

Lecture: 1**Immunology**

Immunology is the study of the immune system, including its responses to microbial pathogens and damaged tissues and its role in disease.

Immune system & Immune Response

The collection of cells, tissues, and molecules that mediate resistance to infections is called the **immune system**, and the coordinated reaction of these cells and molecules to infectious microbes comprises an **immune response**.

Immunity:

Immunity is defined as resistance to disease, specifically infectious disease.

Immunity is classified in to two major groups:

- ❖ **Innate Immunity:** is always present in healthy individuals.
- ❖ **Acquired Immunity:** Acquired (adaptive or specific) immunity is not present at birth. It is learned. The learning process starts when a person's immune system encounters foreign invaders and recognizes non-self-substances (antigens).

Differences between innate & acquired immunity:

Innate Immunity	Acquired immunity
1. Nonspecific to invader	Specific
2. It presented from birth	developed later in life
3. Genetically determination	Non
4. Consist of physical chemical barriers & cells	antibody & lymphocytes
5. No memory cells	memory cells

Innate Immunity

Innate Immunity

Innate immunity, also called natural immunity, is always present in healthy individuals, function to block the entry of microbes and to rapidly eliminate microbes that do succeed in entering host tissues.

It has the following characteristics in common:

1. Are present intrinsically with or without previous stimulation.
2. Have limited specificity for shared microbe and cellular structures (pathogen associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs).
3. It involves several defensive barriers including Anatomical, Humoral, and Cellular barriers:

Anatomical barriers, Humoral Barriers and Cellular Barriers

Anatomical barriers:

1. Mechanical (physical) Factors
2. Chemical Factors
3. Biological Factors

A. Anatomical barriers

1. Mechanical (physical) Factors:

- ***Skin*** is example of the first line of defense against infection.
- ***Coughing and sneezing*** mechanically eject pathogens from respiratory tract.
- ***Tears and urine flow*** also mechanically expels pathogens,
- ***mucus membrane*** secreted by the respiratory and gastrointestinal tract serves to trap microorganisms.

2. Chemical factors:

- ***Low pH*** (3-5) of skin due to fatty acids in sebum which is fungi and bacterial static,
- ***β- defensin in the*** respiratory tract act as antimicrobial.
- ***Enzymes*** like ***lysozyme and phospholipase*** in saliva, tears and breast milk are also antibacterial.
- In the stomach, ***low pH (1.2-3.0) of gastric juice and proteases*** serve as powerful chemical defenses against ingested pathogens.

3. Biological Factors:

Within the genitourinary and gastrointestinal tracts, **normal flora** is found

A person's normal flora is formed when non-pathogenic bacteria colonize epithelial surfaces. Normal flora protects the host by:

- * Competing with pathogenic bacteria for nutrients and attachment sites
- * Production of antibacterial substances.

The use of antibiotics can disrupt the normal flora, making pathogenic organisms more likely to cause disease

B. Humoral Barriers (against penetrating pathogens):

1. Complement System:

It is the major humoral non-specific defense mechanism. Once activated complement can lead to increase vascular permeability, recruitment of phagocytic cells, and lysis and opsonization of bacteria.

2. Coagulation System:

For example

1. **beta-lysine**, a protein produced by platelet during coagulation can lyse many Gr+ bacteria act as antimicrobial.
2. **Interferons**: Are protein that can limit virus replication in cells.
3. **Lysozyme**: breaks down the cell wall of bacteria.
4. **Interleukin-1**, IL-4, IL-6 and TNF: induce fever and production of acute phase proteins, some of which are antimicrobial because they can opsonize bacteria.

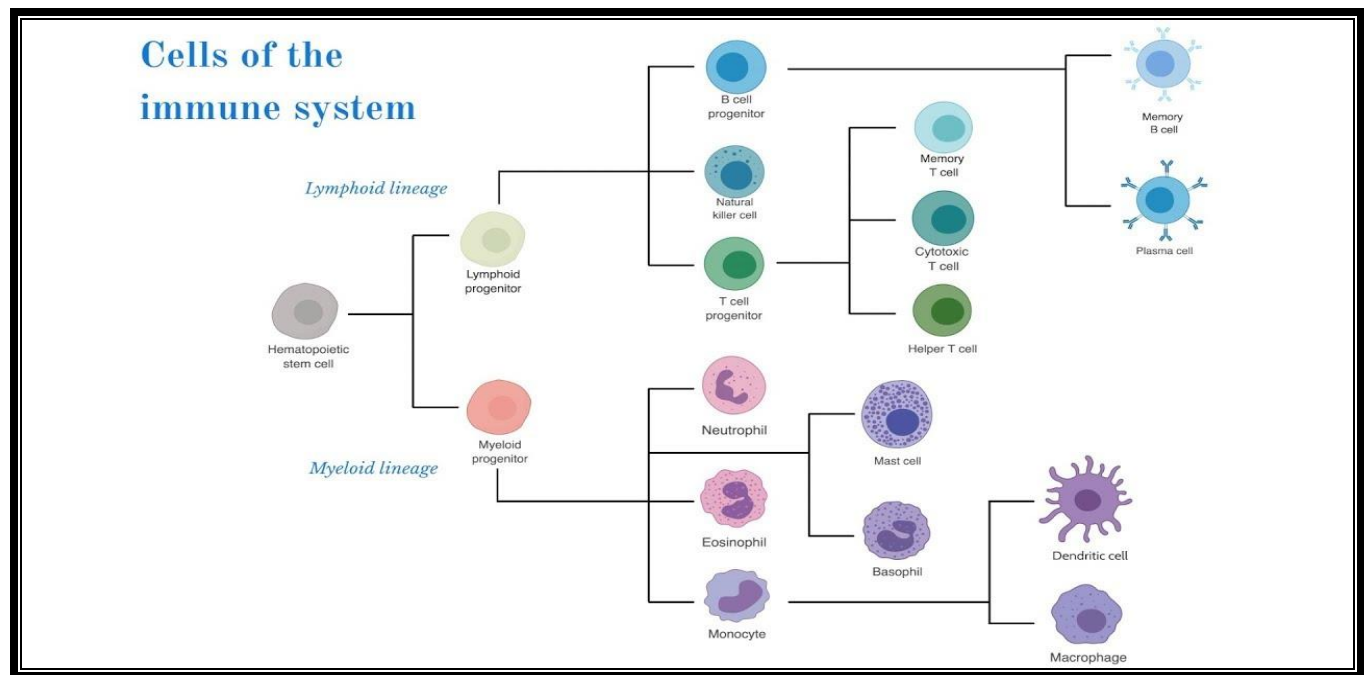
C. Cellular Barrier to infection:

1. **Neutrophil**: polymorphonuclear cells (PMNs) attack invading organisms and kill them intracellularly.
2. **Macrophages**: Tissue macrophages, intracellular killing of microorganisms.
3. **Natural Killer (NK) cells**: Kill virus infected and tumor cells.
4. **Eosinophils**: Have proteins in granules that are effective in killing certain parasites.
5. **Mast cells, platelets (thrombocytes), and endothelial cells.**

Lecture 2:**Cells and organs of the immune system****Immune system:**

Immune system is the collection of organs, cells and molecules which are responsible for defending us against infection.

Stem cells in the bone marrow (B.M) is the origin of all blood cells.

Cells of the Immune System:**Cells of the Innate Immune System:**

Innate immune system consists of many cells and molecules that represent the 2nd line of immunity.

Cells	Molecules
Neutrophil	
Macrophage	C3b
NK cell	INF

Neutrophil:

- Originates from myeloid stem cell in the B.M.
- Constitutes 65% of circulating white blood cells (WBC_s)
- Its nucleus is segmented, with variable number of segment (2-5 lobes). Thus, it is named polymorphonuclear (PMN) leukocyte.
- Its cytoplasm is granular, these granules are stained pale pink (neutral) by using wright stain.
- These cytoplasmic granules are the lysosomes which contain many degradative enzymes, such as:
 1. Myeloperoxidase (green)
 2. Lysosomes
 3. Lactoferrin.
 4. Proteinase, nuclease, and lipase

} these enzymes kill.
the pathogen (antigen).

Neutrophil has the following receptors on its surface:

- TLR (toll-like receptor): used for binding to antigen
- FcR (Fc receptor): used, for binding to Fc fragment of IgG and IgM (antibodies).
- C3bR (C3b receptor): used for binding to C3b complement component.

The function of neutrophil in innate immunity, it acts as phagocyte (engulf and kill pathogen {antigen} by phagocytosis.

The number of neutrophils in blood is increased (neutrophilia) in acute pyogenic bacterial infection.

Macrophage:

- Originates from myeloid stem cell as monocyte. Monocyte stay to blood for three days and then go to tissue to become macrophage.
- Monocyte constitutes 5-7 % Of circulating (WBC_s).
- Monocyte-macrophage has single large kidney-shaped nucleus (mono-one).

- Its cytoplasm has fine granules (so it appears granular) and these cytoplasmic granules are the lysosomes which contain many degradative enzymes.

Macrophage has the following receptors on its surface:

1. TLR (toll-like receptor): used for binding to antigen
2. FcR (Fc receptor): used for binding to Fc fragment of IgG and IgM (antibody).
3. C3bR (C3b receptor): used for binding to C3b complement component.

The function of macrophage in innate immunity:

Phagocytosis: it acts as phagocyte: engulf and kill pathogen [antigen}.

Cytokine production: when the macrophage engulf antigen, it produces several cytokines, such as:

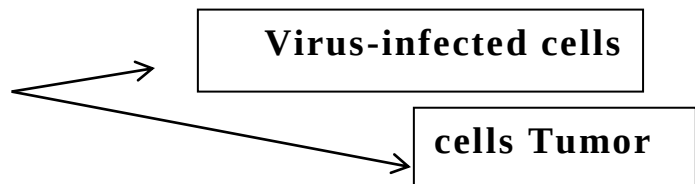
1. IL-1 (Interleukin-1): which induce inflammation
2. TNF (tumor necrosis factor): which has an inflammatory function
3. IL-8: Attracts neutrophil, NK cell to the site of infection
4. NO (nitric oxide): kill extra-cellular pathogen

The number of monocytes in blood is increased (monocytosis) in chronic granulomatous bacterial infection and in viral infection.

❖ Natural Killer Cells (NK cells):

- Originates from lymphoid stem cell.
- Constitutes 10% Of circulating lymphocytes
- It is a large granular lymphocyte (T-cell and B-cell are granular)

- It is specialized in killing of



- NK cell has the following receptors-on its surface:
- MHC-I receptor: used for binding to MHC-I molecule
- FcR: used for binding to Fc fragment of IgG or IgM (antibodies)

Note: MHC-I molecule is present on all normal nucleated cells of the body, and any cell become abnormal (virus-infected cell or tumor cell) loses the MHC-I molecule.

The function of NK cell in innate immunity: NK cell makes a daily survey to all body cells to check the presence of MHC-I molecule. Cells that not have MHC-I molecule (virus-infected cell or tumor cell) are killed by two mechanisms:

- Cytotoxin production → cell death
- Perforin: make pores in cell membrane of abnormal cell
- Granzyme: destructs the internal component of abnormal cell
- Fas-Fas Ligand interaction → programmed cell death (apoptosis)

Fas: is a molecule presented on the surface of all body cells (normal and abnormal cells)

Fas Ligand: is a molecule produced by NK cells after recognition of abnormal cells by MHC-I receptor.

Attachment of Fas Ligand molecule (on NK cell) to Fas molecule (on abnormal cell) result in generation of chemical signals that go to the nucleus of abnormal cell and leads to programmed death (apoptosis) of the abnormal cell.

Molecules of the Innate Immunity:

C3b complement: it will be explained in the complement lecture.

Interferon (INF):

- INF is a glycoprotein synthesized by a virus-infected cells.
- INF is released from virus-infected cell to protect the neighboring non-infected cells from viral infection.
- INF inhibits viral replication within the non-infected cells → kill the viruses inside the cell, i.e. it has no effect on viruses outside the cell (free viruses)
- Function of INF in innate immunity: the virus-infected cell secretes INF. INF binds to a receptor on the surface of non-infected neighboring cells → generation of a chemical signal which go to the nucleus of non-infected cell → induce synthesis of three enzymes (synthetase, ribonuclease and kinase). When the virus enters these non-infected cells, these enzymes will destroy the viral mRNA

inhibit viral protein synthesis → inhibit viral replication → kill the virus inside the cell.

Lecture 3:

Lymphoid Organs

Lymphoid Tissue: Specialized connective tissues and organs where the lymphocytes form the major cellular components.

The immune system consists of primary organs (central lymphoid organ) and secondary organs (Peripheral lymphoid organ).

The Primary Lymphoid Organ

The **primary lymphoid organs** are primary lymphoid tissues involved in the production and early selection of lymphocytes and consisting of:

1- **The Thymus:**

Thymus is bi-lobed organ situated above the heart, each lobe divided into outer part Cortex and inner part Medulla. Thymic function decline with ages, most active during the neonatal and pre-adolescent periods, the growth continue until reaches its maximum size at puberty and then undergo atrophy with aging and replaced by adipose tissue.

Cortex contain immature T-cells, highly dividing, highly dying thymocyte, about 95 % of T-cell will be die, 1-5 % will remain and migrate down to the medulla. There are cortical epithelial cells which also helps the thymocyte in their proliferation and maturation by secreting thymic hormones (Thymopoietin, Thymoline and Thymosine).

Medulla contain mature, less dividing, less dying thymocytes. That is mean thymus deals with proliferation and maturation of T-Lymphocytes and the letter (T) is related to it.

2- **Bone Marrow:**

Is the flexible tissue found in the interior of bones, it is the site of B-cell origin and development in human and mice.

Secondary lymphoid organs: plays a major role in mounting immune response to antigen,

- 1- Encapsulated organs: include spleen, lymph nodes, appendix, tonsil, skin (Langerhans cells) and liver. These organs act as filters or traps for foreign antigens, and that plays a major role in mounting immune response to Ags in the blood stream which is come into contact with macrophages and lymphocytes (B & T cells).

- 2- Un-capsulated organs: called mucosa associated lymphoid tissue (MALT), is found in various body sites which includes Payer's patches (aggregates of cells) in small intestine, and numerous lymphoid follicles in intestine, respiratory tract and genitourinary tract as well.

Primary & Secondary Lymphoid Organs

Features	Primary Lymphoid organ	Secondary Lymphoid Organ
Component	Bone marrow, fetal liver, thymus	Spleen, lymph nodes, and mucosa-associated lymphoid tissue (MALT) including tonsils, adenoids, respiratory, genitourinary, and gastrointestinal tracts
Proliferation and differentiation	Antigen-independent	Antigen-dependent
Product	Immunocompetent cells (B cells and T cells)	Effector cells (antibody-secreting plasma cells for humoral immune response and T helper and T cytotoxic cells for cell-mediated immune response)
Event	Development and maturation of B Cells and T cells	Induction of immune response: encounter of antigens and antigen-presenting cells (APC) with mature B and T cells, generation of effector cells, and memory cells

Inflammation

Inflammation is the recruitment of circulating blood leukocytes (e.g., phagocytes and lymphocytes) and various plasma proteins (e.g., complement, antibodies, fibrinogen) to sites of infection, where they function to destroy the microbes and repair damaged tissue.

Some of the many causes of inflammation include:

1. Infection and microbes,
2. Immune reactions between antigen and antibody.
3. Trauma, burns, physical or chemical agent and tissue necrosis.
4. Other causes of inflammation include: temperature extremes, oxygen

The duration of inflammation lasts from a few minutes to a few years. Depending on:

- ✓ the extent of the injury
- ✓ the type of injury
- ✓ the vascularity of the tissue

Signs of Inflammation

- ✓ Erythema
- ✓ Heat (fever)
- ✓ Edema
- ✓ Pain
- ✓ Loss of Function

These are signs of inflammation and it caused by:

1. Vasodilation: increase in the diameter of blood vessels (capillary) which is responsible for redness (erythema) and increase temperature (heat).
2. Increase permeability in the walls of blood vessels leading to escape of fluid and cells into space & surrounding tissues (swelling and edema).

Fever (pyrexia): this occurs due to bacterial products such as endotoxins. The functions of fever are:

1. Inhibit multiplication of bacteria.
2. Interfere with nutrition of bacteria.
3. Increase stimulates immune reaction like phagocytosis and specific immune response.

Functions of Inflammation

- ✓ Inactivate injurious agent
- ✓ Break down and remove dead tissue
- ✓ Initiate healing of tissue

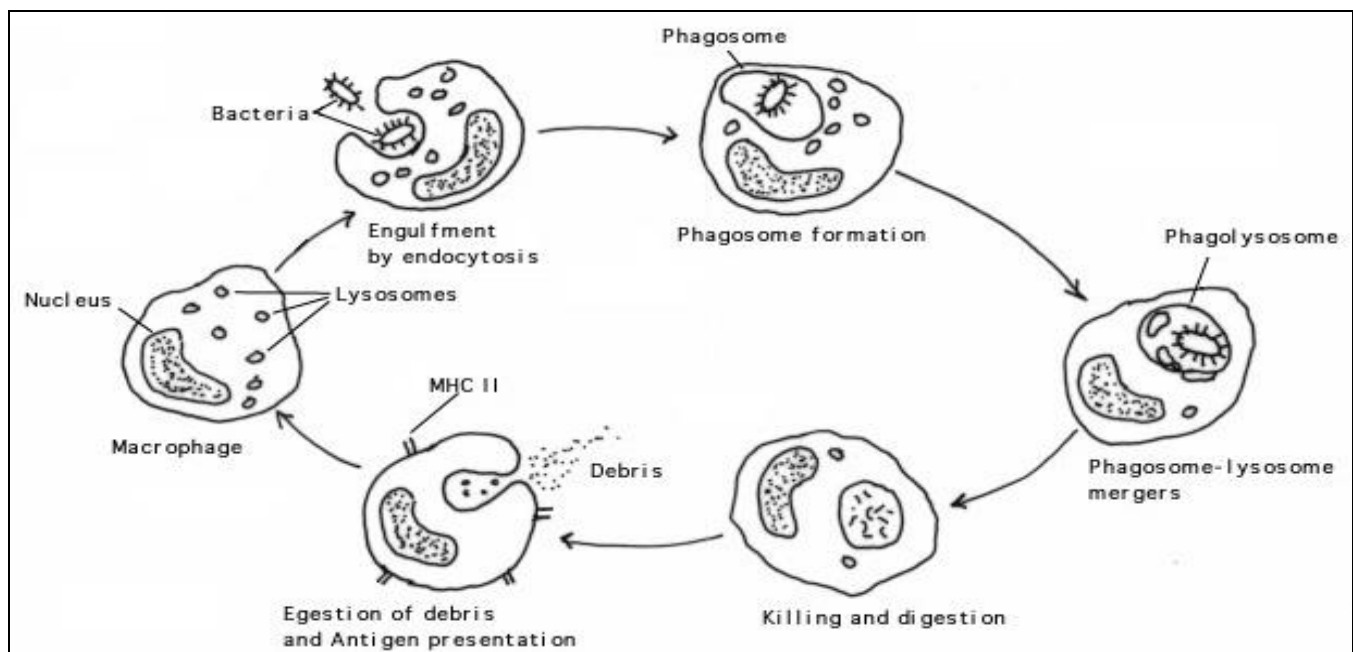
Lecture 4:

Phagocytosis and Phagocytic cells

The primary method used by the body to remove free microorganisms in the blood and tissue fluids, by engulfing, ingestion particles and destroying them enzymatically (lysozyme, myeloperoxidase, Protease) by phagocytic cells like macrophages.

Stages of Phagocytosis:

1. Recognition and attachment of microbes by phagocytes.
2. Ingestion of microbes and other materials by phagocytes.
3. Enzymatic destruction of microbes and other materials by fusion of lysosome with phagosome creates phagolysosome,
4. Waste material is expelled or assimilated.



Types of phagocytic cells:

1. **Polymorphonuclear cells (PMNs):** predominate in acute pyogenic infection, short-lived, circulating, granulated contains lysozyme and myeloperoxidase (degradative enzymes) and produce toxic substance (NO) which accelerate destruction of infectious microorganisms.

2. Macrophages / Monocytes:

Monocytes are less abundant in the blood than neutrophils, numbering 500 to 1000 per μL . They also ingest microbes in the blood and in tissues. During inflammatory reactions, monocytes enter extravascular tissues and differentiate into cells called **macrophages**, which, unlike neutrophils, survive in these sites for long periods. Macrophages present in tissues in different forms **such as**:

- Alveolar macrophages in lungs.
- Kupffer cells in Liver.
- Splenic macrophages in white pulp of spleen.
- Microglial cells in brain.
- Mesangial cells in kidneys.
- Osteoclasts in bones.
- Langerhans cells in skin and mucous membrane

These cells long-lived also migrate, engulf, and kill the bacteria multiply within the cells, do not possess myeloperoxidase, secrete plasminogen enzyme.

Antigen-Presenting Cells (APCs):

APCs are specialized cells located in the epithelium of the skin, gastrointestinal tract, and respiratory tract which capture antigens, transport them to peripheral lymphoid tissues, and display (present) them to lymphocytes. APCs connect the innate immunity with the acquired immunity.

Types of APCs:

1. Dendritic cells (DCs)
2. Macrophages.
3. B cells.

Steps of Antigen Presentation:

➤ **Uptake:**

APCs phagocytize exogenous pathogens such as bacteria, parasites, and toxins in the tissues then they digest phagocytized pathogens and begin to express the antigen in the form of a peptide on their MHC complexes, which enables them to present the antigen to naive T cells.

➤ **Degradation:**

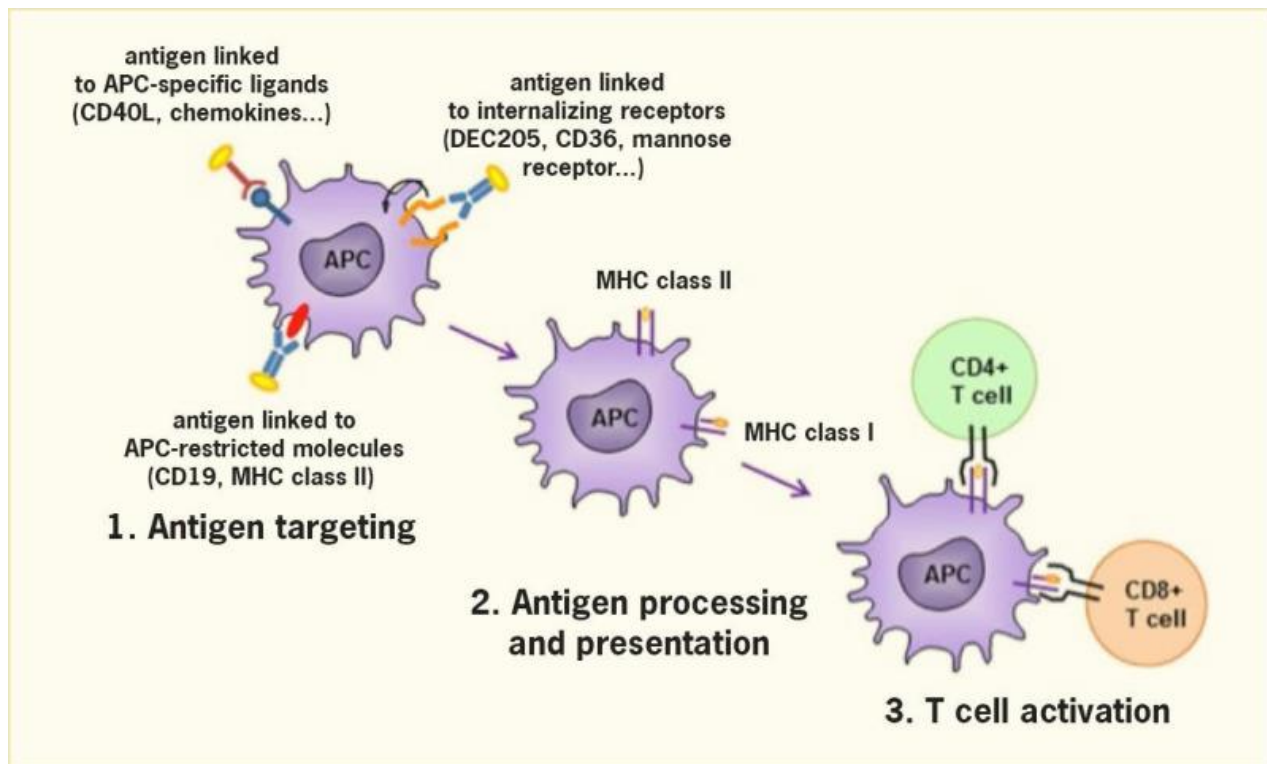
Controlled processing of antigens to peptides through proteolysis.

- **Peptide-MHC Complex Formation:**
Loading of the processed peptides onto MHC molecules.
- **Presentation of the Peptide-MHC Complex:**
Movement of MHC-Peptide Complexes on surface of cells for recognition by T-Cells.
- **Recognition of Antigens by T-cell and B-cells:**

Note:

Intracellular antigens are presented on the MHC-I molecule resulting in the activation of cytotoxic T-cells.

Extracellular antigens are presented on the MHC-II molecules and lead to the activation of helper T-cells.



Lecture:5**Adaptive (acquired or specific immunity) immunity**

Adaptive immunity, also called specific immunity or acquired immunity, requires expansion and differentiation of lymphocytes in response to microbes before it can provide effective defense; that is, it adapts to the presence of microbial invaders.

Whereas the mechanisms of innate immunity recognize structures shared by classes of microbes, the cells of adaptive immunity (lymphocytes) express receptors that specifically recognize a much wider variety of molecules produced by microbes as well as noninfectious substances.

Characteristics of Acquired Immunity:

1. Specificity Ensures that distinct antigens elicit specific responses.
2. Diversity: Enables immune system to respond to a large variety of antigens
3. Memory: Leads to enhanced responses to repeated exposures to the same antigens
4. Clonal expansion: Increases number of antigen-specific lymphocytes from a small number of naive lymphocytes
5. Contraction and homeostasis: Allow immune system to respond to newly encountered antigens.
6. Non-reactivity to self: Prevents injury to the host during responses to foreign antigens

Adaptive immunity can be subdivided into two major types depending on how immunity was introduced.

- ✓ Naturally acquired immunity occurs through contact with a disease-causing agent.
- ✓ Artificially acquired immunity develops for example by vaccination.

Both naturally and artificially acquired immunity can be further subdivided into **active** or **passive**, depending on whether immunity is induced in the host or passively transferred from immune host. and each of these types can in turn be naturally or artificially acquired.

Passive immunity

Passive immunity is protection by products produced by an animal or human and transferred to another human, usually by injection. Passive immunity often provides effective protection, but this protection wanes (disappears) with time, usually within a few weeks or months. They are divided into two:

I. Naturally acquired passive immunity: Refers to antibodies transferred from mother to fetus across the placenta and to the newborn in colostrum and breast milk during the first few months of life.

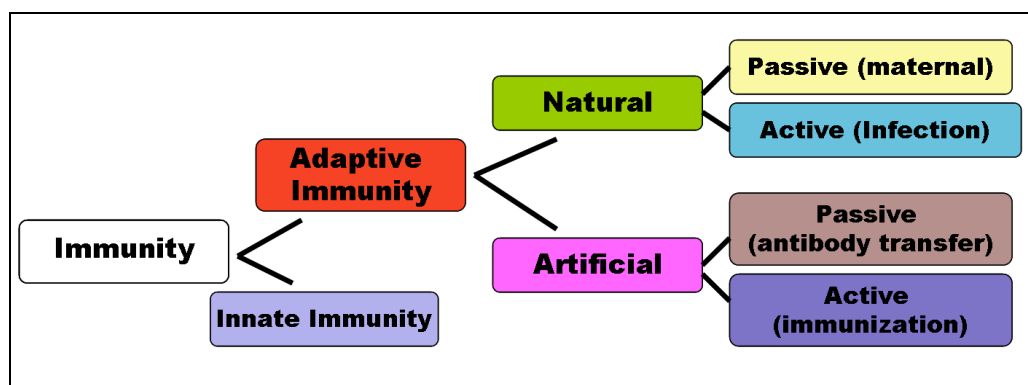
II. Artificially acquired passive immunity: Is introduction of antibodies that are formed by an animal or a human to an individual to prevent or treat infection.

Active immunity

Active immunity is protection that is produced by the person's own immune system. This type of immunity usually lasts for many years, often during a lifetime.

I. Naturally acquired active immunity: Is immunity that comes from infections encountered in daily life.

II. Artificially acquired active immunity: It is stimulated by the use of vaccines to artificially establish a state of immunity.



Comparison between Active and Passive Acquired Immunity:

	Active	Passive
1. Source	Self	other human or lower animal
2. Effectiveness	High	moderate to low
3. Method	infection or vaccination or immunization	through placenta or injection γ -globulin
4. Time to develop	7-10 days	immediately
5. Duration	long time (may be years)	short time (few days or several weeks)
6. Easy of reactivity	easy by booster dose	dangerous (may cause anaphylaxis)

Cells of the Adaptive Immune system:

Lymphocytes: produced in the primary lymphoid organs then migrates by the circulation to the secondary lymphoid organs, is a type of white blood cells. The three major types of lymphocytes are T-cell, B-cell

a- **T- Lymphocytes:** classified according to their function into:

I- Cells with **CD4⁺ marker** are called **helper T-cells**, become activated by antigen presenting macrophages and then help in generating T-cytotoxic cells and cooperating with B-cells in production of antibodies.

II- Cells with **CD8⁺ marker** are **cytotoxic T-cells** (Tc cells), cytotoxic against tumor cells and host cells infected with intracellular pathogens on the basis of MHC presenting foreign proteins.

III- **T-suppressor / regulator cells** which suppress T and B-cell responses. T-regulatory are subpopulation of T-helper cells, serve as regulator of T-cells responses by secreting inhibitory cytokines that decrease the activity of both T & B-cells.

a- **B-lymphocytes:** develop from stem cells in the bone marrow, these cells produce antibodies with specificity for antigen after determination into plasma cells and make it easier for immune cells to destroy the antigens.

Lecture: 6

The Antigen

An antigen is defined as an organism, a molecule, or part of a molecule that is recognized by the immune system. **or**

Any substance (usually foreign) that binds specifically to an antibody or a T-cell receptor; often is used as a synonym for **immunogen**.

An Immunogens:

Immunogen is a specific type of antigen which is capable of inducing an immune response and binds to the products of the immune response,

Note: *the antigen is able to combine with the products of the immune response once they are made but it may not have the ability to induce it.*

*Note: all immunogens are antigens, but some antigens (e.g., **haptens**) are not immunogens.*

The foreign substances that induce an immune response possess two properties:

1. **Immunogenicity:** is the ability of a substance (immunogen) to induce a specific immune response, resulting in the formation of antibodies or cell mediated immune response.
2. **Antigenicity:** is the property of a substance (antigen) that causes it to react specifically with the final products of the immune response (secreted antibodies and/or surface receptors on T-cells)

Hapten:

Hapten is a molecule or substance with low molecular weight (non-immunogenic) that cannot induce an immune response on its own such as antibiotics, analgesics, penicillin and other low- molecular weight compounds

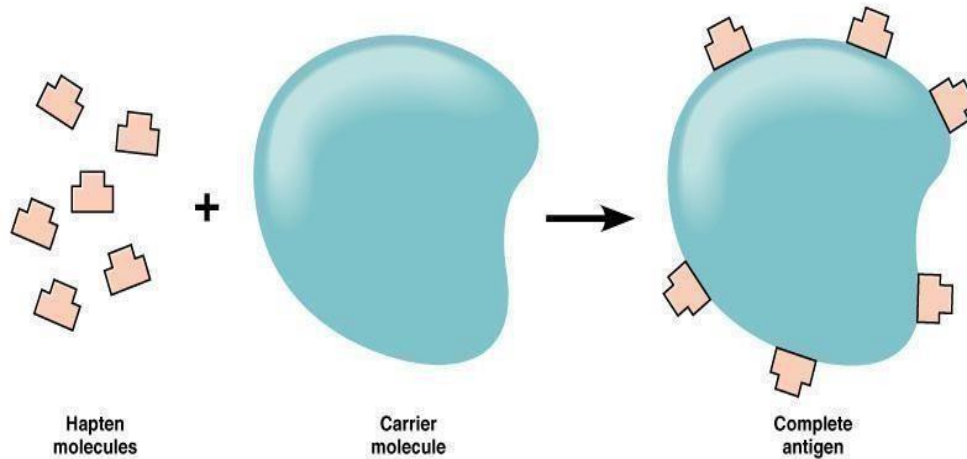
However, if a hapten is combined with larger macromolecules (usually proteins) which serve as carriers then a response can be induced.

Hapten-carrier conjugate A covalent combination of a small molecule (**hapten**) with a large carrier molecule or structure.

Carrier An immunogenic molecule containing antigenic determinants recognized by T cells including albumins, globulins, or synthetic polypeptides.

Conjugation of a carrier to a non-immunogenic **hapten** renders the hapten immunogenic.

Ex: Serum Protein such as Albumin or Globulin.



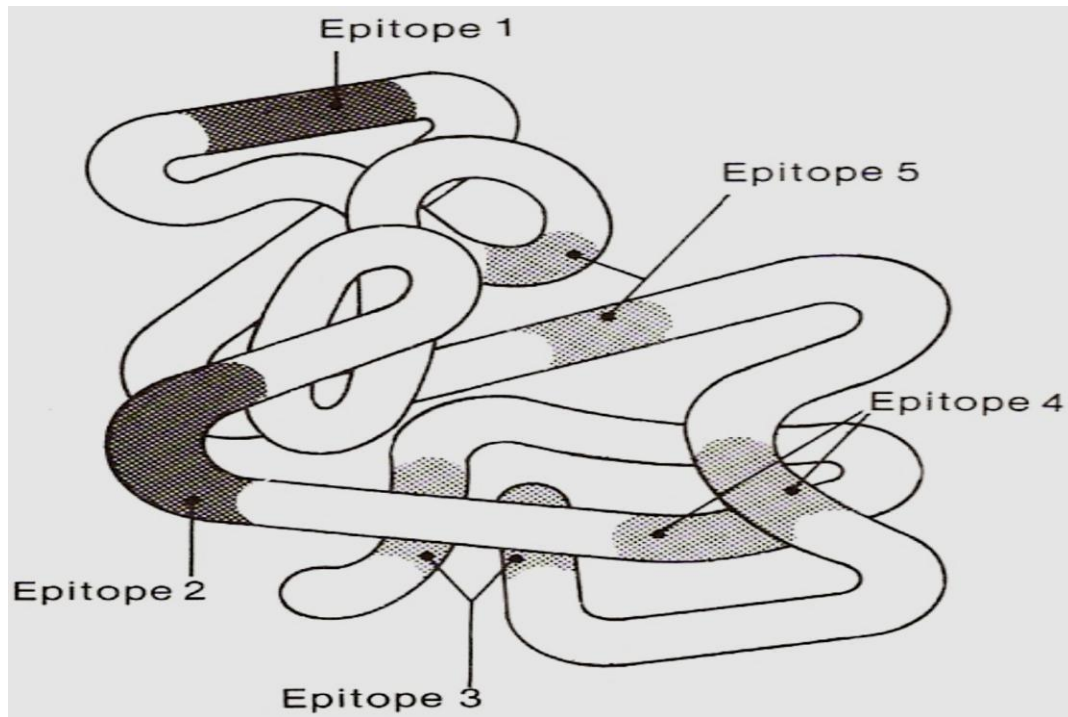
An adjuvant: Factors that are added to a vaccine mixture to enhance the immune response to antigen by activating innate immune cells. Dead mycobacterium were among the original adjuvants, but more refined preparation include alum, cytokines, and/or lipids are used.

Adjuvants function in one or more of the following ways;

- 1- By prolonging retention of the immunogen,
- 2- By increasing the effective size of the immunogen,
- 3- By stimulating the local influx of macrophages and/or other immune cells to the injection site and promoting their subsequent activities.

The Epitope (antigenic determinant or antigenic specificity):

- Epitopes (also called **determinant groups or antigenic determinants**) are the sites either on or within the antigen with which antibodies react.
- Antibodies are specific for epitopes.
- A particular antigen molecule may have many different epitopes or determinant, each of which can be a target for antibody binding.
- The epitopes on an antigen can be linear or conformational



Types of Antigens:

1. **Heterophil Antigens:** are identical antigens found in the cells of different species.
2. **Graft Antigen:** divided in three types:
 - **Autoantigens:** These are the antigens belonging to host itself.
 - **Alloantigens or Isoantigens:** are antigens found in different members of the same species (the red blood cell antigens A and B are examples).
 - **Xenoantigen:** An antigen found in more than one species. Certain proteins of brain, kidney, thyroglobulin and lens protein of one species share specificity with that of another species
3. **Bacterial Antigen:**
4. **Super antigens (SAGs)** : are a class of antigens that cause non-specific activation of T-cells at the T cells receptor (TCR) resulting in polyclonal T cell activation and massive secretion of cytokine (e.g. IL-2, IL-1, IL-6, TNF α), then resulting in toxic shock syndrome. SAGs are produced by some pathogenic viruses and bacteria most

likely as a defense mechanism against the immune system. SAgS including retroviral protein and bacterial toxins (staphylococcal enterotoxins, toxic shock syndrome toxin).

Factors Influencing Immunogenicity:

1. Chemical complexity:

- Proteins are usually very good immunogens.
- Pure polysaccharides and lipopolysaccharides are good immunogens.
- Nucleic acids are usually poorly immunogenic.
- Lipids are non-immunogenic, although they may be haptens.

2. Foreignness

- An antigen must be foreign to the host with which it makes contact to serve as an immunogen.
- The degree of immunogenicity is dependent upon the degree of foreignness.
- The greater the phylogenetic difference between species, the more foreign something becomes with high immunogenicity.

3. Molecular weight

- High molecular weight increase immunogenicity that induces immune response.
- The best immunogens are in the range more than 10000 Dalton (Da.) and the most active immunogen is with 100000 Da., while the small molecules with 5-10,000 Dalton (Da.) are generally poor immunogens.

4. Degradability

- The molecules with the ability to biodegrade are the best immunogens to be presented by MHC molecules to activate T-cells (Ag processing by Ag-presenting cells (APC)).
- Macromolecules that cannot be degraded and presented with MHC molecules are poor immunogens. Example such as polystyrene

5. Rout of immunization

The rout of antigen administration plays an important role in immunogenicity. According to high immunogenicity the routs divided as following:

- Intravenous (iv): into a vein (non-favorite route because it is minimizing the immune response).
- Intradermal (id): into the skin.
- Subcutaneous (sc): beneath the skin (best route).
- Intramuscular (im): into a muscle (the best route because it is prolonging the period of immune response).
- Intraperitoneal (ip): into the peritoneal cavity (best route)

6. **Stability:** Ag with high stability acts as immunogen due to its ability to activate the immune response while the opposite is not immunogens because they are haven't a rigid structure enough to be stably bound by antibodies such as lipids and gelatin.

7. Antigen dose:

- Too high or low dose of Ag will fail to activate enough immune response and cause immunologic unresponsiveness state (Tolerance).
- Appropriate dose of Ag cause optimum antigenicity.

*Lecture: 7***Immunoglobulins (Antibodies)**

Antibody: Antibodies are glycoproteins, which are sensitized, and secreted by plasma cells in response to specific antigenic stimulation and it forms about 20% of plasma protein.

Functions of Antibodies:

1. Neutralizing viruses and toxins or toxoids, which neutralize the antigen.
2. Agglutinin: immobilize motile bacteria and aggregate cells forming clumps.
3. Precipitins: which form complexes with soluble antigens forming precipitates.
4. Lysine: antibodies together with complement lyse the antigenic cells.
5. Opsonins: antibodies combine with surface components of microbes and other cells so that they are more readily phagocytized.
6. Activation & fixation of complement: IgM and most IgG subclasses can activate complement system resulting to cell lysis.

Basic Structure of Immunoglobulins

Immunoglobulin is a Y shaped molecule consists of four amino acid chains:

- Two heavy (H) chains (50 000 Daltons)
- Two light (L) chains (25000 Daltons).

The antibody molecule could be anatomically divided into two fragments:

- **Fab** fragments (for Ag binding).
- **FC** fragment (for Ag elimination).

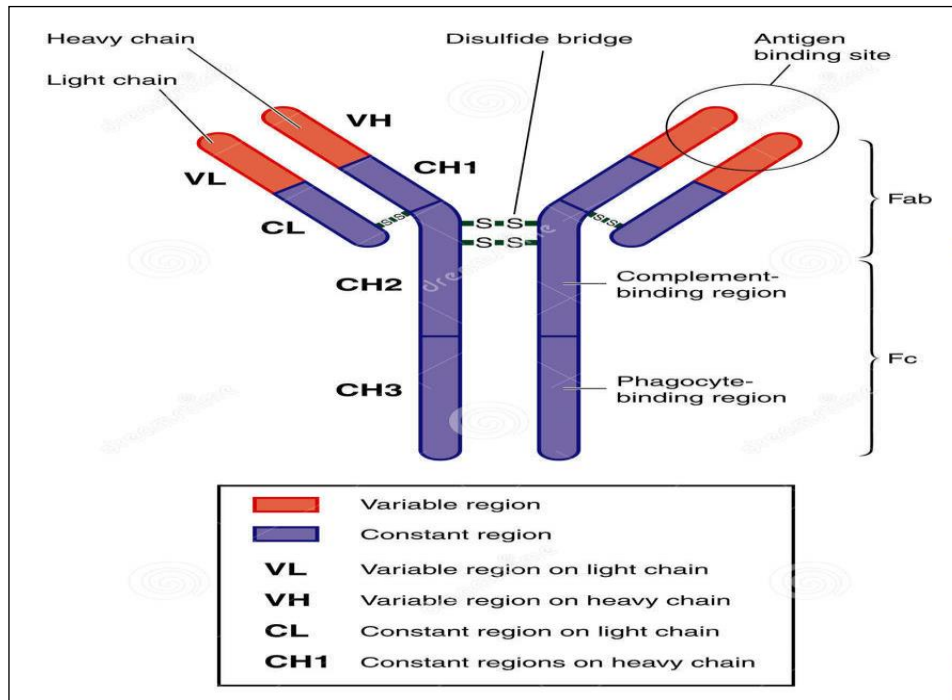
Each Chains contain two regions:

- Variable (V) region: contain variable amino acid sequence.
- Constant (C) region: contain constant amino acid sequence

Each chain has many domains:

- Each heavy chain has four domains (CH1, CH2, CH3, and VH).
- Each light chain has two domains (CL and VL)

The constant region of the light chain is determining the mechanisms to destroy antigens. This region of light chain is either Kappa (K) or Lambda (λ) with ratio 2:1.



Light Chain:

1. Variable region: contains the following structures:

- **Hot spots (hyper variable area):** these are three areas in the variable region which characterized by high variability in amino acid sequencing to form the Ag binding site.
- V_L domain.

2. Constant region: contains the C_L domain

Note: There are two types of the light chain: **Kappa and Lambda**

Heavy Chain:

1. Variable region: contain the following structure

- Hot spots (hyper variable area): these are three areas in the variable region which characterized by high variability in amino acid sequencing to form Ag binding site.
- V_H domain.

2. Constant region: contain the following structure:

- $CH1$ domain.
- $CH2$ domain: it is the site of binding to complements.
- $CH3$ domain: it is the site of binding to phagocyte and NK cell.

Note: Antibodies are divided into 5 classes (isotypes) based on their constant region structure (amino acid sequences) of heavy chain and immune function:

1. Immunoglobulin Gamma (γ) IgG.
2. Immunoglobulin Alpha (α) IgA.
3. Immunoglobulin Mu (μ) IgM.
4. Immunoglobulin Delta (δ) IgD.
5. Immunoglobulin Epsilon (ϵ) IgE.

In addition, there are subtypes of IgA (IgA1, IgA2), while IgG have 4 subtypes (IgG1-IgG4).

Hinge Region:

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

Note: in case if IgM and IgE there is additional domain in the heavy chain (CH4 domain)

Areas:

- 1-Switch area  located between V_L and C_{H1} domains.
It is the site of class switching (transformation from IgM to IgG or IgA or IgE)
- 2.Hinge area  located between C_{H1} and C_{H2} domains.
It is the site of opening of Immunoglobulin (transformation from inactive to active Ig)
- 3.Paratope light  a cleft formed by the three hot spots of
Chain and adjacent three hot spots chain. It is the site of Ag binding.

Properties of Human Immunoglobulins

Properties	IgG	IgA	IgM	IgD	IgE
Structure	Monomer	Monomer, Dimer	Pentamer	Monomer	Monomer
Number of binding sites	2	2,4	10	2	2
Biological function	Long term immunity	Secretory Ab on mammary mammalian	Produced on end response to Ag	Receptor on B-cell	Ab of allergy and worm infections
Molecular weight x1000 Da	150	150, 60	900	150	190
Complement fixation	+	0	++++	0	0
Serum concentration approximate (mg/dl)	1000	200	120	3	0.05
Serum half-life (days)	23	6	5	3	2
Placental transfer	+	0	0	0	0
Binding to Fc receptor on	Phagocyte	Phagocyte	B-cell	B-cell	Mast cell and basophil

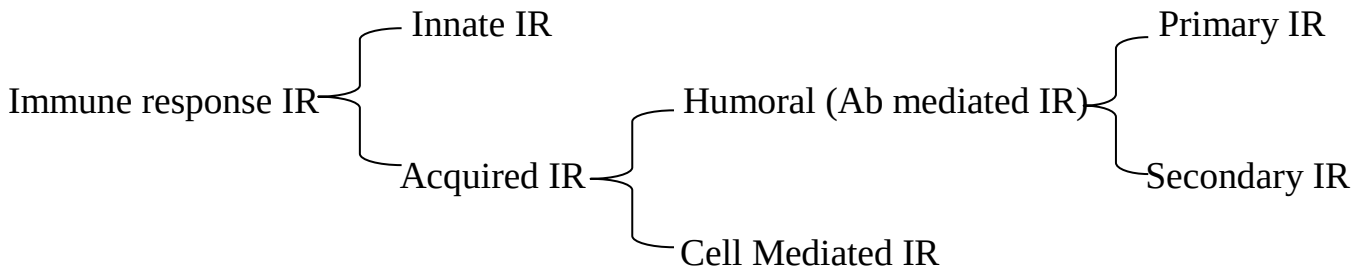
Lecture 8:

Immune Response

Immune response (IR): a protective response of the body's immune system to an antigen, especially a microorganism or that causes disease.

Mechanisms of IR:

1. Recognize, processed and presentation of antigen.
2. Cooperation of cells.
3. Destruction of specific Ag.



Adaptive Humoral Immune Response:

For activation, B-cells need the binding of antigen to specific surface Ig on B-cell leading to their proliferation and differentiation into effective plasma cells and memory cells. Plasma cells produce and secrete antibody specific for the antigen.

Humoral or Ab mediated immunity develops mainly against:

- Extracellular pathogens such as staphylococci, streptococci, encapsulated pathogens (eg: Pneumococcus, Neisseria).
- Pathogens that secrete exotoxins and endotoxins (eg: Corynebacterium diphtheria).
- Viruses

Functions:

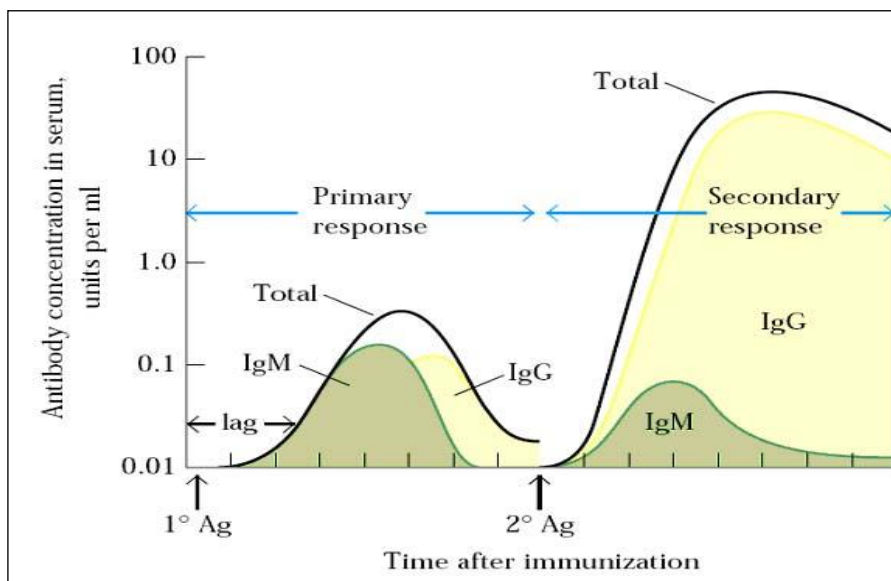
1. Antibodies neutralize exotoxins and some viruses (eg: rabies, Hepatitis A and B).
2. IgM and IgG can activate the complement system which can opsonize pathogens leading to ingestion by phagocytic cells, or can cause lysis of the organisms.

Note: Exposure to an Ag by an individual for the first time is called primary IR and re-exposure to the same antigen is called secondary IR which develops due to immunologic memory.

There are two types of adaptive humoral IR:

1. Primary humoral IR characterized by:

- ✓ Latent phase (lag phase): in the first few days (7-10 days), is a period for T and B cells to bind Ag then activated and differentiated. Ab levels in the serum is zero (no Ab production).
- ✓ Exponential phase (log phase): increased of serum Ab level logarithmically and reaches a peak.
- ✓ Steady phase (plateaus phase): production and degradation of Abs are balanced.
- ✓ Decline phase: the IR begins to shut down, and Abs concentration in the serum decreases rapidly due to combination with Ags.



2. Secondary IR (anamnestic or memory IR):

In the second exposure to same Ag, there is a rapid Ab response (the lag phase is only 3-5 days) this is related to memory cells produced after the first contact.

Comparison between Primary & Secondary IR:

Primary IR	Secondary IR
The lag phase is often longer, weeks or months	Very short (3-4days) due to memory presence cells
IgM predominant with low IgG	IgG predominant with low IgM
Antibody titer is low	Antibody titer is high
Cell (native B cell)	Memory B cell
Ab level decline rapidly	Ab level tend to remain high for longer

Cell-mediated immunity

- The main defense is against intracellular pathogens like Mycobacterium, viruses. It may be involved in tumor immunity. Immune response in this case is due to activation of T-cells leads to production of cell-mediated immunity by a direct attack on foreign cells.
- Two different types of T-cells are generated, T-helper (CD^{+4}) and cytotoxic T-cells (CD^{+8}), their killing are specific.
- Helper T-cells are killed the target cells specifically by producing lymphokines, while cytotoxic T-cells are responsible for direct usually of virally-infected cells.

Regulation of the immune response:

Regulatory mechanisms can act at the recognition, activation or effector phases of an immune response and include:

1. Recognition of antigen in the absence of co-stimulation —————> anergy
2. Recognition of antigen with CTLA-4 engagement of B7 —————> down regulation of T cell activation.
3. Secretion of inhibitory cytokines like IL-10 and TGF- β .
4. Idiotype/anti-idiotypic interactions leading to stimulation or inhibition of immune responses.
5. Dose and route of antigen exposure.

Regulation by antibody:

Soluble antibody can compete with antigen receptors on B cells and block or prevent B cell activation.

Antigen antibody complexes can bind to Fc receptors on B cells, sending an inhibitory signal to B cells. In this case the regulation is occurring at the recognition level.

Antibody can also regulate activation (enhance) by maintaining a source of antigen for APC through the activation of complement system. Complement activation allows for ligation to the complement receptor on the APC.

Regulation by cytokines:

- **Proinflammatory cytokines:** IL1-alpha, IL1-beta, IL6, and TNF-alpha induce the immune response.
- **Anti-inflammatory cytokines:** IL-1ra, IL-4, IL-6, IL-10, IL-11, IL-13, and TGF- β , inhibit the synthesis of proinflammatory cytokines and enhance the stimulation of Th2 cells and other inhibitory mechanisms.

Regulation by regulatory T cells (Tregs):

- Regulatory T cells (Tregs) are a recently described populations of cells that can regulate immune responses.
- They do not prevent initial T cell activation; rather, they inhibit a sustained response and prevent chronic and potentially damaging responses.
- They do not have characteristics of Th1, Th2 or TH17 cells but they can suppress both Th1 and Th2 responses.

Lecture 9:**Major Histocompatibility Complex (MHC)**

MHC is a set of molecules displayed on cell surfaces that are responsible for lymphocyte recognition and antigen presentation. It is controlling the immune response through recognition of "self" and "non- self".

MHC genes:

The MHC genes consist of three classes located in the short arm of chromosome 6. In human these genes are called human leukocyte antigens (HLA). According to Mendelian rule Half of these genes are inherited from father and another half of genes from mother.

Class I:

Includes three loci (A, C, and B) responsible for production of MHC I molecule.

Class II:

Includes three loci (DP, DQ, and DR) responsible for production of MHC II molecule.

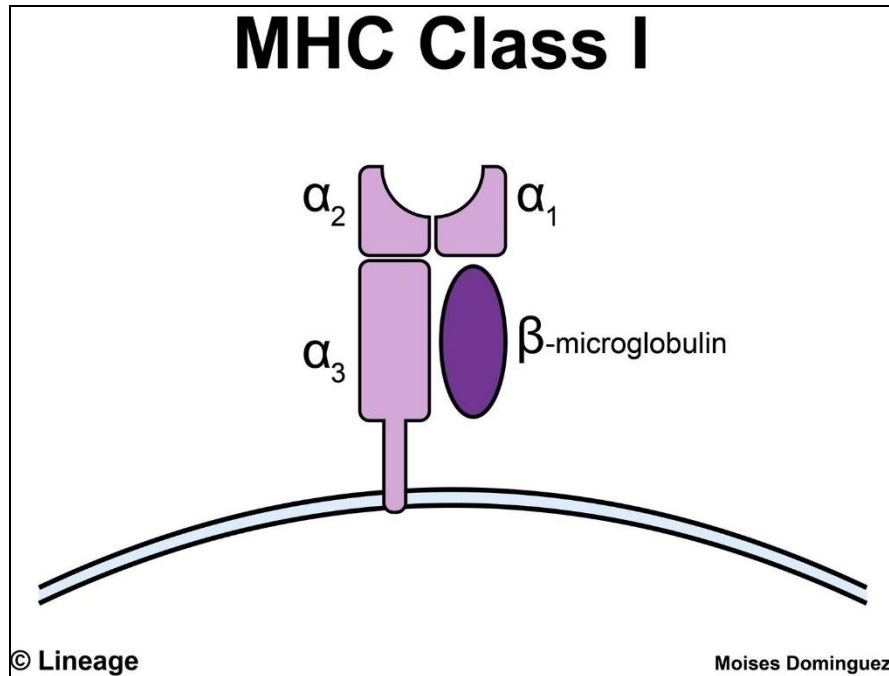
Class III:

Genes found in this region responsible for production of complement proteins C2 and C4 as well as TNF, and Leucotrin (LT).

Nomenclature of MHC (HLA) genes: HLA + locus + allele. For example: HLA - B 27, HLA - DR 4.

MHC class I molecules:

- Glycoproteins found on surfaces of all nucleotide human cells.
- Play a role in innate and adaptive immunity.
- It consists of two chains (heavy and light). The heavy chain has three parts (extracellular, transmembranous, and intracellular). The extracellular part contains three domains ($\alpha 1$, $\alpha 2$, $\alpha 3$). The light chain has only extracellular part that contains only one domain ($\beta 1m$).

**The Function of MHC I in immunity:**

In Innate immunity: The MHC I molecule is regarded as an indicator for the normality of the body cells during the NK cell survey.

Adaptive immunity:

Class I MHC molecules involve in presenting intracellular or endogenous pathogens or antigens → activation of CD^{+8} T cell (cytotoxic T cell).

Class I pathway of antigen presentation

- Under normal condition the MHC class I molecules forms a complex with the self-peptides or self-antigens.
- When a virus infected a normal cell, the viral DNA moves inside the cell and produce viral proteins in the cytosol.
- In the cytosol there is a protein complex called the proteasome that degrade the viral protein into smaller peptides (8-15 amino acid long).
- These fragmented peptides are transported into the endoplasmic reticulum by a peptide delivery system called the transporter associated with antigen processing (TAP). TAP is made up of two domains or subunits called TAP 1 and TAP 2.

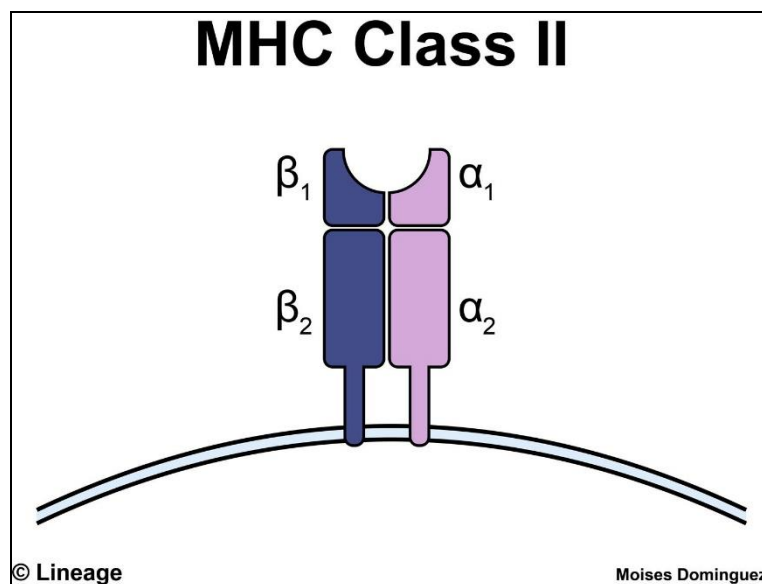
- Inside the endoplasmic reticulum the α and β chains of MHC class I molecules are synthesized and moves towards the TAP. As a result, the peptides bind to the class I MHC molecule forming the P-MHC class I complex.
- The complex moves to the Golgi apparatus then moves towards the surface of the plasma membrane.
- T cell receptors recognize the antigen peptide complex resulting in the activation and differentiation of CD^{+8} T cell which kills the virus infected cells.

MHC class II Molecules:

- Class II MHC molecules are heterodimers and characterized by two non-covalently connected polypeptide chains.
- Found only in antigen presenting cells (APC) dendritic cell, macrophage, and B cell.
- Play a role in adaptive immunity only.

The Structure of MHC II Molecule:

- It consists of two chains (heavy and light).
- The heavy chain has three parts (extracellular, transmembranous, and intracellular). The extracellular part contains two domains (α_1 , α_2).
- The light chain also has three parts (extracellular, transmembranous, and intracellular). The extracellular part contains two domains (β_1 , β_2).



The Function of MHC II in immunity:

MHC class II molecules are responsible for presenting exogenous or extracellular pathogen or antigen to the helper T cell (Th) resulting in to the activation and differentiation of helper T cell (CD⁺₄).

Class II pathway of antigen presentation

- The pathway starts only after the engulfment of the antigen by the antigen presenting cells.
- Inside the cell, the antigen carries a covering called an endosome. The endosome is fused with the lysosome present in the cytoplasm and forms endolysosome. As a result, the foreign protein is degraded by the proteolytic enzyme present inside the lysosome and small peptides are formed.
- The class II MHC molecules are synthesized and formed in the endoplasmic reticulum. The α and β chain of the molecule is also associated with the invariant chain. This association helps to restrict the binding of self-antigen with the class II MHC molecule.
- The invariant chain- MHC complex is then transported to the Golgi apparatus and from the Golgi apparatus to another vesicle.
- Inside the vesicle, the invariant chain is digested and only a small fragment is attached with the molecule.
- The vesicle containing the MHC class II molecule is then fused with the vesicle containing fragmented peptides.
- The fragmented peptide is then bound with the MHC class II forming MHC class II-peptide complex is then transported to the surface of the cell.
- The T cell recognizes the peptide bound with the MHC class II molecule then differentiated into CD⁺₄ helper T-cells which help in the Abs production.

Lecture: 10

Complement System

The complement system is a collection of circulating and membrane-associated proteins (C1 – C9) that are important in defense against microbes. Many complement proteins are proteolytic enzymes, and complement activation involves the sequential activation of these enzymes.

Properties of complement:

1. Inactivated by heating at 56°C for 30 minutes.
2. Activated by microbes.
3. Cause destruction (lysis) of target cell.

Complement Activation:

Three major steps appeared in complement activation :

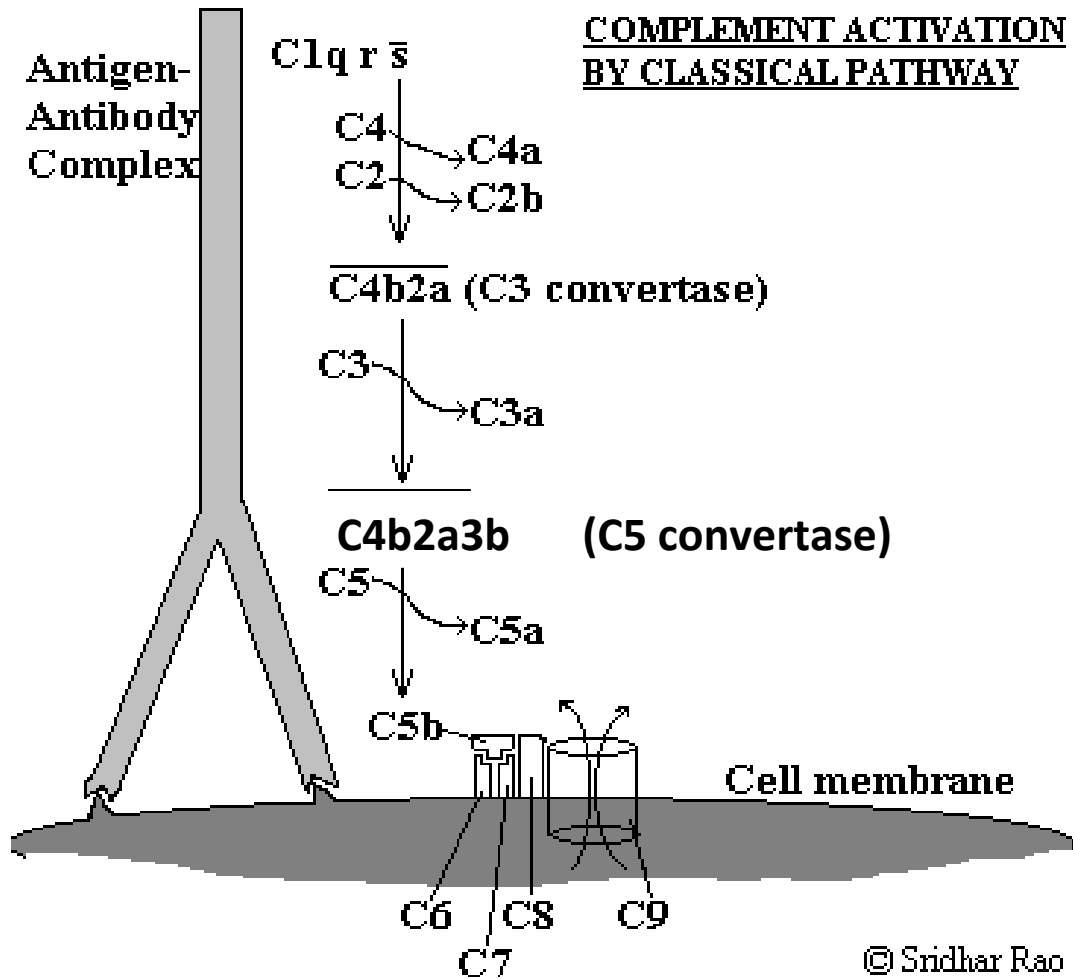
1. Recognition.
2. Enzyme activation.
3. Cell membrane change.

There are 3 major pathways for complement activation the classical, alternate pathway and lectin pathway.

The Classical path way

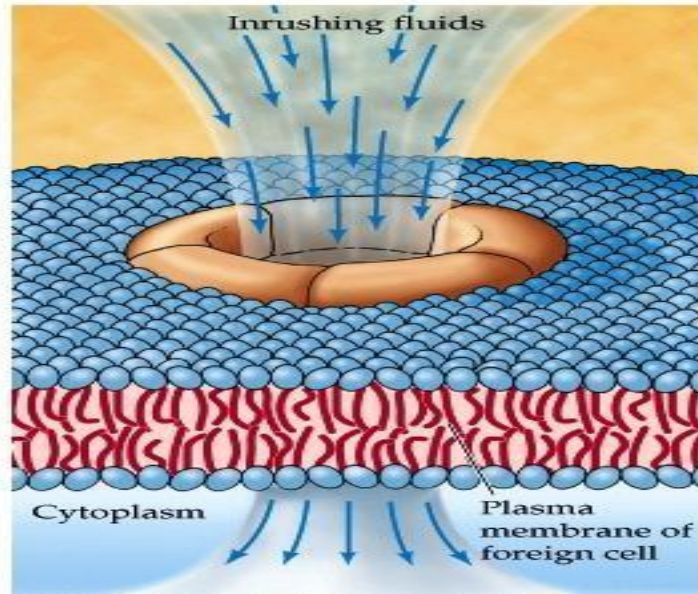
- ✓ The classical pathway is triggered by immune complexes (Ag + Ab IgG or IgM) in the presence of complement components C1, C4, C2, C3, Ca^{+2} and Mg^{+2} cations.
- ✓ While IgG1, IgG2 and IgG3 (most effective) can activate complement, IgG4 is not able to activate at all.

The classical pathway is illustrated in the following diagram:



Biological activity of the classical pathway products

Component	Biological activity
C3a	Anaphylatoxin: can activate basophils and mast cells to degranulate resulting in increased vascular permeability and contraction of smooth muscle cells which may lead to anaphylaxis.
C3b	Opsonin: promote phagocytosis by binding to complement receptors. Activation of phagocytic cells.



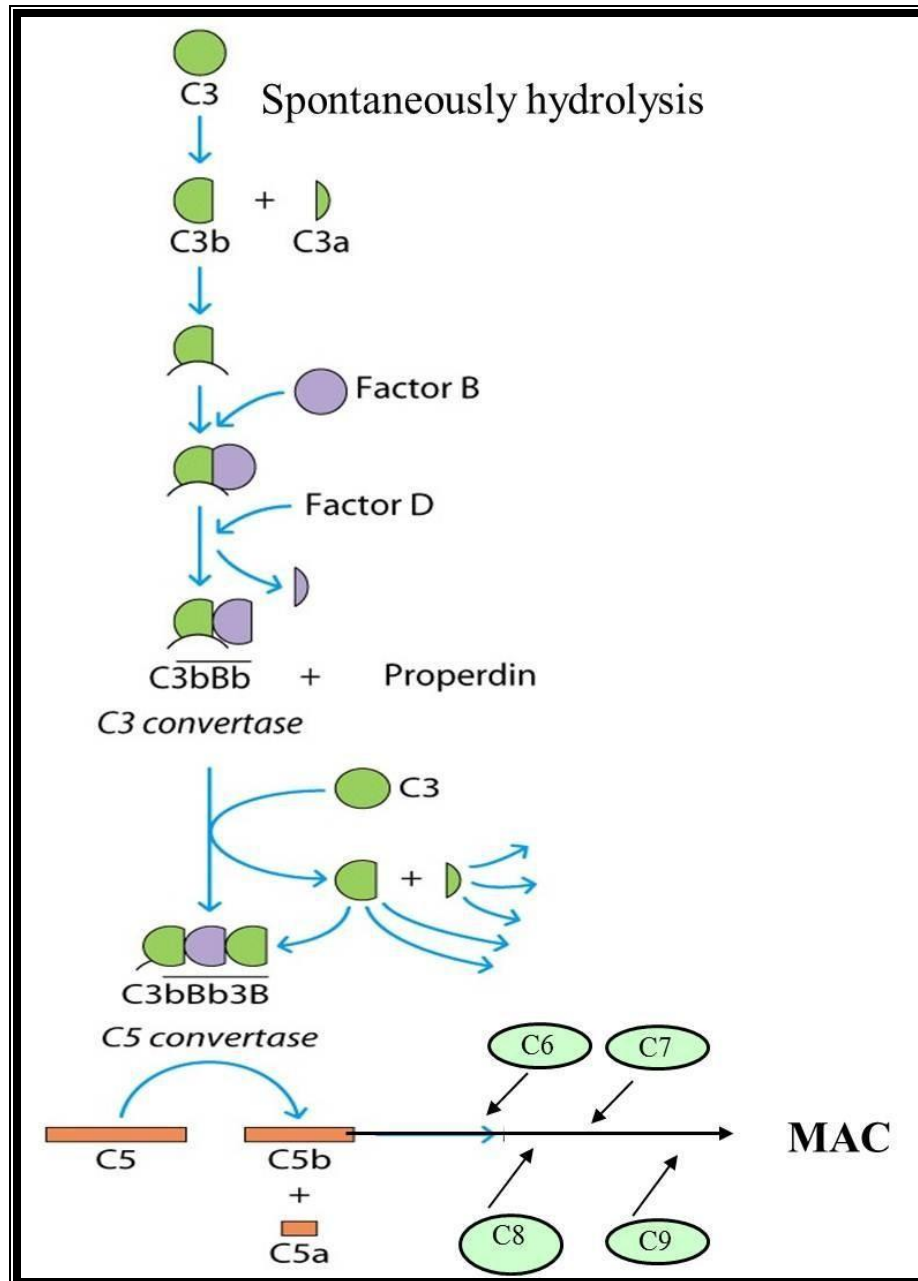
Alternative Pathway

- ✓ Alternate pathway is a non-antibody-initiated pathway.
- ✓ This pathway does not require C1, C2 and C4.
- ✓ Activated by bacterial cell wall (lipopolysaccharide), fungi, viruses envelop, parasites (Trypanosoma) and some other proteins (eg: proteases).
- ✓ It begins with the spontaneous activation of C3 in serum and requires Factors B and D and Mg^{+2} , all present in normal serum.

It activated as the following:

1. Factor B: binds to the c3b (in the Ag_c3b complex) to form C3bB complex.
2. Factor D: cleaves the factor B into Ba and Bb. The Bb binds to c3b to form c3bBb complex (C3 convertase).
3. Factor P: (properdin) binds to Bb to form c3bBbp complex convert c3 from inactive form to active form (by cleaving the c3 into c3a and c3b).
4. Additional c3b will bind to the complex to form c3bBbpc3b complex. (c3bBbpc3b) acts as c5 convertase and convert c5 from inactive form to active form by cleaving the c5 into c5a and c5b.
5. C5b insert into the cell membrane of the Ag.
6. C6 C7 C8 C9 will be inserted subsequently to the cell membrane of Ag to form C5b,6,7,8,9 complex. (C5b,6,7,8,9 is called membrane attack complex or MAC)
7. MAC leads to formation of pore in the cell membrane cell lysis (cytolysis)

Note: the "b" fragments complement participate in the activation pathway. whereas, the (a) fragments are split off and have other activities (i.e. act as anaphylatoxins or chemotaxins).



The lectin pathway:

Lectin: are proteins that binds to mannose residues (glycoproteins or carbohydrate on the surface of pathogens).

- The lectin pathway is homologous to the classical pathway, but with the opsonin, mannose-binding lectin (MBL), instead of C1q.
- This pathway is activated by binding of MBL to mannose residues on the pathogen surface, which activates the MBL-associated serine proteases, MASP-1, and MASP-2 (very similar to C1r and C1s, respectively), which can then split C4 into C4a and C4b and C2 into C2a and C2b.
- C4b and C2a then bind together to form the classical C3-convertase, as in the classical pathway.
- Represent the second pathway depending on bacterial, fungal, viruses and parasites carbohydrates in activation.
- It is the more important pathway in early infection especially in babies between (6-18) months (in the period between decreases antibodies passively transferred from mother and full development of their adaptive immune system).

The Role of Activated Complement in Immune Response

- 1- Viral neutralization.
- 2- Cytotoxic function (lysis).
- 3- Opsonization.
- 4- Immune complexes (IC) clearance.
- 5- Inflammatory function.

Lecture: 10

CYTOKINES

Cytokine is a soluble protein synthesized by immune cell and acts on other immune cell (i.e chemical messages between immune cells) to regulate the immune response.

Features of the cytokines:

1. They have low molecular weight
2. Produced in very low concentrations
3. Have short life (i.e cytokines remain for few minutes)
4. Produced on need (i.e no continuous production of cytokines)

Classification of the cytokines:

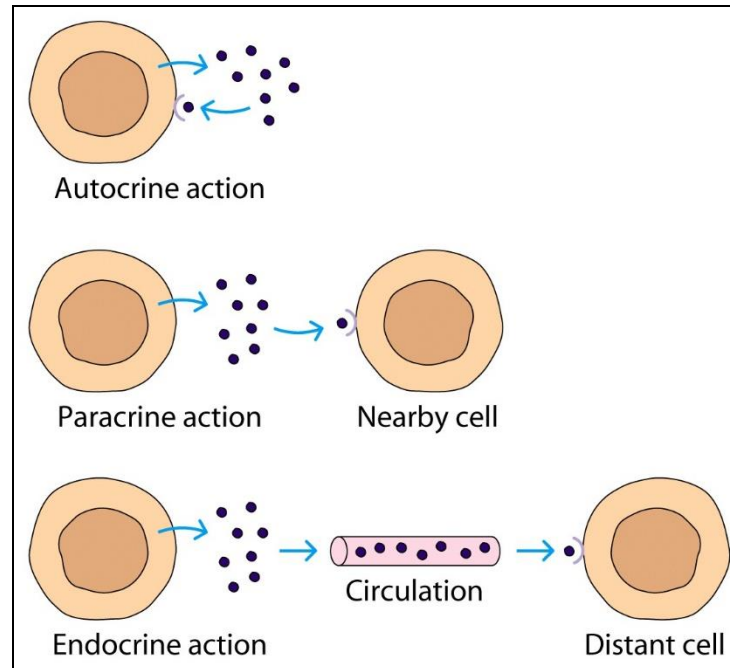
Cytokines are classified according to producing cell, according to site of action, and according to their function.

According to the producing cell:

- Monokines: cytokines produced by monocytes (macrophages).
- Lymphokines: cytokines produced by lymphocytes.

According to site of action:

- Autocrine cytokines: cytokines which act on the same cell those produce them.
- Paracrine cytokines: cytokines which act on the (neighboring cell).
- Endocrine cytokines: cytokines which act on the far away cell.



According to the function of cytokine:

- Chemokines: cytokines which act as chemotactic for other immune cells.
- Interleukins (IL): cytokines produced by one leukocyte and act on another leukocyte (i.e. act as chemical messages between leukocytes).

The Role of cytokines in immune response

Regulate the adaptive Immune response: IL-1, IL-2, IL-4, IL-5 and interferon gamma ($\text{INF}\gamma$) activate lymphocytes and macrophages.

Facilitate the innate immune response: example: gamma INF activate macrophages and NK cells.

Induce inflammation: IL-1 and TNF produced by macrophages causes inflammation

Affect WBCs movement: IL-8 attracts neutrophils and macrophages to the site of infection (chemotaxis)

Stimulate hemopoiesis in the bone marrow: IL-3 and G-CSF stimulate production of WBCs in the bone marrow.

Cytokine	Main Source	Important function
IL-1	1-Dendritic cell 2- macrophage	1- Activation of naive helper T cell (THo) 2. Induce inflammation 3. Cause fever
IL-2	1.Activated Th. 2.Th1	1- Proliferation and differentiation of Th into Th1, Th2 2. Activation of cytotoxic T cell (Tc)
IL-4	Th2	1.Proliferation of B cell 2.Class switching to IgG and IgE
IL-5	Th2	1- Differentiation of B cell
IL-6	1. Macrophage 2. Endothelial cells	1. Induce acute phase protein. 2. Proliferation and Abs secretion of B-Cells
INF alpha	Macrophages	1. Antiviral activity 2. Increase MHC I expression 3. Activate NK cell

Lecture: 11

IMMUNITY TO BACTERIA

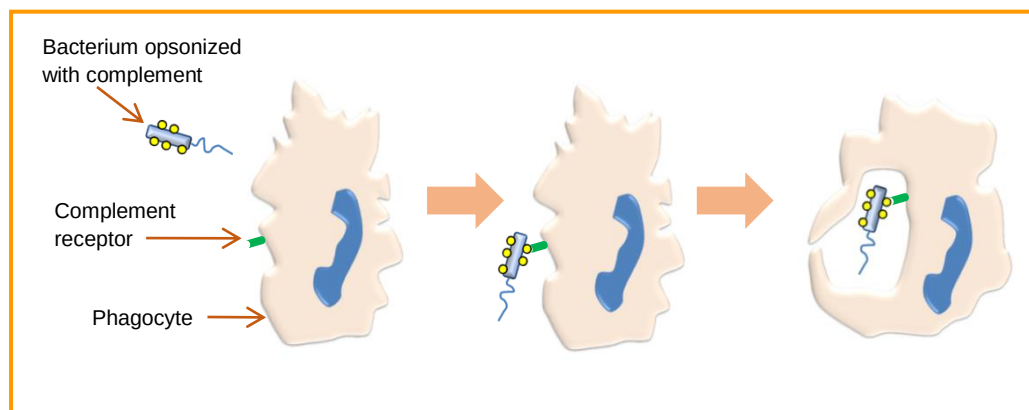
Immunity to extracellular bacteria

Extracellular bacteria are those that multiply and reside outside the host cell. These bacteria mainly affect the cells in two ways. They either attack by causing inflammation and tissue damage or by producing toxins.

Innate Immunity to Extracellular Bacteria

1. Stimulation of phagocytes:

Phagocytes take the help of surface receptors and Fc receptors to identify extracellular bacteria and its opsonization with the help of antibodies, respectively. Most of these receptors are associated with promotion of phagocytic activity and microbicidal activity. After bacteria are ingested by phagocytosis, they are killed by various processes that occur inside the cell, and broken into small fragments by enzymes. Phagocytes present the fragments on their surface via **class II major histocompatibility (MHC class II) molecules**. Circulating **helper T cells** recognize these bacterial fragments and begin to produce proteins called **cytokines**.



2. Induction of inflammatory response:

Antigen presenting cells like dendritic cells in addition to phagocytes are stimulated by microbes and these cells secrete cytokines which are responsible for causing leukocyte infiltration at the site of inflammation.

3. Activation of complement system:

Both gram positive and gram-negative bacteria stimulate alternative pathway of complement system and mannose expressing bacteria stimulate lectin pathway of complement system by binding to mannose binding lectin.

Adaptive Immunity to Extracellular Bacteria

- Circulating helper T cells recognize bacterial fragments presented by antigen presenting cells and begin to produce cytokines.
- Two major groups of helper T cells are known as **Th1 and Th2** cells. These cell types differ in the types of cytokines they secrete.
- Th1 cells predominantly produce interferon-g (IFN-g), which promotes cell-mediated immune mechanisms.
- Th2 cells produce mostly interleukin-4 (IL-4), which promotes humoral immunity by activating B cells. B cells make antibodies that stick to extracellular bacteria and prevent their growth and survival.
- The antibodies also defend the body by neutralization, opsonization, phagocytosis and stimulation of complement system. Bacterial specific CD4⁺ helper T cells also induces inflammation and phagocytic activity. Besides this, these antigens may cause some mutational disorders and also the affected individual may have reduced immune response towards microbial infections.

Immune Evasion by Extracellular Bacteria

1. Polysaccharide antigens or encapsulated bacteria are more lethal as compared to a strain devoid of capsule because they resist phagocytosis.

2. Capsulated bacteria inhibit alternate pathway of complement system due to the presence of sialic acid.
3. Genetic edition of surface antigens: E.g., surface antigen of some specific bacteria is contained in their pili. Pili contains a protein antigen called “pilin” and this pilin undergoes gene variation. Pili are the structures of bacteria responsible for bacterial adhesion to host cells.

Immunity to Intracellular Bacteria

Innate Immunity to Intracellular Bacteria

- Phagocytes and natural killer cells provide innate immunity to the intracellular bacteria.
- Some bacteria survive and multiply easily in the phagocytes, the phagocytes need to be stimulated by the secretions of these bacteria in order to clear the infection.
- The secretions from these bacteria are recognized by Toll-Like Receptors (TLRs) and cytoplasmic proteins of the NOD-like receptor (NLR) family so that they stimulate the phagocytes to degrade the invading bacteria.
- Some intracellular bacteria like *Listeria monocytogenes* need cell mediated immunity in order to be eliminated from the body.

Adaptive Immunity to Intracellular Bacteria

- Some bacteria (*Salmonella* spp.) engulfed during phagocytosis avoid the killing mechanisms of the phagocyte to survive inside cells.
- These bacteria cannot be detected by complement or antibody but, instead, are eliminated using a cell-mediated response.
- Infected macrophages present bacterial peptides on their cell surface using MHC class II molecules resulting in the activation of Th-1 cells
- The Th1 cell releases IFN-g which stimulates killing mechanisms, (such as production of lysozyme) inside the infected macrophage to digest and destroy the invading bacterium.
- IFN-g also increases antigen presentation by cells, making the bacterium more visible to the immune system and more prone to attack.

Dodging of Immune System by Intracellular Bacteria

Intracellular bacteria tend to dodge the immune system in many ways comprising evading into the cytosol or preventing phagolysosome fusion and by overpowering the reactive oxygen species by their microbicidal activity. These bacteria have the potential to cause chronic infections because they can survive the phagocyte mediated elimination and thrive for years in the body and may show reversion of the disease.

Lecture: 12

Immune Responses to Viruses

Immune response against viruses can be achieved by cytotoxic cells, interferons and antibodies.

Via cytotoxic cells

When a virus infects a person (host), it invades the cells of its host in order to survive and replicate. Once inside, the cells of the immune system cannot ‘see’ the virus and therefore do not know that the host cell is infected. So, cells employ a system that allows them to show other cells what is inside them – they use or **MHC class I** to display pieces of protein from inside the cell upon the cell surface. If the cell is infected with a virus, these pieces of peptide will include fragments of proteins made by the virus to activate **cytotoxic T cell**

Cytotoxic T cells have specialized proteins on their surface that help them to recognize virally-infected cells. These proteins are called **T cell receptors (TCRs)** that can specifically recognize a particular antigenic peptide bound to an MHC molecule. If the T cell receptor detects a peptide from a virus, it warns its T cell of an infection. The T cell releases **cytotoxic factors** to kill the infected cell and, therefore, prevent survival of the invading virus

Some viruses stop MHC molecules from getting to the cell surface to display viral peptides. If this happens, the T cell doesn't know there's a virus inside the infected cell.

However, another immune cell specializes in killing cells that have a reduced number of MHC class I molecules on their surface – this cell is a **NK cell**. When the NK cell finds a

cell displaying fewer than normal MHC molecules it releases toxic substances, which kill the virally-infected cell.

Mechanisms of cytotoxic IR against Virus

1. Apoptosis:

Once inside the target cell, they initiate a process known as programmed cell death or **apoptosis**, causing the target cell to die.

2. Cytokines include **tumor necrosis factor that transfer a signal from the T cell to the infected, or other neighboring cells, to enhance the killing mechanisms.**

Cytotoxic factors (granules) of Cytotoxic cells are:

- 1. Perforin**, a protein that can make pores in cell membranes; these pores allow entry of other factors into a target cell to facilitate destruction of the cell.
- 2. Granulysin** is a substance released by cytotoxic T cells (CD8) and NK cell. It functions to create holes in the target cell membrane and destroy it. Granulysin is able to induce apoptosis in target cells and also has antimicrobial action.
- 3. Enzymes called **granzymes**** are also stored in cytotoxic cells, and released from, the granules. Granzymes enter target cells through the holes made by perforin.

Via interferons

Virally infected cells produce and release small proteins called **interferons**, which

- 1. Prevent replication of viruses**, by directly interfering with their ability to replicate within an infected cell.
- 2. They also act as signaling molecules** that allow infected cells to warn nearby cells of a viral presence - this signal makes neighboring cells increase the numbers of MHC class I molecules upon their surfaces, so that T cells

surveying the area can identify and eliminate the viral infection as described above.

Via antibodies

1. Firstly, the antibodies **neutralize** the virus, meaning that it is no longer capable of infecting the host cell.
2. Secondly, many antibodies can work together, causing virus particles to stick together in a process called **agglutination**. Agglutinated viruses make an easier target for immune cells than single viral particles.
3. The activation of phagocytes. A virus-bound antibody binds to receptors, called Fc receptors, on the surface of phagocytic cells and triggers **phagocytosis**, by which the cell engulfs and destroys the virus.
4. Finally, antibodies can also activate the complement system, which opsonizes and promotes phagocytosis of viruses. Complement can also damage the envelope (phospholipid bilayer) that is present on some types of viruses.

Immunity to Fungi

Innate recognition of fungi by the immune system

- Fungi are recognized by cells of the innate immune system (e.g. dendritic cells and macrophages) which bind components of fungal cell walls using pattern recognition receptors (PRRs) on their surface.
- C-type lectin receptors (CLRs, e.g. Dectin-1) are particularly important PRRs in anti-fungal immunity, although several other PRRs are also involved including the Toll-like receptors (TLRs), e.g. TLR2.
- When PRRs bind fungi, they signal using their intracellular tails or associated molecules resulting in phagocytosis, initiation of killing mechanisms (e.g. production of reactive oxygen species) and also help drive the development of adaptive immunity.

- Adaptive immunity to fungi is only partially understood, although it seems that CD4⁺ T-cells that make IFN γ (Th1) or IL-17 (Th17) provide the best protection during fungal infections, as these help drive effective killing by innate effector cells such as neutrophils and macrophages.

Lecture 12:

Tumor Immunity

- **A tumor** is a solid mass of tissue that forms when abnormal cells group together. Tumors can affect bones, skin, tissue, organs and glands. Tumor could be malignant or benign. Malignant tumors are distinguished from benign tumors by their progressive growth and invasiveness.
- When a tumor continues to grow and to invade healthy tissue, it is considered to be a cancer.
- **Metastasis** is a characteristic of many malignant tumors (cancers). Metastatic cells become dislodged from the main tumor, invade blood or lymphatic vessels, and travel to other tissues, where they continue to grow and to invade.

Tumor antigens

Tumor cells express antigens on their surfaces that are often the targets of immune responses.

- Many tumor antigens are cellular peptides presented by MHC molecules that stimulate antigen-specific T-cell proliferation.
- Some antigenic molecules on tumor cells are variant forms of normal proteins that result from mutation of the gene encoding the protein.
- Others are normally found only on cells of certain developmental stages or lineages and are antigenic when expressed out of their usual context.
- Other tumor antigens are simply molecules found at higher-than-normal concentration on tumor cells, whereas a few others are proteins encoded by genes unique to tumors.

Types of tumors Antigens:

1. **Tumor-specific transplantation antigens (TSTAs):** TSTAs are not found on normal somatic cells but result from mutations of genes and the resulting altered proteins that are expressed by the tumor cells.
2. **Tumor-associated transplantation antigens (TATAs):** TATAs are not unique to tumor cells; rather, their expression on tumor cells is altered. For example, the tumor antigen may be found in excessive amounts or may be expressed on a cell type where it would not normally exist. Human breast cancer cells often have high levels of the growth factor receptor HER2/neu, which is found in very low concentrations on normal cells but is overexpressed on approximately 20% to 30% of primary breast tumors.

The immune surveillance theory

The immune surveillance theory suggests that cancer cells frequently arise within the body but are normally eliminated before they multiply sufficiently to become clinically detectable. Accordingly, through the workings of an effective immune system that patrols the body and mounts responses against abnormal cells, most transformed cells never become true cancers. Tumors arise only if they are able to escape immune surveillance. Evidence supporting the immune surveillance theory comes from immunosuppressed and immunodeficient individuals who have increased tumor incidence.

Innate Immunity to Tumor:

1. NK cells:

NK cells have a limited ability to discriminate between tumor cells and normal cells. NK recognition of targets occurs via killer activation receptors (KARs) and killer inhibitory receptors (KI Rs). KI Rs recognize human MHC class I molecules: H LA-B and H LA-C. Another inhibitory NK receptor, CD94, recognizes another class I molecule called HLA- E. When a KAR is engaged by binding to its carbohydrate ligands on target cells, the "kill" signal to the NK cell is activated.

2. Cytokines:

Tumor necrosis factor (TNF) is one such antitumor cytokine. When injected into animals with tumors, TNF-a and TNF-b can stimulate necrosis of the tumor cells.

TNF- α also inhibits angiogenesis, the growth of new blood vessels by decreasing blood flow to the tumor.

Interferons are another group of cytokines with antitumor activity. IFN- α , IFN- β , and IFN- γ have all been shown to increase MHC-I expression on tumor cells (which often down regulate MHC-I expression to evade the immune response). Increasing the MHC-I expression can increase susceptibility of the tumor cells to CTLs.

Adaptive Immunity to Tumor:

Specific antigen-dependent immune responses can develop to antigens on tumor cells. Although they are not always effective in halting progression of a tumor, evidence exists that both humoral and cell mediated immune responses can be induced in response to the presence of malignant cells.

- Antibodies are known to be generated against certain tumor-specific antigens present on the surface of malignant cells.
- CTLs can sometimes kill tumor cells by direct contact.
- DTH reactions involve Th 1 cells recruiting and activating macrophages, which attack and kill tumor cells.