B cell proliferation.
3. B cells proliferate and produce plasma cells. The plasma cells bear antibodies with the identical antigen specificity as the antigen receptors of the activated B cells. The antibodies are released and circulate through the body, binding to antigens.
4. B cells produce memory cells. Memory cells provide future immunity.

S.N.	Characteristics	Humoral Immunity	Cell-mediated Immunity
1.	Definition	The immunity mediated by macromolecules found in the extracellular body fluids is called humoral immunity. ("humor" a medieval term for body fluid)	The immunity that identifies and destroys infected cells in the body is called cell-mediated immunity.
2.	Mediator	The main cell involved in humoral immunity are B-cells.	The main cell involved in cell-mediated immunity are T-cells.
3.	Components	B cells, T cells, and macrophages.	Helper T cells, cytotoxic T-cells, natural killer cells, and macrophages.
4.	Pathogen	The humoral immunity protects against extracellular pathogens and also their toxin.	The cell-mediated immunity protects against intracellular pathogens.
5.	Pathogen recognition	Recognize antigens or pathogens that	It responds to any cell that displays aberrant MHC markers, including cells invaded by pathogens, tumor cells, or transplanted cells.

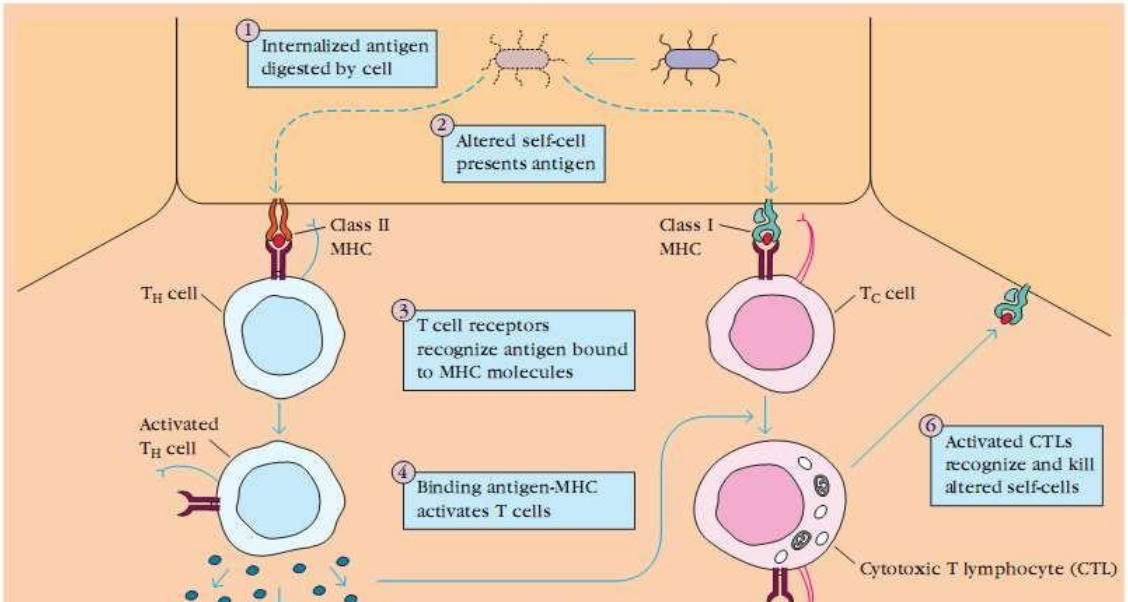
		are circulating in the lymph or blood.	
6.	Antigen detectors	Phagocytes and antibodies themselves are used to detect antigens.	Receptors and MHC molecules on the cell surfaces are used to detect antigens.
7.	Antigen Binding	B-cells produce antibodies and the antibodies bind to antigens.	T-cell receptors on cells bind to T-cells which in turn bind to antigens.
8.	Antigen Processing	Do not require the processing of antigens.	Antigens must be processed and presented for T-lymphocyte mediated response.
9.	Receptor Involved	It involves B-cell receptors (BCRs).	It involves T-cell receptors (TCRs).
10.	Accessory surface receptors/molecules	Igα, Igβ, Fc receptors, CD40, CD21	CD3 molecular complex Dimer of ∑ chain, CD4, CD8, CD2, CD28, integrins
11.	Type of T-cell involved	Only the T helper cell (CD4+) is involved.	Both CD4+ and CD8+ T cells are involved.

12.	Antibodies formation	Antibodies are formed in a humoral response.	Antibodies are not formed in a cell-mediated immune response.
13.	Onset	The onset is rapid.	The onset is delayed.
14.	Result	The end result of the activation is the differentiation of plasma B-cells, secreting antibodies.	The end result of the activation is the secretion of cytokines.
15.	Protection against	Extracellular bacterial or viral pathogens.	It protects against fungus, viruses, and intracellular bacterial pathogens.
16.	Immunological surveillance	It does not provide immunological surveillance.	It provides immunological surveillance.
17.	Hypersensitivity reactions	Hypersensitivity type I, II, and III is mediated by humoral immunity.	Hypersensitivity type IV is mediated by cell-mediated immunity.
18.	Role in Organ transplantation and Grafting	It may be involved in early graft	It participates in rejections of organ transplants.

		rejection due to preformed antibodies.	
19.	Immunity against cancer	It does not provide immunity against cancer.	As it destroys the tumor and cancerous cells, it provides protection against cancer.
20.	Assessment method	From plasma level of antibodies	Skin test for the development of delayed-type of hypersensitivity

The cell-mediated response involves mostly T cells and responds to any cell that displays aberrant MHC markers, including cells invaded by pathogens, tumor cells, or transplanted cells. The following chain of events describes this immune response:

1. Self cells or APCs displaying foreign antigens bind to T cells.
2. Interleukins (secreted by APCs or helper T cells) costimulate activation of T cells.
3. If MHC-I and endogenous antigens are displayed on the plasma membrane, T cells proliferate, producing cytotoxic T cells. Cytotoxic T cells destroy cells displaying the antigens.
4. If MHC-II and exogenous antigens are displayed on the plasma membrane, T cells proliferate, producing helper T cells. Helper T cells release interleukins (and other cytokines), which stimulate B cells to produce antibodies that bind to the antigens and stimulate nonspecific agents (NK and macrophages) to destroy the antigens.



Differences between Primary and Secondary Immune Response

In a primary immune response, naive **B cells** are stimulated by antigen, become activated, and differentiate into antibody-secreting cells that produce antibodies specific for the eliciting antigen. A secondary immune response is elicited when the same antigen stimulates memory B cells, leading to the production of greater quantities of specific antibodies that are produced in the primary response. Some of the differences between Primary and Secondary Immune Response are as follows:

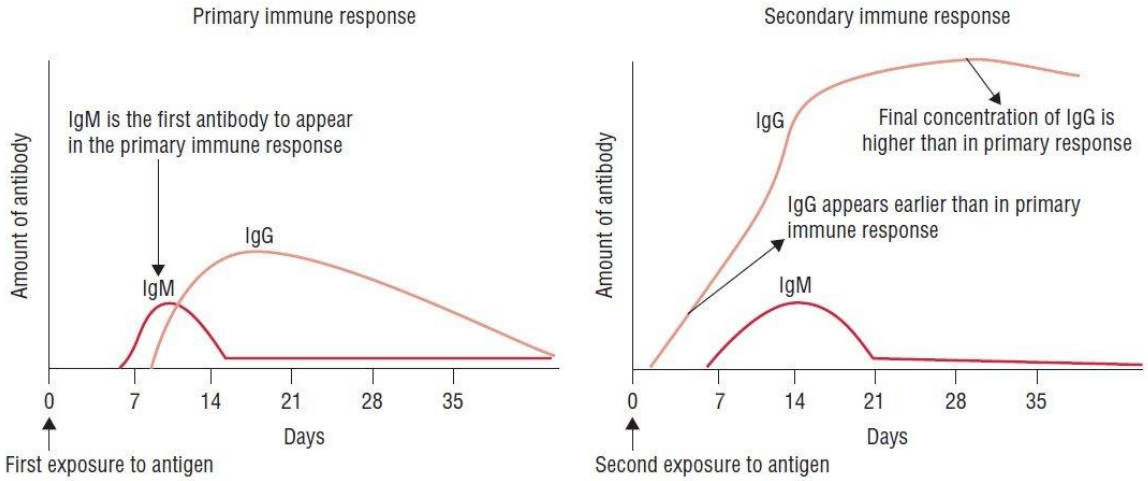
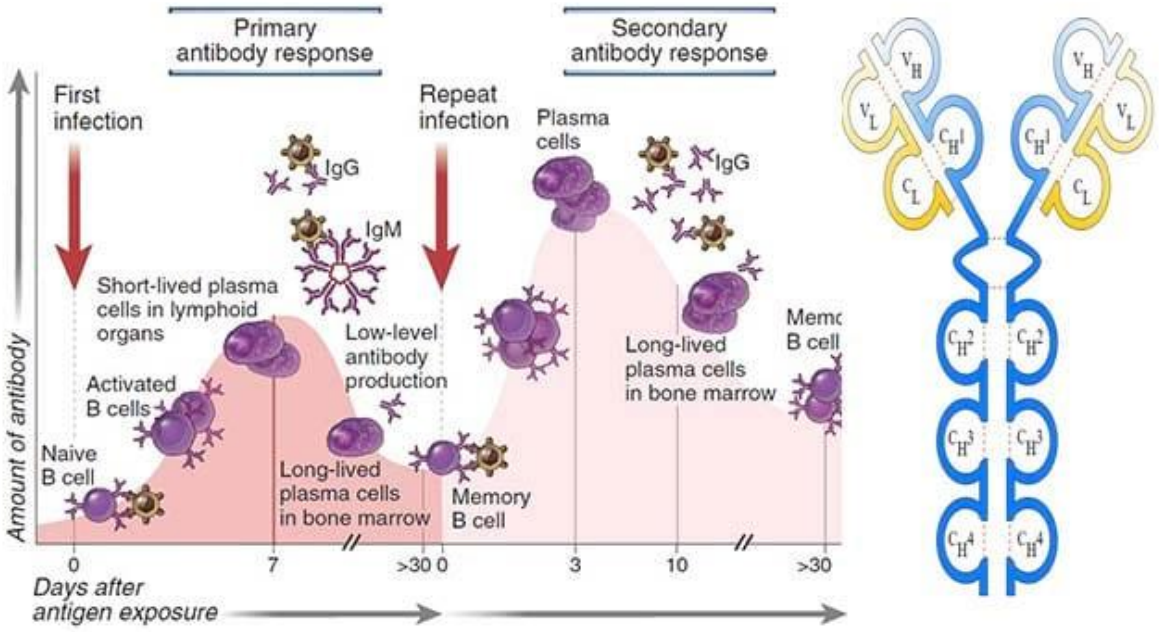


Figure: A schematic diagram showing a primary and secondary response,

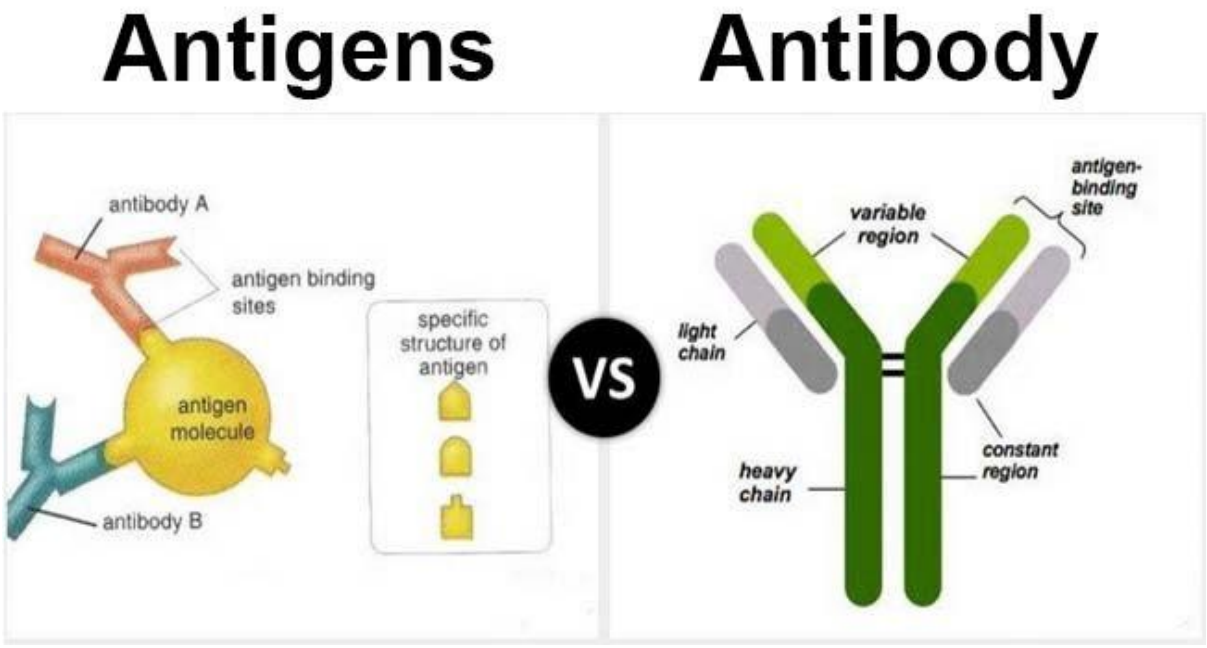


S.N.	Characteristics	Primary Immune Response	Secondary Immune Response
1	Definition	Primary Immune Response is the reaction of the immune system when it contacts an antigen for the first time.	Secondary Immune Response is the reaction of the immune system when it contacts an antigen for the second and subsequent times.
2	Appearance	Appears mainly in the lymph nodes and spleen.	Appears mainly in the bone marrow and then, in the spleen and lymph nodes.
3	Occurrence	This occurs in response to the primary contact of the antigen.	This occurs in response to the second and subsequent exposure to the same antigen.
4	Antibody Peak	The antibody level reaches its peak in 7-10 days.	The antibody level reaches its peak in 3-5 days.
5	Affinity of Antibody	Low affinity to their antigens.	High affinity to their antigens.
6	Responding Cells	Naive B cells and T cells	Memory B cells
7	Antibodies	Both thymus-dependent and thymus-independent antibodies are involved in the primary immune response.	Only thymus-dependent antibodies are involved in the secondary immune response.
8	Lag Phase	Long (4-7 days)	Short (1-4 days)

9	Types of Antibodies	A large amount of IgM and a small amount of IgG are produced during the primary immune response.	A large amount of IgG, a small amount of IgM, IgA, and IgE are produced during the secondary immune response.
10	Amount of Antibody	Few antibodies are produced in the primary immune response.	100-1000 times more antibodies are produced in the secondary immune response.
11	Strength of the Response	The primary immune response is usually weaker than secondary immune response.	The secondary immune response is stronger.
12	Antibody level	Antibody level declines to the point where it may be undetectable.	The antibody level tends to remain high for longer.

Unit -II

Mind Map



I. Antigens

Antigens are specifically defined as molecules that interact specifically with **immunoglobulin receptor of B-cell** or antigen is any substance that may be specifically bound by an antibody molecule or T cell receptor. Antigen is a substance which when introduced into living animal evokes specific immune response either by producing specific antibody or by sensitized T-cell. Antigen may be soluble substance, toxin or substance present in bacteria, virus, RBC and other types of cell. It is a substance usually protein in nature and sometimes polysaccharide, that generates a specific immune response and induces the formation of a specific antibody or specially sensitized T cells or both. Antigens are "targeted" by antibodies.

An antigen may be a substance from the environment, such as chemicals, bacteria, viruses, or pollen or may also be from inside the body. In general, two main divisions of antigens are recognized: **foreign antigens (or hetero-antigens)** and **autoantigens (or self-antigens)**.

Foreign originates from outside the body. Examples include parts of or substances produced by viruses or microorganisms (such as bacteria and protozoa), as well as substances in snake venom, certain proteins in foods, and components of serum and red blood cells from other individuals.

Autoantigens originates within the body. Normally, the body is able to distinguish self from non-self, but in persons with autoimmune disorders, normal bodily substances provoke an immune response, leading to the generation of autoantibodies. Ribonucleoprotein antigens in lupus-related diseases and mitochondrial antigens in primary biliary cirrhosis (PBC) etc. are examples of autoantigens.

Although all antigens are recognized by specific lymphocytes or by antibodies, only some antigens are capable of activating lymphocytes. Antigens that stimulate immune responses are called **immunogens.** An antigen that induces an immune response i.e., stimulates the lymphocytes to produce antibody or to attack the antigen directly is called an immunogen. A substance that induces specific immune response can be called as immunogen.

Antibody binds to only a portion of the antigen, which is called a **determinant or an epitope**. **Epitope** is immunologically active regions of an immunogen (or antigen) that binds to antigen-specific membrane receptors on lymphocytes or to secreted antibodies. It is also called **antigenic determinants**. The small area of chemical grouping on antigen molecule which determines specific immune response and reacts specifically with antibody is known as epitope. Antigens typically contain multiple determinants, some of which may be repeated and each of which can be bound by an antibody. The presence of multiple identical determinants in an antigen is referred to as **polyvalency or multivalency**.

The spatial arrangement of different epitopes on a single protein molecule may influence the binding of antibodies in several ways. When determinants are well separated, two or more antibody molecules can be bound to the same protein antigen without influencing each other; such determinants are said to be **non-overlapping**. When two determinants are close to one another, the binding of antibody to the first determinant may cause steric interference with the binding of antibody to the second; such determinants are said to be **overlapping**.

Immunogenicity is defined as the property of a substance (immunogen) that endows it with the capacity to provoke a specific immune response. Immunogenicity is the ability to induce a humoral/ cell-mediated immune response. Antigenicity is defined as the property of a substance (antigen) that allows it to react with the products of a specific immune response (antibody or Tcell receptor). Antigenicity is the ability to combine specifically with the final products (Antibodies or receptors in T-Cell) of humoral /cell mediated immune response.

Definition

Antigens are molecules or molecular structures that are foreign to the body and generally induce an immune reaction in the form of the production of antibodies against them.

- In simple words, antigens can be anything that doesn't belong to the body and are foreign.
- Even though antigens are usually defined by the induction of an immune response, all antigens might not induce an immune response. The antigens that induce a response are termed immunogens.
- The ability of antigens to elicit an immune response depends on the presence of specific regions on the antigens called antigenic determinants. The determinants bind to receptor molecules with the complementary structure on immune cells to elicit a response.
- Antigens are indicated by the term 'Ag', and these can occur in different forms like pollen, viruses, chemicals, or bacteria.
- The concept of antigen arose from the fact that our body can distinguish between the components of the body and foreign particles.
- In response to these antigens, the body induces the production of antibodies that act against the said antigens.
- Most antigens in humans are proteins, peptides, or polysaccharides; however, lipid and nucleic acids can also act as antigens when combined with proteins or polysaccharides.
- In addition, antigens might also be intentionally introduced into the body in the form of vaccines in order to induce the adaptive immune system of the body against the antigen.

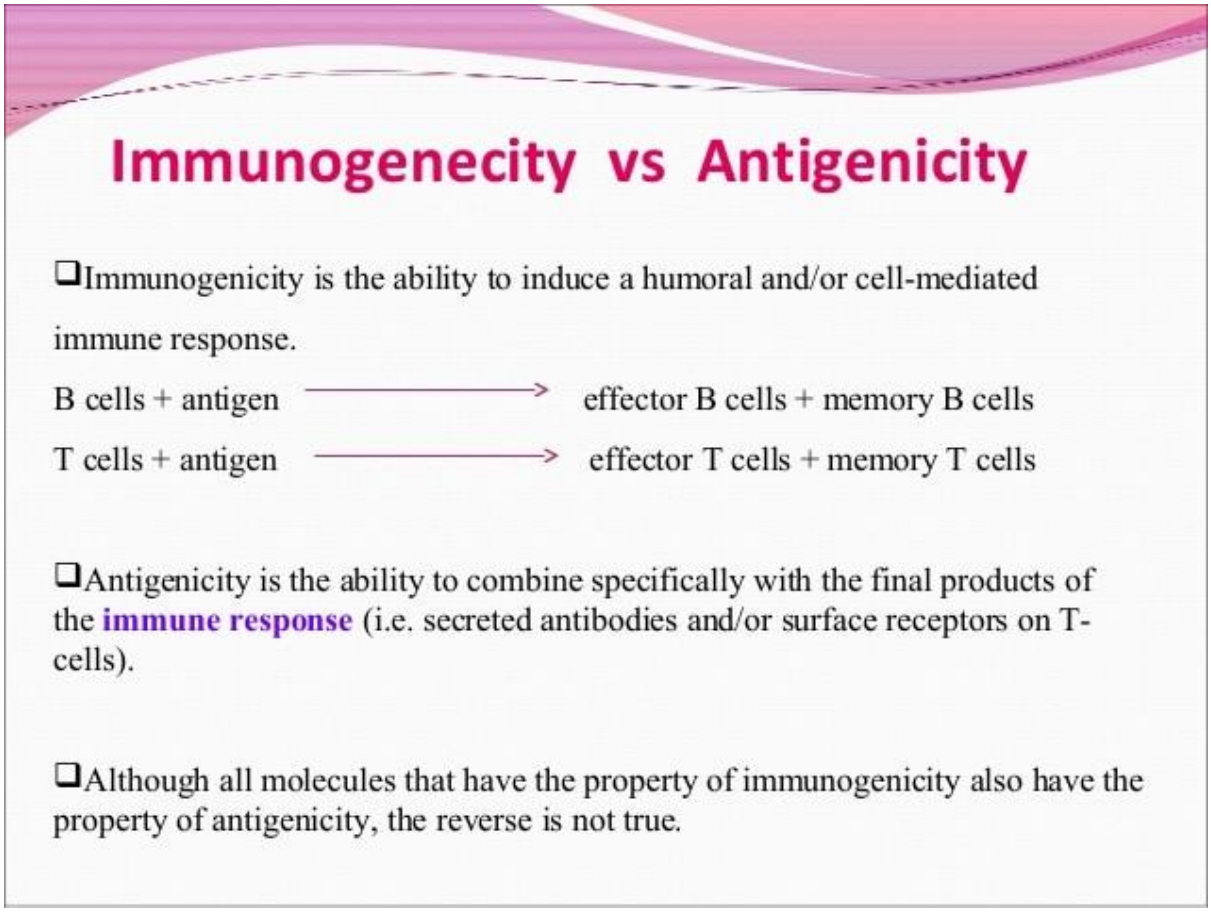
II. ANTIGENICITY AND IMMUNOGENICITY

Antigenicity is defined as the property of a substance (antigen) that allows it to react with the products of a specific immune response (antibody or T-cell receptor). On the other hand, immunogenicity is defined as the property of a substance (immunogen) that endows it with the capacity to provoke a specific immune response. From these definitions it follows that all immunogens are antigens; the reverse, however, is not true, as dis-cussed later.

B-cell immunogens are usually complex, large molecules that are able to interact with B-cell surface receptors (membrane immunoglobulins) and deliver the initial activating signal leading to clonal expansion and differentiation of antibody-producing cells. T-cell immunogens can be best defined as compounds that can be processed by antigen-presenting cells into short polypeptide chains that combine with MHC molecules; the peptide-MHC complexes are able to interact with specific T-cell receptors and deliver activating signal to the T cells carrying such receptors.

Landsteiner, Pauling, and others discovered in the 1930s and 1940s that small aromatic groups, such as amino-benzene sulfonate, amino-benzene arsenate, and amino-benzene carboxylate, unable to induce antibody responses by themselves, would elicit antibody formation when chemically coupled to immunogenic proteins. The injection of these complexes into laboratory animals resulted in the production of antibodies specific for the different aromatic groups. The aromatic groups were designated as “haptens” and the immunogenic proteins as “carriers.” The immune response induced by a hapten-carrier conjugate included antibodies able to recognize the hapten and the carrier as separate entities. The hapten-specific antibodies are also able to react with soluble hapten molecules, free of carrier protein. Thus, a hapten is an antigen, but not an immunogen. In practical terms, it must be noted that the designations of antigen and immunogen are often used interchangeably

Experiments comparing the specificity of hapten-specific antibodies induced with isomers of aromatic groups were critical for the definition of antibody specificity. Experiments comparing the effects of different hapten-carrier combinations or preimmunization with carrier proteins on hapten-specific responses helped to define T-B lymphocyte cooperation. Later, the principles established with hapten-carrier conjugates were expanded to the induction of immune responses directed against many small molecular weight compounds and even poorly immunogenic polysaccharides, all of which may induce strong responses after conjugation to an immunogenic carrier protein. This knowledge helped explain the pathogenesis of some hyper sensitivity disorders and was the basis for the development of improved immunization protocols.



III. Properties of Antigen

Antigens have different properties which determine the immunogenicity of the antigens and thus are essential in order to understand the immune reaction against them. Since these properties determine the immunogenicity, these are considered properties required to form a good antigen. The following are some of the properties of antigens;

1. Foreign Nature

- All antigens that induce an immune response in the host are foreign to the body of the recipient.
- The host body recognizes the antigen to be different from the normal body components.
- The immunogenicity of the antigen increases with the increase in the degree of foreignness. In the case of biological antigens, the foreignness increases with the increase in the phylogenetic gap between the two species.
- However, there are some exceptions in that some proteins occurring within the host might also induce an immune response, as in the case of autoantigens.

- Similarly, proteins and other molecules from other species might also not induce an immune response if they lack antigenic determinants or epitopes.

2. Chemical Nature

- The most potent and commonly encountered antigens are proteins followed by polysaccharides.
- However, other molecules like lipids and nucleic acids can also act as antigens when complex with proteins and polysaccharides.
- In the case of proteins, the antigen should contain immunogenic regions with at least 30% of amino acids like lysine, glutamine, arginine, glutamic acids, aspargine, and aspartic acid, along with a high number of hydrophilic or charged groups.
- The level of immunogenicity also increases with the heterogenicity of the molecules. Homopolymers are usually less immunogenic than heteropolymers.

3. **Molecular Size**

- The molecular size of the antigens is also crucial in the immunogenicity of the molecules.
- It has been established that antigens should have a minimum size of greater than 5000 Da before they can be considered immunogenic.
- However, low molecular weight substances can demonstrate immunogenicity when coupled with large-sized carriers.
- The low molecular weight substances are termed haptens that are considered 'partial antigens' with at least one antigenic determinant.

4. Molecular Rigidity and Complexity

- The rigidity and complexity of molecules are essential factors that determine immunogenicity.
- In general, rigid molecules are good antigens as they can raise antibodies to certain structures when compared to the less rigid ones.
- The complexity of the structure is also an essential factor as a peptide antigen with a repeating unit of a single amino acid is less immunogenic than a molecule with two or more repeating amino acids units.

5. **Antigenic Determinants and Cross-reactivity**

- Antigenic determinants are regions in an antigen molecule that is involved in the reaction with antibodies.
- Usually, antigens with two or more antigenic determinants can induce antibody production. Thus, a smaller antigen usually doesn't induce antibody production as it is not possible for a small molecule to have more than one antigenic determinant.
- Cross-reactivity of antigens is also an essential factor where antibodies induced by a different antigen can interact with another antigen.

III. Antigen Structure

- The molecular structure of an antigen is characterized by its ability to bind to the antigen-binding site of an antibody.
- Antibodies differentiate between different antigens on the basis of the specific molecular structures present on the surface of the antigen.
- Most antigens are proteins or polysaccharides. These can include coats, capsules, flagella, toxins, and fimbriae of bacteria, viruses, or other microorganisms. Besides, secretions and other chemicals of the same nature can also act as antigens.
- Lipids and nucleic acids of these microorganisms are only antigenic when these are combined with proteins or polysaccharides.
- The structure of antigens might be different depending on the nature of the antigen, their size, and immunogenicity.
- All immunogenic antigens have a specific structural component called epitope or antigenic determinant.
- The number of epitopes differs in different antigens and determines the number of antibodies a single antigen can bound to.
- The structural components of interaction in antigens are different, which determines the classes of antibodies they bound to.
- The region on antibodies that interacts with antigens is called a paratope. It has been established that the structure of epitope and paratope can be defined with a lock and key metaphor as the structures are specific and fit with one another.

IV. Types of Antigen

Antigens can be classified into two groups on the basis of their origin;

a. Exogenous Antigens

- Exogenous antigens are the antigens that are originated outside the body of the host and, thus, are foreign to the host.
- These antigens might enter the body through inhalation, ingestion, or injection and then circulate throughout the body via bodily fluids.
- The uptake of exogenous antigens is primarily mediated by phagocytosis via Antigen Processing Cells (APCs) like macrophages, dendritic cells, etc.
- Many antigens like intracellular viruses might begin as exogenous antigens and later become endogenous.

b. Endogenous Antigens

- Endogenous antigens are antigens that originate within the body of the host during metabolism or as a result of intracellular viral or bacterial infection.
- Endogenous antigens are usually the cells of the body or fragments, compounds, or antigenic products of metabolism.
- These are usually processed in the macrophages and are later detected by cytotoxic T-cells of the immune system.
- Endogenous antigens include antigens that are xenogenic or heterologous, autologous, and idiotype or allogenic.
- Endogenous antigens might result in autoimmune diseases as the host immune system detects its own cells and particles as immunogenic.

c. Autoantigens

- Autoantigens are proteins or protein complexes of the host that are attacked by the host's immune system, resulting in autoimmune disease.
- Autoantigens can be deadly to the host as the body's own cells should not be targeted by the immune system.
- The immunological tolerance to such antigens is lost as a result of genetic and environmental factors.

d. Tumor Antigens (Neoantigens)

- Tumor antigens or neoantigens are presented by Major Histocompatibility Complex (MHC) I and II on the surface of tumor cells.

- The antigens are produced as a result of a tumor-specific mutation during the malignant transformation of normal cells.
- These antigens usually do not induce an immune response as the tumor cells develop ways to evade antigen presentation and immune defense.

e. Native Antigens

- Native antigens are antigens that are not processed by any antigen-presenting cells (APC), and thus immune cells like T-cells cannot bind to these antigens.
- However, B-cells can be activated such antigens even without any processing.

2. Types of antigens on the basis of immune response

- Antigens can be classified into two distinct groups on the basis of immune response;

a. Complete antigens/ Immunogens

- Complete antigens or Immunogens are antigens that elicit a specific immune response.
- These antigens can induce an immune response by themselves without any carrier particles.
- These are usually proteins, peptides, or polysaccharides with high molecular weight (greater than 10,000 Da).

b. Incomplete antigens/ Haptens

- Incomplete antigens or haptens are antigens that cannot generate an immune response by themselves.
- These are usually non-protein substances that require a carrier molecule to form a complete antigen.
- Haptens have a low molecular weight (usually less than 10,000 Da) and fewer antigenic determinant sites.
- The carrier molecule bonded to the hapten is considered a non-antigenic component and is a protein or a polysaccharide molecule.

V. Examples of Antigen

1. Blood group antigens

- Blood group antigens are proteins or sugars present on the surface of different components in the red blood cell membrane.
- The antigens in the ABO blood group are the sugar that is produced by a serried of reactions that catalyzes the transfer of sugar units.
- The type of sugar in the red blood cell is determined by the type of enzyme involved, which in turn is determined by the person's DNA.
- The antigens of the Rh blood group are proteins that are also determined by the host's DNA. The RhD gene encodes the D antigen, which occurs as a large protein on the red blood cell.
- These antigens can be distinguished by antigen-antibody reactions that help determine different blood groups in humans.

2. **Bacterial Capsule**

- A bacterial capsule is a polysaccharide layer occurring outside the cell envelope that induces an immunogenic reaction in the host.
- The capsule is a well-organized layer that cannot be removed easily and thus is considered a possible cause of bacterial pathogenicity.
- In some bacteria, the capsule can also be involved in evading phagocytosis as a capsule-specific antibody is required to cause phagocytosis.
- Bacterial capsules are also used as antigens used in vaccines where the polysaccharide component of the capsule is conjugated with protein carriers.
- The exact structure, function, and involvement of capsules in bacteria differ in different bacterial species.

VI. Haptens

- Haptens Derived from Greek word "Haptein" which means " To fasten".
- The term Hapten was first coined by Karl Landsteiner.
- Many low molecular weight organic molecules that are not antigenic by themselves but become antigenic if they bond to a layer carrier molecule such as a protein.
- These low molecular weight by itself, they require carries molecule to act as a complete Antigen.
- The carrier molecules is a non-antigenic component and helps in provoking the immune response.

- Example: Serum protein such as Albumin or Globulin.
- Low molecular weight (less than 10,000).
- Haptens can react specifically with its corresponding antibody.
- Examples: Capsular polysaccharide of pneumococcus, Polysaccharide "C" of beta *helaemolytic streptococci*, cardiolipin antigen, etc.
- Haptens may be complex or simple.

Complex Haptens

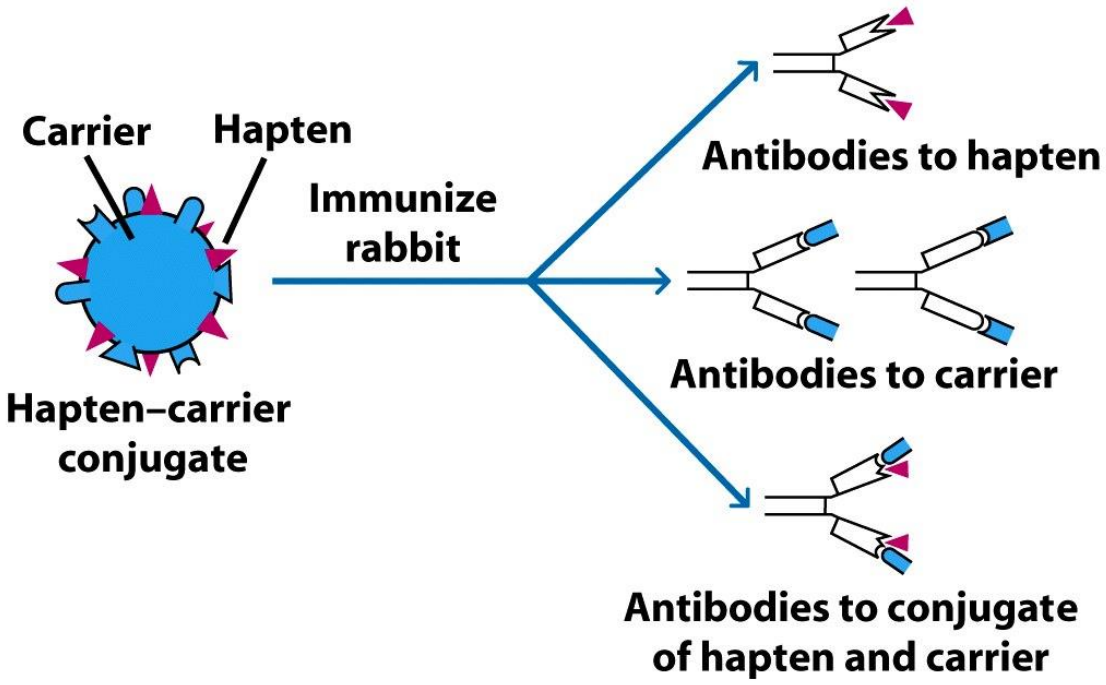
1. Polyvalent
2. Precipitate with specific antibodies.

Simple Haptens

1. Univalent
2. Non – Precipitate with specific antibodies.

Examples for Haptens:

1. Aniline (an organic compound) and it's derivatives (o-, m, p- benzoic acid) (first researched Hapten). 2. Hydralazine - A blood pressure lowering drug.
3. Fluorescein – A fluorescent dye.
4. Penicillin – An antibiotic.



Injection with:	Antibodies formed:
Hapten (DNP)	None
Protein carrier (BSA)	Anti–BSA
Hapten–carrier conjugate (DNP-BSA)	Anti–DNP (major) Anti–BSA (minor) Anti–DNP/BSA (minor)

Figure 4-1
Kuby IMMUNOLOGY, Sixth Edition

VII. Epitopes

- An epitope is the part of an antigen that the host's immune system recognizes, eliciting the immune response to an invading pathogen. It specifically binds to the corresponding antigen receptor on the immune cell (such as a B cell) and binding only occurs if the structures are complementary.
- Once epitope and receptor bind together in this puzzle-like combination, the production of antibodies is stimulated. These antibodies are specifically targeted to the epitopes that bind to the antigen receptors. In this manner, the epitope is also the region of the antigen that is recognized by the specific antibody which then removes the antigen from the host organism after binding to it. The region on the antibody which binds to the epitope is known as the paratope.

- Many antigens have a number of distinct epitopes on their surfaces. Each of these epitopes can interact with different antigen receptors on immune cells. The blood serum of an immunized person usually contains a mixture of antibodies that can bind with different epitopes on the surface of an antigen. Antibodies that target with the same epitope can have different abilities to bond with it.
- The epitope is also referred to as the *antigenic determinant*. It is usually a non-self protein. However, in the case of autoimmune diseases, sequences in the host can be recognized as epitopes by the immune system. Epitopes are generally around five or six amino acids in length.

Types of epitope

There are three types of epitope: conformational, linear, and discontinuous. This classification is based upon their structure and their interaction with the antibody's paratope. Conformational epitopes are formed through the interaction of amino acid residues which are disconnected from each other. The number of conformational epitopes is unknown.

Linear epitopes are determined not just by their primary structure (sequence of amino acids) but also by other residues present. More distant amino acid residues of the antigen as well as those which flank the primary structure affect the linear epitope's three-dimensional conformation.

Discontinuous epitopes consist of parts of the protein which are brought together by protein folding rather than being close to each other in the structure. This class of epitope can contain both conformational and linear parts. Data from various studies have provided evidence that most antigen-antibody binding occurs at discontinuous epitope sites. Protective antibodies (for example, those in vaccines) particularly rely on this.

T- and B- cells provide an immunologic response based on pathogen-specific memory. B-cell epitopes are the portion of the antigen that antibodies or immunoglobulin binds to. T-cell epitopes are found on the surface of an antigen-presenting cell and are bound to major histocompatibility complex molecules. Epitopes can be cross-reactive.

III. Adjuvants

Adjuvants are substances that when mixed with an antigen and injected with it enhance the immunogenicity of that antigen.

The word adjuvants was derived from the Latin word " Adjuvare" which means " To help".

Adjuvants are often used to boost the immune response when an antigen has low immunogenicity or when only small amount of an antigen are available.

Adjuvants may be added to a vaccine to modify the immune response.

The antibody response of mice to immunization with Bovine Serum Albumin (BSA) Can be increased five fold or more if the BSA is administered with an adjuvant.

Commonly used Adjuvants

1. Aluminium potassium sulfate (alum) (first Aluminium and now completely replaced by Aluminium hydroxide and Aluminium phosphate for commercial vaccines).
2. Freunds Incomplete Adjuvant (oil in water emulsions).
3. Freunds Complete Adjuvant (Inactivated and dried Mycobacterium tuberculosis).
4. Plant Saponins
5. Cytokines
6. Quil A (detergent)
7. Mineral oil and food based oil (pea nut)

Mechanism of Immune stimulation by Adjuvants

1. Extend the presence of antigen in the blood.
2. Helps the antigen presenting cells absorb antigens.
3. Activates Macrophages and lymphocytes.
4. Support the production of cytokines.

IV. Antibodies: Structure and function.

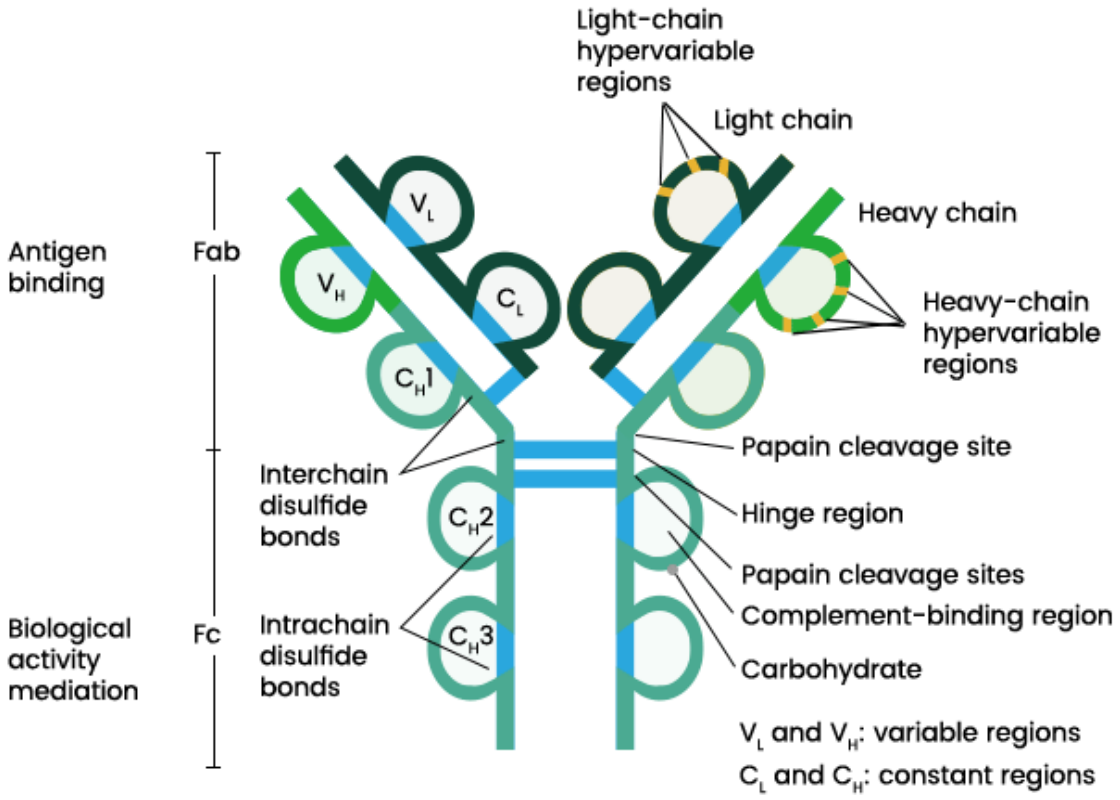
Antibody Definition

Antibodies, also known as immunoglobulins, are proteins produced by lymphocytes as a result of interaction with antigens. Antibodies are a part of the humoral immune of the adaptive immune system where each antibody identifies a specific antigen and protects the body against it.

- Antibodies are glycoproteins that bind to antigens with a high degree of specificity and affinity.
- **B lymphocytes** are stimulated by the binding of antigen, which results in the secretion of millions of antibodies in the bloodstream.
- The produced antibodies circulate through the bloodstream and neutralize antigens that are identical to those that triggered the immune response.
- The binding of antibodies to microorganisms or other such antigens can result in the microorganism being immobile or preventing them from penetrating the cells.

- Antibodies carry out two principal functions in the immune system. The first function is the recognition and binding to foreign bodies. The second more important function is to trigger the elimination of the attached foreign material.
- Since millions of antibodies are produced during an immune response, some of these remain in circulation in the blood for several months. This provides an extended immunity against the particular antigen.
- Each antibody is a Y-shaped protein where each tip of the Y contains a paratope that recognizes an epitope of a particular antigen.
- Antibodies can be classified into different classes based on different structures and functions.

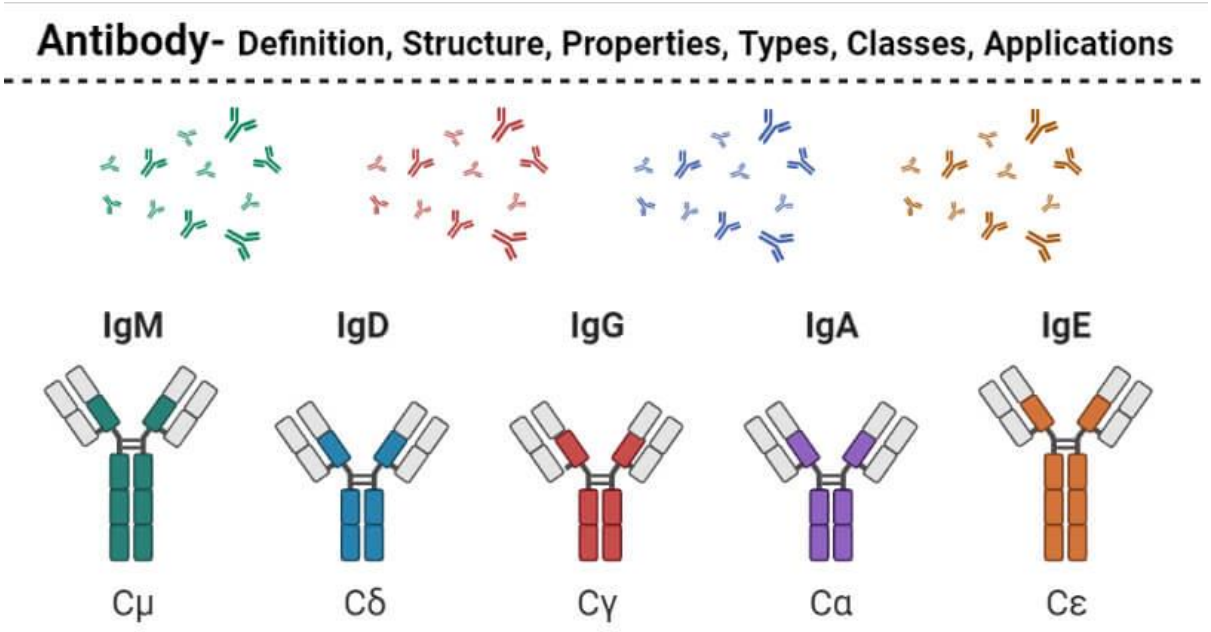
Antibody Structure



- Antibodies are globular plasma proteins with a basic Y-shaped structure and four polypeptides.
- There are two identical heavy chains and two identical light chains connected together by disulfide bonds. The light chains consist of polypeptides of the size 22,000 Da, and the heavy chains consist of polypeptides of the size 50,000 Da.

- Each heavy chain is connected to a light chain by a disulfide bond, and the two heavy chains are connected to the light chains by two sulfide bonds.
- There are five different types of heavy chains in mammals that are designated by letters: α, δ, γ, ε and μ. There are two types of light chains designated by λ and κ.
- An antibody is composed of a variable region and a constant region. The variable region changes to various structures depending on the differences in the antigens. The constant regions are constant and do not change with antigens.
- The variable region varies between clones and is involved in antigen recognition. The constant region is conserved among clones and is required for the structural integrity and effector functions.
- The heavy and light chain in an immunoglobulin molecule consists of an amino-terminal variable region with 100-110 amino acids.
- Each heavy chain has one variable domain and one constant domain. The light chain, in turn, consists of one variable domain and three constant domains.
- The rest of the chain consists of the constant region, which limited variation that determines the light chain subtypes.
- The heavy chains in some antibodies contain a proline-rich hinge region. The hinge region separates the antigen-binding and effector domains.
- The region allows the movement of the domains enabling them to bind to antigens separated by varying distances.

Antibody Types or Classes



- Antibodies can be classified into five different classes; IgG, IgM, IgA, IgD, and IgE. All the antibodies have the basic four-chain antibody structure, but they have different heavy chains.
- The differences in the immunoglobulins are more pronounced in the Fc regions of the antibody, which leads to the triggering of different effector functions.
- The structural differences in the antibodies also result in differences in the polymerization state of the monomer unit.
- The following are the five different classes of antibodies;

Immunoglobulin G (IgG)

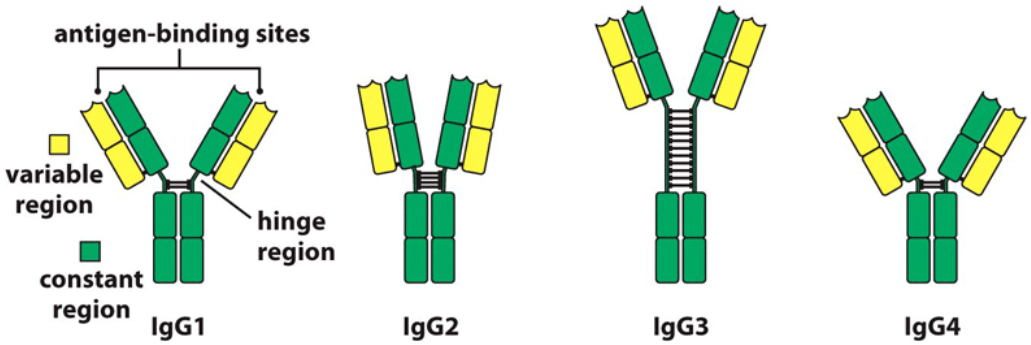
IgG is the most abundant immunoglobin, which accounts for about 80% of the total serum antibodies. The concentration of IgG in the blood is about 10mg/ml.

Structure of IgG

- The basic structure of IgG is composed of a Y-shaped protein where the Fab arms are linked to the Fc arms by an extended region of polypeptide chain called the hinge.
- The region is exposed and sensitive to attack by proteases that cleave the molecule into distinct functional units arranged in a four-chain structure.
- An IgG molecule consists of two identical γ heavy chains, usually of the size 50kDa.
- The light chains in IgG exist in two forms; κ and λ, where the κ form is more prevalent than λ, in the case of humans.
- The Fc regions of the molecule have a highly conserved N-glycosylation site in the heavy chain.

Immunoglobulin G (IgG)

- *Structure, Subclasses and Functions*



IgG Antibody Isotype Comparison

Property	IgG1	IgG2	IgG3	IgG4
Molecular Weight (kDa)	150	150	170	150
Amino acids in hinge region	15	12	62	12
Inter-H chain disulfide bonds	2	4	11	2
Half life (days)	14-21	14-21	7	14-21
Mean adult serum level (g/l)	6.98	3.8	0.51	0.56
Relative abundance (%)	60	32	4	4

Properties of IgG

- The IgG antibodies exist in the serum in the monomeric form, and these can cross the placenta from the mother to the fetus.
- Each IgG antibody has two paratopes that bind to two different epitopes on different antigens.
- IgG has four subclasses classified on the basis of the subclasses of the γ heavy chains.
- IgG antibodies participate predominantly in secondary immune response as these are generated as a result of class switching and maturation of the response.

Subclasses of IgG

- IgG antibodies have been classified into four subclasses; IgG1, IgG2, IgG3, and IgG4.

- These are named in the order of their abundance in serum, with IgG1 being the most abundant.

IgG1

- IgG1 is the most abundant subclass of IgG antibodies with γ1 heavy chains.
- IgG1 is primarily induced by soluble protein antigen and membrane proteins but is often accompanied by lower levels of the other subclasses.
- A deficiency of IgG1 can lead to a decreased total IgG level as it is the most abundant subclass.

IgG2

- IgG2 is the second most abundant IgG in the human serum, and it is composed of γ2 heavy chains.
- IgG2 is almost entirely responsible for the response against bacterial capsular polysaccharide antigens.
- IgG2 is the only subclass of IgG antibodies that cannot cross the placenta during pregnancy.
- The deficiency of IgG2 can result in a weak defense against pathogenic microorganisms.

IgG3

- IgG3 is the third most abundant IgG occurring in human serum with the γ3 heavy chains.
- These are particularly effective in the induction of effector functions. It is a potent proinflammatory antibody with a shorter half-life.
- IgG3 is also the most effective complement activator and has a high affinity for FcR on phagocytic cells, aiding in opsonization.

IgG4

- IgG4 is the least abundant IgG antibody in human serum, which consists of γ4 subclasses of heavy chains.
- IgG4 is induced by allergens, and it is formed after repeated or long-term exposure to antigen in a non-infectious setting.
- IgG4 can cross the placenta and transfer from the mother to the fetus. IgG4 deficiencies are quite rare, but the increased levels of IgG4 in the serum have been associated with a number of problems in different organs.

Functions of IgG

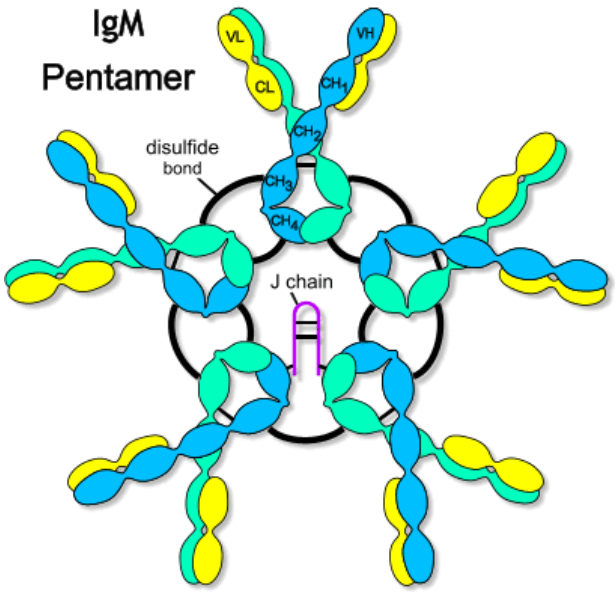
- IgG antibodies provide long-term protection against various agents like bacteria, viruses, and bacterial toxins.
- IgG is one of the most potent complement activators when compared to all other antibodies.
- The binding ability of IgG to antigens is more effective as it enhances phagocytosis.

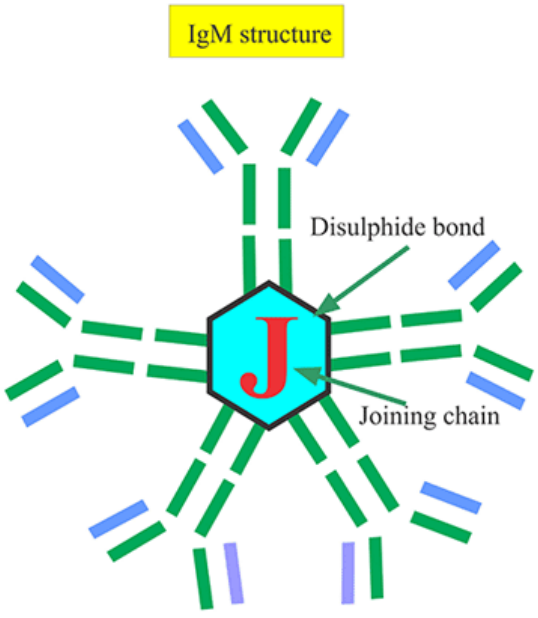
Immunoglobulin M (IgM)

IgM is the third most abundant immunoglobulin in serum, with a concentration of 1.5 mg/ml. It is the largest antibody and is the first antibody to appear in response to the initial exposure to antigen.

Structure of IgM

- IgM is secreted in a pentameric form with five distinct units, where each are composed of two μ heavy chains and two light chains.
- A J chain might be present in the hexameric form of the molecule, but it isn't always present. The J chain is usually added just before the secretion of the pentamer as it helps in the polymerization of the monomers.
- Each of the monomers has two antigen-binding sites, resulting in 10 binding domains in a single molecule. However, all the domains cannot be occupied at the same time due to limitations in space.
- The pentameric form of IgM has a molecular weight of 900 kDa.





Properties of IgM

- IgM is the largest and the only pentameric antibody in humans. It is also the first antibody to be produced in response to the initial exposure to an antigen.
- IgM is the first immunoglobulin to be synthesized by the fetus, beginning at about 20 weeks of age.
- IgM is a pentameric molecule with 10 antigen-binding sites and 5 Fc portions held together by disulfide linkages.
- The monomeric form of IgM occurs as the major antibody receptor on the surface of B lymphocytes.
- IgM is relatively short-lived and usually disappears earlier than IgG.
- The large size of the molecules do not allow effective diffusion of the antibody, and thus, it is found in very low concentration in the intracellular fluids.

Functions of IgM

- IgM is very effective against viruses as less IgM than IgG is enough to neutralize viral infections.
- IgM is also a better agglutinin as it takes 100 to 1000 more molecules of IgG than that of IgM for the same level of agglutination.
- IgM is involved in activating the classical pathway of complement in the immune system due to the presence of two Fc regions in close proximity.

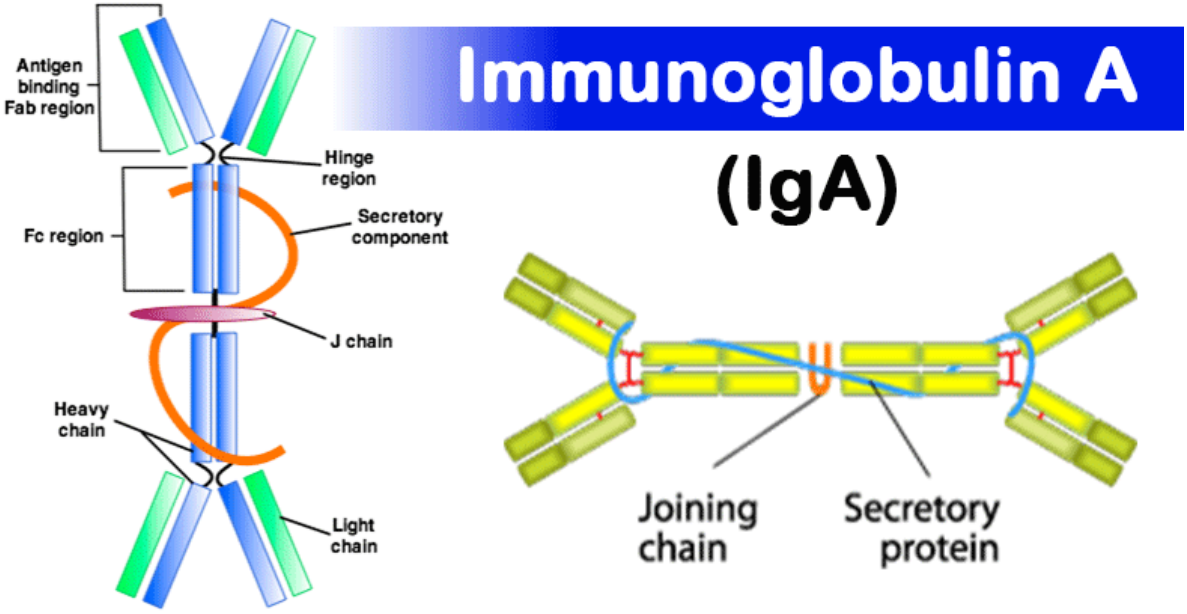
Immunoglobulin A (IgA)

IgA or sIgA is the main immunoglobulin found in the mucous membrane in the form of secretory antibodies. The concentration of IgA is found in small quantities in blood, but it is found in high concentrations in tears, saliva, and sweat

Structure of IgA

- The molecular size of IgA is 160 kDa with a four-chain monomeric structure, however, it can occur in dimeric and trimeric forms.
- The heavy chain of IgA is divisible into three constant domains, CH1, CH2, and CH3, and a variable VH domain.
- The hinge region occurs between the CH1 and CH2 domains held together by disulfide linkages.
- sIgA has a secretory component as an additional component with a polypeptide chain of 75 kDa and extracellular proteolytic fragment.

- The molecule also has a J-chain linked to the chains via disulfide bridges. The secretory and J chain facilitates the transport of IgA across epithelial cells and protects the molecule from proteolytic digestion by enzymes.



Properties of IgA

- IgA is the second most abundant immunoglobulin in humans, with a concentration of 2-4 mg/ml. It accounts for about 10-15% of the total serum concentration but is the most abundant antibody in external secretions.
- IgA is the first line of defense as it works by inhibiting bacterial and viral adhesion to epithelial cells and by neutralizing viral and bacterial toxins intracellularly.
- The secretory IgA mostly occurs in dimeric form with two monomeric units linked together by a joining peptide.

Subclasses of IgA

- IgA has been classified into two subclasses; IgA1 and IgA2.
- IgA1 is the monomeric form, and IgA2 is the dimeric or polymeric form.
- IgA1 occurs in serum IgA (about 80%), which is produced in the bone marrow and released on the mucosal surfaces.
- The IgA in most locally secreted products is polymeric with a relative release of dimeric IgA2.
- One of the most prominent differences between IgA1 and IgA2 is the hinge region which is quite extended in IgA1.

Functions of IgA

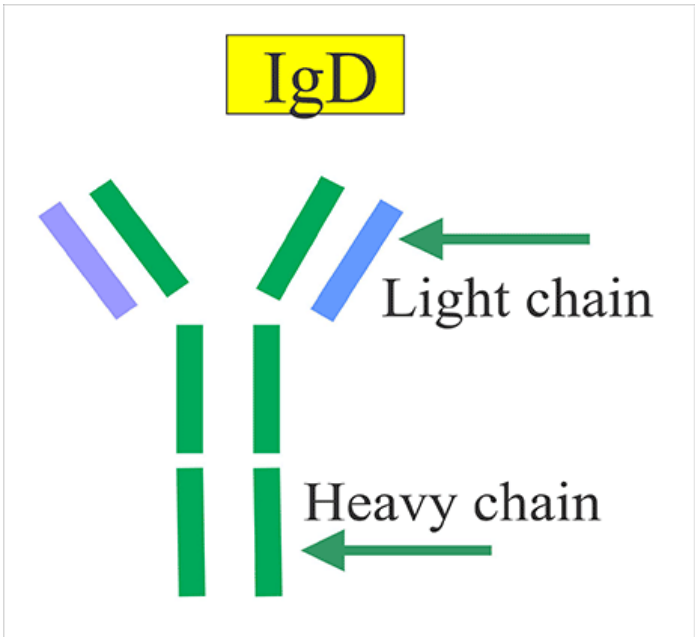
- IgA is the first line of defense as it protects the body from the entry and colonization of mucosal surfaces by different foreign particles.

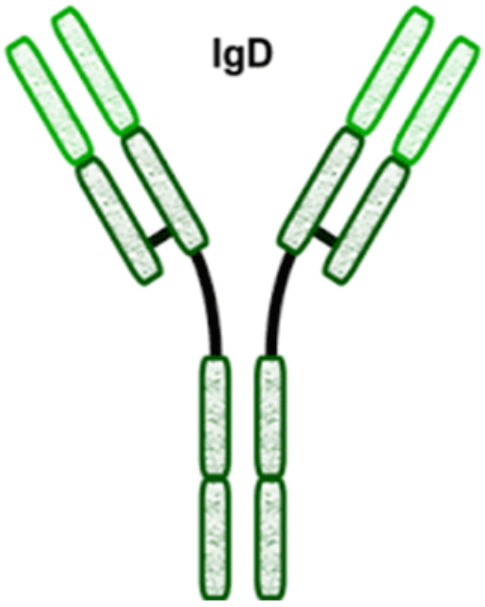
Immunoglobulin D (IgD)

IgD is a monomeric antibody that occurs on the surface of immature B lymphocytes. It is produced in a secreted form in a small amount in the blood serum.

Structure of IgD

- IgD has a structural diversity throughout evolution in the vertebrates as it is flexible to complement the function of IgM.
- It is a glycoprotein with two identical δ heavy chains and two identical light chains.
- IgD found on the surface of B lymphocytes has some extra amino acids at C-terminal in order to anchor to the membrane.
- The light and heavy chains are linked together by disulfide links, but they have additional intrachain disulfide links that divide the chains into domains.
- The IgD molecule also has an extended hinge region which increases the flexibility of the molecule but decreases its resistance against proteolytic cleavage.





Properties of IgD

- IgD is found in low concentration in serum, and its exact function in the immune system is not yet clearly understood.

- It represents about 0.25% of the total serum immunoglobulins with a relative molecular mass of 185 kDa and a half-life of 2.8 days.
- It also accounts for about 1% of the proteins present in the plasma membranes of B lymphocytes. Here, it usually coexpressed with another cell surface antibody, IgM.

Functions of IgD

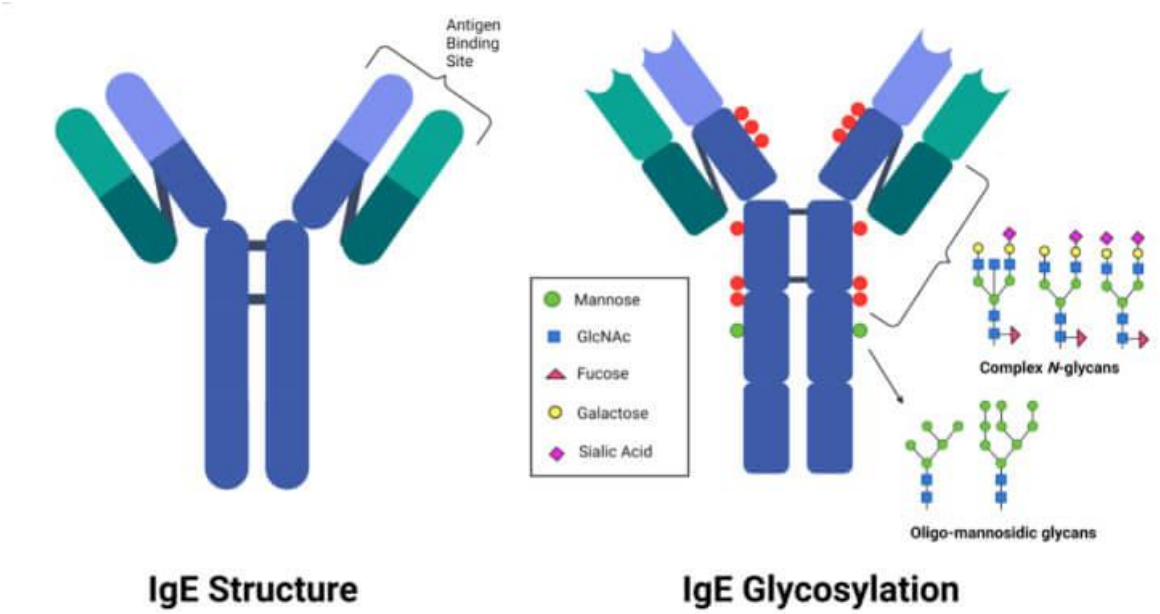
- The most important function of IgD is antigen receptor on B cells. It also regulates B cell function if it encounters an antigen.
- It is also secreted in some amounts in the blood, mucosal secretions, and the surface of innate immune effector cells.

Immunoglobulin E (IgE)

IgE is a type of immunoglobulin found only in mammals and synthesized by plasma cells. It occurs in a monomeric form with two ε heavy chains and two light chains.

Structure of IgE

- IgE has a typical antibody structure with ε heavy chains that have a high carbohydrate content.
- IgE has two identical antigen-binding sites consisting of both light and heavy chains and a valency of 2.
- Like all antibodies, heavy and light chains are further divided into variable and constant regions.
- The heavy chains consist of five domains, out of which one is variable, and four are constant.

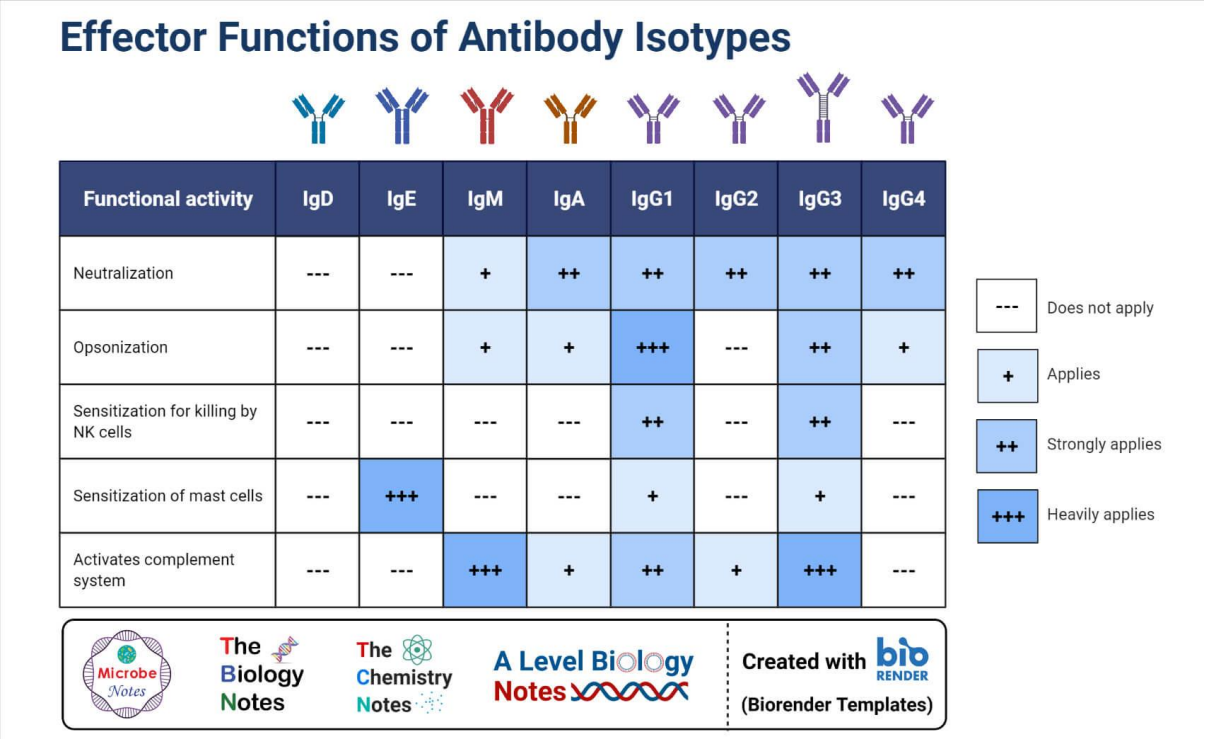


IgE Structure IgE Glycosylation

Functions of IgE

- IgE is mostly associated with allergic reactions where it binds to reintroduced antigens and triggers the release of pharmacologically active agents.
- It also plays an essential role in response to allergens and antigen preparation used in desensitization immunotherapy.

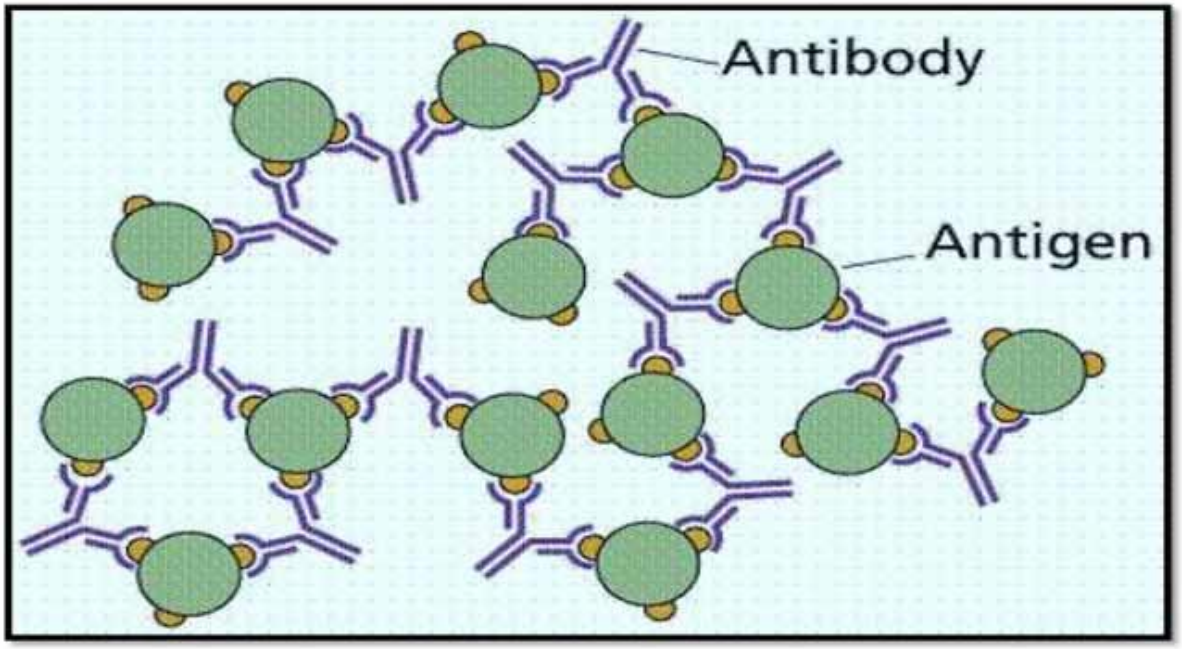
Applications of Antibodies



- Antibodies can be used to treat immune deficiencies as a means of passive immunity.
- The development of monoclonal antibodies has been used to treat several diseases like multiple sclerosis, rheumatoid arthritis, and different cancers.
- Antibodies can also be used in medical diagnostics as many biochemical assays allow the detection of antibodies for the diagnosis of diseases.
- Different classes of immunoglobulins can be used to analyze the antibody profile of patients.
- Antibodies can also be used as workhorse agents in biomedical research to study the workings of different antigens and their relationship with the host.

Unit – III

Mind Map



I. Antigen – Antibody interactions: Avidity and affinity.

The interactions between antigens and antibodies are known as ***antigen–antibody reactions***. The reactions are highly specific, and an antigen reacts only with antibodies produced by itself or with closely related antigens. Antibodies recognize molecular shapes (epitopes) on antigens. Generally, the better the fit of the epitope (in terms of geometry and chemical character) to the antibody combining site, the more favorable the interactions that will be formed between the antibody and antigen and the higher the affinity of the antibody for antigen. The affinity of the antibody for the antigen is one of the most important factors in determining antibody efficacy *in vivo*

The antigen- antibody interaction is bimolecular irreversible association between antigen and antibody. The association between antigen and antibody includes various non-covalent interactions between epitope (antigenic determinant) and variable region (V_H/V_L) domain of antibody.

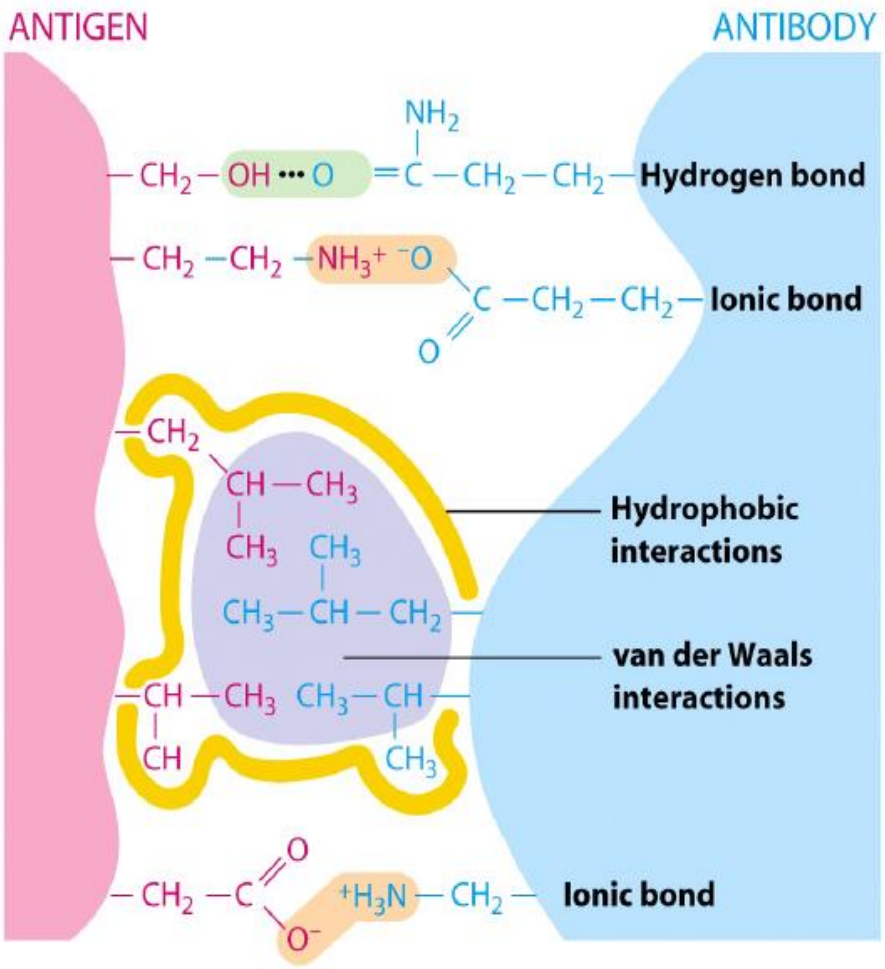
Chemical Bonds Responsible for the Antigen–Antibody Reaction

The interaction between the Ab-binding site and the epitope involves exclusively non-covalent bonds, in a similar manner to that in which proteins bind to their cellular receptors, or enzymes bind to their substrates. The binding is reversible and can be prevented or dissociated by high

ionic strength or extreme pH. The following intermolecular forces are involved in Ag–Ab binding:

1. **Electrostatic bonds:** This result from the attraction between oppositely charged ionic groups of two protein side chains; for example, an ionized amino group (NH_4^+) on a lysine in the Ab, and an ionized carboxyl group (COO^-) on an aspartate residue in the Ag.
2. **Hydrogen bonding**: When the Ag and Ab are in very close proximity, relatively weak hydrogen bonds can be formed between hydrophilic groups (e.g., OH and C=O, NH and C=O, and NH and OH groups).
3. **Hydrophobic interactions**: Hydrophobic groups, such as the side chains of valine, leucine, and phenylalanine, tend to associate due to Van der Waals bonding and coalesce in an aqueous environment, excluding water molecules from their surroundings. As a consequence, the distance between them decreases, enhancing the energies of attraction involved. This type of interaction is estimated to contribute up to 50% of the total strength of the Ag–Ab bond.
4. **Van der Waals bonds:** These forces depend upon interactions between the "electron clouds" that surround the Ag and Ab molecules. The interaction has been compared to that which might exist between alternating dipoles in two molecules, alternating in such a way that, at any given moment, oppositely oriented dipoles will be present in closely apposed areas of the Ag and Ab molecules.

Each of these non-covalent interactions operates over very short distance (generally about 1 Å) so, Ag-Ab interactions depends on very close fit between antigen and antibody.



Strength of Ag-Ab interactions

1. **Affinity**

- Combined strength of totalnon-covalent interactions between single Ag- binding site of Ab and single epitope is affinity of Ab for that epitope.
- Low affinity Ab: Bind Ag weakly and dissociates readily.
- High affinity Ab: Bind Ag tightly and remain bound longer.

2. **Avidity**

- Strength of multiple interactions between multivalent Ab and Ag is avidity. Avidity is better measure of binding capacity of antibody than affinity. High avidity can compensate low affinity.

Avidity & affinity
Measuring affinity of Ab to Ag

Association between CDR and monovalent Ag can be expressed as:

Ag + Ab ⇆ Ag-Ab;

A + B ⇆ C+ D

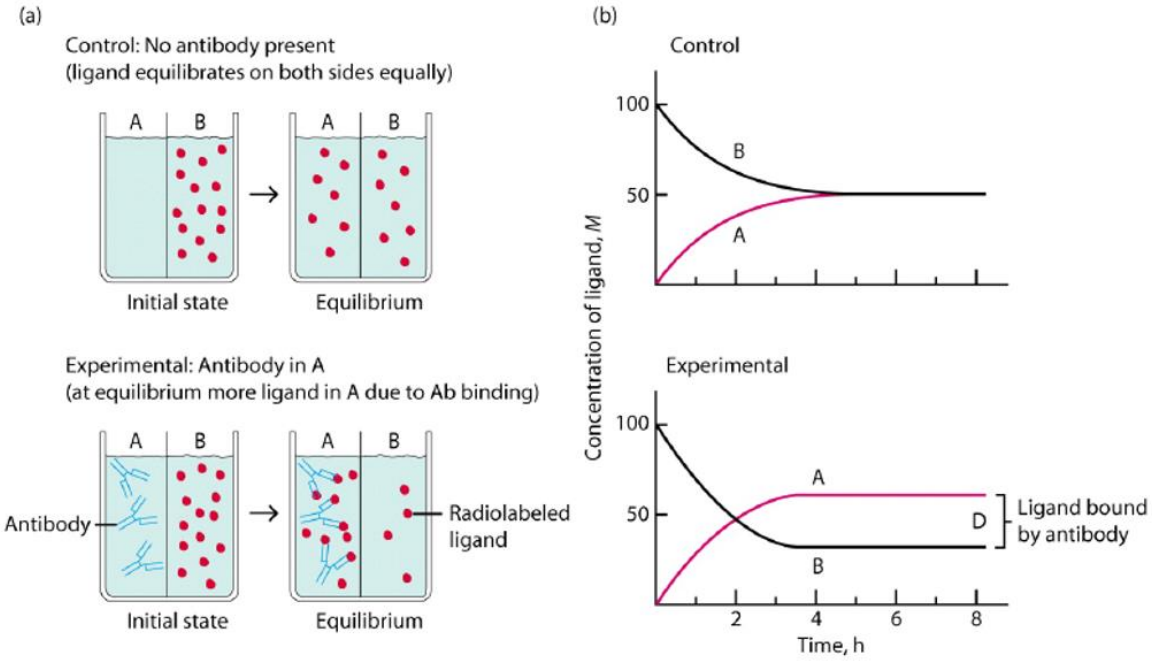
$\mathbf{k_1}$ = forward (association) rate constant whereby $\mathbf{k_1/k_{-1} = K_a}$ (Assoc/equilibrium constant)

$\mathbf{k_{-1}}$ = reverse (dissociation) rate constant

$\mathbf{K_a} = \frac{\mathbf{[Ag\text{-}Ab]}}{\mathbf{[Ag]\,[Ab]}}$ value of K_a depends on k_1;
for small haptens, k_1 is high
for large protein Ag's, k_1 is lower

Rate of reaction = Ka x [C]x[D]/[A]x[B]

K_a determined by equilibrium dialysis



3. **Cross reactivity**

- Antibody elicited by one Ag can cross react with unrelated Ag if they share identical epitope or have similar chemical properties.

II. Basic principles and applications of precipitation reactions (radial immuno diffusion, double immuno diffusion and immuno electrophoresis)

Precipitation definition

It is a type of antigen-antibody reaction, in which the antigen occurs in a soluble form. When a soluble antigen reacts with its specific antibody, at an optimum temperature and P^H in the presence of electrolyte antigen-antibody complex forms insoluble precipitate. This reaction is called a precipitation reaction. A lattice is formed between the antigens and antibodies; in certain cases, it is visible as an insoluble precipitate. Antibodies that aggregate soluble antigens are called **precipitins.**

The interaction of antibody with soluble antigen may cause the formation of insoluble lattice that will precipitate out of solution. Formation of an antigen-antibody lattice depends on the valency of both the antibody and antigen. The antibody must be **bivalent**; a precipitate will not form with monovalent Fab fragments. The antigen must be **bivalent or polyvalent**; that is it must have at least two copies of same epitope or different epitopes that react with different antibodies present in polyclonal sera. Antigen and antibody must be in an appropriate concentration relative to each other.

1. Antigen access: Too much antigen prevents efficient crosslinking/lattice formation.
2. Antibody access: Too much antibody prevents efficient crosslinking/lattice formation.
3. Equivalent Antigen and Antibody: Maximum amount of lattice (Precipitate) is formed

Prozone phenomenon

Antigen and antibody reaction occurs optimally only when the proportion of the antigen and antibody in the reaction mixture is equivalent. On either side of the equivalence zone, precipitation is actually prevented because of an excess of either antigen or antibody. The zone of antibody excess is known as the ***prozone phenomenon*** and the zone of antigen excess is known as **post zone phenomenon**.

In the prozone phenomenon, there is too much antibody for efficient lattice formation. This is because antigen combines with only a few antibodies and no cross-linkage is formed. In the post zone phenomenon, small aggregates are surrounded by excess antigen and again no lattice network is formed. Thus, for precipitation reactions to be detectable, they must be run in the zone of equivalence.

When instead of sedimenting, the precipitate remains suspended as floccules, the reaction is known as ***flocculation***.

Precipitation reactions are based on the interaction of antibodies and antigens. They are based on two soluble reactants that come together to make one insoluble product, the precipitate. These reactions depend on the formation of lattices (cross-links) when antigen and antibody exist in optimal proportions. Excess of either component reduces lattice formation and subsequent precipitation. Precipitation reactions differ from agglutination reactions in the size and solubility of the antigen. Antigens are soluble molecules and larger in size in precipitation reactions. There are several precipitation methods applied in the clinical laboratory for the diagnosis of disease. These can be performed in semi-solid media such as agar or agarose, or non-gel support media such as cellulose acetate.

Applications of the precipitation reaction

1. Detection of unknown antibody to diagnose infection e.g. VDRL test for syphilis.
2. Standardization of toxins and antitoxins.
3. Identification of Bacteria e.g. Lancified grouping of streptococci.
4. Identification of bacterial component e.g Ascoli's thermoprecipitin test for *Bacillus anthracis.*

Examples of Precipitation

Immunodiffusion precipitation test

- Immunodiffusion is an immunological technique used for the detection and quantification of antibodies and antigens, which are mostly immunoglobulins and nuclear antigens.
- In this technique, antigen and antibodies are applied simultaneously in two adjacent wells.
- As the antigen and antibody diffuse towards each other, precipitates are seen in the form of lines as the antigen and antibodies interact with each other.
- It is also possible to compare the concentration of different antigens by placing multiple antigens in multiple wells.
- Based on the formation of precipitation lines, the presence of different antigens and, in turn, the presence of viruses or bacteria can be detected.

Immuno-diffusion

Immuno-diffusion is a technique for the detection or measurement of antibodies and antigens by their precipitation which involves diffusion through a substance such as agar or gel agarose. Simply, it denotes precipitation in gel.

It refers to any of the several techniques for obtaining a precipitate between an antibody and its specific antigen.

This can be achieved by:

1. a) Suspending antigen/antibody in a gel and letting the other migrate through it from a well or,
2. b) Letting both antibody and antigen migrate through the gel from separate wells such that they form an area of precipitation.

Based on the method employed, immuno-diffusion may be:

1. **Radial immunodiffusion**
2. **Ouchterlony Double Diffusion**

Radial immunodiffusion (RID) or **Mancini method** is also known as **Mancini immunodiffusion** or **single radial immunodiffusion assay**. It is a single diffusion technique whereby a solution containing the antigen is placed into wells in a gel or agar surface evenly impregnated with antibody. The diameter of the ring that precipitates around the well as a result of antigen antibody reaction corresponds to the amount of antigen in the solution.

Objectives of Radial Immunodiffusion

The Mancini immunodiffusion test may be carried out with one or more of the following objectives:

1. To detect antigen-antibody complexes.
2. Describe the circumstances under which antigen-antibody complexes precipitate out.
3. Determine relative concentration of antigens.

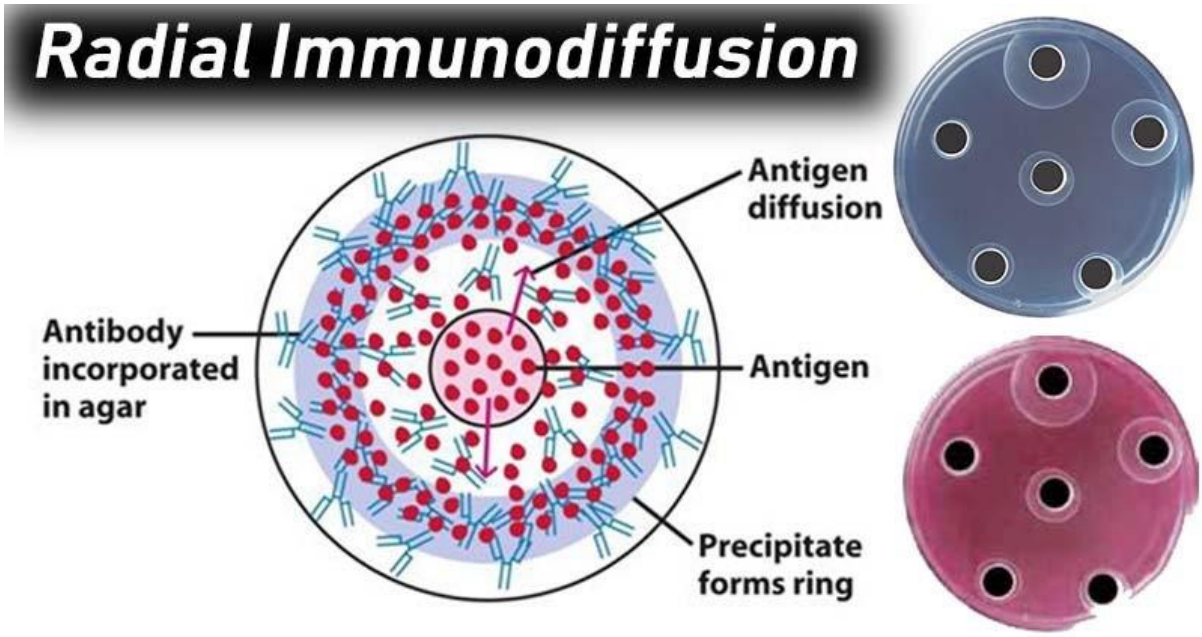
Principle of Radial Immunodiffusion

Radial immuno-diffusion is a type of precipitation reaction. It is thus based on the principles of the precipitin curve which states that antigen-antibody interact forming visible cross-linked precipitate when the proper ratio of antigen to antibody is present. In the test, antibody is incorporated into agar and poured into a glass plate to form a uniform layer. Circular wells are cut into the agar and antigen is introduced into the wells. Specific antigens to the impregnated antibodies diffuse through the agar in all directions from the well and react with the antibody present forming visible precipitate or a precipitin ring. Ring shaped bands of precipitates from concentrically around the well indicating reaction. The diameter of the precipitate ring formed, corresponds to the amount of antigen in the solution.

Procedure of Radial Immunodiffusion

1. An agar containing an appropriate antiserum (antibody) is poured in plates.
2. Carefully circular wells are cut and removed from the plates.
3. A single or series of standards containing known concentration of antigen are placed in separate wells, while control and "unknown" samples are placed in other remaining wells.
4. As the antigen diffuses radially, a ring of precipitate will form in the area of optimal antigen – antibody concentration.
5. The ring diameters are measured and noted.
6. A standard curve is prepared using the ring diameters of the standards versus their concentrations. This curve is then used to determine the concentration of the control and unknown samples.

Result Interpretation of Radial Immunodiffusion



1. The presence of a precipitin ring around the antigen wells indicate specific antigen-antibody interaction.
2. Absence of precipitin ring suggest absence of reaction.
3. The greater the amount of antigen in the well, the farther the ring will form from the well.

Applications of Radial Immunodiffusion

- Immuno-diffusion techniques are mostly used in immunology to determine the quantity or concentration of an antigen in a sample.
- Estimation of the immunoglobulin classes in sera.
- Estimation of IgG, IgM antibodies in sera to influenza viruses.

Other applications include:

- To determine relative concentrations of antibodies in serum.
- Estimate serum transferrin and alpha-feroprotein.
- To compare properties of two different antigens.
- To determine the relative purity of an antigen preparation
- For disease diagnosis
- Serological surveys

Ouchterlony Double Immunodiffusion technique

- Immuno-diffusion is a technique for the detection or measurement of antibodies and antigens by their **precipitation** which involves diffusion through a substance such as agar or gel agarose. Simply, it denotes precipitation in gel.
- It refers to one of the several techniques for obtaining a precipitate between an antibody and its specific antigen.
- Immunodiffusion reactions are classified based on the:

1. Number of reactants diffusing (Single diffusion/Double diffusion)
2. Direction of diffusion (One dimension/Two dimension)

- They thus may be of the following types:

1. Single diffusion in one dimension
2. Single diffusion in two dimensions
3. Double diffusion in one dimension
4. Double diffusion in two dimensions

Double Immuno-diffusion

- Double immunodiffusion is an agar gel immunodiffusion.
- It is a special precipitation reaction on gels where antibodies react with specific antigens forming large antigen-antibody complexes which can be observed as a line of the precipitate.
- In double immunodiffusion, both the antibody and antigen are allowed to diffuse into the gel.

- After application of the reactants in their respective compartments, the antigen and the antibody diffuse toward each other in the common gel and a precipitate is formed at the place of equivalence.

Double diffusion in one dimension

The method also called Oakley–Fulthrope procedure involves the incorporation of the antibody in agar gel in a test tube, above which a layer of plain agar is placed. The antigen is then layered on top of this plain agar. During incubation, the antigen and antibody move toward each other through the intervening layer of plain agar. In this zone of plain agar, both antigen and antibody react with each other to form a band of precipitation at their optimum concentration.

Double diffusion in two dimensions

It is more commonly known as Ouchterlony double diffusion or passive double immunodiffusion. In this method, both the antigen and antibody diffuse independently through agar gel in two dimensions, horizontally and vertically.

Objectives

The Ouchterlony double immunodiffusion test may be carried out with one or more of the following objectives:

1. To detect antigen-antibody complexes.
2. Describe the circumstances under which antigen-antibody complexes precipitate out.
3. Detect the presence of an antigen-specific antibody.
4. To test the similarity between antigens.

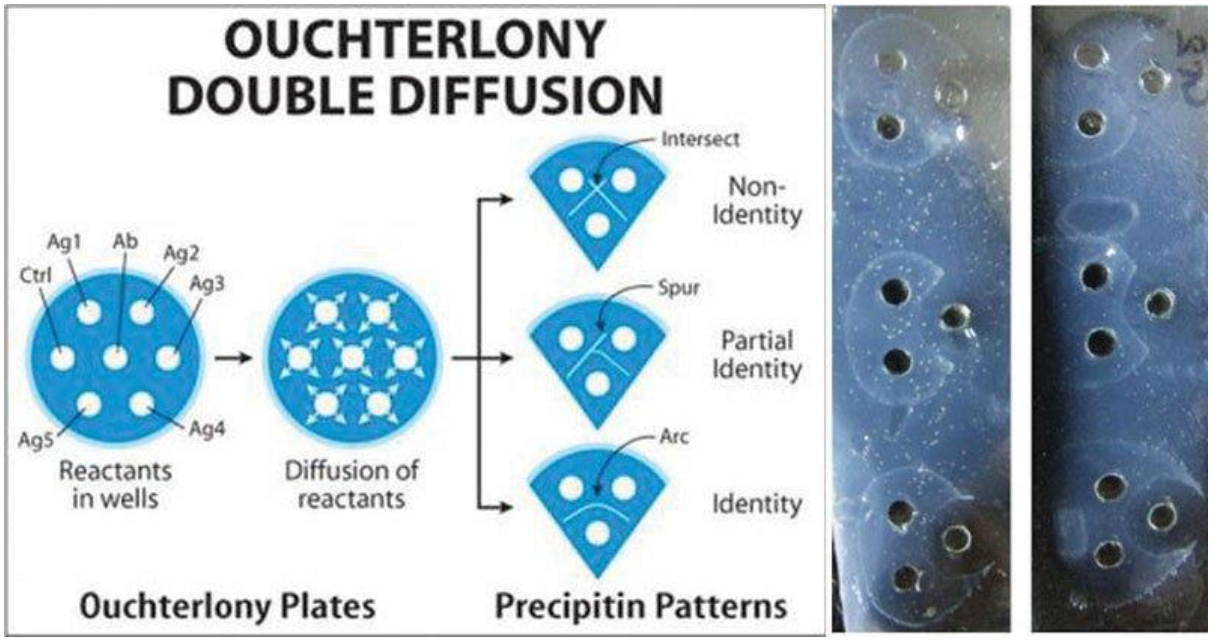
Principle

In the test, an antigen solution or a sample extract of interest is placed in wells bore on gel plates while sera or purified antibodies are placed in other remaining wells (Mostly, an antibody well is placed centrally). On incubation, both the antigens in the solution and the antibodies each diffuse out of their respective wells. In case of the antibodies recognizing the antigens, they interact together to form visible immune complexes which precipitate in the gel to give a thin white line (precipitin line) indicating a reaction.

In case multiple wells are filled with different antigen mixtures and antibodies, the precipitate developed between two specific wells indicate the corresponding pair of antigen-antibodies.

Results

- The presence of an opaque precipitant line between the antiserum and antigen wells indicates antigen-antibody interaction.
- Absence of precipitant line suggests the absence of reaction.
- When more than one well is used there are many possible outcomes based on the reactivity of the antigen and antibody selected.



- The results may be either of the following:
- **A full identity (i.e. a continuous line):** Line of precipitation at their junction forming an arc represents serologic identity or the presence of a common epitope in antigens.
- **Non-identity (i.e. the two lines cross completely):** A pattern of crossed lines demonstrates two separate reactions and indicates that the compared antigens are unrelated and share no common epitopes.
- **Partial identity (i.e. a continuous line with a spur at one end):** The two antigens share a common epitope, but some antibody molecules are not captured by the antigen and traverse through the initial precipitin line to combine with additional epitopes found in the more complex antigen.
- The pattern of the lines that form can determine whether the antigens are the same.

Applications

1. It is useful for the analysis of antigens and antibodies.

2. It is used in the detection, identification, and quantification of antibodies and antigens, such as immunoglobulins and extractable nuclear antigens.
3. Agar gel immunodiffusions are used as serologic tests that historically have been reported to identify antibodies to various pathogenic organisms such as *Blastomyces*.
4. Demonstration of antibodies in serodiagnosis of smallpox.
5. Identification of fungal antigens.
6. Elek's precipitation test in the gel is a special test used for demonstration of toxigenicity of *Corynebacterium diphtheriae.*

Immunoelectrophoresis-

- Immunoelectrophoresis refers to precipitation in agar under an electric field.
- It is a process of a combination of immuno-diffusion and electrophoresis.
- An antigen mixture is first separated into its component parts by electrophoresis and then tested by double immuno-diffusion.
- Antigens are placed into wells cut in a gel (without antibody) and electrophoresed. A trough is then cut in the gel into which antibodies are placed.
- The antibodies diffuse laterally to meet diffusing antigens, and lattice formation and precipitation occur permitting determination of the nature of the antigens.
- The term "immunoelectrophoresis" was first coined by Grabar and Williams in 1953.

Principle of Immunoelectrophoresis

When an electric current is applied to a slide layered with gel, the antigen mixture placed in wells is separated into individual antigen components according to their charge and size. Following electrophoresis, the separated antigens are reacted with specific antisera placed in troughs parallel to the electrophoretic migration and diffusion is allowed to occur. Antiserum present in the trough moves toward the antigen components resulting in the formation of separate precipitin lines in 18-24 hrs, each indicating reaction between individual proteins with its antibody.

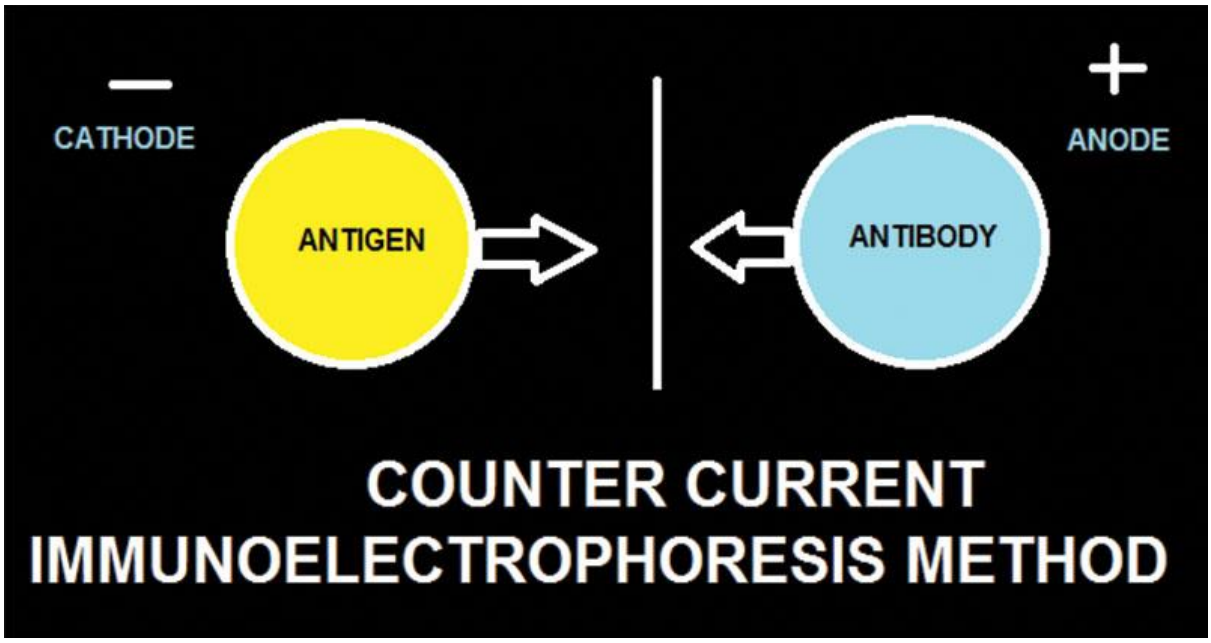
Applications of Immunoelectrophoresis

1. The test helps in the identification and approximate quantization of various proteins present in the serum. Immunoelectrophoresis created a breakthrough in protein identification and in immunology.
2. Immunoelectrophoresis is used in patients with suspected monoclonal and polyclonal gammopathies.

3. The method is used to detect normal as well as abnormal proteins, such as myeloma proteins in human serum.
4. Used to analyze complex protein mixtures containing different antigens.
5. The medical diagnostic use is of value where certain proteins are suspected of being absent (e.g., hypogammaglobulinemia) or overproduced (e.g., multiple myeloma).
6. This method is useful to monitor antigen and antigen-antibody purity and to identify a single antigen in a mixture of antigens.
7. Immunoelectrophoresis is an older method for qualitative analysis of M-proteins in serum and urine.
8. Immunoelectrophoresis aids in the diagnosis and evaluation of the therapeutic response in many disease states affecting the immune system.

Counter Current Immunoelectrophoresis:

It is a modification of immunoelectrophoresis in which antigen and antibody move in opposite directions and form precipitates in the area where they meet in concentrations of optimal proportions.It is also referred to as countercurrent or crossed-over immunoelectrophoresis.The technique is similar to the Ouchterlony method, the only difference being that the antigen movement is facilitated by electrophoresis. It is thus also called 'voltage facilitated double immunodiffusion'.



Counter current immunoelectrophoresis is mostly carried out with one or more of the following objectives:

1. To rapidly check any antisera for the presence and specificity of antibodies for a particular antigen.
2. To detect antigens and/or antibodies in serum for diagnosis of a particular disease

Counter-current immunoelectrophoresis depends on the movement of antigen towards the anode and of antibody towards the cathode through the agar under the electric field. The test is performed on a glass slide in agarose gel of high electro-endosmotic flow. A pair of wells is punched out where one well is filled with antigen and the other with the antibody. Electric current is then passed through the gel. The migration of antigen and antibody is greatly facilitated under the electric field, and the line of precipitation as precipitin arcs is made visible in 30–60 minutes, which indicates a positive reaction.

Applications

The counter-current immuno-electrophoresis has many uses:

1. It is a rapid and a highly specific method for detection of both antigen and antibodies in the serum, cerebrospinal fluid, and other body fluids in the diagnosis of many infectious diseases including bacterial, viral, fungal, and parasitic.
2. The test was very popular in the past for detecting various antigens such as alpha-fetoprotein in serum and capsular antigens of *Cryptococcus* and *Meningococcus* in cerebrospinal fluid.
3. Still today, it is commonly used for Hepatitis B surface antigen (HBsAg), fetoprotein, hydatid and amoebic antigens in the serum, and cryptococcal antigen in the CSF.
4. It is a rapid sensitive method for detecting pneumococcal capsular antigens in sputum.

Rocket Immunoelectrophoresis is an adaptation of radial immunodiffusion developed by Laurell. It is also known as electroimmunoassay or electroimmunodiffusion. It is called as "rocket electrophoresis" due to the appearance of the precipitin bands in the shape of cone-like structures (rocket appearance) at the end of the reaction. In rocket immunoelectrophoresis, antigen migrates in an electric field in a layer of agarose containing an appropriate antibody. The migration of the antigen toward the anode gives rise to rocket-shaped patterns of precipitation. The area under the rocket is proportional to antigen concentration.

Objectives of Rocket Immunoelectrophoresis

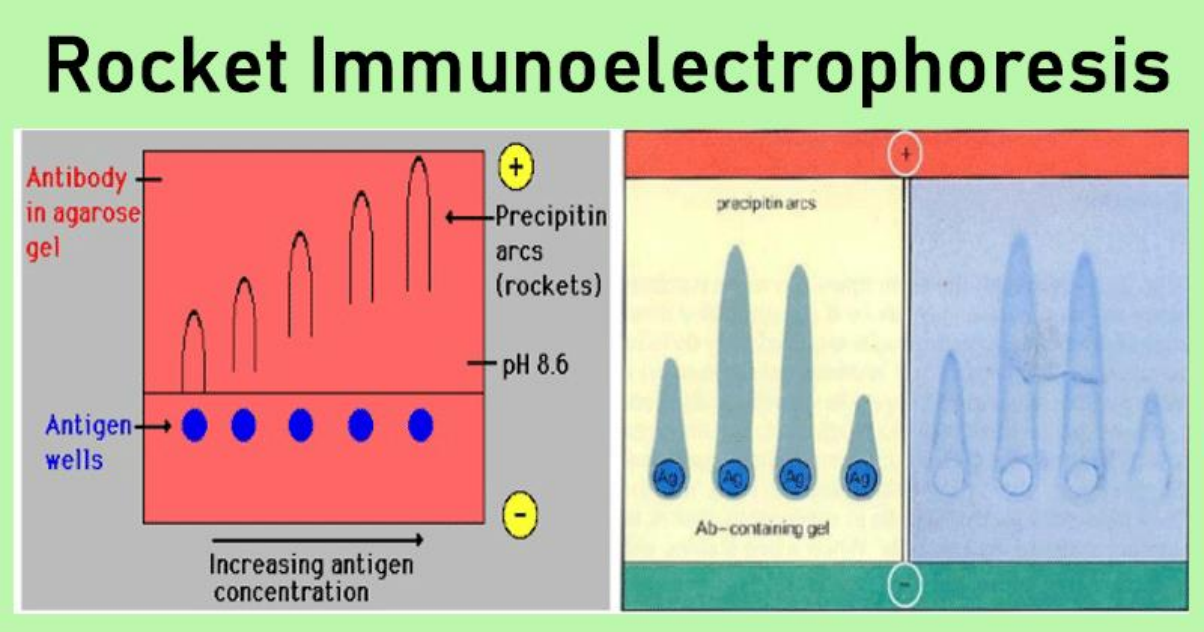
1. To detect antigen-antibody complexes.
2. Determine the concentration of antigen in an unknown sample.

Principle of Rocket Immunoelectrophoresis

Rocket immunoelectrophoresis is a quantitative one-dimensional single electro-immunodiffusion technique. In this method antibody is incorporated in the gel at a pH value at which the antibodies remain essentially immobile. Antigen is placed in wells cut in the gel. Electric current is then passed through the gel, which facilitates the migration of negatively charged antigens into the agar. As the antigen moves out of the well and enters the agarose gel, it combines with the antibody to form immune complex which becomes visible. During the initial phase there is considerable antigen excess over antibody and no visible precipitation occurs. However, as the antigen sample migrates further through the agarose gel, more antibody molecules are encountered that interact with the antigen to form immune complex. This results in formation of a precipitin line that is conical in shape, resembling a rocket.

The greater the amount of antigen loaded in a well, the further the antigen will have to travel through the gel before it can interact with sufficient antibody to form a precipitate. Thus, the height of the rocket, measured from the well to the apex and area are directly proportional to the amount of antigen in the sample.

Result Interpretation of Rocket Immunoelectrophoresis



- A precipitation ‘rocket’ spreading out from the loading well indicate positive reaction or specific antigen-antibody reaction due to the presence of antibody specific to the antigen.
- The absence of the precipitation indicates no reaction or the absence of any corresponding antibody – antigen.

- The height of the rocket, and its area are directly proportional to the amount of antigen in the sample, that is, the height of the precipitin peak depends on the concentration of antigens loaded in the corresponding wells.

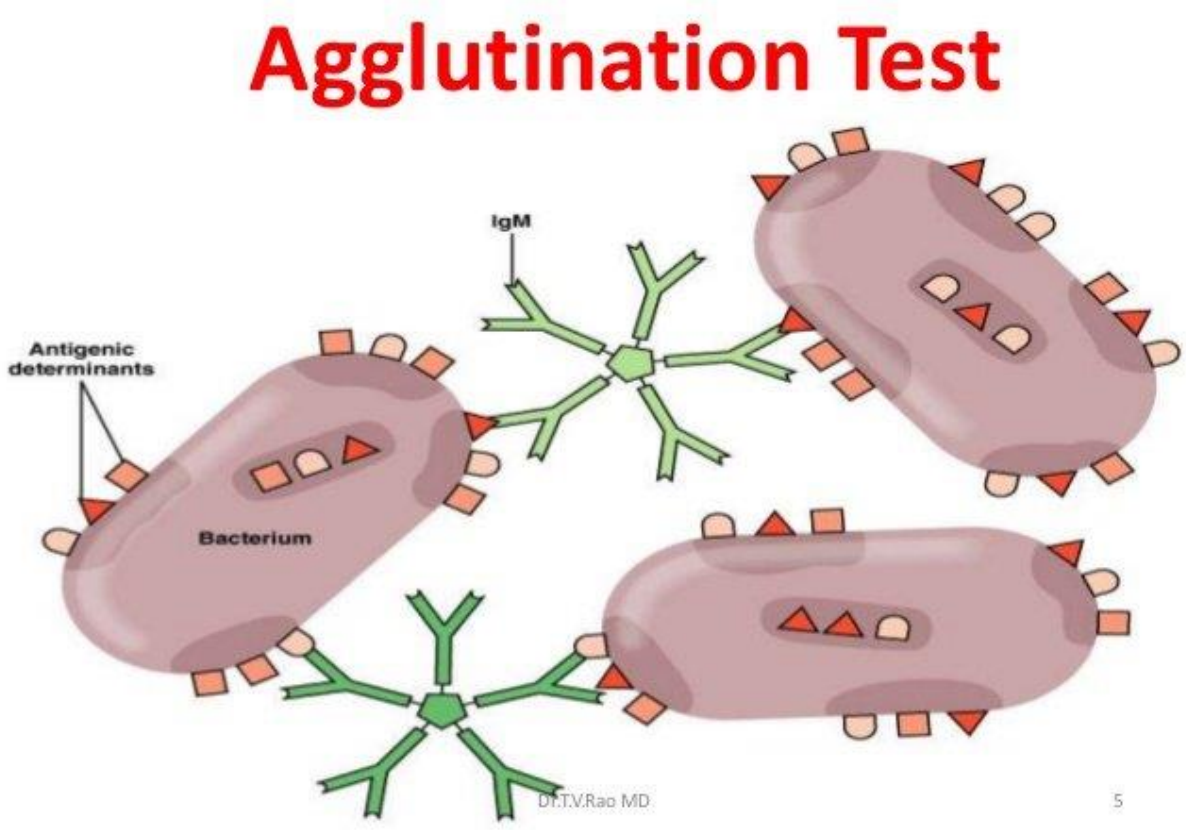
Applications of Rocket Immunoelectrophoresis

1. Rocket electrophoresis is used mainly for quantitative estimation of antigen in the serum.
2. The method has been used for quantization of human serum proteins before automated methods became available.
3. Determining the concentration of a specific protein in a protein mixture.
4. In estimation of immunoglobulin protease activity.
5. Studies dealing with antigenic relationships between organisms.
6. In enzyme activity electrophoresis.

III. Agglutination reactions

Agglutination is an antigen-antibody reaction in which a particulate antigen combines with its antibody in the presence of electrolytes at a specified temperature and pH resulting in the formation of visible clumping of particles. It occurs optimally when antigens and antibodies react in equivalent proportions. This reaction is analogous to the precipitation reaction in that antibodies act as a bridge to form a lattice network of antibodies and the cells that carry the antigen on their surface. Because cells are so much larger than a soluble antigen, the result is more visible when the cells aggregate into clumps.

When particulate antigens react with specific antibody, antigen-antibody complex forms visible clumping under optimum P^H and temperature. Such a reaction is called agglutination. Antibodies that produce such reactions are called **agglutinins**.



What is agglutination?

Agglutination is the visible expression of the aggregation of antigens and antibodies. Agglutination reactions apply to particulate test antigens that have been conjugated to a carrier. The carrier could be artificial (such as latex or charcoal particles) or biological (such as red blood cells). These conjugated particles are reacted with patient serum presumably containing antibodies. The endpoint of the test is the observation of clumps resulting from that antigen-antibody complex formation. The quality of the result is determined by the time of incubation with the antibody source, amount and avidity of the antigen conjugated to the carrier, and conditions of the test environment (e.g., pH and protein concentration). Various methods of agglutination are used in diagnostic immunology and these include latex agglutination, flocculation tests, direct bacterial agglutination, and hemagglutination.

Agglutination differs from precipitation reaction in that since agglutination reaction takes place at the surface of the particle involved, the antigen must be exposed and be able to bind with the antibody to produce visible clumps. In agglutination reactions, serial dilutions of the antibody solution are made and a constant amount of particulate antigen is added to serially diluted antibody solutions. After several hours of incubation at 37°C, clumping is recorded by visual

inspection. The titer of the antiserum is recorded as the reciprocal of the highest dilution that causes clumping. Since the cells have many antigenic determinants on their surface, the phenomenon of antibody excess is rarely encountered.

Prozone phenomenon

The condition of excess antibody, however, is called a **prozone phenomenon.** At a high concentration of antibody, the number of epitopes are outnumbered by antigen-binding sites. This results in the univalent binding of antigen by antibody rather than multivalently and thus, interferes in the crosslinking of antigen (Lattice formation).

Occasionally, antibodies are formed that react with the antigenic determinants of a cell but does not cause any agglutination. They inhibit the agglutination by the complete antibodies added subsequently. Such antibodies are called **blocking antibodies**. Anti-Rh antibodies and anti-brucella antibodies are few examples of such blocking antibodies.

Agglutination tests are easy to perform and in some cases are the most sensitive tests currently available. These tests have a wide range of applications in the clinical diagnosis of non-infectious immune disorders and infectious diseases. Agglutination reactions have a wide variety of applications in the detection of both antigens and antibodies in serum and other body fluids. They are very sensitive and the result of the test can be read visually with ease.

Applications of Agglutination Reactions

1. Cross-matching and grouping of blood.
2. Identification of Bacteria. E.g. Serotyping of *Vibrio cholera*, Serotyping of *Salmonella* Typhi and Paratyphi.
3. Serological diagnosis of various diseases. E.g Rapid plasma regains **(RPR)** test for Syphilis, Antistreptolysin O **(ASO)** test for rheumatic fever.
4. Detection of unknown antigen in various clinical specimens. E.g. detection of **Vi** antigen of *Salmonella* Typhi in the urine.

Examples of Agglutination

Haemagglutination assay

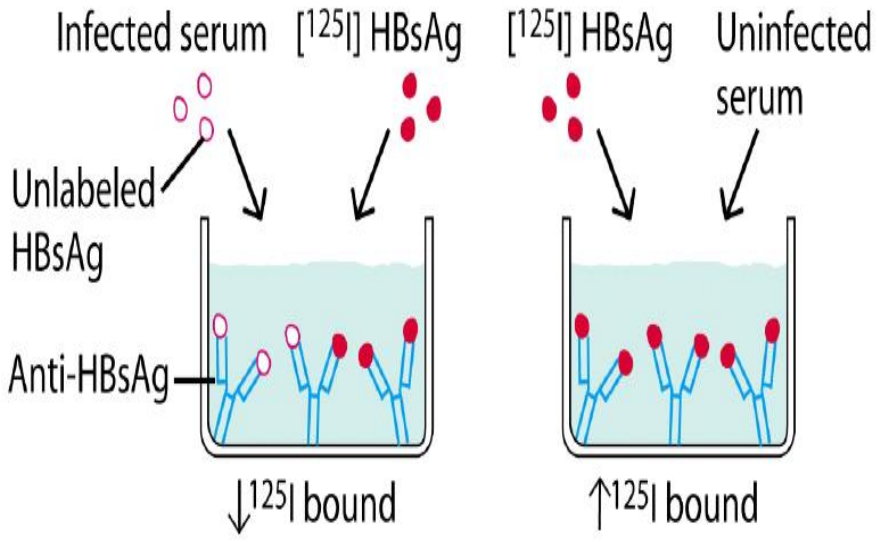
- Haemagglutination assay is a diagnostic technique used for the detection of viruses, bacteria, and antibodies.
- The antigens present in various viruses or bacteria bind with the sialic acid receptors present on the surface of red blood cells, creating a network of RBCs and viral particles.
- The formation of these lattices depends on the concentration of viruses/ bacteria and RBCs.

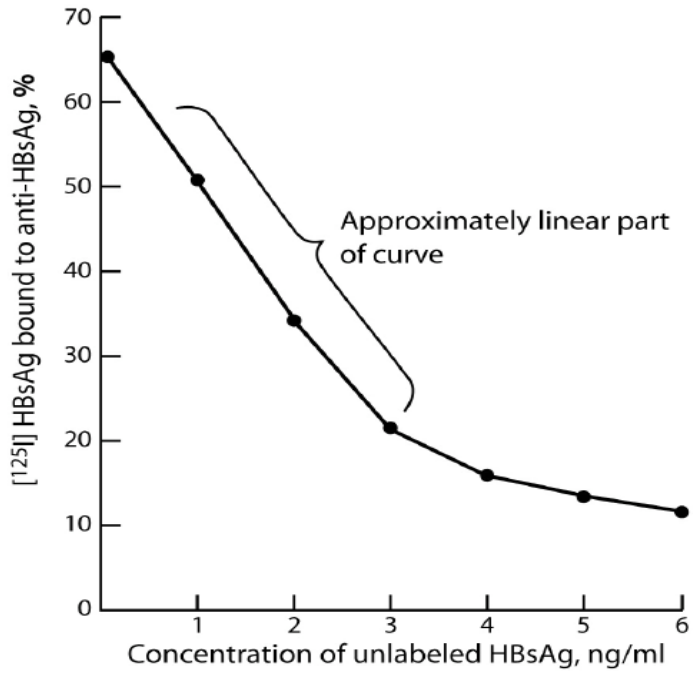
- When the concentration of the antigen is too low, the RBCs are not arranged in lattices but settle down at the bottom of the container.
- Haemagglutination is based on the same principle as the one used by viruses during infection.
- Control is placed in one of the wells to compare the concentration of antigen present in the sample.
- Based on the amount of agglutination formed in the well, the concentration of the antigen can be determined. The antigen concentration can then be used to determine the concentration of the organism present in the sample.

IV. RIA (Radio Immuno Assay)

When radioisotopes instead of enzymes are used as labels to be conjugated with antigens or antibodies, the technique of detection of the antigen-antibody complex is called radioimmunoassay (RIA). Radioimmunoassay (RIA) is an *in vitro* assay that measures the presence of an antigen with very high sensitivity. RIA was first described in 1960 for the measurement of endogenous plasma insulin by **Solomon Berson and Rosalyn Yalow** of the Veterans Administration Hospital in New York.

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





Principle of Radioimmunoassay

It involves a combination of three principles.

1. An immune reaction i.e. antigen, antibody binding.
2. A competitive binding or competitive displacement reaction. (It gives specificity)
3. Measurement of radio emission. (It gives sensitivity)

Immune Reaction:

When a foreign biological substance enters into the body bloodstream through a non-oral route, the body recognizes the specific chemistry on the surface of foreign substance as antigen and produces specific antibodies against the antigen so as nullify the effects and keep the body safe. The antibodies are produced by the body's immune system so, it is an immune reaction. Here the antibodies or antigens bind move due to chemical influence. This is different from principle of electrophoresis where proteins are separated due to charge.

Competitive binding or competitive displacement reaction:

This is a phenomenon wherein when there are two antigens that can bind to the same antibody, the antigen with more concentration binds extensively with the limited antibody displacing others. So here in the experiment, a radiolabelled antigen is allowed to bind to high-affinity

antibody. Then when the patient serum is added unlabeled antigens in it start binding to the antibody displacing the labeled antigen.

Measurement of radio emission:

Once the incubation is over, then washings are done to remove any unbound antigens. Then radio emission of the antigen-antibody complex is taken, the gamma rays from radiolabeled antigen are measured.

The target antigen is labeled radioactively and bound to its specific antibodies (a limited and known amount of the specific antibody has to be added). A sample, for e.g. blood-serum, is added in order to initiate a competitive reaction of the labeled antigens from the preparation, and the unlabeled antigens from the serum-sample, with the specific antibodies. The competition for the antibodies will release a certain amount of labeled antigen. This amount is proportional to the ratio of labeled to an unlabeled antigen. A binding curve can then be generated which allows the amount of antigen in the patient's serum to be derived. That means as the concentration of unlabeled antigen is increased, more of it binds to the antibody, displacing the labeled variant. The bound antigens are then separated from the unbound ones, and the radioactivity of the free antigens remaining in the supernatant is measured.

Antigen-antibody complexes are precipitated either by crosslinking with a second antibody or by means of the addition of reagents that promote the precipitation of antigen-antibody complexes. Counting radioactivity in the precipitates allows the determination of the amount of radiolabeled antigen precipitated with the antibody. A standard curve is constructed by plotting the percentage of antibody-bound radiolabeled antigen against known concentrations of a standardized unlabeled antigen, and the concentrations of antigen in patient samples are extrapolated from that curve.

The **extremely high sensitivity** of RIA is its **major advantage**.

Uses of Radioimmunoassay

1. The test can be used to determine very small quantities (e.g. nanogram) of antigens and antibodies in the serum.
2. The test is used for quantitation of hormones, drugs, HBsAg, and other viral antigens.
3. Analyze nanomolar and picomolar concentrations of hormones in biological fluids.

The **limitations of the RIA** include:

1. The cost of equipment and reagents
2. Short shelf-life of radiolabeled compounds

3. The problems associated with the disposal of radioactive waste.

V. ELISA

Enzyme-linked immunosorbent assay (ELISA)

Enzyme-linked immunosorbent assay (ELISA) utilizes an enzyme system to show specific combination of an antigen with its antibody. It is a method of quantifying an antigen immobilized on a solid surface. ELISA uses a specific antibody with a covalently coupled enzyme. The amount of antibody that binds the antigen is proportional to the amount of antigen present, which is determined by spectrophotometrically measuring the conversion of a clear substance to a colored product by the coupled enzyme. The ELISA technique was first conceptualized and developed by **Peter Perlmann** and **Eva Engvall** at Stockholm University, Sweden.

Enzyme system of ELISA consists enzyme which is labeled to a specific antibody or antigen and a chromogenic substrate which is added after antigen-antibody reaction. The substrate is hydrolysed by the enzyme attached to antigen-antibody complexes. An ELISA test uses components of the immune system (such as IgG or IgM antibodies) and chemicals for the detection of immune responses in the body. The ELISA test involves an enzyme (a protein that catalyzes a biochemical reaction). It also involves an antibody or antigen (immunologic molecules). Examples of the uses of an ELISA test includes to diagnose infections such as HIV (human immunodeficiency virus) and some allergic diseases like food allergies. ELISA tests are also known as an immunosorbent assay.

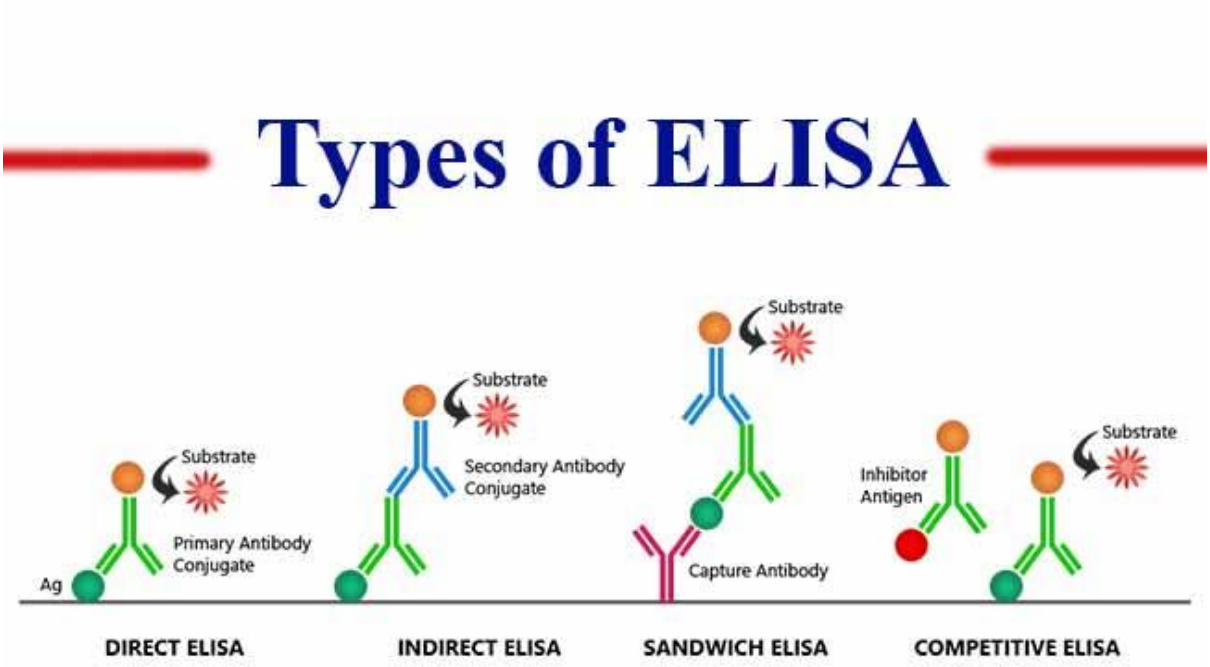
Following the antigen– antibody reaction, chromogenic substrate specific to the enzyme (**o-phenyldiamine dihydrochloride for peroxidase, p-nitrophenyl phosphate for alkaline phosphatase**, etc.) is added. The substrate is acted upon (usually hydrolysed) by enzyme attached to antigen-antibody complex to give color change. The color in reaction can be read visually or The reaction is detected by reading the optical density (estimated colorimetrically) using microassay plate reader i.e. ELISA reader. Usually, a standard curve based on known concentrations of antigen or antibody is prepared from which the unknown quantities are calculated.

he antigen or antibody is coated on solid surface such as in plastic tube or well of microtiter plate. Thus, after the antigen and antibody have combined (Antigen-antibody complex formed) they remain firmly attached to solid surface during subsequent washing stages.

Enzyme immunoassays (EIAs) can be used for detection of either antigens or antibodies in serum and other body fluids of the patient. In EIA techniques, antigen or antibody labeled with

enzymes are used. **Alkaline phosphatase, Horseradish peroxidase, and β-galactosidase** are the enzymes used in the EIA tests.

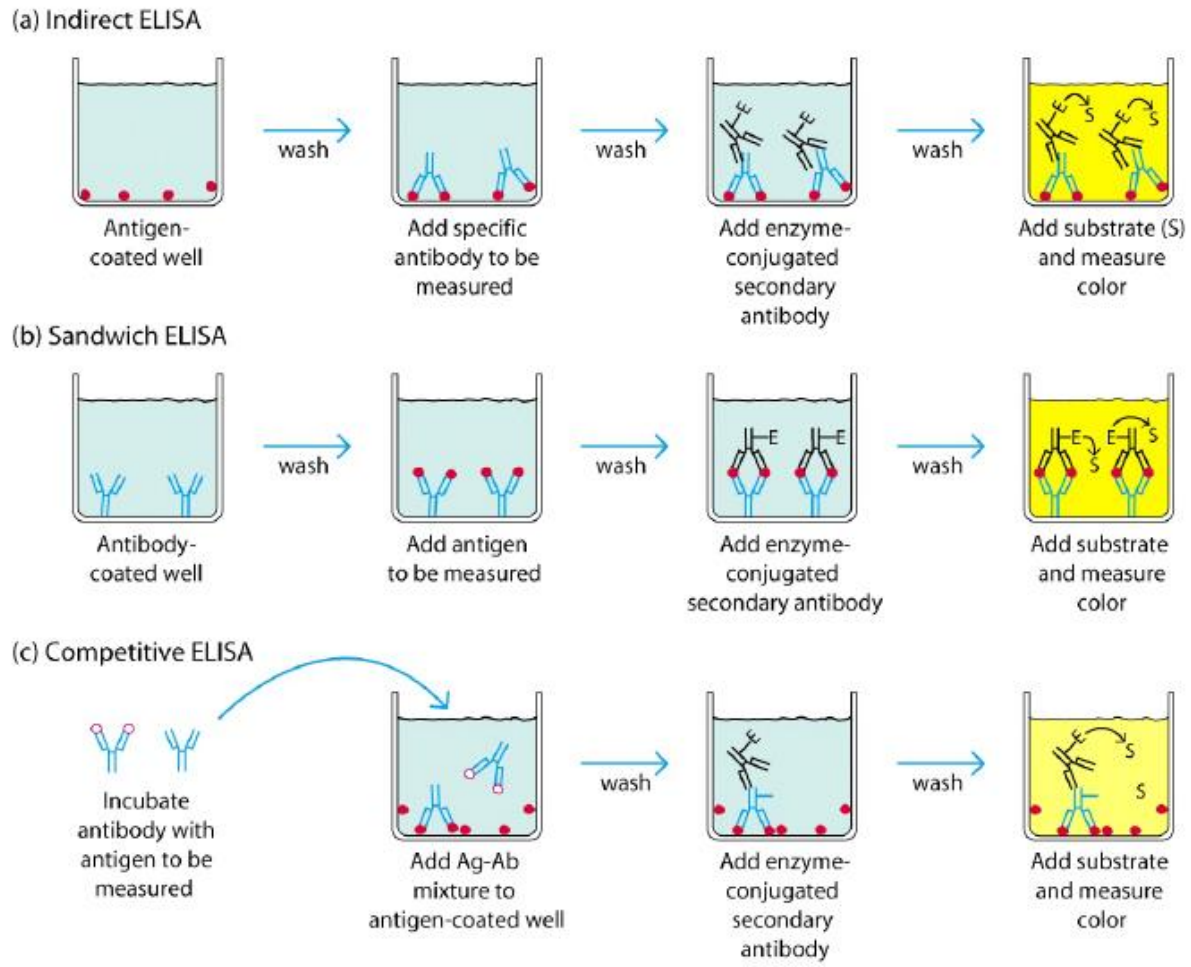
There are different types of ELISAs available for the detection and quantitation of either the antigen or antibodies in serum and other body fluids. There are four types of ELISA tests:



Direct ELISA
Antigen is attached to a polystyrene plate. Enzyme-labeled antibody is added that can react with the antigen and a substrate that can be measured.
Indirect ELISA
Antigen is attached to a polystyrene plate. Addition of primary antibody followed by an enzyme-labeled antibody that can react with both the primary antibody and substrate.
Sandwich ELISA
A capture antibody is attached to the polystyrene plate, then antigen is added that specifically attaches or captures the antigen. A second antibody, also specific for the antigen but not the same as the capture antibody is added and "sandwiches" the antigen. This second antibody is then followed by an enzyme-labeled antibody specific for the second antibody that can react with a substrate that can be measured.

Competitive ELISA

This test is like the sandwich ELISA but involves the addition of competing antibodies or proteins when the second antibody is added. This results in a decrease in the substrate signal that is generated. This test is considered to give good, highly specific results.



VI. Western blotting

Western Blot Definition

Western blot, also known as immunoblotting, is the process of separating proteins and identifying them in a complex biological sample.

- The use of polyacrylamide gel electrophoresis is a prerequisite for western blotting in order to separate proteins prior to their identification.
- The process of western blotting involves the transfer of proteins separated by SDS PAGE into an absorbent membrane. The proteins can then be identified on the membrane by different means.

- Western blotting has revolutionized the field of immunology with the use of antibody probes against membrane-bound proteins.
- The immunodetection of proteins has a wide application in biochemistry and other sciences as it can detect and characterize a multitude of proteins.
- The sensitivity of the process depends on the efficiency of transfer retention of proteins during processing and the final detection.
- Western blotting or protein blotting depends on the specificity of interaction between the protein of interest and the probe used for the detection of the protein.
- Unlike Southern blotting that utilizes radio-labeled nucleic acid probes, western blotting usually uses a second antibody tagged with an enzyme.
- Western blotting has a number of advantages over other similar techniques as the process only requires the use of a small amount of reagents, and the same protein transfer can be used for multiple analyses.

Principle of Western Blot

- The principle of western blotting is the interaction between the proteins and the probes used for the detection of the proteins.
- The proteins used for western blotting are separated by gel electrophoresis to obtain them on a gel matrix.
- The proteins are then transferred to a nitrocellulose or polyvinylidene fluoride (PVDF) membrane, where they are immobilized. The transfer of the protein is known as blotting.
- The protein on the membrane can either be detected by the use of a reporter-labeled primary antibody directed against the protein or a reporter-labeled secondary antibody directed at the primary antibody.
- The reporter or probe present on the antibody can be an enzyme that produces a color reaction or a luminescent signal at the antigen-antibody binding site that produces a fluorescent signal in the presence of a particular substrate.
- The signal or color generated by the probe requires a detection system that is appropriate for the signal or intensity generated.

Procedure of Western Blot

The process of western blotting consists of the following steps;

1. Sample Preparation

- The most commonly used samples for western blot are cell lysates which are collected by the process of extraction.
- The extraction can be achieved by different means like mechanical destruction, chemical extraction, or the use of enzymes.
- The extraction of often performed at cold temperature in the presence of protease inhibitors in order to prevent the denaturation of the proteins.

2. Gel Electrophoresis

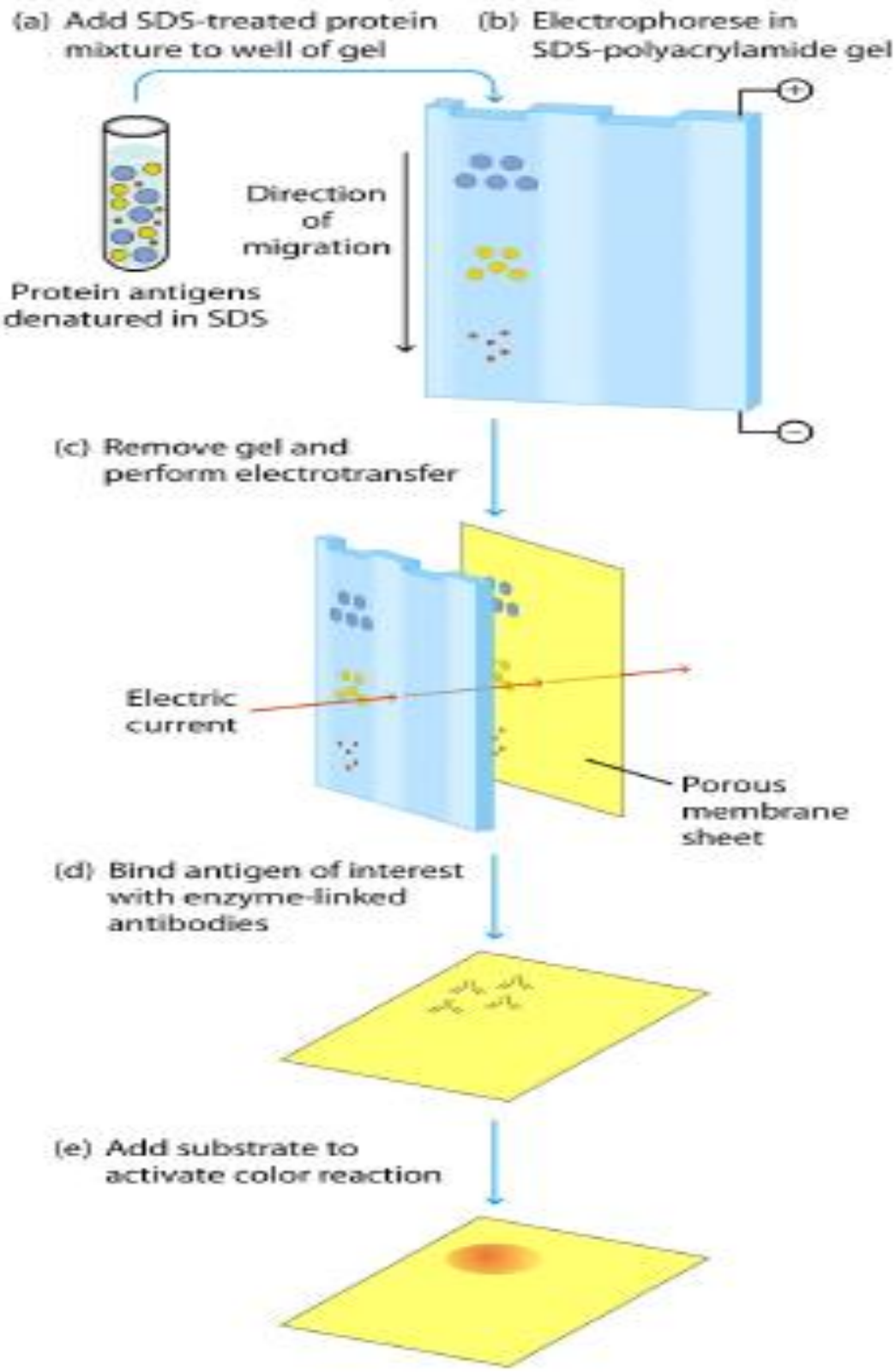
- The protein sample is diluted with the sample buffer and is heated and shaken for 10 minutes at 70°C.
- The sample is then centrifuged at 5000g.
- The gel case is removed from the pouch and is placed in the buffer tank against the rubber seal with the gel walls facing the inside of the tank reservoir.
- The running buffer is poured onto the upper reservoir while ensuring that no buffer leakage occurs on the lower tank.
- Each of the wells is then loaded with an equal volume of heat-denatured sample, and one of the lanes is reserved for the protein ladder.
- The lid is placed on the tank, and it is connected to the power supply.
- The run is allowed to run at 200 V constant for 50 minutes.

3. Protein Transfer

- The transfer buffer is prepared by adding 10% methanol to the buffer.
- The transfer case is taken and laid out. It is then covered with a transfer buffer.
- A foam sponge is taken and laid on the backside, over which goes the filter paper. These should be placed to ensure that both of them are wet and slightly submerged.
- The gel is taken out from the tank and placed on the wet filter paper.
- The nitrocellulose membrane is wet with the transfer buffer and is placed on top of the gel in a way that there are no bubbles between the gel and the membrane.
- The transfer case is placed into the transfer tank, which is further filled with transfer buffer.
- The tank is then connected to power at 100V for 1 hour.
- Once the transfer is complete, the transfer case is removed, and the nitrocellulose membrane is removed from the gel.

4. Immunodetection

- The membrane is washed with Tris-buffered saline for 5 minutes in a Petri dish.
- The 10% nonfat dry milk is mixed with the Tris buffer, and the membrane is covered with the mixture for 30 minutes at room temperature.
- The membrane is washed with the Tris buffer to remove any excess mixture remaining on the membrane.
- With the help of the forceps, the membrane is transferred to a new Petri dish onto which the primary antibody is added.
- The membrane with the antibody is incubated for 3 hours at room temperature. The membrane is washed after incubation with the Tris buffer.
- The membrane is transferred again to a new Petri dish, where a secondary HRP-conjugated antibody is added. The membrane is incubated for 1 hour. The concentration of secondary antibodies often remains at 1 µg/ml, but this also depends on the dilution.
- The membrane is washed again with Tris buffer to remove excess antibodies from the surface.
- The membrane is incubated with the substrate for 5 minutes, and the observation is made.



Result Interpretation of Western Blot

- The result of western blotting depends on the type of probes used during the process.

- If an enzyme-conjugated secondary antibody is used, the reaction between the substrate and the enzyme produces a color.
- The soluble dye is converted into an insoluble form, resulting in a different color on the membrane.
- In order to stop the development of a blot, the dye is removed by washing the membrane.
- The protein levels can then be evaluated by spectrophotometry.

Applications of Western Blot

1. Western blotting is an excellent method with high sensitivity in order to detect a particular protein even in low quantity.
2. Western blotting has been used in the clinical diagnosis of different diseases. The confirmatory test for HIV involves a western blot by detecting anti-HIV antibodies in the serum.
3. The technique has been used to quantify proteins and other gene products in gene expression studies.
4. Since western blotting detects the proteins by their size and ability to bind to the antibody, it is appropriate for evaluating the protein expressions in cells and further analysis of protein fractions during protein purification.
5. Western blotting is also used for the analysis of different biomarkers like growth factors, cytokines, and hormones.

Unit - IV

I. **Antigen presentation: Class I and II MHC molecules**

Major Histocompatibility Complex (MHC)

Both T and B cells use surface molecules to recognize antigen, they accomplish this in very different ways. In contrast to antibodies or B-cell receptors, which can recognize an antigen alone, T-cell receptors only recognize pieces of antigen that are positioned on the surface of other cells. These antigen pieces are held within the binding groove of a cell surface protein called the **Major histocompatibility complex (MHC) molecule** encoded by a cluster of genes collectively called the MHC locus. These fragments are generated inside the cell following antigen digestion, and the complex of the antigenic peptide plus MHC molecule then appears on the cell surface. MHC molecules thus act as a cell surface vessel for holding and displaying fragments of antigen so that approaching T cells can engage with this molecular complex via their T-cell receptors. The MHC in humans is known as **human leukocyte antigens (HLA) complex**.

HLA complex

In humans, the HLA complex of genes is located on short arm of chromosome 6 containing several genes that are critical to immune function. The HLA complex of genes is classified into three classes as follows:

Human HLA complex

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

Major Histocompatibility Complex Class I (MHC-I)

Class I molecules consist of a heavy polypeptide chain of 44 kDa non-covalently linked to a smaller 12 kDa peptide called β_2-microglobulin. The largest part of the heavy chain is organized into three globular domains (α_1, α_2 and α_3) which protrude from the cell surface; a hydrophobic section anchors the molecule in the membrane and a short hydrophilic sequence carries the C-terminus into the cytoplasm. The heavy chain has a variable and constant region.

The variable region is highly pleomorphic. The polymorphism of these molecules is important in the recognition of self and non-self. The constant region of the heavy chain binds with the CD8 proteins of the cytotoxic T cells.

X-ray analysis of crystals of a human class I molecule shows both β_2 -microglobulin and the α_3 region resemble classic Ig domains in their folding pattern. The α_1 and α_2 domains interact to form a platform of eight antiparallel β strands spanned by two long α-helical regions. The structure forms a deep groove, or cleft with the long α helices as sides and the β strands of the β sheet as the bottom. This **peptide-binding groove** is located on the top surface of the class I MHC molecule, and bind a peptide of 8 to 10 amino acids.

Class I proteins are involved in **graft rejection and cell-mediated cytolysis**.

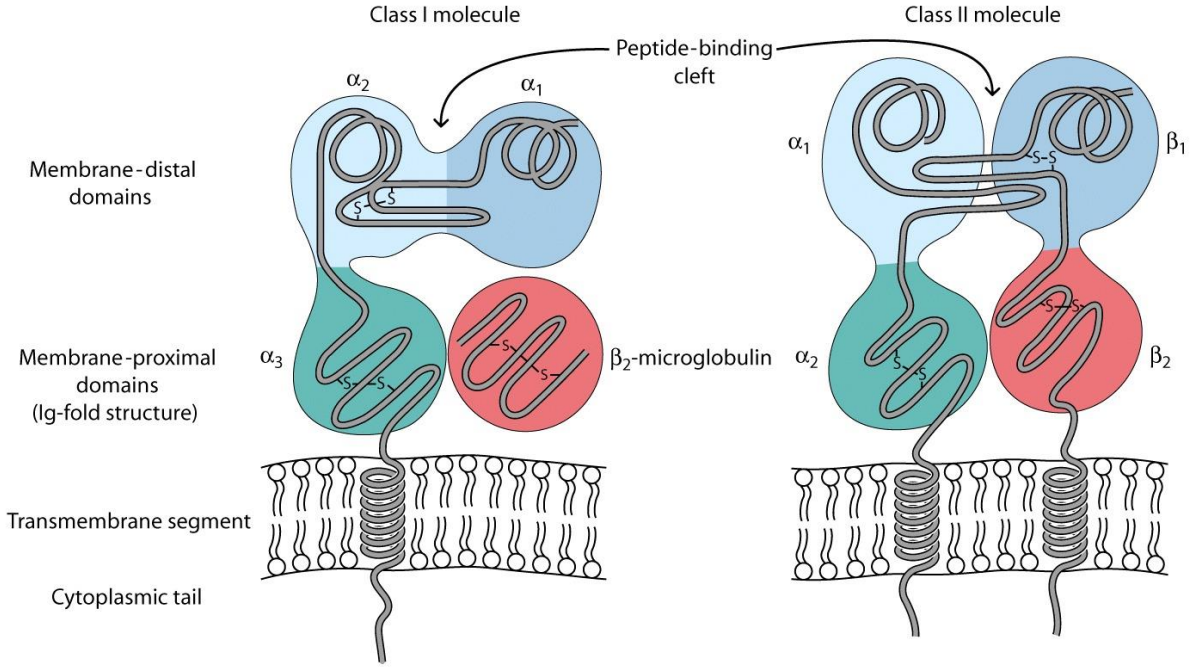


TABLE 7-2 Peptide binding by class I and class II MHC molecules

	Class I molecules	Class II molecules
Peptide-binding domain	α1/α2	α1/β1
Nature of peptide-binding cleft	Closed at both ends	Open at both ends
General size of bound peptides	8–10 amino acids	13–18 amino acids
Peptide motifs involved in binding to MHC molecule	Anchor residues at both ends of peptide; generally hydrophobic carboxyl-terminal anchor	Anchor residues distributed along the length of the peptide
Nature of bound peptide	Extended structure in which both ends interact with MHC cleft but middle arches up away from MHC molecule	Extended structure that is held at a constant elevation above the floor of MHC cleft

Major Histocompatibility Complex Class II (MHC-II)

Class II MHC molecules are also transmembrane glycoproteins, consisting of α and β polypeptide chains of molecular weight 33-kDa α chain and a 28-kDa β chain, which associate by noncovalent interactions. Class II molecule contains two external domains: α_1 and α_2 domains in one chain and β_1 and $\beta_2$domains in the other. There is considerable sequence homology with class I. Structural studies have shown that the α_2 and β_2 domains, the ones nearest to the cell membrane assume the characteristic Ig fold, while the α_1 and β_1 domains form the peptide-binding groove for processed antigen. The peptide-binding groove of class II molecules is composed of a floor of eight antiparallel β strands and sides of antiparallel α helices, where peptides typically ranging from 13 to 18 amino acids can bind.

Class II proteins are primarily responsible for the **graft-versus-host response and the mixed leukocyte response.**

Mechanism of Major Histocompatibility Complex I

- MHC class I glycoproteins present antigens of endogenous origin to TCRs of CD8+ T cells.
- Endogenous peptides derive from degradation of intracellular proteins, including viral or tumor antigens in infected or transformed cells, through the proteasome.
- Degradation products translocate from the cytoplasm to the endoplasmatic reticulum (ER) where they are loaded on MHC class I molecules via the peptide-loading complex that includes the ER transporter associated with antigen processing (TAP1/2), tapasin, the oxidoreductase ERp57, and the chaperone protein calreticulin.
- Cellular components involved in the presentation of endogenous antigens, from proteasome subunits to the peptide-loading complex, are collectively referred to as antigen-processing machinery (APM).
- CD8+ T lymphocytes express CD8 receptors, in addition to the T-cell receptors (TCR).
- When a Cytotoxic T cell CD8 receptor docks to a MHC class I molecule and the TCR fits the epitope within the MHC class I molecule, the CD8+ T lymphocytes triggers the cell to undergo programmed cell death by apoptosis.
- This helps mediate cellular immunity which is the primary means to address intracellular pathogens, such as viruses and some bacteria.

Functions of Major Histocompatibility Complex I

1. **Antigen Processing and Presentation**

Nucleated cell normally present peptides, mostly self peptides derived from protein turnover and defective ribosomal products. Also, during viral infection, intracellular microorganism infection, or cancerous transformation, such proteins degraded inside the cell by proteasomes are also loaded onto MHC class I molecules and displayed on the cell surface.

2. **Transplant Rejection**

During transplant of an organ or stem cells, MHC molecules themselves act as antigens and can provoke immune response in the recipient causing transplant rejection. Since, the MHC variation in the human population is high and no two individuals except identical twins express the same MHC molecules, they can mediate transplant rejection.

Mechanism of Major Histocompatibility Complex II

- MHC class II molecules present antigens of exogenous origin to CD4+ T cells.
- Phagocytes such as macrophages and immature dendritic cells take up entities by phagocytosis into phagosomes which fuse with lysosomes and the acidic enzymes cleave the uptaken protein into many different peptides.
- During synthesis of class II MHC, the molecules are transported from the endoplasmic reticulum (ER) via the Golgi to endosomal compartments. The α and β chains produced are complexed with a special polypeptide known as the invariant chain (Ii). The Ii prevents endogenous peptides from binding to the groove of MHC class II molecules.
- After removal of Ii in the acidic endosomal compartments, peptides are able to bind to the MHC groove.
- A particular peptide exhibiting immunodominance loads onto MHC class II molecules.
- Peptide-loaded MHC class II molecules are then transported to the membrane surface for antigen presentation.
- The peptide:MHC class II complex is then recognized by the cognate T cell receptor (TCR) of helper T cells.

Functions of Major Histocompatibility Complex II

- The TCR–peptide: MHC class II engagement is crucial to the induction and regulation of adaptive immunity by selecting the mature CD4+ T cell repertoire in the thymus and activating these lymphocytes in the periphery.
- The secure attachment to the MHC molecule with the presented peptide ensure stable peptide binding which enhance T cell recognition of the antigen, T cell recruitment, and a proper immune response.

- Since they sample and present antigens from exogenous sources, MHC class II molecules are critical for the initiation of the antigen-specific immune response.

Distribution of MHC

Essentially, all nucleated cells carry classical class I molecules. These are abundantly expressed on lymphoid cells, less so on liver, lung and kidney, and only sparsely on brain and skeletal muscle. In the human, the surface of the villous trophoblast lacks HLA-A and -B and bears HLA G, which does not appear on any other body cell. Class II molecules are also restricted in their expression, being present only on antigen presenting cells (APCs) such as B-cells, dendritic cells and macrophages and on thymic epithelium. When activated by agents such as interferon g, capillary endothelia and many epithelial cells in tissues other than the thymus, they can develop surface class II and increased expression of class I.

They function as cell surface markers enabling infected cells to signal cytotoxic and helper T-cells.

Importance of MHC

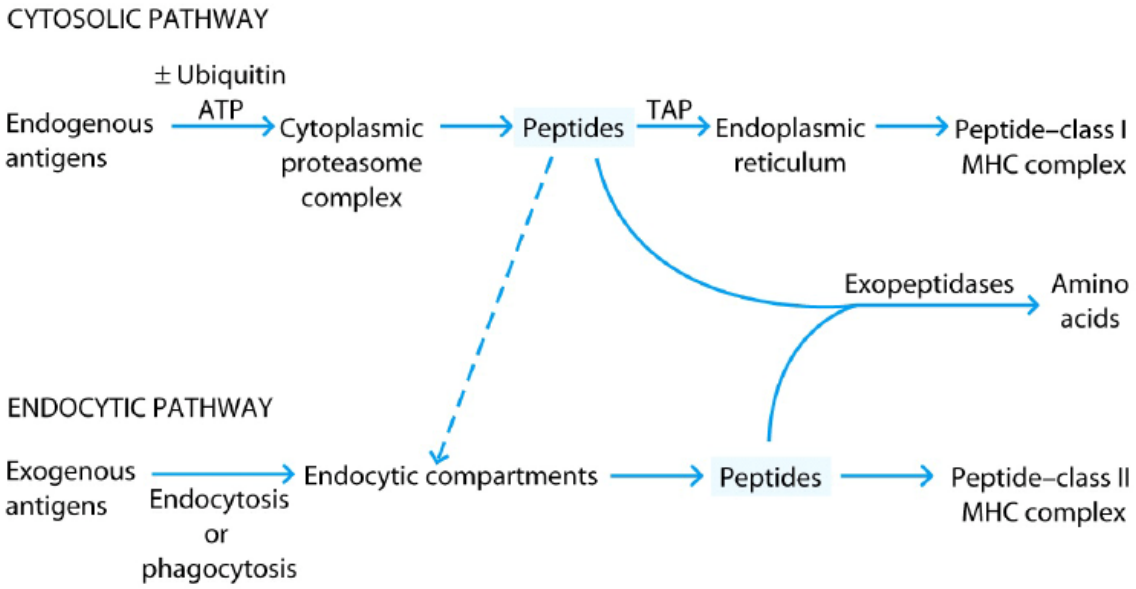
1. Antibody molecules interact with antigen directly but the T-Cell Receptor (TCR) only recognizes antigen presented by MHC molecules on another cell, the Antigen Presenting Cell. The TCR is specific for antigen, but the antigen must be presented on a self-MHC molecule.
2. The TCR is also specific for the MHC molecule. If the antigen is presented by another allelic form of the MHC molecule in vitro (usually in an experimental situation), there is no recognition by the TCR. This phenomenon is known as **MHC restriction**.

Peptide antigens associated with class I MHC molecules are recognized by $CD8^+$ cytotoxic T lymphocytes, whereas class II-associated peptide antigens are recognized by $CD4^+$ helper T cells.

III. Cytosolic and endocytic pathways.

Ag is processed thru 2 separate pathways:

- MHC I interact with peptides from **cytosolic** degradation
- MHC II interacts with peptides from **endocytic** degradation



Processing Endogenous Ag: The Cytosolic Pathway

- Cellular [c]'s of proteins are constantly regulated; most have a brief half-life and are "turned over" the same holds true for endogenous Ag's!
- Processing of endogenous Ag involves 3 activities:

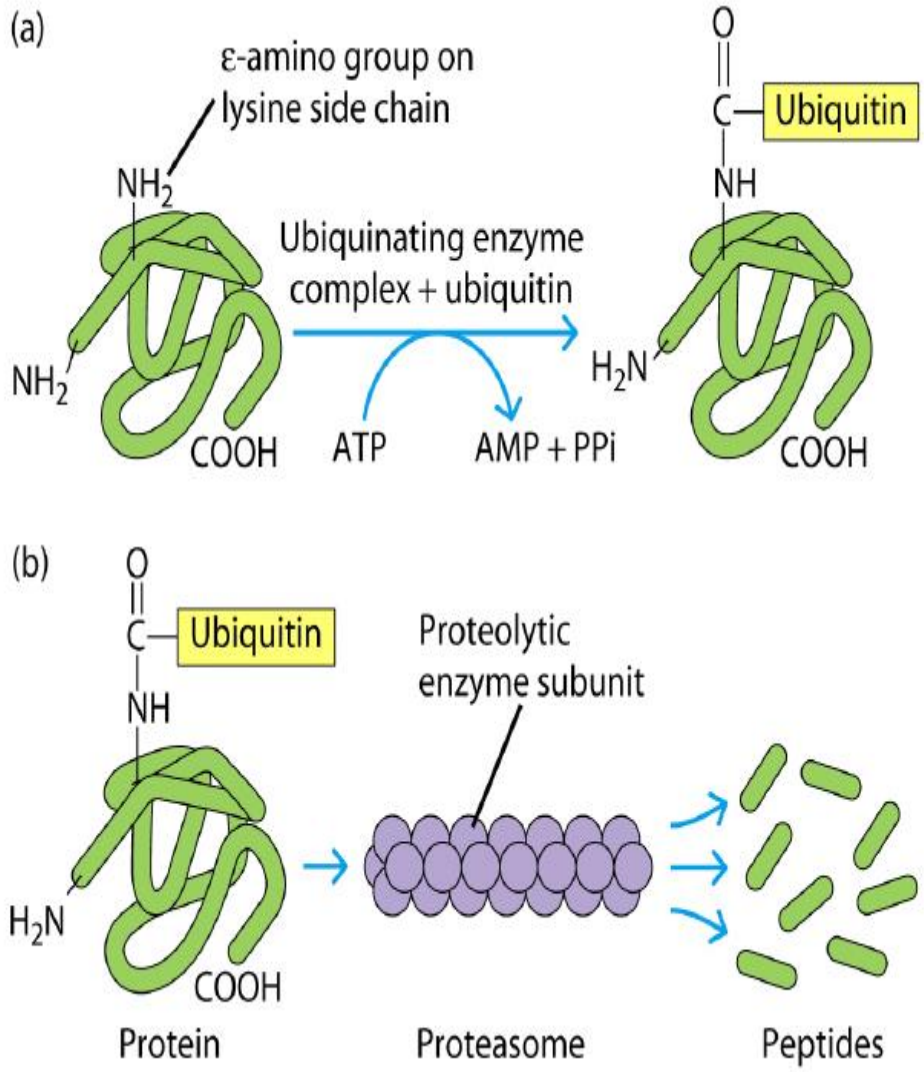
Peptide generation from proteolysis

Transport to ER

Peptide binding to MHC I

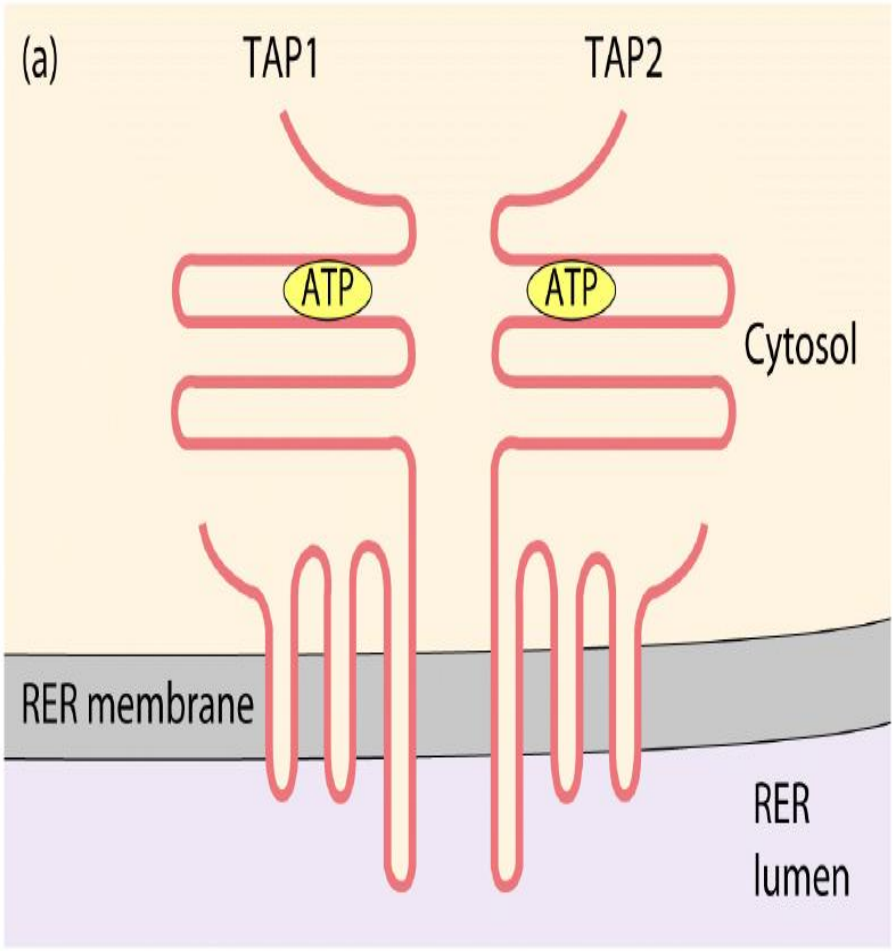
a) Peptide generation from proteolysis

- Proteins targeted for lysis combine w/ a small protein → **ubiquitin**
- **Ubiquitin-protein complex** is degraded by a proteosome
- Specific proteosomes generate peptides which can bind to MHC I



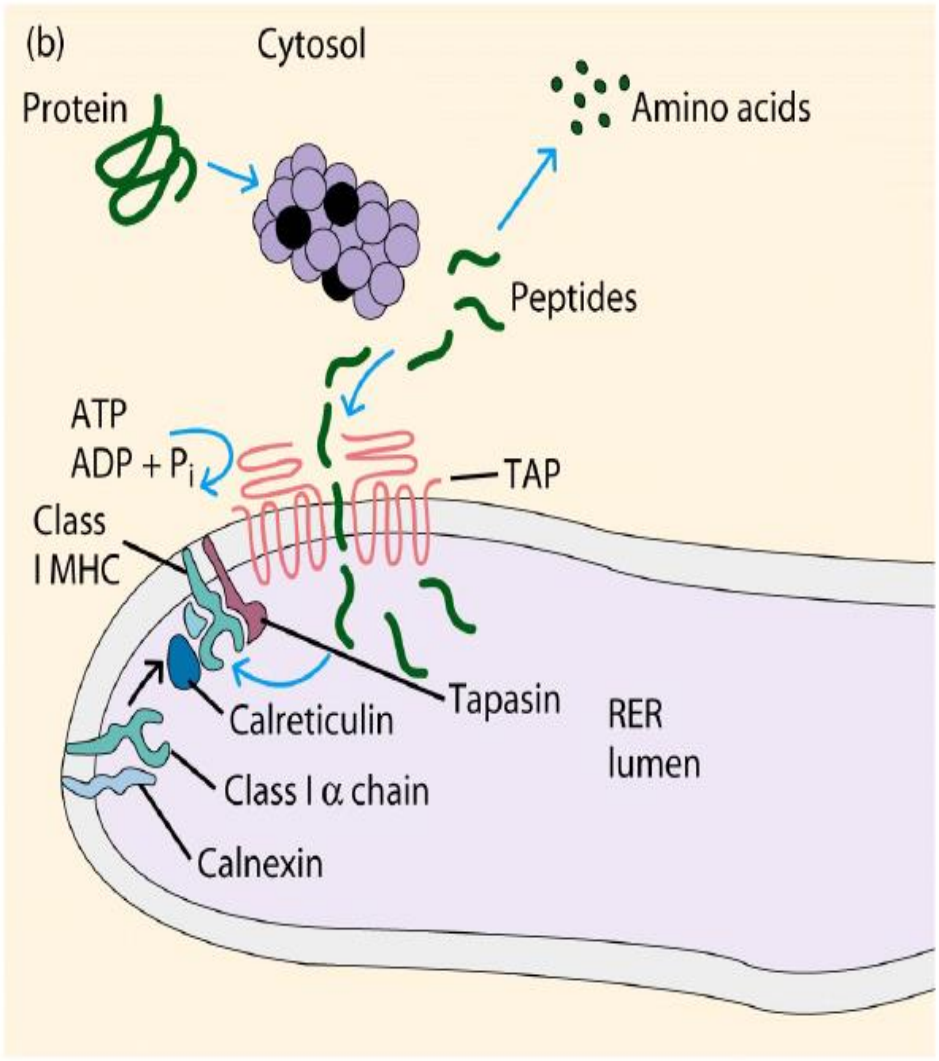
b) Transport to ER

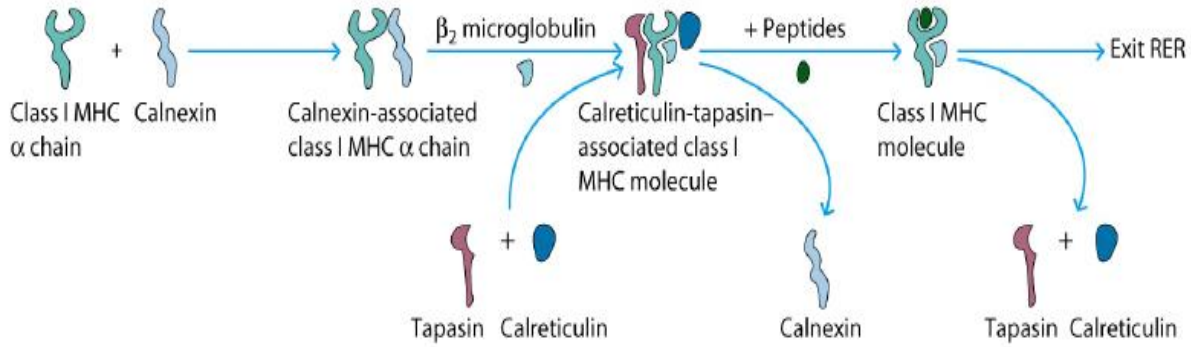
- Peptides from proteolysis bind to a "transporter protein assoc w/ Ag processing" (TAP)
- TAP is a heterodimer which uses ATP to help transport peptides (8-10 aa's) to lumen of ER
- Usually basic aa's @ COOH end of peptide chain



c) Peptide binding to MHC I

3. MHC I assembly occurs w/ the aid of **chaperone proteins** to promote folding (**calnexin** + MHC I α chain)
4. **Tapasin + calreticulin** brings TAP/ peptide close to MHC assembly
5. Allows MHC I to bind to peptides
6. **MHC I-Ag** exits ER to Golgi to plasma membrane





Processing of Exogenous Ag's: The Endocytic Pathway

- Exogenous Ag's are typically phagocytized/ endocytized by MØ and APC's

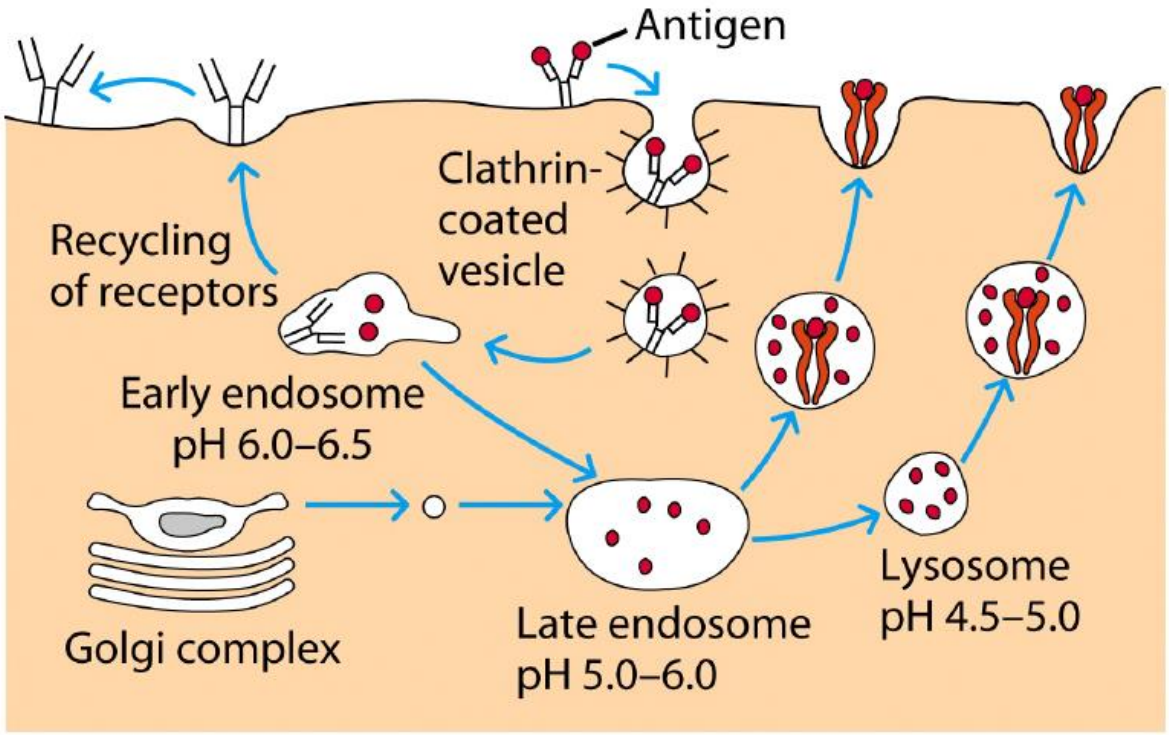
Foreign Ag is degraded w/i endocytic vacuole of endocytic pathway. The pathway includes:

Early endosomes (pH 6-6.5)

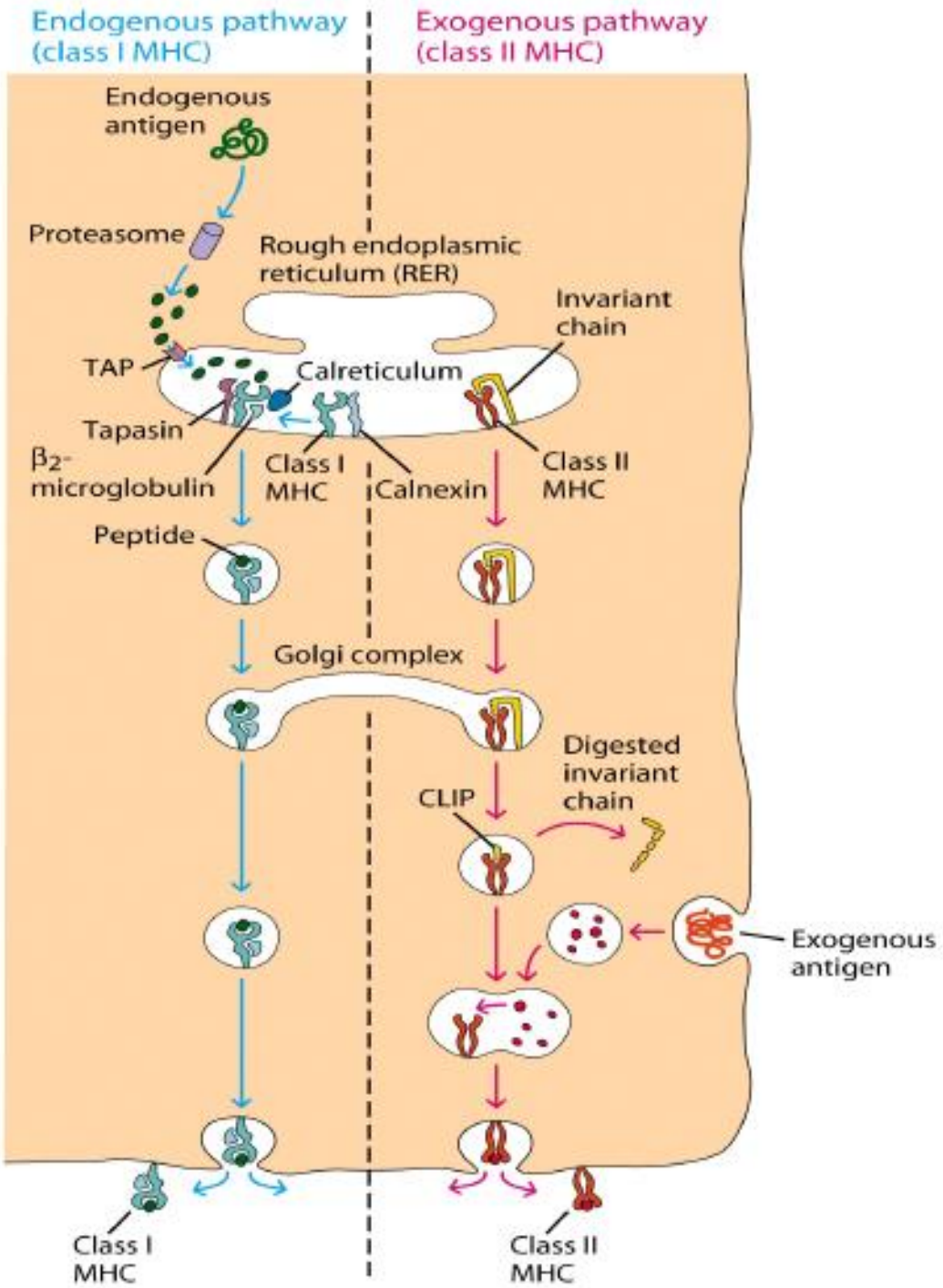
Late endosomes or endolysosome (pH 5-6)

Lysomes (pH 4.5 – 5)

- Ag is degraded into 13-18 aa polypeptides which bind to MHC II
- Eventually endocytic vacuole returns to PM → recycling surface receptors

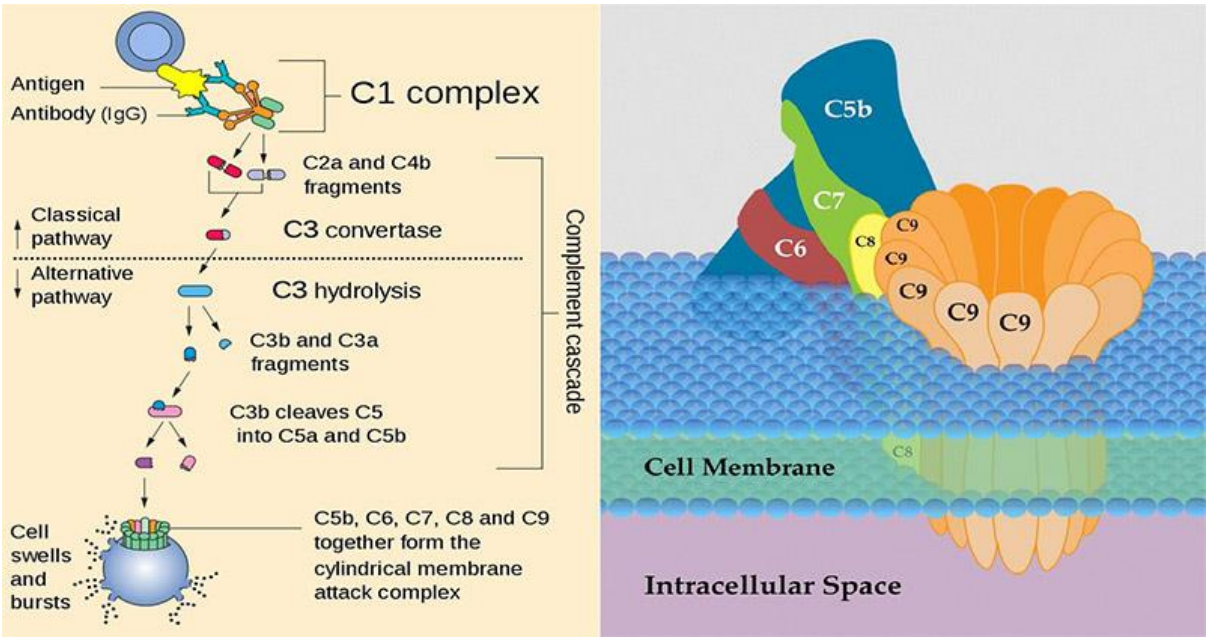


- within ER, α and β chains of MHC II combine w/ a protein – "**the invariant chain**" (IC, CD74)
- the IC binds to MHC @peptide binding cleft + then exits the ER to Golgi apparatus
- as proteolytic activity continues, the IC is degraded to a small fragment (CLIP- class II associated invariant chain peptide)
- another MHC II (HLA-DM (found in endosomes)) substitutes Ag for CLIP w/i lysosome
- MHC II – Ag complex is transported to the Plasma Membrane


Endogenous pathway
(class I MHC)
Exogenous pathway
(class II MHC)
Endogenous
antigen
Proteasome
Rough endoplasmic
reticulum (RER)
Invariant
chain
TAP
Calreticulum
Tapasin
β2-
microglobulin
Class I
MHC
Calnexin
Class II
MHC
Peptide
Golgi complex
Digested
invariant
chain
CLIP
Exogenous
antigen
Class I
MHC
Class II
MHC

III. Complement pathways – classical and alternative

Complement refers to a set of serum proteins that cooperates with both the innate and the adaptive immune systems to eliminate blood and tissue pathogens. Various complement components bind and opsonize bacteria, rendering them susceptible to receptor-mediated phagocytosis by macrophages, which express membrane receptors for complement proteins. Other complement proteins elicit inflammatory responses, interface with components of the adaptive immune system, clear immune complexes from the serum, and/or eliminate apoptotic cells. Finally, a Membrane Attack Complex (MAC) assembled from complement proteins directly kills some pathogens by creating pores in microbial membranes. Paul Ehrlich coined the term ***complement***, defining it as "the activity of blood serum that completes the action of antibody."



Complement is a complex system of enzymes, regulatory proteins, and cell surface receptors that are involved in host defense, inflammation, and modulation of immune responses. The system provides a fast-acting mechanism for the identification and removal of foreign substances, providing protection before the adaptive immune system can come into play. It is also involved in a wide variety of homeostatic processes including the clearance of immune complexes, effete cells, and cellular debris from damaged tissues.

The **complement system** is a part of the **immune system** that enhances (**complements**) the ability of antibodies and phagocytic cells to clear microbes and damaged cells from an organism, promotes inflammation, and attacks the pathogen's plasma membrane.

The complement system consists of serum and cell surface proteins that interact with one another and with other molecules of the immune system in a highly regulated manner to generate products that function to eliminate microbes**.** Complement proteins are plasma proteins that are normally inactive; they are activated only under particular conditions to generate products that mediate various effector functions of complement.

Properties of Complement

Complement shows the following properties:

1. It is present in sera of all mammals including humans and in lower animals including birds, amphibians, and fishes.
2. These are heat-labile substances that are inactivated by heating serum at 56°C for 30 minutes.
3. These are glycoproteins and are synthesized primarily by liver cells and to a very less extent by macrophages and many other cell types. The rate of synthesis of the various complement glycoproteins increase when complement is activated and consumed.
4. The complement usually does not bind to the antigen or antibody but only to antigen–antibody complex.
5. The importance of the complement lies in the fact that it contributes to both the acquired and innate immunity of an individual.

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

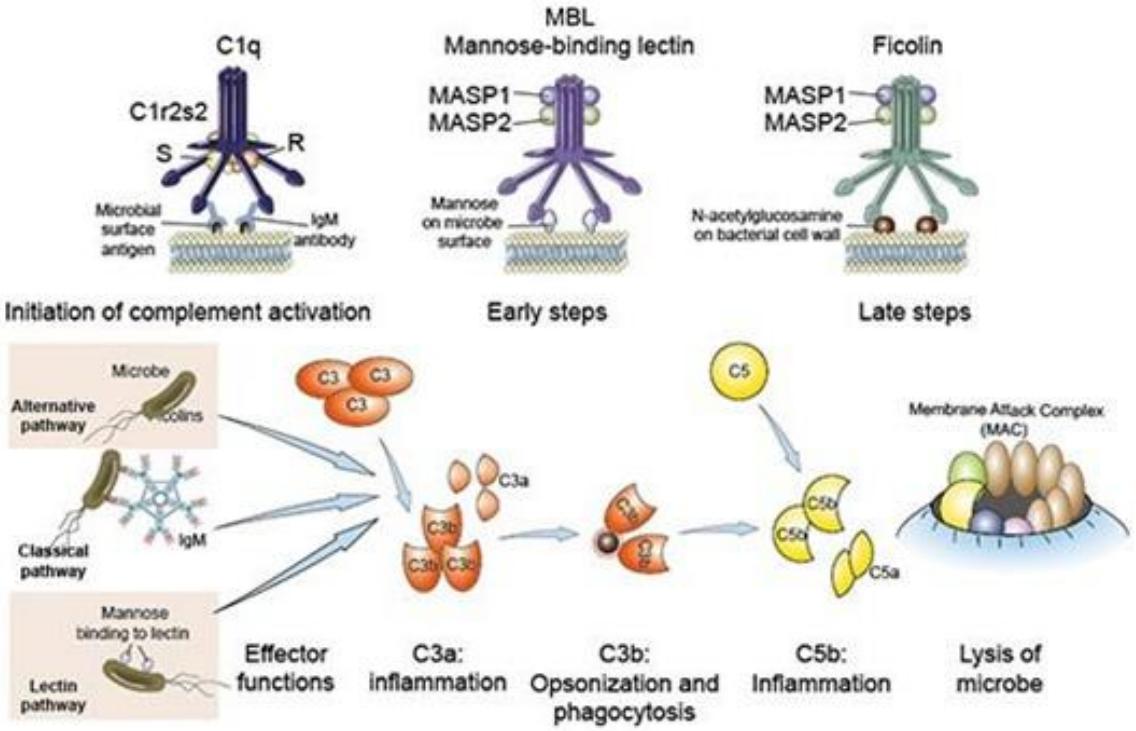
1. It causes **lysis of cel**ls (such as bacteria, viruses, allografts, and tumor cells).
2. It **generates mediators** that participate in triggering specific cell functions, inflammation, and secretion of immunoregulatory molecules.
3. It **facilitates opsonization**, the process by which bacteria are more readily and more efficiently engulfed by phagocytes.
4. It **causes immune clearance**, in which immune complexes from the circulation are removed and are transported to spleen and liver.

Nomenclature of Complement

Complement components are designated by numerals, viz., C1–9. These components circulate in plasma in the form of proenzymes that are functionally inactive. Activation involves cleavage by proteolysis into peptide fragments. The fragments are designated with lowercase suffixes—for example, C3 is cleaved into two fragments, C3a and C3b. Normally, the large fragment is designated “b”, and the small fragment “a”. But for historical reasons, with respect

to the fragments of C2, the large fragment is designated C2a and the small one is designated C2b.

Activation of complement involves the sequential proteolysis of proteins to generate enzyme complexes with proteolytic activity**.** Proteolytic cascades allow tremendous amplification because each enzyme molecule activated at one step can generate multiple activated enzyme molecules at the next step. The products of complement activation become covalently attached to microbial cell surfaces, to antibodies bound to microbes and to other antigens, and to apoptotic bodies. Complement activation is inhibited by regulatory proteins that are present on normal host cells and absent from microbes**.** The regulatory proteins are an adaptation of normal cells that minimize complement-mediated damage to host cells. Microbes lack these regulatory proteins, which allows complement activation to occur on microbial surfaces.



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

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

The complement system is activated by microbes and by antibodies that are attached to microbes and other antigens.

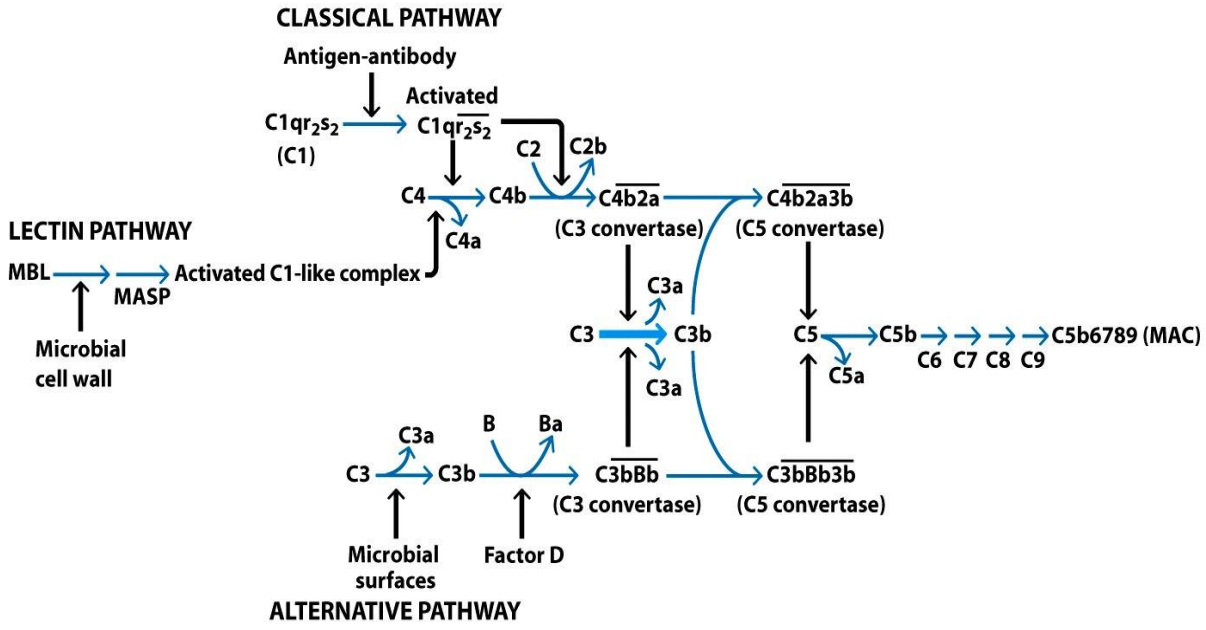


Figure 7-2
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Classical pathway

It is activated by certain isotypes of antibodies bound to antigens; the **alternative pathway**, which is activated on microbial cell surfaces in the absence of antibody; and the **lectin pathway**, which is activated by a plasma lectin that binds to mannose residues on microbes. Although the pathways of complement activation differ in how they are initiated, all of them result in the generation of enzyme complexes that are able to cleave the most abundant complement protein, C3. **The classical pathway,** so called because it was discovered first, uses a plasma protein called C1q to detect antibodies bound to the surface of a microbe or other structure. Once C1q binds to the Fc portion of the antibodies, two associated serine proteases, called C1r and C1s, become active and initiate a proteolytic cascade involving other complement proteins. The classical pathway is one of the major effector mechanisms of the humoral arm of adaptive immune responses. Innate immune system soluble proteins called pentraxins, can also bind C1q and initiate the classical pathway.

The alternative pathway

It was discovered later but is phylogenetically older than the classical pathway, is triggered when a complement protein called C3 directly recognizes certain microbial surface structures, such as bacterial LPS. C3 is also constitutively activated in solution at a low level and binds to cell surfaces, but it is then inhibited by regulatory molecules present on mammalian cells. Because microbes lack these regulatory proteins, the spontaneous activation can be amplified

on microbial surfaces. Thus, this pathway can distinguish normal self from foreign microbes on the basis of the presence or absence of the regulatory proteins.

The lectin pathway

It is triggered by a plasma protein called mannose-binding lectin (MBL), which recognizes terminal mannose residues on microbial glycoproteins and glycolipids, similar to the mannose receptor on phagocyte membranes described earlier. MBL is a member of the collectin family (discussed later) with a hexameric structure similar to the C1q component of the complement system. After MBL binds to microbes, two zymogens called MASP1 (mannose-associated serine protease 1, or mannan-binding lectin-associated serine protease) and MASP2, with similar functions to C1r and C1s, associate with MBL and initiate downstream proteolytic steps identical to the classical pathway.

The central event in complement activation is proteolysis of the complement protein C3 to generate biologically active products and the subsequent covalent attachment of a product of C3, called C3b, to microbial cell surfaces or to antibody bound to antigen.

Complement activation depends on the generation of two proteolytic complexes: the **C3 convertase,** which cleaves C3 into two proteolytic fragments called C3a and C3b; and the **C5 convertase**, which cleaves C5 into C5a and C5b.

The initiating pathways have several things in common. They are triggered by

1. The binding of one of their components to the activator.
2. A cascade of enzyme activation.
3. Generation of biological effects.

Each initiating pathway is triggered by a different type of activator, usually a cell, microbe, or molecular aggregate that presents charge patterns that are “recognized” by components of the individual initiating pathway.

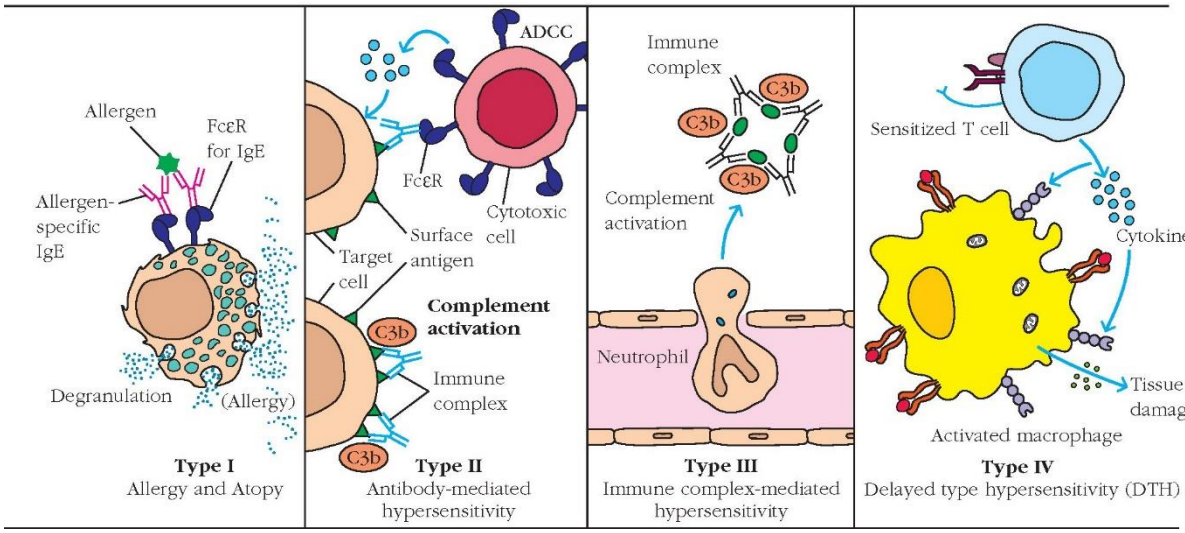
Each complement pathway has unique proteins for the initiating step, but shares the same or related proteins for the intermediate steps, and uses the same components in the last step, culminating in the same activities. Complement activation represents the dynamic interplay among the different pathways, the control processes, and other protein systems and cells in the local environment.

Complement activation promotes phagocytosis because C3b becomes covalently linked to microbes, and phagocytes (neutrophils and macrophages) express receptors for C3b. Peptides produced by proteolysis of C3 (and other complement proteins) stimulate inflammation. The

C5 convertase assembles after the prior generation of C3b, and this convertase contributes both to inflammation (by generation of the C5a fragment) and to the formation of pores in the membranes of microbial targets. The pathways of complement activation differ in how C3b is produced but follow a common sequence of reactions after the cleavage of C5.

Unit – V

Mind Map



I. Vaccines: Active and Passive immunization.

- **A vaccine** is a medical preparation given to provide immunity from a disease.
- Vaccines use a variety of different substances ranging from dead microorganisms to genetically engineered antigens to defend the body against potentially harmful microorganisms.
- Effective vaccines change the immune system by promoting the development of antibodies that can quickly and effectively attack disease-causing microorganisms when it enters the body, preventing disease development.
- A vaccine may contain live-attenuated or killed microorganisms or parts or products from them capable of stimulating a specific immune response comprised of protective antibodies and T cell immunity.
- A vaccine should stimulate a sufficient number of memory T and B lymphocytes to yield effector T cells and antibody-producing B cells from memory cells.
- The viral vaccines should also be able to stimulate high titers of neutralizing antibodies.
- Injection of a vaccine into a nonimmune subject induces active immunity against the modified pathogens.
- **Vaccination** is immunization against infectious disease through the administration of vaccines for the production of active (protective) immunity in humans or other animals.

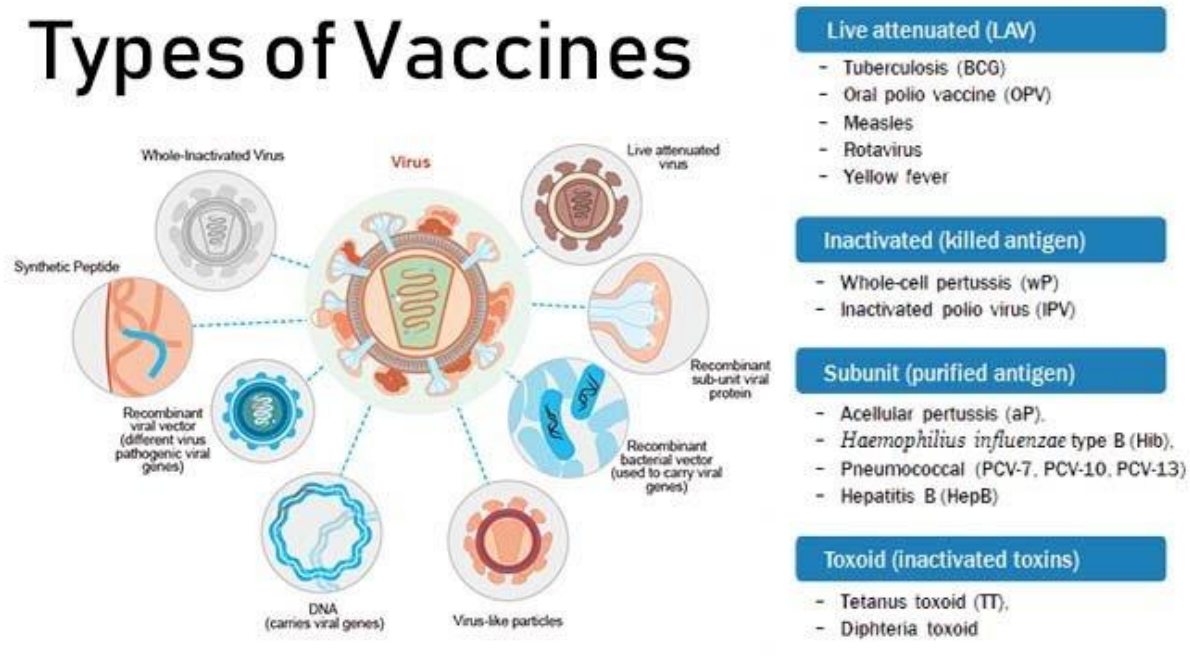


Figure: Types of Vaccines. Image Source: GenScript

There are 4 main types of vaccines:

1. Live Attenuated vaccines (LAV)
2. Inactivated vaccines (Killed Antigen)
3. Subunit and Conjugate Vaccines (Purified Antigen)
4. Toxoid vaccines (Inactivated Toxins)

A. Live Attenuated Vaccines

- In some cases, microorganisms can be attenuated or disabled so that they lose their ability to cause significant disease (pathogenicity) but retain their capacity for transient growth within an inoculated host.
- Some agents are naturally attenuated by virtue of their inability to cause disease in a given host, although they can immunize these individuals.
- The first vaccine used by Jenner is of this type: vaccinia virus (cowpox) inoculation of humans confers immunity to smallpox but does not cause smallpox.
- Attenuation can often be achieved by growing a pathogenic bacterium or virus for prolonged periods under abnormal culture conditions.
- This selects mutants that are better suited for growth in the abnormal culture conditions than in the natural host.

- For example, an attenuated strain of *Mycobacterium bovis* called **Bacillus Calmette-Guerin (BCG) Vaccine** was developed by growing *M. bovis* on a medium containing increasing concentrations of bile.
- After 13 years, this strain had adapted to growth in strong bile and had become sufficiently attenuated that it was suitable as a vaccine for tuberculosis.
- Due to variable effectiveness and difficulties in follow-up monitoring, BCG is not used in the United States.
- The Sabin form of the polio vaccine and the measles vaccine both consist of attenuated viral strains.

Examples:

- Vaccinia (smallpox)
- Measles, mumps, rubella (MMR combined vaccine)
- Varicella (chickenpox)
- Influenza (nasal spray)
- Rotavirus
- Zoster (shingles)
- Yellow fever

B. Inactivated vaccines (Killed Antigen)

- Another common means to make a pathogen safe for use in a vaccine is by treatment with heat or chemicals.
- This kills the pathogen, making it incapable of replication, but still allows it to induce an immune response to at least some of the antigens contained within the organism.
- It is critically important to maintain the structure of epitopes on surface antigens during inactivation.
- Heat inactivation is often unsatisfactory because it causes extensive denaturation of proteins; thus, any epitopes that depend on higher orders of protein structure are likely to be altered significantly.
- Chemical inactivation with formaldehyde or various alkylating agents has been successful.
- **The Salk polio vaccine** is produced by formaldehyde inactivation of the poliovirus.

Examples:

- Polio (IPV)
- Hepatitis A

- Rabies

C. Subunit and Conjugate Vaccines (Purified Antigen)

- These subunit vaccines are composed of antigens purified from microbes which are usually administered with an adjuvant.
- Vaccines composed of bacterial polysaccharide antigens are used against pneumococcus and *Haemophilus influenzae*.
- Because polysaccharides are T-independent antigens, they tend to elicit low-affinity antibody responses and are poorly immunogenic in infants (who do not mount strong T cell-independent antibody responses).
- High affinity antibody responses may be generated against polysaccharide antigens even in infants by coupling the polysaccharides to proteins to form conjugate vaccines.
- These vaccines elicit helper T cells to simulate germinal center reactions, which would not occur with simple polysaccharide vaccines.
- Such vaccines work like hapten-carrier conjugates and are a practical application of the principle of T-B cell cooperation.

Examples:

- Hepatitis B
- Influenza (injection)
- Haemophilus influenzae type b (Hib)
- Pertussis (part of DTaP combined immunization)
- Pneumococcal
- Meningococcal
- Human papillomavirus (HPV)

D. Toxoid vaccines (Inactivated Toxins)

- Toxoid vaccines use a toxin (harmful product) made by the germ that causes a disease.
- They create immunity to the parts of the germ that cause a disease instead of the germ itself.
- That means the immune response is targeted to the toxin instead of the whole germ.
- Like some other types of vaccines, you may need booster shots to get ongoing protection against diseases.

Examples:

- Diphtheria, tetanus

II. Hypersensitivity reactions and its types

Introduction

- **Hypersensitivity** is increased reactivity or increased sensitivity by the animal body to an antigen to which it has been previously exposed.
- The term is often used as a synonym for allergy, which describes a state of altered reactivity to an antigen.
- Hypersensitivity has been divided into categories based upon whether it can be passively transferred by antibodies or by specifically immune lymphoid cells.
- The most widely adopted current classification is that of Coombs and Gell that designates immunoglobulin-mediated (immediate) hypersensitivity reactions as types I, II, and III, and lymphoid cell-mediated (delayed-type) hypersensitivity/cell-mediated immunity as a type IV reaction.
- "Hypersensitivity" generally represents the "dark side," signifying the undesirable aspects of an immune reaction, whereas the term "immunity" implies a desirable effect.
- A hypersensitive response (HR) is an anti-pathogen response in plants produced by avr-R system activation that leads to alterations in Ca+ flux, MAPK activation, and NO and ROI formation.
- There is rapid necrosis of plant cells in contact with the pathogen.
- This process prevents spread of the pathogen and releases hydrolytic enzymes that facilitate injury to the pathogen's structural integrity.

Causes of Hypersensitivity

Immune responses that are the cause of hypersensitivity diseases may be specific for antigens from different sources:

- Autoimmunity: reactions against self antigens.
- Reactions against microbes.
- Reactions against non-microbial environmental antigens.

Mechanism of Hypersensitivity

Hypersensitivity diseases are commonly classified according to the type of immune response and the effector mechanism responsible for cell and tissue injury. These mechanisms include some that are predominantly dependent on antibodies and others predominantly dependent on

T cells, although a role for both humoral and cell-mediated immunity is often found in many hypersensitivity diseases.

Immediate (type I) hypersensitivity

It is caused by IgE antibodies specific for environmental antigens and is the most prevalent type of hypersensitivity disease. Immediate hypersensitivity diseases, commonly grouped under allergy or atopy, are often caused by activation of interleukin-4 (IL-4), IL-5, and IL-13 producing Th2 cells and the production of IgE antibodies, which activate mast cells and eosinophils and induce inflammation.

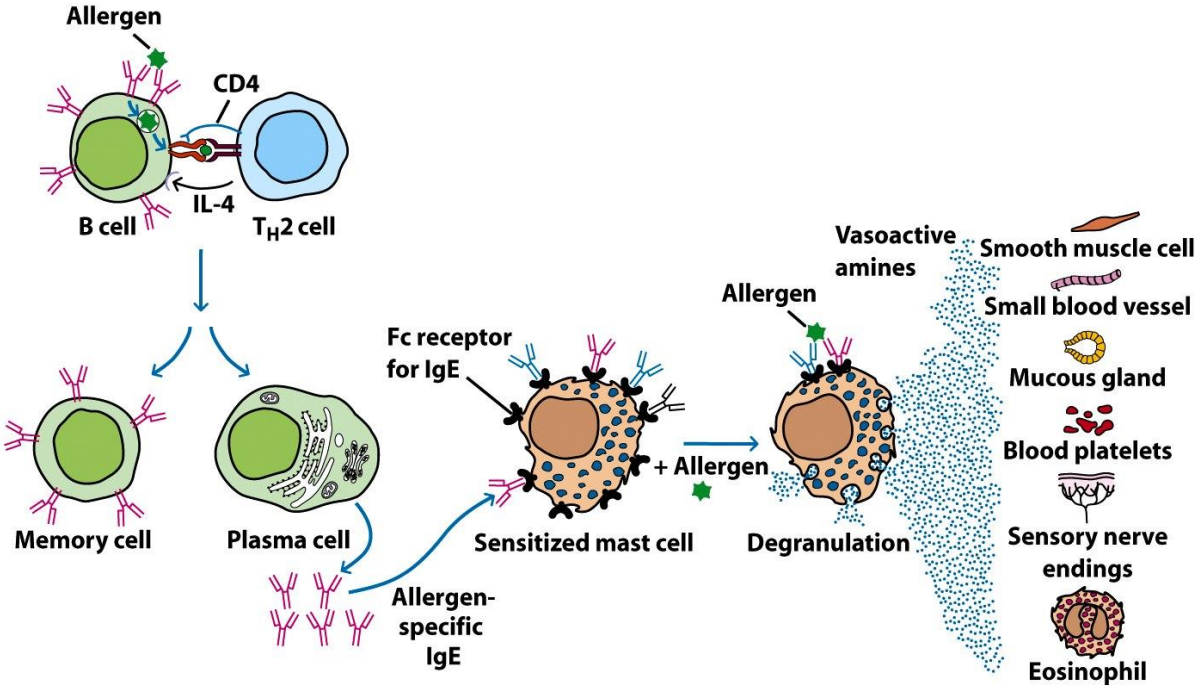


Figure 15-2
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Antibody-mediated (type II) hypersensitivity

IgG and IgM antibodies specific for cell surface or extracellular matrix antigens can cause tissue injury by activating the complement system, by recruiting inflammatory cells, and by interfering with normal cellular functions.

Immune complex-mediated (type III) hypersensitivity

IgM and IgG antibodies specific for soluble antigens in the blood form complexes with the antigens, and the immune complexes may deposit in blood vessel walls in various tissues, causing inflammation, thrombosis, and tissue injury.

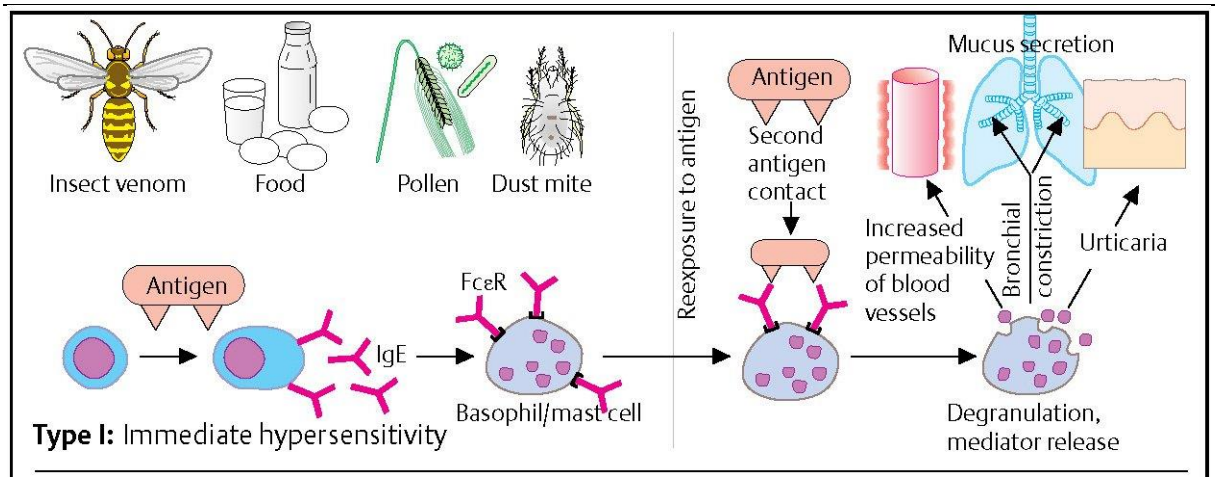
T cell-mediated (type IV) hypersensitivity

In these disorders, tissue injury may be due to T lymphocytes that induce inflammation or directly kill target cells. In most of these diseases, the major mechanism involves the activation of CD4+ helper T cells, which secrete cytokines that promote inflammation and activate leukocytes, mainly neutrophils and macrophages. CTLs contribute to tissue injury in some diseases.

Types of Hypersensitivity Reactions

The Gell's and Coombs' classification of hypersensitivity reactions considers four types of reactions. Type I, II, and III reactions are basically mediated by antibodies with or without participation of the complement system; type IV reactions are cell-mediated. While in many pathological processes mechanisms classified in more than one of these types of hypersensitivity reactions may be operative, the subdivision of hypersensitivity states into four broad types aids considerably in the understanding of their pathogenesis.

Type I: Immediate reaction



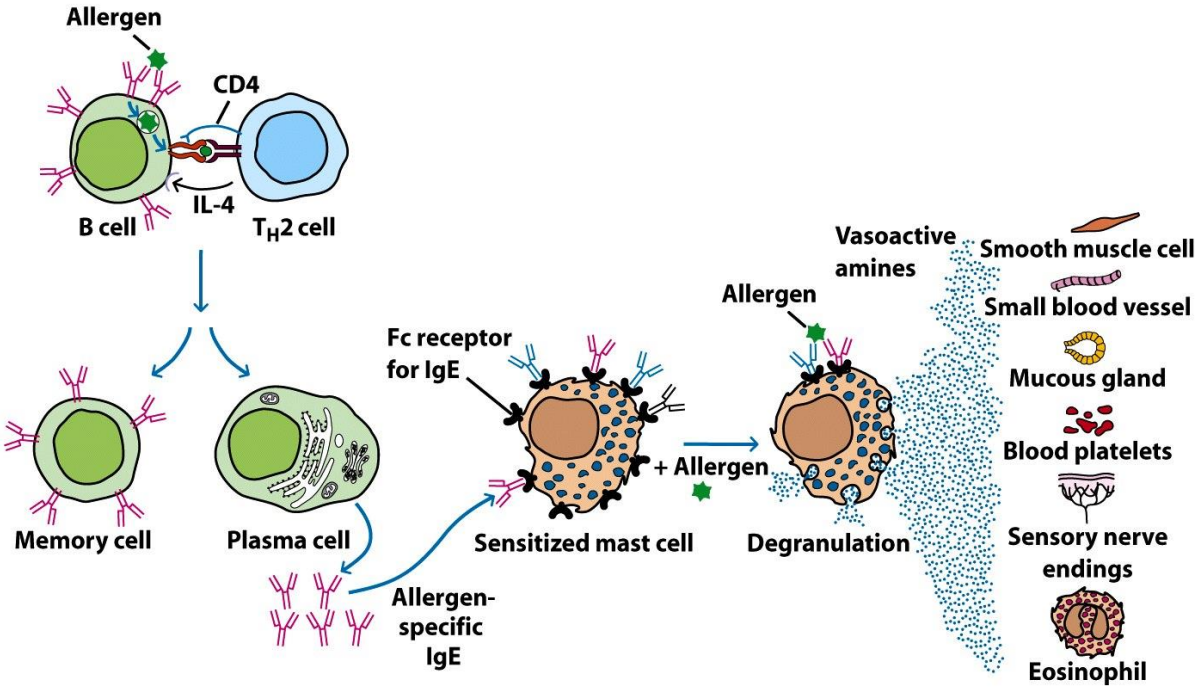


Figure 15-2
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Some antigens (allergens), such as insect venom, foods, pollen, and dust mite, can induce the formation of IgE antibodies in individuals with a corresponding predisposition. The IgE antibodies bind via Fc receptors to mast cells (sensitization). If the individual is re-exposed to the allergen, cross-linkage of the membrane-bound IgE occurs. This results in the immediate release of mediators (e.g., histamine, kininogen), which induce vasodilation, smooth-muscle contraction, mucus secretion, edema, and/or skin blisters. Most allergens are small proteins that can easily diffuse through the skin or mucosa. They are frequently proteases and are active at very low doses. IL-4 favors differentiation of TH2 cells. The exact mechanism that leads B cells to produce IgE is not known.

Type II: Antibody-mediated cytotoxic reaction

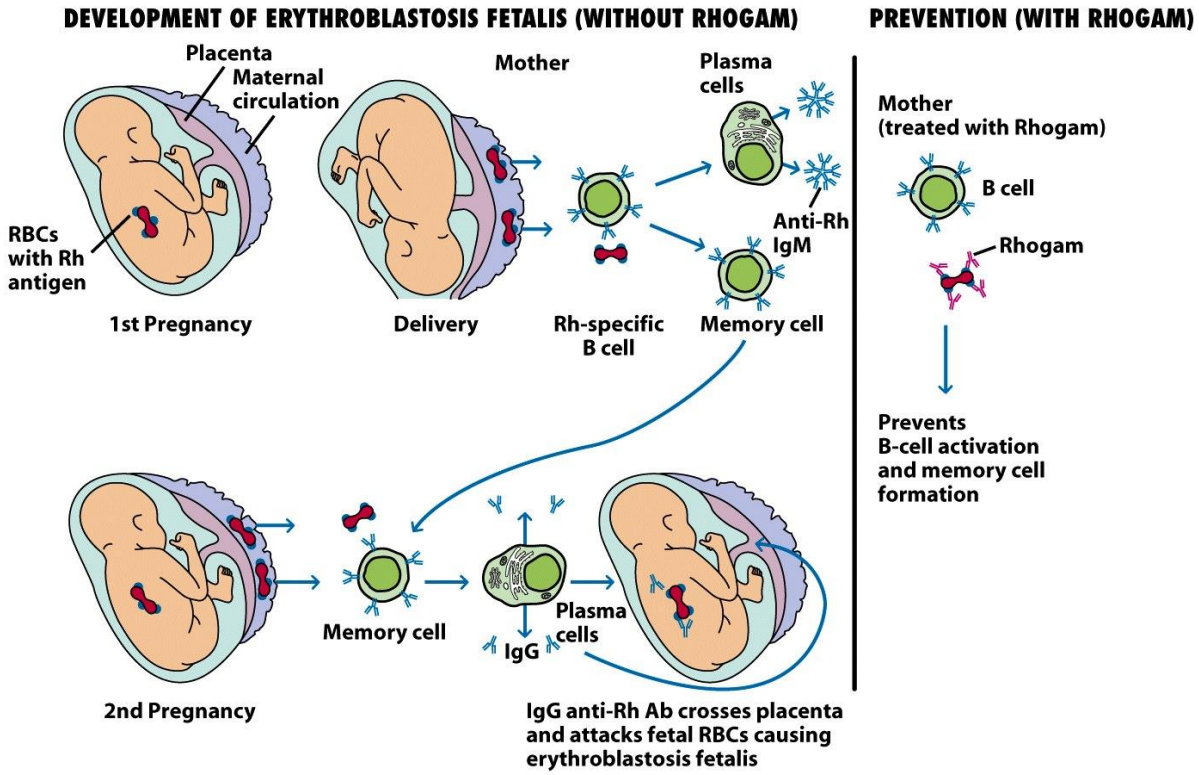
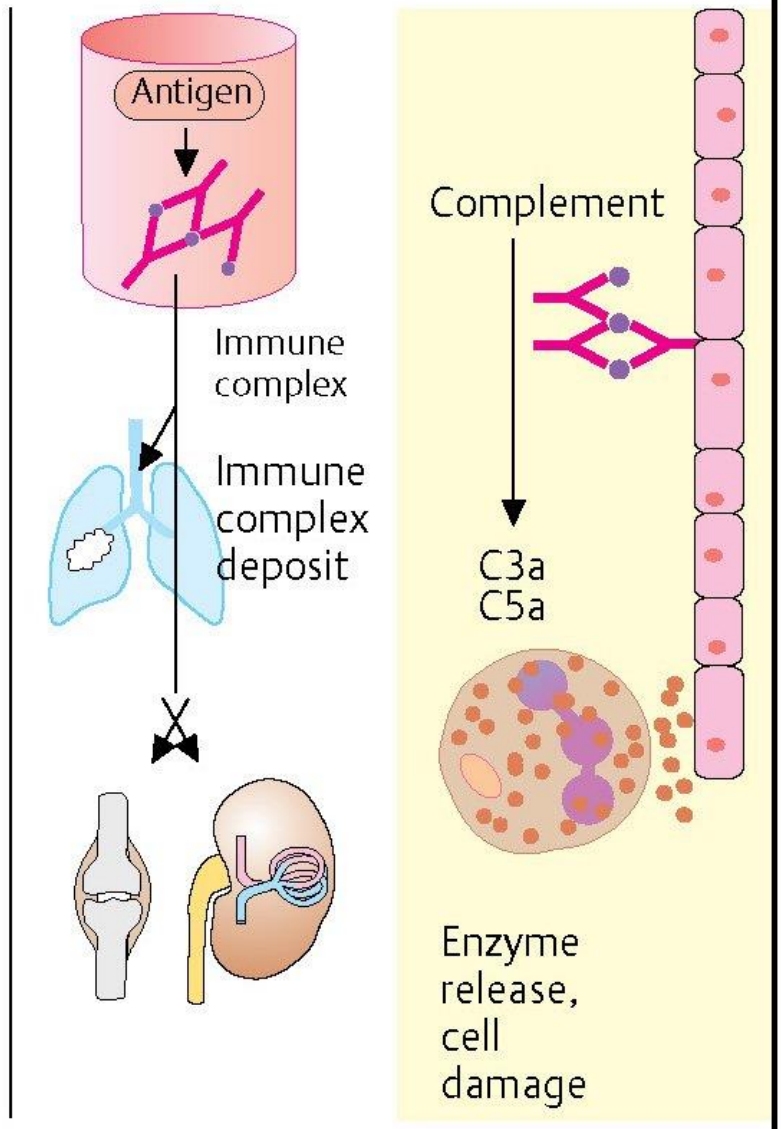


Figure 15-14
Kuby IMMUNOLOGY, Sixth Edition

The immunization of individuals to erythrocyte antigens during pregnancy is a typical example of a type II reaction. Children who inherit the RhD erythrocyte antigen from their father can induce immunization against the RhD+ antigen in their RhD-mother. Sensitization usually occurs at birth when fetal blood cells come into contact with the maternal immune system. In any subsequent pregnancies, maternal anti-RhD antibodies of the IgG type can pass into the placenta and cause severe hemolysis of fetal RhD+ erythrocytes. Other examples: Drugs (e.g., penicillin) can passively bind to erythrocytes. Antibodies directed against penicillin then lead to lysis of the erythrocytes. The formation of antibodies directed against the basement membrane (BM) of the glomerulus can develop during the course of kidney inflammation. Lung damage accompanied by pulmonary hemorrhage and renal inflammation (glomerulonephritis) may occur due to cross-reaction of these antibodies with the basement membrane of the lung (Good-pasture's syndrome).

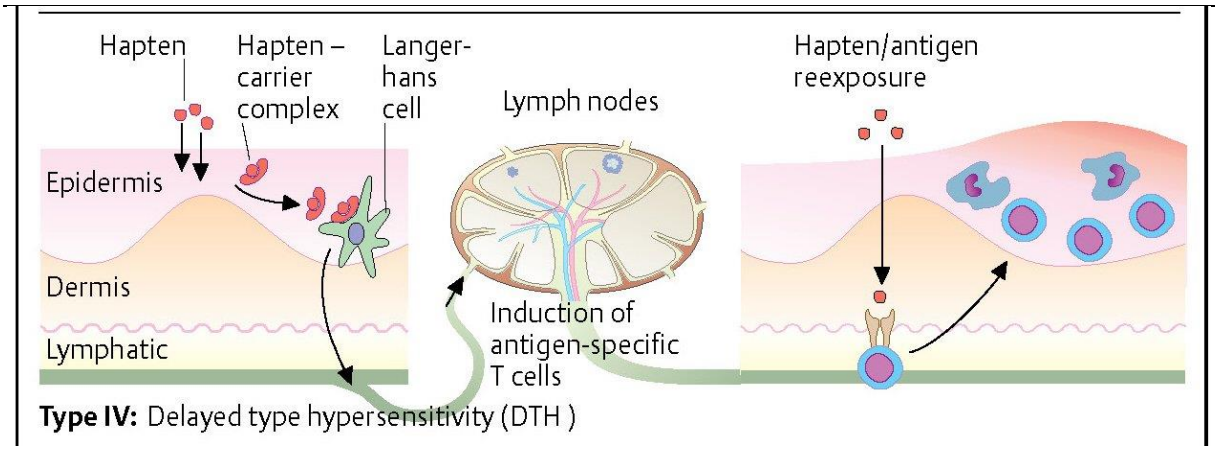
Type III: Immune complex-mediated reaction



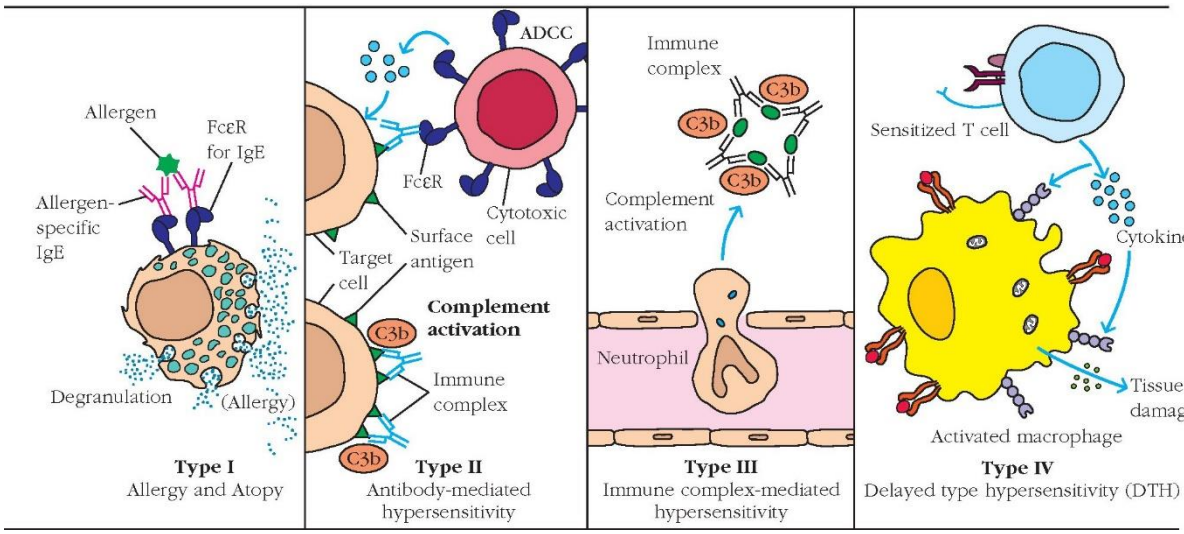
Antibody-antigen complexes (immune complexes) can form during an immune response. Immune complexes can settle in vessel walls, the basement membrane of the lungs and/or kidneys, and in the joints (synovia). They can induce inflammatory processes in these structures by binding complement factors C3a and C5a (anaphylatoxins). A particular type III reaction is the Arthus reaction: when an antigen has penetrated the skin of an individual who has preformed IgG antibodies, the immune complexes can bind to Fc receptors of most cells inducing degranulation inflammatory cells are recruited and complement is activated, leading

to the release of C5a and local inflammation, platelet accumulation, and eventually to blood vessel occlusion with necrosis.

Type IV: Delayed-type hypersensitivity reaction



Haptens are molecules of very small molecular weight (often < 1 kDa). They are too small to function as antigens, but they can penetrate the epidermis and bind to certain proteins in the skin (carrier proteins). Hapten-carrier complexes are bound by antigen-presenting cells of the skin (Langerhans cells), which then migrate to regional lymph nodes. T-cell stimulation then occurs at the lymph node. The so-called sensitization phase lasts ca. 10-14 days. If the individual is reexposed to the hapten, antigen-specific T cells migrate to the skin, where they accumulate and proliferate. They also cause edema formation and local inflammation with the help of cytokines. Compounds containing nickel or chrome and chemicals such as those found in rubber are typical triggers of type IV hypersensitivity reactions.



Here is the comparison table:

Alternative Name

Type I	Type II	Type III	Type IV
Allergic hypersensitivity	Cytotoxic hypersensitivity	Immune complex hypersensitivity	Cell-mediated hypersensitivity/ Delayed type of hypersensitivity

Principle

Type I	Type II	Type III	Type IV
Antibody-mediated degranulation of granulocytes leading to the destruction of cells.	Antibody-mediated destruction of healthy cells.	Antigen-antibody complex-mediated destruction of cells.	T lymphocytes mediated the destruction of cells.

Primary Mediator

Type I	Type II	Type III	Type IV
IgE	IgG/IgM	IgG/IgM	Specific subsets of CD4+ helper T cells or CD8+ cytotoxic T cells.

Other components as mediators

Type I	Type II	Type III	Type IV

Mast cells, Basophils, histamine & other pharmacological agents	Complement, Neutrophils	Complement, phagocytes and K cells	Dendritic cells, macrophages, and cytokines

Reaction time

Type I	**Type II**	**Type III**	**Type IV**
Immediate or within a few hours	5-8 hours	2-8 hours	After 24 hours only, mostly 48-72 hours after contact

Antigen

Type I	**Type II**	**Type III**	**Type IV**
Free in circulation (Soluble)	Fixed on cells	Free in circulation (Soluble)	Soluble or cell-bound

Antigen origin

Type I	**Type II**	**Type III**	**Type IV**
Exogenous	Endogenous or exogenous	Exogenous or endogenous	Exogenous or endogenous

Antibody

Type I	**Type II**	**Type III**	**Type IV**
Fixed on mast cells and basophils	Free in circulation	Free in circulation	Not applicable

Mechanism

Type I	Type II	Type III	Type IV
Allergen-specific IgE antibodies bind to mast cells via their Fc receptor. When the specific allergen binds to the IgE, cross-linking of IgE induces degranulation of mast cells.	IgG or IgM antibody binds to a cellular antigen, leading to complement activation and cell lysis. IgG can also mediate ADCC with cytotoxic T cells, natural killer cells, macrophages, and neutrophils.	Antigen-antibody complexes are deposited in tissues. Complement activation provides inflammatory mediators and recruits neutrophils. Enzymes released from neutrophils damage tissue.	Th2 cells secrete cytokines, which activate macrophages and cytotoxic T cells.

Complement activation

Type I	Type II	Type III	Type IV
No	Yes	Yes	No

Appearance

Type I	Type II	Type III	Type IV
Weal & flare	Lysis & necrosis	Erythema & edema	Erythema & induration

Transfer with serum

Type I	Type II	Type III	Type IV

Passive transfer possible with serum	Passive transfer	Passive transfer	Cannot be transferred with serum; but possible with T cells transfer

Desensitization

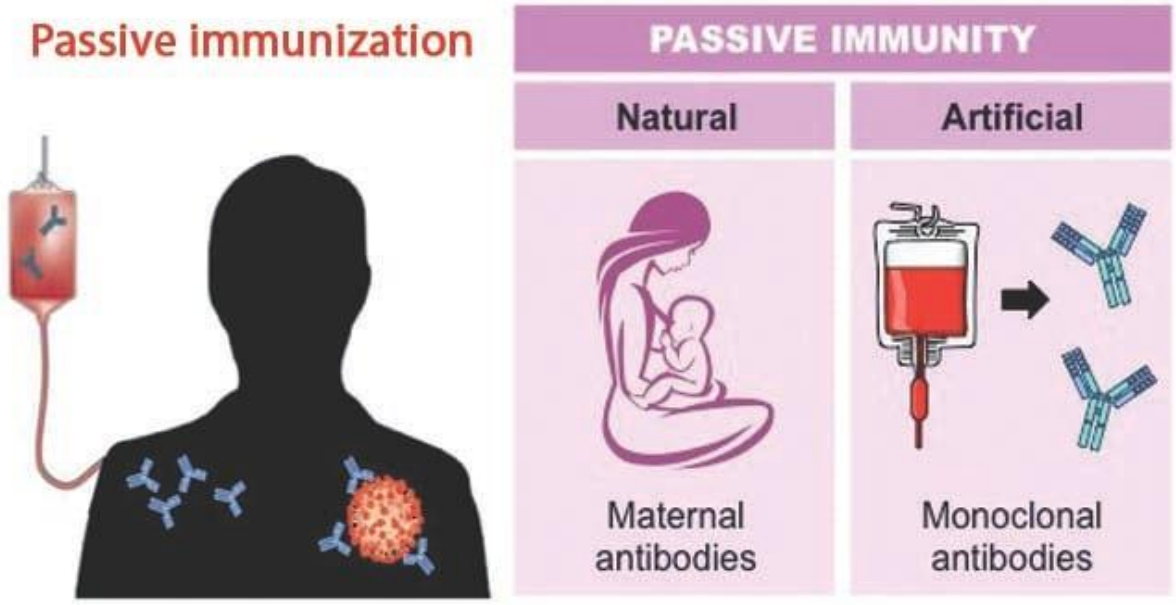
Type I	**Type II**	**Type III**	**Type IV**
Easy but short-lived	Easy but short-lived	Easy but short-lived	Difficult but long-lived.

Examples

Type I	**Type II**	**Type III**	**Type IV**
Asthma, Rhinitis, Atopic eczema, Bee sting reaction	Rhesus incompatibility (Rh hemolytic disease), Transfusion Reactions, Cell Destruction due to autoantigens, Drug-Induced Hemolytic Anemia	Glomerulonephritis, Systemic Lupus Erythematosus, Farmer's lung arthritis, Vasculitis	The tuberculin reaction, Granuloma formation, Allergic contact dermatitis, Type-1 diabetes

III. Passive immunization

- Immunization is the process whereby a person naturally acquires or is induced to acquire immunity or resistance to an infectious disease.
- An individual can acquire such immunity either passively or actively and thus immunization may be active or passive immunization.



Passive immunization

- In **passive immunization,** a person receives antibodies or lymphocytes that have been produced by another individual's immune system while in active immunization the individual's own immune system is stimulated to produce antibodies and lymphocytes.
- **Passive immunization** is hence the administration of preformed antibodies, usually IgG.
- It may arise naturally, such as when a fetus receives antibodies from the mother across the placenta or when a breast-feeding infant ingests antibodies in the mother's milk.
- However, passive immunization also can be conferred artificially by means of preformed antibodies administered through intravenous or intramuscular routes.
- These antibodies may be derived from individuals who have high titres to particular microbes and are used to provide rapid protection.
- Antibodies given to immune deficient patients are usually IgG-derived from pooled normal plasma or purified blood products of immune people.
- Antibodies preformed in animals has also been used against some diseases, the most common being that of the horses. However, the danger of immune complex formation and conditions like serum sickness with repeated administration must be checked for.
- Passive immunization is done either as prophylaxis or as a post exposure measure.
- Passive immunization is also used to provide protection in immune compromised individuals who are unable to make the appropriate antibody response.

- It may also be handy under some conditions in which a person is incapable of making any antibody at all, i.e., severe combined immunodeficiency.
- Pre-formed antibodies have to be given on a continuous basis, ideally every three weeks, since they are continuously catabolized and only effective for a short period.
- Infections in which passive immunization is important include diptheria, tetanus, rabies etc., in events of accidental exposure to certain pathogens such as hepatitis B or at other instances such as snake bites.

Advantages of Passive immunization

- Passive immunization with preformed antibodies leads to prompt availability of large amounts of antibody. It is thus quick acting, producing an immune response within hours or days, faster than a vaccine.
- It helps to prevent or slow down the course of disease.
- It is beneficial to high-risk individuals, such as people with immune system deficiencies.

Drawbacks of Passive immunization

- The protection offered by passive immunization is short-lived, usually lasting only a few weeks or months since it do not lead to the formation of long-lasting memory immune cells.
- In passive immunity it is possible to initiate hypersensitivity reactions if the antibody is from another species.
- Antibody treatment cannot be used for routine cases of diseases.
- Antibodies can be difficult and costly to produce.
- Many antibody treatments must be given via intravenous injection, which is a more time-consuming and potentially complicated procedure than the injection of a vaccine.

Active Immunization

- Immunization is the process whereby a person naturally acquires or is induced to acquire immunity or resistance to an infectious disease.
- An individual can acquire such immunity either passively or actively and thus immunization may be active or passive immunization.
- In active immunization, the immune system is stimulated to produce antibodies against a particular infectious agent and thus the immune system of the individual to which immunity is to be conferred is actively involved in the process.

- It may arise naturally, such as when an individual is exposed to an antigen or pathogen.

For example, an individual who recovers from a first case of the measles is immune to further infection by the measles-causing virus, because the virus stimulates the immune system to produce antibodies that specifically recognize and neutralize the pathogen the next time it is encountered.

- However, active immunization also can be conferred artificially by means of vaccines. Vaccines consist of a nontoxic antigen preparation that infers protective immunity by inducing a memory response to an infectious microorganism.
- This results in immunity which may either be antibody mediated immunity and/or cellular immunity.
- Vaccines consist of microbial products with or without adjuvant which do not cause infection under normal conditions but rather provide a long term immunological protection against the offending microbe.
- Depending upon the type of disease, a vaccine may contain live attenuated or killed microorganisms or parts or products from them capable of stimulating a specific immune response comprised of protective antibodies and T cell immunity.
- The purpose of vaccination is to ensure that a large enough number of antibodies and lymphocytes capable of reacting against a specific pathogen or toxin are available before exposure to it occurs.
- Artificial active immunization can be induced via two different routes :

Systemic immunization which involves injecting the vaccine subcutaneously or intramuscularly into the deltoid muscle. Examples include systemic vaccines for measles, mumps and rubella, and against *Pneumococcus*, *Meningococcus* and *Haemophilus* infections.

Mucosal immunization which involves on the mucosal route as the site of choice for immunization either orally or through the nasal associated immune tissue (NALT) such as the oral immunization against Polio. Although not commonly in practice, recent vaccination approaches have focused on this route.

- Active immunization is mostly performed as a prophylaxis measure.
- Some infections in which active immunization is performed include Hepatitis A infection, Influenza, Measles, Mumps, Rubella, Yellow fever etc.

Advantages

- The protection offered by active immunization is long-lived since it leads to the formation of long-lasting memory immune cells.

- Active immunization may be reactivated quickly by a recurrence of the infection or by revaccination.
- It is less costly in preparation and to administer than passive immunization techniques.

Drawbacks

- The protective response takes time to establish ranging from few days to weeks which makes it inefficient as a post exposure remedy.
- Since active immunization is dependent on the individuals' immune responses, it may not be suitable for protection of immuno-compromised or immuno-deficient individuals.

Question Bank

UNIT – 1 (2 MARK)

1. What is immunology?
2. Define vaccine.
3. What is variolation?
4. What is immune response?
5. Differentiate between innate and adaptive immunity.
6. Bone marrow is a primary lymphoid organ – justify.
7. Herd immunity.
8. Phagocytes.
9. Cytokines.
10. Robert Koch.
11. Natural killer cells.
12. Contribution of Alexander Fleming.
13. Phagocytosis.
14. Active immunity.
15. Lymphoid organ.
16. Erythropoiesis.
17. Autoimmune disease.
18. Blood transfusion.
19. Bone marrow.
20. Immunity.
21. Complement.

UNIT – 1 (5 MARK)

1. What is haematopoiesis? Explain it.
2. Briefly explain the structure and function of spleen.
3. Describe the cells of immune system.
4. Describe about innate immune mechanism.

5. Enumerate the structure and function of any two secondary lymphoid organs.
6. Discuss briefly the scope of immunology.
7. Write a brief account on history of immunology.
8. Write a note in secondary lymphoid organs.
9. Describe the mechanism of phagocytosis.
10. Write short note on significant contribution of immunologist.
11. Give a brief account on cell mediated immunity.
12. With neat diagram explain the structure and function of thymus.
13. Give an account of T helper cells and T suppressor cells.
14. Mention briefly about immune response of cell.
15. Explain the basic factor involved in innate immunity.

UNIT – 1 (10 MARK)

1. Describe the mechanism of innate immunity.
2. Describe the history and scope of immunotechnology.
3. Compare and contract T and B lymphocytes.
4. Give a detailed account on innate and adaptive immunity.
5. Write a detailed account on humoral and cell mediated immunity.
6. Give a detailed account on lymphoid organs.
7. Write an essay on cell mediated immunity.
8. Write an essay on the mechanism of immunity against pathogen.
9. Outline the functions of cells in the immune response

UNIT – 2 (2 MARK)

1. What is hapten?
2. What is paratope?
3. Define epitope.
4. What is antigenicity?
5. What are hapten give example?
6. Draw the structure of IgM.

7. Valace of IgM.
8. Adjuvants.
9. Differentiate immunogen and antigen.
10. What is antigenicity and immunogenicity.
11. Valance of an antigen.
12. Define immunogen.
13. Immunogenicity.
14. Antigen.
15. IgG.

UNIT – 2 (5 MARK)

1. Discuss about the properties of immunogen.
2. Describe the difference between immunogenicity and antigenicity.
3. What is antigen? Explain the characteristics of antigen.
4. Write the difference between antigen and hapten.
5. What is an epitope? Describe its function.
6. List the salient features of an antigen.
7. Write short note on hapten.
8. List out the properties of immunogen.
9. Write the function of different types of antibody.
10. Write a note on different type of immunoglobulin and their functions.
11. List down some of the essential factors for an antigen to express it antigenicity.
12. Explain in detail about antigenicity.
13. Describe the role of immunogenicity in drug development
14. Define the following:
    - Haptens
    - Epitopes and
    - Adjuvants
15. Differentiate immunogenicity and antigenicity.
16. Explain salient features of haptens.

17. Explain general properties of antigen.
18. What is adjuvant explain it.

UNIT – 2 (10 MARK)

1. Discuss about the general structure and function of immunoglobulin.
2. Explain the structure and function of IgG.
3. Describe the structure and function of different type of antibody.
4. Explain the ultra-structure of antibody with neat diagram.
5. With neat diagram explain the structure and the biological properties of IgM.
6. Briefly describe the structure and function of antibodies.
7. Explain detail about structure and function of antigen.
8. Describe the structure and function of IgA.

UNIT – 3 (2 MARK)

1. What is RIA?
2. What is agglutination?
3. Define affinity.
4. Distinguish between avidity and affinity.
5. Discuss any two application of ELISA.
6. Application of western blotting.
7. Scatchard equation.
8. Expand ELISA.
9. Hemagglutination.
10. Avidity.
11. Sandwich ELISA.
12. Zone of equivalence.
13. Electrophoresis.
14. Enzyme conjugate.
15. NBT.
16. Immunodiffusion.

UNIT – 3 (5 MARK)

1. Write the principle and application of double immunodiffusion.
2. Write about the principle and application of immuno electrophoresis.
3. Describe the principle and application of ELISA.
4. Explain briefly the principle and application of RIA.
5. Describe the different type of agglutination reaction.
6. Write a short note on radio immuno assay.
7. Write a short note on different type of ELISAs.
8. Give a brief account on immunodiffusion.
9. Describe the salient feature of antigen antibody interaction.
10. Describe the principle and application of immunodiffusion.
11. Write the factors that influence the strength of antigen antibody interactions.
12. Write note on western blotting.
13. Differentiate about avidity and affinity.
14. Principle and application of radial immunodiffusion.

UNIT – 3 (10 MARK)

1. Discuss the principle and application of agglutination reaction.
2. Explain the principle and application of radial and double immunodiffusion.
3. Explain in detail about principle, methodology and application of ELISA.
4. Explain in detail about antigen antibody reaction.
5. Explain the principle and application of radio immuno assay and western blotting.
6. Describe the principle and application of blotting technique.

UNIT – 4 (2 MARK)

1. What is MHC?
2. What is organ transplantation?
3. Phagocytosis.
4. Write any two function of complement system.

5. Difference between class I and class II MHC.
6. Advantage of polymorphism in MHC.
7. Membrane Attack Complex.
8. Differentiate HLA and H2 complex.
9. Opsonins.
10. Antigen Presenting Cells or APC.
11. Graft rejection.
12. What are immune associated antigens.
13. HLA.
14. Macrophage.
15. B-cells.
16. Cytosolic.
17. Functions of MHC class IV molecules.

UNIT – 4 (5 MARK)

1. Discuss about cytosolic pathway.
2. Describe the classical pathway.
3. Write the role of MHC molecule I and II in antigen presentation.
4. Briefly describe the structure of HLA.
5. Why MHC class II deficiency patient have the following abnormalities
6. (i) Lack of CD4 TH cells (ii) low level Ig in the blood
7. Describe the role of complement in humoral immune response. How do inborn complement defect affect antibody production and function.
8. Write the biological significance of complement.
9. Describe the function of MHC molecule.
10. Describe the endocytic pathway of antigen presentation and processing.
11. Illustrate the alternative pathway of complement.
12. Discuss about antigen-antibody interactions.
13. What is complement and write its functions.
14. Explain class I and II MHC molecule.
15. Briefly describe the endocytic pathway.

UNIT –4 (10 MARK)

1. What is HLA? explain the structure and function of HLA.
2. What is complement? Briefly explain the classical pathways.
3. Discuss the cytosolic pathway of antigen presentation by MHC molecule.
4. Enumerate the role of MHC in the immune system.
5. Explain the alternative pathway of complement activation.
6. Write the biological significance of complements. Describe the sequence of complement activation.
7. Explain about the mechanism of presentation of antigen through class I and II MHC molecule.
8. Discuss about the classical and alternative pathway of complement.
9. Explain cytosolic and endocytic pathway.
10. What is MHC? Explain the structure of MHC.

UNIT – 5 (2 MARK)

1. What is passive immunity?
2. Define Arthus reaction.
3. What is vaccine?
4. Define allergy.
5. What is mast cell?
6. Mention any two pharmacologic mediators of hypersensitivity reaction.
7. Expand – BCG.
8. Delayed type hypersensitivity.
9. DNA vaccine.
10. Allergens.
11. Vaccine.
12. Define the term allergens in suitable example.
13. Mantoux reaction.
14. Memory T cells.
15. Heat shock proteins.

16. DBT vaccine.
17. Asthma.
18. Active immunity.

UNIT – 5 (5 MARK)

1. Describe the passive immunization.
2. Describe the different type of allergen.
3. Briefly explain the active immunization method.
4. Discuss about the different type of allergen.
5. Mention the production and application of any two types of vaccines.
6. Discuss the steps involved in the development of delayed type hypersensitivity.
7. Write short note on active immunization.
8. Comment on modern vaccine.
9. Short note on delayed type hypersensitivity.
10. Outline the different type of hypersensitivity reaction.
11. What is hypersensitivity give an account on immediate hyper sensitivity.
12. Give an account on 'erythroblastosis fetalis.'
13. Write the essay on the anaphylaxis.
14. Comment on edible vaccine.
15. What are the types of vaccines that are available in market for humans?
16. Outline the types of active immunity.
17. Briefly describe type two hypersensitivity Reaction.

UNIT – 5 (10 MARK)

1. Briefly explain the type II hypersensitivity reaction.
2. Describe in detailed about type I hypersensitivity reaction.
3. Write in detail on active and passive immunization.
4. What is vaccine? Write an essay to explain about the various type of vaccine.
5. Give an account on vaccines and its preparation and application.
6. Explain in detail about hypersensitivity reaction and its types.
7. Write an essay about passive immunization.