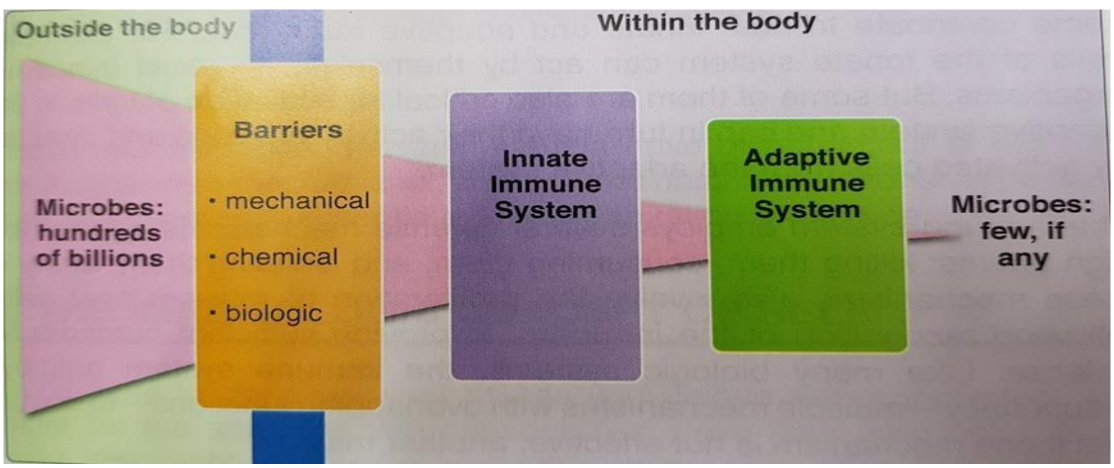
Lec-1

Introduction

The immune system is a complex system that is responsible for protecting us against infections and foreign substances.

There are three lines of defense:

- The first is to keep invaders out (through skin, mucus membranes, etc,(
- The second line of defense consists of non-specific ways to defend against pathogens that have broken through the first line of defense (such as with inflammatory response and fever).
- The third line of defense is mounted against specific pathogens that are causing disease (B cells produce antibodies against bacteria or viruses in the extracellular fluid, while T cells kill cells that have become infected).



- Antigen(Ag): any substance (usually foreign) that binds specifically to component of adaptive immunity.
- Immunogen: is any substance capable of eliciting an immune response. All immunogen are antigen, but some Ags are not immunogene
- Antibod (Ab): secreted immunoglobulin from plasma cell produced in response to and counteracting a specific Ags. Antibody combine chemically

with substance, which the body recognize as alien, such as bacteria, virus and any foreign substance in the blood. Immunoglobulin (Ig) an antibody or heavy or light polypeptide chain that is a part of an antibody molecules

- Vaccination /immunization: deliberated induction of protective immunity to pathogen.
- Allergen: non-infectious antigen that induce hypersensitivity reaction, most commonly IgE mediated type 1 reaction.
- Epitope (Ag determinant): the portion of Ag is recognized and bound by an Ab or T cell receptor.
- Pathogen: disease-causing organisms.

❖ **Types of immunity:** Immunity is divided into two subdivisions which include:

(A) Innate (Natural or non-specific immunity).
(B) Adaptive (Specific immunity).

Table (1) Attributes comparison between innate and adaptive immunity

Characteristic	Non-specific Immunity	specific Immunity
Response time	Immediately through (Minutes/hours)	Required large time to response (days)
Specificity	Low specific for recognize of molecules and molecular	Highly specific for recognized to microbial and non-microbial structure
Memory	No have memory	Have memory
Soluble components of blood or tissue fluids	Many antimicrobial peptides and proteins	Antibodies
Major cell types	Phagocyte, natural killer and dendritic cells.	T, B and antigen presenting cells (APCs)

❖ **The innate immune system components have four elements:**

*** Physical factors:**

Skin, Mucous membrane, Cilia and The low pH of gastric.

****Chemical factors:**

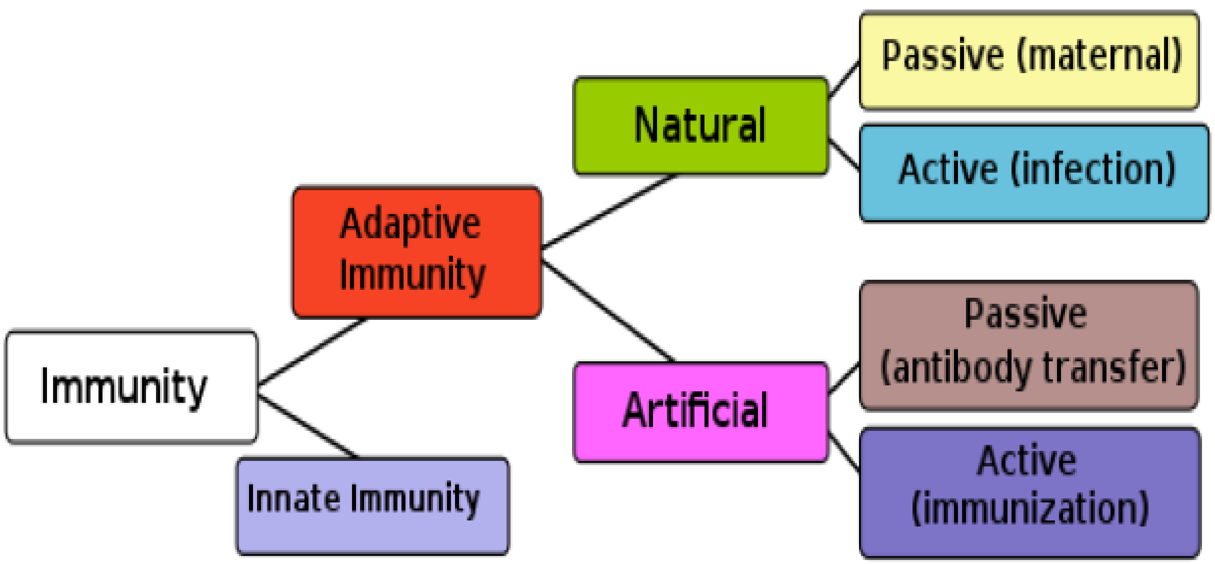
1.**Tears and saliva ,Fatty acids** in sweat, **Lysozyme and phospholipase** found in tears, saliva and nasal, **Defensins** (low molecular weight proteins) found in the lung and gastrointestinal tract have antimicrobial activity, **Surfactants** in the lung, **Properdin** which is a material active against some viruses, **Lactoferrin** and **transferrin**, Interferons, **Lysozyme**, **Interleukin** and **beta-lysin (β-lysin)**

***** Biological factors**: The normal flora in the skin and gastrointestinal tract

****** Cellular barriers:**

Part of the inflammatory response is the recruitment of neutrophiles, eosinophils, macrophages / monocytes, Natural killer (NK) and lymphokine activated killer (LAK) cells into infection sites; also epithelial, endothelial and fibroblast cells.

- **Adaptive immunity:** It's immune typing which a person develops it during life times (not inherited); also it's the second line defense that protects body against pathogenic microorganisms



1. Passive immunity: It's an immunity in which antibodies produced elsewhere are given to the individual. They are divided into two:

i. Naturally acquired passive immunity: refers to antibodies transferred from mother to fetus across the placenta and to 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.

2. Active immunity: It is a product of the individual's own immune system in response to a foreign antigen.

i. Naturally acquired active immunity: is immunity that comes from infections encountered in daily life.

ii. Artificially acquired active immunity: It is stimulated by initial exposure to specific foreign macromolecules through the use of **vaccines** to artificially establish a state of immunity

Lec-2

Immune system: Cells, Tissues and Organs

1-Origin of cells of immune system:

All components of the blood, including immune cells are originated from hematopoietic stem cells these cells are highly differentiated into progenitor cells to give different cells. The formation and development of hematopoietic stem cells begins in the early embryonic stages, lately these cells migration to liver, spleen, and differentiated in bone marrow in one of two pathways.

1-Myeloid progenitor cells can differentiate into:

- Megakaryocytes, which are differentiated into platelets.
- Erythroblasts, which are multiply and differentiated into RBCs.
- Myeloblasts, which can differentiate into neutrophils, eosinophil, and basophil.
- Monoblasts, which can differentiate into monocyte and dendritic cells.

2- Lymphoid progenitor can be differentiating into:

- T-lymphocytes, which are responsible for the cellular immune response
- B-lymphocytes, which produce antibodies (humoral immune response .
- Natural killer cells (NKs).

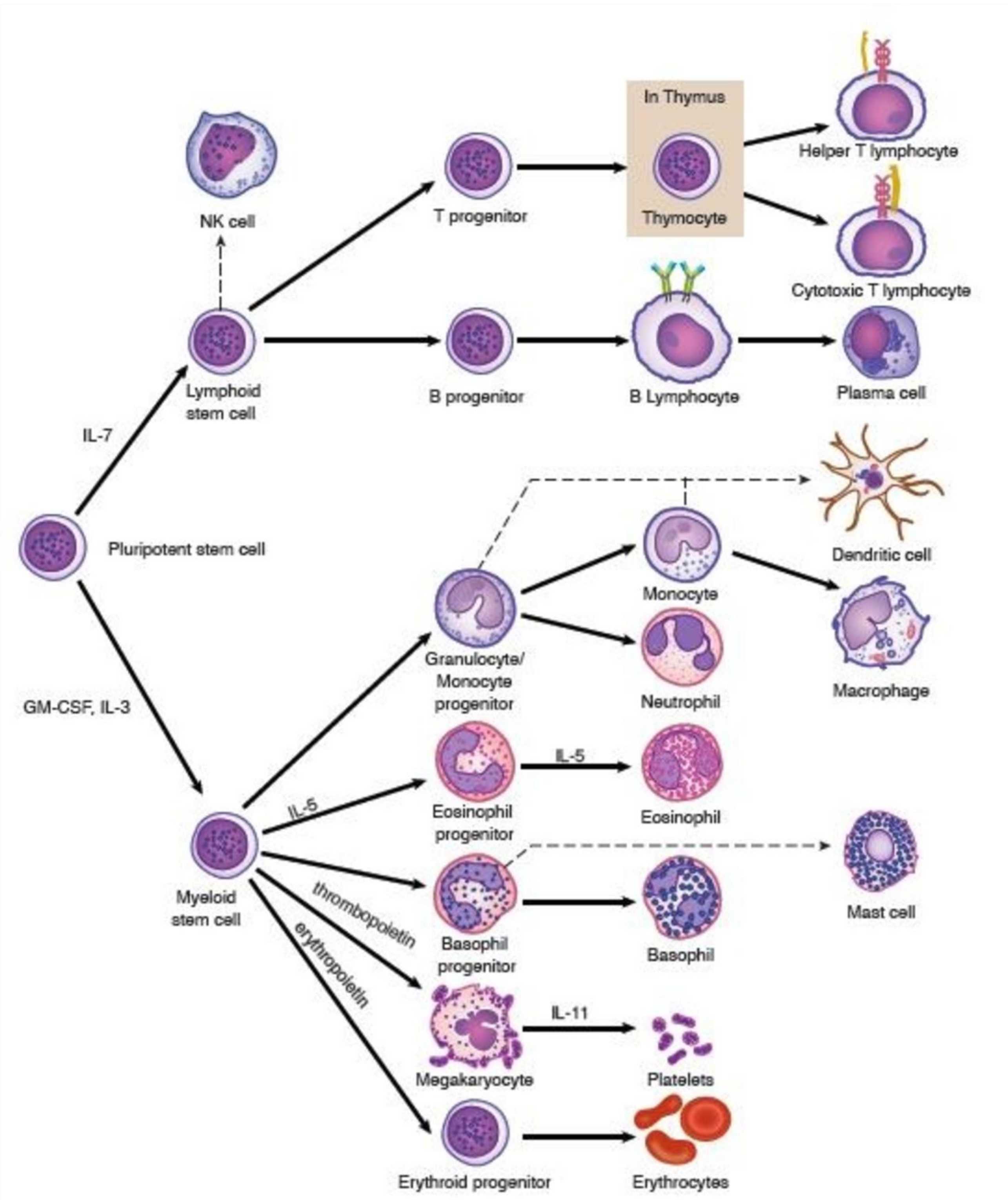
Lymphoid system:

Some stem cells are migrate to primary lymphoid organs which are includes

- Thymus and Bone marrow to continue of proliferate and differentiate of them.
- Bone marrow is the source of progenitor cells; it also plays a role in differentiate of progenitor cells into B-lymphocytes.

The Mature T and B lymphocytes leave their differentiated site and migrate to peripheral or secondary lymphoid organs which are include:

- Lymph nodes**,** Spleen**,** Mucosa-associated lymphoid tissue (MALT)**,** Gut-associated lymphoid tissues (GALT) **and** Tonsils



In Thymus
Helper T lymphocyte
NK cell
T progenitor
Thymocyte
Cytotoxic T lymphocyte
Lymphoid stem cell
B progenitor
B Lymphocyte
Plasma cell
IL-7
Pluripotent stem cell
Dendritic cell
Monocyte
Granulocyte/ Monocyte progenitor
Neutrophil
Macrophage
GM-CSF, IL-3
IL-5
Eosinophil progenitor
Eosinophil
IL-5
Myeloid stem cell
Mast cell
thrombopoietin
erythropoietin
Basophil progenitor
Basophil
IL-11
Megakaryocyte
Platelets
Erythroid progenitor
Erythrocytes

The Lymphocytes cells:

Lymphocytes are one of white blood cells classes; derived from stems cells and maturate either in the bone marrow or thymus; comprise 20-40% of all leukocytes, distributed into blood, lymph and lymphoid organs. *divide in three major types of lymphocyte, **B lymphocyte, T lymphocyte and NK cells.***

Different lymphocytes are identified by protein markers on their surface called "cluster of differentiation" or "CD" system like CD45 which is found in all leukocytes the most common CD is CD4 and CD8

1-T- Lymphocyte:

Naming and maturation:

"T-cell" is an abbreviation of "thymus dependent lymphocyte". it arises in the bone marrow as T-cell precursors, then migrate and passage through the thymus to complete their maturation which includes rearrangements and coding of the variable part of the TCR (T Cell Receptor) by enzymes and hormones activity. It requires to react with many receptors include antigens receptors, Fc fragment of antibodies, RBCs receptors, and Virus receptor.

- Surface markers (TCR): they are two kinds of TCR, TCR 1 and 2 with other markers/receptors present on their surface.
- Subsets of T Cells: There are **two major** types of T cells:

I- Helper T cells (TH):

- Are identified by the presence of CD4 marker.
- They recognize antigen when presented along with Class II MHC molecules.
- They are subdivided into the TH1 and TH2 subsets on the basis of the kinds of cytokines they produce. TH1 cells produce interleukin-2 (IL-2), interferon-gamma (IFNγ), and tumor necrosis factor-beta (TNF-β) while TH2 cells produce IL-4, IL-5, IL-6, IL-10 and TGF-β (Transforming growth factor beta). They are promotes differentiation of B-cells and cytotoxic T-cells, Activates macrophages, and secrete cytokines.

II- Cytotoxic T cells (TC):

- Are identified by the presence of CD8 marker.
- They recognize antigen when presented along with Class I MHC molecules.
- They have a role in down regulation of immune response, and Kill infected cells.

2- B - Lymphocyte:

They are called B cells they are developed and mature occurs in bone marrow. The early stages of B cell maturation occur in the fetal liver and continue in the bone marrow throughout life.

- Surface markers: The most important surface markers on the surface of mature B cell are: CD32 (receptor of Immunoglobulin), CD35 (Receptor for complement component), and markers that distinguish B cells such as CD20, CD21 and CD22.

Functions of B-cells:

1. Direct recognition and presentation of antigen.
2. Secrete large amounts of antibodies after differentiation into plasma cells.
3. It has memory can survive 20 years or more (Memory B cells). Subsets of B cells: There are two major types of B cells, T-independent cells and T dependent cells.

3- Natural Killer cells

- Naming: Also called Large Granular Lymphocytes (LGLs), they are large lymphocyte which kill variety of target cells (such as tumor cells, bacteria / virus infected cells, and transplanted cells) without participation of MHC molecules.
- Surface markers: have CD16 (receptor of Immunoglobulin), CD8, CD56,

Functions:

1. Recognition of various target cells.
2. Cytolysis of target substance and cytokines secretion as IFN-γ.
3. Kill antibody-coated target cells by antibody-dependent cell cytotoxicity (ADCC).
4. Also participate in Graft in organs transplants as bone marrow.

Antigens and immunogens

Antigen (Ag): Molecules that can be recognized by the immunoglobulin receptor of B cells or by the T-cell receptor when complexed with major histocompatibility complex (MHC) (Antigens are substances that react with antibodies. while ***immunogens*** are molecules that induce an immune response.

- The antigens that are not immunogenic but can take part in immune reactions are termed as **haptens**

Determinants of Antigenicity

A number of factors have been identified that make a substance immunogenic. Some of the important determinants of antigenicity
include:

1. Molecular size: In general, protein molecules with large molecular weight are highly antigenic,Substances with molecular weights of about 100,000 Da and more are highly immunogenic, while substances with molecular weights of less than 5000 Da are generally not immunogenic.

2. Foreignness: To be immunogenic, a molecule must be recognized as nonself, i.e., foreign. The molecule is considered self or non self by the immune system depending on whether or not the molecule was exposed to the immune system during fetal development.

3. Chemical-structural complexity

A. Proteins: Major Immunogens are proteins which usually are best immunogens.

B. Polysaccharides: they are good immunogens.

C. Nucleic Acids: they are usually poorly immunogenic.

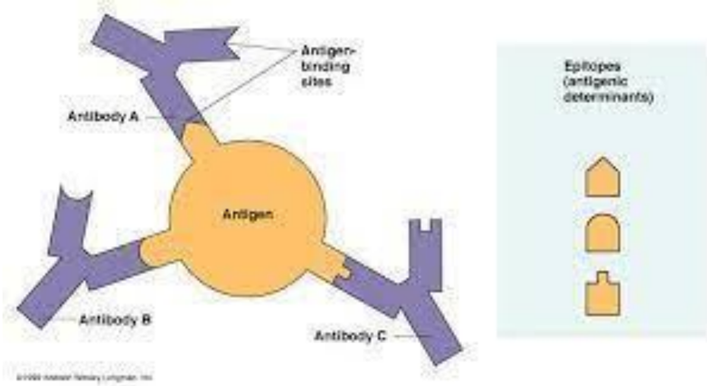
D. Lipids: lipids are non-immunogenic, although they may be haptens.

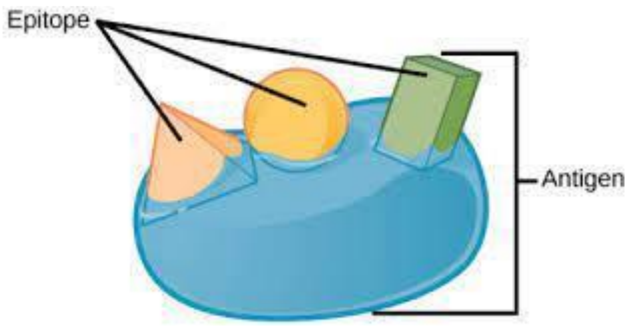
4. Stability: Highly stable and nondegradable substances (e.g., some plastics, metals, or chains of D-amino acids) are not immunogenic. This is because internalization, processing, and presentation by antigen-presenting cells (APCs) are always essential to mount an immune response. Therefore, very stable substances (such as silicon) have been successful as non-immunogenic materials for reconstructive surgeries, such as breast implants. On the other hand, if a substance is very unstable, it may break up before an APC can be internalized, and hence become immunogenic. In addition, large, insoluble complexes are more immunogenic than smaller, soluble ones. This is because macrophages find it easier to phagocytose, degrade, and presentthe insoluble complexes than the soluble complexes.

5. Dosage and route of the antigen: The dose of antigen and the route by which it comes into contact with the immune system also influence immunogenicity of the antigen. Very low doses of antigen do not stimulate immune response, either because too few lymphocytes are contacted or because a nonresponsive state is elicited. Conversely, an extremely high dose also fails to elicit tolerance.

C. Epitopes: An epitope is defined as the immunologically active region of an immunogen that binds to receptors on lymphocytes or secreted antibodies. There are two types of epitopes: B-cell epitopes and T-cell epitopes. The foreign substances that induce an immune response possess two properties:

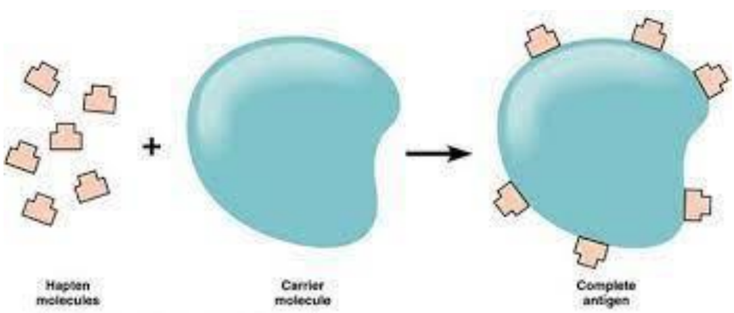
1. Immunogenicity: Antigen ability to stimulate and evoke specific immune response.
2. Immunoreactivity: Antigen ability which can combine with Antibodies.



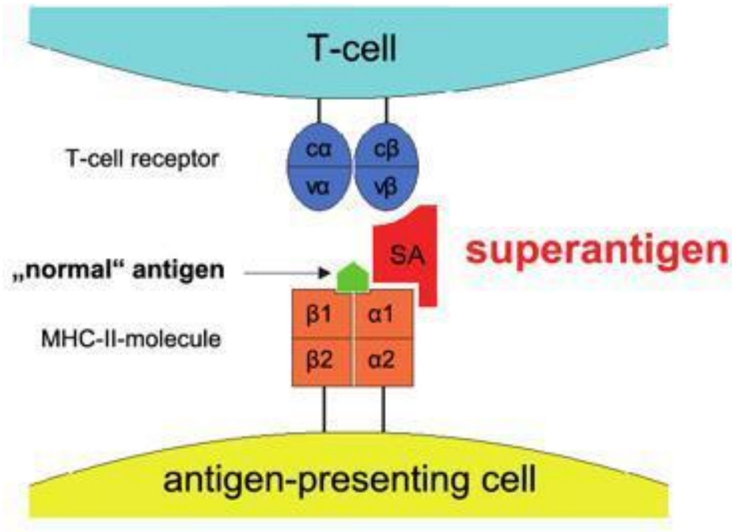


D. Allergen: is a substance that causes allergic reaction after exposure via ingestion, inhalation, injection or contact with skin.

Hapten: A small molecule cannot induce an immune response when administered by themselves but which can when coupled to a carrier molecule such as proteins. Haptens have the property of antigenicity but not immunogenicity.



Super antigens: Super-antigens are a class of molecules that can interact with APCs and T lymphocytes in a nonspecific way. The superantigens act differently by interacting with MHC class II molecules of the APC and the Vb domain of the T-lymphocyte receptor. This interaction results in the activation of a larger number of T cells (10%) than conventional antigens (1%), leading to massive cytokine expression and immunomodulation.



Types of antigens: it can be dividing into:

➢ According to origin: which includes:

1- Exogenous antigens: are antigens can entry the body from outside by inhalation, ingestion, or injection.

2- Endogenous antigens: are antigens can be generated within cell either a result of normal cell metabolism, or because of viral or intracellular bacterial infection.

3- Auto-antigens: is usually a normal protein or complex of proteins (sometimes DNA or RNA) that is recognized by immune system of patients.

➢ According to the cellular response generated:

A. T-independent Antigens: antigens which can directly stimulate B cells to produce antibody without requirement for T cells help such as polysaccharides antigens.

B. T-dependent Antigens: are an antigen doesn't directly stimulate the production of antibody without helps of T cells and contain a few copies of many different antigenic determinants such as proteins.

Properties	T cell-dependent antigens	T cell-independent antigens
Activation of B cells	Can only activate B cells in the presence of Th cells	Can activate B cells in the absence of Th cells
Structural properties	Complex	Simple
Presence in most pathogenic microbes	Yes	No
Antibody class-induced	IgG, IgM, IgA, IgD, IgE	IgM
Immunological memory response	Yes	No

Immunological adjuvants: Is a substance (various additives or vehicles) that mixed with an immunogen to enhance immune response against the immunogen.

Adjuvants appear one or more of the following effects:

1. Antigens persistence is prolonged.

2. Local inflammation is increased.

3. Co-stimulatory signals are enhanced.

4. Proliferation of lymphocytes is stimulated.

Lec-4

Phagocytosis

Phagocytosis: is the engulfment and degradation of microbes and other particulate matter by cells such as macrophages, dendritic cells, neutrophils and inactivated B cells. The main functions of phagocytic cells include migration, chemotaxis, ingestion, and microbial killing. They Identified either by morph. or by presence of CD14 marker

Types of phagocytic cells:

The phagocytic cells can be divided into:

A. Monocytes/ Macrophages.

B. Polymorph nuclear granulocytes (PMNs).

C. Dendritic cell and B-cell

❖ When they enter to tissue, they are called macrophages which have different names according to organs such as:

Location	Cells
Blood stream	Monocyte
Liver	Kupffer cells
Kidney	Mesengial cell
Lungs	Alveolar Macrophage
Brain	Microglial cell
Spleen	Sinus Macrophage
Peritoneal cavity	Serosal Macrophage

Initiation of Phagocytosis

1- Recognition and attachment of microbes by phagocytes

Phagocytosis is initiated when a phagocytes binds a cell or molecules that has penetrated the body barriers. The binding occurs at various receptors on the phagocyte surface. These include

- (PRRs and TLR) that recognize microbe related receptor,
- complement receptor (CR) that recognize certain fragments of complement that adhere to microbial surface
- Fc receptor that recognize immunoglobulins that have bound to microbial surfaces or other
- Mannosyl-fucosyl receptors (MER), Phosphatidyl serine receptors (PSR), CD14 and CD68, Fc receptors, and complement receptors.

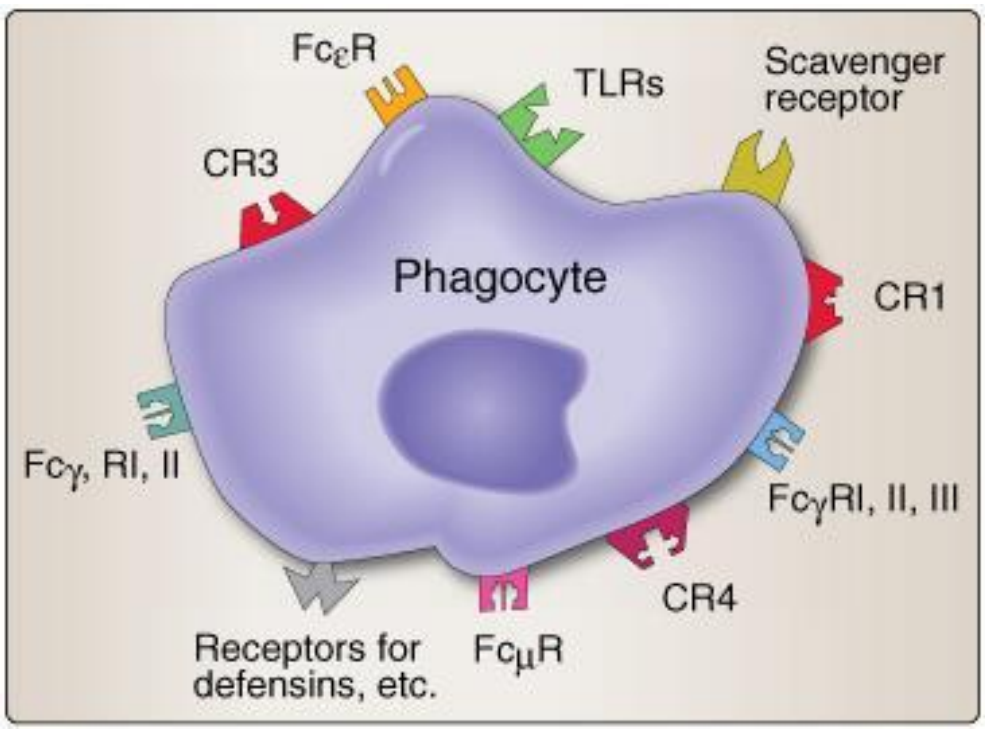


Fig -1 (phagocyte receptors)

2- Ingestion of microbes and other materials

Fallowing attachments to the cell membrane, a microorganism is engulfed by extensions of the cytoplasm and cell membrane called pseudopodia and is drawn into the cell by internalization or endocytosis**.**

The attachment and ingestion of microbes trigger changes within phagocytes. It increases the size, become more aggressive in seeking additional microbes to bind and ingest, and elevates production of certain molecules

3- Destruction of microbes and other materials

The phagosome fuse with lysosomes to form phagolysosomes. Lysosomes employ several mechanisms for killing and degrading ingested matter.

Granulocytes contain granules composed of lysozyme, other hydrolytic enzymes, several cationic proteins, the defensins (antimicrobial components), lactoferrin, and toxic nitrogen oxides.

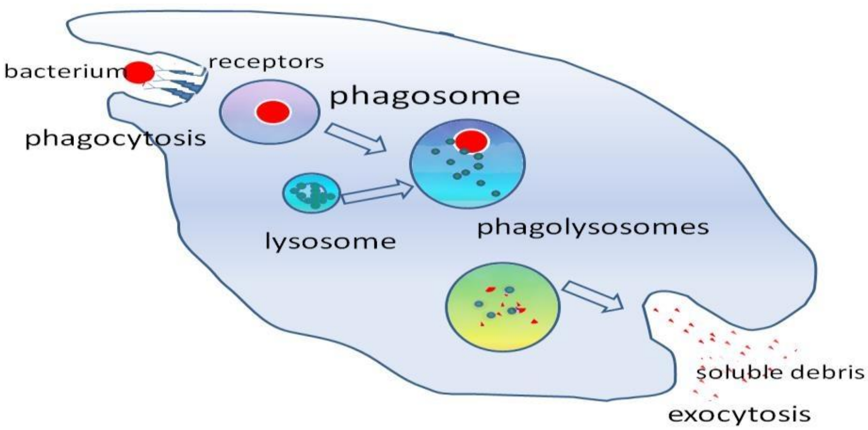


Fig-2 (steps of phagocytosis)

4- Secretion of cytokines and chemokines

Once activated, phagocytes secrete cytokines and chemokines that attract and activate other cells of the innate immune response. Cytokines or (chemical messenger) induce the production of of proteins that lead to elevation body temperature and other cytokines increase the permeability of local vascular epithelial to enhance the movement of cells and soluble molecules from vasculature into the tissue.

Factors Affecting Phagocytosis

Phagocytosis is made more efficient by the presence of antibodies (opsonins) that coat the surface of bacteria and facilitate their ingestion by phagocytes. **Opsonization** can occur by three mechanisms:

1- Antibody alone can act as opsonin.

2- Antibody plus antigen can activate complement via the classic pathway to yield opsonin.

3- Opsonin may be produced by a heat-labile system in which immunoglobulin or other factors activate C3 via the alternative pathway.

Antibodies

Antibodies are globulin proteins (immunoglobulins) that are synthesized in serum and tissue fluids, which react specifically with the antigen that stimulated their production. The antibodies are the gamma globulins. The most important function of antibodies is to confer protection against microbial pathogens.

Immunoglobulins are produced and secreted by antibody forming cell (AFCs) which is the differentiated B-lymphocytes and is called plasma cells in response to an exposure and binding to an antigen Antibodies confer protection in the following ways:

1. They prevent attachment of microbes to mucosal surfaces of the host.

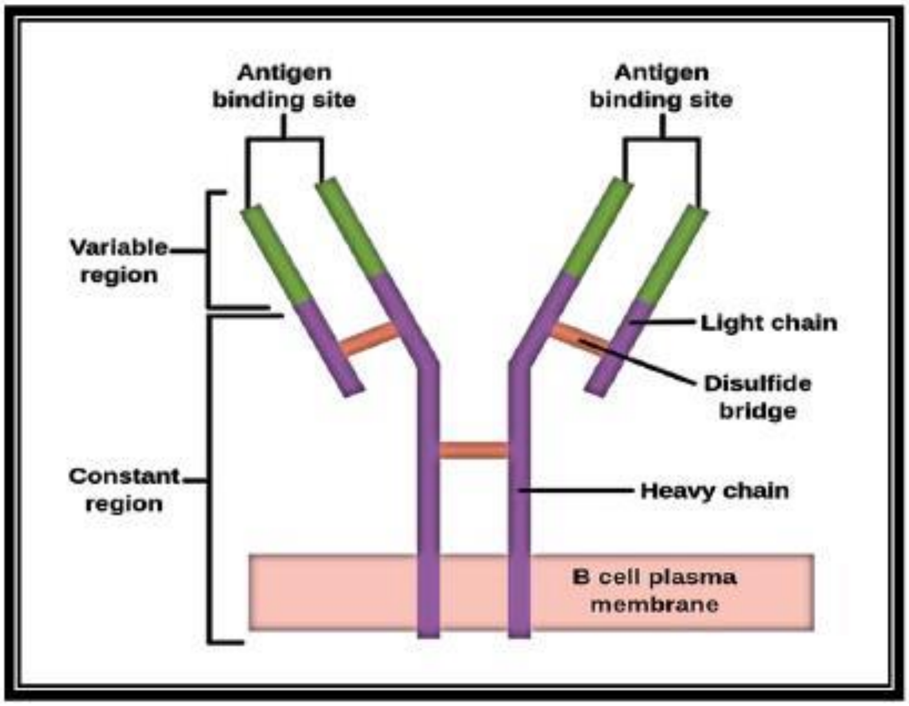
2. They reduce virulence of microbes by neutralizing toxins and viruses.

3. They facilitate phagocytosis by opsonization of microbes.

4. They activate complement, leading to complement-mediated activities against microbes.

5. Binding to various cells e.g. phagocyticcells, lymphocytes, mast cells, and basophiles which lead to activate some functions of these cells.

6. Antibody-dependent cell mediated cytotoxicity (ADCC) kills cells: The linking of Ab bound by Fc receptors to target cells (e.g. virus infected cell) can directly to cytotoxic activities of the effector cells against target cells such as killer cells, this process is called Antibody-dependent cell mediated cytotoxicity .



Basic structure of immunoglobulins:

A. Basic units: Four chain structures from two identical light chains (L) and two identical heavy chains (H).

B. Disulfide bonds:

1. Inter-chain disulfide bonds - The two light chains and the two heavy chains are held together by inter-chain disulfide bonds.

2. Intra-chain disulfide binds within each of the polypeptide chains there are also intrachain disulfide bonds.

3. The heavy and light chains are bound by non-covalent interactions such as salt linkage, hydrogen bonds, and hydrophobic interaction to form a heterodimer.

C. Variable (V) and Constant (C) Regions: heavy chains and light chains can be divided into two regions based on variability in the amino acid sequences. These are:

1. Light Chain - VL and CL.

2. Heavy Chain - VH and CH.

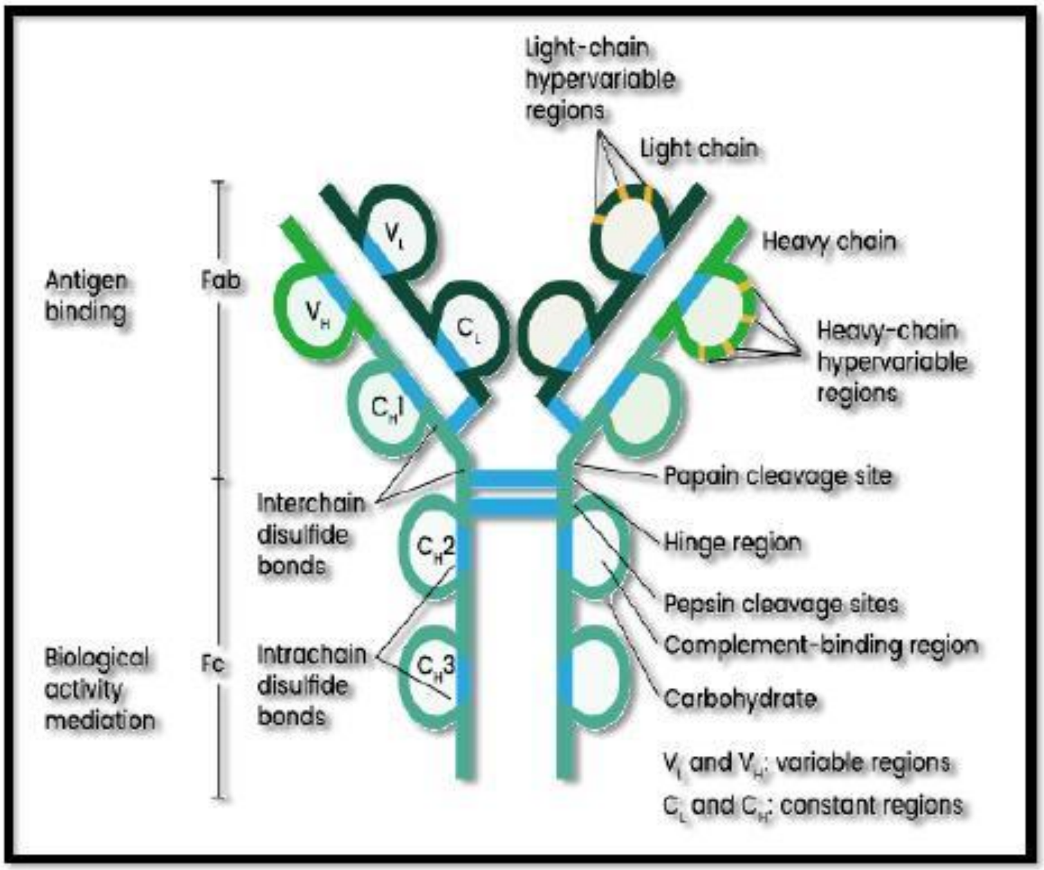
D. Hinge Region: This is the region at which the arms of the antibody molecule form a Y. It is called the hinge region because there is some flexibility in the molecule at this point.

E. Oligosaccharides: Carbo-hydrates are attached to the CH2 domain in most immunoglobulins. However, in some cases carbohydrates may also be attached at other locations.

Structure of the variable region:

A. Hypervariable (HVR) or complementarity determining regions (CDR): In immunoglobulins there is three regions have variability in amino acids sequencing it was called hypervariable regions (HVR) or complementarity determining regions (CDR) which are found in both of H and L chains.

B. Framework regions: The regions between CDR in the variable region are called the framework regions. Based on similarities and differences in the framework regions the immunoglobulin heavy and light chain in variable regions can be divided into groups and subgroups.



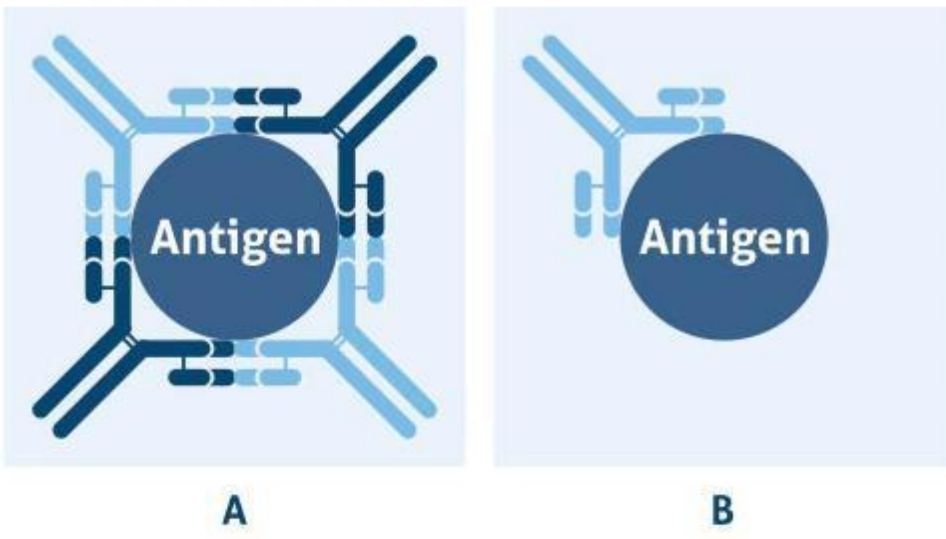
Treatment of Immunoglobulins

with Proteolytic Enzymes The immunoglobulin molecule can be broken into a number of “sections” or “fragments” by action of proteolytic enzymes.

The proteolytic enzyme **papain cleaves just above the** inter-chain disulfide bonds linking the heavy chains, whereas the enzyme pepsin cleaves just below these bonds, thereby generating different digestion products. For example, peptide bonds in the “hinge” region are broken on treatment of antibody molecule with papain, resulting in production of two identical Fab fragments and one Fc fragment. The Fab fragments produced during cleavage monovalently bind to the antigen. Treatment with pepsin cleaves immunoglobulin but at a different site, producing an Fc fragment and two Fab fragments.

Antibody Types

There are two types of antibodies available to scientists: polyclonal and monoclonal. **Polyclonal antibodies** contain a heterologous mixture of IgGs against the whole antigen, whereas **monoclonal antibodies** are composed of a single IgG against one epitope



A) Polyclonal antibodies bind to the same antigen, but different epitopes.

C) monoclonal antibodies bind to the same epitope on a target antigen.

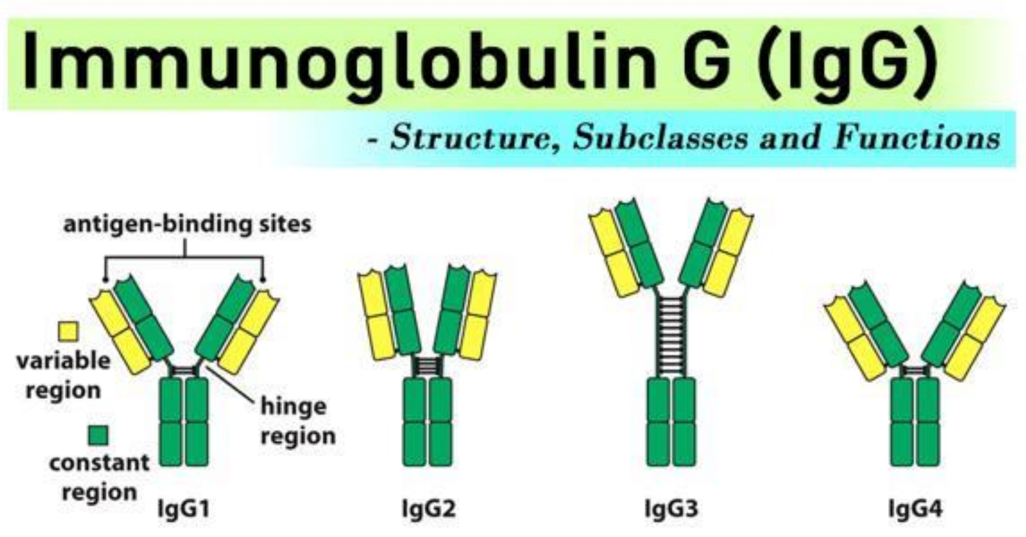
Polyclonal antibodies	monoclonal antibodies
Refer to a mixture of immunoglobulin molecules that are secreted against a particular antigen.	Refer to a homogenous population of antibodies that are produced by a single clone of plasma B cells.
Produced by different clones of plasma B cells.	Produced by the same clone of plasma B cells.
A heterogeneous antibody population.	A homogenous antibody population
Interact with different epitopes on the same antigen.	Interact with a particular epitope on the antigen.

Immunoglobulines Classes

As we know from the previous lecture that Abs are immunoglobulins (Igs) produced by B-cells. There are five subtypes of Ig called (IgG, IgM, IgA, IgD, and IgE). They differ in their constant region and activity.

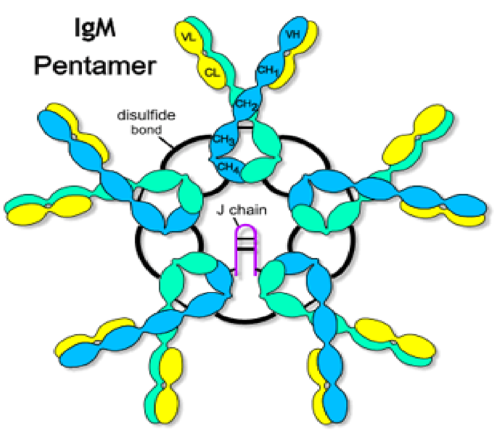
1. IgG:

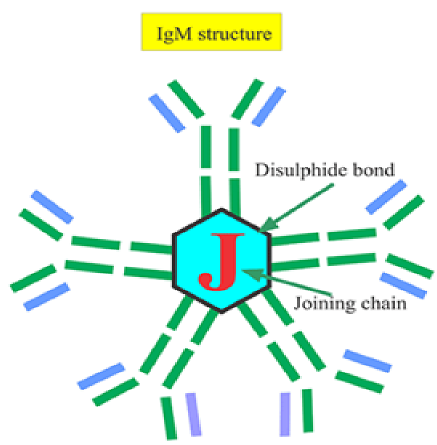
- The major antibody in the serum.
- Placental transfer: IgG1 is the only class of Ig that crosses the placenta. IgG2 does not cross well.
- IgG is responsible for opsonization and activation of complement system (classical pathway). Macrophages, monocytes, PMNs and some lymphocytes have Fc receptors for the Fc region of IgG. Then, the antibody has prepared the antigen for eating by the phagocytic cells.
- Produce in the secondary immune response.
- The concentration in the serum is 12.4 µg/ml.
- Half-life of IgG is 23 days.
- IgG is monomere and the heavy chain is with Gamma (γ) type.
- It is has four isotypes: IgG1, IgG2, IgG3, IgG4.



2. IgM:

- IgM exists as a pentamer (consist of five parts binds together by J chain). the heavy chain is with Meo (μ) type
- Not opsonized factor
- Half-life of IgM is 5 days; the molecular weight with 900kDa
- The best active immunoglobulin in complement system fixation.
- Produce in the primary immune response
- IgM is exist in two forms: membrane-bound antibody on B cells and in a secreted form (pentamer).





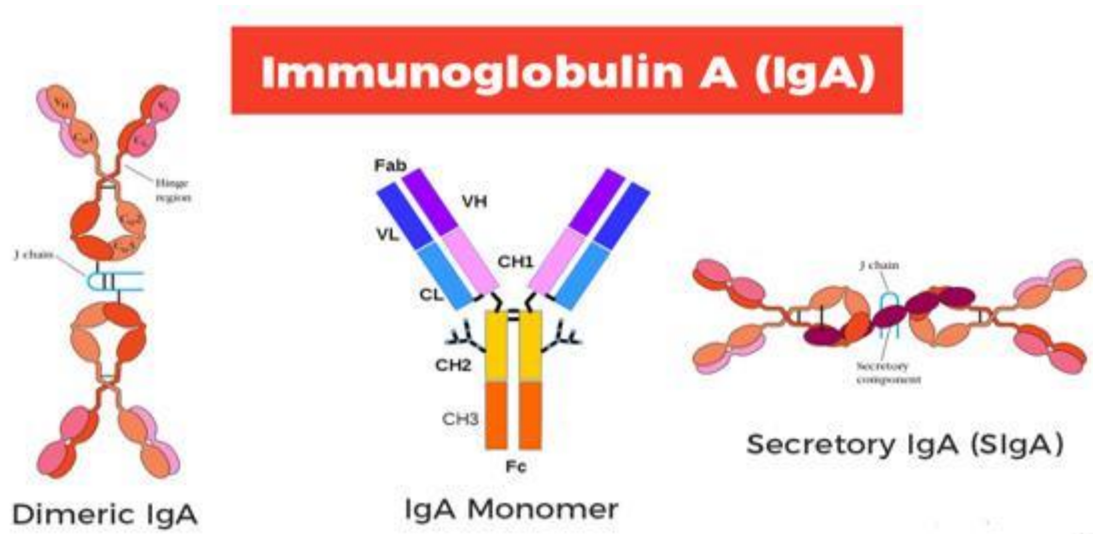
3. IgA: IgA is exist in two forms:

1. Serum IgA

It is present in the serum and is a monomeric 7S molecule with a molecular weight of 60,000 Da. It has a half life of 6–8 days. It has two subclasses, IgA1 and IgA2, which are two α-chain isotypes α-1 and α-2, respectively

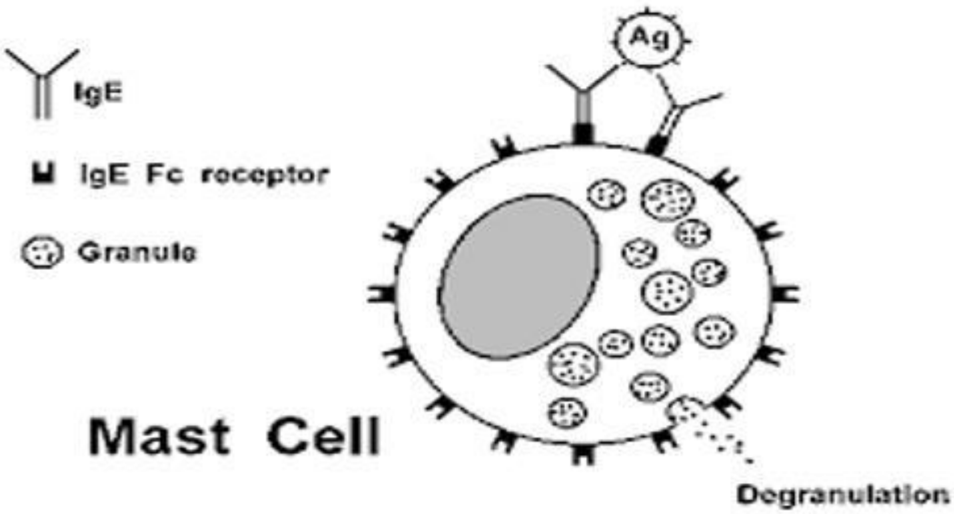
2. Secretary IgA (SIgA): found in fluids (Milk, saliva, mucous, tears).

- ❖ The function of IgA is neutralize bacteria and viruses. The concentration in the serum is 2.8 µg/ml.
- ❖ Half-life of IgA is 6 days; molecular weight with 160kDa.
- ❖ The heavy chain is with Alpha (α) type.
- ❖ There are two types of IgA: IgA1, IgA2.
- ❖ It is exist as monomeric or dimeric (J chain is associated with it).



4. IgE:

- ➢ Involved in allergic reactions
- ➢ IgE also plays a role in parasitic helminthes diseases, asthma and anaphylaxis
- ➢ Eosinophils have Fc receptors for IgE and binding of eosinophils to IgE coated helminths results in killing of the parasite.
- ➢ Produce in lymphoid tissues especially in respiratory duct and intestinal.
- ➢ Binds to basophil and mast cells via Fc lead to degranulation of mast cells and release of histamine and chemotactic factors of eiosinophil.
- ➢ Half-life of IgE is 2 days and the molecular weight is 190kDa; the concentration in the serum is 1.8-20 µg/ml.
- ➢ It is exist as monomeric and the heavy chain is with Epsilon (ε) type.



5.IgD:

- Found on B cell surface where it functions as a receptor for antigen.
- Half-life of IgD is 5 days; did not transfer to placenta.
- Destroyed by heating and digestive enzymes.
- The role of IgD in immunity continues to remain elusive

The inflammation:

Inflammation: is the repair of damage or destruct of tissue which is occur by wounds or invader pathogens, it may be divided into acute or chronic inflammation.

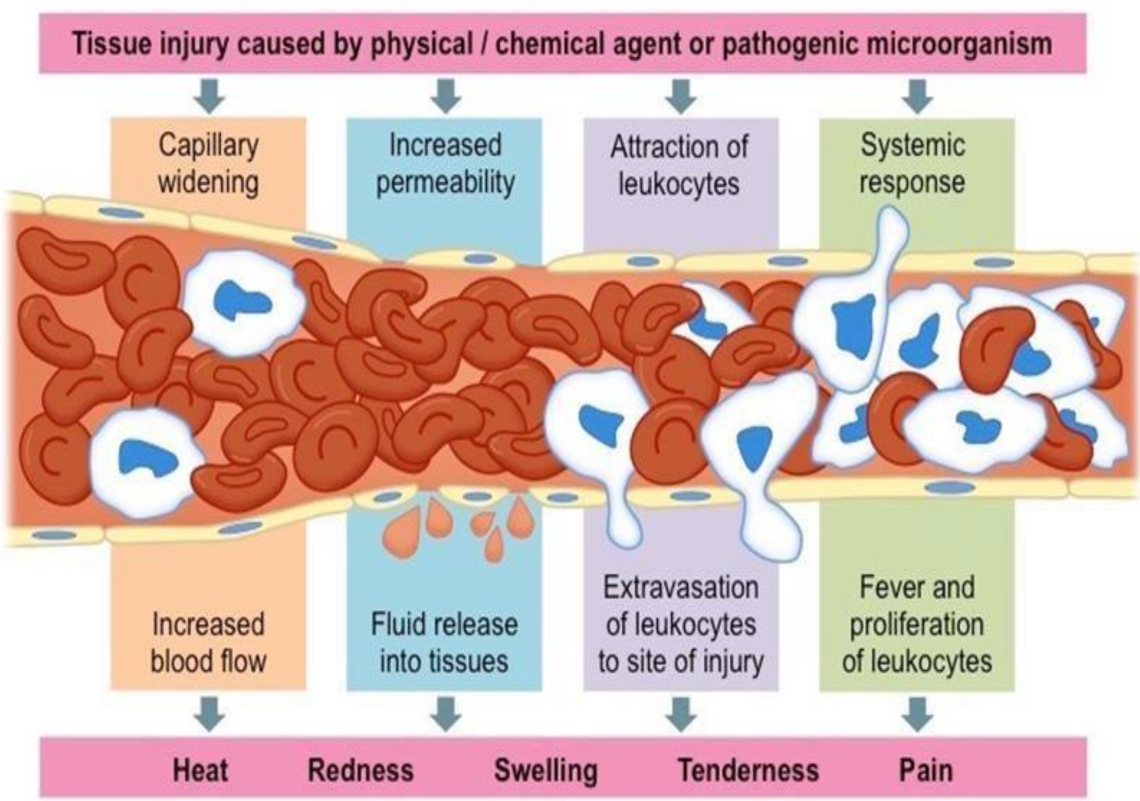
I. Function of inflammation:

1. To mobilize and attract of immune components to the site of injury.

2. To set mechanism for repair tissue damage, localize, and clear harmful substances.

3. To destroy microbes and block their further invasion.

II. Inflammatory reactions: Is one of the most effective defense mechanism in human and other animals which include vascular and cellular reaction into the presence of invading microorganisms or injury.



III. Inflammatory Response:

Any injury to tissue, such as that following establishment and multiplication of microorganisms, elicits an inflammatory response. The innate immune response of macrophages includes the release of cytokines, including interleukin-1 (IL-1) and tumor necrosis factor- (TNF-). The other mediators released from activated macrophages include prostaglandins and leukotrienes. These inflammatory mediators begin to elicit changes in local blood vessels.

The inflammation process may be divided in to the following stages:

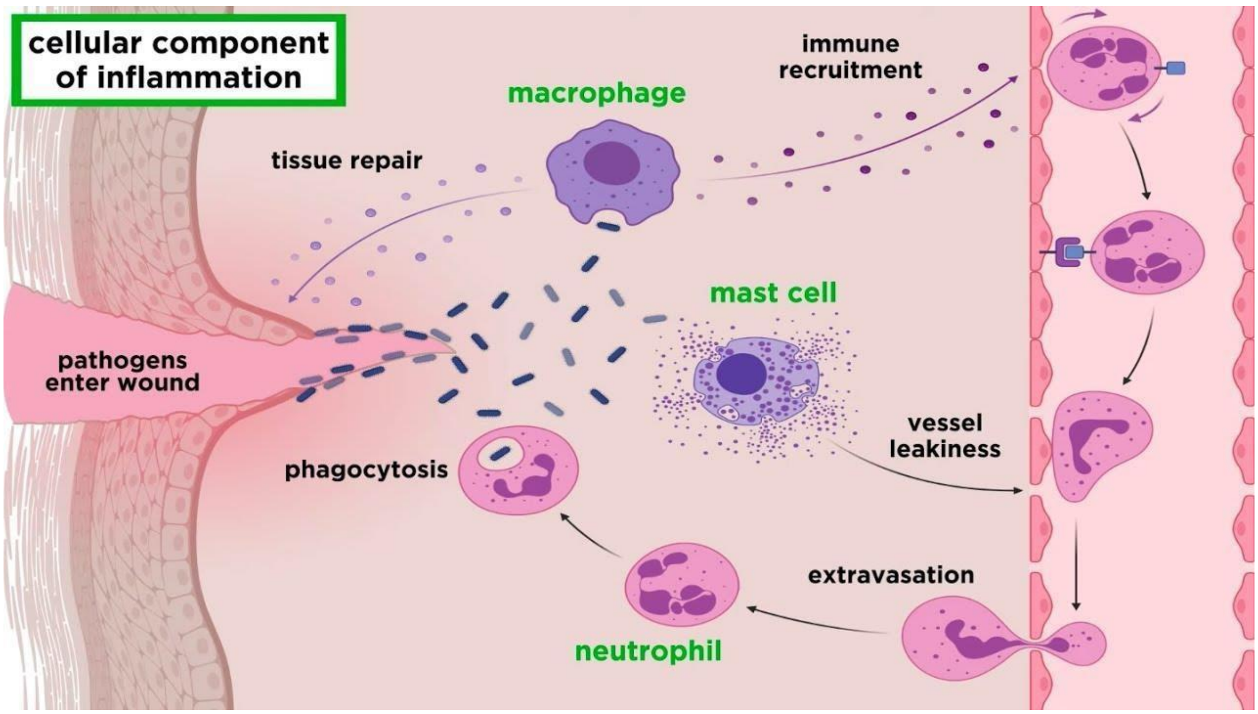
- Initiation (Damage to tissue): When pathogens or non-pathogens particles breach the external barriers of innate immunity which includes skin and mucous membrane that leads to tissue injury.
- Tissue response: the early event of tissue response is releasing of chemical factors from damage cells in injured tissue, these factors are promote to vasodilation (increase the diameter of blood vessels as arterioles, veins, and capillaries) with constriction in the smooth muscles, redness (erythema) and temperature elevate, that leading to an increase permeability facilitate an influx of fluid called (exudates) from blood vessels in to the tissue which includes C-reactive protein (CRP), enzymes of clotting system, activated complement, that resulting accumulate of exudates contributing to tissue swelling, and called of this phenomenon (edema).
- Leukocyte response: influx of leukocytes from vessels in to tissue is facilitated by the increase capillary permeability, the emigration of phagocytes includes:

1. Migration and adherence of phagocytic cells to endothelial wall of the blood vessels.

2. Diapedesis or extravasations that which mean pass or emigration of the cells in the inflamed region.

3. Migration of chemotaxis through the tissue to the site of the inflammatory response.

4. The phagocytic cells accumulate at the site of inflamed and begin to phagocytose of invading pathogens (e.g. bacteria) and damage tissue. The accumulation of dead cells, digested materials and fluid forms a substance called (pus).



- Tissue repair (resolution): The final stage of inflammation is tissue repair, when all harmful agents or substances have been removed or neutralized at the injury site. The ability of a tissue to repair itself depends on the part of the tissue involved. Skin, being a relatively simple tissue has a high capacity for regeneration.
- Cure: include fill, cover, and shrink the wound.

Fever: Is an important systemic component of inflammation, regulatory by thermostat of control center in hypothalamus, normal elevated of body temperature which is initiated under the effect of pyogenes which may be:

1. Exogenous: which are products of infectious agents like fungi, bacteria, viruses.

2. Endogenous: liberates form phagocytic cells as a natural part of immune response like some cytokines .

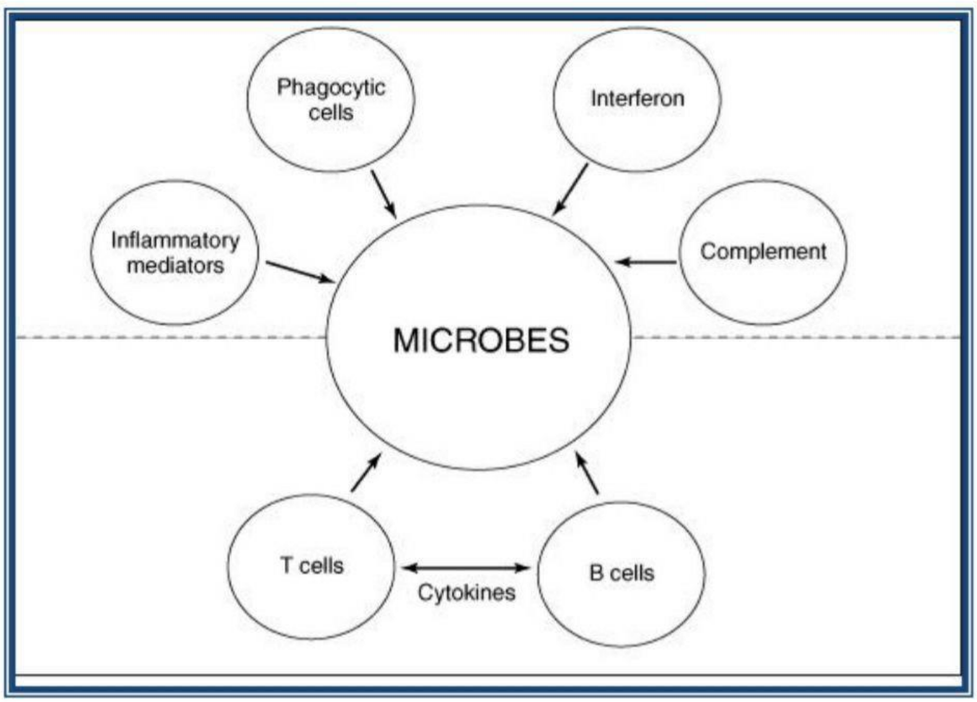
Benefits of fever:

1. Inhibit multiplication of sensitive microorganisms to temperature e.g. viruses.

2. Increase of metabolism and stimulate immune response like phagocytosis and other specific immune response.

Interferons:

Viral infection induces the expression of antiviral proteins known as interferons. These proteins, called interferon- (IFN-) .And interferon- (IFN α, IFN B-), are distinct from the interferon (IFN-Y) produced by activated T lymphocytes. The alpha and beta interferons help control viral replication by inhibiting protein synthesis in cells.



The complement system (part I)

Complement is the name given to a system of nonspecific proteins present in normal human & animal serum which has got the ability to lyse or damage cells (bacteria , viruses, …etc) & to stimulate some Ag-Ab reactions. The complement system helps the ability of antibodies and phagocytic cells to clear pathogens from an organism. It is a part of innate and adaptive immune system.

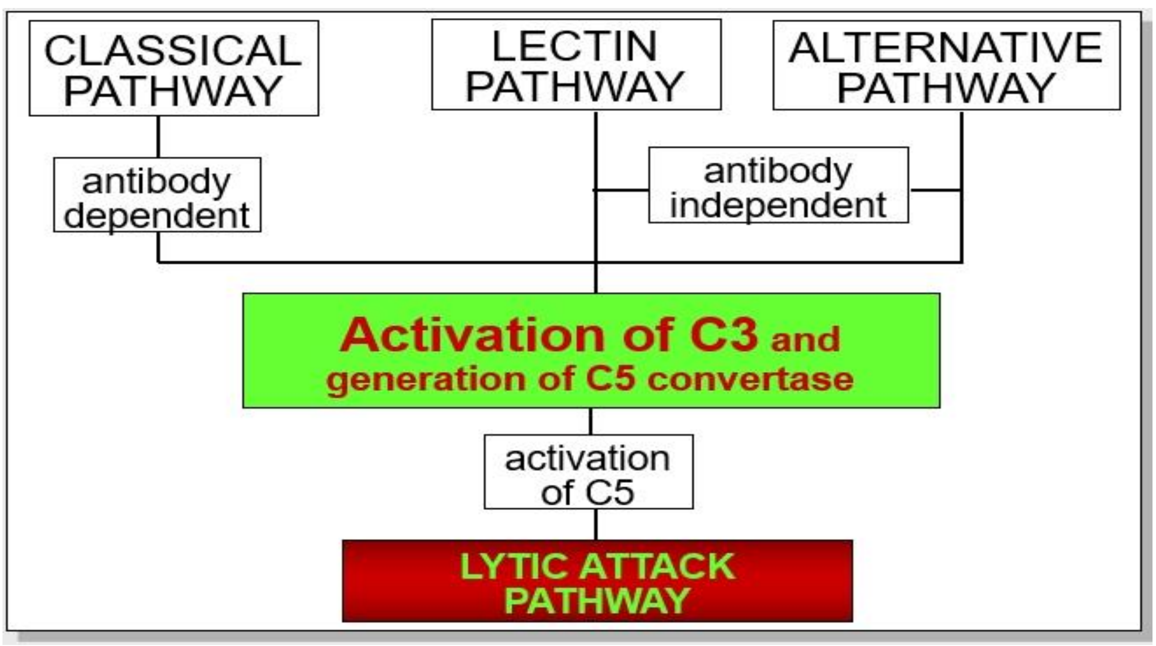
- The complement system consists of a number of small proteins (Over 25 proteins and protein fragments, make up the complement system) found in the blood.
- The proteins and glycoproteins that constitute the complement system are synthesized by hepatocytes and other cells such as tissue macrophages, blood monocytes, and epithelial cells of the genito-urinal tract and gastrointestinal tract.
- The end-result of this activation cascade is massive amplification of the response and activation of the cell-killing membrane attack complex (MAC).
- The complement proteins constitute about 5 % of the serum proteins & which are normally present in circulation inactive form.
- Cleavage of components generates a small fragment which is released, and a larger molecule which attaches to cell surface and continues in reaction sequence.
- The complement works as a **cascade system.**

Cascade is when one reaction triggers another reaction which trigger others and so on. These types of systems can grow exponentially very fast.

❖ The following are the basic functions of complement:

- opsonization to enhance phagocytosis
- phagocyte attraction and activation
- lysis of bacteria and infected cells
- regulation of antibody responses
- clearance of immune complexes &clearance of apoptic cells

Three biochemical pathways activate the complement system: the **classical** complement pathway, the **alternative** complement pathway, and the **lectin** pathway.



The classical complement pathway typically requires **antigen-antibody complexes for activation (specific immune response),** whereas the ***alternative*** and ***mannose-binding lectin*** *pathways* can be activated by C3 hydrolysis or antigens without the presence of antibodies (**non-specific immune response**).

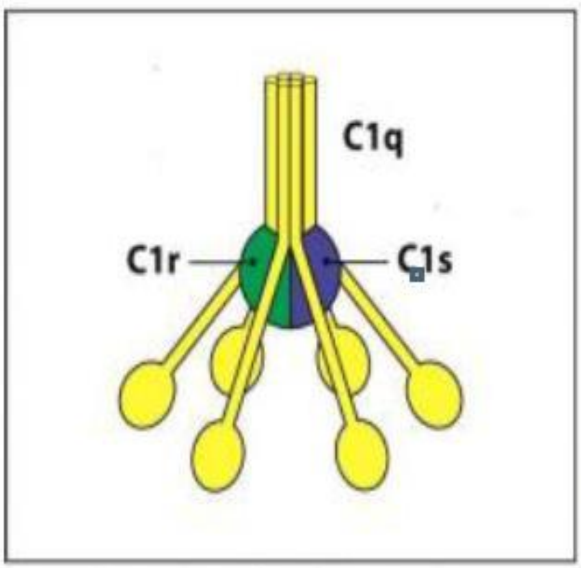
Classical pathway:

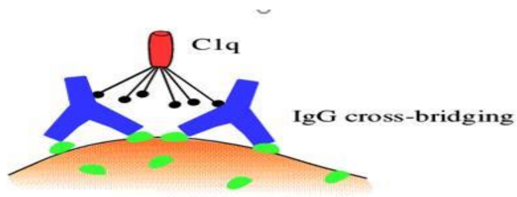
The classical complement pathway is one element of adaptive immunity.

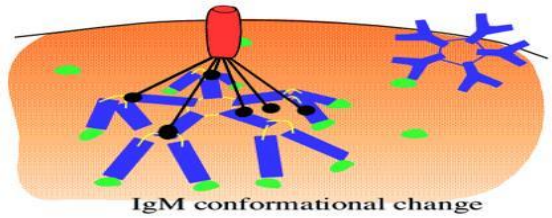
- The classical pathway is triggered by activation of the C1-complex.

The C1-complex is composed of 1 molecule of C1q, 2 molecules of C1r and 2 molecules of C1s, or C1qrs.

- The classical pathway is triggered when C1q binds to IgM or IgG complexed with antigens.
- A single IgM can initiate the pathway, while multiple IgGs are needed.
- Such binding leads to conformational changes in the C1q molecule, which leads to the activation of two C1r molecules. C1r is a serine protease. They then cleave C1s (another serine protease).



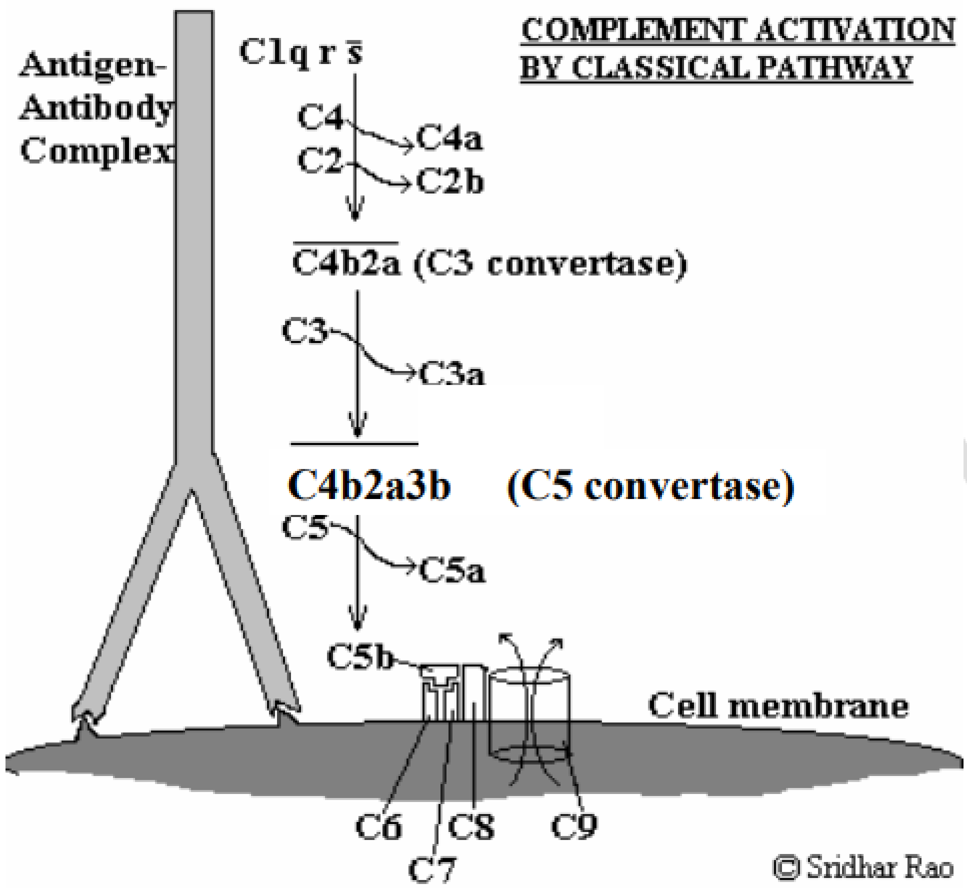




- ❖ The C1r2s2 component now splits C4 and then C2, producing C4a, C4b, C2a, and C2b.
- ❖ **C4b and C2b bind to form the classical pathway C3-convertase** (C4b2b complex), which promotes cleavage of C3 into C3a and C3b; C3b later joins with C4b2b (the C3 convertase) to make C5 convertase (C4b2b3b complex), and so on for the other components of classical pathway C6, C7, C8 and C9.
- ❖ C5b initiates the membrane attack pathway, which results in the membrane attack complex (MAC) that consisting of C5b, C6, C7, C8, and polymeric C9.

Membrane attack complex (MAC) forms a transmembrane channel in which cytoplasm can rush out of and water rushes in, which causes osmotic lysis of the target cell.

- ➢ C3a binds to the surface of pathogens, leading to greater internalization by phagocytic cells by opsonization.
- ➢ C5a is an important chemotactic protein, helping recruit inflammatory cells.
- ➢ Both C3a and C5a have anaphylatoxin activity, directly triggering degranulation of mast cells as well as increasing vascular permeability and smooth muscle contraction.


COMPLEMENT ACTIVATION
BY CLASSICAL PATHWAY
Antigen-
Antibody
Complex
C1q r s̄
C4
C4a
C2
C2b
C4b2a (C3 convertase)
C3
C3a
C4b2a3b (C5 convertase)
C5
C5a
C5b
Cell membrane
C6
C7
C8
C9
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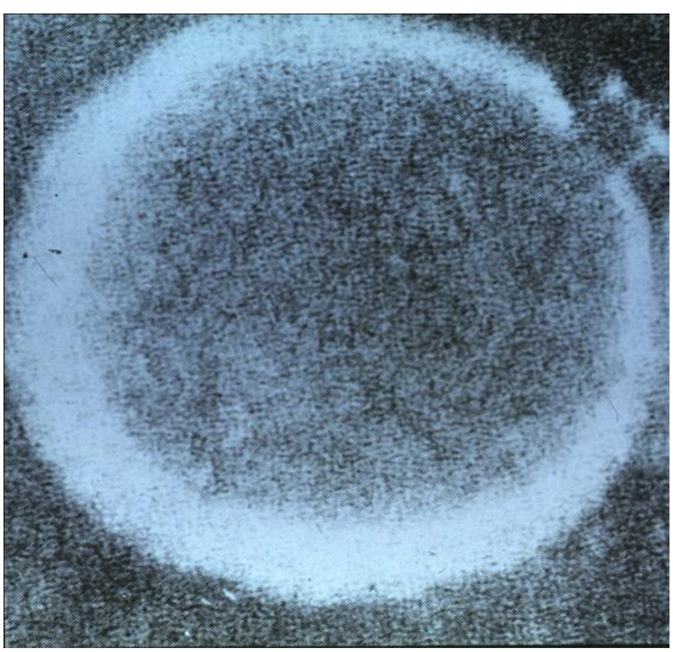


The complement system (part II)

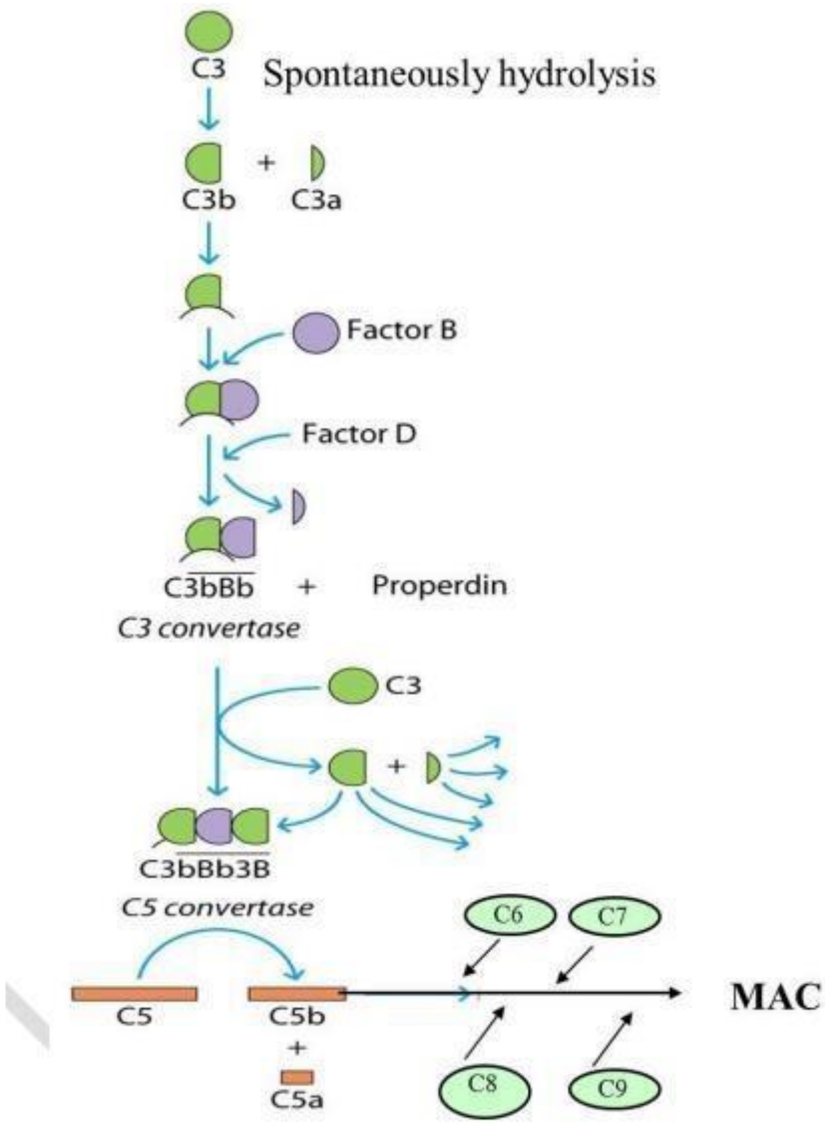
Alternative pathway:

- ❖ The alternative complement pathway is one element of innate immunity. This pathway doesn't depend on antibodies (antibody-independent).
- ❖ Triggering by cell wall components of bacteria, fungi, viruses and some parasites, like: lipopolysaccharides (LPS) in gram –ve bacteria and teichoic acid in gram +ve bacteria.
- ❖ The alternative pathway does not rely on pathogen-binding antibodies like the other pathways.

Steps of activation of alternative pathway: The initial component of the alternative pathway involves four serum proteins: C3b, factor B, factor D, and properdin.

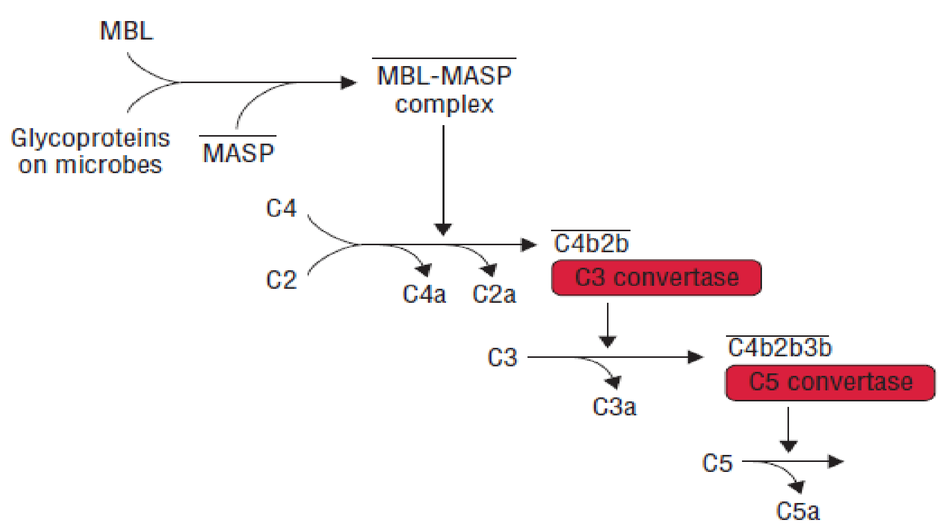
1. The alternative pathway activated at a low level, as a result of spontaneous C3 hydrolysis due to the breakdown of the internal thioester bond (C3 is mildly unstable in aqueous environment). To form C3b
2. The C3b is able to bind to foreign surface antigen (ex. LPS)
3. C3b that is generated from C3 by a C3 convertase enzyme complex in the fluid phase is rapidly inactivated by factor H and factor I.

4. The surface-bound C3b may now bind with another plasma protein (factor B) to form C3bB. This complex in the presence of factor D will be cleaved into Ba and Bb.
5. Bb will remain associated with C3b to form C3bBb, which is the alternative pathway C3 convertase.
6. The C3bBb complex is stabilized by binding factor P (properdin).
7. The stabilized C3 convertase, C3bBbP, then acts enzymatically to cleave much more C3.
8. The alternative complement pathway is able to distinguish self from non-self on the basis of the surface expression of complement regulatory proteins.
9. Host cells don't accumulate cell surface C3b (and the proteolytic fragment of C3b called iC3b) because this is prevented by the complement regulatory proteins, while foreign cells, pathogens and abnormal surfaces may be heavily decorated with C3b and iC3b.
10. Once the alternative C3 convertase enzyme is formed on a pathogen or cell surface, it may bind covalently with another C3b, to form C3bBbC3bP, the C5 convertase. This enzyme then cleaves C5 to C5a, a potent anaphylatoxin, and C5b. The C5b then recruits and assembles C6, C7, C8 and multiple C9 molecules to assemble the membrane attack complex. This creates a hole or pore in the membrane that can kill or damage the pathogen or cell.


C3
Spontaneously hydrolysis
C3b
C3a
Factor B
Factor D
C3bBb
+
Properdin
C3 convertase
C3
C3bBb3B
C5 convertase
C6
C7
C5
C5b
+
C5a
C8
C9
MAC

Lectin pathway

- The lectin pathway, as the name suggests, is triggered by lectins. Lectins are the proteins that recognize and bind to specific carbohydrate targets.
- The mannose-binding lectin (MBL) is one such protein that takes part in the lectin pathway of complement activation. MBL is a large serum protein that binds to mannose, fructose, and glucosamine on bacterial and other cell surfaces with mannose-containing polysaccharides (*mannans*).
- The binding of MBL to a pathogen results in the secretion of two MBL-associated serine proteases: MASP-1 and MASP-2. MASP-1 and MASP-2 are similar to C1r and C1s, respectively, and MBL is similar to C1q.
- Formation of the MBL/MASP-1/ MASP-2 tri-molecular complex results in activation of MASPs and subsequent cleavage of C4 into C4a and C4b. Subsequently, it proceeds to produce MAC in the same way as that occurs in the classical and alternative pathways.



Lectin pathway of activation of the complement

It is the more important pathway in early infection especially in babies between (6-18) months (in the period between decreases antibodies passively transferred)

Regulation of Complement System

Many components of the activated complement system are capable of attacking host cells besides foreign cells and microorganisms. Thus it is very important that the host cells to protected from autologus attack. A series of regulatory proteins involved in the regulation mechanism.

1. C1 inhibitor (C1-INH) Important regulator of classic pathway a serine protease inhibitor (serpin)

Irreversibly binds to and inactivates C1r and C1s

2. Factor H Regulate alternative pathway Reduce amount of C5 convertase available

3. Properdin Protects C3b and stabilizes C3 convertase

4. Factor I Cleaves cell-bound or fluid phase C3b and C4b → inactivates C3b and C4b

5. Decay accelerating factor (DAF) CD55 Glycoprotein on surface of human cells Prevents assembly of C3bBb or accelerates disassembly of preformed convertase > no formation of MAC Acts on both classical and alternative

Role of complement system in disease:

- Mutations in the C1 inhibitor gene or deficiency can cause hereditary angioneurotic edema (HANE).
- Deficiency in C3 leads to increased susptibility to bacterial infections.
- Mutations in the MAC components of complement, especially C8, are often implicated in recurrent Neisserial infection
- Acquired deficiency of DAF results in an increase in complement mediated hemolysis.

Hypersensitivity (part I)

Hypersensitivity refers to an inappropriate reactions produced by the normal immune system, resulting in tissue damage. It is a harmful immune response in which tissue damage is induced by inappropriate immune responses in a sensitized individual on re-exposure to the same antigen. Both the humoral and cell-mediated arms of the immune response may participate in hypersensitivity reactions.

Gell and Coombs (1963) classified hypersensitivity reactions into four categories based on the time elapsed from exposure of antigen to the reaction and the arm of immune system involved. Types I, II, and III are antibody-mediated and are known as immediate hypersensitivity reactions, while type IV is cell-mediated (i.e., mediated by cell-mediated immunity) and is known as delayed hypersensitivity reactions.

Type	Name	Mechanism	Disease examples
Type I	Immediate hypersensitivity	IgE-mediated degranulation of mast cells following antigen binding and cross-linking of IgE	Allergic asthma, allergic rhinitis, anaphylaxis
Type II	Antibody-mediated hypersensitivity	IgM/IgG antibody:antigen interactions on target cell surfaces	Drug-induced thrombocytopenia, myasthenia gravis, Graves disease, haemolytic anaemia of newborn
Type III	Immune complex-mediated hypersensitivity	Immune complex formation and deposition in tissues leading to local or systemic inflammatory reactions	Rheumatoid arthritis, SLE, Goodpasture's syndrome, Arthus reaction, serum sickness
Type IV	Delayed-type hypersensitivity	Sensitized T_H1 cells activated to release cytokines upon binding to antigen, resulting in macrophage and cytotoxic T cell accumulation	Contact dermatitis, chronic transplant rejection

Type I (Anaphylactic) Hypersensitivity

- Type I hypersensitivity reaction is commonly called allergic or immediate hypersensitivity reaction. This reaction is always rapid, occurring within minutes of exposure to an antigen, and always involves IgE-mediated degranulation of basophils or mast cells. IgE is responsible for sensitizing mast cells and providing recognition of antigen for immediate hypersensitivity reactions.
- The short time lag between exposure to antigen and onset of clinical symptoms is due to the presence of preformed mediators in the mast cells. Thus, the time taken for these reactions to initiate is minimal, so the onset of symptoms seems to be immediate. **Type I reaction can occur in two forms: anaphylaxis and Angioedema.**

Anaphylaxis

Anaphylaxis is an acute, potentially fatal, and systemic manifestation of immediate hypersensitivity reaction.

- It occurs when an antigen (allergen) binds to IgE on the surface of mast cells with the consequent release of several mediators of anaphylaxis.
- On exposure to the antigen, TH2 cells specific to the antigen are activated, leading to the stimulation of B-cells to produce IgE antibody.
- The IgE then binds to Fc portion of mast cells and basophils with high affinity.
- On re-exposure to the antigen, the allergen cross-links the bound IgE, followed by activation of IgE and degranulation of basophils and mast cells to release pharmacologically active mediators within minutes.

- Severe *systemic* reaction; massive histamine release; systemic vasodilation, risk of edema that can block airways, circulatory shock

Triggers:. Typical allergens include:

■ Plant pollen, proteins (e.g., foreign serum and vaccines),

■ Certain food items (e.g., eggs, milk, seafood, and nuts),

■ Drugs (e.g., penicillin and local anesthetics),

Signs and symptoms of Anaphylaxis:

respiratory distress; if in GI tract, nausea, vomiting, diarrhea, cramps, bloating; ultimately, hypotension, fainting, shock

Angioedema

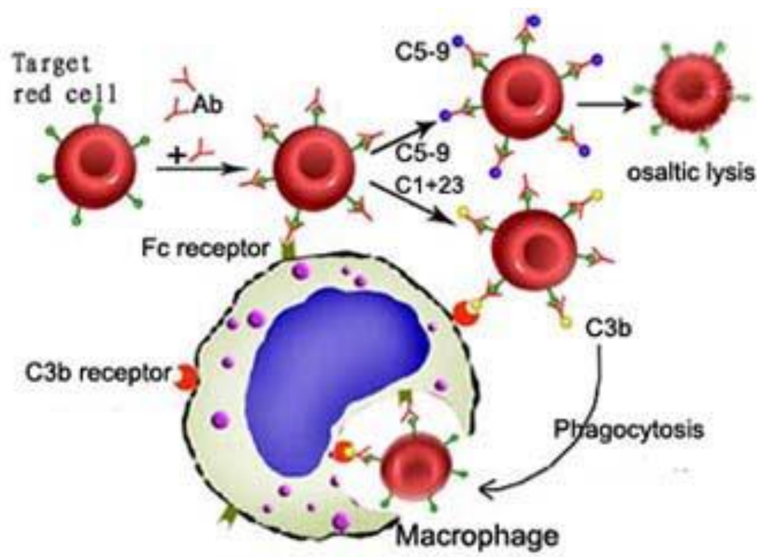
- **Rapid onset of *localized* swelling** Skin, genitals, extremities, GI tract, respiratory tract (can obstruct airway)
- **Triggers**: Nuts, chocolate, fish, eggs, milk, preservatives, aspirin, some hypertensive drugs
- **Signs and symptoms of Angioedema**: Depends on location; may not be itchy, usually resolves within 72 hours

Type II (Cytotoxic) Hypersensitivity

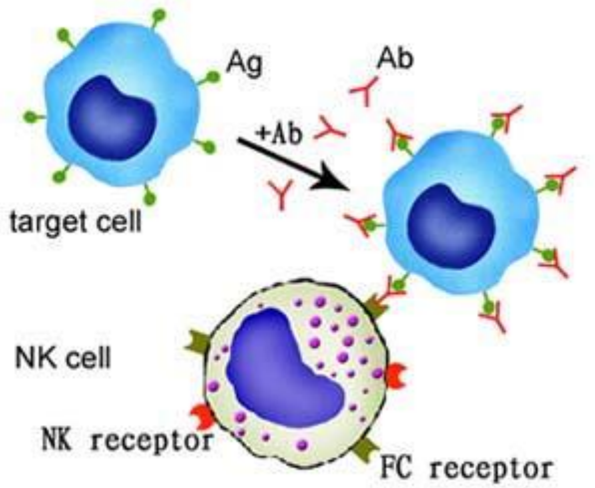
Type II cytotoxic reaction is mediated by antibodies directed against antigens on the cell membrane that activates complement thereby causing antibody-mediated destruction of cells.

- The cell membrane is damaged by a membrane attack complex during activation of the complement.

- The reactions involve combination of IgG or IgM antibodies with the cell-fixed antigens or alternately circulating antigens absorbed onto cells.
- Antigen–antibody reaction leads to complement activation, resulting in the formation of membrane attack complex. This complex then acts on the cells, causing damage to the cells, as seen in complement-mediated lysis in Rh hemolytic disease, transfusion reaction, or hemolytic anemia.
- **Antibody-dependent cell-mediated cytotoxicity (ADCC):** Itis another mechanism, which involves the binding of cytotoxic cells with Fc receptors in the Fc binding part of the antibodies coating the target cells. The antibody coating the target cell can also cause its destruction by acting as an opsonin.



Complement-dependent reactions that lead to cell lysis or susceptible to phagocytosis



Antibody-dependent cell-mediated cytotoxicity

Hypersensitivity (part II)

Type III hypersensitivity reactions Immune Complex Disease

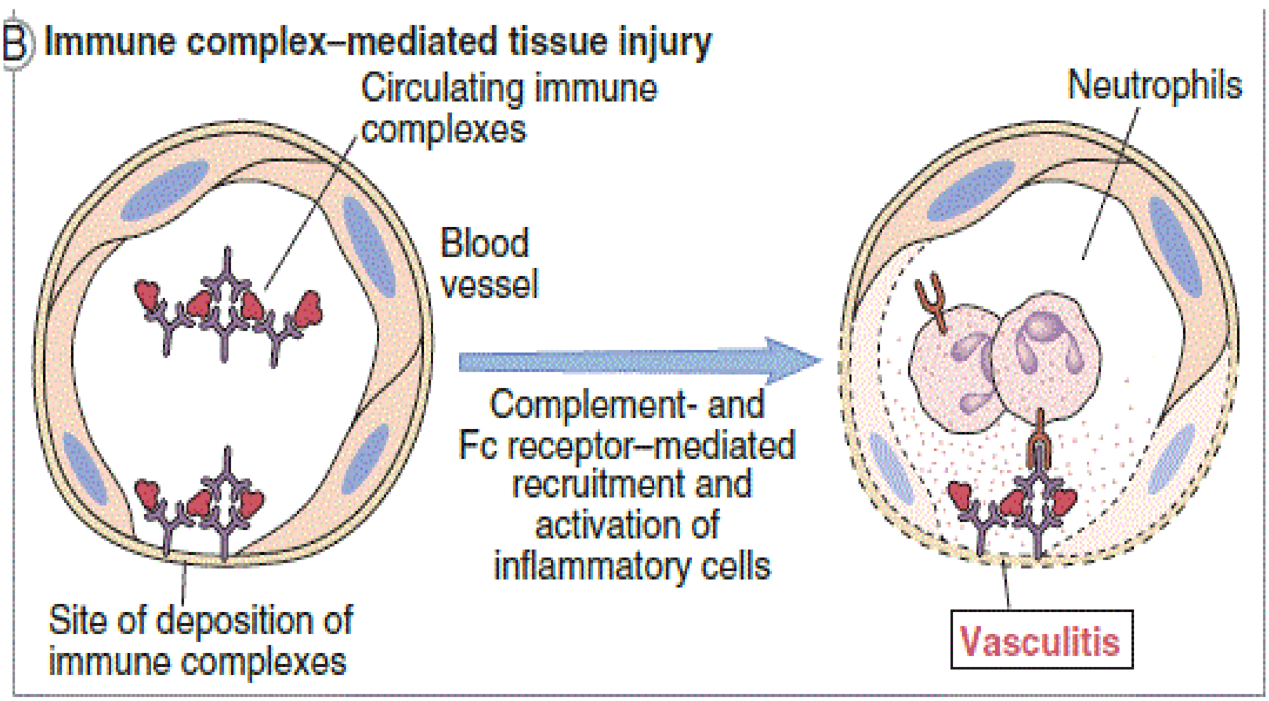
This type caused by formation of immune complexes in circulation and deposit it in blood vessels. Under normal conditions complexes are bound by complement to RBCs and then eliminated by phagocytes.

- In Type III hypersensitivity, immune complexes are not cleared out and they become deposited in tissues. The antigens that form immune complexes may be *exogenous*, such as a foreign protein that is injected or produced by an infectious microbe, or *endogenous*
- Clinical example

1- Poststreptococcal glomerulonephritis

2- systemic lupus erythematosus

3- serum sickness



There are two types of reactions:-

- Local reaction due to local Ag to particular organ such as the kidney (glomerulonephritis), joints (arthritis), or the small blood vessels of the skin if the complexes are deposited or formed locally (local Arthus reaction)

Arthus reaction

- If an animal or human is injected intra-dermally with an antigen to which large amounts of circulating antibodies exist(intravenous injections).
- Antigen will diffuse into the walls of local blood vessels and large immune complexes will precipitate close to the injection site.
- This initiates an inflammatory reaction that peaks approximately 4 to 10 hours post injection and is known as an Arthus reaction.
- Inflammation at the site of an Arthus reaction is characterized by swelling and localized bleeding, followed by fibrin deposition

Systemic reaction

when immune complexes are formed in the circulation and are deposited in many organs such as:

- SERUM SICKNESS: is induced by systemic administration of a protein antigen, which elicits an antibody response and leads to the formation of circulating immune complexes

- A condition may develop when a patient is injected with a large amount of antitoxin that was produced in an animal . Like horse anti-tetanus or anti-diphtheria serum.
- After about 10 days, the immune system produces antibodies, combine with these proteins to form immune complexes.
- The immune complex precipitate and enter the walls of blood vessels and activate complement cascade, initiating inflammatory response .
- These symptoms include fever, weakness, generalized vasculitis (rashes) with edema and erythema, arthritis and sometimes glomerulonephritis

Type IV hypersensitivity (Delayed type)

- Type IV hypersensitivity reaction is called delayed type hypersensitivity (DTH) because the response is delayed. .
- The reaction is characterized by large influxes of nonspecific inflammatory cells, in particular, macrophages.
- It differs from the other types of hypersensitivity by being mediated through cell-mediated immunity
- This reaction occurs due to the activation of specifically sensitized T lymphocytes rather than the antibodies.
- Initially described by Robert Koch in tuberculosis as a localized reaction., this form of hypersensitivity was known as tuberculin reaction.
- Later, on realization that the reaction can be elicited in various pathologic conditions, it was renamed as delayed type hypersensitivity.

Type IV hypersensitivity (Delayed type)

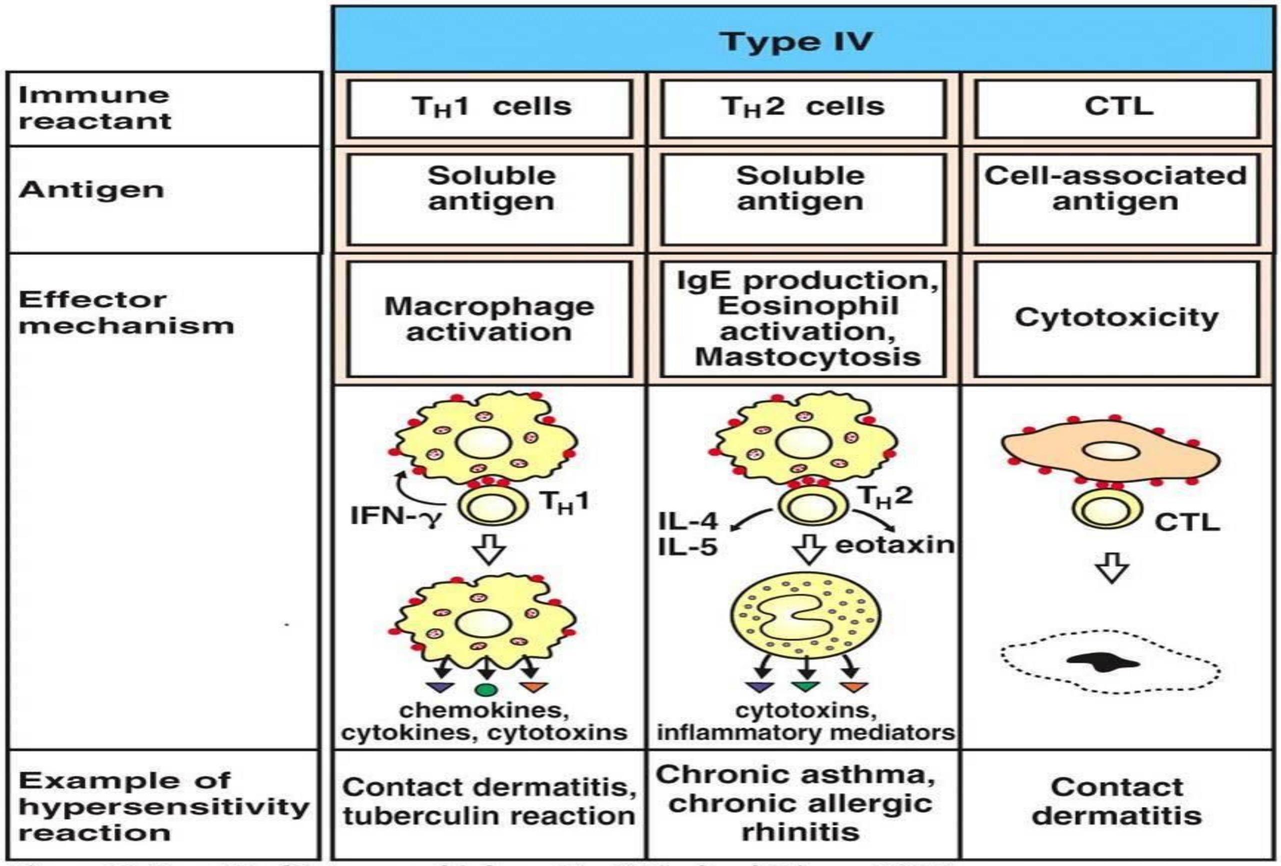


Figure 12-2 part 2 of 2 Immunobiology, 6/e. (© Garland Science 2005)

There are two stage of (DTH) mechanism:

- Sensitization stage

Proliferation and Differentiation of CD4+ T Cells when antigens are generated by dendritic cells during this stage. These T cells can activate macrophages and trigger inflammatory response.

- Effector stage

Responses of differentiated effector T cells. Secondary contact yields what we call DTH. T memory cells are activated and produce cytokines. Th1 secrete IFN-ɤ, TNF-α, and TNF-β which cause tissue destruction and inflammation. Activated Th17 cells secrete IL-17, IL-22, chemokines, and several other cytokines.

TYPES OF DTH REACTIONS

- DTH reactions are of two types: contact hypersensitivity and tuberculin-type hypersensitivity reactions.
- CONTACT HYPERSENSITIVITY

• Contact hypersensitivity is a manifestation of DTH occurring after sensitization with certain substances.

• These include drugs such as sulfonamides and neomycin, plant products such as poison ivy and poison oak, chemicals such as formaldehyde and nickel, and cosmetics, soaps and other substances.

• This reaction manifests when these substances acting as haptens enter the skin and combine with body proteins to become complete antigens to which a person becomes sensitized.

- On second exposure to the same antigen, the immune system responds by attack of cytotoxic T cells that cause damages, mostly in the skin.
- The condition manifests as itching, erythema, vesicle, eczema, or necrosis of skin within 12- -48 hours of the second exposure.

The classic example of DTH is the **Mantoux test** or **Mendel-Mantoux test** (**tuberculin sensitivity test or PPD test** for purified protein derivative) is a screening tool for tuberculosis (TB).

- When a patient previously exposed to mycobacterium tuberculosis is injected with a small amount of tuerculin (PPD) intradermally, there is little reaction in the first few hours. Gradually, induration and redness develop and reach a peak in 48-72 h.
- A positive skin test indicates that the person presence of current disease.