

SHRIMATI INDIRA GANDHI COLLEGE
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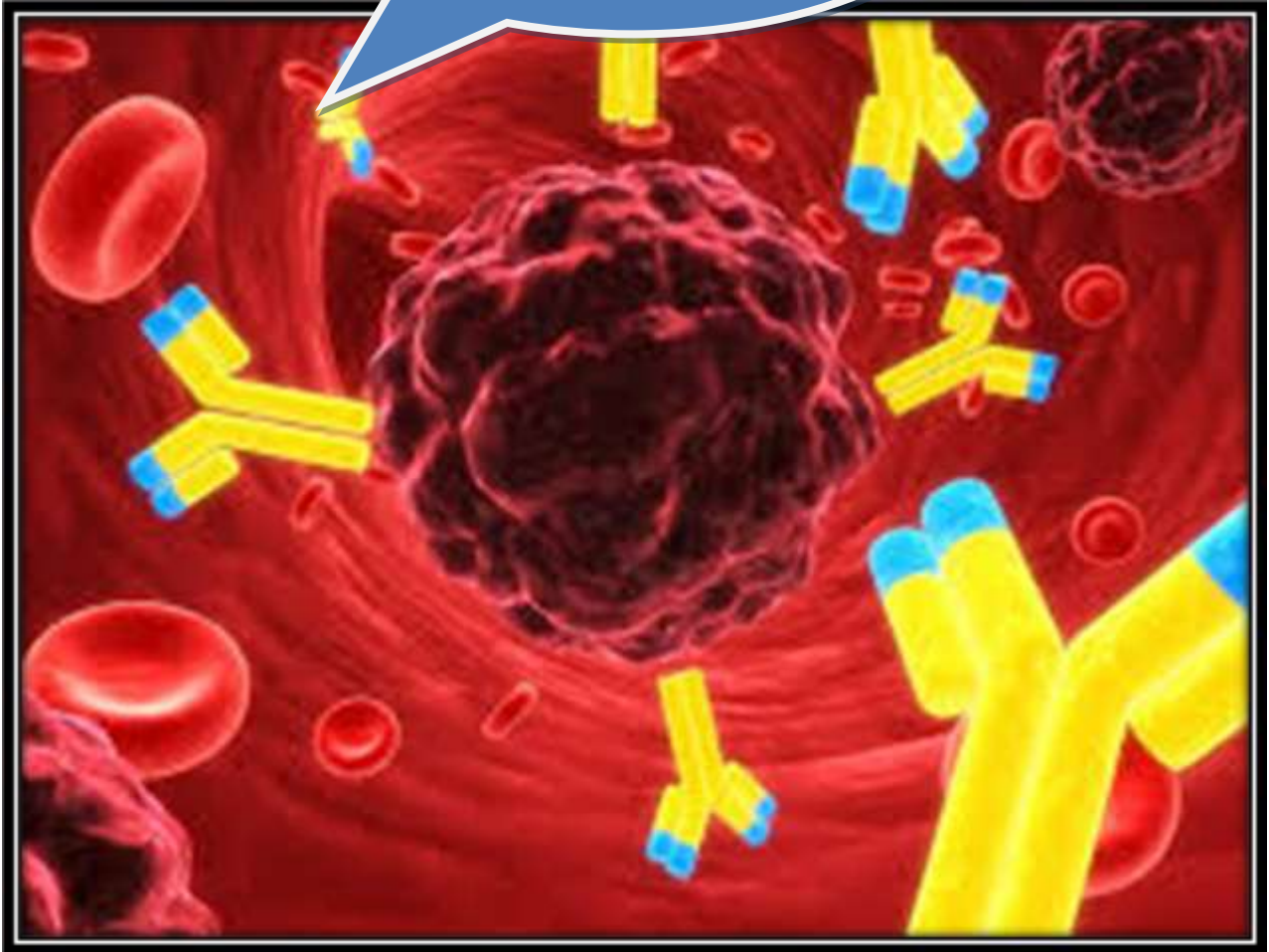
TUTORIAL MATERIAL
IMMUNOLOGY

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DEPARTMENT OF BIOCHEMISTRY
SHRIMATI INDIRA GANDHI COLLEGE

**IMMUNOLOGY
TUTORIAL
MATERIAL FOR
SLOW LEARNER**



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UNIT I -Immune System**Short Answer:**

1. What is immunology?

It deals with the study of processes by which the body defends itself from the invasion and attack of foreign organisms.

2. What is mean by Attenuation?

The process of weakening or reducing the virulence of pathogenic organisms without losing the capacity to induce immunity.

3. Define immunity?

Immunity is defined as the resistance to infection. This is carried out by the process of recognition and disposal of non-self or foreign material that enters the body.

4. What is mean by adoptive immunity?

This is a type of passive immunity produced by injecting immunologically competent lymphocytes and not by injecting antibodies. This method is adopted in the treatment of tuberculosis and leprosy.

Answers the Question elaborately:**1. Write a note on immune System**

- ✓ The immune system is the collection of cells, tissues and molecules that functions to defend us against infectious microbes. The coordinated reaction of the immune system against infections (and other foreign substances) is known as the immune response.
- ✓ Immunity is the state of protection against foreign organisms or substances (antigens).
- ✓ The immune system can be classified into two types such as the innate immune system and the adaptive immune system.
- ✓ An **immunogen** is a substance that induces a specific immune response.
- ✓ The immune system produces both humoral and cell-mediated responses.
- ✓ An **antigen** (Ag) is substance that reacts with the products of a specific immune response; i.e., antibodies or specific T cells.
- ✓ The **antigenic determinant** (or **epitope**) is that particular part of an antigen that combines the components of the specific immune response. This is important because it implies that (often ?) the truly antigenic part of an organism, might only be a very small part of the whole.

- A **hapten** is a substance, almost always very small in molecular terms, that by itself is non-immunogenic, but that can react with the products of a specific immune response. If a hapten is administered by itself, it cannot induce an immune response (thus, haptens are not immunogens). If the hapten combines with a carrier, then the combination is immunogenic. Also, free haptens can react with products of the immune response after such products have been formed (thus, haptens possess antigenicity). An **antibody** (Ab) is a specific protein (immunoglobulin) which is produced in response to an immunogen and which reacts with an antigen.
- Abnormalities of the immune system that result in defective immune responses make individuals susceptible to infections by viruses, bacteria, fungi and parasites.

2. Write a note on Immunological Cell

Lymphoid cells

Lymphocytes

Lymphocytes are the central cells of the immune system, responsible for adaptive immunity and the immunologic attributes of diversity, specificity, memory, and self/non self recognition. Lymphocytes constitute 20%–40% of the body's white blood cells and 99% of the cells in the lymph. These lymphocytes continually circulate in the blood and lymph and are capable of migrating into the tissue spaces and lymphoid organs, thereby integrating the immune system to a high degree.

Lymphocytes are two types

- **T lymphocytes (T cells)**
 - Mature in the thymus
 - Directly attack and destroy foreign cells
- **B lymphocytes (B cells)**
 - Mature in the bone marrow
 - Produce plasma cells that produce antibodies

Macrophages, Dendritic cells and Reticular cells is the antigen presenting cells, it initiate the immune response

Lymphocytes are one of the five kinds of **white blood cells** or **leukocytes** circulating in the blood.

Although mature lymphocytes all look pretty much alike, they are extraordinarily diverse in their functions. The most abundant lymphocytes are:

- **B lymphocytes** (often simply called **B cells**) and
- **T lymphocytes** (likewise called **T cells**).

B cells are produced in the **bone marrow**.

- The precursors of T cells are also produced in the bone marrow but leave the bone marrow and mature in the **thymus** (which accounts for their designation).
- Each B cell and T cell is **specific** for a particular **antigen**. What this means is that each is able to **bind to** a particular molecular structure.

The specificity of binding resides in a **receptor** for antigen:

- the B cell receptor (**BCR**) for antigen and
- the T cell receptor (**TCR**) respectively.
- BCRs bind intact antigens (like diphtheria toxoid, the protein introduced into your body in the **DTP vaccine**). These may be
 - soluble molecules present in the extracellular fluid;
 - intact molecules that the B cell plucks from the surface of **antigen-presenting cells** like **macrophages** and **dendritic cells**.
- The bound antigen molecules are engulfed into the B cell by **receptor-mediated endocytosis**.
- The antigen is digested into fragments
- which are then displayed at the cell surface nestled inside a **class II histocompatibility molecule**.
- **Helper T cells** specific for this structure (i.e., with complementary TCRs) bind the B cell and
- secrete **lymphokines** that:

- stimulate the B cell to enter the cell cycle and develop, by repeated mitosis, into a **clone** of cells with identical BCRs;
- switch from synthesizing their BCRs as integral membrane proteins to a soluble version;

T Cells

- The surface of each T cell also displays thousands of identical **T cell receptors (TCRs)**.
- There are two types of T cells that differ in their TCR:
 - **alpha/beta** ($\alpha\beta$) T cells. Their TCR is a [heterodimer](#) of an alpha chain with a beta chain. Each chain has a variable (V) region and a constant (C) region. The V regions each contain 3 **hypervariable regions** that make up the antigen-binding site.
 - **gamma/delta** ($\gamma\delta$) T cells. Their TCR is also a heterodimer of a gamma chain paired with a delta chain.

The TCR (of alpha/beta T cells) binds a bimolecular complex displayed at the surface of **some other cell** called an [antigen-presenting cell](#) (APC). This complex consists of:

- a fragment of an antigen lying within the groove of a
- [histocompatibility molecule](#)

The complex has been compared to a "hot dog in a bun".

Most of the T cells in the body belong to one of two subsets. These are distinguished by the presence on their surface of **one or the other** of two [glycoproteins](#) designated:

- **CD4**
- **CD8**

Which of these molecules is present determines what types of cells the T cell can bind to.

- **CD8⁺** T cells bind epitopes that are part of [class I histocompatibility molecules](#). Almost all the cells of the body express class I molecules.
- **CD4⁺** T cells bind epitopes that are part of [class II histocompatibility molecules](#). Only specialized antigen-presenting cells express class II molecules. These include:

- [dendritic cells](#)
- phagocytic cells like [macrophages](#) and **B cells**

3. Describe in detail about Lymphoid organs?

The organs concerned with immune reaction are called lymphoid organ. They contain lymphoid cells (lymphocytes). The lymphoid organs and lymphoid cells constitute the lymphoid system.

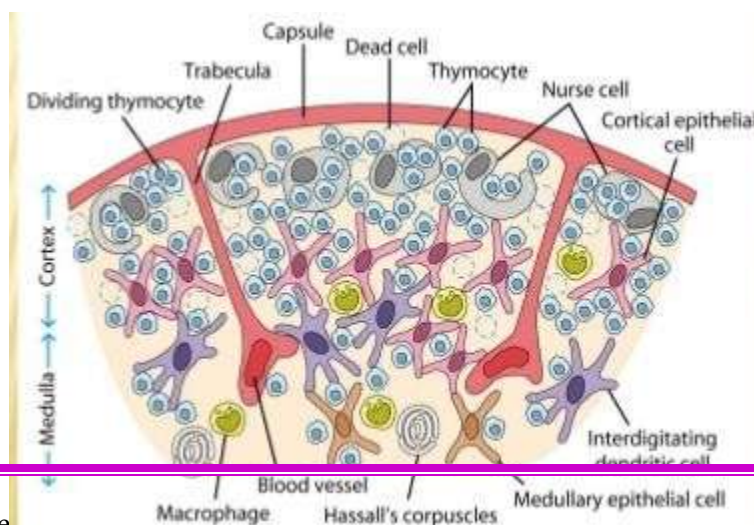
The primary or central lymphoid organs generate lymphocytes from immature progenitor cells. The thymus and the bone marrow constitute the primary lymphoid organs involved in the production and early clonal selection of lymphocyte tissues. Bone marrow is responsible for both the creation of T cells and the production and maturation of B cells.

Secondary or peripheral lymphoid organs, which include lymph nodes and the spleen, maintain mature naive lymphocytes and initiate an adaptive immune response. The peripheral lymphoid organs are the sites of lymphocyte activation by antigens. Activation leads to clonal expansion and affinity maturation. Mature lymphocytes recirculate between the blood and the peripheral lymphoid organs until they encounter their specific antigen.

The primary or central lymphoid organs

Thymus

- It is the site of T cell maturation
- Most active in younger children; atrophies with age
- Does not contain reticular fibers
- Lack B cells, therefore no germinal centers are present in the thymus



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

Both the cortex and medulla of the thymus are crisscrossed by a three-dimensional stromal-cell network composed of epithelial cells, dendritic cells, and macrophages. The medulla consists of MHC class II antigen. In addition there are some peculiar structure in the medulla called **Hassall's corpucles**. The function of the thymus is to generate and select a repertoire of T cells that will protect the body from infection.

Function

- The function of the thymus is to generate and select a repertoire of T cells that will protect the body from infection.
- It bring about cell mediated immunity.
- It bring about graft rejection.

Bone marrow

Bone marrow is the spongy tissue inside your **bones**. It's home to blood vessels and stem cells that help produce red and white blood cells and Platelets. **Bone marrow** is responsible for both the creation of T cells and the production and maturation of B cells.

There are two types of bone marrow:

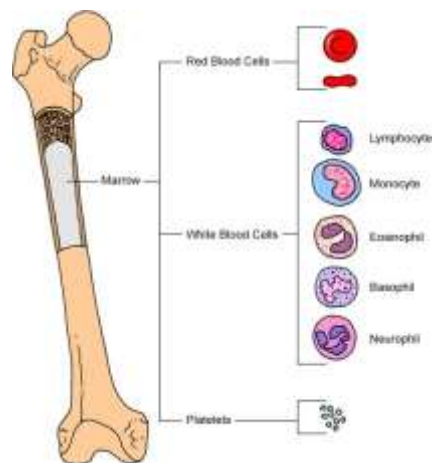
1. Vascular and adipose region
2. Haemopoitic region

The **Vascular region** is the circulatory system that supplies nutrient and removes waste from the actively growing blood cells .The other half of the bone marrow which is actively involved in the haemoposis is known as the red marrow.

Red marrow (consisting mainly of with proliferating and differentiating blood cells in connective tissue matrices bordered by venous sinuses, [Red blood cells](#), [platelets](#) and most [white blood cells](#) (Plasma cells and macrophages) arise in red marrow) .In adult animals much of the red marrow is replaced by fatty tissue and become **Yellow marrow**.

Main function of bone Marrow.

1. It is the site of origin of all T and B cells, Phagocytes, platelets, erythrocytes and other leukocytes in adults. All the cells of the blood, including lymphocytes, are produced from hemopoietic stem cells (HSC) to give rise all elements of the blood.
2. In addition to hematopoiesis. Bone marrow is the site of removal of aged and defective erythrocyte.
3. Site of differentiation and maturation of B-lymphocytes. In bone marrow, the B-cells develop their receptors during different stages in bone marrow



Secondary or peripheral lymphoid organs

Lymph nodes

- Only the lymph nodes filter lymph
- Cluster along the lymphatic vessels of the body
- Lymph is filtered through the lymph nodes before it is returned to the bloodstream
- Lymph nodes are embedded in connective tissue

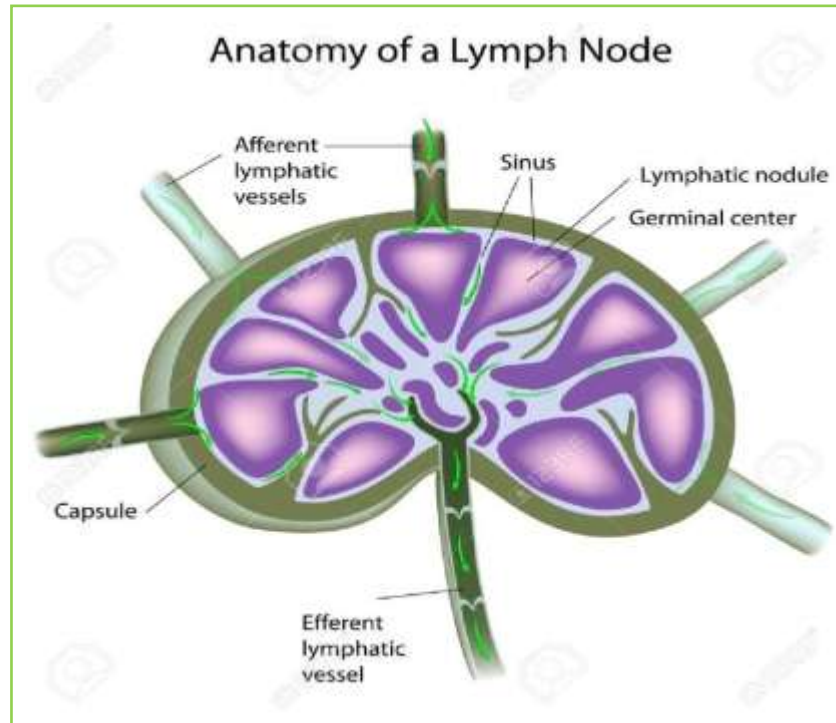
- Large clusters of lymph nodes appear near the body surface in the inguinal, axillary, and cervical regions.
- Functions of lymph nodes
 - Filters lymph
 - Assist in activating the immune system

Anatomy of a lymph node

Lymph nodes are the sites where immune responses are mounted to antigens in lymph. They are encapsulated beanshaped structures containing a reticular network packed with lymphocytes, macrophages, and dendritic cells.

Lymph nodes is coveredby a capsule.The capsule penetrate into the lymph node to form septa called trabeculae(Strands of connective tissue which divide the node into compartments). Morphologically, a lymph node can be divided into three roughly concentric regions: the cortex, the paracortex, and the medulla, each of which supports a distinct microenvironment. The outermost layer, the cortex, contains lymphocytes (mostly B cells), macro-phages, and follicular dendritic cells arranged in primary follicles. After antigenic challenge, the primary follicles enlarge into secondary follicles, each containing a germinal center.

Beneath the cortex is the paracortex, which is populated largely by T lymphocytes and also contains interdigitating dendritic cells thought to have migrated from tissues to the node. These interdigitating dendritic cells express high levels of class II MHC molecules,which are necessary for presenting antigen to TH cells.



The innermost layer of a lymph node, the medulla, is more sparsely populated with lymphoid-lineage cells; of those present, many are plasma cells actively secreting antibody molecules.

- Circulation in the lymph nodes
- Afferent lymphatic vessels – lymph enters here
- Once inside the nodes, the lymph moves through a series of sinuses and then exits at the hilus
- Efferent lymphatic vessels – lymph exits here

As antigen is carried into a regional node by the lymph, it is trapped, processed, and presented together with class II MHC molecules by interdigitating dendritic cells in the paracortex, resulting in the activation of TH cells. The initial activation of B cells is also thought to take place within the T-cell-rich paracortex. Once activated, TH and B cells form small foci consisting largely of proliferating B cells at the edges of the paracortex.

Some B cells within the foci differentiate into plasma cells secreting IgM and IgG. These foci reach maximum size within 4–6 days of antigen challenge. Within 4–7 days of antigen challenge, a few B cells and TH cells migrate to the primary follicles of the cortex. It is not known what causes this migration. Within a primary follicle, cellular interactions between follicular dendritic cells, B cells, and TH cells take place, leading to development of a secondary follicle with a central germinal center. Some of the plasma cells generated in the germinal center move to the medullary areas of the lymph node, and many migrate to bone marrow.

Afferent lymphatic vessels pierce the capsule of a lymph node at numerous sites and empty lymph into the subcapsular sinus. Lymph coming from the tissues percolates slowly inward through the cortex, paracortex, and medulla, allowing phagocytic cells and dendritic cells to trap any bacteria or particulate material (e.g., antigen-antibody complexes) carried by the lymph.

After infection or the introduction of other antigens into the body, the lymph leaving a node through its single efferent lymphatic vessel is enriched with antibodies newly secreted by medullary plasma cells and also has a fiftyfold higher concentration of lymphocytes than the afferent lymph.

Spleen

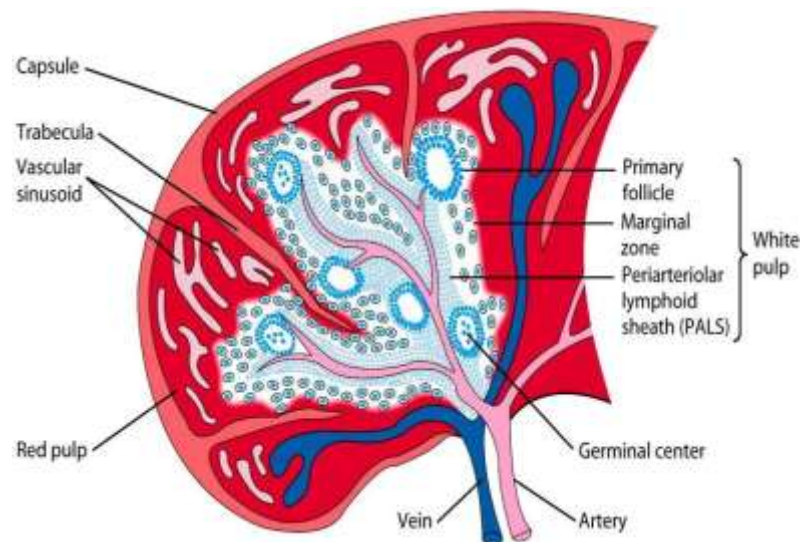
The spleen plays a major role in mounting immune responses to antigens in the blood stream. It is a large, ovoid secondary lymphoid organ situated high in the left abdominal cavity. While lymph nodes are specialized for trapping antigen from local tissues, the spleen specializes in filtering blood and trapping blood-borne antigens; thus, it can respond to systemic infections.

Functions of the spleen

- Cleanses the blood by removing old RBCs and platelets, as well as debris from the blood.
- Stores the breakdown products of RBCs
- Site of erythrocyte production in the fetus

Anatomy of the spleen

- Surrounded by a fibrous capsule
- Contains both T cells, B cells, RBCs and macrophages



- Divided histologically into two regions

Red pulp

- It consists of a network of sinusoids populated by macrophages and numerous red blood cells (erythrocytes) and few lymphocytes;
- it is the site where old and defective red blood cells are destroyed and removed

White pulp

The splenic white pulp surrounds the branches of the splenic artery, forming a periarteriolar lymphoid sheath (PALS) populated mainly by T lymphocytes. Primary lymphoid follicles are attached to the PALS. These follicles are rich in B cells and some of them contain germinal centers. The marginal zone, located peripheral to the PALS, is populated by lymphocytes and macrophages.

Function

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

Lymphocytes in the blood also enter sinuses in the marginal zone and migrate to the PALS. The initial activation of B and T cells takes place in the Tcell- rich PALS. Here interdigitating dendritic cells capture antigen and present it combined with class II MHC molecules to TH cells. Once activated, these TH cells can then activate B cells.

The activated B cells, together with some TH cells, then migrate to primary follicles in the marginal zone. Upon antigenic challenge, these primary follicles develop into characteristic secondary follicles containing germinal centers (like those in the lymph nodes), where rapidly dividing B cells (centroblasts) and plasma cells are surrounded by dense clusters of concentrically arranged lymphocytes.

Tonsils – the simplest lymphoid organs; named according to their location

- Palatine tonsils

- Lingual tonsils
- Pharyngeal tonsil
- Tubal tonsils
- Aggregates of lymphoid follicles
 - Location of these follicles make them ideal because they are able to:
 - Destroy bacteria and prevent pathogens from slipping through the intestinal wall
 - Generate many –memory lymphocytes for long-term immunity
 - Examples
 - **Peyer’s patches** – found in the distal portion of the small intestine
 - **Appendix** – an off-shoot of the cecum (the first part of the large intestine)
- **Mucosa-associated lymphatic tissue (MALT)** – protects the digestive and respiratory tracts from foreign material
 - Include the tonsils, Peyer’s patches, appendix, and lymphoid follicles found in the bronchi

Clinical Disorders of the Lymphatic System

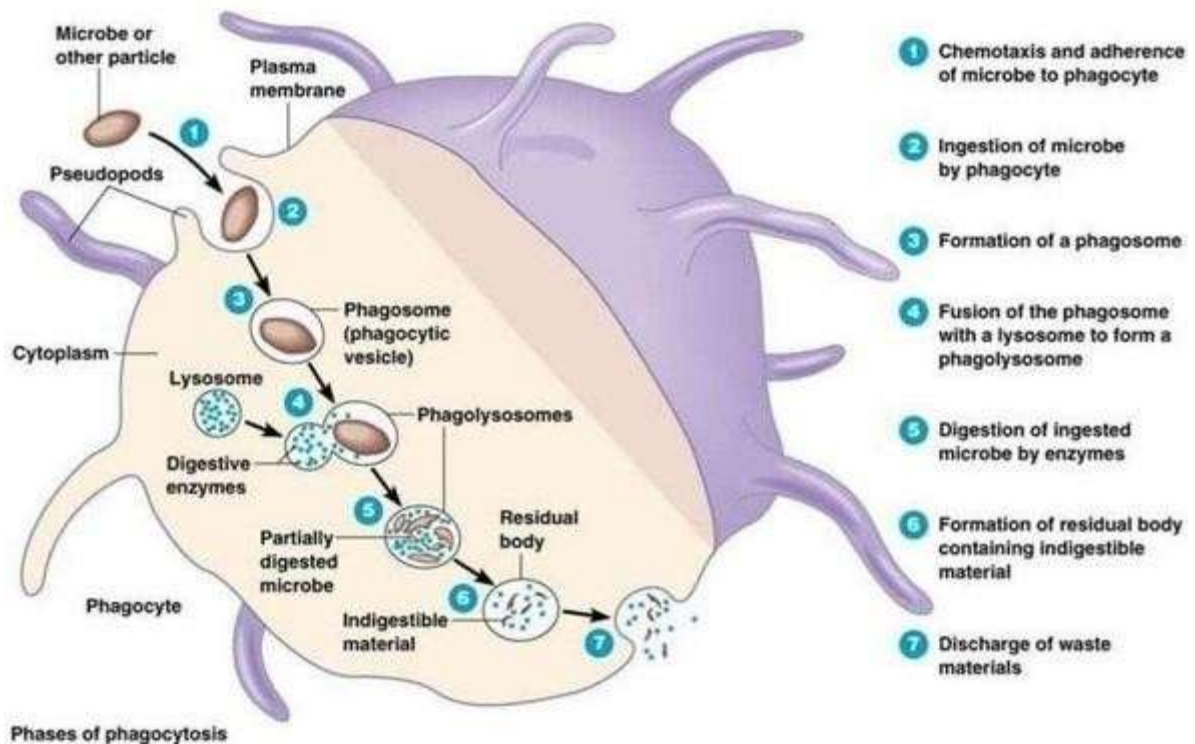
- Lymphangitis – inflammation of the lymphatic vessels
- Lymphedema – blockage of the lymphatic vessels
- Buboes - infected lymph nodes

4. Write a note on Phagocytosis?

- Microbes that penetrate the first line of defense face the second line of defense, which depends mainly on **phagocytosis**, the ingestion of invading organisms by certain types of white cells. The main phagocytic cells are polymorphonuclear neutrophils and macrophages.
- Phagocyte function is intimately associated with an effective inflammatory response and also with certain antimicrobial proteins.
- Phagocytes attach to their prey via surface receptors found on microbes but not normal body cells.

- Adherence induces membrane protrusions, called pseudopodia, Fusion of the pseudopodia encloses the material within a membrane-bounded structure called a phagosome, a phagosome moves toward the cell interior, where it fuses with a lysosome to form a phagolysosome.

Stages of phagocytosis



- Microbes are destroyed within lysosomes in two ways.
 - Lysosomes contain nitric oxide and other toxic forms of oxygen, which act as potent antimicrobial agents.
 - Lysozymes and other enzymes degrade mitochondrial components.
- Some microbes have adaptations that allow them to evade destruction by phagocytes.

- The outer capsule of some bacterial cells hides their surface polysaccharides and prevents phagocytes from attaching to them.
- Other bacteria are engulfed by phagocytes but resist digestion, growing and reproducing within the cells.

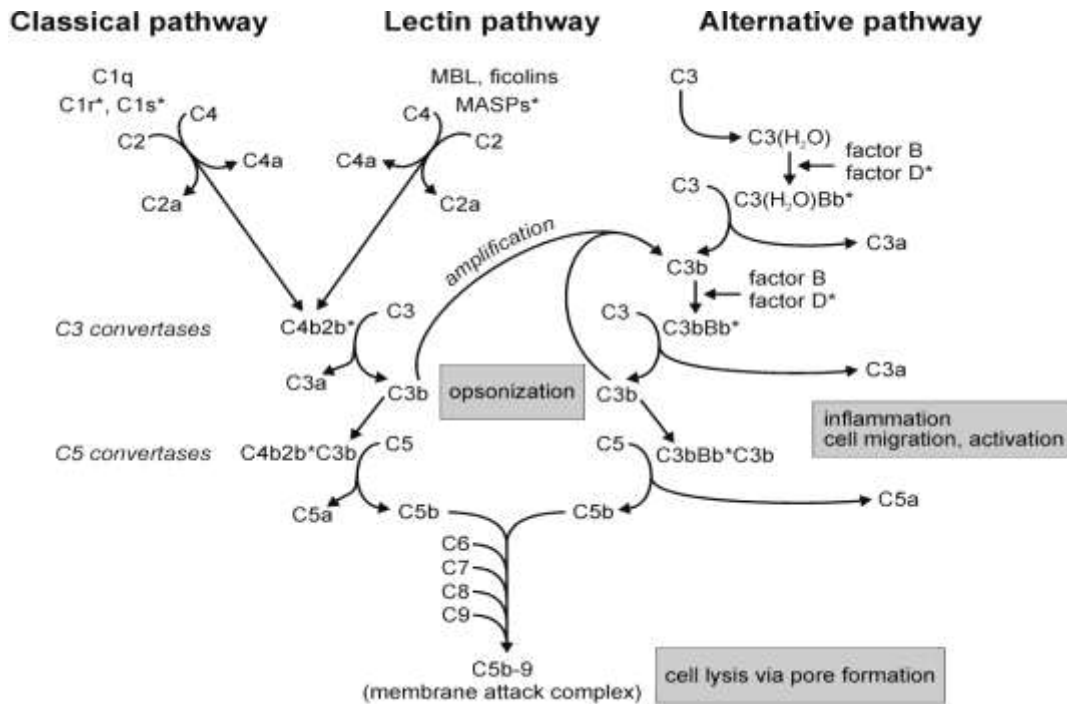
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- **Answers the Question elaborately:**

1.Explain in detail about Complement:

- The complement system is the major effector of the humoral branch of the immune system.
- The proteins and glycoproteins that compose the complement system are synthesized mainly by liver hepatocytes, and blood monocytes, tissue macrophages ,and epithelial cells of the gastrointestinal and genitourinary tracts.
- The complement system comprises a group of serum proteins,many of which exist in inactive forms.
- Complement activation occurs by the classical, alternative,or lectin pathways, each of which is initiated differently.The three pathways converge in a common sequence of events that leads to generation of a molecular complex that causes cell lysis.
- Activation of the alternative and lectin pathways is antibody-independent. These pathways are initiated by reaction of complement proteins with surface molecules of microorganisms.
- The classical pathway is initiated by antibody binding to a cell target; reactions of IgM and certain IgG subclasses activate this pathway.

- complement deficiencies range from increases in susceptibility to infection to tissue damage caused by immune complexes.



Function of complement

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

Unit II-Immunity and Immune response

Answers the Question elaborately:

1.Explain in detail about the immune response?

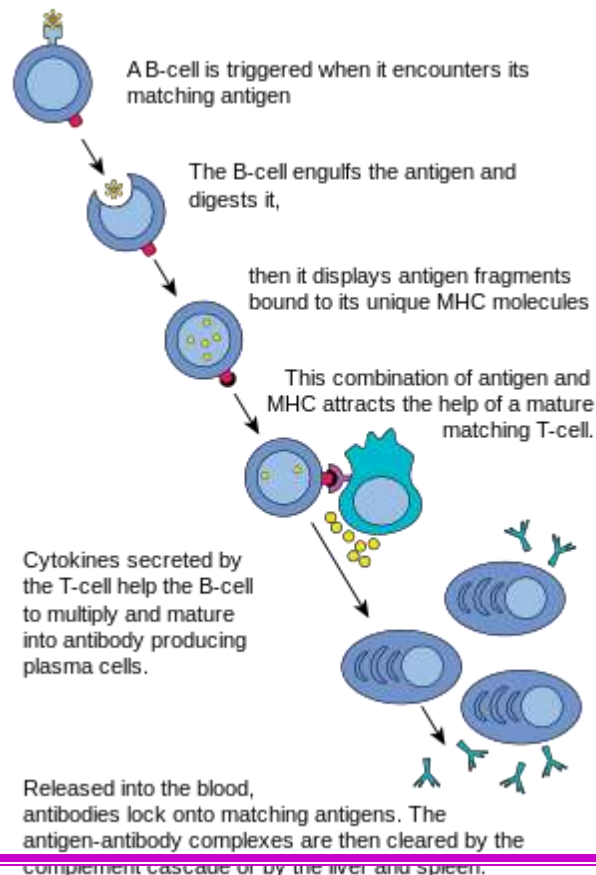
- Introduction of a foreign substance may produce,
 - specific antibody(Humoral immune response)
 - cell mediated immunity

Humoral immune response:

- **The Humoral immunity** refers to antibody mediated immune response .In the humoral response, cells derived from B-lymphocytes secrete defensive proteins called antibodies that bind to microbes and target them for elimination. The Humoral response is suited for elimination of exogenous antigens. Humoral immunity is named because it involves substances found in the [humours](#), or [body fluids](#).

The humoral immune response is mediated by B cell.

Steps involved in Humoral immune response:



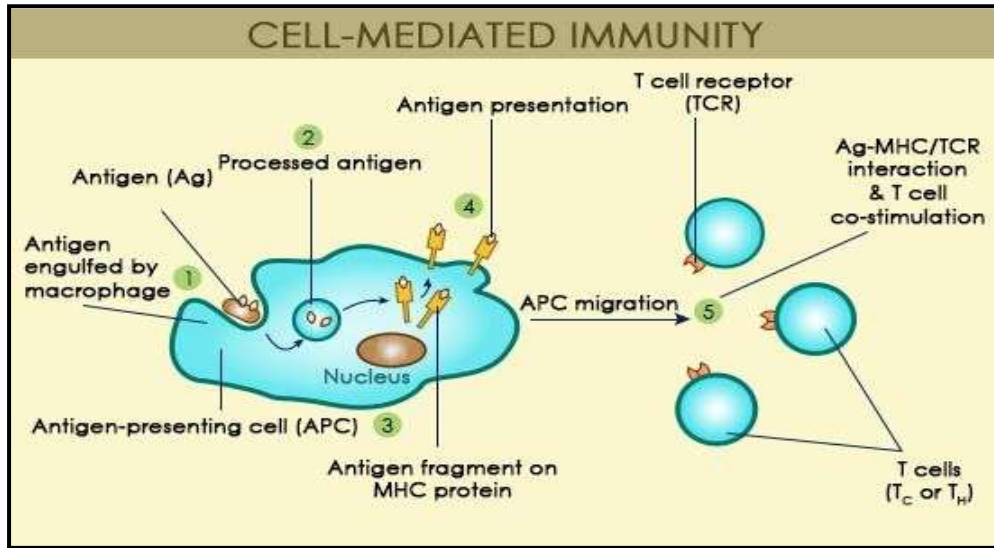
- **B cell activation**-Antigen binding to Native B cell receptor
- **Clonal Selection**-Activated B cell undergo cell division. The phenomenon of selective proliferation of B cell in response to their interaction with the antigen is called Clonal Selection.
- **Differentiation**-Activated B cell produce Plasma cell and Memory cell.
- **Ab production**-Plasma cell produce Antibodies that bind to antigen and Memory cell for immunological memory or secondary response.

2. Write a note on Cell mediated immunity ?

- Cell-mediated immunity is an immune response that does not involve antibodies, but rather involves the activation of phagocytes, antigen-specific cytotoxic T-lymphocytes, and the release of various cytokines in response to an antigen.
- T cells interact with other immune cells via a receptor called T cell receptor (TCR), which can only interact with antigenic molecule bound to class II molecule on the surface of antigenic presenting cell (APCs).
- The cell-mediated immunity involves the activation of NK cells, T lymphocytes, macrophages, and cytokines in response to an antigen.

Cellular immunity protects the body by:

- activating antigen-specific cytotoxic T-lymphocytes that are able to induce apoptosis in body cells displaying epitopes of foreign antigen on their surface, such as virus-infected cells, cells with intracellular bacteria, and cancer cells displaying tumor antigens;
- activating macrophages and natural killer cells, enabling them to destroy pathogens; and
- stimulating cells to secrete a variety of cytokines that influence the function of other cells involved in adaptive immune responses and innate immune responses.



Steps:

1. The process starts when an antigen presenting cell, usually a macrophage, ingests foreign material and incorporates a piece of the antigen into the surface of the cell membrane.
2. Everywhere the macrophage then travels, it can present this antigen to any T-Helper cells that it encounters.
3. The T-Helper cells have a receptor in their membrane cleverly called the T-Cell Receptor. This is a protein receptor with a specific shape. If the antigen on the macrophage matches with a T-cell receptor on the T-Helper cell, it becomes an active T-cell. There are literally millions of different T-helper cells with different receptor shapes. Only when the macrophage finds the specific, appropriate one will the process continue. That's why it takes some time before the immune system starts to respond the infection. Image you as a single person being tasked with speaking with everyone on campus until you spoke with JoePa. It's possible but it would take some time.
4. Once the appropriate T-helper cell is activated, it can now proceed to stimulate another T-cell.
5. The Cytotoxic T-cell, once stimulated by a t-helper cell, will search out and destroy any cells in the body that are misbehaving. Any cell with an altered MHC, self identity protein will be targeted. If a cell has the wrong MHC, like a transplanted kidney cell it can be attacked. When cells are invaded by viruses or become cancerous, the MHC markers become altered as the cell is fixated on other inappropriate tasks. This altered marker can also be recognized by Cytotoxic T-cells and destroyed. In this way the problem is eliminated.
6. Once the active infection or tumor or transplant is destroyed many of the T cells, both helper and cytotoxic, will slowly die off. The problem is over, they're no longer needed. However, a number of them remain as Memory cells. These cells will respond to the same antigen if it is ever encountered again. In this way, following viral infections, for example, are easier to fight as there are multiple T- helper cells ready to respond. The macrophage does not have to search the whole body for a single T-helper cell, there are now many. Again using the earlier analogy,

instead of you yourself trying to find JoePa, imagine you and all your friends splitting up and looking in different buildings on campus. The job would be easier and faster.

Unit III- Hypersensitivity and autoimmunity

Short answers

1) Define Hypersensitivity

Hypersensitivity is defined as the violent reaction of the immune system leading to severe symptoms and even death in a sensitized animal when it is re-exposed to the same antigen for the second time. It is also called allergy.

2) What are the Factors causing hypersensitivity?

Hypersensitivity is caused by numerous factors, these factors causing allergy are called allergens. They may two factors;

a) Extrinsic factor(introduced into the body from outside)

b) Intrinsic factor(factors which remain within the body). They are the following:

1) Drugs.

2) Airborne particles.

3) Food stuffs.

4) Infectious organisms.

5) Blood transfusion.

3) What are the Types of hypersensitivity?

Hypersensitivity is classified in two ways. They are;

1) Based on the time taken for the reaction.

a) Immediate hypersensitivity.

b) Delayed hypersensitivity.

2) Based on the different mechanisms of pathogenesis.

a) Type I- Anaphylactic hypersensitivity.

b) Type II- Antibody-dependent cytotoxic hypersensitivity.

c) Type III- Immune complex-mediated hypersensitivity.

Answers the Question elaborately:

1.Describe about Hypersensitivity?

- Hypersensitive or allergy reactions are inflammatory reactions within the humoral or cell-mediated branches of the immune system that lead to wide tissue damage or even death.
- It mediated by Several compound is called as Mediator.
- The mediators can be classified as either primary or secondary.
- Primary mediators are histamine, proteases, eosinophil chemotactic factor, neutrophil chemotactic factor and heparin.
- Secondary mediators include platelet-activating factor, leukotrienes, prostaglandins, bradykinins, and various cytokines.

It classified in to four types

- Type I hypersensitive reaction is mediated by IgE antibodies.
- Type II hypersensitive reaction occurs when antibody(IgD) reacts with antigenic determinants present on the surface of cells, leading to cell damage or death through complement mediated lysis or antibody-dependent cell-mediated cytotoxicity(ADCC).
- Type III hypersensitive reaction is mediated by the formation of immune complexes and the ensuing activation of complement.
- Type IV hypersensitive reaction involves the cell-mediated branch of the immune system.

Types of Hyper Sensitivity

Type of hypersensitivity	Pathologic Immune Mechanisms	Mechanisms of tissue injury and disease
Immediate hypersensitivity: Type I	IgE antibody	Mast cells and their mediators (vasoactive amines, lipid mediators, cytokines)
Antibody mediated: Type II	IgM, IgG antibodies against cell surface or extracellular matrix antigens	Opsonization and phagocytosis of cells Complement- and Fc receptor-mediated recruitment and activation of leukocytes (neutrophils, macrophages) Abnormalities in cellular functions, e.g., hormone receptor signaling
Immune complex mediated: Type III	Immune complexes of circulating antigens and IgM or IgG antibodies	Complement- and Fc receptor-mediated recruitment and activation of leukocytes
T cell mediated: Type IV	1. CD4+ T cells (delayed-type hypersensitivity) 2. CD8+ CTLs (T cell-mediated cytotoxicity)	1. Macrophage activation, cytokine-mediated inflammation 2. Direct target cell killing, cytokine-mediated inflammation
Abbreviations: CTL, cytolytic T lymphocyte		

2.Explain in detail about Autoimmune Disease

- They result in an improper response of the immune system against self-components termed **autoimmunity**.
- Autoimmune diseases can be divided into organ-specific and systemic diseases.
- The organ-specific diseases involve an autoimmune response directed mainly against a single organ or gland.
- The systemic diseases are directed against a broad spectrum of tissues and have manifestations in a variety of organs resulting from cell-mediated responses and cellular damage caused by auto-antibodies or immune complexes.

Autoimmune disease may be due to,

- failure of suppression - ie. a T-cell defect
- tissue damage altering self-antigens with a sustained response
- classical example is post-streptococcal GN
- infection altering cell surface markers in a genetically susceptible individual
- IDDM probably included in this group

Organ-Specific Autoimmune Diseases

- Addison's disease-Adrenal cells
- Goodpasture's syndrome-Renal and lung basement membranes
- Graves' disease-Thyroid-stimulating hormone receptor
- Hashimoto's thyroiditis-Thyroid proteins and cell
- Myasthenia gravis-Acetylcholine receptors
- Pernicious anemia-Gastric parietal cells; intrinsic factor
- Hashimoto's thyroiditis-Thyroid proteins and cells
- Autoimmune hemolytic anemia-RBC membrane proteins
- Spontaneous infertility-Sperm.

Systemic Autoimmune Diseases

- Multiple sclerosis-Brain or white matter
- Rheumatoid arthritis-Connective tissue, IgG
- Scleroderma-Nuclei, heart, lungs, gastrointestinal tract, kidney
- Sjogren's syndrome-Salivary gland, liver, kidney, thyroid
- Systemic lupus erythematosus (SLE)- DNA, nuclear protein, RBC and platelet membranes

Treatment

- Immunosuppressive drugs.
- Thymectomy.
- Plasmapheresis for diseases involving immune complexes.
- vaccination with T cells specific for a given autoantigen
- Administration of synthetic blocking peptides that compete with autoantigen for binding to MHC molecules
- Treatment with monoclonal antibodies
- Induction of tolerance

Unit –IV- Immunoglobulin and Vaccine

Short answers

1. What is mean by antigen?

A substance that can produce a specific immune response when it is introduced into the tissues of an animal and that can react specifically with antibodies or sensitized cells is known as an antigen.

2. What is mean by immunogenicity?

The ability of a material to induce an immune response is referred to as immunogenicity and such materials are known as immunogens.

3. Comment on epitopes and paratopes?

The part of the antigens with which the antibody reacts is known as the epitopes or antigenic determinants. The portion of the antibody molecule that binds to the epitope is called the paratope.

4. What is mean by haptens?

Haptens is called an incomplete antigens. Haptens can combine with an antibody but cannot initiate an immune response, not immunogenic by itself unless it is bound to a carrier before introduction into the body.

5. Comment on vaccines?

Vaccine is an antigenic preparation of microorganisms such as bacteria, viruses or rickettsiae administered for prevention treatment of infectious disease.

6. What is mean by immunoglobulins?

Immunoglobulins are immunologically active serum proteins formed to an antigen and react specifically with that antigen.

Answers the Question elaborately:

1. Explain the Structure and functions immunoglobulin?

- **Immunoglobulin (Ig):** Immunoglobulins are glycoprotein molecules that are produced by plasma cells in response to an immunogen and which function as antibodies. The immunoglobulins derive their name from the finding that they migrate with globular proteins when antibody-containing serum is placed in an electrical field.

Basic structure of Immunoglobulin: Although different immunoglobulins can differ

structurally, they all are built from the same basic units.

- **Heavy and Light Chains**

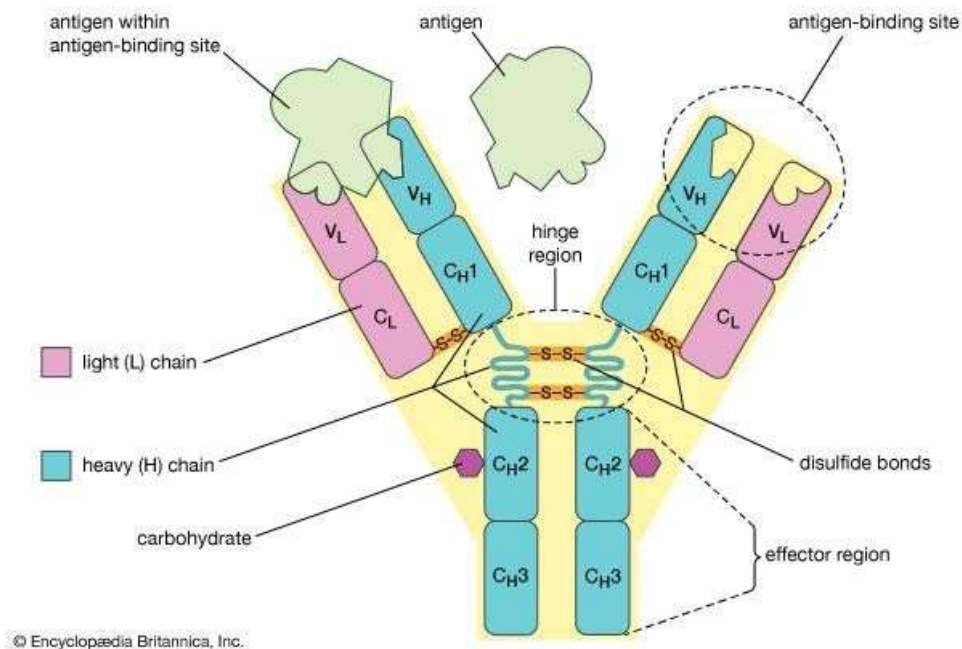
- All immunoglobulins have a four chain structure as their basic unit. They are composed of two identical light chains (23kD) and two identical heavy chains (50-70kD)

- **Disulfide bonds**

- **Inter-chain disulfide bonds** - The heavy and light chains and the two heavy chains are held together by inter-chain disulfide bonds and by non-covalent interactions. The number of inter-chain disulfide bonds varies among different immunoglobulin molecules.
- **Intra-chain disulfide binds** - Within each of the polypeptide chains there are also intra-chain disulfide bonds.

- **Variable (V) and Constant (C) Regions**

- When the amino acid sequences of many different heavy chains and light chains were compared, it became clear that both the heavy and light chain could be divided into two regions based on variability in the amino acid sequences. These are the:
 - **Light Chain** - VL (110 amino acids) and CL (110 amino acids)
 - **Heavy Chain** - VH (110 amino acids) and CH (330-440 amino acids)



- **Hinge Region**

- This is the region at which the arms of the antibody molecule forms a Y. It is called the hinge region because there is some flexibility in the molecule at this point.

- **Domains**

- Rather, it is folded into globular regions each of which contains an intra-chain disulfide bond. These regions are called domains.
 - **Light Chain Domains** - VL and CL
 - **Heavy Chain Domains** - VH, CH1 - CH3 (or CH4)

- **Oligosaccharides**

- Carbohydrates are attached to the CH2 domain in most immunoglobulins. However, in some cases carbohydrates may also be attached at other locations.

Antigen-binding site

- • Antigen binding occurs at 3 HYPERVARIABLE regions, known as COMPLEMENTARITY DETERMINING REGIONS (CDR's)

- • These have specific residue positions
- • The region of binding is a large undulating 3D structure (~750Å = 10-10m), so is highly specific and there are a significant number of interactions between the antibody and antigen surface

Forces involved

- • Hydrogen bonds
- • Ionic bonds
- • Hydrophobic interactions
- • Van der Waals interactions

Antibody Affinity

- The strength of the total non-covalent interactions between a **single antigen binding site** and a **single epitope** on the antigen.
- The affinity association constant K can be calculated:
- K varies from 10⁴ to 10¹¹ L/mol

Antibody Avidity

- The overall strength of **multiple interactions** between an antibody with **multiple binding sites** and a complex antigen with **multiple epitopes**
- • This is a better measure of binding capacity in biological systems
- • **Monovalent** interactions have a low affinity
- • **Bivalent** interactions have a high affinity
- • **Polyvalent** interactions have a very high affinity

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Cross-Reactivity

- Antibodies elicited in response to one antigen can also recognise a different antigen, for example:
 1. Vaccination with cowpox induces antibodies which are able to recognise smallpox
 2. ABO blood group antigens are glycoproteins on red blood cells. Antibodies made against microbial agents on common intestinal bacteria may cross-react with the glycoproteins, which poses a problem for blood transfusions.

Immunoglobulin Fragments: Structure and function relationship

Immunoglobulin fragments produced by proteolytic digestion have proven very useful in elucidating structure/function relationships in immunoglobulins.

- **Fab - fraction antigen binding:** this is the highly variable area which determines specificity
- **Fc - fraction crystalline:** which determines what happens once the Ab-Ag interaction has occurred
- **F(ab')₂**

2.write a note on types of Ig?

A. Immunoglobulin classes: The immunoglobulins can be divided into five different classes, based on differences in the amino acid sequences in the constant region of the heavy chains. All immunoglobulins within a given class will have very similar heavy chain constant regions. These differences can be detected by sequence studies or more commonly by serological means (i.e. by the use of antibodies directed to these differences).

1. IgG - Gamma heavy chains
2. IgM - Mu heavy chains
3. IgA - Alpha heavy chains

4. IgD - Delta heavy chains
5. IgE - Epsilon heavy chains

Structure and some properties of IG classes and subclasses

IgG

- **Structure**
 - All IgG's are monomers (7S immunoglobulin). The subclasses differ in the number of disulfide bonds and length of the hinge region.
- **Properties**
- IgG is the most versatile immunoglobulin because it is capable of carrying out all of the functions of immunoglobulin molecules.
 - IgG is the major Ig in serum - 75% of serum Ig is IgG
 - IgG is the major Ig in extra vascular spaces
 - Placental transfer - IgG is the only class of Ig that crosses the placenta. Transfer is mediated by a receptor on placental cells for the Fc region of IgG. Not all subclasses cross equally well; IgG2 does not cross well.
 - Fixes complement - Not all subclasses fix equally well; IgG4 does not fix complement
 - Binding to cells - Macrophages, monocytes, PMNs and some lymphocytes have Fc receptors for the Fc region of IgG. Not all subclasses bind equally well; IgG2 and IgG4 do not bind to Fc receptors. A consequence of binding to the Fc receptors on PMNs, monocytes and macrophages is that the cell can now internalize the antigen better. The antibody has prepared the antigen for eating by the phagocytic cells. The term opsonin is used to describe substances that enhance phagocytosis.

IgM

- **Structure**

- IgM normally exists as a pentamer (19S immunoglobulin) but it can also exist as a monomer. In the pentameric form all heavy chains are identical and all light chains are identical. Thus, the valence is theoretically 10. IgM has an extra domain on the mu chain (CH4) and it has another protein covalently bound via a S-S bond called the J chain. This chain functions in polymerization of the molecule into a pentamer.

- **Properties**

- IgM is the third most common serum Ig.
- IgM is the first Ig to be made by the fetus and the first Ig to be made by a virgin B cells when it is stimulated by antigen.
- As a consequence of its pentameric structure, IgM is a good complement fixing Ig. Thus, IgM antibodies are very efficient in leading to the lysis of microorganisms.
- As a consequence of its structure, IgM is also a good agglutinating Ig. Thus, IgM antibodies are very good in clumping microorganisms for eventual elimination from the body.
- IgM binds to some cells via Fc receptors.
- B cell surface Ig
- Surface IgM exists as a monomer and lacks J chain but it has an extra 20 amino acids at the C-terminus to anchor it into the membrane. Cell surface IgM functions as a receptor for antigen on B cells. Surface IgM is noncovalently associated with two additional proteins in the membrane of the B cell called Ig-alpha and Ig-beta. These additional proteins act as signal transducing molecules since the cytoplasmic tail of the Ig molecule itself is too short to transduce a signal. Contact between surface immunoglobulin and an antigen is required before a signal can be transduced by the Ig-alpha and Ig-beta chains. In the case of T-independent antigens, contact between the antigen and surface immunoglobulin is sufficient to activate B cells to differentiate into antibody secreting plasma cells. However, for T-dependent antigens, a second signal provided by helper T cells is required before B cells are activated.

IgA

- **Structure**

- When IgA exists as a dimer, a J chain is associated with it.
- When IgA is found in secretions it also has another protein associated with it called the secretory piece or T piece; sIgA is sometimes referred to as 11S immunoglobulin. Unlike the remainder of the IgA which is made in the plasma cell, the secretory piece is made in epithelial cells and is added to the IgA as it passes into the secretions. The secretory piece helps IgA to be transported across mucosa and also protects it from degradation in the secretions.

- **Properties**

- IgA is the 2nd most common serum Ig.
- IgA is the major class of Ig in secretions - tears, saliva, colostrum, mucus. Since it is found in secretions secretory IgA is important in local (mucosal) immunity.
- Normally IgA does not fix complement, unless aggregated.
- IgA can binding to some cells - PMN's and some lymphocytes.

IgD

- **Structure**

- IgD exists only as a monomer.

- **Properties**

- IgD is found in low levels in serum; its role in serum uncertain.
- IgD is primarily found on B cell surfaces where it functions as a receptor for antigen. IgD on the surface of B cells has extra amino acids at C-terminal end for anchoring to the membrane. It also associates with the Ig-alpha and Ig-beta chains.
- IgD does not bind complement.

IgE

- **Structure**

IgE exists as a monomer and has an extra domain in the constant region.

- **Properties**

- IgE is the least common serum Ig since it binds very tightly to Fc receptors on basophils and mast cells even before interacting with antigen.
- Involved in allergic reactions - As a consequence of its binding to basophils and mast cells, IgE is involved in allergic reactions. Binding of the allergen to the IgE on the cells results in the release of various pharmacological mediators that result in allergic symptoms.
- IgE also plays a role in parasitic helminth diseases. Since serum IgE levels rise in parasitic diseases, measuring IgE levels is helpful in diagnosing parasitic infections. Eosinophils have Fc receptors for IgE and binding of eosinophils to IgE-coated helminths results in killing of the parasite.
- IgE does not fix complement.

Clinical implications of human immunoglobulin classes

IgG

- **Increases in:** a) Chronic granulomatous infections; b) Infections of all types; c) Hyperimmunization; d) Liver disease; e) Malnutrition (severe); f) Dysproteinemia; g) Disease associated with hypersensitivity granulomas, dermatologic disorders, and IgG myeloma; h) Rheumatoid arthritis
- **Decreases in:** a) Agammaglobulinemia; b) Lymphoid aplasia; c) Selective IgG, IgA deficiency; d) IgA myeloma e) Bence Jones proteinemia; f) Chronic lymphoblastic leukemia

IgM

- **Increases (in adults) in:** a) Waldenström's macroglobulinemia; b) Trypanosomiasis; c) Actinomycosis; d) Carrión's disease (bartonellosis); e) Malaria; f) Infectious mononucleosis; g)

Lupus erythematosus; h) Rheumatoid arthritis; I) Dysgammaglobulinemia (certain cases).

- **Note:** In the newborn, a level of IgM above 20 ng./dl is an indication of in utero stimulation of the immune system and stimulation by the rubella virus, the cytomegalovirus, syphilis, or toxoplasmosis.
- **Decreases in:** a) Agammaglobulinemia; b) Lymphoproliferative disorders (certain cases); c) Lymphoid aplasia; d) IgG and IgA myeloma; e) Dysgammaglobulinemia; f) Chronic lymphoblastic leukemia

IgA

- **Increases in:** a) Wiskott-Aldrich syndrome; b) Cirrhosis of the liver (most cases); c) Certain stages of collagen and other autoimmune disorders such as rheumatoid arthritis and lupus erythematosus; d) Chronic infections not based on immunologic deficiencies; e) IgA myeloma
- **Decreases in:** a) Hereditary ataxia telangiectasia; b) Immunologic deficiency states (e.g., dysgammaglobulinemia, congenital and acquired agammaglobulinemia, and hypogammaglobulinemia); c) Malabsorption syndromes; d) Lymphoid aplasia; e) IgG myeloma; f) Acute lymphoblastic leukemia; g) Chronic lymphoblastic leukemia

IgD

1. **Increases in:** a) Chronic infections; b) IgD myelomas

IgE

- **Increases in:** a) Atopic skin diseases such as eczema; b) Hay fever; c) Asthma; d) Anaphylactic shock; e) IgE-myeloma
- **Decreases in:** a) Congenital agammaglobulinemia; b) Hypogammaglobulinemia due to faulty metabolism or synthesis of immunoglobulins

Selective Immunoglobulin Distribution

- • IgG and IgM in blood
- • IgG in extracellular fluid
- • Dimeric IgA in secretions across epithelia, including breast milk
- • Maternal IgG in foetus via placental transfer
- • IgE with mast cells below epithelium
 - • Brain devoid of antibodies

Isotypes and Allotypes

Isotypes are antibodies who are present in everybody, with a constant region.

Allotypes are antibodies that contain single amino acid mutations, giving allelic polymorphisms which vary in the population

Humoral and cell-mediated immunity defend against different types of threats

- The immune system can mount two types of responses to antigens: a humoral response and a cell mediated response.
- **Humoral immunity** involves B cell activation and clonal selection and results in the production of antibodies that circulate in the blood plasma and lymph.
- Circulating antibodies defend mainly against free bacteria, toxins, and viruses in the body fluids.
- In **cell-mediated immunity**, activation and clonal selection of cytotoxic T lymphocytes allows these cells to directly destroy certain target cells, including -nonself cancer and transplant cells.
- The humoral and cell-mediated immune responses are linked by cell-signaling interactions, especially via **helper T cells**.

Helper T lymphocytes function in both humoral and cell-mediated immunity.

- When a helper T cell recognizes a class II MHC molecule-antigen complex on an antigen-presenting cell, the helper T cell proliferates and differentiates into a clone of activated helper T cells and memory helper T cells.

- A surface protein called CD4 binds the side of the class II MHC molecule.
- This interaction helps keep the helper T cell and the antigen-presenting cell joined while activation of the helper T cell proceeds.
- Activated helper T cells secrete several different cytokines that stimulate other lymphocytes, thereby promoting cell-mediated and humoral responses.
- Dendritic cells are important in triggering a primary immune response.

They capture antigens, migrate to the lymphoid tissues, and present antigens, via class II MHC molecules, to helper T cells.

- Macrophages present antigens to memory helper T cells, while B cells primarily present antigens to helper T cells in the course of the humoral response.

In the cell-mediated response, cytotoxic T cells counter intracellular pathogens.

- Antigen-activated cytotoxic T lymphocytes kill cancer cells and cells infected by viruses and other intracellular pathogens.
- Fragments of nonself proteins synthesized in such target cells associate with class I MHC molecules and are displayed on the cell surface, where they can be recognized by cytotoxic T cells.
- This interaction is greatly enhanced by the T surface protein **CD8** that helps keep the cells together while the cytotoxic T cell is activated.
- When a cytotoxic T cell is activated by specific contacts with class I MHC-antigen complexes on an infected cell, the activated cytotoxic T cell differentiates into an active killer, which kills its target cell—the antigen-presenting cell—primarily by secreting proteins that act on the bound cell.
- The death of the infected cell not only deprives the pathogen of a place to reproduce, but also exposes it to circulating antibodies, which mark it for disposal.

- Once activated, cytotoxic T cells kill other cells infected with the same pathogen.
- In the same way, cytotoxic T cells defend against malignant tumors.
 - Because tumor cells carry distinctive molecules not found on normal cells, they are identified as foreign by the immune system.
 - Class I MHC molecules on a tumor cell present fragments of tumor antigens to cytotoxic T cells.
 - Interestingly, certain cancers and viruses actively reduce the amount of class I MHC protein on affected cells so that they escape detection by cytotoxic T cells.
 - The body has a backup defense in the form of natural killer cells, part of the nonspecific defenses, which lyse virus-infected and cancer cells.

In the humoral response, B cells make antibodies against extracellular pathogens.

- Antigens that elicit a humoral immune response are typically proteins and polysaccharides present on the surface of bacteria or transplanted tissue.
- The activation of B cells is aided by cytokines secreted by helper T cells activated by the same antigen.
 - These B cells proliferate and differentiate into a clone of antibody-secreting plasma cells and a clone of memory B cells.
- When antigen first binds to receptors on the surface of a B cell, the cell takes in a few of the foreign molecules by receptor-mediated endocytosis.
- The B cell then presents antigen fragments to a helper B cell.
- Many antigens (primarily proteins), called **T-dependent antigens**, can trigger a humoral immune response by B cells only with the participation of helper T cells.
- Other antigens, such as polysaccharides and proteins with many identical polypeptides, function as **T-independent antigens**.
 - These include the polysaccharides of many bacterial capsules and the proteins of the bacterial flagella.

- These antigens bind simultaneously to a number of membrane antibodies on the B cell surface.
- This stimulates the B cell to generate antibody-secreting plasma cells without the help of cytokines.
- While this response is an important defense against many bacteria, it generates a weaker response than T-dependent antigens and generates no memory cells.
- Any given humoral response stimulates a variety of different B cells, with each giving rise to a clone of thousands of plasma cells.
 - Each plasma cell is estimated to secrete about 2,000 antibody molecules per second over the cell's 4- to 5-day life span.
 - A secreted antibody has the same general Y-shaped structure as a B cell receptor, but lacks a transmembrane region that would anchor it to a plasma membrane.
- Antigens that elicit a humoral immune response are typically the protein and polysaccharide surface components of microbes, incompatible transplanted tissues, or incompatible transfused cells.
 - In addition, for some humans, the proteins of foreign substances such as pollen or bee venom act as antigens that induce an allergic, or hypersensitive, humoral response.
- Antibodies constitute a group of globular serum proteins called **immunoglobins (Igs)**.
- There are five major types of heavy-chain constant regions, determining the five major classes of antibodies.
 - Two classes exist primarily as polymers of the basic antibody molecule: IgM as a pentamer and IgA as a dimer.
 - The other three classes—IgG, IgE, and IgD—exist exclusively as monomers,
- The power of antibody specificity and antigen-antibody binding has been applied in laboratory research, clinical diagnosis, and disease treatment.
 - Some antibody tools are polyclonal, the products of many different clones of B cells, each specific for a different epitope.
 - Others are monoclonal, prepared from a single clone of B cells grown in culture.

- These cells produce **monoclonal antibodies**, specific for the same epitope on an antigen.
- These have been used to tag specific molecules.
- For example, toxin-linked antibodies search and destroy tumor cells.
- The binding of antibodies to antigens is also the basis of several antigen disposal mechanisms.
 - In **viral neutralization**, antibodies bind to proteins on the surface of a virus, blocking the virus's ability to infect a host cell.
 - In **opsonization**, the bound antibodies enhance macrophage attachment to and phagocytosis of the microbes. Neither the B cell receptor for an antigen nor the secreted antibody actually binds to an entire antigen molecule.
- Antibody-mediated **agglutination** of bacteria or viruses effectively neutralizes and opsonizes the microbes.
 - Agglutination is possible because each antibody molecule has at least two antigen-binding sites.
 - IgM can link together five or more viruses or bacteria.
 - ◦ These large complexes are readily phagocytosed by macrophages.
 - • In **precipitation**, the cross-linking of soluble antigen molecules—molecules dissolved in body fluids—forms immobile precipitates that are disposed of by phagocytosis.
 - • The **complement system** participates in the antibody-mediated disposal of microbes and transplanted body cells.
 - The pathway begins when IgM or IgG antibodies bind to a pathogen, such as a bacterium.
 - The first complement component links two bound antibodies and is activated, initiating the cascade.

Ultimately, complement proteins generate a **membrane attack complex (MAC)**, which forms a pore in the bacterial membrane, resulting in cell lysis.

- Whether activated as part of innate or acquired defenses, the complement cascade results in the lysis of microbes and produces activated complement proteins that promote inflammation or stimulate phagocytosis.

UNIT V-IMMUNOLOGICAL TECHNIQUES

Short answers

1) Comment on antigen-antibody reaction?

The interaction between an antigen and antibody is called antigen-antibody reaction.

2) What is meant by immune complex?

When antigen and antibody are brought together, the antibodies bind with antigen to form a complex molecule called immune complex or antigen-antibody complex.

3) Define agglutination?

Agglutination is an antigen-antibody reaction where the antibody of serum causes the cellular antigens to adhere to one another to form clumps.

It is the clumping of a particular antigen and its antibody.

4) What is meant by opsonization?

Opsonization is the process by which a particulate antigen becomes more susceptible to phagocytosis by combination with an opsonin.

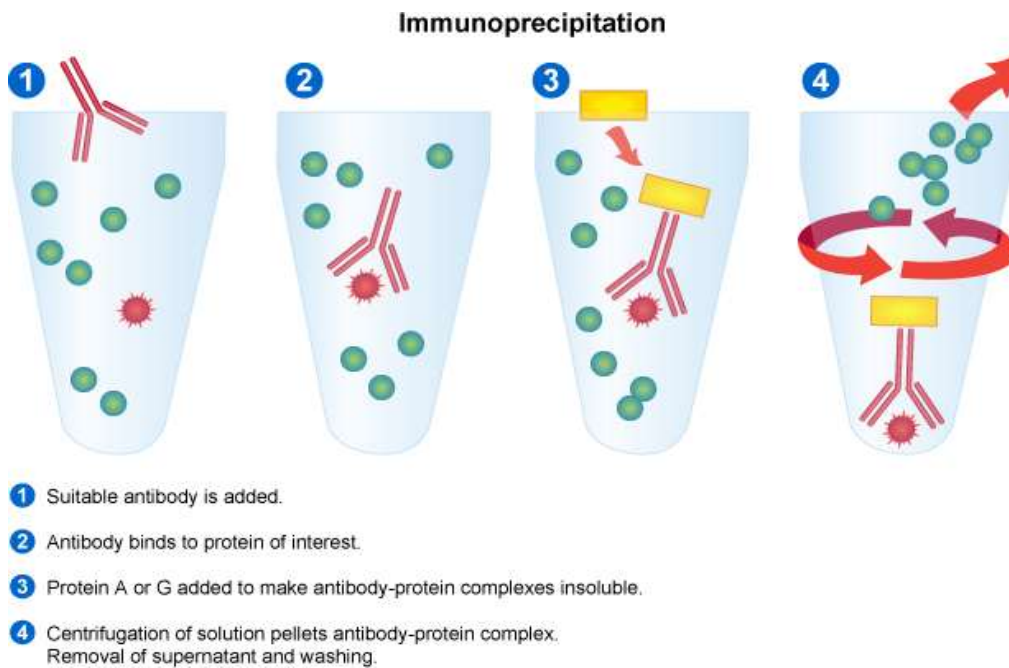
5) Define immunofluorescence?

When antibodies are mixed with fluorescent dyes such as fluorescein or rhodamine, they emit radiation. This phenomenon of emitting radiation by antibodies labeled with fluorescent dyes is called immunofluorescence.

Answers the Question elaborately:

1.Explain in detail about Immunoprecipitation ?

Immunoprecipitation (IP) is the technique of precipitating a protein antigen out of solution using an antibody that specifically binds to that particular protein. This process can be used to isolate and concentrate a particular protein from a sample containing many thousands of different proteins. Immunoprecipitation requires that the antibody be coupled to a solid substrate at some point in the procedure.



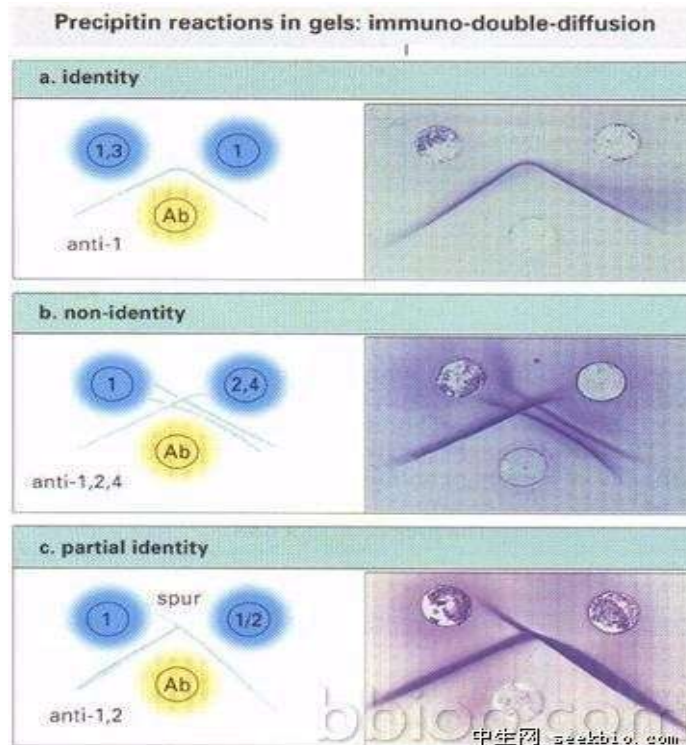
2. Write a note on Immunodiffusion?

Immunodiffusion refers to the movement of antigen or antibody or both antigen and antibody molecules in a support medium by diffusion.

Procedure

Molten agar is poured on glass slide or petridish and allowed to solidify. Two small wells at few millimeter distance apart are punched out. Antigen and corresponding antibody solutions are dropped in opposite wells. The slide/petridish is then kept in a moist chamber for 18-24 hours.

The antigen and antibody move through the agar and combine to form antigen-antibody complex. The antigen-antibody complex is visible as a precipitation line.



Reaction of identity:

Antigens in two wells are identical (Ag₁ and Ag₂). So the reaction with the antibody results in a precisely similar precipitin lines. The precipitin lines don't cross, but form a continuous line. Fusion of the two precipitin lines indicates that the antibody is reacting with epitopes commonly present on the two antigens.

ii. Reaction of non-identity:

Two non-identical antigens (Ag₁ and Ag₃) are in the antigen wells while the antibody well has antibodies to both the antigens. The two antigen-antibody precipitin lines formed differ from each other. Hence the two precipitin lines completely cross (intersect) each other.

iii. Reaction of partial identity:

The antigenic determinants in the antigens of two wells are partially shared (Ag₁ and Ag_{1a}). Hence the antibody reacts with both the antigens and forms lines that do not form a complete cross. The precipitin line crosses in only one direction. The extended precipitin line is referred to as a spur. The spur indicates that the antibody is also precipitating an additional epitope that is not present in one of the antigens.

Single Radial Immunodiffusion

Either antigen or antibody remains fixed and the other reactant moves (Diffusion technique of Oudin). The antibody is mixed with molten agar and poured into a petri dish. After solidification of the agar, wells are cut, and filled with different concentrations of antigen .

The precipitate is seen as a white circular line around each well. The diameter of the circular ring is directly proportional to the antigen concentration (i.e., more the antigen concentration, the diameter of the precipitate is more).

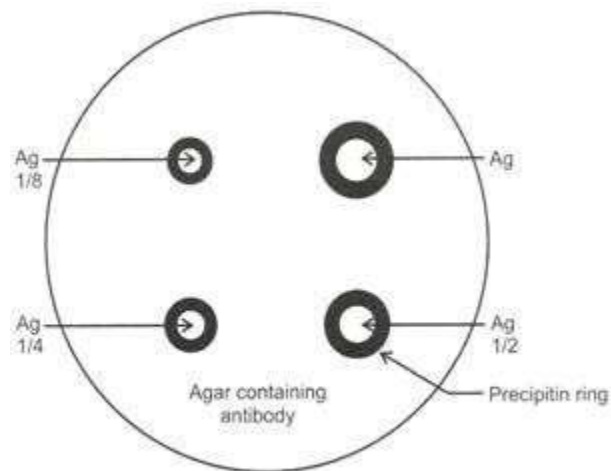
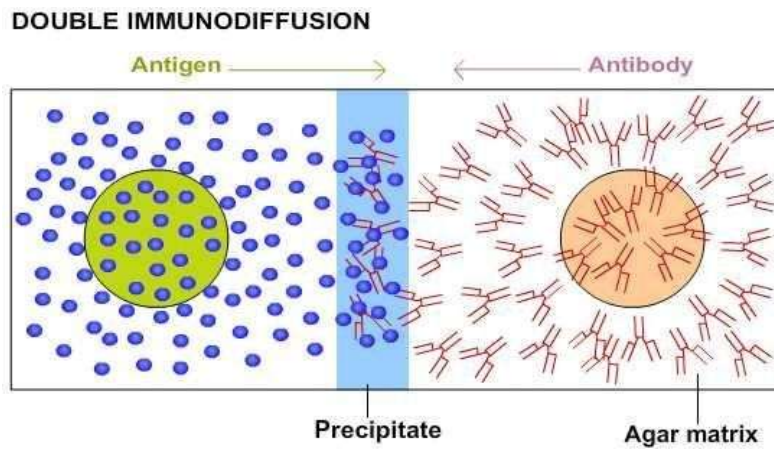


Fig. 27.4: Single radial immunodiffusion

A standard curve can be plotted in a graph sheet by plotting the known antigen concentrations in the X axis and the diameters in the Y axis. The test antigen, whose concentration is unknown, can be determined by interpolating the standard curve with the diameter of the precipitate formed by the test antigen.

Double immunodiffusion

Ouchterlony double immunodiffusion (also known as agar gel **immunodiffusion** or passive **double immunodiffusion**) is an immunological technique used in the detection, identification and quantification of antibodies and antigens, such as immunoglobulins and extractable nuclear antigens.

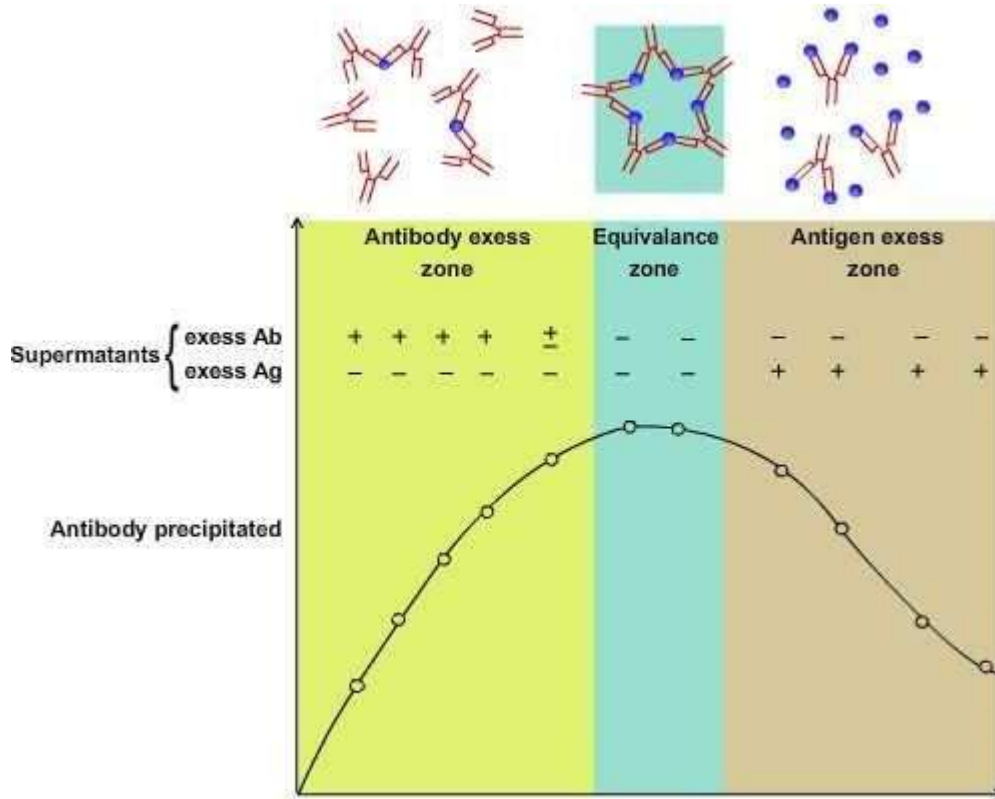


- ❖ The Ouchterlony double diffusion (ODD) technique is one of the simplest techniques extensively used to check antisera for the presence of antibodies for a particular Ag and to determine its titre.
- ❖ This method has been widely used for detection and qualitative diagnostic procedures. The method is called "double" referring to the fact that in this procedure, antigen and antibody are allowed to migrate towards each other in a gel and a line of precipitation is formed where the two reactants meet.
- ❖ This precipitation reaction is highly specific. The method is even today widespread and used by people working with diagnosis or protein detection or comparing antigens or antisera.

The technique involves cutting wells into an Agarose solidified in a glass plate. The wells are filled with antibody or antigen and the plate is incubated. When homologous antigen and antibody diffuse toward each other from the individual wells, a precipitin line will form somewhere between the two wells.

- ❖ Precipitation occurs because the antigen is multivalent i.e., has several antigenic determinants per molecule to which antibodies can bind. Antibodies have at least two antigen binding sites, thus large aggregates or lattices of antigen and antibody are formed.
- ❖ Precipitation will not occur if excess antigen is present or if excess antibody is present. Cross-linking and lattice formation will only occur when antigen and antibody concentrations are optimal. An increasing amount of antigen is added to a constant amount of antibody in solution. This is called the antibody-excess zone (Prozone phenomenon). The Ag and Ab concentrations are relatively higher near their respective wells. As they diffuse farther from the wells, their concentration decreases. An antigen will react with its specific antibody to form an Ag-Ab complex. As more antigens are added, the amount of protein precipitated increases until the antigen/antibody molecules are at an optimal ratio. This is known as the equivalence

zone or equivalence point. When the amount of antigen in solution exceeds the amount of antibody, the amount of precipitation will decrease. This is known as the antigen excess zone.



2. Write a note on immunoelectrophoresis?

Immunoelectrophoresis was first coined by Grabar and Williams in 1953. After electrophoretic separation of serum proteins in an agar gel, the serum proteins were allowed to diffuse against antibodies, leading to a pattern of precipitation arcs in the agar representing the major constituents among the serum proteins.

Immunoelectrophoresis is a general name for a number of biochemical methods for separation and characterization of proteins based on electrophoresis and reaction with antibodies. All variants of immunoelectrophoresis require immunoglobulins, also known as antibodies, reacting with the proteins to be separated or characterized.

Principle

On the basis of the difference in surface charge between the different protein molecules of antigens, several antigens can be separated from a mixture by electrophoresis in agar gel.

Procedure

Immunoelectrophoresis combines electrophoresis with immunodiffusion.

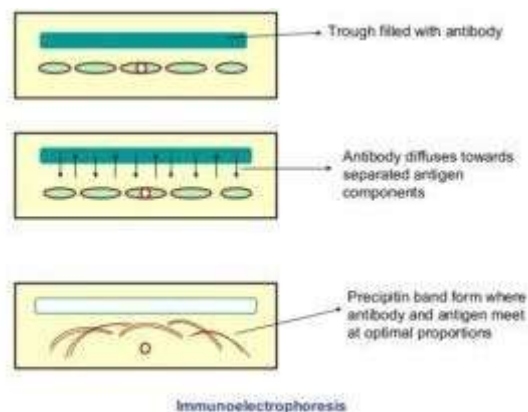
Stage 1

Antigen mixture is placed in a well cut agar gel on slide or plate. Electric current is passed through the agar for sometimes. Antigen migrate and get separated from each other according to their charges.

Stage 2

A trough is cut in the gel parallel to the direction of migration of antigen and filled with antibody.

Allow to diffuse for some time. Antigen antibody diffuse and formed precipitation are where antibodies encounters the antigens in optimal proportion.



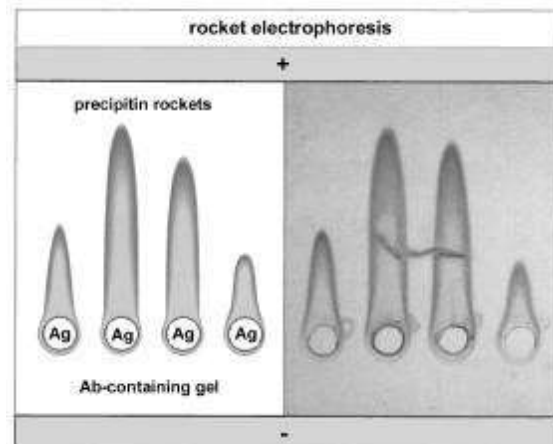
APPLICATIONS

- Useful in determination of presence and absence of serum protein.
- Example: Albumin, Immunoglobulin.

- Useful in detection of unuseful protein like human myeloma.
- Detect high antibody concentration.

4. Explain in detail about the rocket immuno-electrophoresis

Rocket immuno-electrophoresis, a technique using antibodies, can also be utilized to detect allergenic proteins. The antibodies are contained in a gel, while samples are migrated through this gel by means of electrophoresis. Antigen-antibody complexes will form in the gel resulting in rocket-shaped precipitates. The formation of such complexes will only take place at a constant antigen/antibody ratio. Therefore, the height of the rocket is proportional to the amount of antigen in the sample.



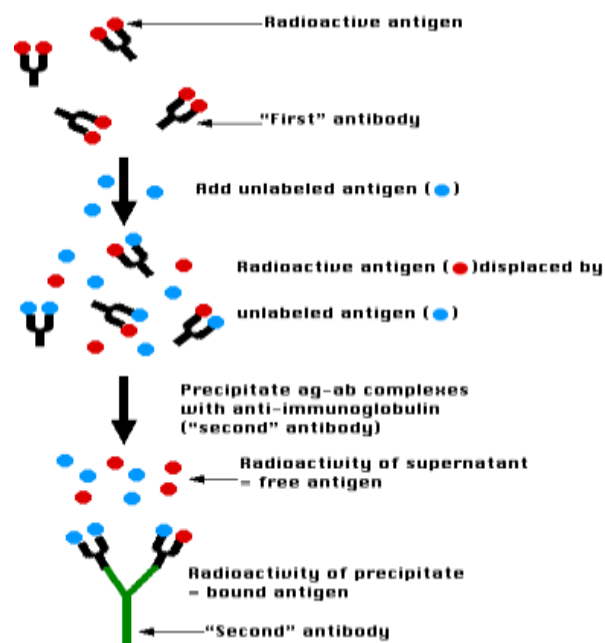
- ❖ This is a qualitative method.
- ❖ Antigen antibody complexes precipitate to form cone like structure (look like a rocket)
- ❖ Length of cone indicator the concentration of antigen.
- ❖ Negatively charged antigen are need for electrophoretic movement within agar matrix.

5. Describe about the Radioimmunoassay(RIA)?

Radioimmunoassay (RIA) is a very sensitive [in vitro assay](#) technique used to measure concentrations of [antigens](#) by use of antibodies.

- The labeled antigen is mixed with antibody at a concentration that saturates the antigen-binding sites of the antibody.
- Then test samples of unlabeled antigen of unknown concentration are added in progressively larger amounts.

- The antibody does not distinguish labeled from unlabeled antigen, so the two kinds of antigen compete for available binding sites on the antibody. As the concentration of unlabeled antigen increases, more labeled antigen will be displaced from the binding sites.
- The decrease in the amount of radiolabeled antigen bound to specific antibody in the presence of the test sample is measured in order to determine the amount of antigen present in the test sample.
- The antigen is generally labeled with a gamma-emitting isotope such as I^{125} , but beta-emitting isotopes such as tritium ($3H$) are also routinely used as labels.
- The radiolabeled antigen is part of the assay mixture; the test sample may be a complex mixture, such as serum or other body fluids, that contains the unlabeled antigen.

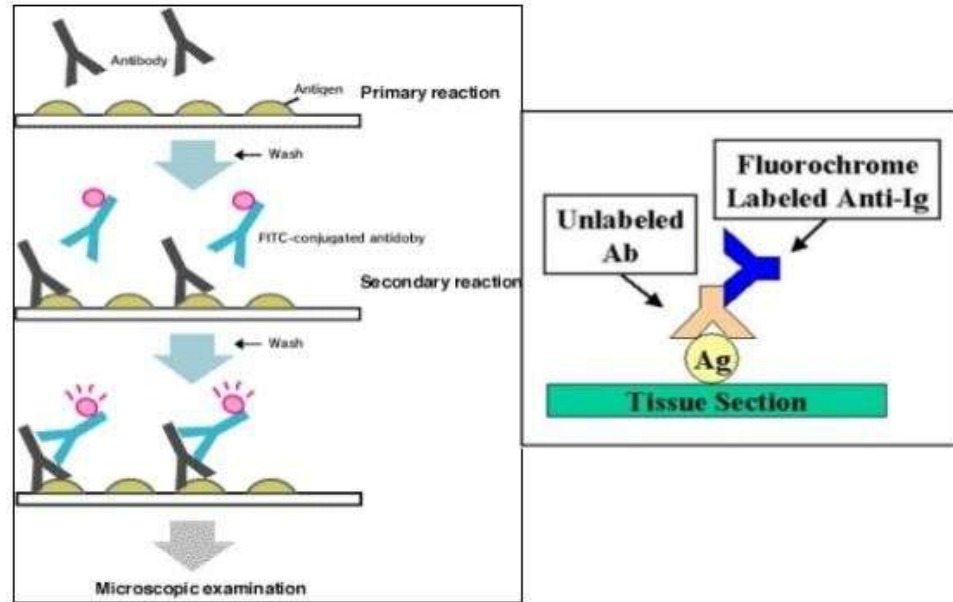


Application:

RIA has many uses, including narcotics (drug) detection, blood bank screening for the hepatitis (a highly contagious condition) virus, early cancer detection, measurement of growth hormone levels, tracking of the leukemia virus, diagnosis and treatment of peptic ulcers, and research with brain chemicals called neurotransmitters.

6. Write a note on Immunofluorescence?

- a technique used for the rapid identification of an antigen by exposing it to known antibodies tagged with the fluorescent dye fluorescein and observing the characteristic antigen-antibody reaction of precipitation.
- Immunofluorescence is a powerful technique that utilizes fluorescent-labeled antibodies to detect specific target antigens.
- The antibodies are chemically conjugated to fluorescent dyes such as fluorescein isothiocyanate (FITC) or tetramethyl rhodamine isothiocyanate (TRITC).
- These labeled antibodies bind (directly or indirectly) to the antigen of interest which allows for antigen detection through fluorescence techniques.
- There are two major types of immunofluorescence techniques, both based on the antigen and antibody reaction, in which the antibody attaches itself to a specific antigen.
- Techniques including direct immunofluorescence and indirect immunofluorescence.
- **Direct immunofluorescence** uses fluorescent-tagged antibodies to bind directly to the target antigen in the skin.
- **The indirect immunofluorescence** is used to detect circulating autoantibodies in immunobullous diseases.



Application:

- Immunofluorescence can be used on tissue sections, cultured [cell lines](#), or individual cells, and may be used to analyse the distribution of [proteins](#), [glycans](#), and small biological and non-biological molecules.
- Direct immunofluorescence -This technique can be used to detect viral, parasitic, tumor antigens from patient specimens or monolayer of cells.
- Indirect immunofluorescence.-It is often used to detect autoantibodies. Commonly used in the detection of anti-nuclear antibodies (ANA) found in the serum of patients with SLE

7.Explain in detail about the Immunoblotting?

- Immunoblotting techniques use antibodies (or other specific ligands in related techniques) to identify target proteins among a number of unrelated protein species
- They involve identification of protein target via antigen-antibody (or protein-ligand) specific reactions.
- Proteins are typically separated by electrophoresis and transferred onto membranes (usually nitrocellulose).
- The membrane is overlaid with a primary antibody for a specific target and then with a secondary antibody labeled, for example, with enzymes or with radioisotopes.

- When the ligand is not an antibody, the reaction can be visualized using a ligand that is directly labeled. Dot blot is a simplified procedure in which protein samples are not separated by electrophoresis but are spotted directly onto membrane.

Application

- immunoaffinity identification of proteins and analysis of immune responses
- genome-proteome interface technique.

7. Write a note on ELISA

- **Enzyme-linked immunosorbent assay**, commonly known as **ELISA** (or EIA), is similar in principle to RIA but depends on an enzyme rather than a radioactive label
- The enzyme-linked immunosorbent assay (ELISA) depends on an enzyme-substrate reaction that generates a colored reaction product.
- A number of enzymes have been employed for ELISA, including alkaline phosphatase, horseradish peroxidase, and galactosidase.

Indirect ELISA

Sandwich ELISA

Competitive ELISA

Indirect ELISA

- sample containing primary antibody (Ab1) is added to an antigen- coated microtiter well and allowed to react with the antigen attached to the well.
- Free Ab1 is washed away, the presence of antibody bound to the antigen is detected by adding an enzyme-conjugated secondary anti-isotype antibody (Ab2), which binds to the primary antibody.

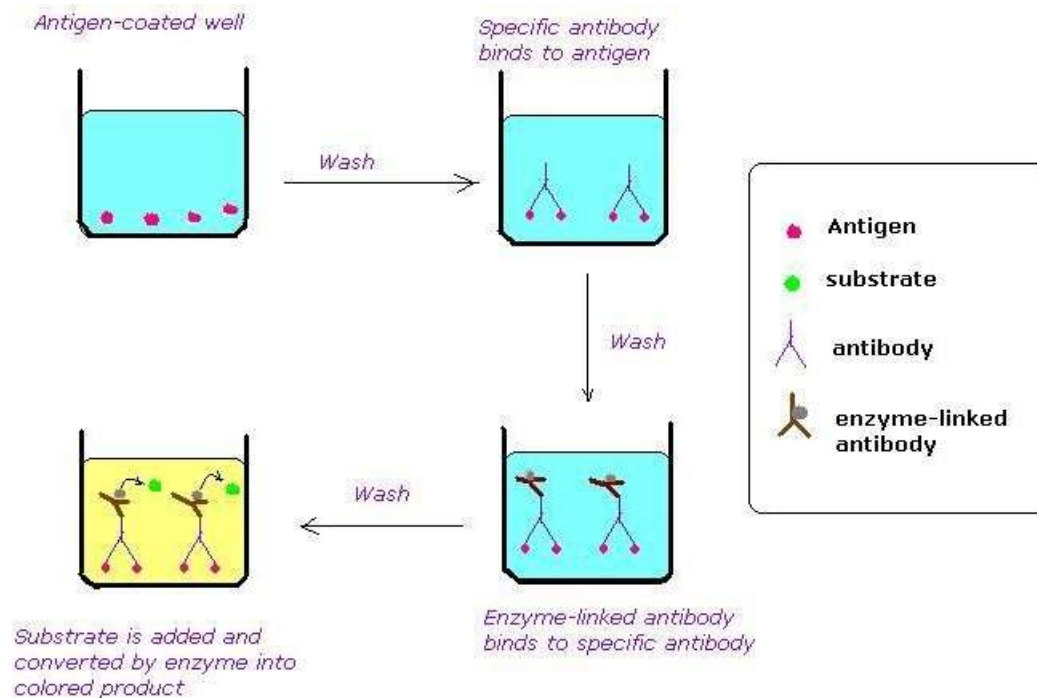


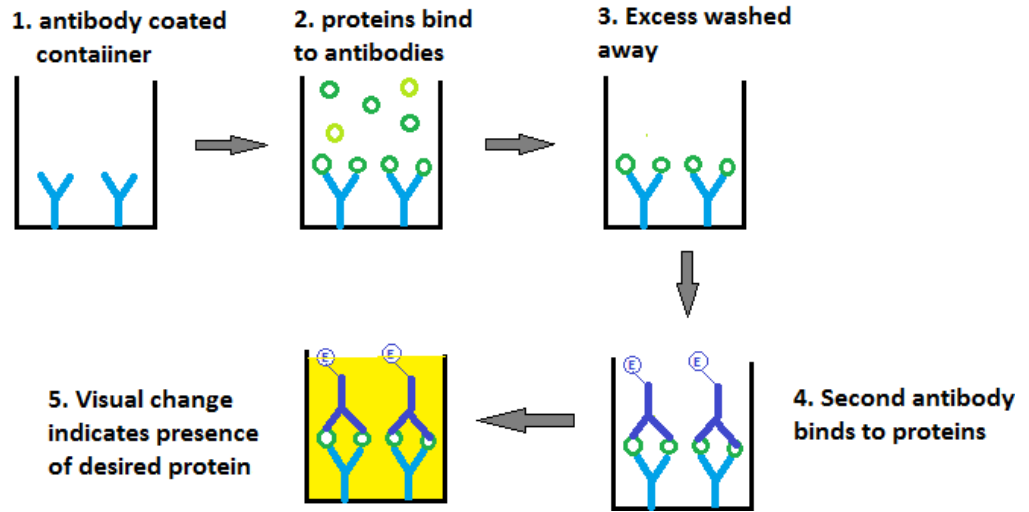
Figure 1: Indirect ELISA

- Any free Ab2 then is washed away, and a substrate for the enzyme is added. The amount of colored reaction product that forms is measured by specialized spectrophotometric plate

Sandwich ELISA

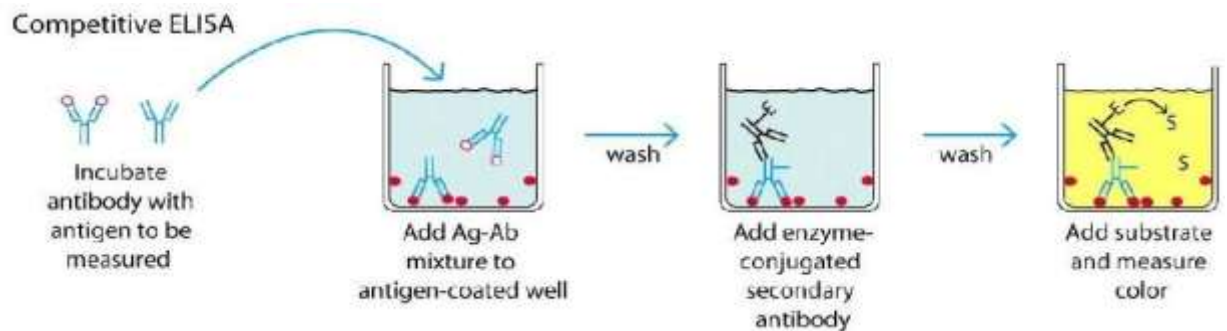
- Antigen can be measured by a sandwich ELISA. In this technique, the antibody (rather than the antigen) is immobilized on a microtiter well.
- A sample containing antigen is added and allowed to react with the immobilized antibody. After the well is washed, a second enzyme-linked antibody specific for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

Sandwich ELISA



Competitive ELISA

- Antibody is first incubated in solution with a sample containing antigen.
- The antigen-antibody mixture is then added to an antigen-coated microtiter well. The more antigen present in the sample, the less free antibody will be available to bind to the antigen-coated well.
- Addition of an enzyme-conjugated secondary antibody (Ab2) specific for the isotype of the primary antibody can be used to determine the amount of primary antibody bound to the well as in an indirect ELISA.



Application

- ELISA is a very sensitive laboratory method and can be quantitative when used in conjunction with standard curves.
- It can quantify the amount of antigen or antibody present in a given sample