Allergy and Hypersensitivity

Dr Rojan Ghanim AL-Allaff

- Generally the immune system is protective,Protective mechanisms may result in severe damages to tissues and may lead to death.

When?

Severe damages may occur when the immune system responded in exaggerated or inappropriate form.

Classification:

1-Type I - immediate

2-Type II - antibody-dependent

3-Type III - immune complex

4-Type IV - cell-mediated or delayed

1- Type I - immediate (or atopic, or anaphylactic) :

- Type I hypersensitivity is an allergic reaction provoked by re-exposure to a specific antigen.
- Exposure may be by ingestion, inhalation, injection, or direct contact.
- The reaction is mediated by IgE antibodies and produced by the immediate release of histamine, tryptase, arachidonate and derivatives by basophils and mast cells.
- This causes an **inflammatory** response leading to an immediate (within seconds to minutes) reaction.
- The reaction may be either local or systemic. Symptoms vary from mild irritation to sudden death from **anaphylactic shock**.

Some examples:

Allergic asthma ,Allergic conjunctivitis ,Allergic rhinitis ("hay fever"),Anaphylaxis

2- Type II - antibody-dependent:

- In type II hypersensitivity, the antibodies produced by the immune response bind to antigens on the patient's own cell surfaces.
- The antigens recognized in this way may either be intrinsic ("self" antigen, innately part of the patient's cells) or extrinsic (absorbed onto the cells during exposure to some foreign antigen, possibly as part of infection with a pathogen .
- IgG and IgM antibodies bind to these antigens to form complexes that activate the classical pathway of complement activation for eliminating cells presenting foreign antigens (which are usually, but not in this case, pathogens).
- As a result mediators of acute inflammation are generated at the site and membrane attack complexes cause cell lysis and death. The reaction takes hours to a day.

Some examples:

Autoimmune haemolytic anaemia,Pernicious anemia ,Transfusion reactions

Hashimoto's thyroiditis

3 . Type III - immune complex

In type III hypersensitivity:

- soluble immune complexes (aggregations of antigens and IgG and IgM antibodies) form in the blood and are deposited in various tissues (typically the skin, kidney and joints)
- This may trigger an immune response according to the classical pathway of complement activation.
- The reaction takes hours to days to develop .

Some Examples:

Serum sickness ,Subacute bacterial endocarditis ,Symptoms of malaria

Systemic lupus erythematosus ,Arthus reaction.

4-Type IV Hypersensitivity

- Type IV hypersensitivity is often called delayed type as the reaction takes two to three days to develop.
- Unlike the other types, it is not antibody mediated but rather is a type of cell-mediated response.

Some clinical examples:

- Contact dermatitis ,tuberculosis ,Transplant rejection

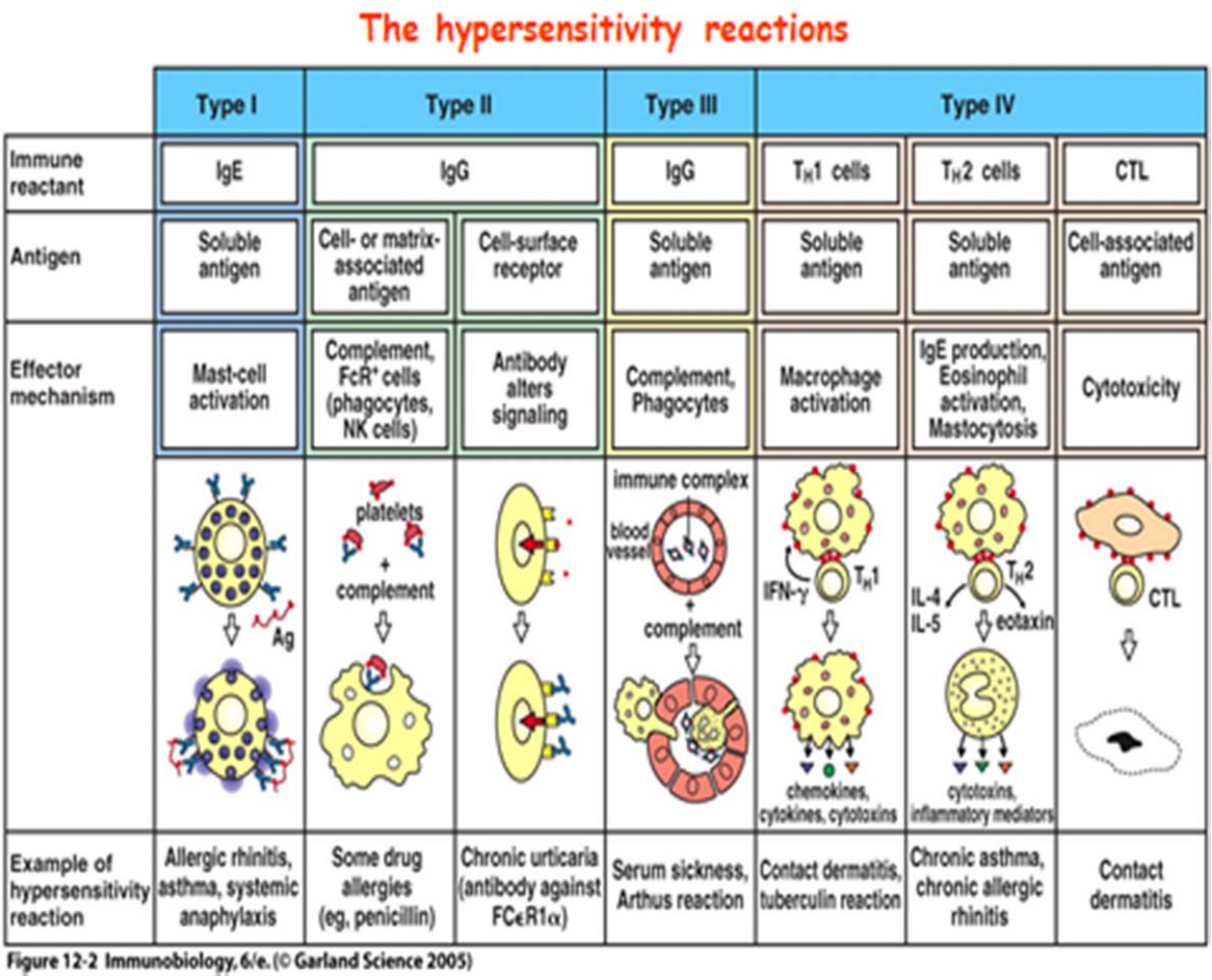
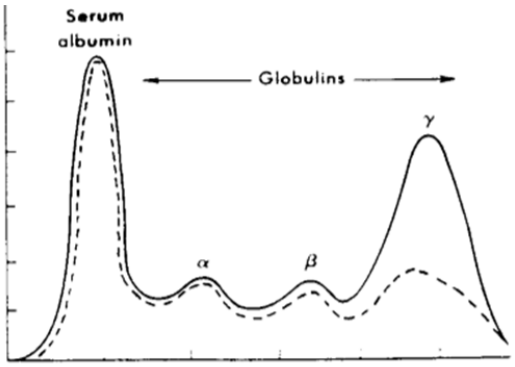


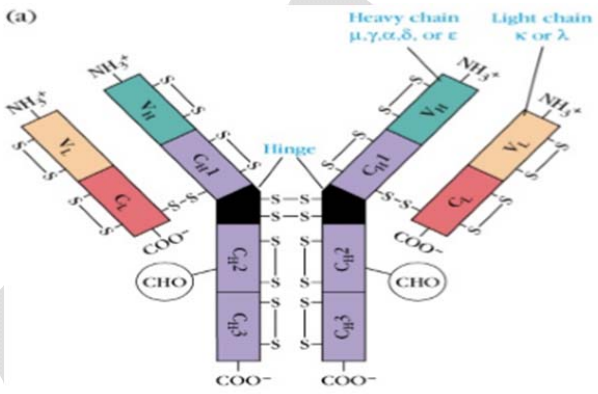
Figure 12-2 Immunobiology, 6/e. (© Garland Science 2005)

Antibody Structure and Function

Dr Rojan Ghanim AL-Allaff

The production of circulating antibodies is one of the major functions of the immune system. Antibodies belong to the general class of glycoproteins called globulins, due to their property of being insoluble in half-saturated ammonium sulfate solutions. Subsets of antibodies have been discovered and they are now known collectively as immunoglobulins.





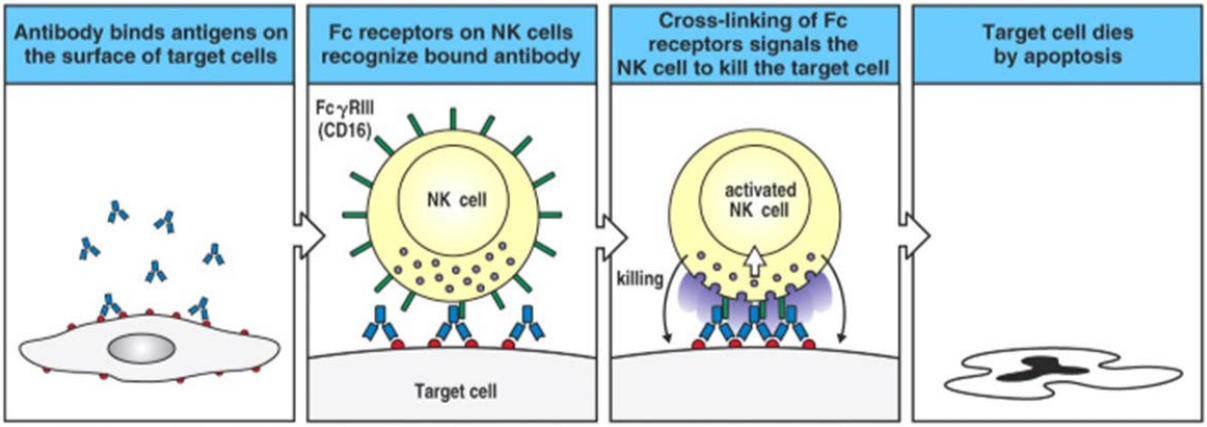
BIOLOGIC PROPERTIES OF IgG and IgM :

1- AGGLUTINATION AND PRECIPITATE FORMATION:IgG & IgM antibodies can cause **agglutination** of particulate antigens like bacteria. With soluble antigens such as toxins, IgG antibodies can form insoluble precipitates. Both types of reactions make the complexes easier for macrophage scavenger cells to ingest and destroy the antigen.

2- PLACENTAL PASSAGE : In humans, IgG is selectively passed through the placenta to provide passive immunity to the fetus. This begins in the 3 rd or 4 th month of pregnancy in humans. Resistance of the neonate to most common infections is almost exclusively via this passive IgG. Active transfer is mediated by the Fc region of IgG molecules.

3- OPSONIZATION: IgG is a powerful opsonizing antibody (from the Greek opsonin, to prepare for eating). The antibody reacts with microbial epitopes and its Fc region is then efficiently bound by specific Fc receptors on macrophages and/or polymorphonuclear cells. The net effect is engulfment of the bacteria into the phagocyte.

4- **ANTIBODY DEPENDENT CELL MEDIATED CYTOTOXICITY (ADCC):** Certain large granular lymphocytes [also called Natural Killer (NK) cells] have Fc receptors on their surface and when antibodies bind cellular antigens (for instance on tumor cells) NK cells can then bind to the antibody via the Fc portion. The NK cell can kill the tumor cells due to the secretion of substances by the NK cell that are cytotoxic to the tumor cells.



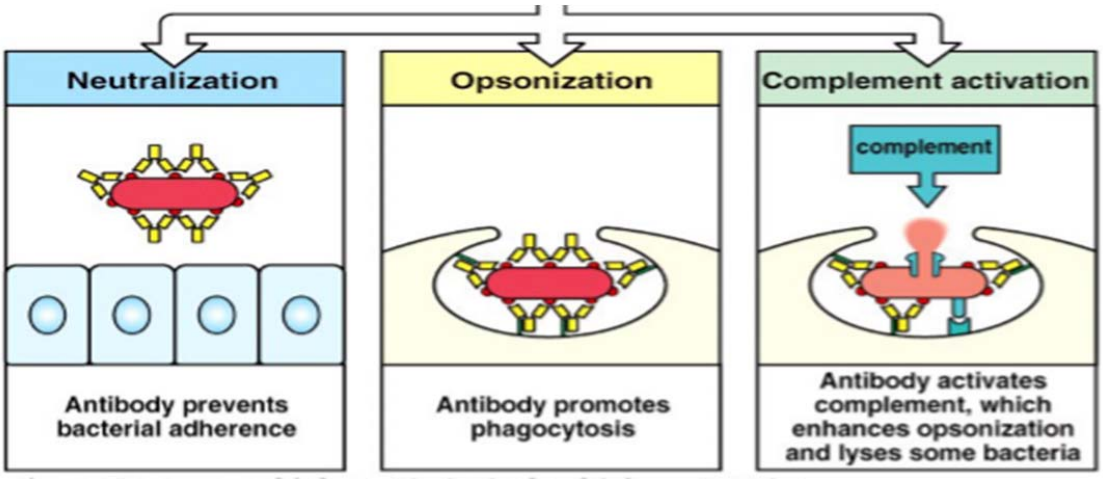
5- **COMPLEMENT ACTIVATION** : IgG and IgM antibodies are efficient activators of the Complement system. many antigen-antibody interactions trigger a series of enzymes collectively known as Complement. Some of the by-products of these reactions can act as opsonins and other components are chemotactic (attract phagocytic cells).

6- **BACTERIAL IMMOBILIZATION**:Motile bacteria can have their movement arrested by IgG and IgM antibodies by crosslinking their flagella or clumping them via their flagella. The antibody can function in this regard like handcuffs in stopping the waving of flagella. The result is that the bacteria are less invasive and less efficient in spreading through tissue.

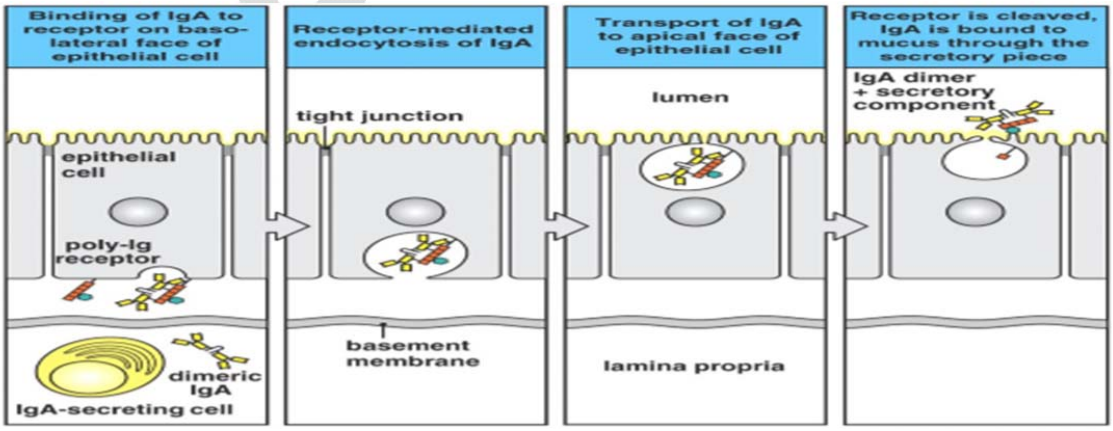
7- **VIRAL NEUTRALIZATION**:Most viruses utilize some form of cellular receptor for initial binding that results in the virus gaining entry into the cell or moving its DNA or RNA into the cell. IgG and IgM antibodies specific for those structures on the virus that bind to the cell receptors will inhibit or eliminate initial binding to the cell, thereby protecting the cell from viral entry. The binding of IgG also facilitates phagocytosis of the organism.

8- **TOXIN NEUTRALIZATION**: Bacterial toxins usually are toxic to cells because the toxin binds to specific cellular receptors to gain entry to the cell and then toxic

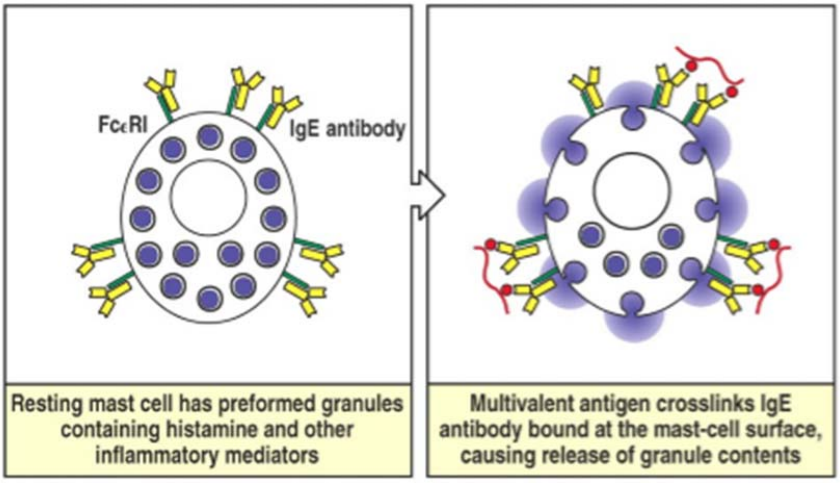
effects occur intracellularly. The strategy that the immune system employs to protect the animal from toxins is to make a variety of antibodies specific for many different epitopes on the toxin to immobilize it in the form of an antigenantibody aggregate and stop the toxin from reaching the cell receptor. The Ab-Ag aggregates can be easily phagocytosed and the toxins degraded and rendered non-toxic by acid proteases in the phagosomes.



BIOLOGIC PROPERTIES OF IgA : IgA appears selectively in the sero-mucous secretions such as saliva, tears, nasal fluids, sweat, colostrum and secretions of the lung, genitourinary and gastrointestinal tracts where it has the job of defending the exposed external surfaces of the body against attack by microorganisms from the environment. Serum IgA I is monomeric and has no known biological function and a short (5.5 day) half life. Secretory IgA (sIgA) is always in the dimeric form Most IgA is transported to epithelial surfaces (see figure below) where it functions as a first-line barrier of protection for these sensitive surfaces.

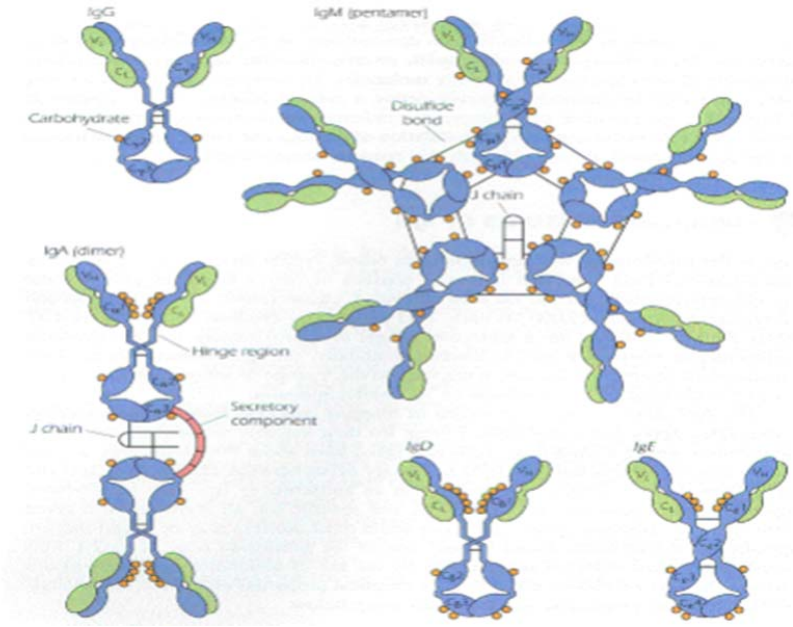


BIOLOGIC PROPERTIES OF IgE: Type I hypersensitivity is mediated by IgE antibodies and occurs when an IgE response is directed against innocuous antigens, such as pollen. The resulting release of pharmacological mediators such as histamine by the IgE-sensitized mast cells produces an acute inflammatory reaction with symptoms such as asthma or rhinitis. The most characteristic manifestation of these types of reactions is hay fever; swollen eyes, runny nose, etc.



STRUCTURAL FEATURES OF IgD: IgD is only found in trace quantities in serum. Its primary biological role is in triggering of lymphocytes. It is co-expressed on the surface of certain subsets of lymphocytes along with IgM.

Schematic structures for the 5 major isotypes of immunoglobulins are shown below:



IMMUNOGENS AND ANTIGENS

Dr Rojan Ghanim AL-Allaff

IMMUNOGEN - Agent capable of binding immune receptors AND inducing an immune response by B cells and T cells

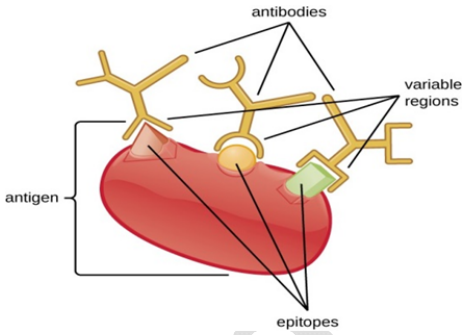
ANTIGENS – Agent that binds with varying degrees of specificity to immune receptors (antibodies on B cells; T cell receptor on T cells)

EPITOPES RECOGNIZED BY T OR B CELLS : Epitopes are the three dimensional arrangements of atoms (sites) on the surface of an antigen that bind to the paratope of an antibody OR the linear peptides that bind the MHC molecules/T cell receptor. Epitopes recognized by B cells generally differ from those recognized by T cells. B cells can mount specific antibody responses without or with help from T cells (T- independent or T- dependent cells) .

Antigen Recognition by B and T Cells

Characteristic	*B cells*	*T cells*
Mechanism	BCR binds Ag	TCR binds Ag+MHC Ag
Antigen nature	Protein/polysaccharide/lipid	Peptide
Epitopes	Surface, linear, conformational	Internal linear peptides

PHYSICOCHEMICAL FORCES INVOLVED IN ANTIGEN-IMMUNE RECEPTOR BINDING:



1. Electrostatic interactions.
2. Hydrogen bonding
3. Van der Waal's forces
4. Hydrophobic bonding

MAJOR CLASSES OF ANTIGENS/IMMUNOGENS:

The following major chemical classes of compounds may be antigenic/immunogenic:

1. Proteins or glycoproteins. Most proteins or glycoproteinsare excellent antigens. The greater the complexity and molecular weight, the better it is as an antigen.

2. Carbohydrates or polysaccharides. Bacterial capsules (i.e.pneumococci) are powerful antigens. The ABO blood group epitope sare carbohydrates.

3. Lipids. Are not routinely antigenic, but if used as a hapten, immune responses can be elicited, i.e.sphingolipids.

4. Nucleic Acids. Are poorly immunogenic themselves, but as haptensare good antigens. Antibodies to DNA are important in patients with systemic lupus erythematosus.

REQUIREMENTS FOR IMMUNOGENICITY

1. Size, dose , route: Usually,compounds of less than 1,000 daltons are non-immunogenic.Compounds between1,000 and 6,000 daltons may or may

not be immunogenic. Those greater than 6,000 daltons are generally immunogenic. Intermediate dose is most immunogenic.Immunogenicity is also a function of route of administration.

2. Chemical Composition: Physicochemical complexity is usually necessary for a compound to be immunogenic. Homopolymers of amino acids usually are not immunogenic (i.e.B.anthracis poly-gamma-D-glutamic acid 50,000 daltons).
3. Foreignness: Was once considered to be an absolute requirement for immunogenicity. It is now clear that certain self-components can be immunogenic to the individual. Foreignness is an excellent general guideline as to whether something might be immunogenic, but it is not a definitive requirement for immunogenicity. Particulate and denatured antigens are often more immunogenic.
4. Adjuvants/Degradability:Adjuvants enhance immune responses by inducing cytokine release or antigen processing. T-dependent immunogens must be enzymatically degraded in order to be immunogenic. Peptides of D-amino acids are non-immunogenic whereas their L-isomers usually are immunogenic.Genes mapping to the Major Histocompatibility Complex (MHC) can profoundly affect the degree of immunogenicity of any substance.

HAPTENS:

Haptens are low molecular weight compounds that are non-immunogenic by themselves but become immunogenic after conjugation to high molecular weight carrier substances that are immunogenic.

CELLS OF THE IMMUNE SYSTEM

Dr. Rojan Ghanim AL-allaff

Reading: Coico and Sunshine. Immunology: A Short Course. John Wiley & Sons, Inc, New York,NY. 6th edition, 2009. Chapter 2; Geha and Notarangelo. Case Studies in Immunology. Garland Publishing, New York, NY. 6th edition, 2012. Case 30: Congenital Asplenia.
Web Resource:
http://www.uth.tmc.edu/pathology/medic/immunology/Immuno/cellsimmsys.html

Immune system cells are derived from pluripotent hematopoietic stem cells in the bone marrow.These cells can be functionally divided into groups that are involved in two major categories of immune responses: innate (natural) and acquired. Innate immunity is present from birth and consists of non-specific components. Acquired immunity by definition requires recognition specificity to foreign (non-self) substances. The major properties of the acquired immune response are specificity, memory, adaptiveness, and discrimination between self and non-self.
The acquired immune response is subdivided into humoral and cellular immunity, based on participation of two major cell types. In Humoral Immunity, B lymphocytes synthesize and secrete antibodies.Cellular Immunity (CMI) involves effector T lymphocytes which secrete immunoregulatory factors following interaction with antigen presenting cells (APCs).

Leukocytes

Leukocytes provide either innate or specific adaptive immunity. These cells are derived from myeloid or lymphoid lineage. Myeloid cells include highly phagocytic, motile neutrophils, monocytes, and macrophages that provide a first line of defense against most pathogens. The other myeloid cells, including eosinophils, basophils, and their tissue counterparts, mast cells, are involved in defense against parasites and in the genesis of allergic reactions. Cells from the lymphoid lineage are responsible for humoral or cell mediated immunity.

Myeloid Cells

Neutrophils: Neutrophils are the most highly adherent, motile, phagocytic leukocytes and are the first cells recruited to acute inflammatory sites. They ingest, kill, and digest pathogens, with their functions dependent upon special proteins, such as adherence molecules, or via biochemical pathways (respiratory burst).

Eosinophils: Eosinophils defend against parasites and participate in hypersensitivity reactions via cytotoxicity. Their cytotoxicity is mediated by large cytoplasmic granules, which contain eosinophilic basic and cationic proteins.

Basophils/Mast cells: Basophils, and their tissue counterpart mast cells, produce cytokines that help defend against parasites, and also cause allergic inflammation. These cells display high affinity surface membrane receptors for IgE antibodies, and have many cytoplasmic granules containing heparin and histamine. The cells degranulate when cell-bound IgE antibodies are crosslinked by antigens, and produce low-molecular weight vasoactive mediators (e.g. histamine).

Monocytes/Macrophages: Monocytes and macrophages are involved in phagocytosis and intracellular killing of microorganisms.Macrophages are differentiated monocytes,which are one of the principal cells found to reside for long periods in the RES reticulo-endothelial system. These monocytes/macrophages are highly adherent, motile and phagocytic; they marshal and regulate other cells of the immune system, such as T lymphocytes; they serve as antigen processing-presenting cells.

Dendritic Cells: Dendritic cells provide a link between innate and adaptive immunity by interacting with T cells in a manner to deliver strong signals for development of memory responses. Dendritic cells recognize foreign agents and pathogens through a series of pattern recognition receptors (non-specific), and are able to present antigen to both T helper and T cytotoxic cells to allow those lymphocytes to mature towards functionality.

Lymphoid Cells

Lymphoid cells provide efficient, specific and long-lasting immunity against microbes/pathogens and are responsible for acquired immunity. Lymphocytes differentiate into three separate lines: (1)thymic-dependent cells or T lymphocytes that operate in cellular and humoral immunity; (2) B-lymphocytes that differentiate into plasma cells to secrete antibodies; and (3) natural killer (NK) cells. T and B lymphocytes produce and express specific receptors for antigens while NK cells do not.

B Lymphocytes: B lymphocytes differentiate into plasma cells to secrete antibodies. The genesis of mature B cells from pre-B cells is antigen-independent. The activation of B cells into antibody producing/secreting cells (plasma cells) is antigen-dependent. Mature B cells can have 1-1.5 x 10^5 receptors for antigen embedded within their plasma membrane. Once specific antigen binds to surface Ig molecule, the B cells differentiate into plasma cells that produce and secrete antibodies of the same antigen-binding specificity. If B cells also interact with T helper cells, they proliferate and switch the isotype (class) of immunoglobulin that is produced, while retaining the same antigen-binding specificity. T helper cells are thought to be required for switching from IgM to IgG, IgA, or IgE isotypes. In addition to antibody formation, B cells also process and present protein antigens.

T Lymphocytes: T lymphocytes are involved in the regulation of the immune response and in cell mediated immunity, and help B cells to produce antibody. Mature T cells express antigen-specific T cell receptors (TCR). Every mature T cell also expresses the CD3 molecule, which is associated with the TCR. In addition mature T cells usually display one of two accessory molecules, CD4 or CD8, which define whether a T cell will be a helper T lymphocyte, or a cytotoxic T lymphocyte (CTL). The TCR/CD3 complex recognizes antigens associated with the major histocompatibility complex (MHC) molecules on target cells (e.g. virus-infected cell).

1- **T Helper Cells:** T helper cells (Th) are the primary regulators of T cell- and B cell-mediated responses. They 1) aid antigen-stimulated subsets of B lymphocytes to proliferate and differentiate toward antibody-producing cells; 2) express the CD4 molecule; 3) recognize foreign antigen complexed with MHC class II molecules on B

cells, macrophages or other antigen-presenting cells; and 4) aid effector T lymphocytes in cell-mediated immunity.

2- **Cytotoxic T Cells:** T cytotoxic cells (CTLs) are cytotoxic against tumor cells and host cells infected with intracellular pathogens. These cells 1) usually express CD8, and, 2) destroy infected cells in an antigen-specific manner that is dependent upon the expression of MHC class I molecules on antigen presenting cells.

3- **T Suppressor/ T Regulatory Cells:** T suppressor cells suppress the T and B cell responses and express CD8 molecules. T regulatory cells (**Tregs**) also affect T cell response, with many cells characterized as CD4+CD25+, TGF-β secretors. Tregs regulate/suppress other T cell activities, and help prevent development of autoimmunity.

4- **Natural Killer T Cells:** Natural killer T cells (NKT) are a heterogeneous group of T cells that share properties of both T cells and natural killer (NK) cells. These cells were identified as T cells that recognize an antigen-presenting molecule (CD1d) able to bind self- and foreign lipids and glycolipids. They constitute only 0.2% of all peripheral blood T cells. The term “NK T cells” was first used in mice to define a subset of T cells that expressed the natural killer (NK) cell-associated marker NK1.1 (CD161). It is now generally accepted that the term “NKT cells” primarily refer to CD1d-restricted T cells co-expressing a heavily biased, semi-invariant T cell receptor (TCR) and NK cell markers. Natural killer T (NKT) cells should not be confused with natural killer (NK cells).

Natural Killer Cells: NK cells are large granular “innate” lymphocytes that nonspecifically kill certain types of tumor cells and virus-infected cells. NK cells share many surface molecules with T lymphocytes. These circulating large granular lymphocytes are able to kill “self” in the absence of antigen-specific receptors. NK cells are especially effective against viral infected cells, and keep the expansion of virus in check until adaptive immunity kicks in. In this regard, they also secrete interferon-gamma, which is an effective immunoregulator. NK cells can also kill via antibody-dependent cellular cytotoxic mechanisms (ADCC) via their Fc receptors. NK cells express a large number of receptors that deliver either activating or inhibitory signals, and the relative balance of these signals controls NK cell activity.

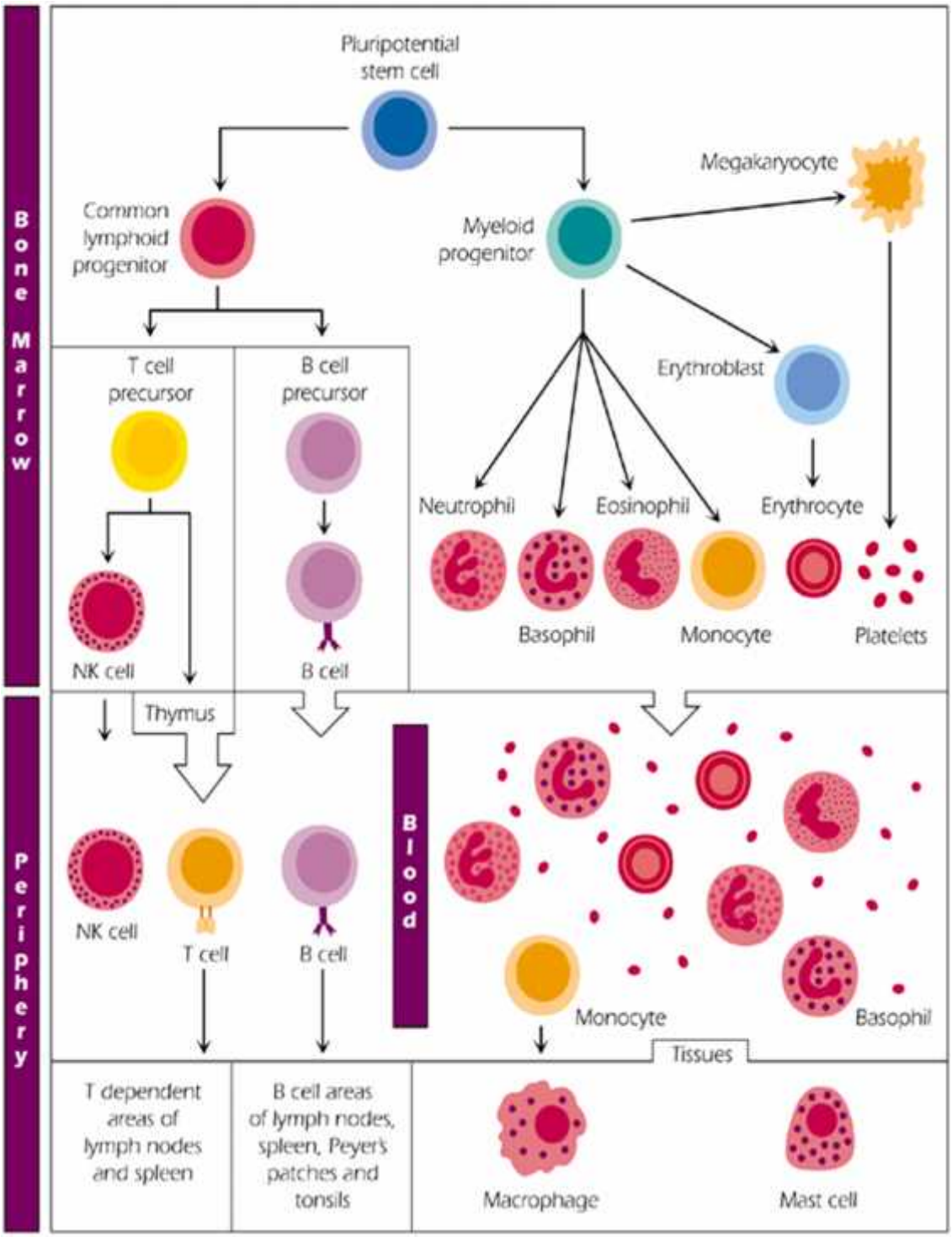


Figure. The developmental pathway of pluripotent bone marrow stem cells.

Coico and Sunshine, 2009. Fig. 2.1.

Complement System

Dr Rojan Ghanim Al-allaff

Complement was discovered by Jules Bordet as a heat-labile component of normal plasma that causes the **opsonisation** and **killing of bacteria**. The complement system refers to a series of >20 proteins, circulating in the blood and tissue fluids. Most of the proteins are normally inactive, but in response to the recognition of molecular components of microorganisms they become sequentially activated in an enzyme cascade – the activation of one protein enzymatically cleaves and activates the next protein in the cascade. Complement can be activated via three different pathways (**Figure 1**), which can each cause the activation of **C3**, cleaving it into a large fragment, **C3b**, that acts as an **opsonin**, and a small fragment **C3a** (anaphylatoxin) that promotes inflammation. Activated C3 can trigger the **lytic pathway**, which can damage the plasma membranes of cells and some bacteria. C5a, produced by this process, attracts **macrophages** and **neutrophils** and also activates **mast cells**.

1-Classical Pathway

This pathway involves complement components **C1**, **C2** and **C4**. The pathway is triggered by **antibody-antigen complexes** binding to **C1**, which itself has three subcomponents **C1q**, **C1r** and **C1s**. The pathway forms a C3 convertase, **C4b2a**, which splits C3 into two fragments; the large fragment, **C3b**, can covalently attach to the surface of microbial pathogens and **opsonise** them; the small fragment, **C3a**, activates **mast cells**, causing the release of vasoactive mediators such as histamine.

2-Alternative Pathway

This pathway involves various factors, **B, D, H** & **I**, which interact with each other, and with C3b, to form a C3 convertase, **C3bBb**, that can activate more C3, hence the pathway is sometimes called 'the amplification loop'. Activation of the loop is promoted in the presence of bacterial and fungal cell walls, but is inhibited by molecules on the surface of normal mammalian cells.

3-Mannose-binding Lectin Pathway

This pathway is activated by the binding of **mannose-binding lectin** (**MBL**) to mannose residues on the pathogen surface. This in turn activates the MBL-associated serine proteases, **MASP-1** and **MASP-2**, which activate **C4** and **C2**, to form the C3 convertase, **C4bC2a**.

Final stage for three pathway:

Lytic Pathway

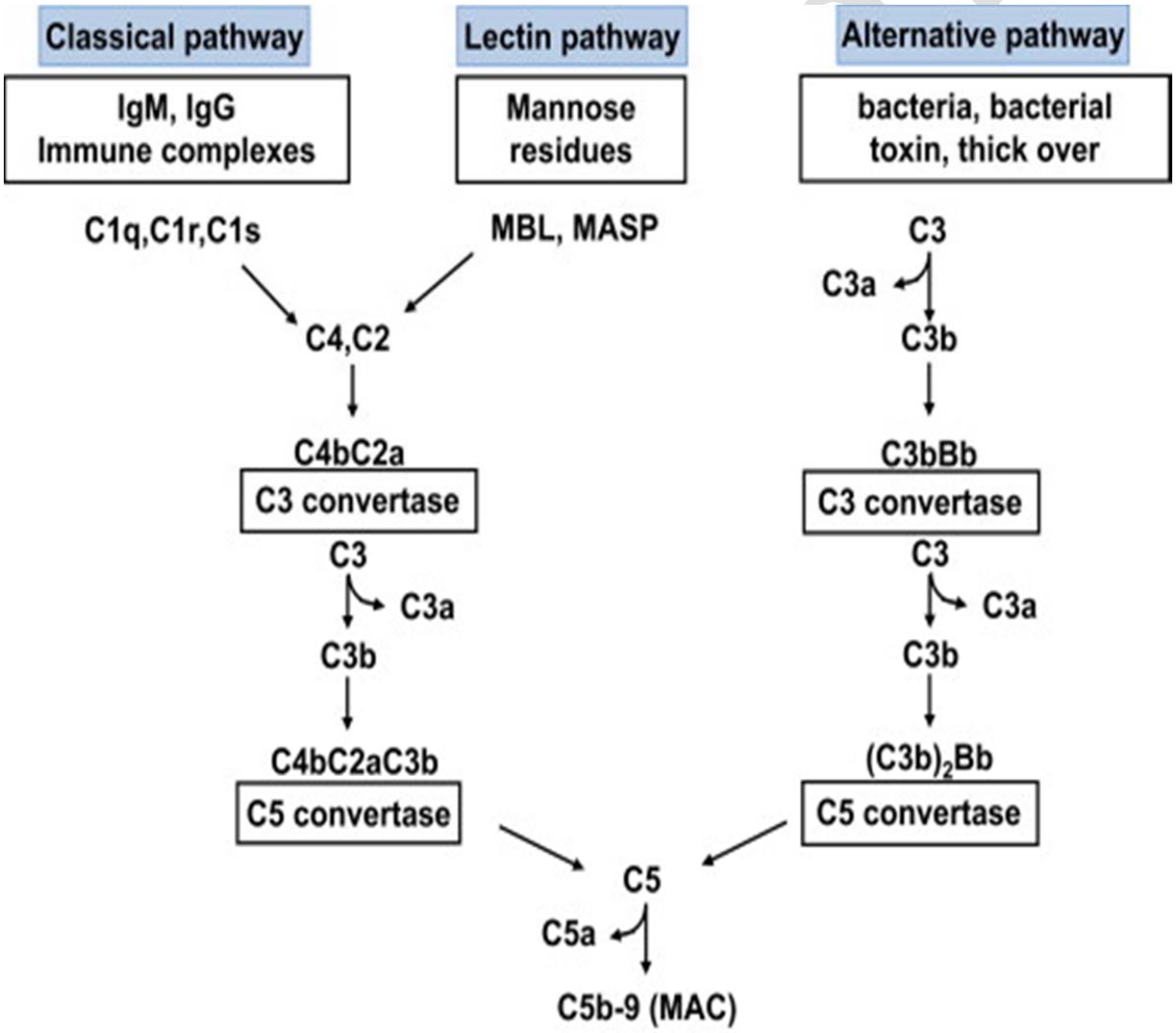
This pathway is initiated by the splitting of **C5**, and attachment of **C5b** to a target. **C6, C7, C8** and **C9** unite with C5b, and this **membrane-attack complex** (**MAC**), when inserted into the outer membrane of some bacteria, can contribute to their death by lysis. Red cells which have antibody bound to the cell surface can also activate the classical and lytic pathways, and become susceptible to lysis.

Role of Complement in Disease

The complement system plays a critical role in inflammation and defence against some bacterial infections. Complement may also be activated during reactions against incompatible blood transfusions, and during the damaging immune responses

that accompany autoimmune disease. Deficiencies of individual complement components or inhibitors of the system can lead to a variety of diseases as severe bacterial infections, severe Neisseria infections,SLE

Figure 1. Complement pathways



Nonspecific and Specific Immunity

Dr Rojan Ghanim AL-Allaff

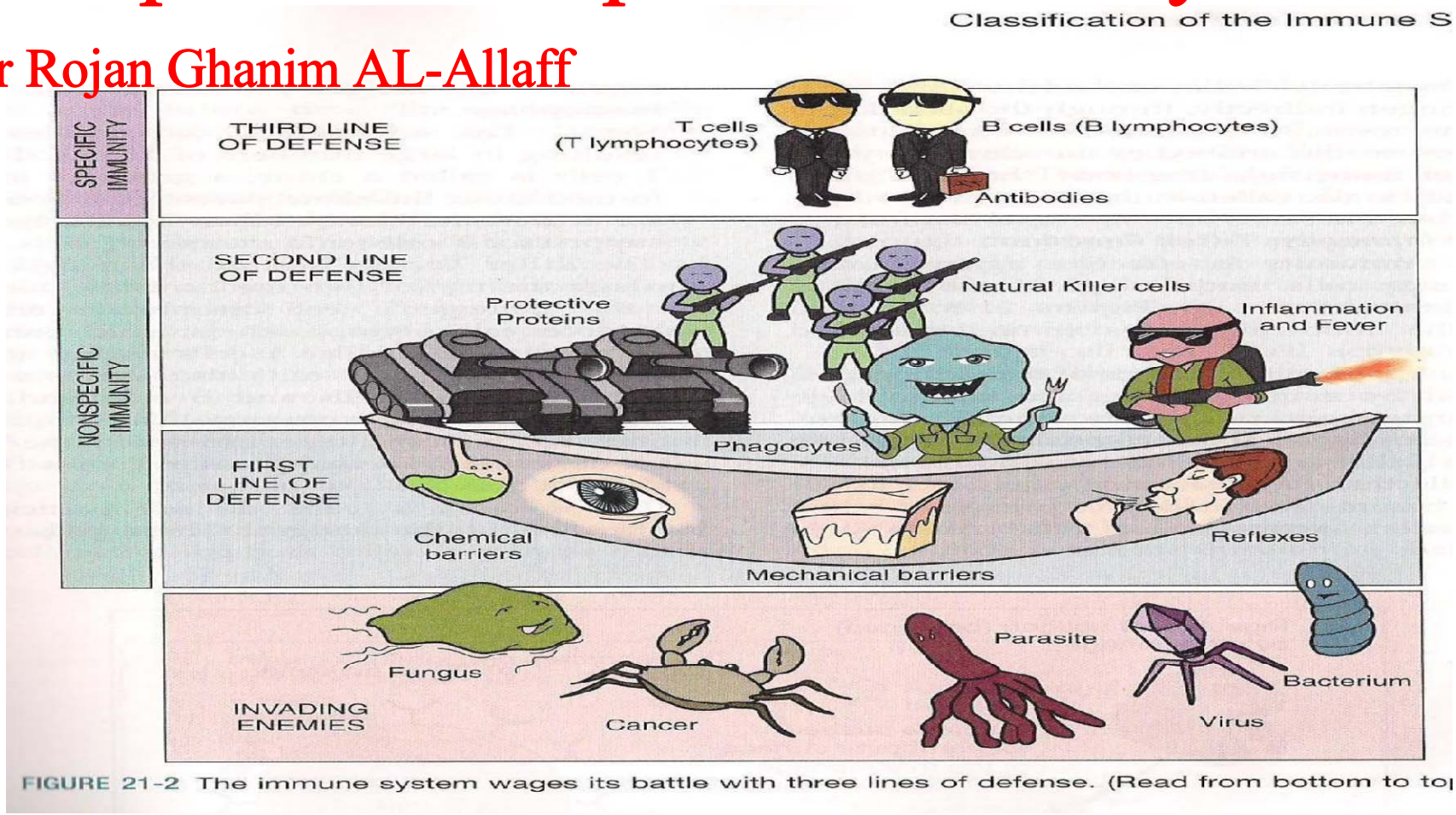


FIGURE 21-2 The immune system wages its battle with three lines of defense. (Read from bottom to to

Defense Mechanisms

State of non-specific and specific protection

Non specific defense mechanisms		Specific defense mechanisms (immune system)
First line of Defense	Second line of Defense	Third line of defense
•Skin •Cilia •Physiological factors	•Phagocytic white blood cells •The inflammatory response •Antimicrobial Substances	•Lymphocytes •antibodies

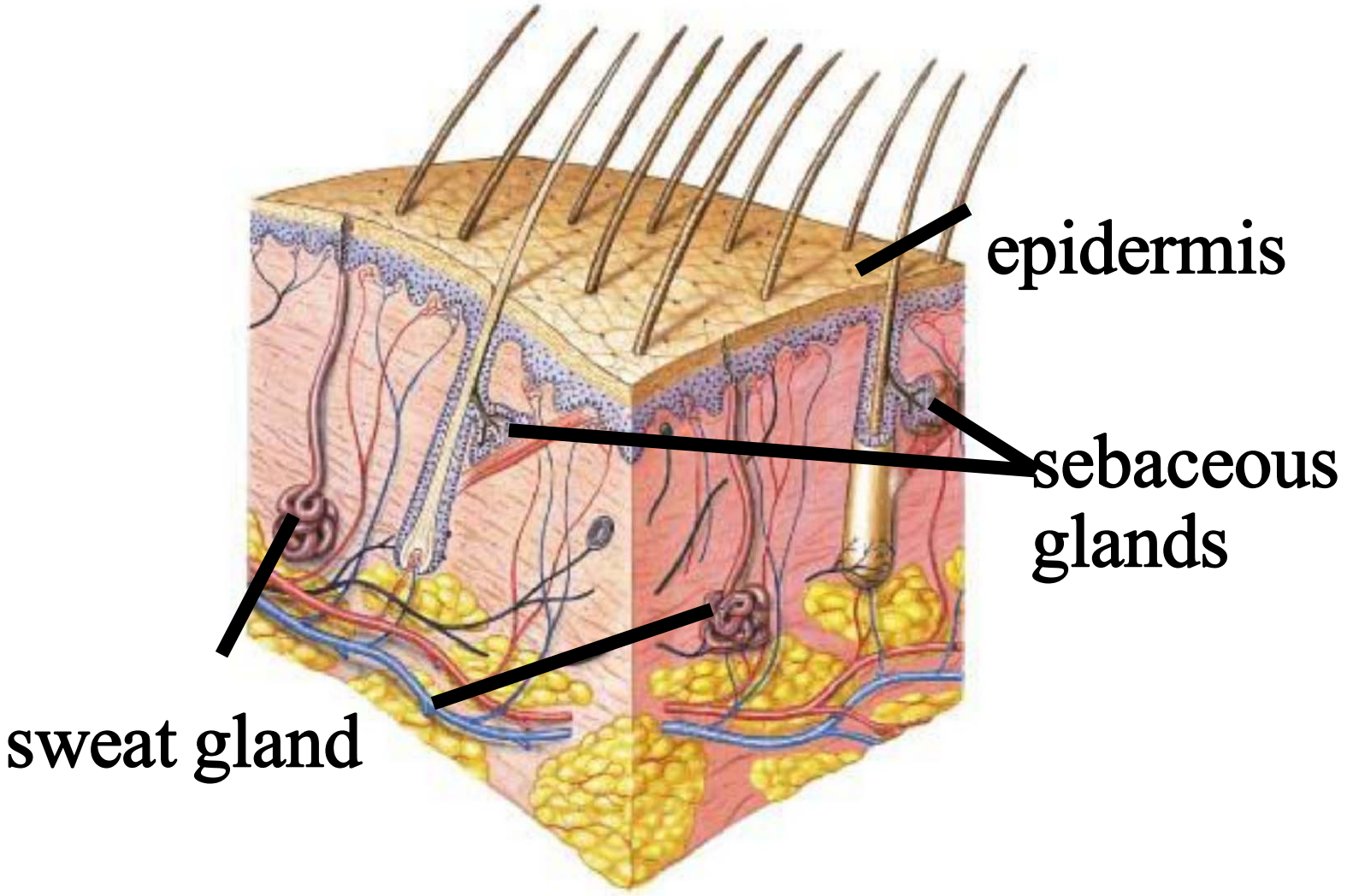
Nonspecific (Natural , Innate) Immunity: first line of defense

- Composed of structural barriers to keep infectious agents out of the body.
  - Intact skin
  - Cilia
  - Physiological factors.

Intact Skin

- The skin and mucous membranes provide an effective barrier against microorganisms. The skin has the thin outer epidermis and the thicker underlying dermis to impede entry, as well as sebaceous glands to produce sebum. Sebum is made of lactic acid and fatty acids, which effectively reduce skin pH to between 3 and 5 to inhibit organism growth. Mucous membranes are covered by cilia which trap organisms in mucous and propel them out of the body.

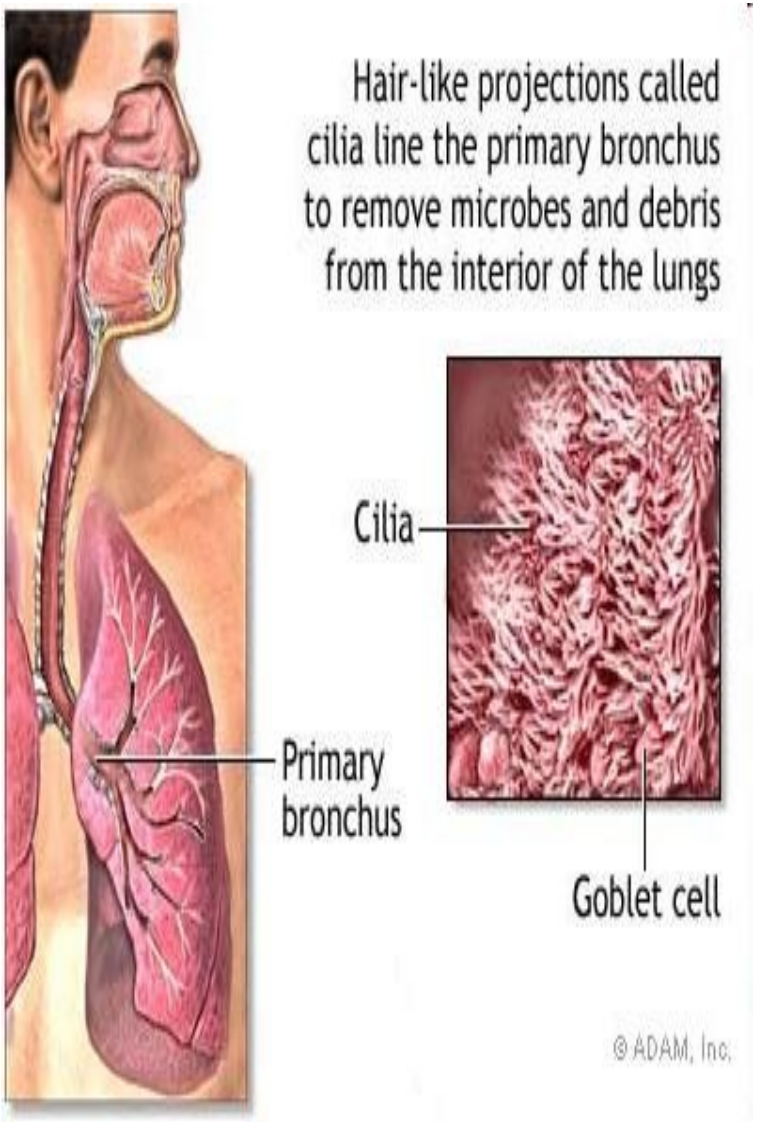
Body Coverings: The Skin



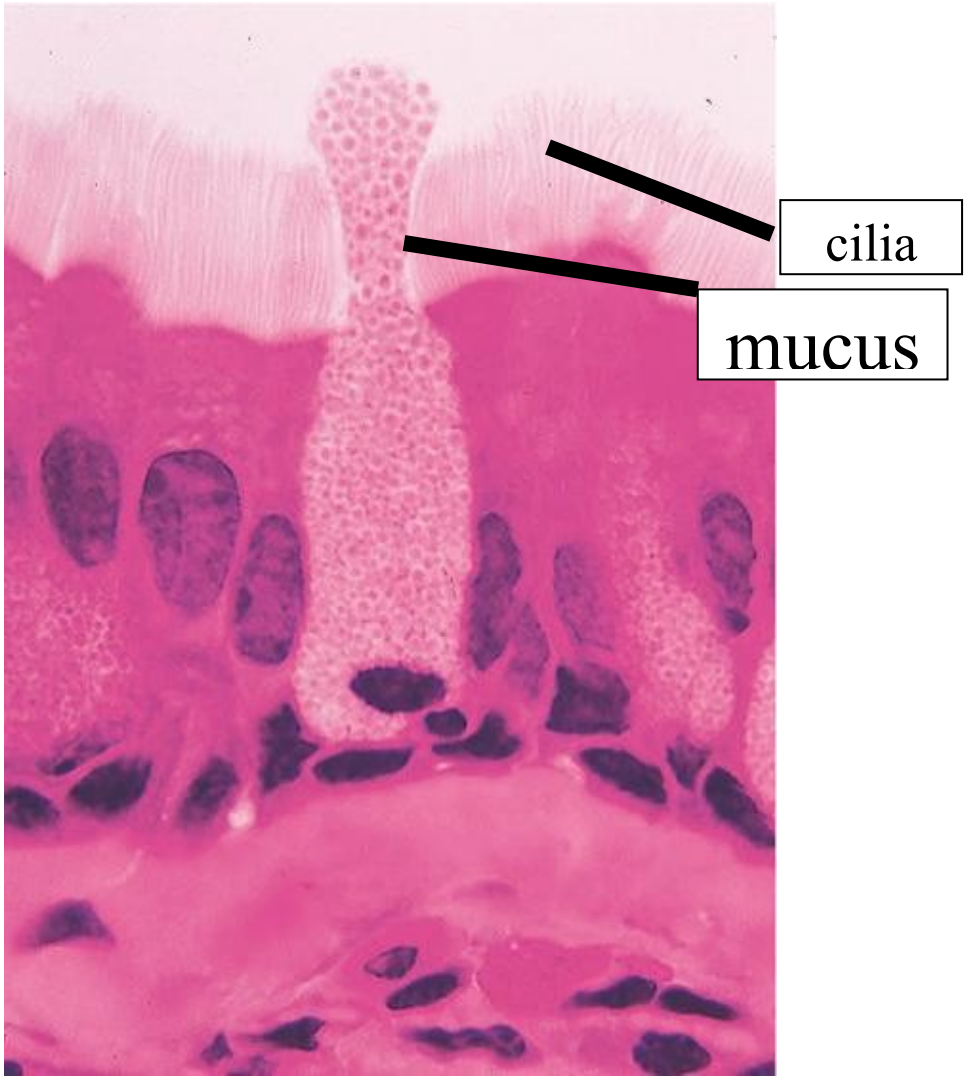
Respiratory Tract

- Upper Respiratory Tract
  - Nasal hairs induce turbulence
  - Mucous secretions trap particles
  - Mucous stream to the base of tongue where material is swallowed
  - Nasal secretions contain antimicrobial substances
  - Upper respiratory tract contains large resident flora
- Lower Respiratory Tract
  - Particles trapped on mucous membranes of bronchi and bronchioles
  - Beating action of cilia causes mucociliary stream to flow up into the pharynx where it is swallowed
  - 90% of particles removed by this way. Only smallest particles (<10μ in diameter) reach alveoli
- Alveoli
  - Alveolar macrophage rapidly phagocytize small particles

Cilia



Mucous Membranes



Alimentary Tract

- General defense mechanisms
  - Mucous secretions
  - Integrity of mucosal epithelium
  - motions of the gut propel contents downward
  - Secretory antibody and phagocytic cells
- Stomach
  - Generally sterile due to low pH
- Small Intestine
  - Upper portion contains few bacteria
  - As distal end of ilieum is reached flora increases
- Colon
  - High numbers of microorganisms
  - 50-60% of fecal dry weight is bacteria

EYE

- Tears contain a high concentration of lysozyme (effective against gram positive microorganisms.

Factors Modify Defense Mechanisms

1. Age
2. Hormones
3. Drugs and chemicals
4. Malnutrition
5. Fatigue and stress
6. Genetic determinants

Nonspecific Immunity, Second line of defense

Phagocytosis:

When the pathogens can penetrate the first line of defense (due to wounds, burns or loss of epithelia)the cell of innate immunity play a role.

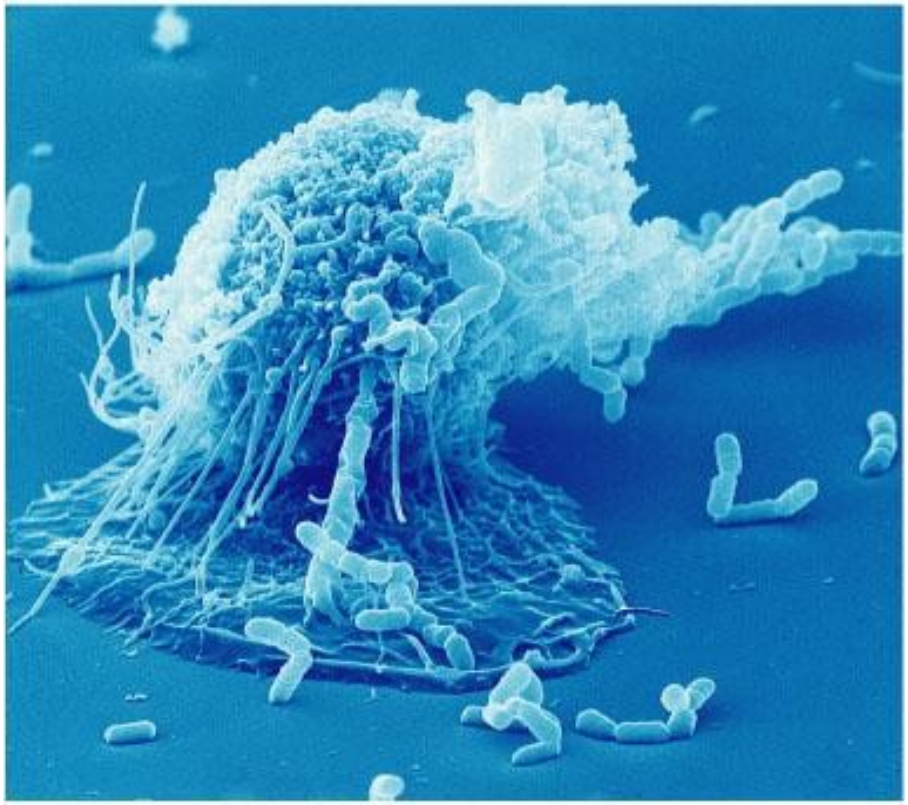
- Phagocytic cells
  - Neutrophils and macrophages
  - Natural Killer (NK) Cells: attack virus infected cells.

  The early responed phagocytic cells neutrophile followed by monocytic macrophages.

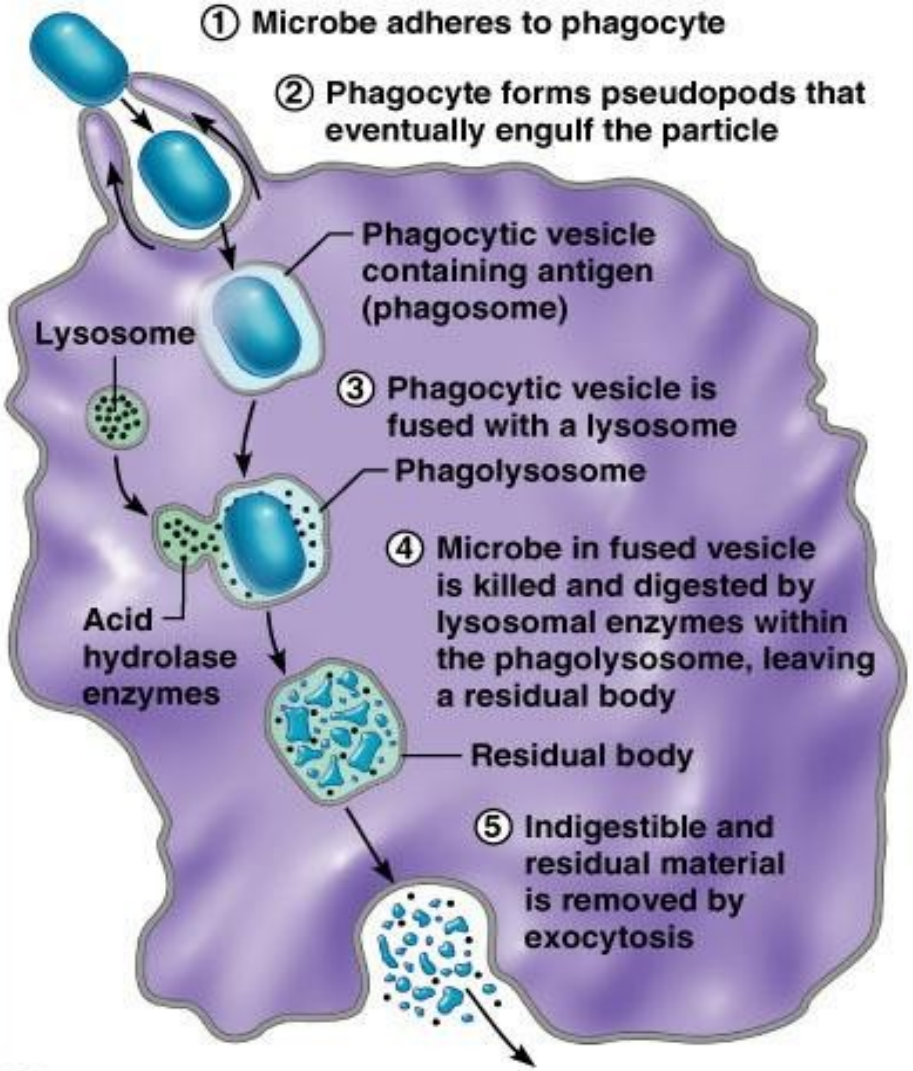
Phagocytosis

1. Initiation is caused by damage to the tissues, either by trauma or as a result of microbial multiplication.
2. Chemotaxis, attraction of leukocytes or other cells by chemicals.
3. Opsonization - Opsonization coating a pathogen by substances so as to enhance phagocytosis.
4. Adherence - firm contact between phagocyte and microorganism.
5. Engulfment into cytoplasm and enclosed in a vacuole.
6. Digestion enzymatic contents in vacuole destroy the microorganism.

Mechanism of Phagocytosis



(a)



(b)

Macrophage

Inflammation

Inflammatory response : is a protective response act to eliminate the initial cause of cell injury as well as the necrotic cells and tissues.

The mission of inflamation were completed by diluting, destroying or neutrilizing harmful agents(microbes and toxins) .

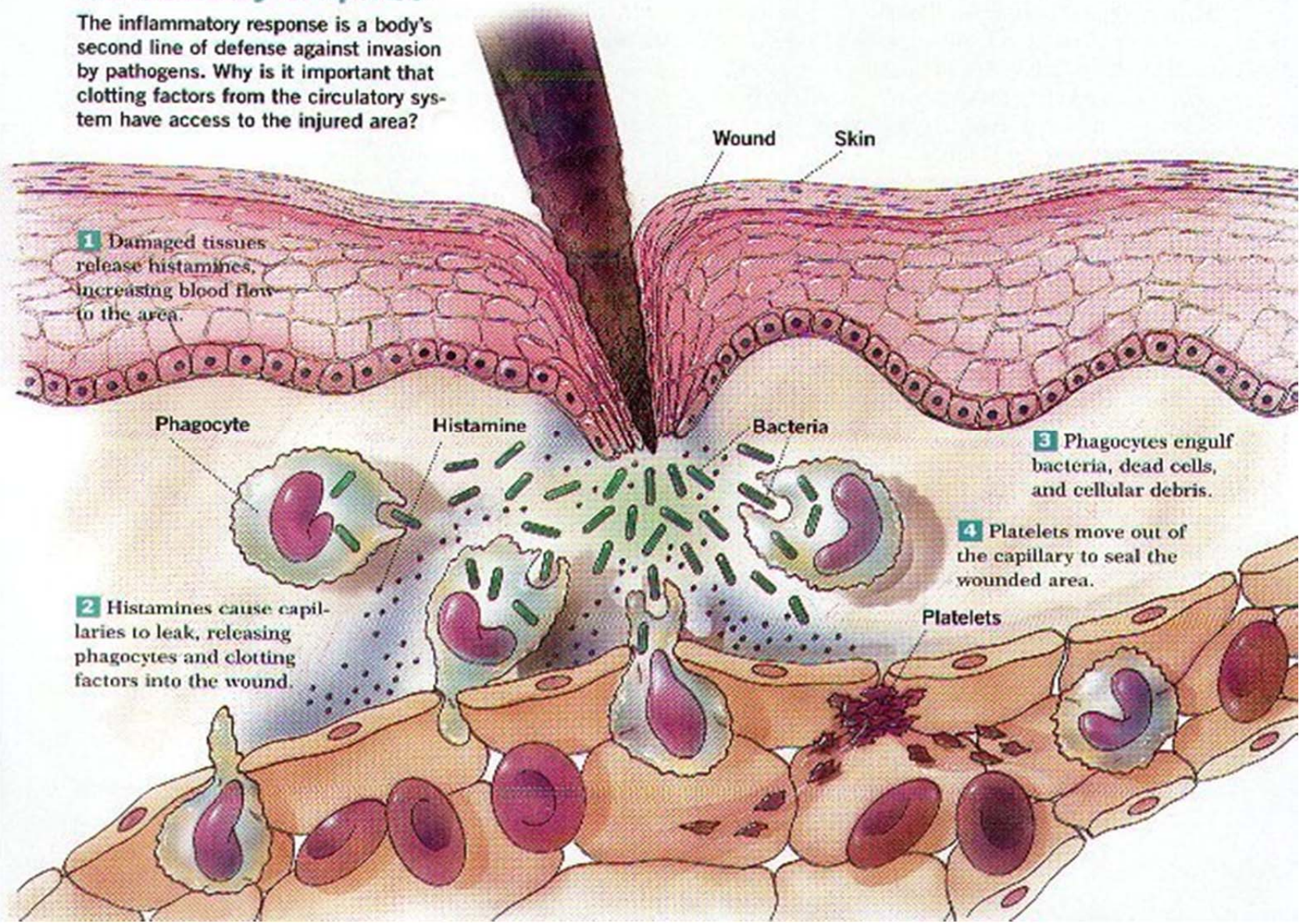
four classic signs of inflammation are redness, swelling, heat and pain.

- Steps of inflammatory response:
  - Dilation of capillaries (hyperemia) to increase blood flow to area
  - Chemotaxis - chemicals released which cause phagocytic white cells to migrate to the area.
  - Increased capillary permeability allowing white cells to go to injured area, a process known as "diapedesis"

– Formation of exudate - same composition as plasma and it contains antibacterial substances, phagocytic cells, and drugs and antibiotics, if present.

Steps of the Inflammatory Response

The inflammatory response is a body's second line of defense against invasion by pathogens. Why is it important that clotting factors from the circulatory system have access to the injured area?



Antimicrobial Substances

- Third major kind of nonspecific cellular and chemical defense
- Include many soluble tissue and serum substances help to suppress the grow of or kill microorganisms
- Includes complement and interferon
- Considered a second line of defense

Complement

The complement system is a group of more than 30 plasma and membrane proteins that play a critical role in host defense. When activated, complement components interact in a highly regulated fashion to generate products that:

a. Recruit inflammatory cells (promoting inflammation).

b. Opsonize microbial pathogens and immune complexes (facilitating antigen clearance).

c. Kill microbial pathogens (via a lytic mechanism known as the membrane attack complex).

d. Generate an inflammatory response.

Complement activation takes place on antigenic surfaces. However, the activation of complement generates several soluble fragments that have important biologic activity.

There are three distinct pathways of activation of complement: the Classical, the MB- lectin, and the Alternative Pathways.

Nomenclature

The components of the classical pathway are designated by the letter C followed by a simple number designation, e.g. C3.

Many complement components are proteases that become active following proteolytic cleavage.

When the components are cleaved during activation, the resulting fragments are given lower case letter designations, such as C3a and C3b.

Components of the alternative pathway are named by capital letters, such as factor B and factor D.

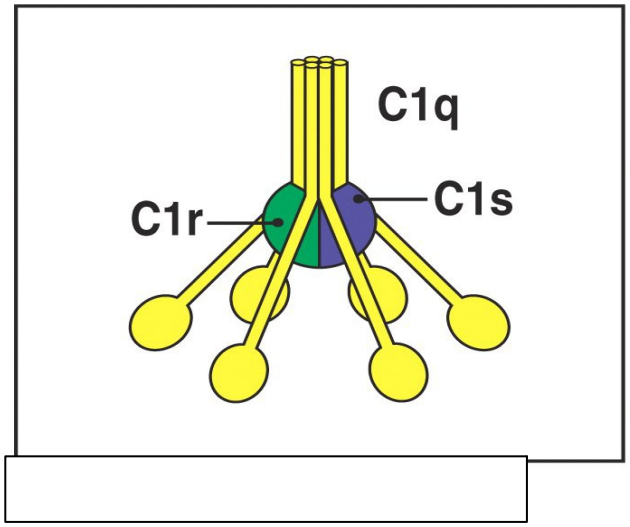
For the MB-lectin pathway, components are designated by acronyms, such as MASP-1 (**M**annan binding lectin-**A**ssociated **S**erine **P**rotease-1)

The lower case "i" is added to indicate that a component is inactive, e.g. iC3b.

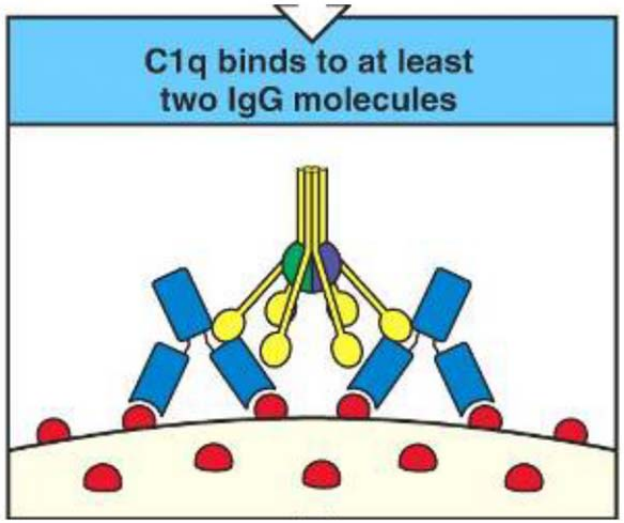
Activation of Complement

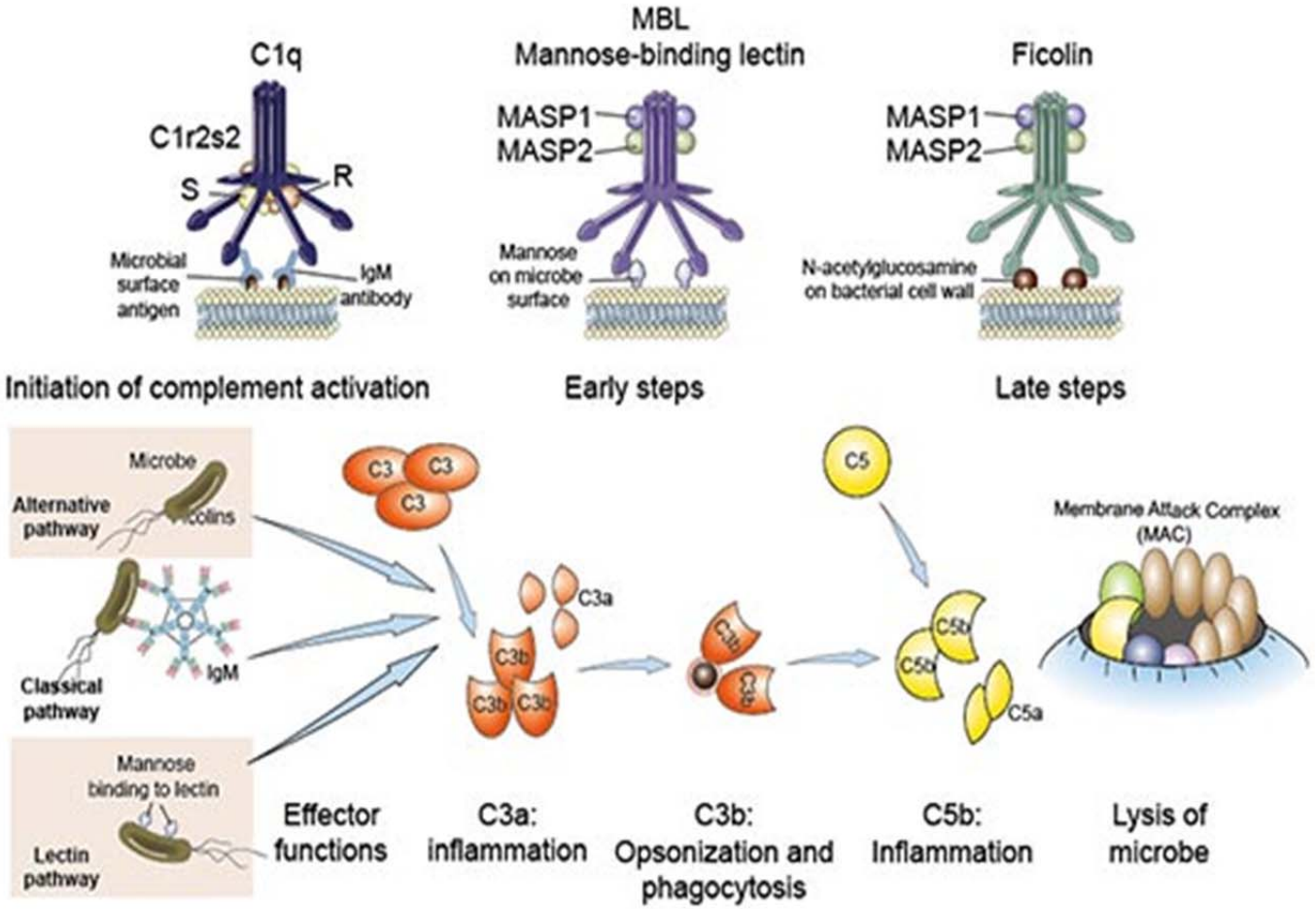
CLASSICAL PATHWAY:

The classical pathway is activated by binding immunoglobulin-M (IgM) or immunoglobulin-g (IgG) on the surface of a target cell. The Fc portion of the Ab binds to C1q, C1r is activated and this in turn activates another molecule of C1r which together activate two molecules of C1s. C1s now cleaves C4 which exposes the binding site for C2 which is also cleaved. The binding of C4b and C2a leads to the formation of a complex referred to as a C3 convertase.



This complex now cleaves C3 forming C3a and C3b, some of which combine with the C3 convertase forming a C5 convertase. This complex now acts on C5, with the resulting C5b binding to C6 initiating the formation of the membrane attack complex (MAC). C5b6 acts on C7, which in turn act on C8 and ultimately on C9 resulting in the formation of the final MAC.




C1q
C1r2s2
S
R
Microbial surface antigen
IgM antibody
MBL
Mannose-binding lectin
MASP1
MASP2
Mannose on microbe surface
Ficolin
MASP1
MASP2
N-acetylglucosamine on bacterial cell wall
Initiation of complement activation
Early steps
Late steps
Microbe
Alternative pathway
Ficolins
Classical pathway
IgM
Mannose binding to lectin
Lectin pathway
C3
C3
C3
C3a
C3b
C3b
C3b
C5
C5b
C5b
C5a
Membrane Attack Complex (MAC)
Effector functions
C3a: inflammation
C3b: Opsonization and phagocytosis
C5b: Inflammation
Lysis of microbe

The alternative pathway

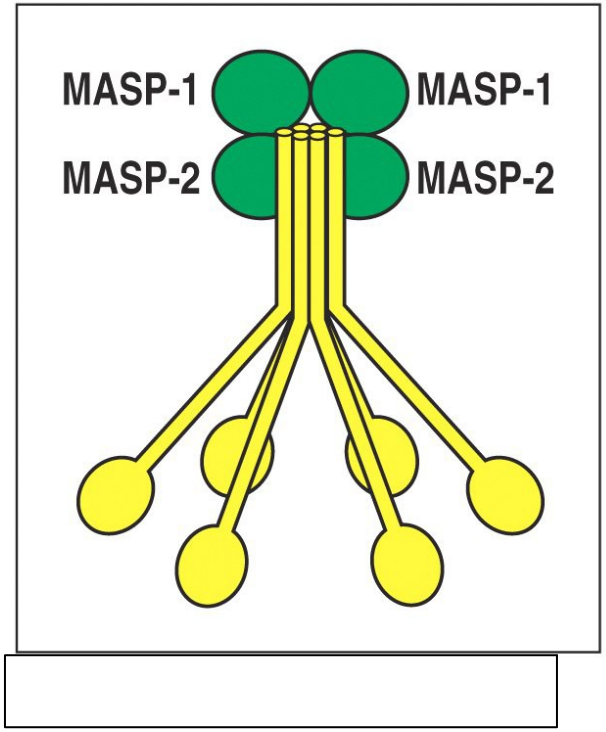
The alternative pathway depends upon the slow hydrolysis of C3, which spontaneously occurs in plasma.

Hydrolyzed C3 can bind and cleave Factor B, and the resulting C3 (H_2O) Bb complex is a C3 convertase that generates additional molecules of C3b When stabilized by Factor P (properdin), the C3bBb complex acts as a C3 convertase, When another molecule of C3b associates with C3bBb (forming C3bBbC3b), a C5 convertase is formed.

From this point (the cleavage of C5), the alternative and classical pathways converge, leading to the formation of a MAC complex

MB-LECTIN PATHWAY:

Activation of the mannan binding lectin pathway is similar to the classical pathway. Except that the MB-lectin pathway is initiated by a protein, Mannan Binding Lectin
(MBL), which is homologous to C1q.



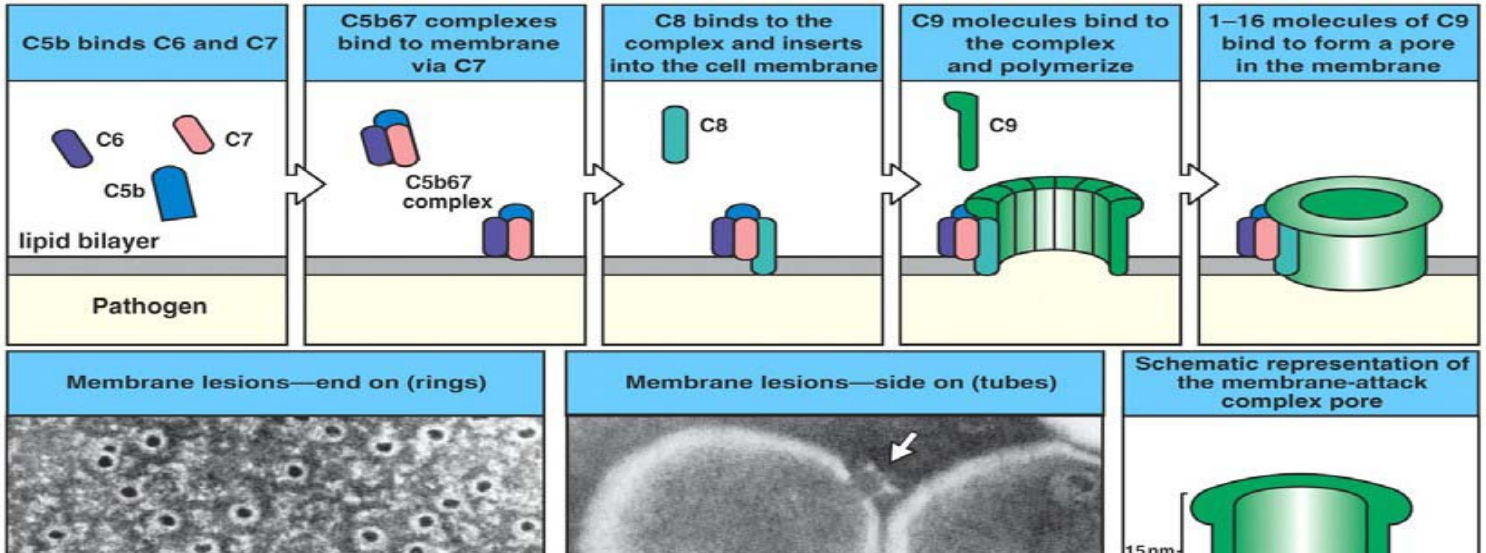
MBL binds to mannose and certain other complex carbohydrates that are found on the surface of many microbial pathogens. See.

MBL is physically associated with two serine proteases, MASP-1 and MASP-2 (mannan binding lectin-associated serine protease-1) that are similar to C1r and C1s.

When MBL binds to the pathogen, MASP-1 and MASP-2 become activated.than

MASP-2 is activated following the binding of MBL, and it mediates the cleavage of C2 and C4. It therefore is C1s-like in its function. MASP-1, on the other hand, does not have activities that are similar to either C1r or C1s, but it can cleave and activate C3, than C5 convertase, cleaves C5 into C5a, which is a soluble inflammatory mediator, and C5b, which is capable of complexing with additional complement components. The generation of C5b initiates the final phase of complement activation, which is the formation of the **M**embrane **A**ttack **C**omplex (MAC).

The MAC is identical for all pathways of complement activation



- Interferons
  - Family of proteins which are important non-specific defense mechanisms against viral infections and cancer.
- Act as messengers that protect other cells in
- the vicinity from viral infection.
- Produced by most body cells, lymphocytes, NK cells
- inhibit viral replication.
- activates macrophages.

Lymphoid Organs

Dr. Rojan Ghanim AL-allaff

The lymphatic organs are tissues in which lymphocytes mature, differentiate and proliferate. Lymphoid organs are comprised of epithelial and stromal cells arranged either into discretely capsulated organs or accumulations of diffuse lymphoid tissue. The primary (central) lymphoid organs are the major sites of lymphopoiesis, where B and T lymphocytes differentiate from stem cells into mature antigen recognizing cells. The secondary lymphoid organs, therefore, are those tissues in which antigen-driven proliferation and differentiation take place.

Historically, the primary lymphoid organ was first discovered in birds, in which B cells undergo maturation in the bursa of Fabricius, an organ situated near the cloaca. Humans do not have a cloaca, nor do they possess a bursa of Fabricius. In embryonic life, B cells mature and differentiate from hematopoietic stem cells in the fetal liver. After birth, B cells differentiate in the bone marrow. Maturation of T cells occurs in a different manner. Progenitor cells from the bone marrow migrate to the thymus where they differentiate into T lymphocytes. The T lymphocytes continue to differentiate after leaving the thymus, and are driven to do so by encounter with specific antigen in the secondary lymphoid organs.

Primary Lymphoid Organs:

Bone marrow :

B cells are generated in the bone marrow.

Fetal Liver and Adult Bone Marrow: Islands of hematopoietic cells in the fetal liver and in the adult bone marrow give rise directly to B lymphocytes.

1. Takes 1-2 weeks to develop from hematopoietic stem cells to mature B cells
2. Sequence of expression of cell surface receptor and adhesion molecules which allows for differentiation of B cells, proliferation at various stages, and movement within the bone marrow microenvironment.
3. Immature B cell leaves the bone marrow and undergoes further differentiation
4. Immune system must create a repertoire of receptors capable of recognizing a large array of antigens while at the same time eliminating self-reactive B cells.
5. Early B cell development constitutes the steps that lead to B cell commitment and expression of surface immunoglobulin, production of mature B cells.
6. Mature B cells leave the bone marrow and migrate to secondary lymphoid tissues .
7. B cells then interact with exogenous antigen and/or T helper cells = antigen dependent phase

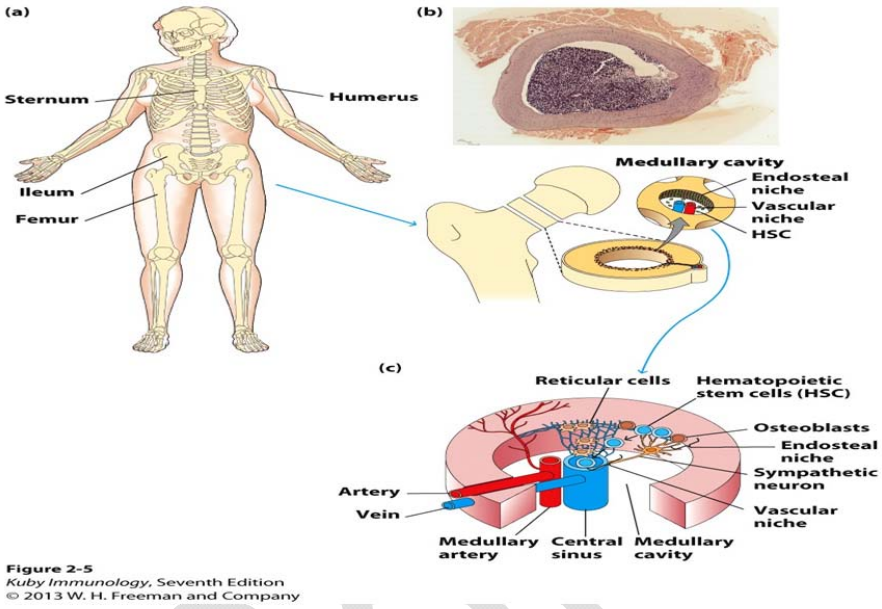


Figure 2-5
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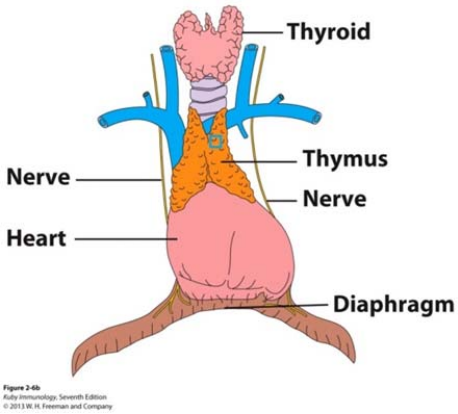
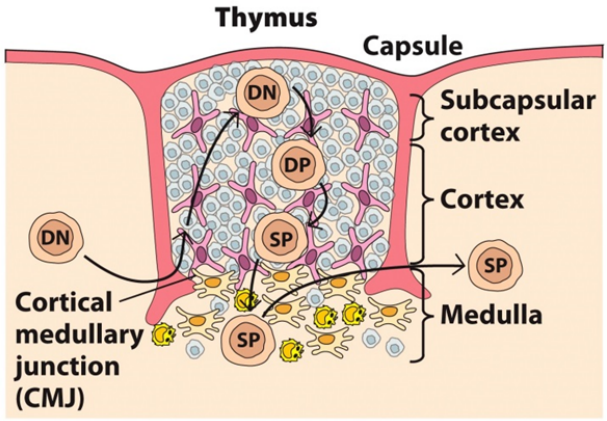
Primary lymphoid organs—Where immune cells develop

Thymus Gland:

The lymphoid organ in which T lymphocytes are educated, mature and multiply. It is a lymphoepithelial organ composed of stroma (thymic epithelium) and lymphocytes, almost entirely of the T-cell lineage. This is where T lymphocytes learn to recognize self antigens as self, and where these cells differentiate and express specific receptors for antigen. Only 5-10% of maturing lymphocytes survive and leave the thymus.

T lymphocyte progenitor produced in the bone marrow enter the thymus via the post capillary venules.Progenitor migrate to the cortex where they proliferate and rearrange their TCR genes. They become double positive and express CD4 and CD8 co-receptors .Double positive thymocyte undergo positive selection. They become single positive and express either CD4 or CD8 co-receptors Single positive thymocyte migrate to the Medula where they undergo negative selection.Mature CD4 and CD8 T cells leave the thymus trhough the post capillary venules.





T cells develop initially in the bone marrow, but then migrate to the thymus to achieve full Maturity

T-lymphocytes selection:

Positive selection:

the elimination of cells with dysfunctional TCR, positively are selected thymocytes that recognize MHC gp with low affinity, then maintain the expression of CD4 or CD8 (depending what class of MHC gp binds to the TCR). These mature T cells (Medullary thymocytes) leave the thymus and migrate to secondary lymphoid organs , **98% of pro-thymocytes in the thymus during its development dies**

Negative selection:

the elimination of autoreactive cells, when thymocytes binds enough strongly by their TCR complex of MHCgp with normal peptides (from autoantigens)which are presented on surface of thymic cells thymocyte receives signals leading to apoptotic cell death .

Secondary lymphoid organs

Dr. Rojan Ghanim AL-allaff

—Where the immune response is initiated

Areas where lymphocytes encounter antigen, become activated, undergo clonal expansion, and differentiate into effector cells. These are connected to each other via the blood and lymphatic circulatory systems,Secondary lymphoid organ areas include:

1. **Spleen**
2. **Lymph nodes**
3. **Mucosa-associated lymphoid tissue (MALT)**
4. **Other diffuse and loosely organized areas**

1-Lymph nodes and Spleen:

Lymph nodes and spleen are the most highly organized secondary lymphoid organs,T-cell and B-cell activity are separated into distinct microenvironments,The cells will actively migrate toward each other during activation events for their required interactions.

Differentiation into effector cells takes place in follicles of secondary lymphoid organs

1-T lymphocytes

- CD4+ T cells differentiate into helper T cells that assist in B-cell differentiation
- CD8+ T cells differentiate into killer (or cytotoxic) T cells that attack and destroy virally infected cells

2-B cells further mature in germinal centers in such tissues

- Antigen affinity is increased
- Class switching can take place

NOTE: Both B and T lymphocytes will develop into long-lived memory cells in these areas, as well

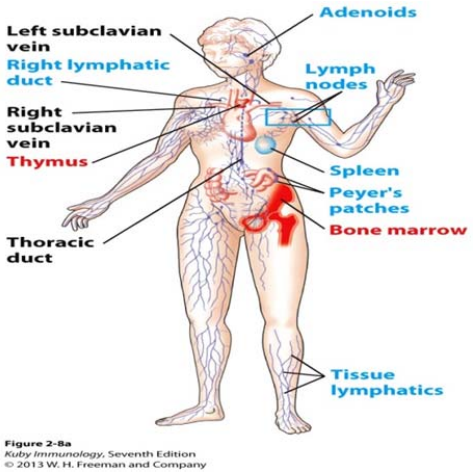


Figure 2-8a
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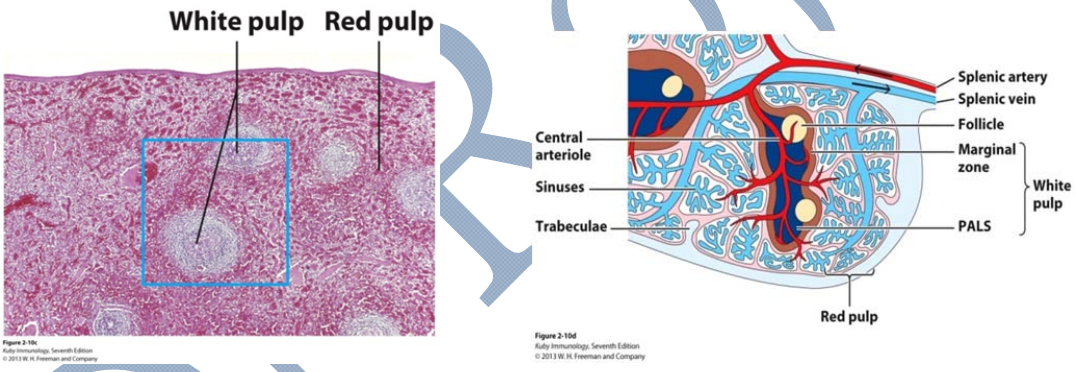
1-Spleen:

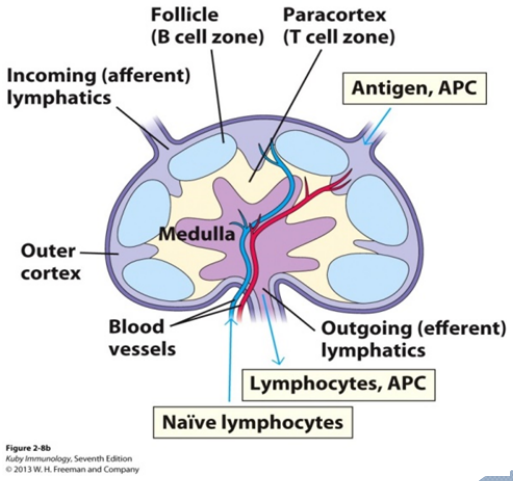
The spleen is the first line of defense against blood-borne pathogens,Red blood cells are compartmentalized in red pulp,White blood cells are segregated in white pulp,A specialized region of macrophages and B cells known as the marginal zone borders the white pulp.



Spleen

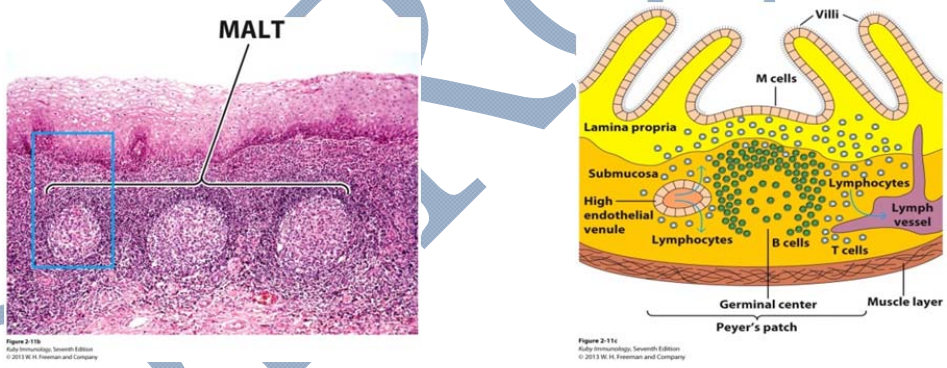
2-Lymph Node:

Lymph nodes form part of the network which filters antigen from tissue fluid or lymph during its passage from the periphery to the thoracic duct. Histologically, the lymph node is composed of a B cell cortex containing primary and secondary follicles, a T cell paracortex, and a central medulla which contains cords of lymphoid tissue**.**



3-Mucosa-associated lymphoid tissue (MALT):

- Important layer of defense against infection at mucosal and epithelial layers
- Organizes responses to antigens that enter mucosal tissues
- Includes a network of follicles and lymphoid microenvironments associated with the intestines (gut-associated lymphoid tissue, or GALT)



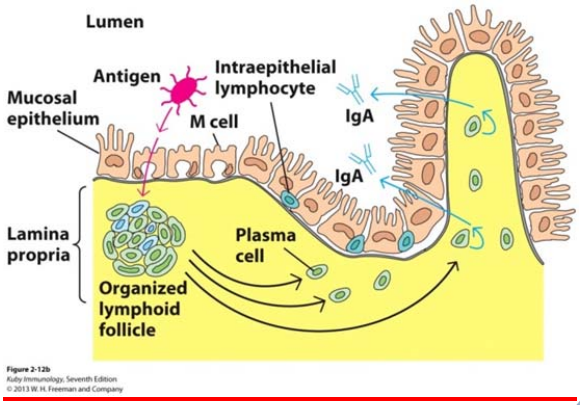
Mucosa-associated lymphoid tissue

4-Other diffuse and loosely organized areas:

GALT (gut-associated lymphoid tissue)or BALT bronchus-associated lymphoid tissue).

M cells in the lining of the gut are unique:

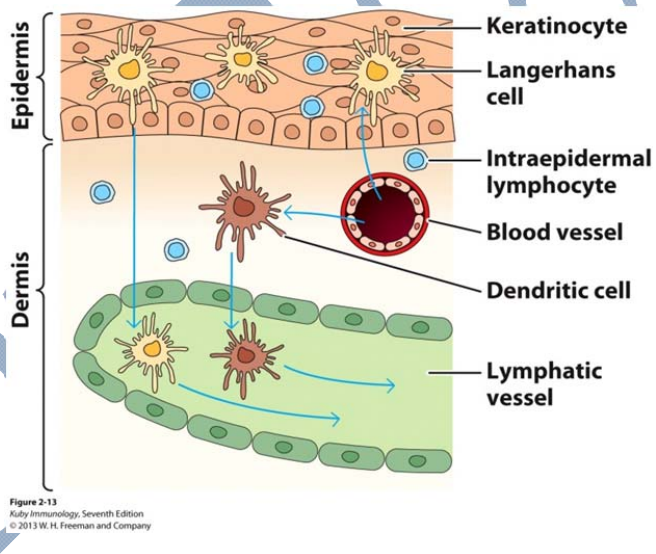
They function to deliver antigen from the intestinal spaces to lymphoid cells in the gut wall.



M cell

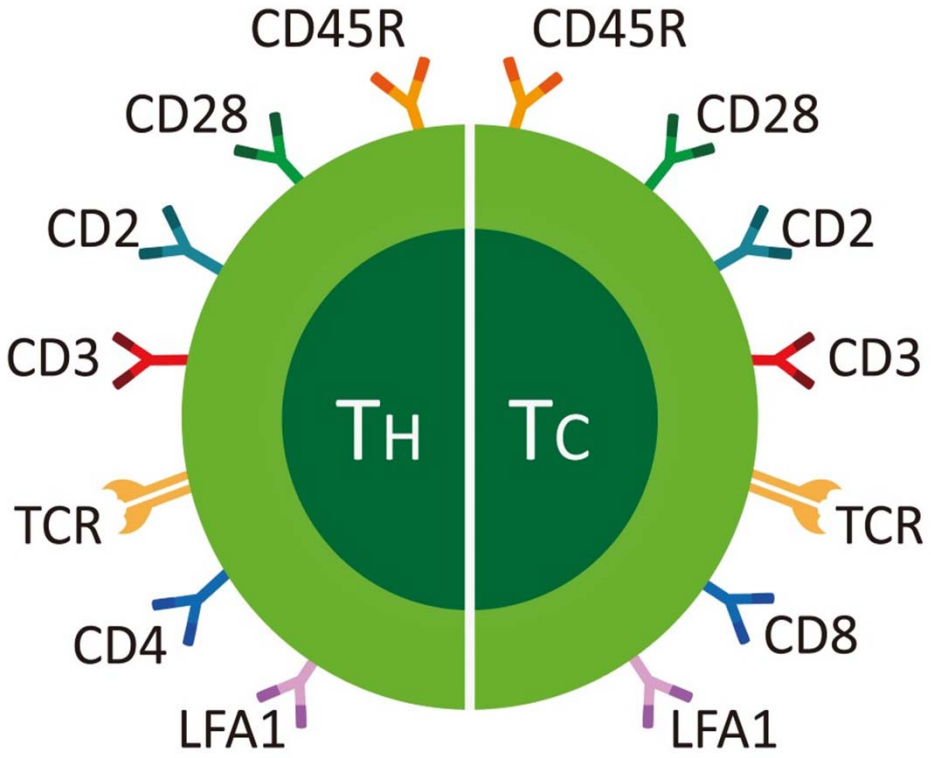
Last but not least, the bone marrow can serve as an important secondary lymphoid organ. In addition to being a site of B cell generation, the bone marrow contains many mature T cells and plasma cells.

Various loosely organized and diffuse lymphoid tissue are also found under the skin, mucosae, and tertiary tissues at sites of infection.



T -LYMPHOCYTES

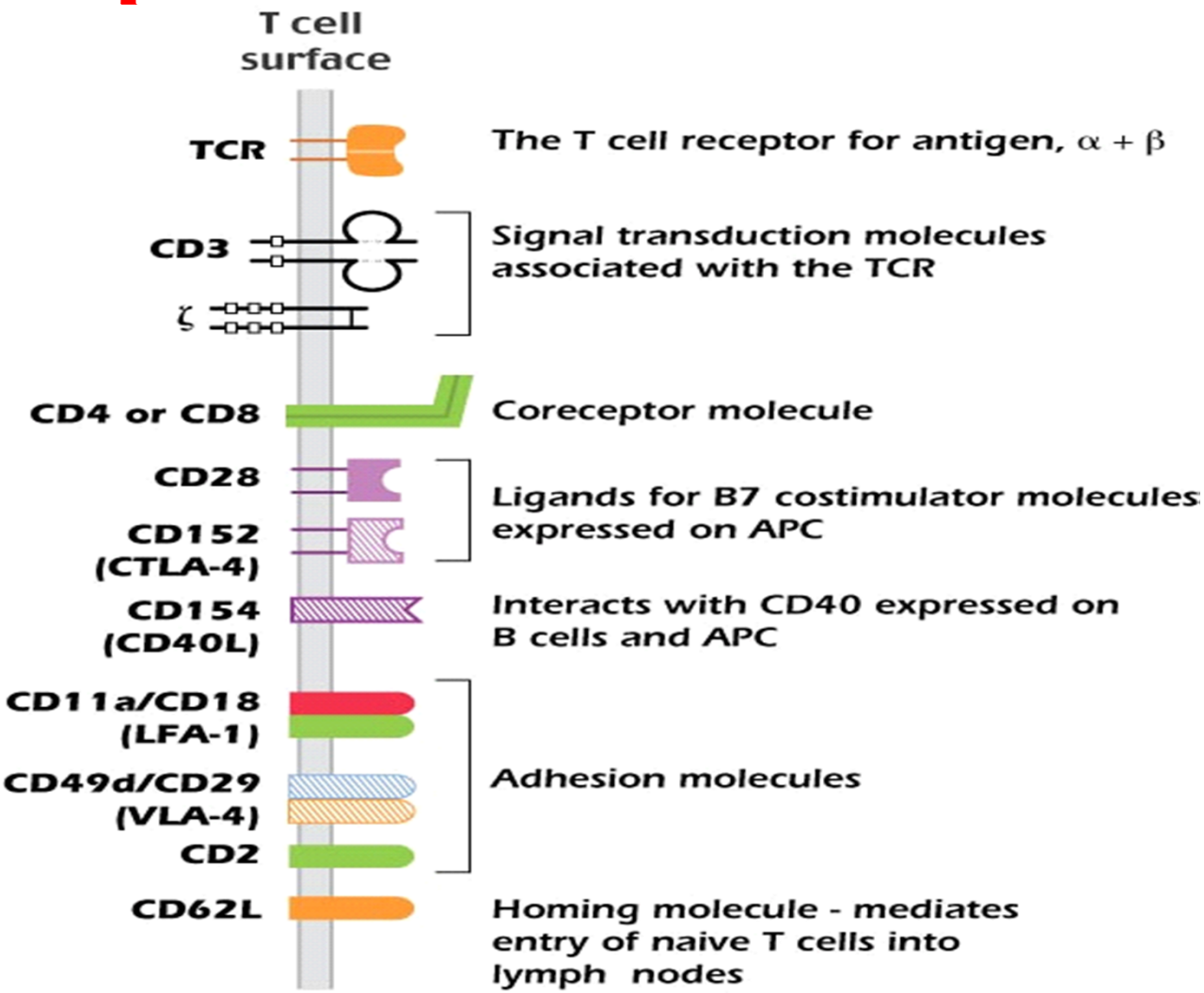
Dr Rojan Ghanim AL-Allaff



T -LYMPHOCYTES

T lymphocytes are involved in the regulation of the immune response and in cell mediated immunity, and help B cells to produce antibody. Mature T cells express antigen-specific T cell receptors (TCR). Every mature T cell expresses the CD3 molecule, which is associated with the TCR. In addition mature T cells usually display one of two accessory molecules, CD4 or CD8. The TCR/CD3 complex recognizes antigens associated with the major histocompatibility complex (MHC) molecules on target cells (e.g. virus-infected cell).

Important T cell markers



TCR-CD3-complex

The TCR heterodimer is tightly associated with the CD3 coreceptor made up of independently encoded 5 subunits .The CD3 complex is required for efficient transport of the TCR to the cell surface. CD3 subunits possess long intracellular tails and are responsible for transducing signals upon TCR engagement with MHC presented antigen.

T HELPER CELLS

T helper cells (Th) are the primary regulators of T cell- and B cell-mediated responses.....They

1) aid antigen-stimulated subsets of B lymphocytes to proliferate and differentiate toward antibody-producing cells.

2) express the CD4 molecule

3) recognize foreign antigen complexed with MHC class II molecules on B cells, macrophages or other antigen-presenting cells.

4) aid effector T lymphocytes in cell-mediated immunity.

Paired Interactions between the APC and CD4 T cell

Paired Interactions between the APC and CD4
T cell
(***Immunological synapse)***

1-Antigen receptor

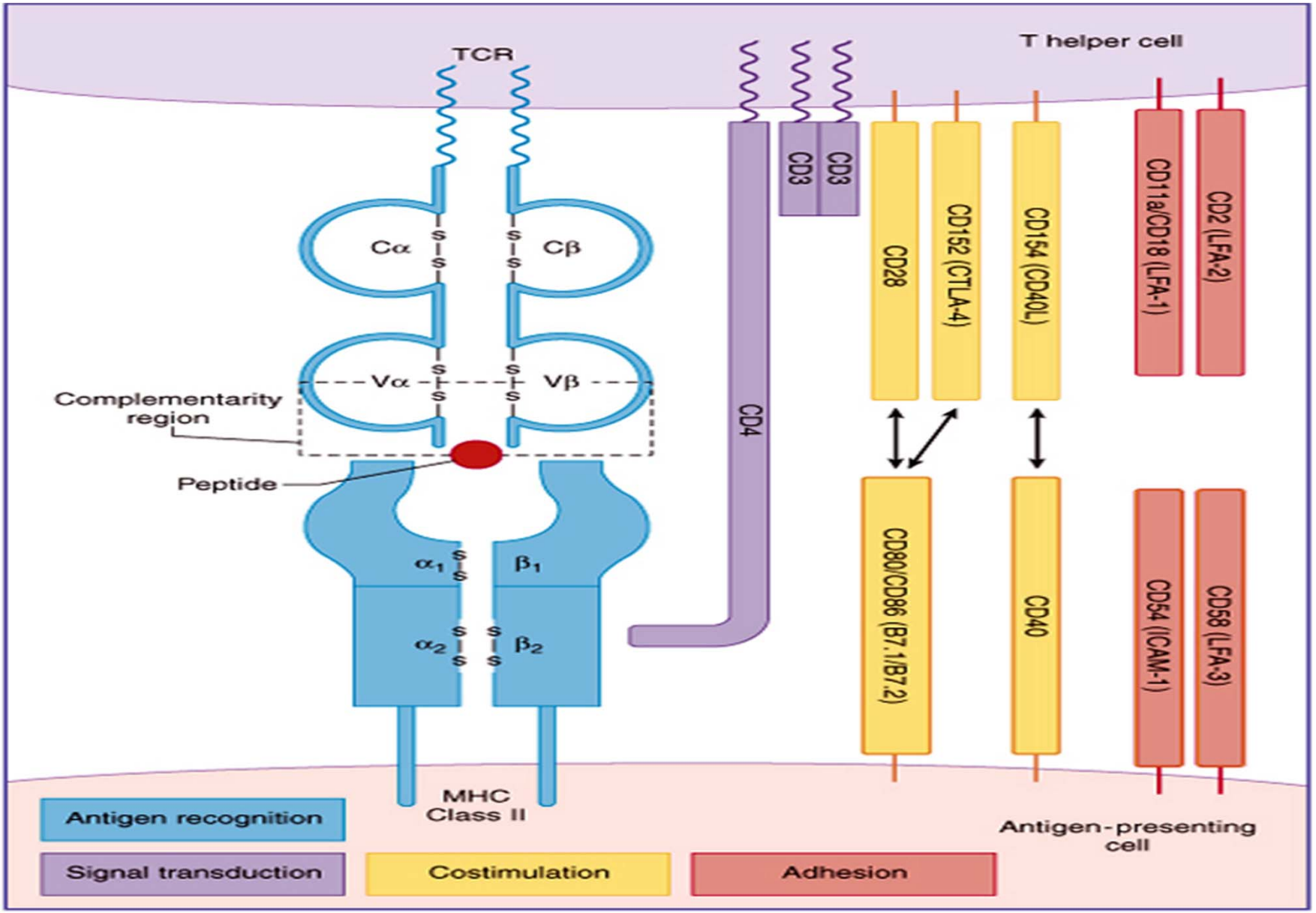
MHC II : TCR - antigen binding
MHC II : CD4 molecule - the co-receptor

2-Costimulatory pairs - second signals

CD40:CD40L (CD154)
CD28/CTLA-4:B7 (CD80, CD86)

3-Adhesion molecules

CD58(LFA-3):CD2
CD54(ICAM-1):CD11a/CD18 (LFA-1)



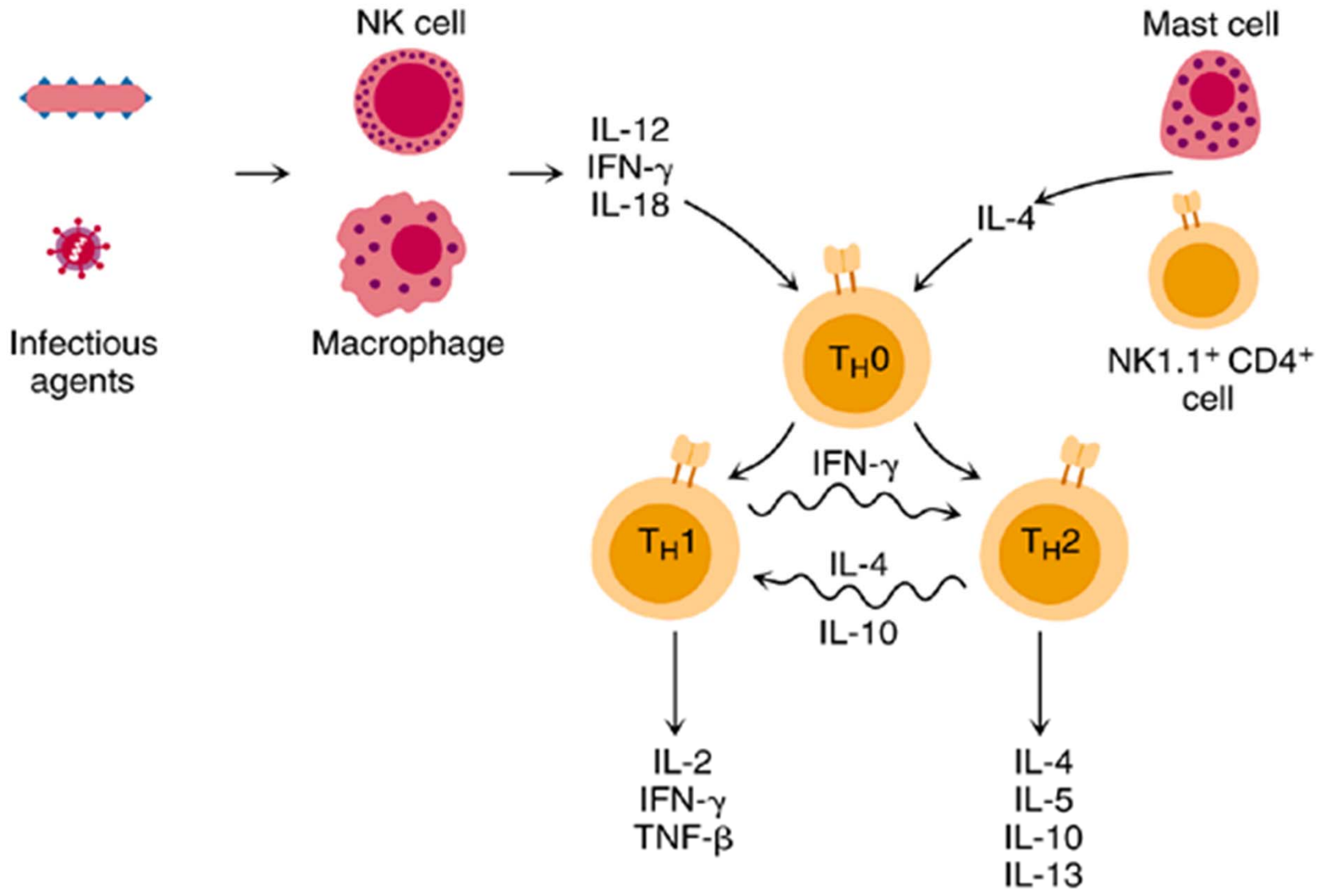
1-THE TH1/TH2 PARADIGM

The TH1/TH2 paradigm was first proposed by Mossman and Coffman to explain the differential effects of T cell help – i.e. T cells helping B cells and T cells helping other T cells. These cells were distinguished functionally rather than morphologically by the differences in cytokine patterns that they produced.

Differential production of specific cytokine patterns by subpopulations of CD4+ cells

- TH1 help other T cells develop immunity against intracellular pathogens (mostly T cell-mediated)

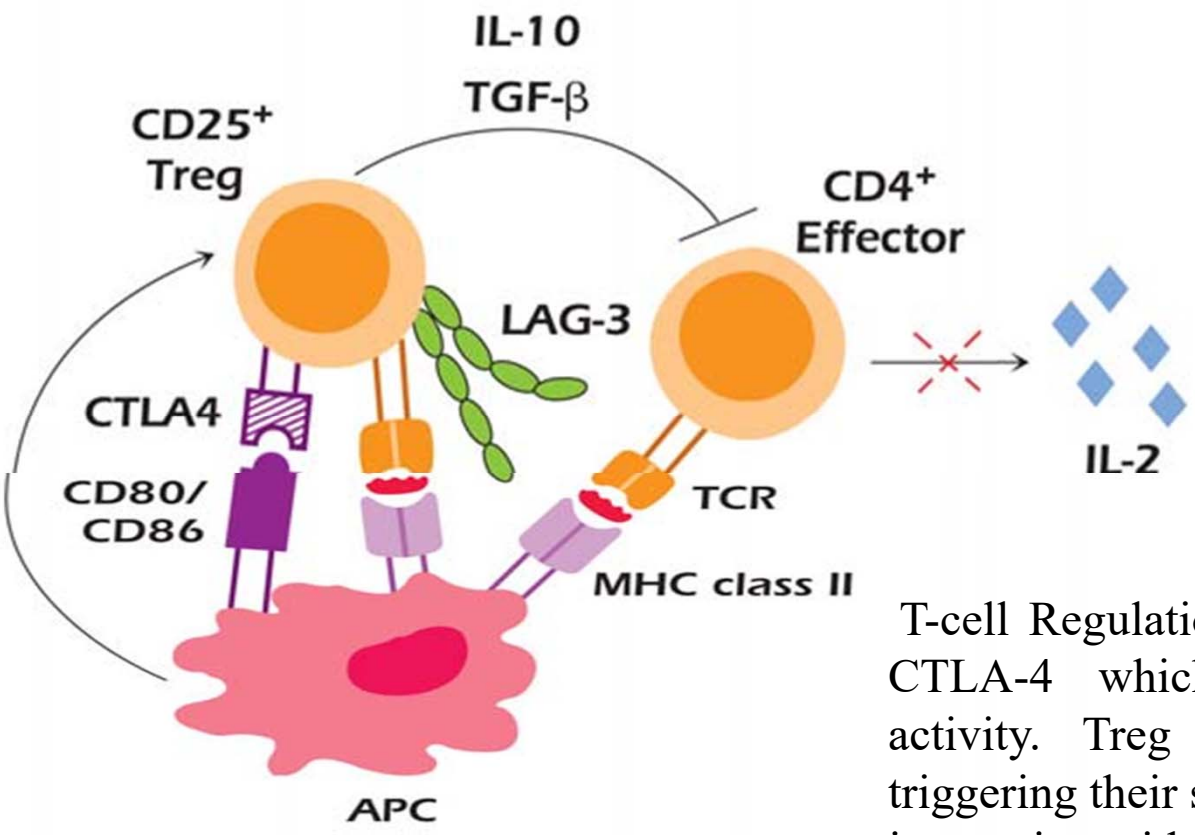
- TH2 help B cells (and other WBC) develop immunity against extracellular pathogens (mostly through IgE, mast cells and eosinophils)



2-REGULATORY T CELLS

(T reg) represent subpopulations of T helper cells that trigger suppressive activities following engagement of their T cell receptor with presenting antigen occupying the MHC on an antigen presenting cell. They typically secrete molecules such as TGF-ß, which function to suppress other T helper cell type activity.

They usually express CD4 and CD25 on their cell surface, and express the transcription factor Foxp3.



T-cell Regulation: Treg receive a signal via CTLA-4 which induces their suppressive activity. Treg may also receive a signal triggering their suppressive activity following interaction with an MHC II molecule.

Treg may then suppress the activation of CD4+ T cells by secreting TGF- β (and IL-10).

Role of Cytotoxic Cell-Mediated Immunity in Host Defense

Host defenses against extracellular infectious agents (e.g., bacteria, protozoa, worms, fungi) typically utilize (1) Antibody, (2) Complement, and/or (3) activated Phagocytes. However,these mechanisms are not adequate for defense against intracellular infectious agents (an infectious agent that invades a host cell). Therefore a different defense system is required.

The mechanisms used are those referred to as

cytotoxic cell mediated immunity.

3-CYTOTOXIC CELLS (CTLS)

directly kill tumor cells and host cells infected with intracellular pathogens. These cells…..

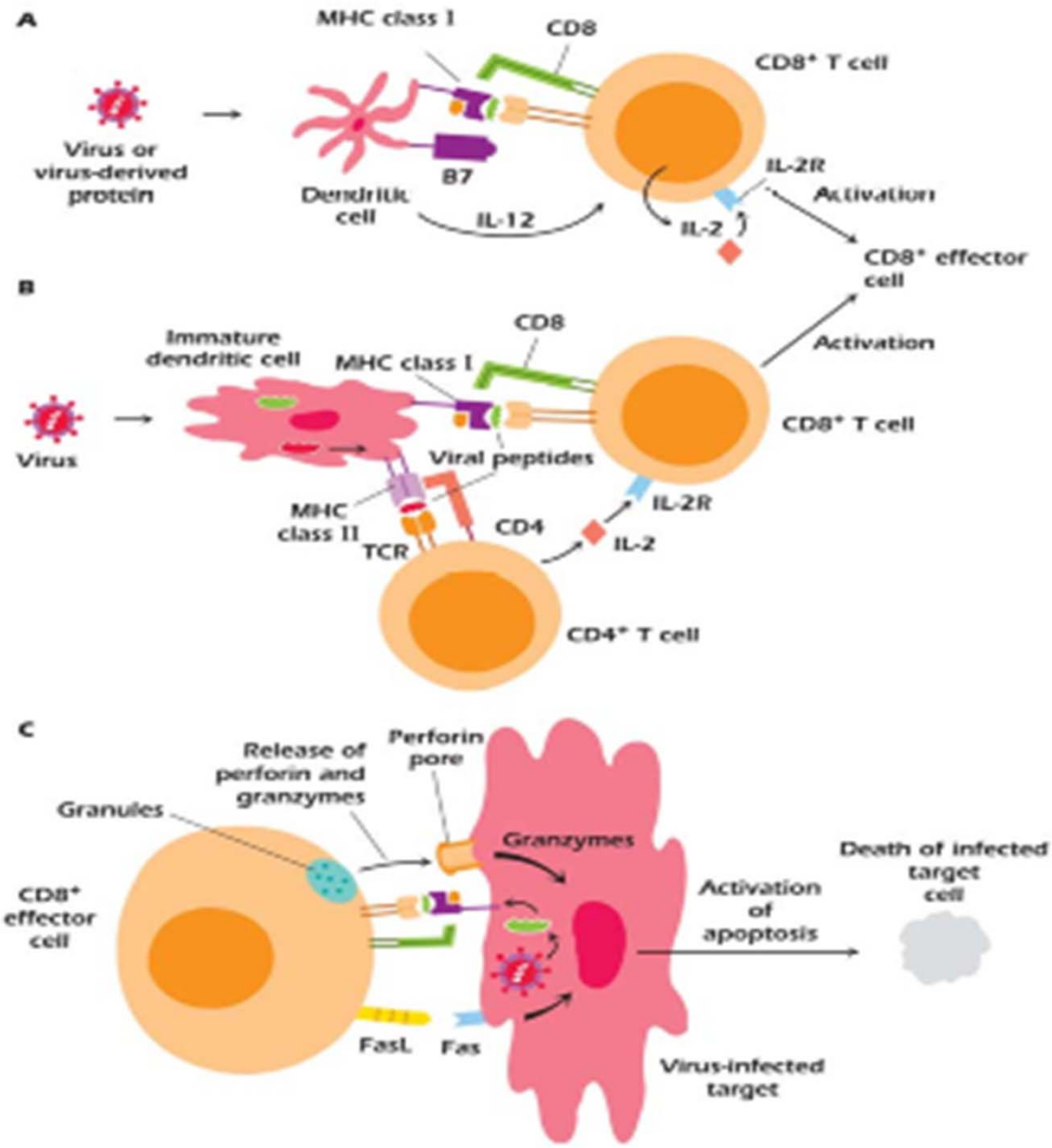
1) usually express CD8.

2) destroy infected cells in an antigen-specific manner that is dependent upon the expression of MHC class I molecules on antigen presenting cells.

Induction of helper function for cytotoxic cell mediated immunity.In many cases,

first CTL encounter with antigen must have help from Helper T cells. The helper cells must recognize antigen presented by MHC Class II molecules on an APC (antigen presenting cell) (dendritic cell or macrophage). The activated Th1 cell secretes IL-2 and IFN-gamma, which activates CTLs.

Activation of Th1 cells also triggers the activation of NK cells and macrophages which then target specific cells.



Generation of CD8+ T cells effector cells and target cell killing. (A) dendritic cells activate CD8+ T cells directly. (B) One pathway for CD4+ T cells to activate CD8+ T cells. (C) Target cell killing by a CD8+ effector T cell. Coico and Sunshine, 2009. Fig 10.10.

THE ROLE OF THE MHC IN THE IMMUNE RESPONSE

Dr. Rojan Ghanim Al -allaff

The Major Histocompatibility Complex (MHC) is a locus on a chromosome comprised of multiple genes encoding histocompatibility antigens that are cell surface glycoproteins. MHC genes encode both class I and class II MHC antigens. These antigens play critical roles in interactions among immune system cells; class I participates in antigen presentation by macrophages to CD8+ lymphocytes (CTL), class II molecules participates in antigen presentation by macrophages to CD4+ lymphocytes (T helper). MHC genes are very polymorphic. The locus also encodes a third category of MHC genes, those of the class III type. The class III MHC molecules include complement proteins, tumor necrosis factor, and lymphotoxin. In man, the MHC locus is designated as HLA (Human Leukocyte Antigen).

MHC molecules gain their name because they were first identified as the targets for rejection of grafts between individuals. When organs are transplanted across MHC locus differences between donor and recipient, graft rejection is prompt. In 1980 the Nobel Prize was awarded to Baruj Benacerraf, Jean Dausset and George D. Snell, for their work involving the major histocompatibility complex and rejection of skin grafts using inbred strains of lab mice. In mice , the MHC locus is designated as H-2. It has since been determined that the function of the MHC is the presentation of antigen fragments (epitopes) to T cells.

Structure of the MHC Class I Molecule:

Each class I locus codes for a transmembrane polypeptide of molecular weight approximately 45 kDa, containing three extracellular domains (1 α, 2 α, 3 α). The molecule is expressed at the cell surface in a noncovalent association with an invariant polypeptide called β2microglobulin (β2M) of 12 kDa. β2M is a member of the Ig superfamily, the complex of class I and β2M appears as a four-domained molecule with the β2M and 3α domain of class I juxtaposed near the cell surface membrane.

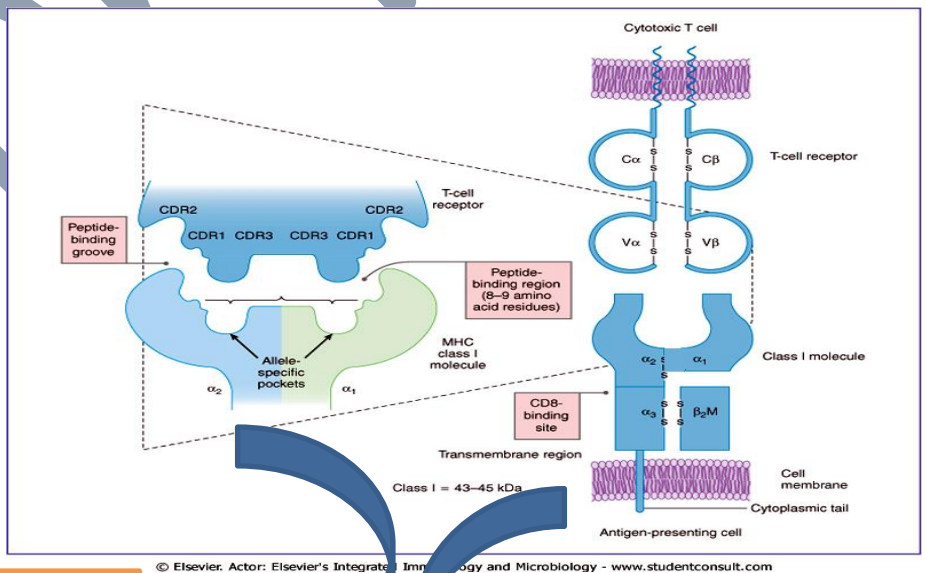


Figure. View of MHC class I showing how a T cell receptor interacts with the class I molecule/β2-M with peptide bound in theFigure. Schematic representation of an intact class I antigen in the plasma membrane. MHC class I showing the peptide binding groove.

Figure. Schematic representation of an intact class I antigen in the plasma membrane. MHC class I showing the association of a class I molecule with B2-M

Structure of the MHC Class II Molecule:

Class II molecules have 2 transmembrane polypeptide chains (α and β, 30-34 and 26-29 kDa respectively); the peptide-binding site is shared by the two domains furthest from the cell membrane. The overall structure of the peptide-binding site is very similar for both class I and class II MHC molecules; the base is made of β-pleated sheet, as in an immunoglobulin domain – the sides of the groove that holds the peptide are α-helices. Peptides bind within the allele specific pockets defined by the 2 transmembrane polypeptide chains, where they are presented to the TCR for recognition. The extracellular domain shows variability in amino acid sequences, yielding grooves with different shapes. These grooves cradle the processed antigen for interaction with the T cell receptor. The CD4 molecule assists inrecognition process, and binds to the invariant portion of the MHC class II molecules.

Like class I genes, class II genes also exhibit polymorphism with multiple allelic formexpressed. In humans, allelic forms are designated different from the mouse. For examples, human class II genes are given numbers such as HLA-D4 or HLA-D7.

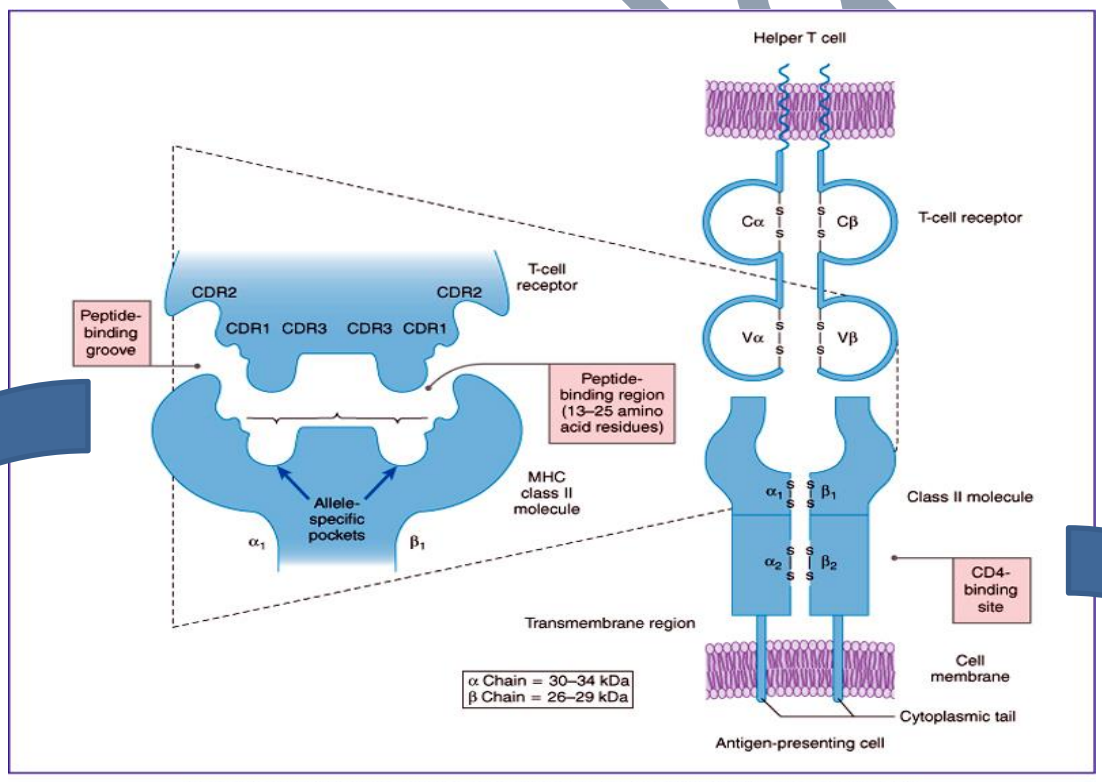
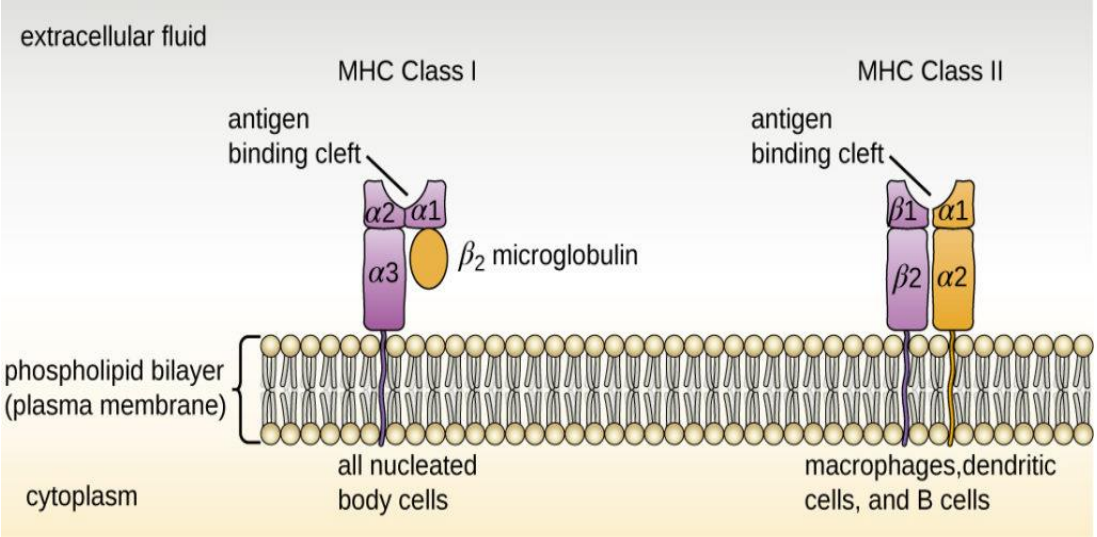


Figure. View of MHC class II showing how a T cell receptor interacts with the class II molecule with peptide bound in the peptide binding groove.

Figure.Schematic representation of an intact class II antigen in the plasma membrane. MHC class II showing the two chain class II molecule.



MHCI and MHC II

MHC Class III Molecules

Class III HLA genes encode complement components that show no structural similarity to either class I or class II molecules. These genes, along with genes encoding tumor necrosis factor (TNF), separate HLA class II and class I genes on the chromosome.

MHC and Antigen Presentation:

There are two major classes of presented antigen (Ag) called **endogenous and exogenous** Ag. MHC class I presents endogenous Ag epitopes to $CD8^+$ T cells and MHC class II present exogenous Ag epitopes to $CD4^+$ T cells. All nucleated cells are capable of presenting MHC class I, but only specialized cells present Ag epitopes on MHC class II.These are macrophages, dendritic cells and B cells. When exogenous Ag enters the body it is phagocytosed, digested and the resulting fragments are presented on MHC class II.

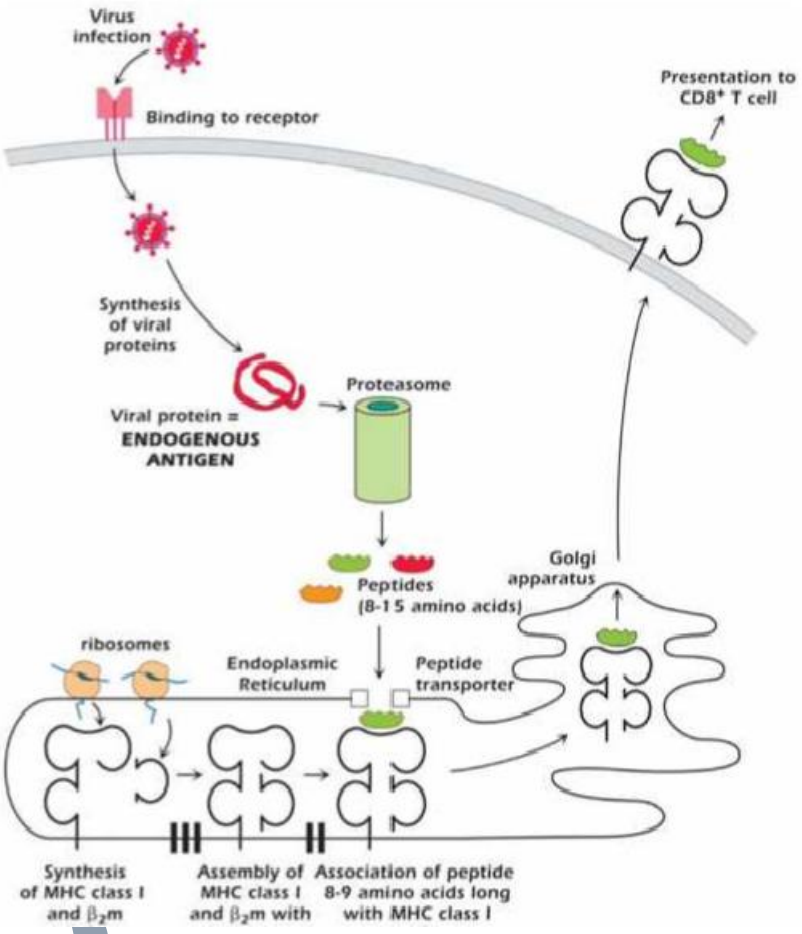
When the $CD4^+$ T cell receptor binds Ag-MHC class II it is activated to proliferate and secrete cytokines which in turn activate the other immune competent cells to generate humoral and/or cellular immunity.

When CD8+ T Cell (CTL) receptor binds Ag-MHC class I it is activated to produce and secrete toxin that kills the cell to which it is bound.

The cells that ingest, digest and present exogenous Ag epitopes on MHC class II are called antigen presenting cells (APCs), and the process of ingestion, digestion and presentation is called antigen processing and presentation. All nucleated cells can display MHC class I, but only APCs display MHC class II.

Endogenous (cytoplasmic) antigen processing and MHC class I presentation:

MHC class I molecules bind peptide fragments derived from proteolytically degraded proteins endogenously synthesized by a cell. Small peptides are transported into the endoplasmic reticulum where they associate with nascent MHC class I molecules before being routed through the Golgi apparatus and displayed on the surface for recognition by cytotoxic T lymphocytes. MHC class I molecules bind small antigenic peptides that are 8-10 amino acid residues in length

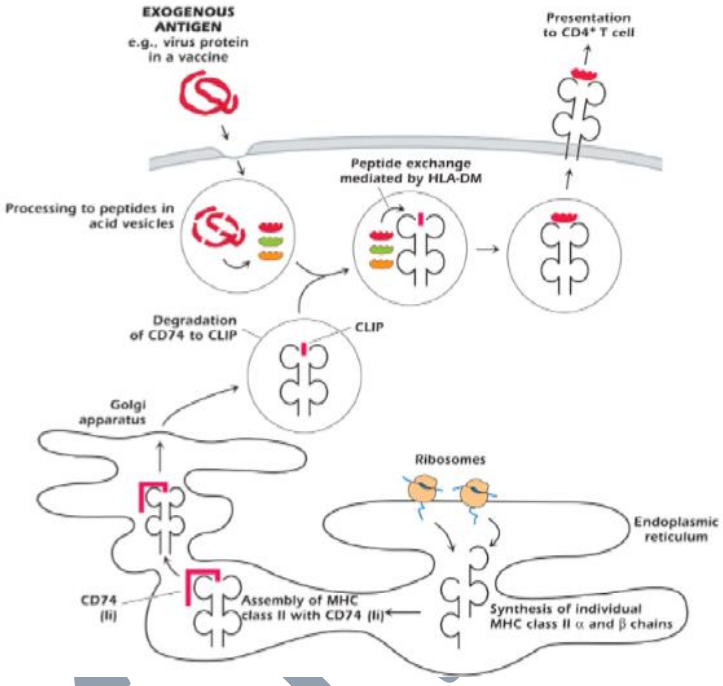


Endogenous (cytoplasmic) antigen processing and MHC class I presentation

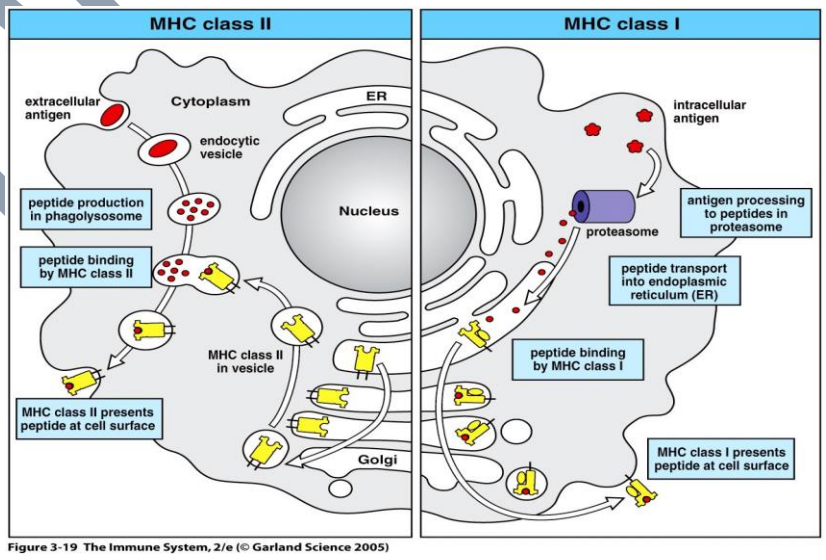
Exogenous (endosomal) antigen processing and MHC class II presentation:

MHC class II molecules bind peptide fragments derived from proteolytically degraded proteins exogenously internalized by "antigen presenting cells," including macrophages, dendritic cells, and B cells. The resulting peptide fragments are compartmentalized in the endosome where they will associate with MHC class II molecules before being routed to the cell surface for recognition by helper T lymphocytes. MHC class II molecules bind larger antigenic peptides usually 13-18 amino acid residues in length (but may be longer).

Like class I molecules, class II MHC molecules are synthesized in the RER. The class II α and β chains reside there as a complex with an additional polypeptide called the invariant chain (Ii). The invariant chain blocks the groove of the class II molecule and prevents endogenous antigens from binding there. The MHC/invariant chain complex is transported to an acidic endosomal or lysosomal compartment that contains a degraded antigen peptide. The invariant chain comes off the complex, exposes the groove of the class II molecule, and allows the antigen peptide to slip into the groove. The class II/antigen peptide complex is then transported to the surface of the APC where it is available for interaction with CD4 TH cells.



MHCclass II



MHC class I and MHC class II