

Debre Birhan University
Department of Biology

Course title: Introduction to immunology

Course code: Biol.4062

Credit hours: 2 theoretical classes/week

(4th Year Students)

Group Assignment

1. Bacterial infection G3,G1
2. Fungal infection G5
3. Viral infection G2
4. Parasitic infection G4
5. Assignment submission date November 19/2017 E.
C.

Format

1. Cover page
 2. Table of contents
 3. Introduction
 4. Body
 5. Conclusion
 6. References
- 1. Cell injury**
 - 2. Immune response**
 - 3. Evasion mechanism**

➤ Questions

1. What is immunology and immunity?
2. What is immune system? Give an example
3. What is the difference between innate and adaptive immunity?
4. How does body defend against infections?
5. Mention body defense mechanisms

Chapter 1

Introduction

Definition of immunology:

- The study of the **cellular and molecular events** that occur after organism encounters microbes and other foreign molecules.
- The study of all **aspects of the immune system** including its structure and function, disorders of the immune system, blood banking, immunization and organ transplantation.
- The branch of **biomedical science** concerned with the response of the organism to antigenic challenge, the recognition of self and non self, and all the biological, serological, and physical chemical effects of immune phenomena.

Development of immunology

- ❖ The discipline of immunology grew out of the observation of individuals who had recovered from certain infectious diseases

Science begins with Observation



Early observers noticed that survivors of certain diseases were resistant to re-infection

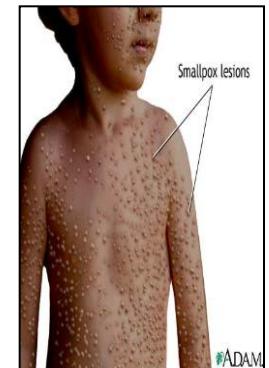
❖ Terms that related to the development of immunology:

✓ **variolation**

✓ **vaccination**

❖ **Variolation** is the process of deliberate dried crusts derived from smallpox pustules in order to against variola virus.

- Chinese and Truks practiced variolation in 15 century that is the first recorded attempts to induce immunity deliberately.
- In 1718, Lady Mary Montagu: was first introduced variolation in Great Britain.



- Edward Jenner invented smallpox vaccine. How?
- In 1796, Edward Jenner noted that milkmaids who had Cowpox lesions (from vaccinia virus) did not get Small pox (Variola virus).
- Edward Jenner introduced an idea that inoculating cowpox pustule fluid into people might protect them from smallpox due to cross protection. To check this idea, he carried out an experiment:
 - ✓ he inoculated an eight-year-old boy with fluid from a cowpox pustule.
 - ✓ Then, the boy was infected with smallpox virus
 - Immediately → the boy developed smallpox
 - After two months → the boy did not get smallpox
- Jenner's technique of inoculating with cowpox to protect against smallpox spread quickly throughout Europe.



Edward Jenner inoculating James Phipps with cowpox

Vaccination

- ✓ inoculation of healthy individual with attenuated (weakened) or killed form of infectious agent to provide protection from diseases.
- The **attenuated vaccine** was discovered by Louis pastuer accidentally.
- Louis Pasteur experiment:
 - 1st one
 - grow the bacteria which cause cholera
 - injected some chickens with **fresh** culture
 - chickens were **developed** cholera.
- Why chickens develop cholera?

2nd one

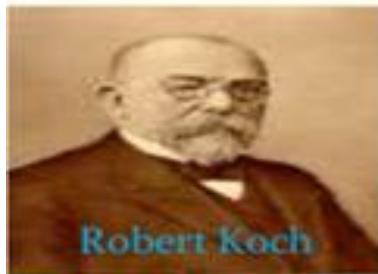
- He grow the bacteria and left for several months
 - He injected some chickens with an **old** culture. The chickens became ill but, they **recovered**.
 - The recovered chicken were injected with fresh culture, they did not develop cholera.
- Pasteur hypothesized and proved that
- **aging** had weakened the **virulence** of the pathogen
 - **attenuated strains** are important to against the **disease**.
 - He called this attenuated strain a **vaccine (from the Latin *vacca*,** meaning “cow”)), in honor of Jenner’s work with cowpox inoculation.

➤ Pasteur extended these findings to other diseases such as **anthrax** and **rabies**

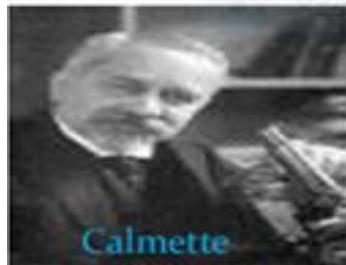
- In 1881, Pasteur was vaccinated tow group of sheep
 - First group with heat-attenuated bacillus
 - Second group with a virulent bacillus.
- ✓ All the vaccinated sheep lived where as unvaccinated sheep died.
- These experiments marked the **advancement** of immunology

- ❖ In 1885, Pasteur administered his first vaccine to human
 - ✓ A young boy was inoculated with **attenuated** rabies virus
 - ✓ The boy had been bitten by a rabid dog.
 - The boy was lived.

- ❖ In 1896 Robert Koch introduced *V. cholerae*, killed whole cell bacterial vaccine



- ❖ 1919- Albert Calmette and Camille Guerin: TB vaccine/BCG- and first used in humans in 1921 (it took 13 yrs and 230 sub culturing)



- 1920s-1950s – many vaccines developed for humans – mostly bacteria such as Diphtheria, Tetanus, Pertussis
- 1950s Jonas Salk – Polio vaccine (killed virus, *injectable*)
- 1960s Sabin develops live attenuated polio vaccine (oral *vaccine*)
- 1960 – 1970s vaccines developed for Measles, Mumps & Rubella
- 1970s attenuated vaccine for Hepatitis B
- 1980s vaccine developed for *Haemophilus influenzae*
- 1990s vaccines developed for Hepatitis A

Definition of terms

Immune system: molecules, cells, and organs responsible for immunity

Immune response

- ✓ collective response of immune system to ‘foreign’ substances

Immunity:

- ✓ The Latin word “Immunis” means “exempt” which is
 - a protective or defense mechanism of our body, which leads us to a healthy life.
 - refers to the state of protection from infectious disease

Immunity can be:

a) Active immunity

- is resistance acquired after contact with **foreign antigens**, e.g, microorganisms
- This contact may consist of :
 - Clinical infections
 - Immunization with **live or killed** infectious agents or their antigens.
 - Exposure to **microbial products** (eg, toxins)

b) Passive immunity

- is resistance based on **antibodies** preformed in another host. E.g.
 - **IgG** passed from the mother to the fetus during pregnancy.
 - **IgA** passed from the mother to the newborn during breast feeding

Active artificial immunity

- when a person is deliberately given antigens

Actives-natural immunity

- when a person is exposed to antigens, but not deliberate.

Passive-artificial immunity

- when a person is deliberately exposed to antibodies

Passive-natural immunity

- transfer of mother's antibodies to fetus or baby.

Antibody (ab): A **protein** produced as a result of interaction with an antigen.

Antigen (ag): any **foreign body** that can be recognized by immune system

Antigency

- is the ability to **combine** specifically with the antibodies and /or cell-surface receptors.

Epitopes: **site on an antigen** recognized by an antibody

cross-reactivity

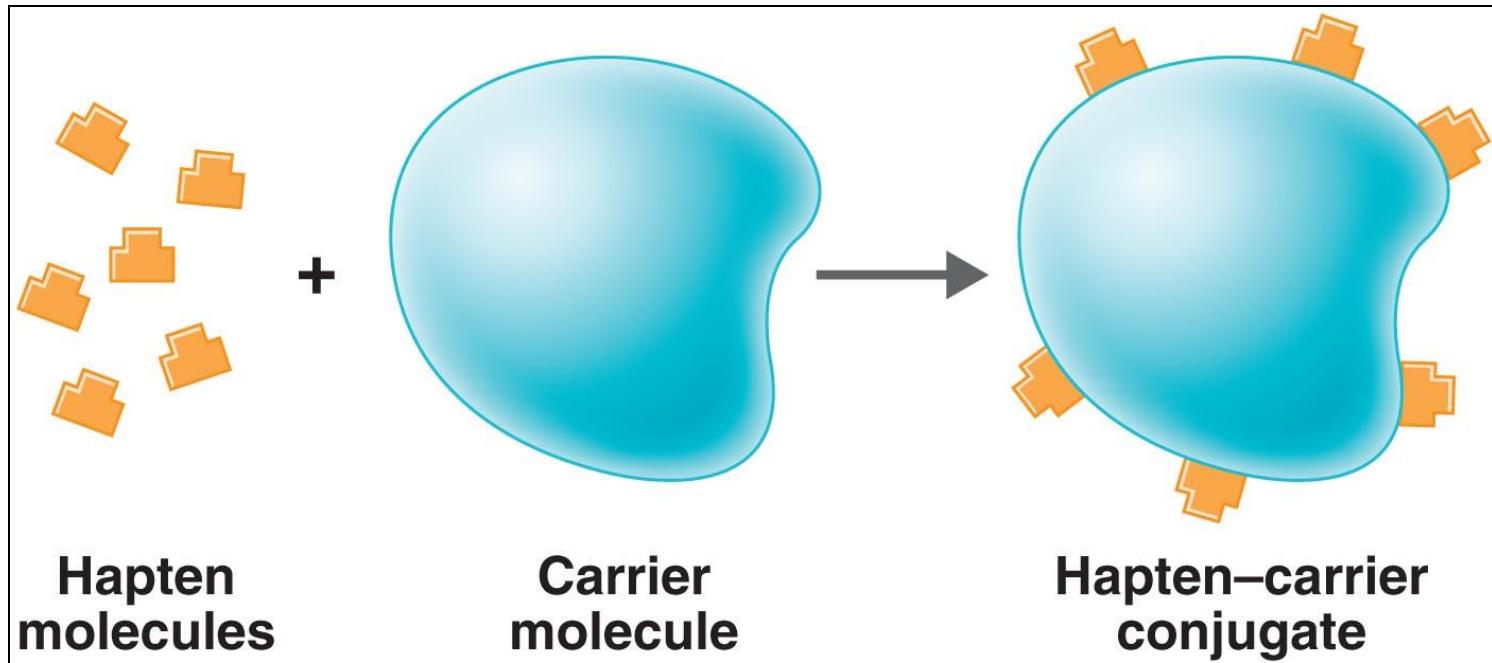
- refers two different antigens **share** an identical or very similar epitope

Immunogenicity: the ability to **induced** the immune response

Hapten:

- Small foreign molecule that is antigenic but lack immunogenic.

- All immunogenic substances are **antigenic** but the reverse is **not**.
- Immunogenic =Haptens + carrier molecule



- **Apoptosis:** A form of programmed cell death
- **Chemotaxis:** Movement of cells up a concentration gradient of chemotactic factors
- **Lymph:** The tissue fluid which drains into and through the lymphatic system.
- **Effector cells:** Cells which carry out an immune function
- **Hematopoietic stem cells:** Self-renewing stem cells that are capable of giving rise to all of the formed elements of the blood
- **Plasma cell:** Terminally differentiated B lymphocyte which actively secretes large amounts of antibody

- **Thymocyte:** Developing T-cell in the thymus.
- **adjuvant:** Any substance which nonspecifically enhances the immune response to antigen
- **Clonal selection.** Antigen selects specific B or T cells to expand into clones
- **Avidity:** is a measure of the overall strength of binding of an antigen with many antigenic determinants and multivalent antibodies
- **Affinity:** is the sum of the attractive and repulsive forces operating between the antigenic determinant (epitope) and the combining site of the antibody.

- Immunogenicity is determined by many factors including
 - ✓ **Foreignness:** The **greater the phylogenetic distance** between two species, the greater the immunogenicity
 - ✓ **Molecular size:-**
 - Larger proteins are more immunogenic
 - generally, substances with a molecular mass greater than 10,000 Dalton are higher immunogenic
 - ✓ **Chemical composition:** **Hetero**polymers are more immunogenic than **homopolymer**s
 - ✓ **Physical form** - In general particulate antigens are more immunogenic than soluble ones and denatured antigens more immunogenic than the native form.

Cells of immune system

- Blood (is a liquid tissue) contains three major components:
 1. Plasma/Serum:soluble factor: complement, antibodies, etc
 2. RBC (erythrocyte) and Platelets (thrombocytes)
 3. Cells of the immune system (white blood cells = Leukocytes)
 - Innate immunity: myeloid cells
 - Adaptive immunity: lymphoid cells

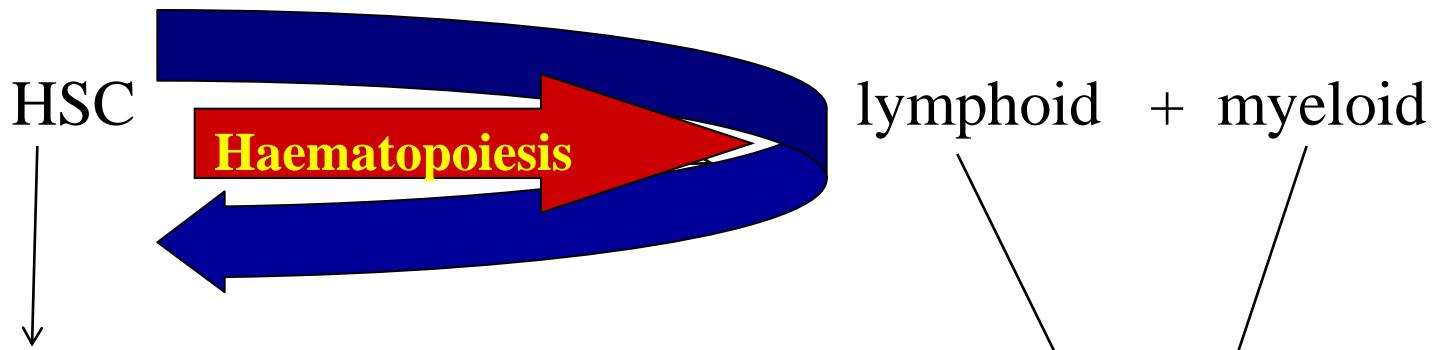
Where do all blood cells originate?

- All blood components produce in bone marrow which contains hematopoietic stem cells.

Haematopoietic stem cells (HSCs)

- Are cells that can differentiate into all of the different mature blood cell types.

Haematopoiesis---(Greek, to make): the formation of blood cellular components



- Multipotent cells (Pulripotent)
- Are self renewing

- unipotent cells (unidirectional)
- non-self renewing

Regulation of hematopoiesis

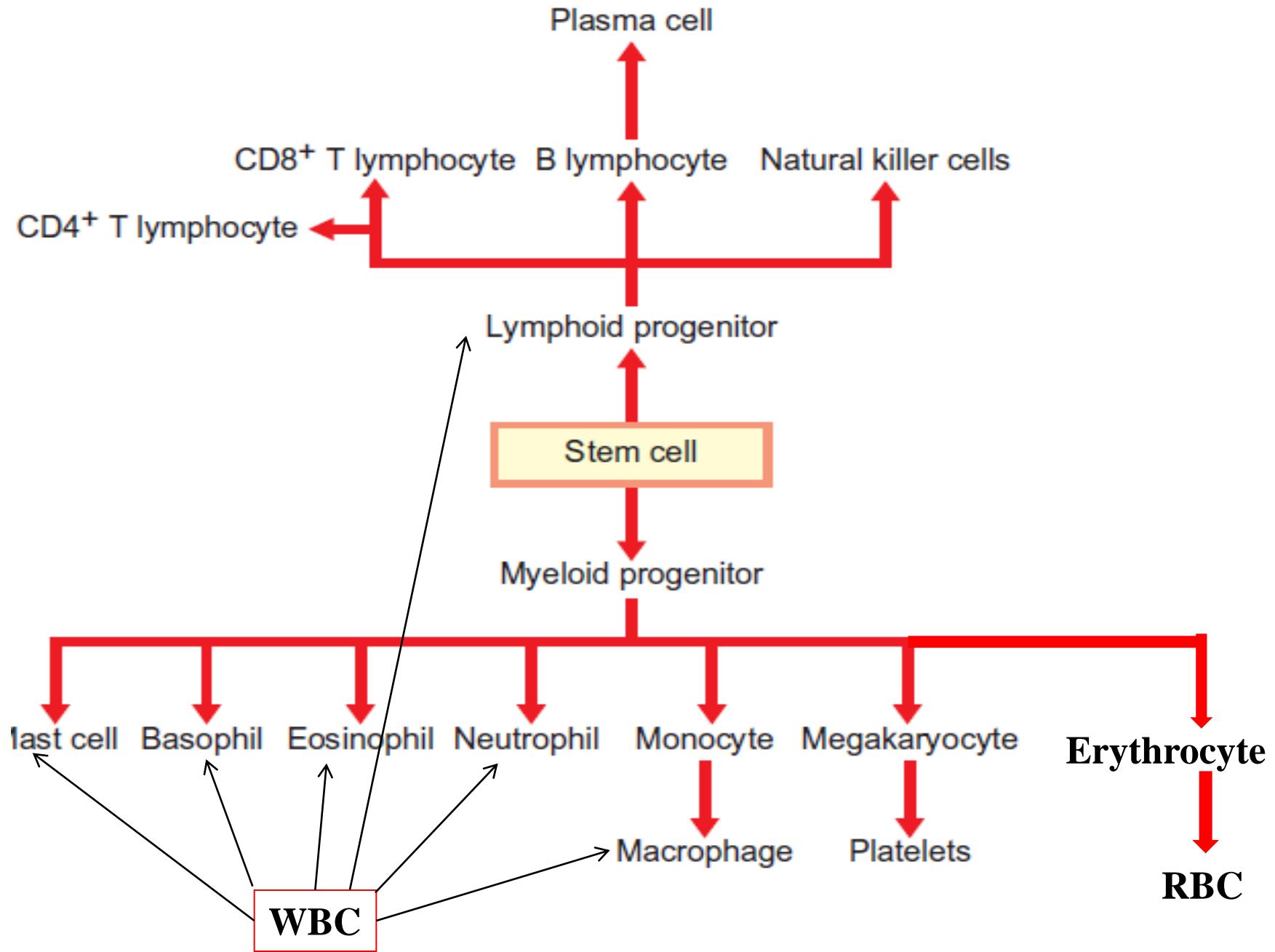
- ❖ proliferation and maturation of precursor cells in the bone marrow are stimulated by **cytokines** and balanced by **apoptosis**
- ❖ Hematopoiesis is a continuous process that generally maintains a steady state in which the production of mature blood cells equals their loss (principally from aging). E. g.:
 - The average erythrocyte has a life span of **120 days**
 - The various white blood cells have different life spans
 - **20–30 years** for some T lymphocytes
 - **a few days**, for neutrophils
- ❖ To maintain steady-state levels, the average human being must produce an estimated 3.7×10^{11} white blood cells per day.

➤ Myeloid (Cells of innate immunity)

- cells used to protect the body from foreign attack without immunized by previous infection or vaccination
 - macrophage, neutrophils, basophils, eosinophils, mast cells, dendritic (DC)

➤ Lymphoid (Cells of adaptive immunity)

- cells used to protect the body which immunized by previous infection or vaccination
 - B lymphocytes
 - T lymphocytes
 - Natural killer (NK) cells



Leukocytes

white blood cells ~ WBC

agranular

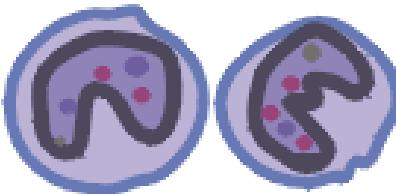
lymphocytes
20 - 25 %



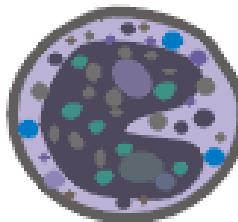
T-cell, B-cell, NK Cell

granular

monocytes
3 - 8%



basophils
.5 - 1% neutrophils
60 - 70% eosinophils
2 - 4%



Macrophage

- Develop from a types of white blood cell known as monocytes
- Pro-monocyte mature and form Monocytes which enter into blood circulation
- When monocytes move from blood to tissue, they convert macrophage
- Monocytes circulate for about 8 hrs
- Monocytes move to tissue when infection occur

➤ Macrophages have different names depending on their location in the body:

- Alveolar macrophages: Lungs
- Histiocytes: Connective tissue
- Kupffer cells: Liver
- Mesengial cells: Kidney
- Microglial cells: brain
- Osteoclast: bone

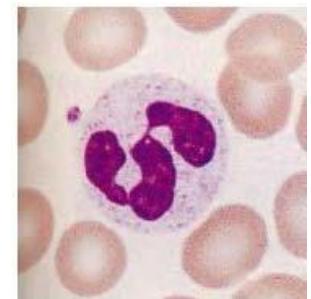
➤ Function of macrophage

- Participate in innate and adaptive immunity
- Phagocytic role
- antigen presenting cells
- Participate in inflammatory response
- Involve in apoptosis
- Recruit neutrophils and other leukocytes into infected site by secreting cytokines

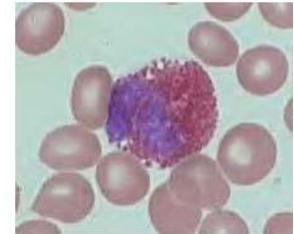
Neutrophils

- are the most **abundant** of the leukocytes, accounting for 50-70% of the WBCs.
- **Short** lived and die at the site of infection, forming **pus**
- They **do not** multiply. Bone marrow produce 80,000 new neutrophils per minute
- They **circulate** in blood and enter into tissue when recruited by Chemotactic factors which caused by infection or injury

- They are **called** neutrophils because their granules stain poorly-they have a **neutral color**
- Their **granules** contain antimicrobial molecules that includes lysozyme, lactoferrin, acid hydrolase, and myeloperoxidase.
- Function of neutrophils
 - Phagocytic role
 - Participate acute inflammatory response (hyper sensitivity reaction)



Eosinophils



- Is the **second abundant** of granulocyte cells
- More resident in tissue and have lobed nuclei
- are **called** eosinophils because their granules stain red with the **acidic dye eosin**
- Their granules contain destructive enzymes such as acid phosphatase, peroxidases, and proteinases for killing infectious organisms.
- Secrete **prostaglandin and various cytokines**
- They are effective when the antigen is coated with antibodies such as Ig G and E
- Their life span is 8-12 days.

Function of eosinophils

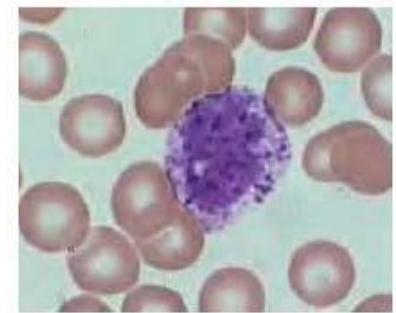
- Phagocytic role---mainly extracellularly (parasites such as helminths) and intracellularly (bacteria, fungi)
- prevent attachment of infectious agents (Increase mucus production)
- Participate in inflammatory response (synthesize inflammatory mediators)

Mast cells

- are resident in mucosal tissues and loose connective tissues
- have large cytoplasmic granules that store pre-formed inflammatory mediators such as histamine and cytokines, and which are discharged when the mast cell is activated (degranulation).
- is the only innate immune cell that is **none motile**
- secreted molecules in mast cells are generally involved in:
 - induction of acute **inflammation**.
 - **expel** parasites (muscle contraction).
 - Involves in **hypersensitivity**

Basophils

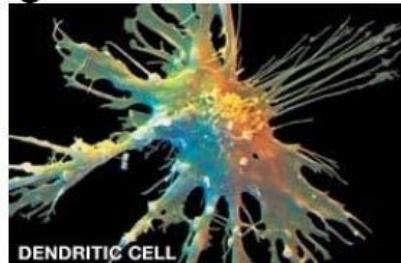
- compromises 0.1% of the WBCs (least abundance)
- stain a darkpurplish blue with the basic dye methylene blue
- have a lobed nucleus
- **short life span** (a few hours to a few days)
- no phagocytosis
- basophils release histamine, leukotrienes, and prostaglandins, chemicals that promotes inflammation



Function

- Provide **chemotactic** factors for neutrophils and eosinophils to infection site
- participate in **hypersensitivity**

Dendritic Cells (DC)



- ✓ are antigen-presenting cells (**APC**)
- ✓ have a distinctive star shaped morphology which resembles **dendrite nerve** cells as a result the name is given
- ✓ are classified into different groups
 - Langerhans cells: skin
 - Interstitial dendritic cells: connective tissue
 - Interdigitating dendritic cells: lymphoid tissues
 - Circulating dendritic cells – circulation
 - Follicular dendritic cells: in Lymph follicles

Organs of immune system

a) Primary lymphoid organs

- Are called central lymphoid organs
- Synthesize and maturation of immunocompetant cells.
- E. g. bone marrow and thymus

Bone Marrow

- A site of **generation** of all circulating **blood cells**
- the site of **B cell** maturation
- harbors numerous antibody secreting plasma cells

Thymus

- A bilobed organ situated in the anterior heart
- It is the site of **T cell** maturation

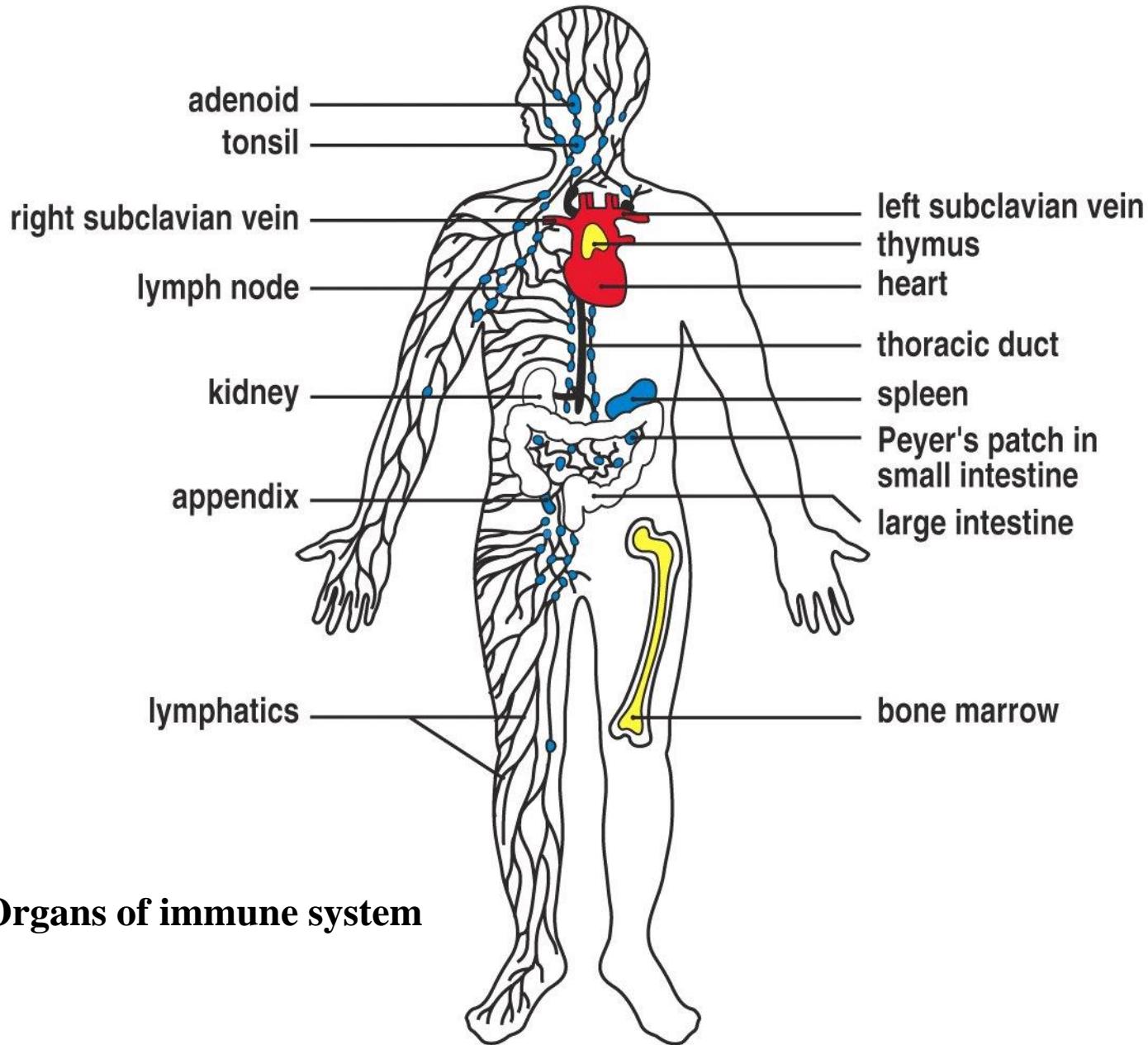


Figure: Organs of immune system

B) Secondary lymphoid organs

- site where mature lymphoid cells encounter with antigen
- Trap antigen from
 - ✓ tissues/ vascular spaces
 - ✓ DCs and macrophages
- E. g.
 - ✓ Lymph nodes
 - ✓ Spleen
 - ✓ Mucosal associated lymphoid tissue (MALT)

Lymph nodes

- ✓ Encapsulated bean shaped structures
- ✓ Located in neck, axillae, groin, and abdominal cavity (cluster)
- ✓ Consists three layers
 - cortex.... mainly consists of B cells and recirculating T and B lymphocytes enter from the blood.
 - Paracortex--- T lymphocytes, dendritic cells and macrophages.
 - Medulla--- B cells, T cells, plasma cells and macrophages.

✓ function

- filter antigens from the **interstitial tissue fluid** and the **lymph** during its passage from the periphery to the thoracic duct.

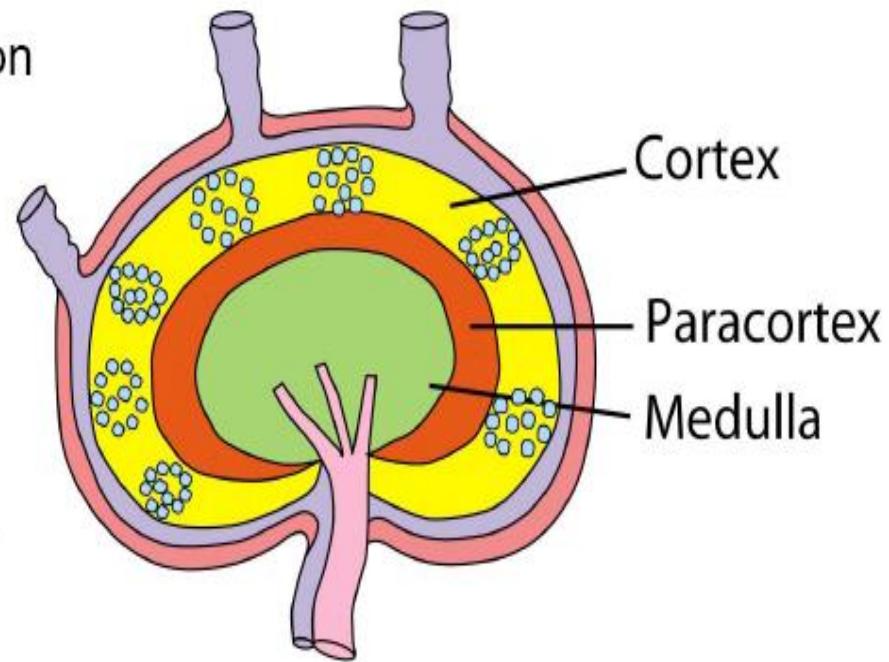
- protection

- ✓ somatic nodes ---skin
- ✓ visceral nodes ---respiratory, digestive and genitourinary tracts

Cortex- B-cell activation

Paracortex- T-cell activation

Medulla- Plasma cells



A lymph node

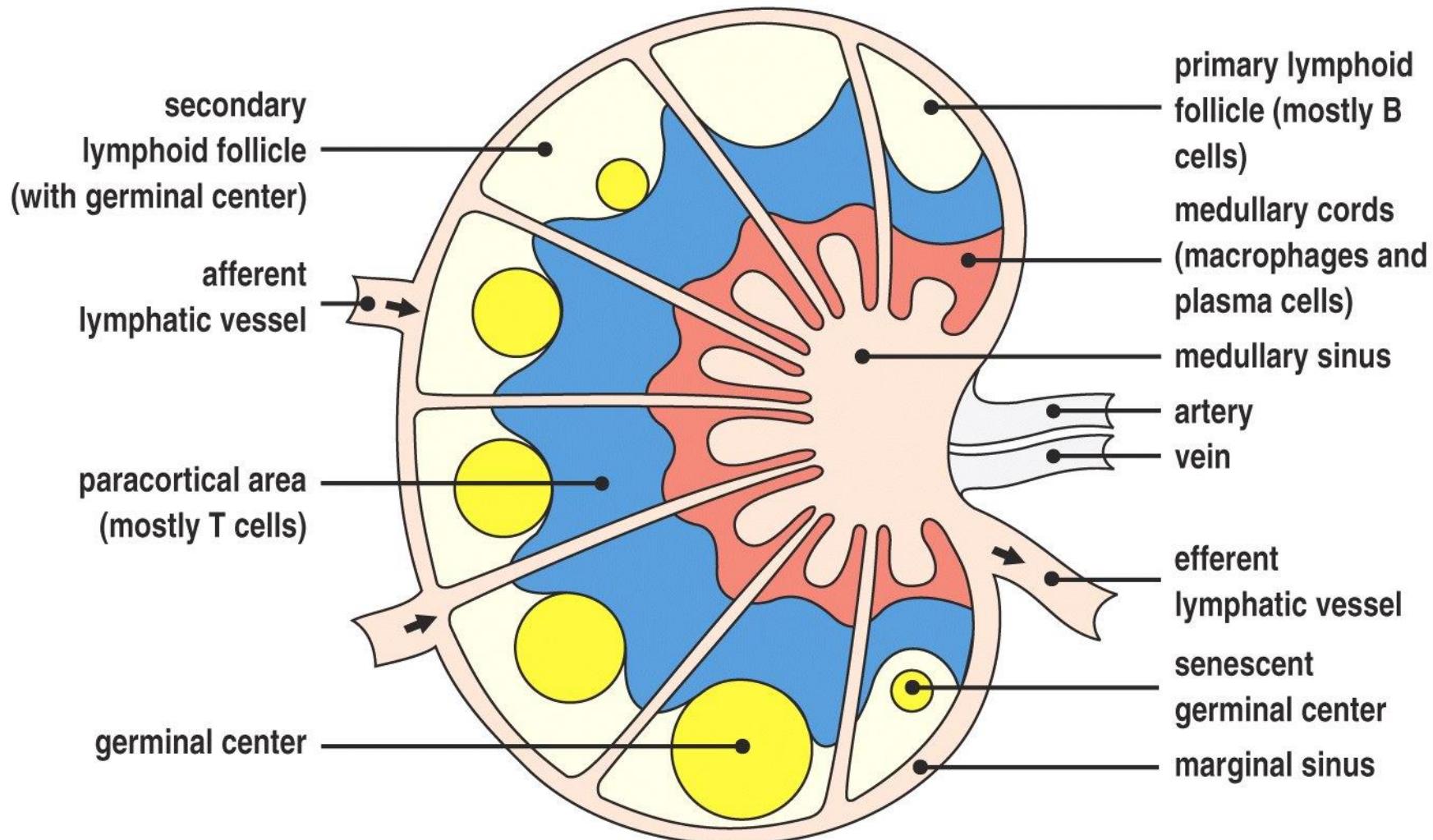


Figure 1-8 part 1 of 2 Immunobiology, 6/e. (© Garland Science 2005)

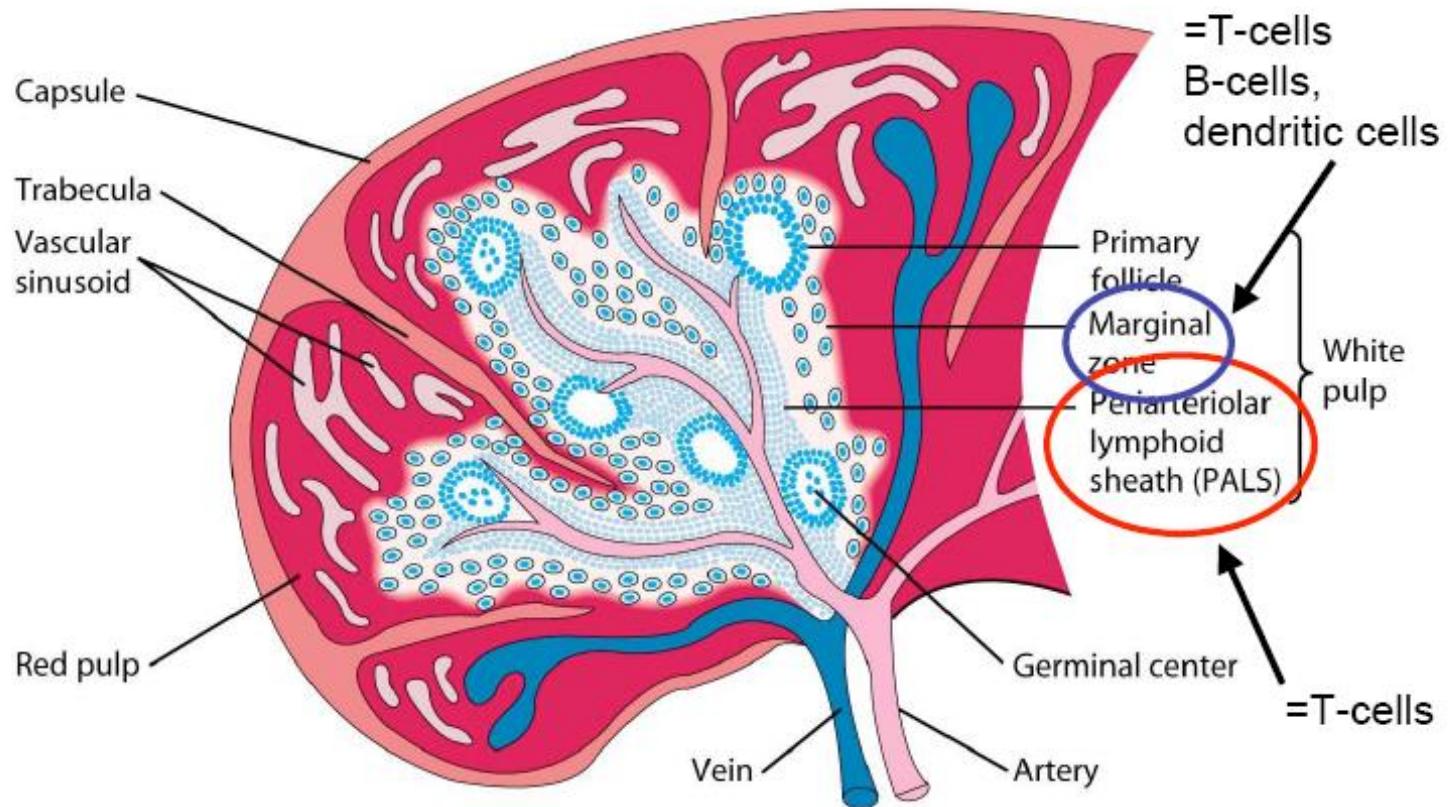
Spleen

- has ovoid shaped
- Is the large organ that located in the left abdominal cavity
- is made up of two different types of tissue:
 - red pulp--- monitor RBCs
 - white pulp--- gather WBCs to provide adaptive immunity

➤Function

- filter antigens from blood
- destruct aged red blood cells
- Receive antigens from macrophages and DCs cells
- defends the body against blood-borne pathogens (phagocytosis)
- against capsulated bacteria such as *Pneumococci* and *Meningococci*.
- synthesize antibody and release into circulation.

The Spleen



periarteriolar lymphoid sheath = PALS

Mucosal-associated lymphoid tissue (MALT).

- Mucous membranes cover the
 - Digestive
 - Respiratory
 - uro- genital parts
- They are the major site of entry for most pathogens (Vulnerable membrane)
- Hence, these sites are protected by lymphoid tissue known as mucosal-associated lymphoid tissue (MALT)

MALT

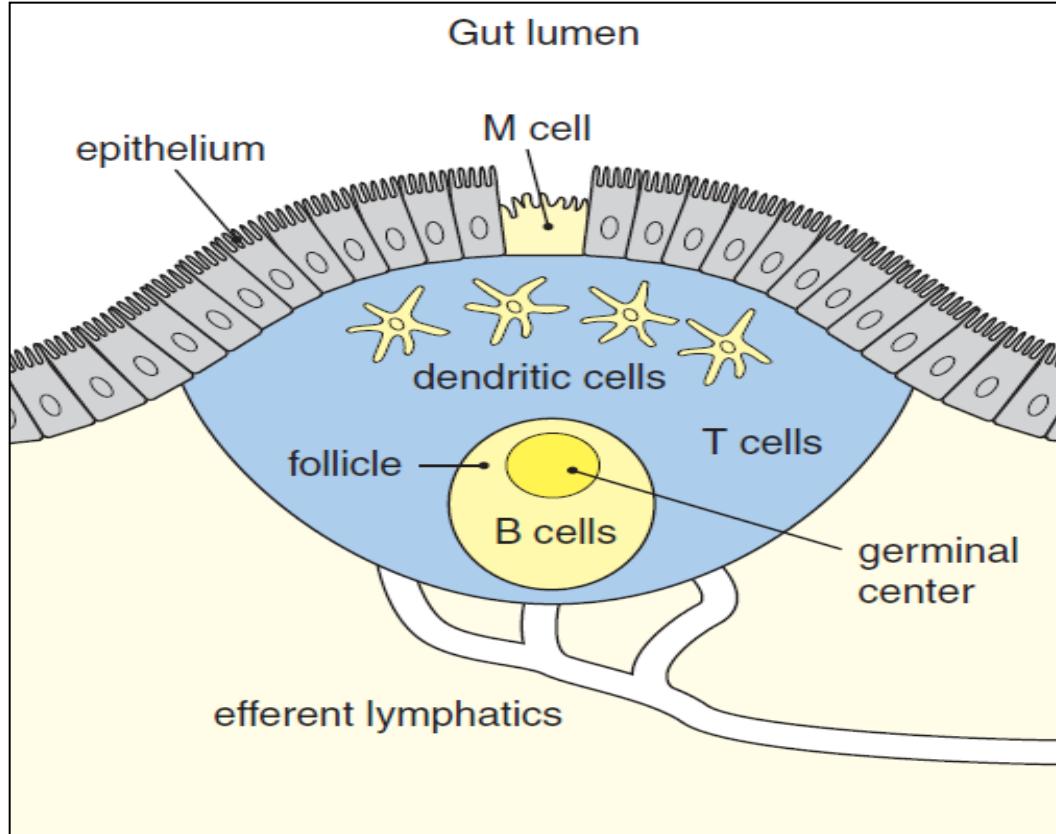
- is a place where **large population of antibody producing** plasma cells whose number exceed in that of plasma cells those found in lymph nodes, spleen, and bone marrow combined.
- Is the **largest** mammalian secondary lymphoid organ system
- comprises about **80%** of all lymphocytes.
- Found associated with mucosal system

➤ Structurally, there are two types of MALT

- Well organized**
- ✓ Tonsils
 - ✓ Gut associated lymphoid tissue (GALT)
 - peyer's patches
 - appendix

- Sparsely organized**
- ✓ BALT---lung
 - ✓ genitourinary tract.
 - ✓ lamina propria of the intestinal

Peyer's patch



- found in the wall of the small intestine
- capture and destroy antigens
- Antigens enter into Peyer's patch through M cell
- Lymphocytes enter into Peyer's patch through blood and leave it through efferent

Tertiary lymphoid tissues

- ✓ which normally contain fewer lymphoid cells than secondary lymphoid organs,
- ✓ Can import lymphoid cells during an inflammatory response.
- ✓ Most prominent of these are
 - cutaneous-associated lymphoid tissues (**CALT**)(e.g. skin)

➤skin

- is an important anatomical barrier to the external environment
- is innate immune defense mechanism
- contains a specialized cutaneous immune systems such as
 - ✓ keranitocytes: cytokines
 - ✓ melanocytes:
 - ✓ langerhans cells (types of DCs)

Function

- ✓ anatomical barrier
- ✓ involve in inflammatory response
- ✓ participate antigen presenting cells

Questions

1. What do mean phagocytosis, and inflammation?
2. How immune system identify the foreign antigen/pathogens from self antigen ?

Characteristics of immune system response

1) Antigen recognition

- ✓ differentiate self from non-self antigen by using cell receptor or marker
- ✓ if the immune system lacks recognition, they may result:
 - self tolerance...does not react with antigens
 - autoimmunity—against its own structure

2) Specificity

- ✓ innate immunity: **broad** and recognize **more than** one epitope
- ✓ adaptive immunity: **narrow** and recognize **one** epitope

3) Immunoregulatory: no attack body cell

4) Memory: for against subsequent infection (**only** adaptive immunes have memory)

Antigen recognition

Innate Immunity:

- PRRs – Pattern Recognition Receptors
 - ✓ are **structures on the cells and or molecules** of the immune system that are capable of recognizing foreign substances/PAMP/
 - ✓ include:
 - ✓ **membrane bound** PRRs: toll-likr receptors (TLRs1-12), receptor kinase, C-type lectin receptor (CLR1 and 2)
 - ✓ **cytoplasimic** PRRs: NOD-like receptors (NLR 1 and 2), RIG like receptor (rlr), RNA helicase
 - ✓ **secreted** PRRs: complement receptors, collectin, ficolins, peptidoglycan recognition proteins

➤ PAMPs – Pathogen Associated Molecular Patterns

- ✓ are **structures on the surface of microbes or secreted**; that are recognized by the host innate immune system
- ✓ are structures that **are shared** by many related microbes
- ✓ are **not shared** with the host
- ✓ are relatively **invariant** (remains unaltered)
- ✓ are **essential** for the survival of the microorganism
- ✓ E. g.
 - flagella
 - peptidoglycan of Gm (+) bacteria
 - lipopolysaccharide (LPS) of Gm (-) bacteria

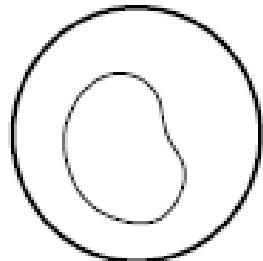
- Innate immune system (i.e. phagocytes) only recognize self cells if they are **damaged or aged**.
- Damaged or aged of self cells express new surface structure which are recognized by innate immune system (phagocytes) and destroy them.

E.g.

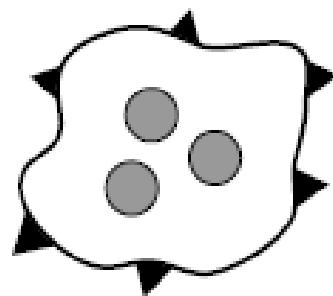
- Loss sialic acid = N-acetyl Glucosamine = aged erythrocytes
- Damaging cell membrane = phosphatidyl serine (PS) = nucleated cells

Self cells

Normal
body cell

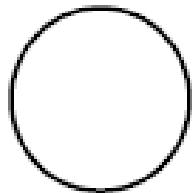


Apoptosing
cell

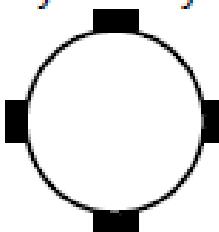


Recognition by
phagocyte

Erythrocyte



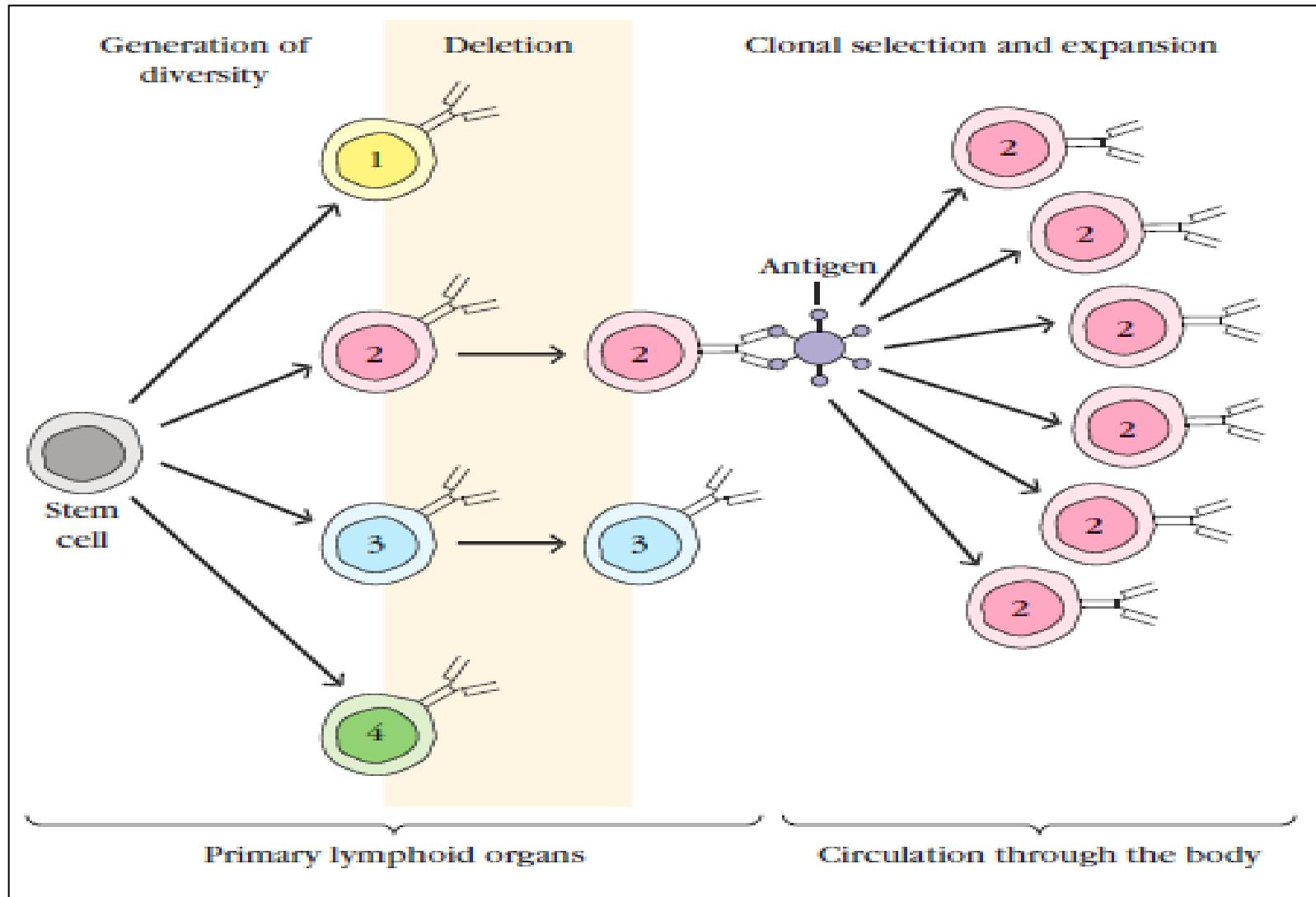
Aged
erythrocyte



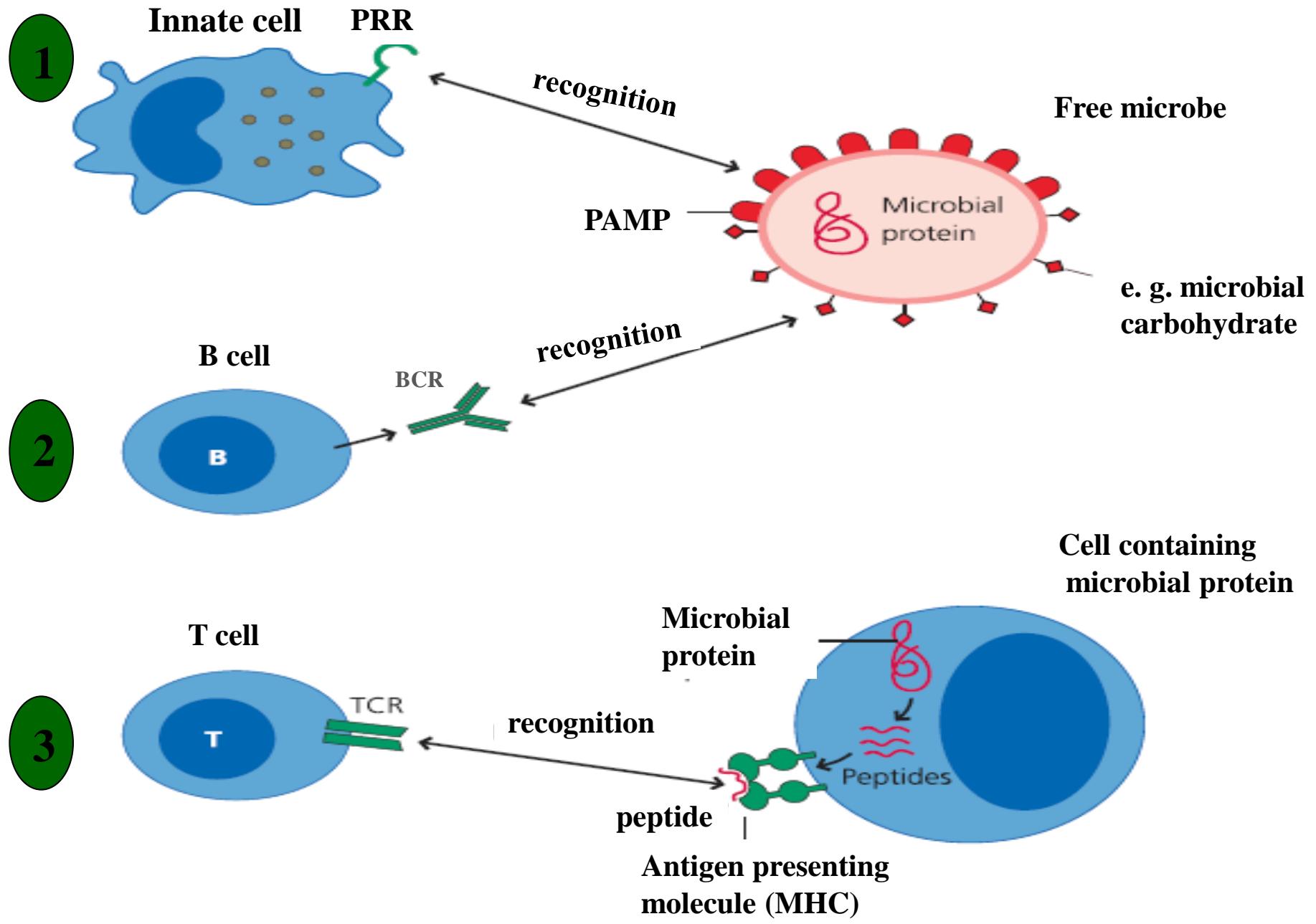
Recognition of aged/damaged self cells by phagocytes

□ Adaptive immunity: BCR and TCR

- recognize a wide **variety of antigens** due to they create a variety of antigen binding sites (**generation of diversity**) through
 - **random combinations** of a set of gene segments (variable (V), diversity (D), and joining (J) genes) that encode different antigen-binding sites (or paratopes), followed by
 - **random mutations** in this area of the genes
- recognize proteins, glycoproteins, polysaccharides, lipopolysaccharides or any pathogen surfaces



- ✓ Expresses many copies of surface receptors that binds to one particular antigen.
- ✓ Clonal selection and production for circulation.



Antigen recognition: innate and adaptive immunities

Table: Examples of PAMP and PRR

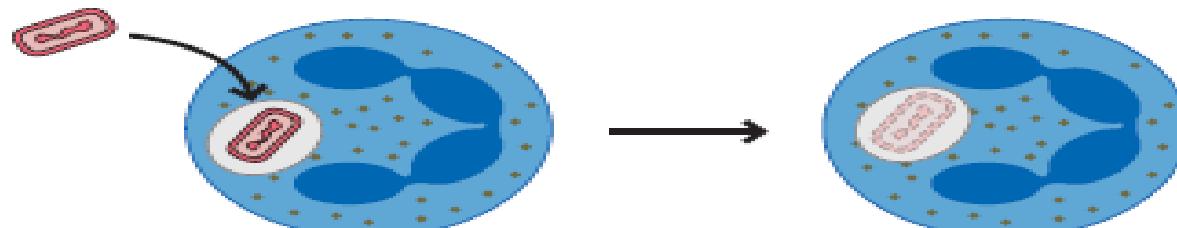
PAMP	PRR	Biological consequence of interaction
LPS (lipopolysaccharide of Gram – bacteria)	TLR-4	Macrophage activation; Secretion of inflammatory cytokines
Flagellin (bacterial flagella)	TLR-5	Macrophage activation; Secretion of inflammatory cytokines
Microbial cell wall components	Complement	Opsonization; Complement activation
Lipoproteins of Gm (+) bacteria, yeast cell wall components	TLR-2	Macrophage activation; Secretion of inflammatory cytokines
Double stranded RNA	TLR-3	Production of interferon (antiviral)
Mannose containing carbohydrate	Mannose binding proteins	Opsonization, complement activation

Immune defense mechanisms

- a) Killing the microbe directly
- b) Killing the cells that harbour microbes
- c) Preventing access

a Direct killing of microbe

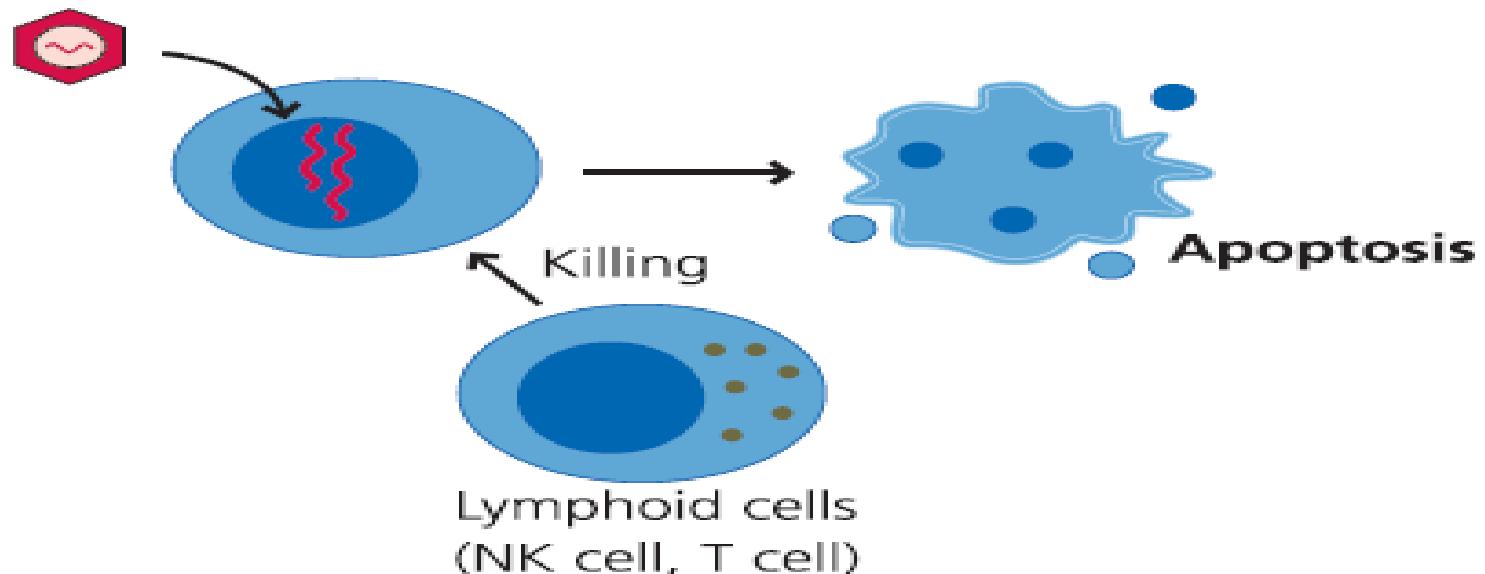
(E.g. bacterium)



Phagocyte
(neutrophil, macrophage)

b Killing infected cell

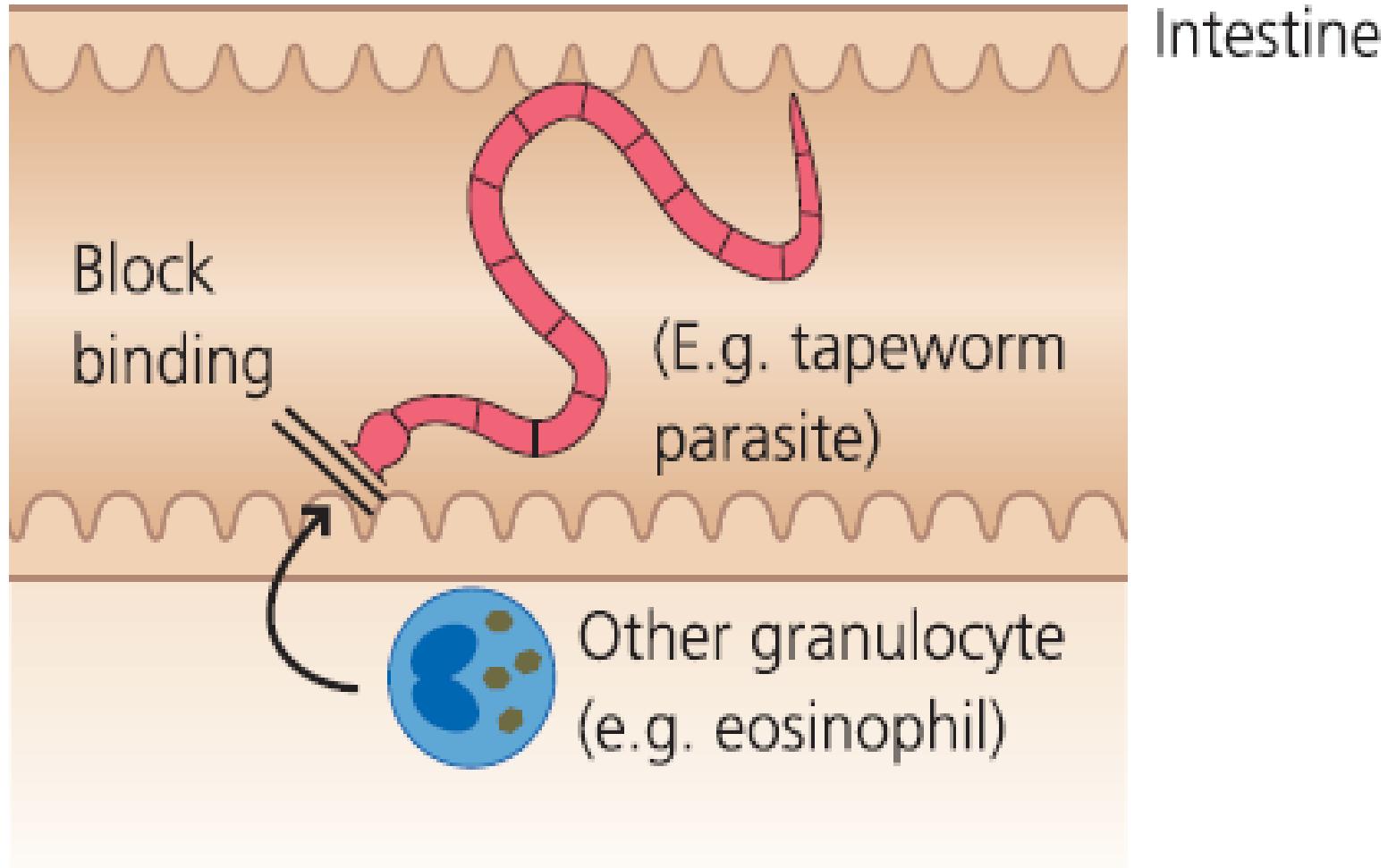
(E.g. virus)



Lymphoid cells
(NK cell, T cell)

Apoptosis

c Expulsion or tissue repair



- Immune System : 2 branches
 - The Adaptive Immune System
 - a specific counter-attack against a “known foreign” invader [previously recognized]
 - The Innate Immune system =
 - a general response to anything other than recognized “self cells”

Chapter 2: Innate immune system

❖ Characteristics of innate immune system

- known as **natural**, **native**, **inborn** or **non-specific**
- evolutionary **older** system
- **first line of defense**
- resistance is **static**, i. e. doesn't improve with repeated exposure, no memory
- no prior exposure is required
- lacks ability to distinguish **fine differences** between foreign substances
- **general response** to all substances

Table: Difference between innate and adaptive immunity

Characters	Innate	Adaptive
Development	Pre-exists before encounter with pathogen	After encounter with pathogen
Response	Rapid (hrs)	Delayed (3 - 5 days)
Memory	Have not memory	Have memory
Antigen recognition, discrimination	PRRs, limited diversity (>100 epitopes), perfect (never recognize self)	BCR, TCR, greater diversity ($>10^{11}$ epitopes), excellent but imperfect (occasional react with self antigen)
Specificity and receptor distribution	Broad (shared microbial molecule), non-clonal	Narrow (specific microbial molecule), clonal
Defense	1 st line	2 nd line
Foundation	Both vertebrate and invertebrate	Vertebrate only

➤ Function of innate immunities:

- Physical barriers
- Hummoral barriers
- Cellular barriers

a) Physical barriers

1) **Mechanical**: skin, epithelial surface, cilia, tears, mucosa

2) **Chemical and physiological**:

- lysozyme in tears, saliva and nasal = breakdown cell wall
- defensins in lung, gastrointestinal tract = lysis cell membrane
- surfactant in the lung: acts as opsonin
- lactoperoxidase: in breast milk: inhibit metabolic enzyme activity (react with sulphhydryl group of enzymes)
- acid in sweat gland and stomach: inhibit/kill microbial growth
- body temperature

3) **Biological** (e.g. normal flora in skin and GIT): control pathogens by

- competing for attachment and nutrients, produce toxic chemicals

b) Hummoral barrier

- Found in serum or formed at the site of infection
- Examples:
 - 1) **Complement:**
 - cytolysis, vasodilation, chemotaxis, opsonization
 - 2) **Lactoferrin and transferin:** binding iron
 - 3) **Interferon:** antiviral activity
 - 4) **Interleukin:** antibacterial activity

c) Cellular barriers

- ❖ Examples: macrophage, neutrophil, eosinophils, basophils, DC, and mast cell
- ❖ Function
 - ✓ phagocytic role
 - ✓ antigen presenting cells
 - ✓ participate in inflammatory response
 - ✓ involve in apoptosis
 - ✓ production of cytokines
 - ✓ prevent attachment of the pathogens

Determinants of innate immunity

- ✓ Species and strains: The **rat** is resistance to diphtheria whereas **guinea-pig and man** are susceptible
- ✓ Hormone: Affect immunity activities (e.g. inflammation)
- ✓ Age: Childhood and elderly individuals are more susceptible than adults
- ✓ Nutrition: Inadequate diet increase susceptibility (e.g. phagocytosis)
- ✓ Genetic factor: **Heterogeneous** individuals are less susceptibility than **homogenous**

➤ Response of innate immune system

- 1) Phagocytosis
- 2) Inflammation
- 3) Complement

Phagocytosis:

- ❖ Phagocytosis is derived from the Greek words “**Eat cell**”
- ❖ A process where pathogen or cell is **engulfed** and **killed** by phagocytic cells
- ❖ It is one form of **endocytosis**
- ❖ Phagocytic cells include:
 - Neutrophils occur predominate **early** in infection
 - Macrophage the chief phagocytic cell mean “ **big eater**”
 - Eosinophils

❖ Major steps of phagocytosis

1. Chemotaxis
2. Adherence
3. Engulfment/ phagosome formation
4. Phagolysosome formation
5. Killing

1. Chemotaxis:

- ✓ phagocytes and other cells are attracted to site of infection by chemicals
- ✓ attracted through chemotaxins (chemical messengers):
 - Complement
 - Proteins from coagulation
 - Products from bacteria and viruses
 - Secretions from immune cells (mast cells, lymphocytes, macrophages, and neutrophils)

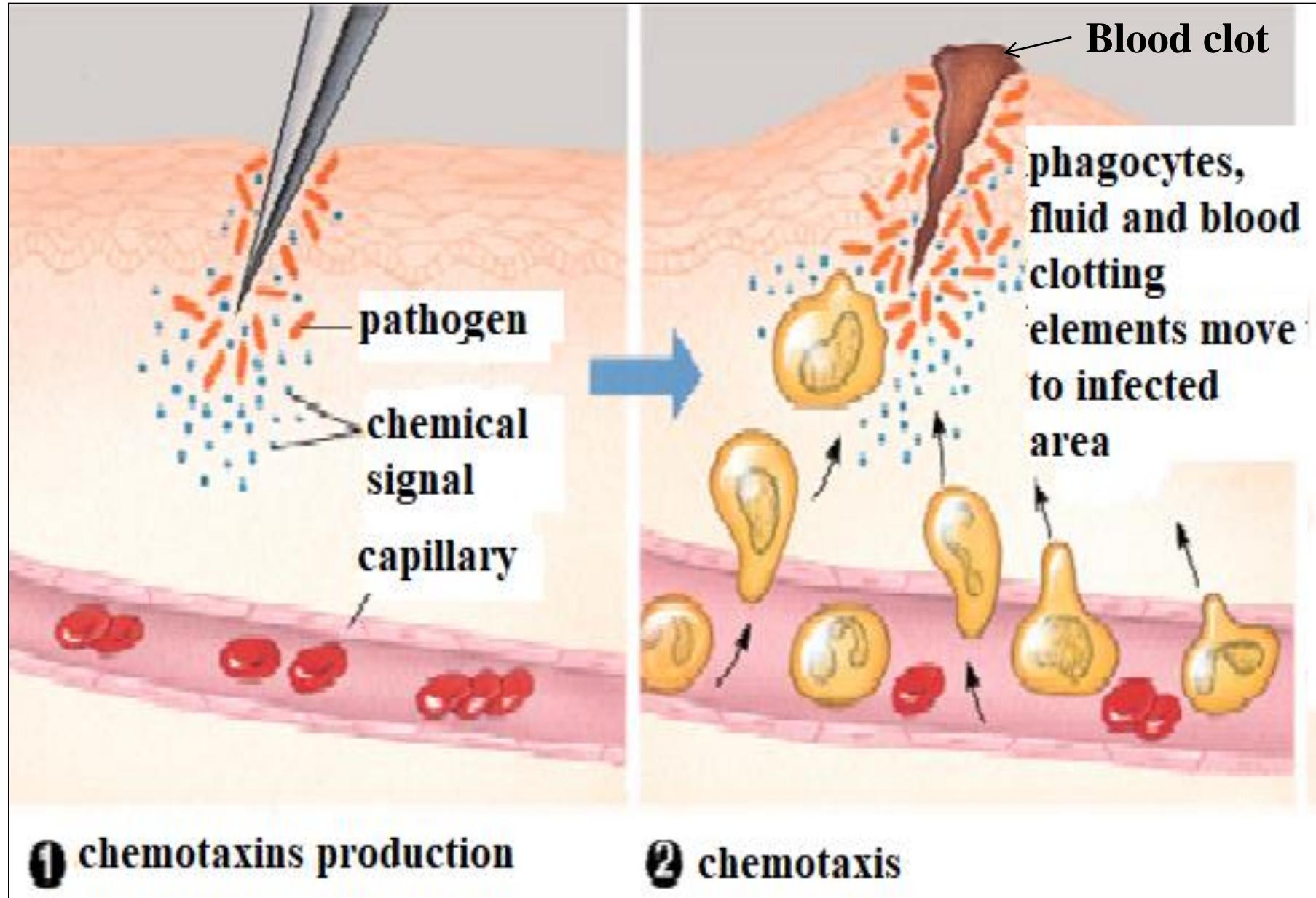


Figure: Phagocytes are attracted to site of infection by chemotaxins

2. Adherence/attachment: Phagocytic cells attach to surface of pathogen.

Opsonins:

- molecule, which enhances phagocytosis by promoting adhesion of the antigen to the phagocyte.
- E.g. Antibodies and complement proteins.

Opsonization:

- is a process by which particulate Ag are rendered more susceptible to phagocytosis (i.e. process of coating antigen with opsonins that facilitates attachment)

3) Engulfment/**formation of phagosome**

- Is process of ingestion antigen by forming phagocytic vesicle

4. **Formation of phagolysosome**

- Is the process of fusing phagosome with cytoplasmic granules (lysosome)

5. **Killing the pathogens: three ways:**

a) **Oxygen independent killing**

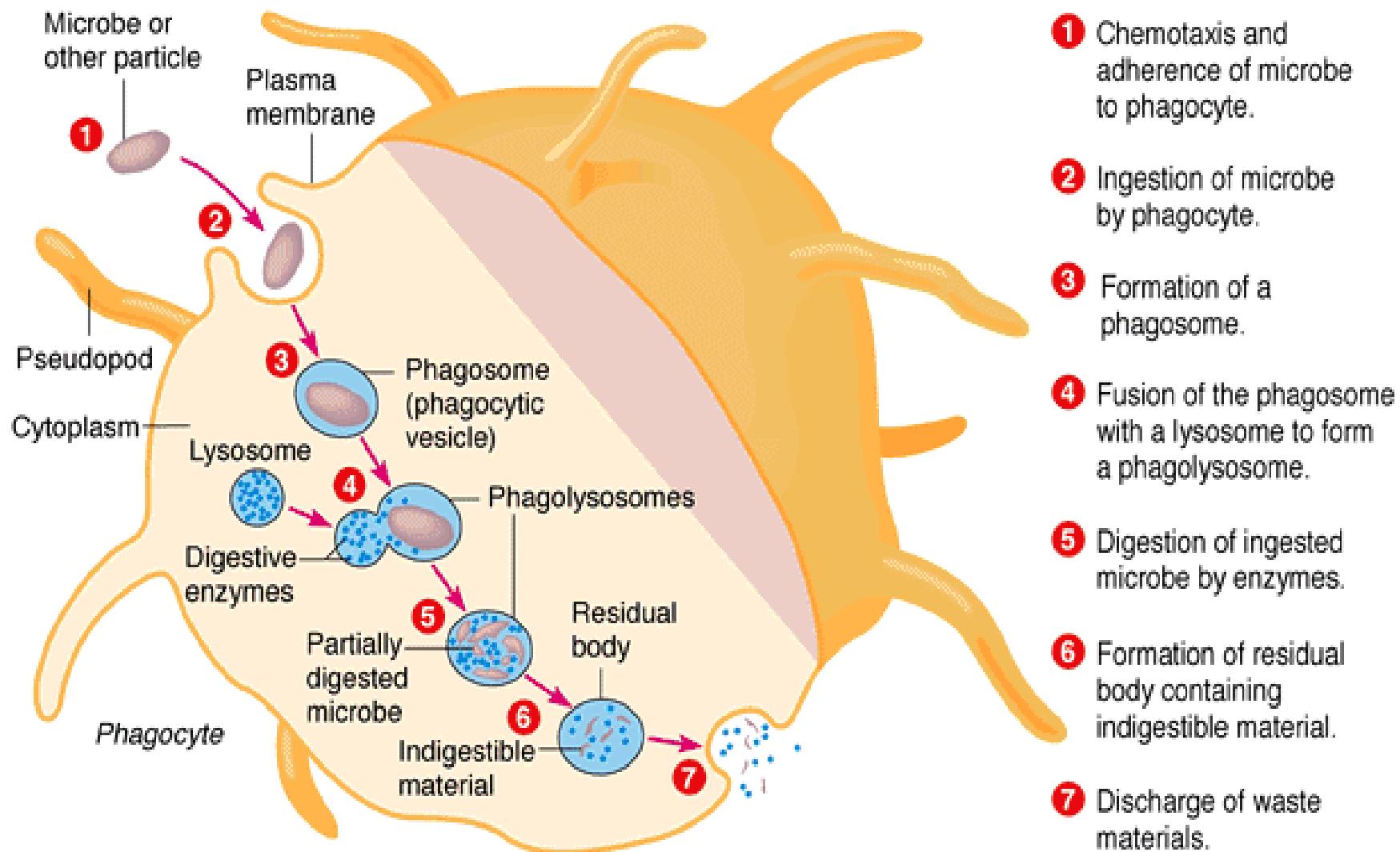
- Lysozyme: lysis cell wall
- Cathepsin and defensins: hydrolyze membrane
- Lactoferrin: binds iron
- lytic enzymes: for protein, lipid, and nucleic acids

b) Oxygen-dependent killing

- **Phagocyte oxidase:** generates oxygen radicals (bind to and damage a variety of microbial products such as membranes, proteins and DNA, leading to killing the pathogens)
- E.g.:
 - O_2^- (superoxide anion)
 - OH^+ (hydroxyl radicals)
 - H_2O_2 (hydrogen peroxide)
 - ClO^- (hypochlorite anion)

c) Reactive nitrogen intermediates (**Nitric oxide synthase**)

- NO (nitric oxide): inhibit the growth of pathogen by binding iron



(a) Phases of phagocytosis

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Process of Phagocytosis

Individual assignment

1. Antigen
2. Factors contributing to the development of autoimmune disease
3. Diagnosis and treatment of autoimmune disease
4. Factors causing acquired immunodeficiency
5. Treatment of acquired immunodeficiency
6. Factors causing innate immunodeficiency
7. Treatment of innate immunodeficiency
8. Vaccination
9. The effects of aging on the innate immune systems
10. The effects of aging on the adaptive immune systems
11. Pattern recognition receptors
12. Regulatory mechanisms within the innate immune system
13. Regulatory mechanisms within the adaptive immune system
14. Immunodiagnosis techniques
15. Major Histocompatibility Complex
16. Functional effects of sex hormones on the immune system
17. The effects of aging on the innate and adaptive immune systems
18. Immunotherapy

Questions

- ❖ Define the following terms
 - Acute inflammation
 - Chronic inflammation
 - Complement
 - When you are sick, your body temperature either increase or decrease. why?

➤ Inflammation:

- ✓ is swelling of localized body parts as result of injury/infection of cells/tissues



- ✓ **This is happened in order to:**

- kill the pathogens and prevent the spread of agents
- dispose of cell debris and pathogens
- repair and replace tissue damaged by pathogen and its products

Inflammation

a) Acute inflammation (short duration)

- ✓ eliminating infectious agents **rapidly** (minute to a day)
- ✓ recruitment of **neutrophils** and **complement**
- ✓ **caused by bacterial pathogens, injured tissue**

b) Chronic inflammation (prolonged duration)

- infectious agents that are **not quickly** eliminated (more than a day)
- recruitment of **macrophages**
- activated lymphocytes, especially **T cells**
- **caused** by viral infection, non-degradable pathogen, autoimmune

- **Inflammatory response is characterized by four major signs:**
- ✓ **Redness**/erythema (rubor): engorged capillaries
 - ✓ **Swelling**/edema (tumor): accumulation of fluid (exudates)
 - ✓ **Pain** (dolor): stretching and distortion of tissue as a result of edema
 - ✓ **Fever**/heat (calor): increase tissue temperature due to interleukin-1 production by macrophage

Major events in inflammatory response:

1. Initiation
2. Vasodilation
3. Migration and phagocytosis
4. Tissue repair

1. Initiation

- components of Mos (i. e. LPS)
- peptide release by pathogens
- released immune cells' products by damaged cells
- hypersensitivity reactions
- physical and chemicals agents (uv, acids)

2. Vasodilation:

- ✓ Increase in diameter of blood vessels that result:
 - facilitate influx of phagocytes and fluids into infected area
 - tissue swelling and redness
 - increase in tissue temperature.

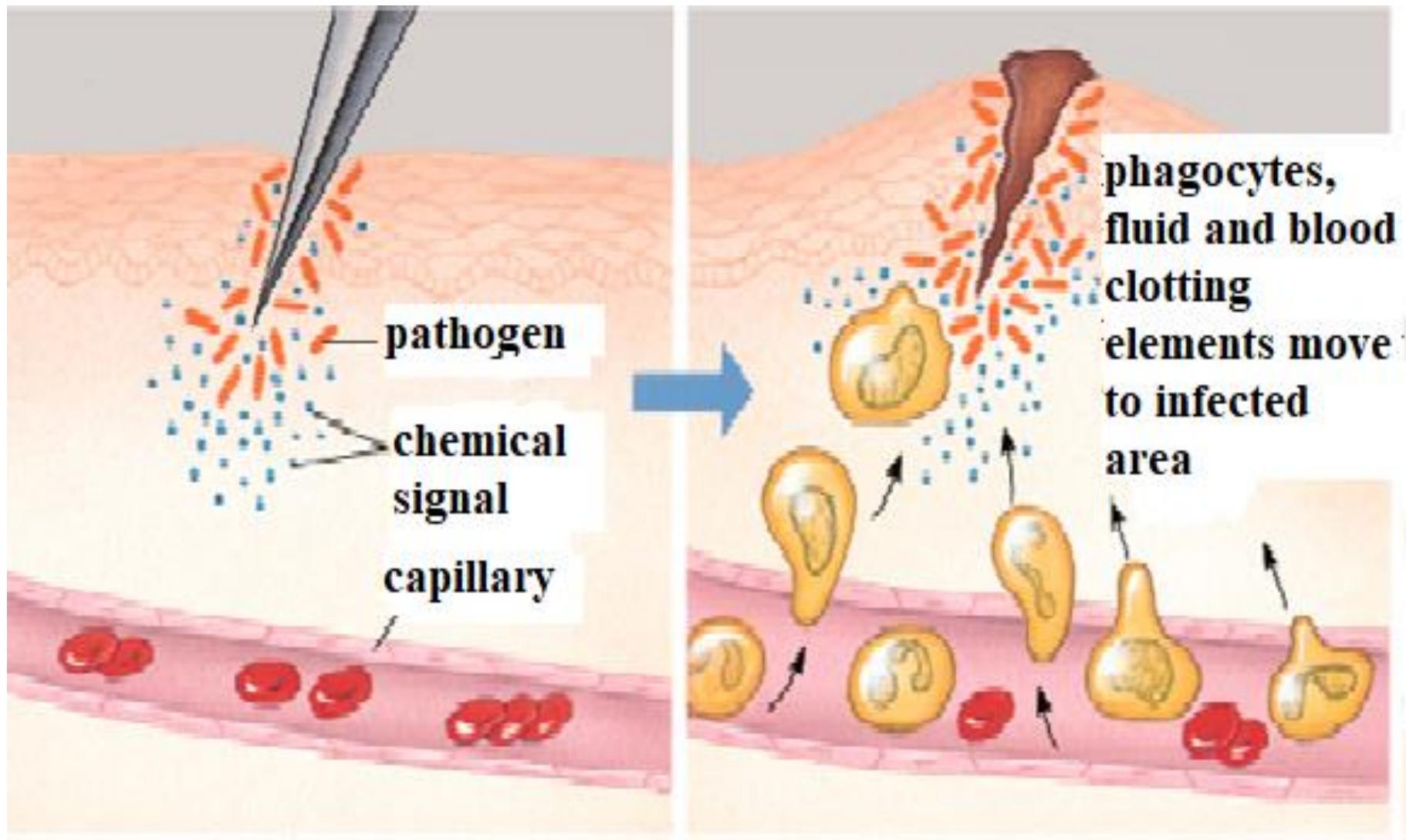
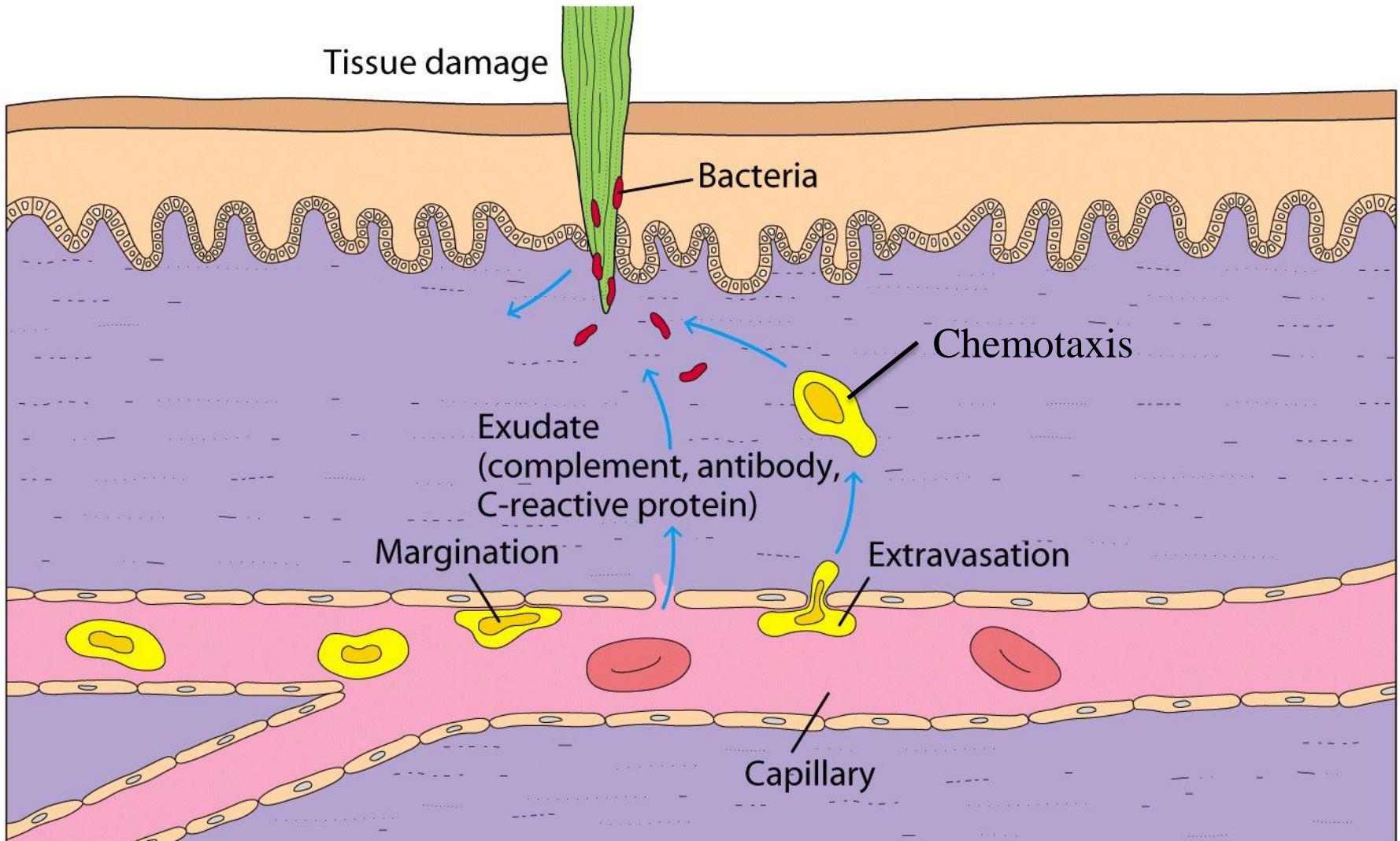


Figure: Vasodilation

➤ Phagocytes migration and phagocytosis

Phases:

- a. **Margination:** phagocytes stick to lining of blood vessels.
- b. **Extravasation (Diapedesis):** phagocytes cross blood vessels and enter surrounding tissue.
- c. **Chemotaxis:** migration of phagocytes from tissue to the site of the infected area.
- d. **Phagocytosis:** engulf and killing foreign materials



➤ **Tissue repair**

- after phagocytosis, tissue repair and regeneration of new tissue begins
- capillaries grow into the fibrin of a blood clot
- fibrin is replaced by fibroblast (wound healing)
- scar tissue forms—accumulation of fibroblasts and capillaries

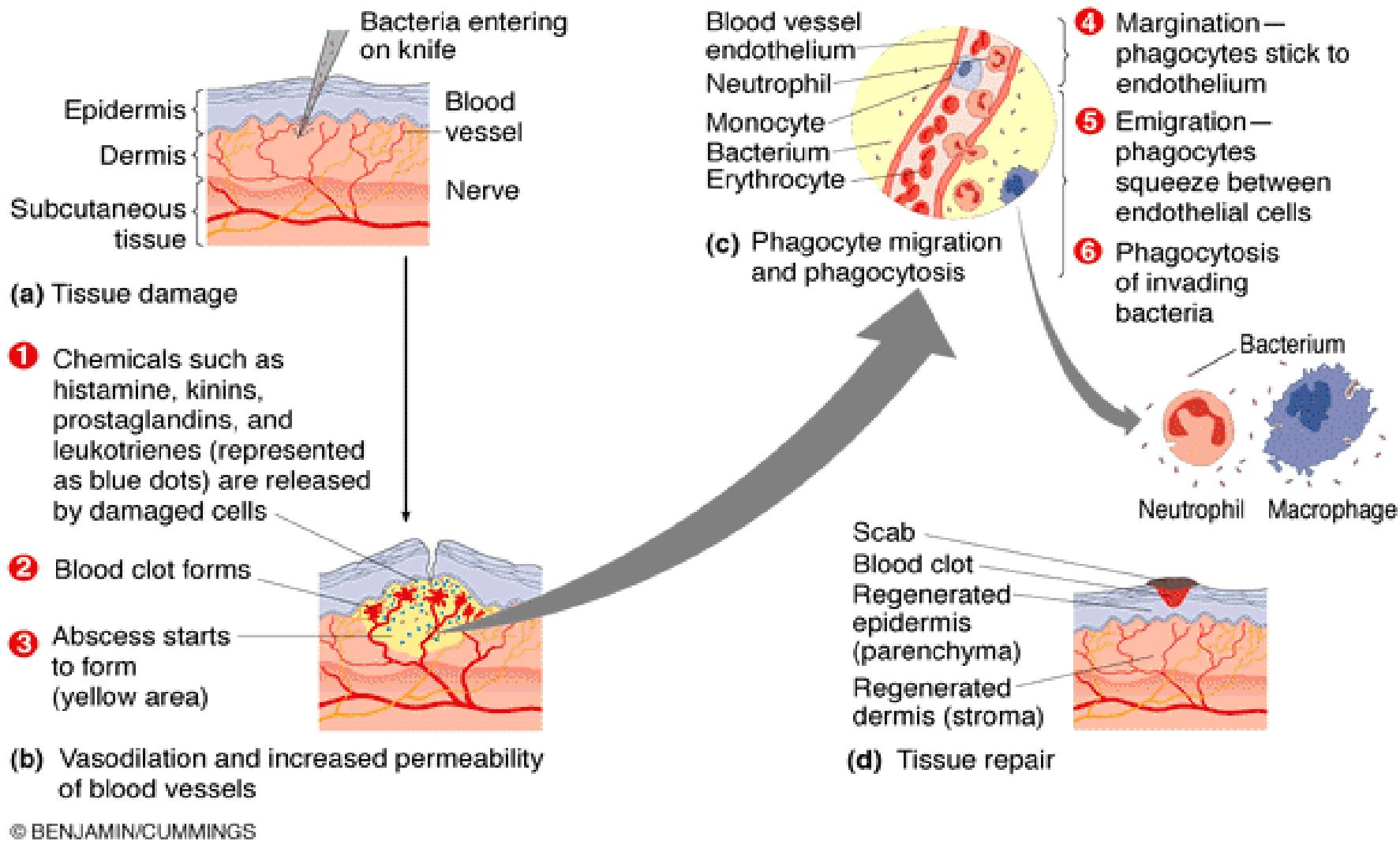


Figure: Process of inflammatory response

Blood clot : clotting factors solidify fibrin at the injury site to prevent spread

Abscess = localized collection of dead cells, body fluids and microbes trapped under the clot

Table: Mediators of inflammation

Mediators	Source	Function
Histamine	Mast cells /basophils	Dilates blood vessels
Kinins (e.g. bradykinin)	Neutrophils, eosinophils	Vasodilatation
Prostaglandins	Neutrophils, eosinophils, platelets	Vasodilatation, increased vascular permeability
Leukotrienes	Neutrophils, mast cells, basophils	Vasodilatation, contraction of smooth muscle, induction of cell adherence and chemotaxis
Chemokines	Macrophages	Attract cells with specific chemokine receptors such as neutrophils and monocytes from the bloodstream.
Blood clotting (Thrombin)	Plasma	Activates enzyme cascade that results in the deposition of insoluble strands of fibrin
Complement (e.g. C3a, C5a)	Plasma	Activate mast cell degranulation
Plasmin enzyme	Plasma	Breakdown fibrin

Complement

❖ Characteristics of complement:

- are group of **serum proteins** (more than 35)
- circulate in the blood in inactive forms as **pro-enzymes (zymogens)**
- become active through **proteolytic cleavage** based
- are synthesized by **liver hepatocytes** (most producer), **blood monocytes**, **tissue macrophages**, and **epithelial cells** of the **gastrointestinal and genitourinary tracts**.
- are heat **sensitive**
- participate in both **innate** and **adaptive** immune system

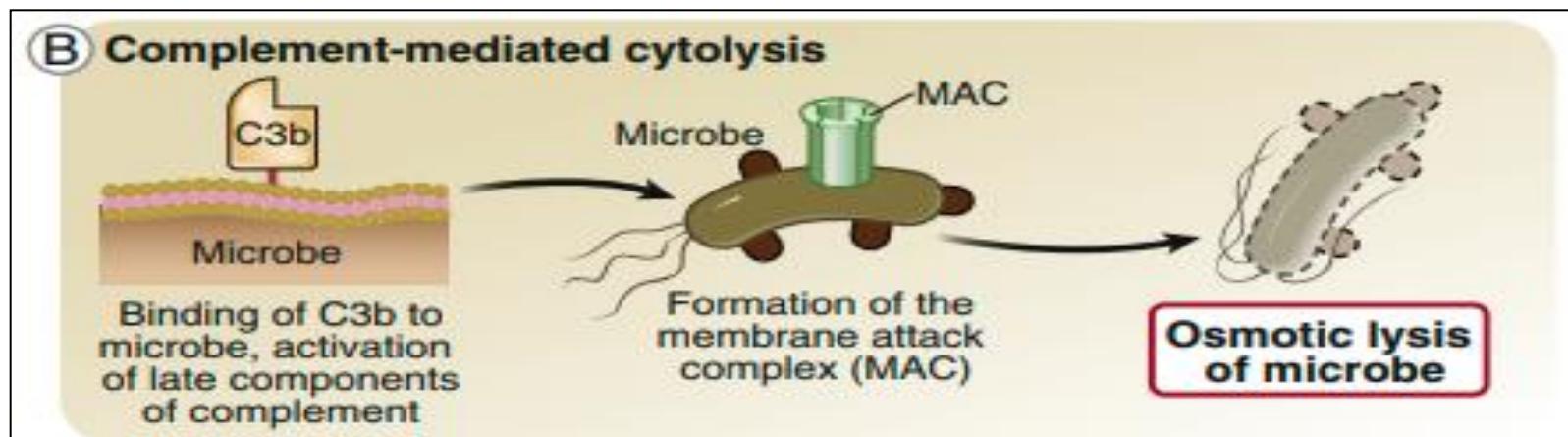
Nomenclature of complement

- Complement components are designated by
 - Numerals (C1–C9)
 - Capital letters (e.g.: factor: B, H, I, D)
 - Small letters: when peptide fragments are produced by activation of complements are denoted by small letters (e. g. C3a, C3b)
 - “**a**” denote smaller fragment and released into microenvironments
 - “**b**” the larger fragment and attached to membrane except C2a
 - the **number** indicates the order of discover

Function (biological effects) of complement

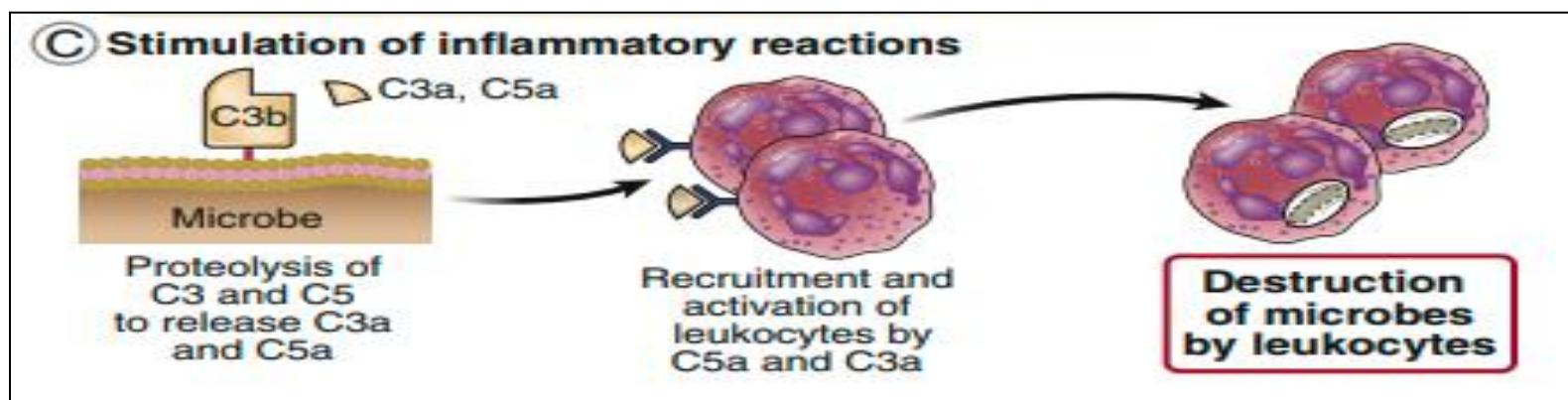
A) Cytolysis [C5b6789 = membrane attack complex]

- Destruction of target cells by lysis of the **cell membrane** such as gram-negative bacteria, parasites, viruses, erythrocytes, and nucleated cells.
 - cytotoxicity --- **lysis** nucleated cells
 - hemolysis --- **lysis** red blood cells
 - bacteriolysis --- **lysis** bacteria



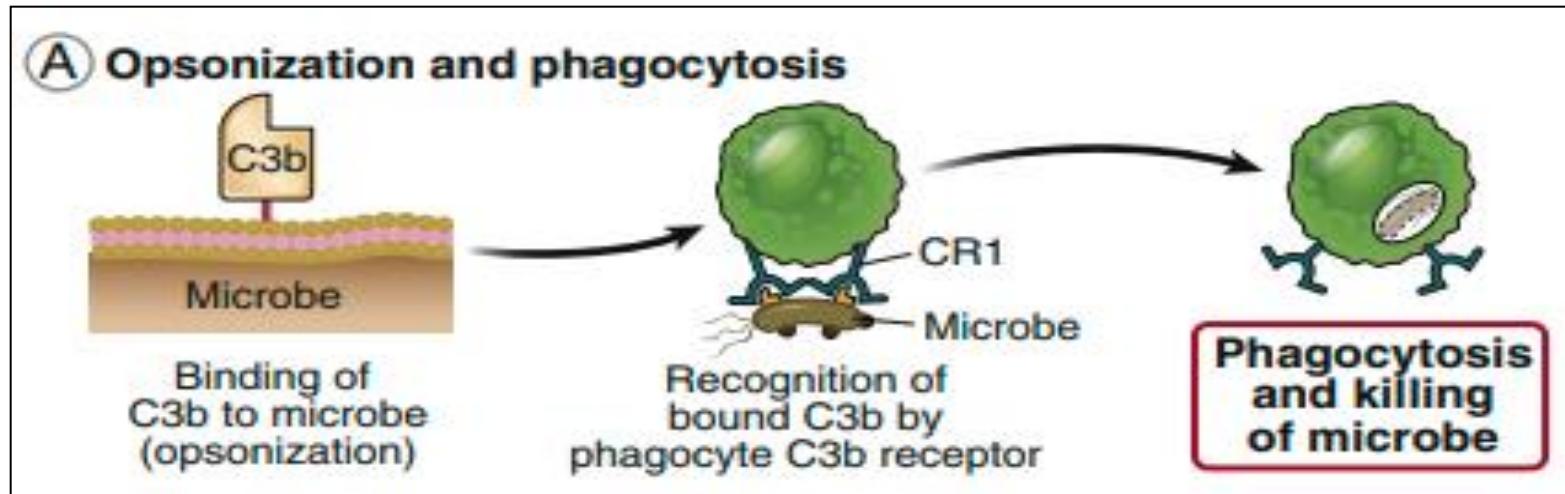
B) Inflammatory response

- ✓ Extravasation and chemotaxis of leukocytes at inflammatory site C3a, C5a, C5b67
- ✓ Degranulation of mast cells and basophils (C3a,C4a, and C5a (anaphylatoxins) and eosinophils (C3a, C5a)



C) Opsonization [C3b, C4b]

- Facilitation of phagocytosis by macrophages or neutrophils



D) Chemotaxis [C5a, C5b67]

- Attract neutrophils to a local site of inflammation.

E) Viral neutralization (C3b, C5b–9 (MAC))

F) Immune complex clearance: remove circulating immune complexes (Ab –Ag complex) from circulating and deposits them in spleen and liver (C3b)

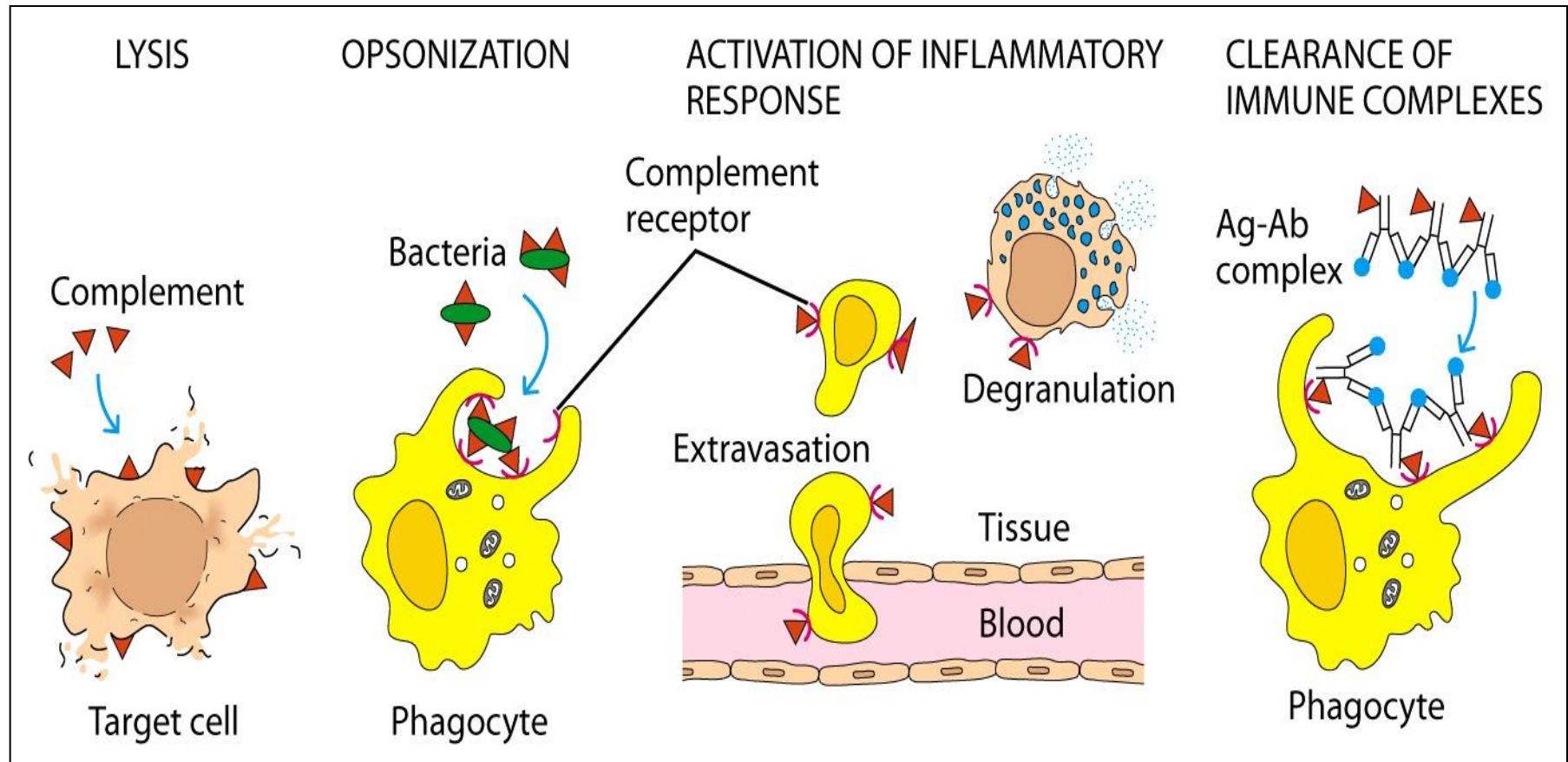


Figure: Summary of the multiple activities of the complement system

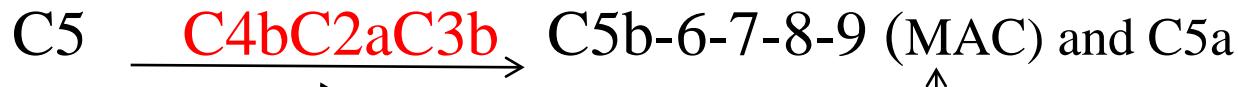
Functional protein classes in the complement system	
Binding to antigen:antibody complexes and pathogen surfaces	C1q
Binding to mannose on bacteria	MBL
Activating enzymes	C1r C1s C2b Bb D MASP-1 MASP-2
Membrane-binding proteins and opsonins	C4b C3b
Peptide mediators of inflammation	C5a C3a C4a
Functional protein classes in the complement system	
Membrane-attack proteins	C5b C6 C7 C8 C9
Complement receptors	CR1 CR2 CR3 CR4 C1qR
Complement-regulatory proteins	C1INH C4bp CR1 MCP DAF H I P CD59

Complement Activation

- Three major pathways for complement activation
 - 1. Classical:
 - Ab dependent activation
 - 2. Lectin
 - mannose dependent activation
 - 3. Alternative
 - auto-activation (spontaneous) or pathogen surface component activation

Classical pathway

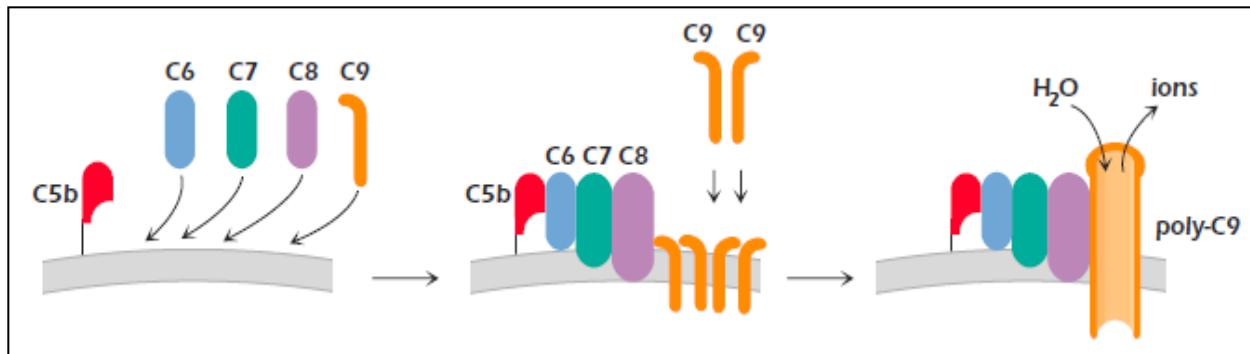
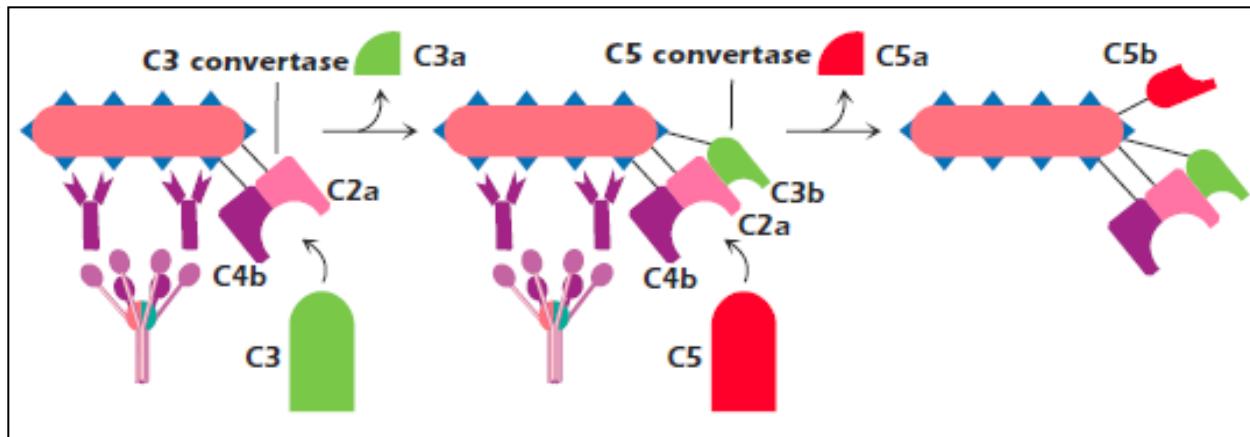
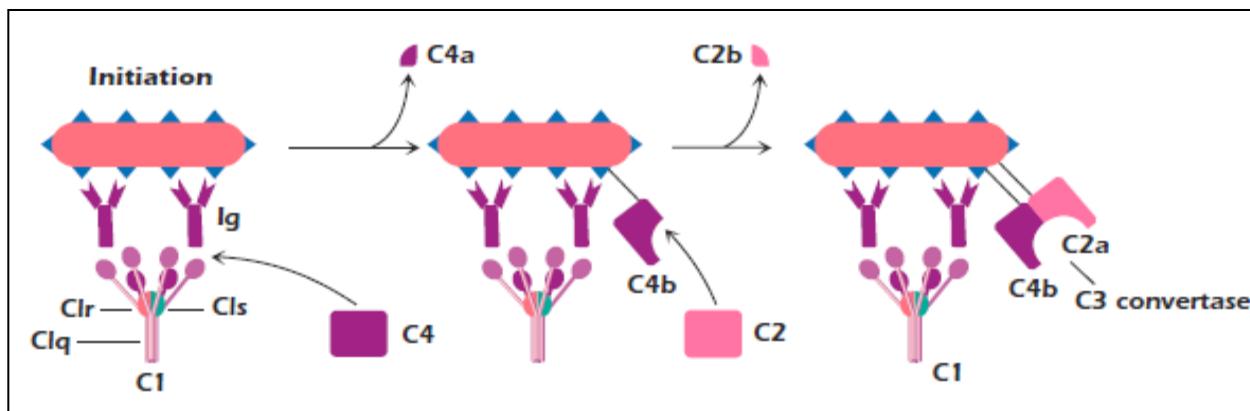
- Antibody (AB) (IgG or IgM)
- Antigen (AG)
- AB + AG \longleftrightarrow
 - C1q binds AB + AG complex = activates C1r
 - C1r activates C1s



C5 convertase

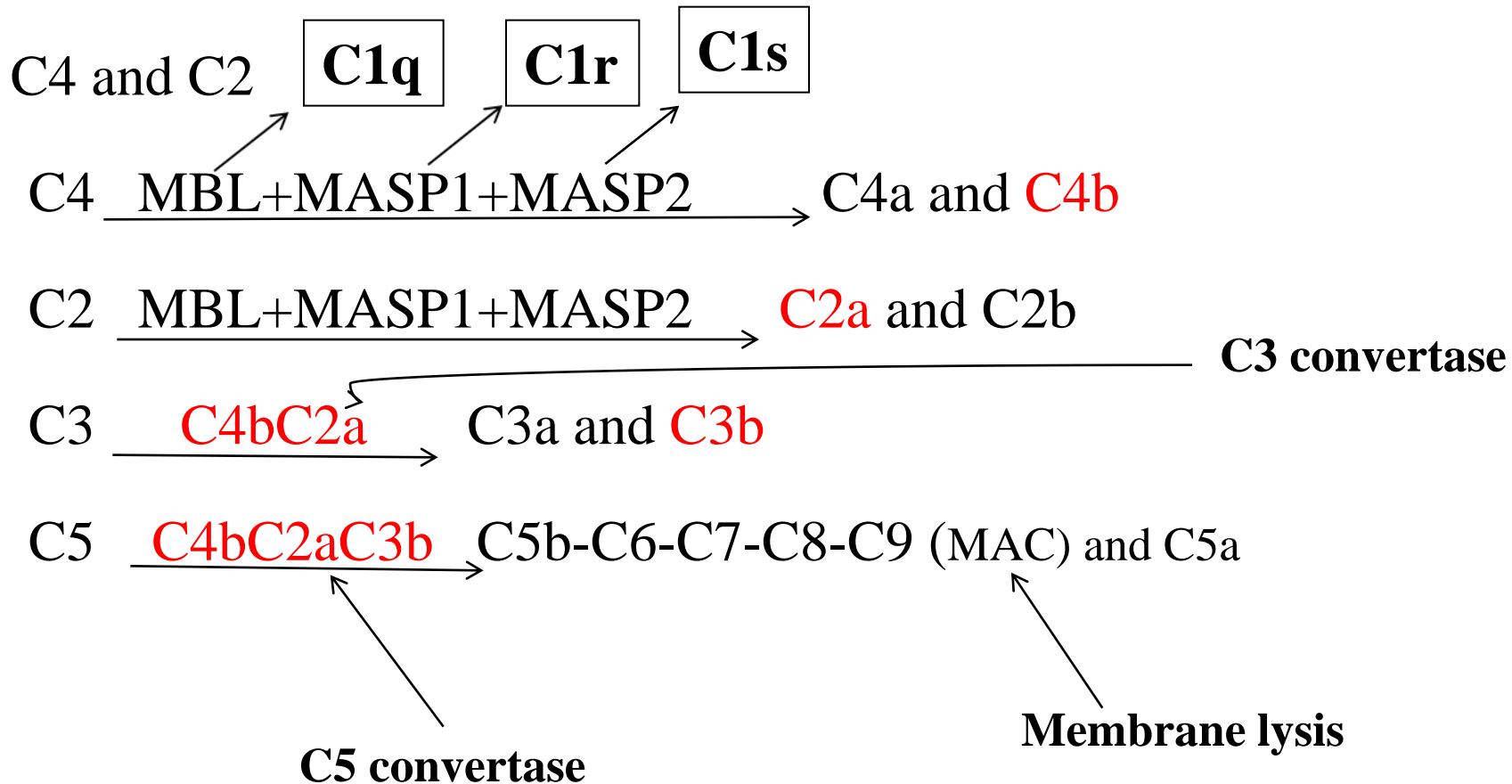
C3 convertase

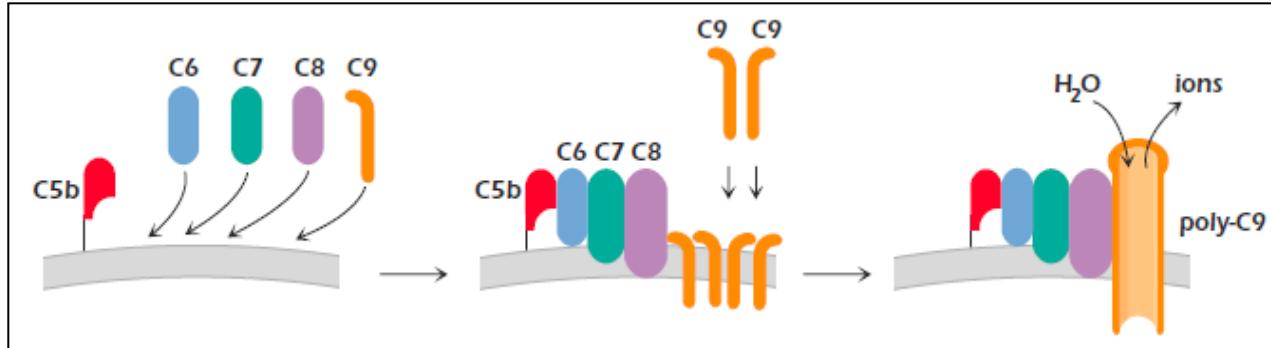
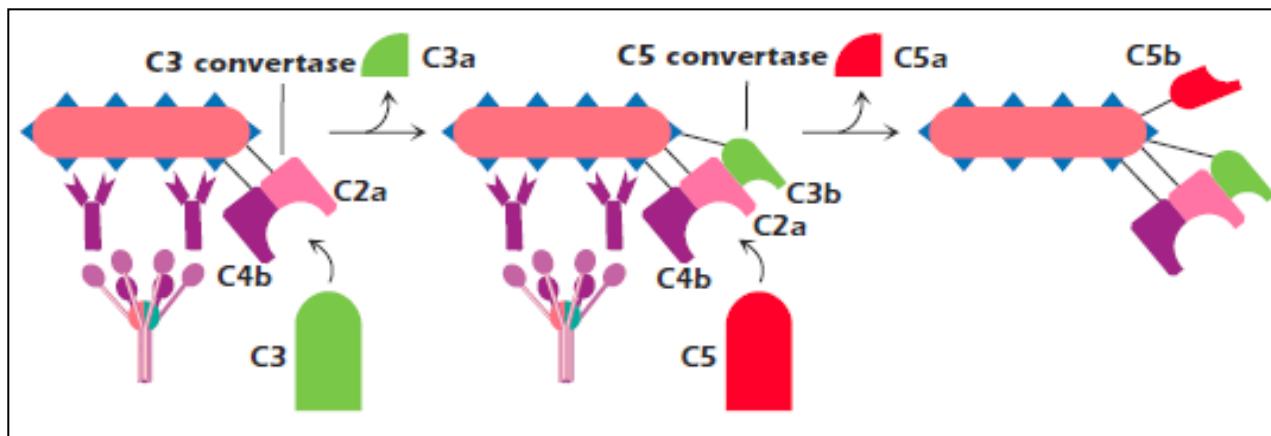
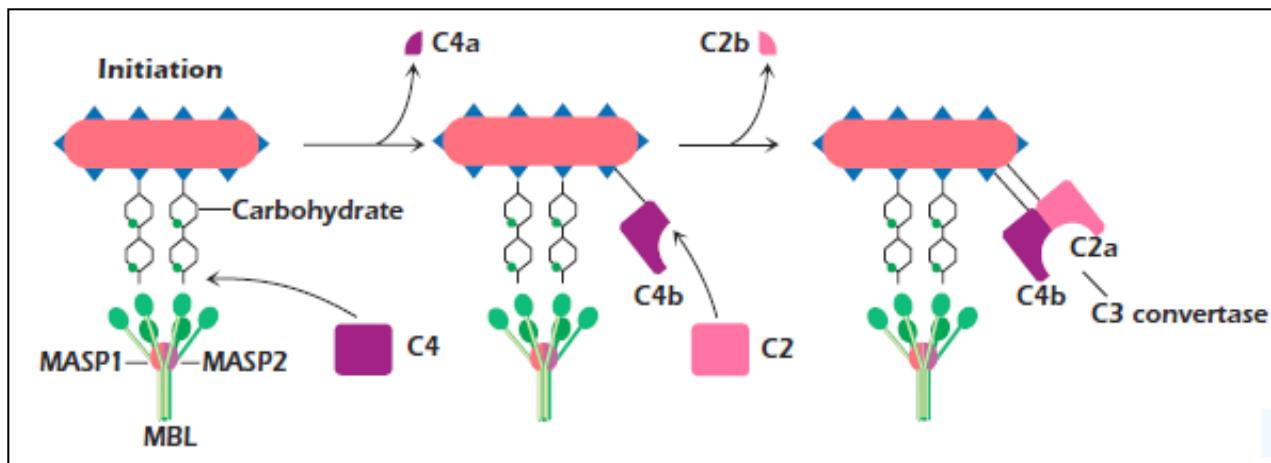
Lysis cell membrane



Lectin pathway

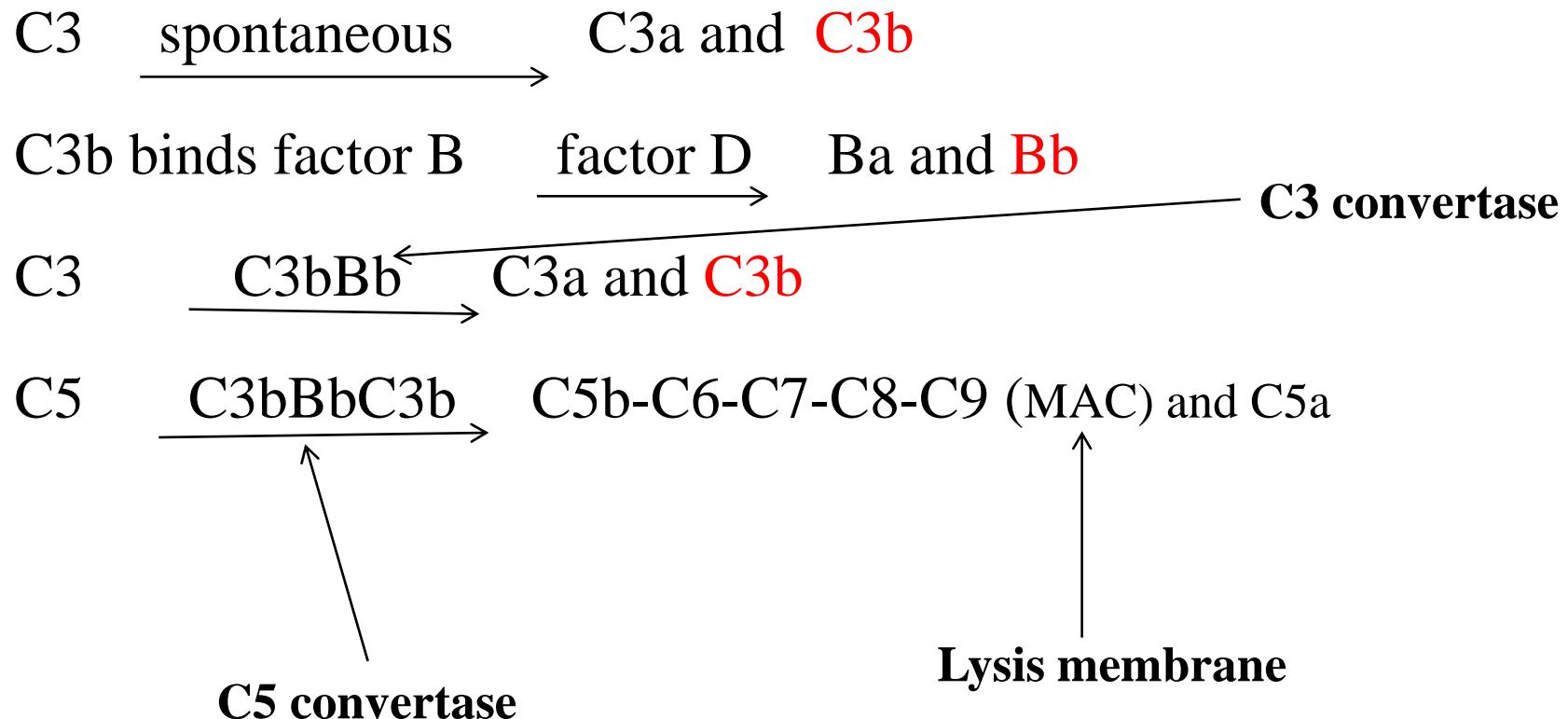
- **MBL** Binds to mannose residues on the surface of Mos
- MASP-1 and MASP-2 attach to MBL which lead to activation of

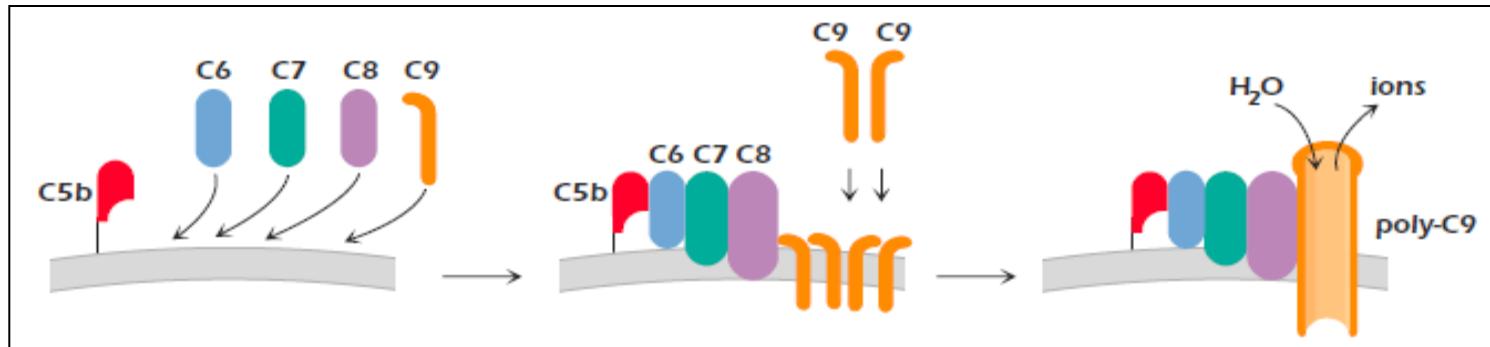
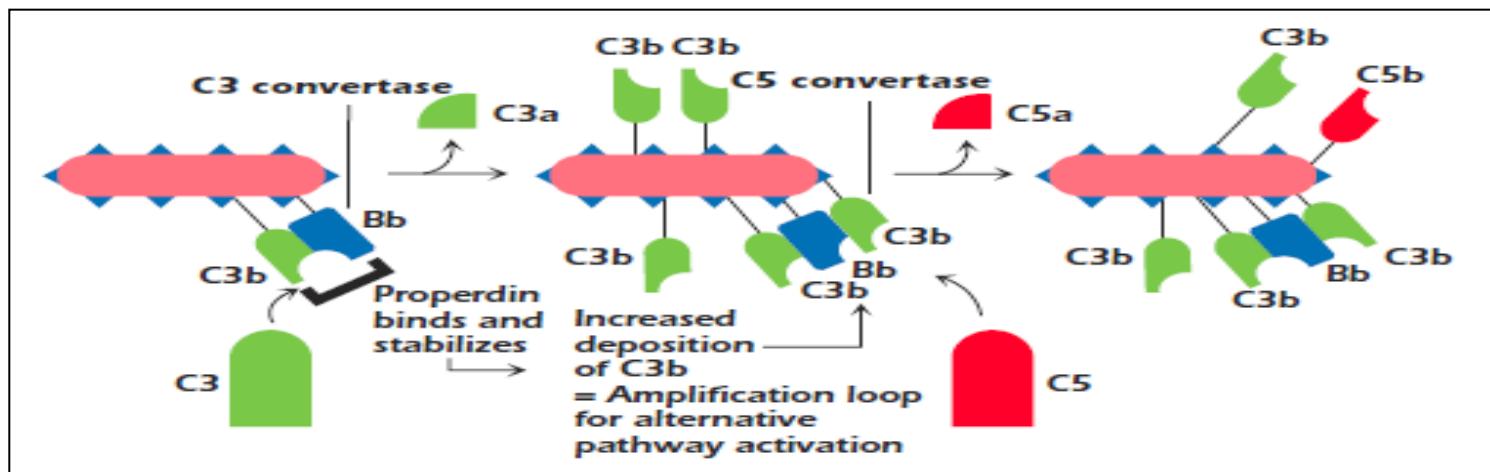
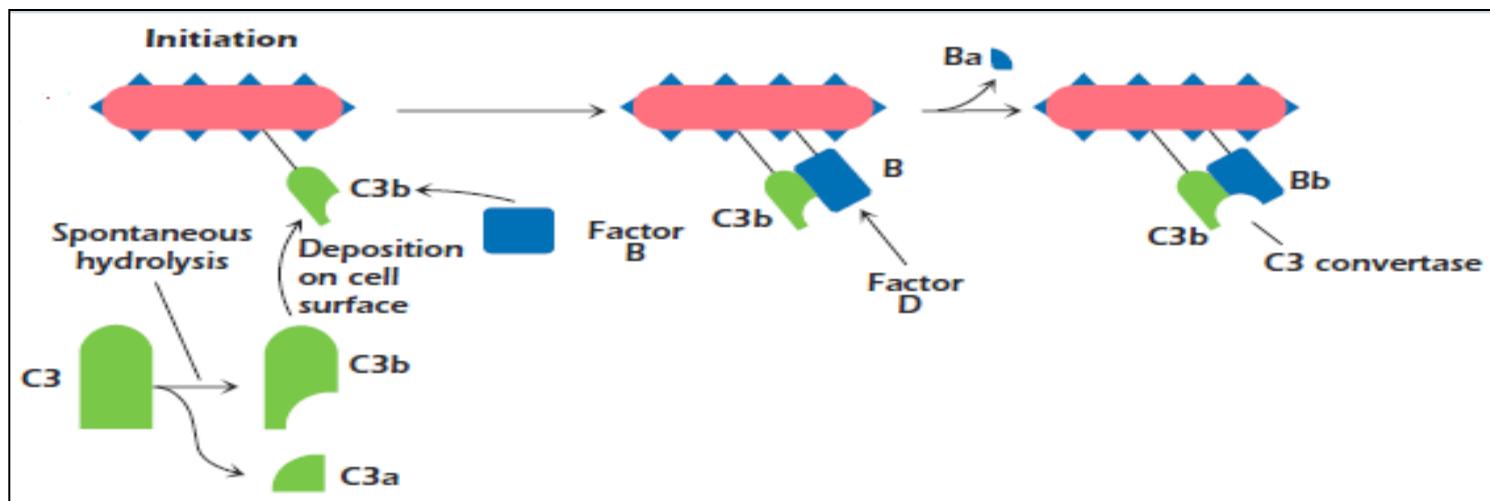




Alternative pathway

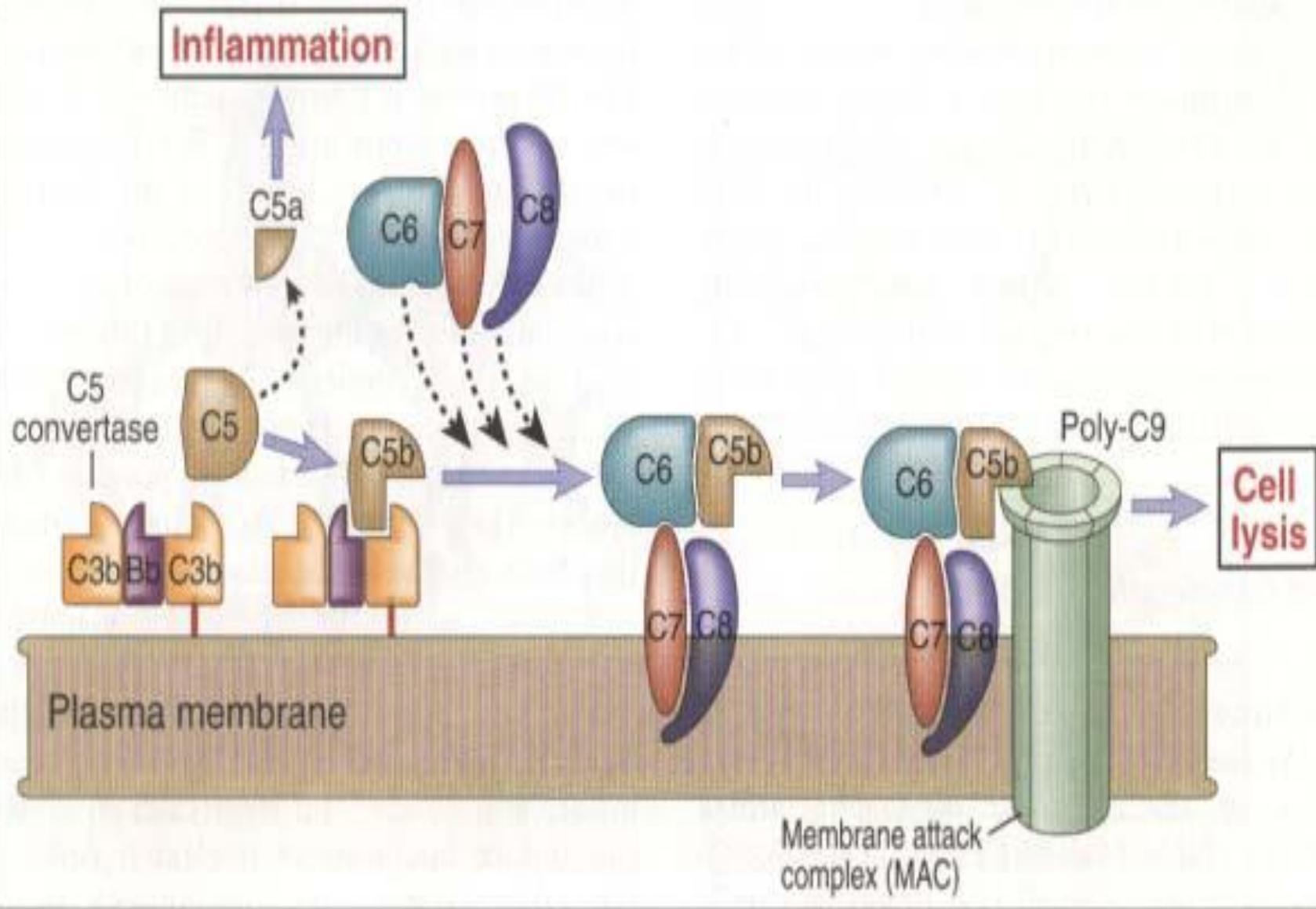
- Activated by microbial cell surface component or spontaneously





Membrane attack complex (MAC)

- Terminal sequence of complement activation
 - Involves C5b, C6,C7,C8, and C9 interact sequentially to form a macromolecular structure, MAC
- Mechanism for membrane damage:
- channel formation---loss of nutrients



Late steps of complement activation and formation of the MAC

Table. Similarity and difference among complement activation pathway

character	classical	lectin	alternative
activation	AB-AG complex	mannose	Surface or spontaneous
Enzyme	C1r and C1s	MASP-1 and 2	Factor D
Starting complement	C4 and C2	C4 and C2	C3 and factor B
C3 convertase	C4bC2a	C4bC2a	C3bBb
C5 convertase	C4bC2aC3b	C4bC2aC3b	C3bBbC3b
MAC	5b6789	5b6789	5b6789

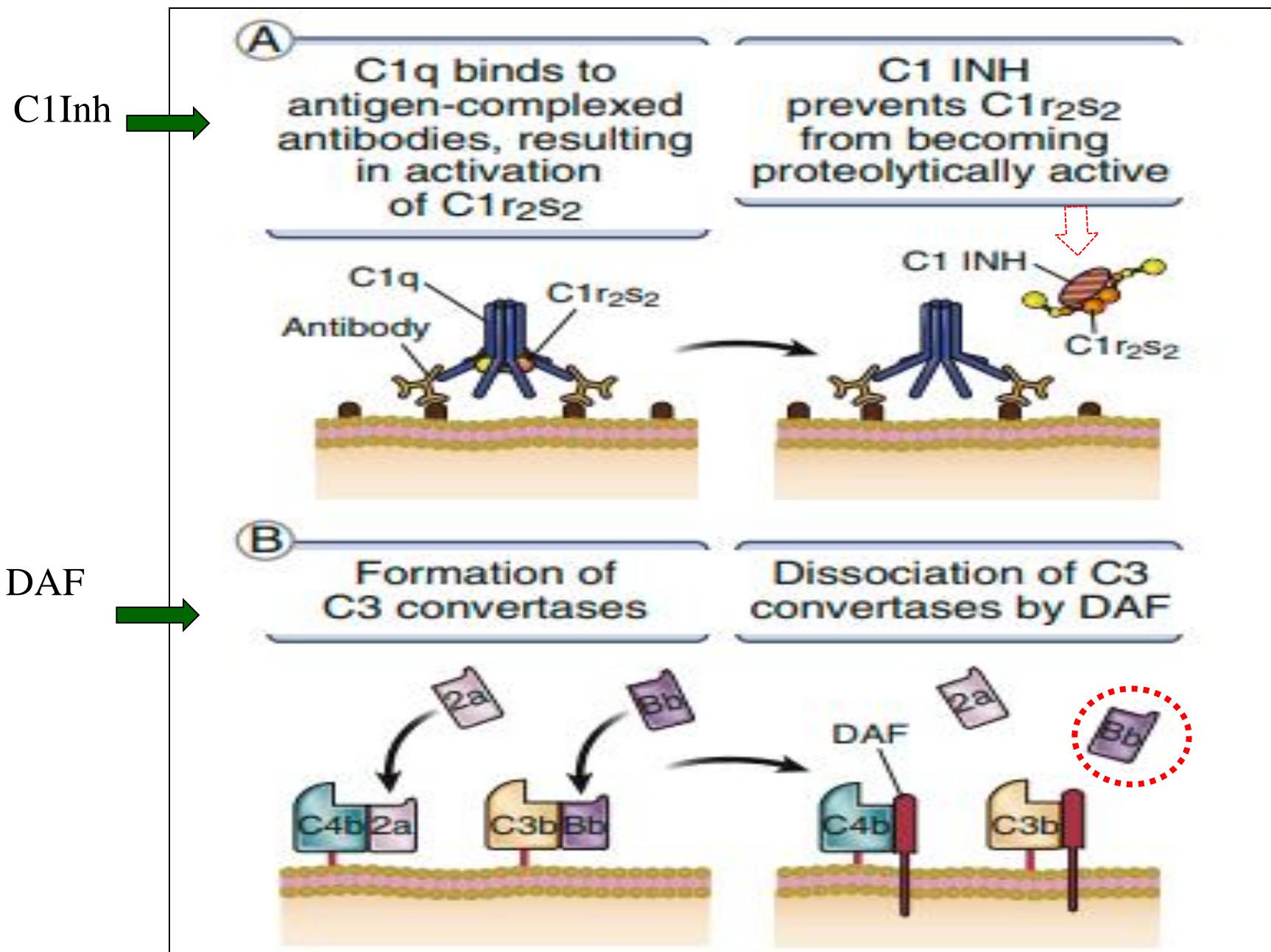
Regulation of the Complement System

Why need regulation?

- To prevent host cell damage by complement activation
- Because many elements of the complement system are capable of attacking host cells as well as foreign materials.
- For example: unregulated production of
 - ✓ C2b results in edema,
 - ✓ C3a/C4a – anaphylaxis,
 - ✓ C3b/C4b – opsonin (activate phagocytic cell).
- Regulatory mechanisms have evolved to restrict complement activity to designated targets.

Table: Proteins that regulate the complement system`

Inhibitors	Pathway	Function
C1 inhibitor (C1Inh)	Classical	Inhibit serine protease (C1r, C1s), dissociate them from C1q
C4b-binding protein (C4bBP)	Classical and lectin	Blocks formation of C3 convertase by binding C4b, displace C2a
Factor H	Alternative	Blocks formation of C3 convertase by binding C3b
Membrane cofactor protein (MCP)	Classical, lectin, alternative	Block formation of C3 convertase by binding C4b or C3b
Decay accelerating Factor (DAF) or CD55	Classical, lectin, alternative	Accelerates dissociation of C2a from C4b2a and Bb from C3bBb
Factor-I	Classical, lectin, alternative	cleaves C4b or C3b using C4bBP, CR1, factor H, DAF, or MCP as cofactor
S protein	Terminal	Prevents C5b678 insertion into cell membrane
CD59	Terminal	Blocking the binding of C5b678 to C9,
Anaphylatoxin inactivator	Effector	Inhibits anaphylatoxin activity (C3a, C4a, and C5a)



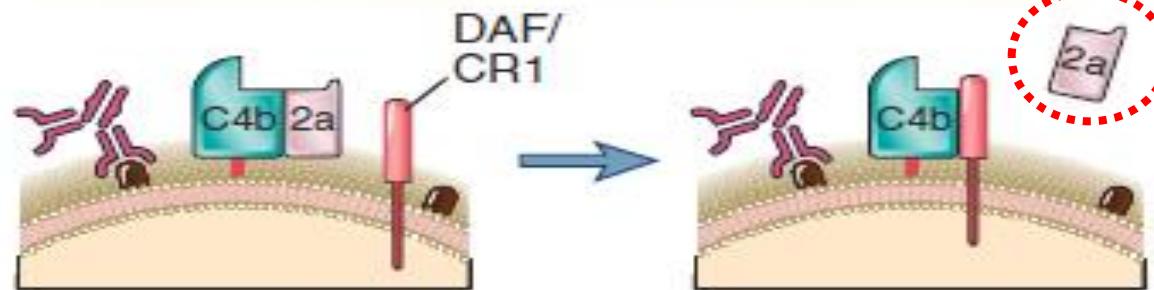
Regulate initiation and C3convertase activity of complement

Formation of C4b2b complex (classical pathway C3 convertase)

DAF, MCP, and CR1 displace C2b from C4b

Classical

A



Formation of C3bBb complex (alternative pathway C3 convertase)

DAF and CR1 displace Bb from C3b

Alternative

B

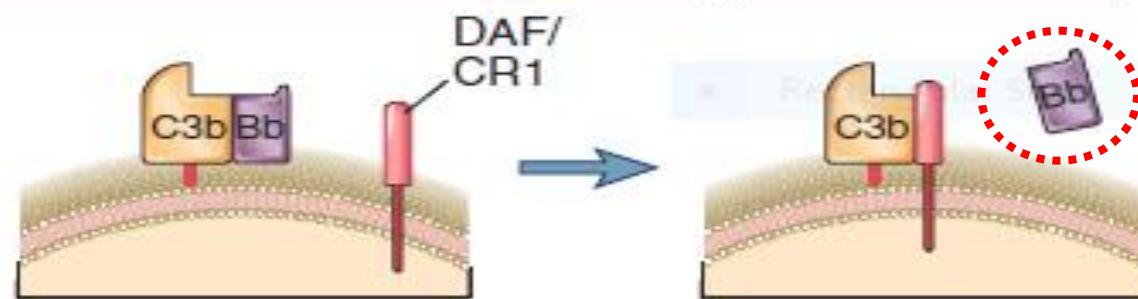
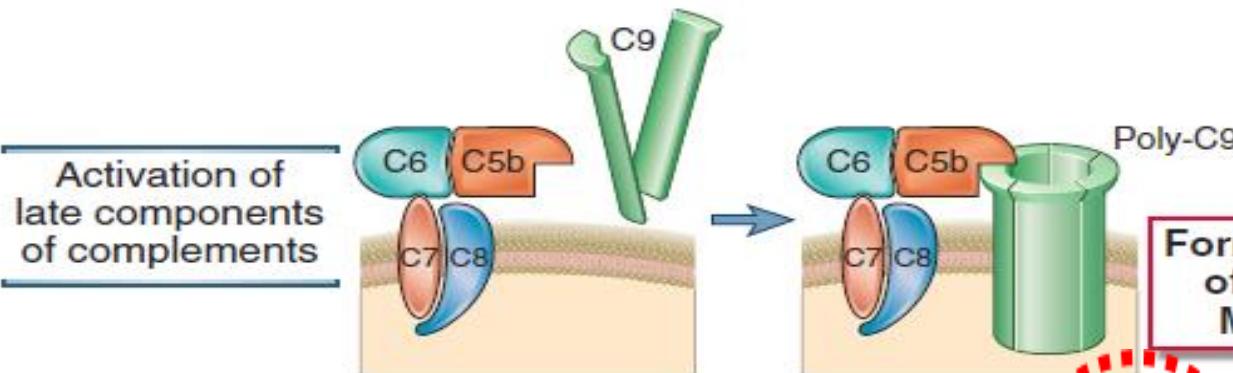
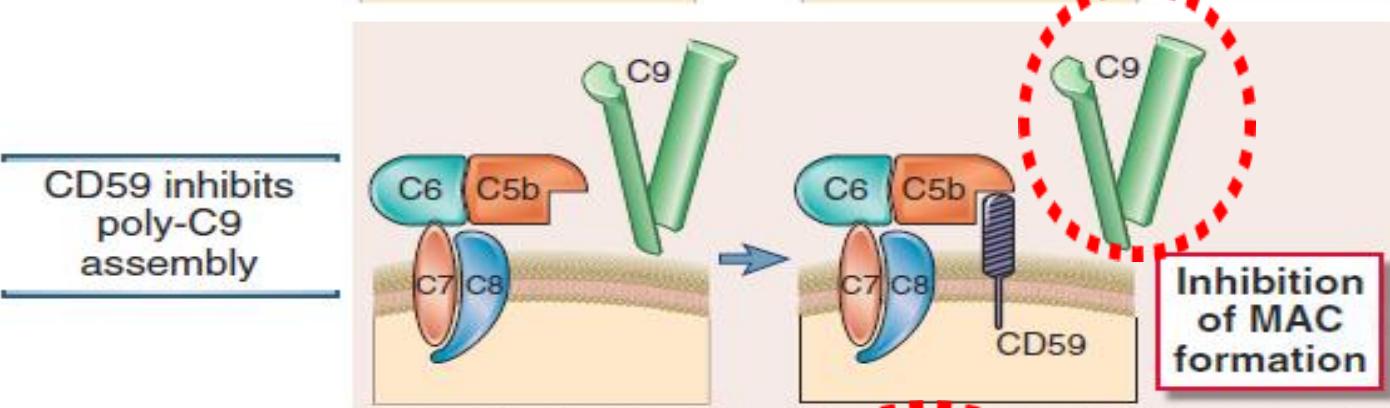


Figure: Inhibition of the formation of C3 convertases

MAC formation



CD59



S protein

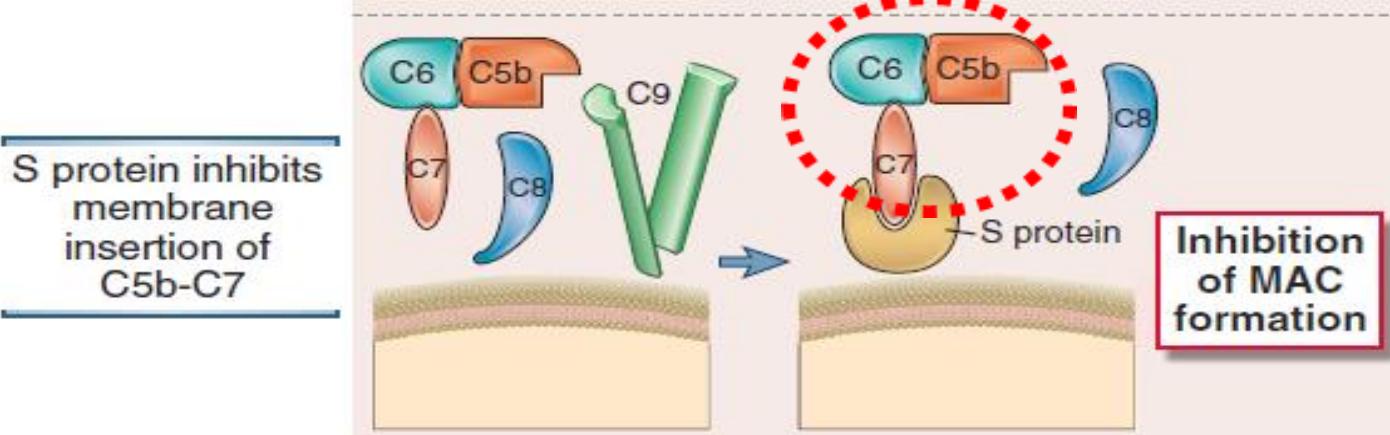


Figure: Regulation of MAC formation

Questions

1. List the basic characters of adaptive immune response
2. Compare and contrast B and T cell receptors

Adaptive immunity

➤ Characteristics of adaptive immunity:

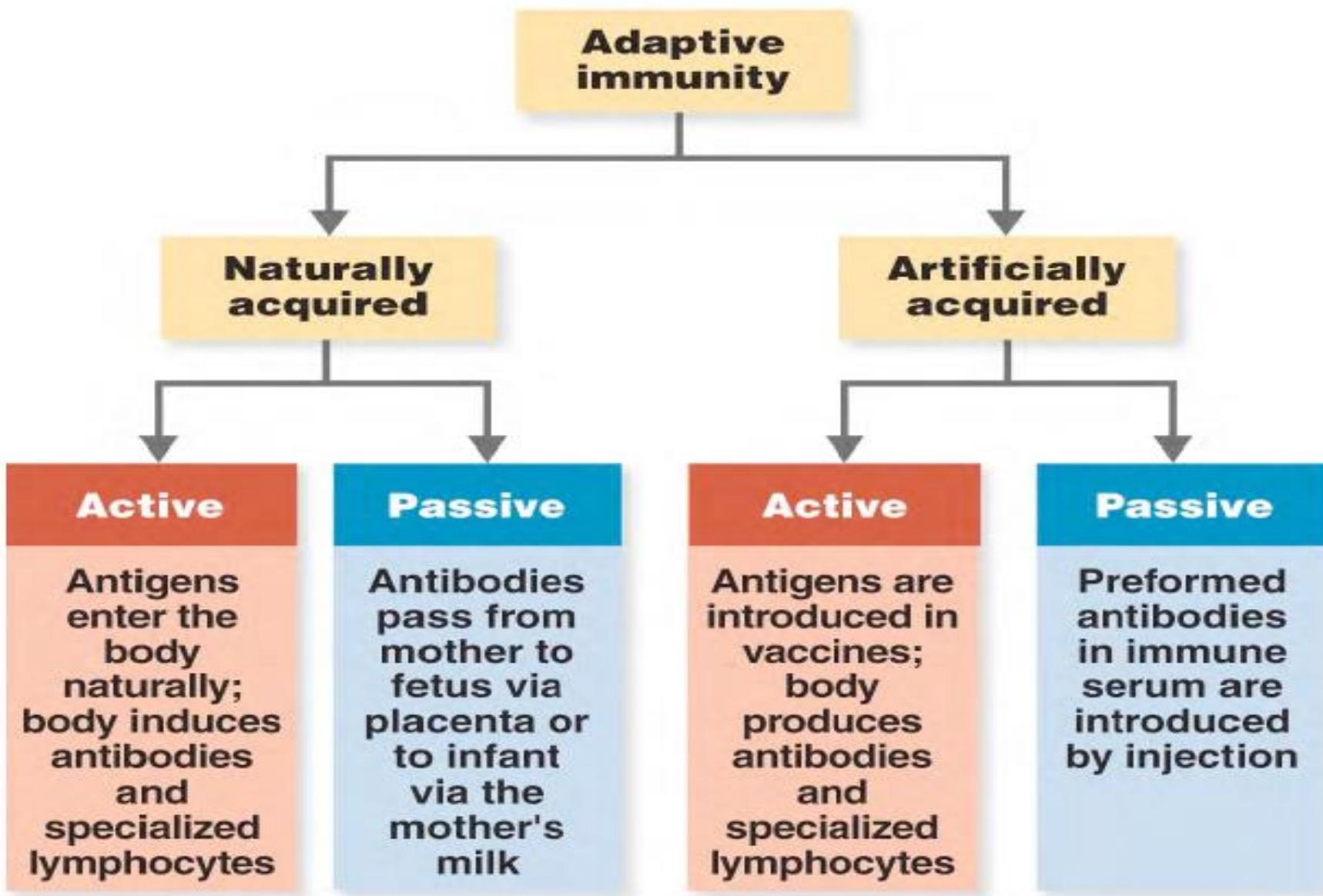
- develop **after birth, acquired**
- **second line** of defense
- is **specific** to the infective agent
- has **memory**
- develops as people are exposed to
 - **infectious agents** or
 - immunized agents (**vaccination**)
- e. g. B cell, T cell

- The cardinal features of adaptive immune responses include:
 - Specificity
 - Diversity
 - Memory
 - Specialization
 - Self-limitation
 - Discrimination

- ***Specificity*** - Insures that distinct antigens elicit **specific responses**
- ***Diversity*** - respond to **a large variety** of antigens
 - The total number of antigenic specificities of the lymphocytes called **Lymphocyte repertoire**
 - It is estimated that the mammalian immune system can discriminate **10^9 to 10^{11}** distinct antigenic determinants
- ***Memory*** – Enhances the ability to respond in a **more rapid**
- ***Specialization*** - Generate responses that are **different** to **different microbes**, maximizing the efficacy of antimicrobial defense mechanisms.

- ***Self-limitations-*** Allows the immune system to return to its resting or basal state so that it will respond to another stimuli (**homeostasis**)
- ***Non-reactivity to self-*** Ability to recognize, respond and eliminate many **non-self antigens** while **not reacting** harmfully to self antigens.
 - This immunological unresponsiveness is referred as ***Tolerance.***
 - Abnormalities in the induction or maintenance of self-tolerance lead to immune responses against self-antigens resulting disease conditions called ***Autoimmune diseases***

Four Types of Adaptive Immunity



Adaptive immune response

➤ General phases

1. **Recognition of antigens:** Develop specific antigen receptor
2. **Activation phase:** two-signal: From pathogens and host immune
3. **Effector phase:** elimination of the antigens
4. **Homeostasis/decay phase**
 - ✓ decline in the immune response
 - ✓ apoptosis (Death of progeny of antigen-stimulated lymphocytes)
5. **Memory development:** The initial activation of lymphocytes generates long-lived memory cells, which may survive for years after the infection and mount rapid responses to a repeat encounter with the antigen.

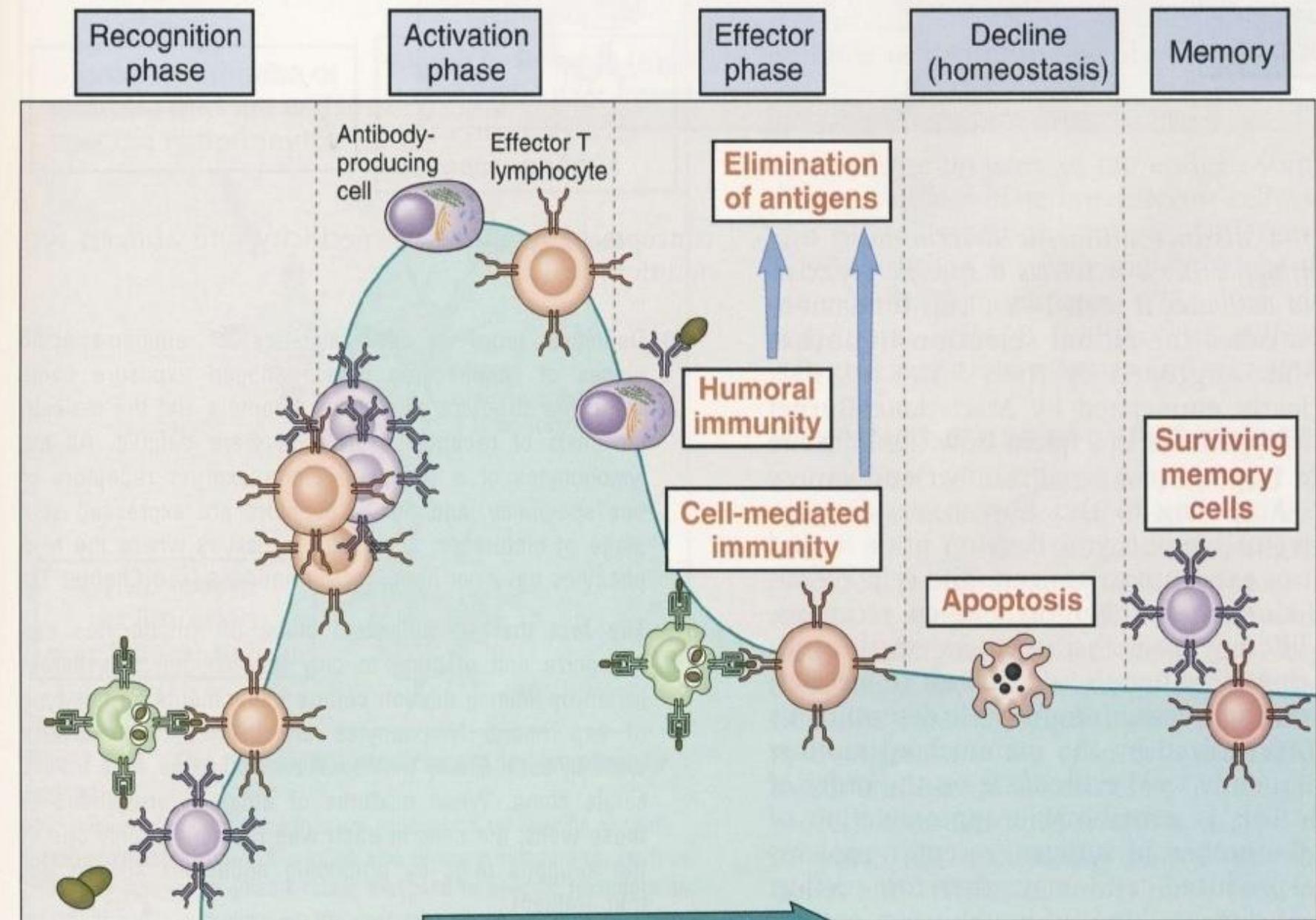


Figure: General phases of adaptive immune response

➤ Requirements for Adaptive immune responses:

- T cells
- B cells
- Antigens
- Antigen-Presenting Cells (APC)
- Major Histocompatibility Complex (MHC)
- Cytokines

- T cells
 - arise from bone marrow and mature in thymus gland
 - Activation, proliferation and differentiation occur in secondary lymphoid organs
- Interaction of T cell receptor (TCR) with an antigenic peptide in MHC complex begins activation of mature peripheral T cell.
- Activation leads to the proliferation and differentiation of T cells into various types of effector cells (CD4 and CD8 T cells) and memory T cells (CD4 and CD8 cells).
 - CD4 exhibiting class II MHC restriction, and
 - CD8 exhibiting class I MHC restriction.

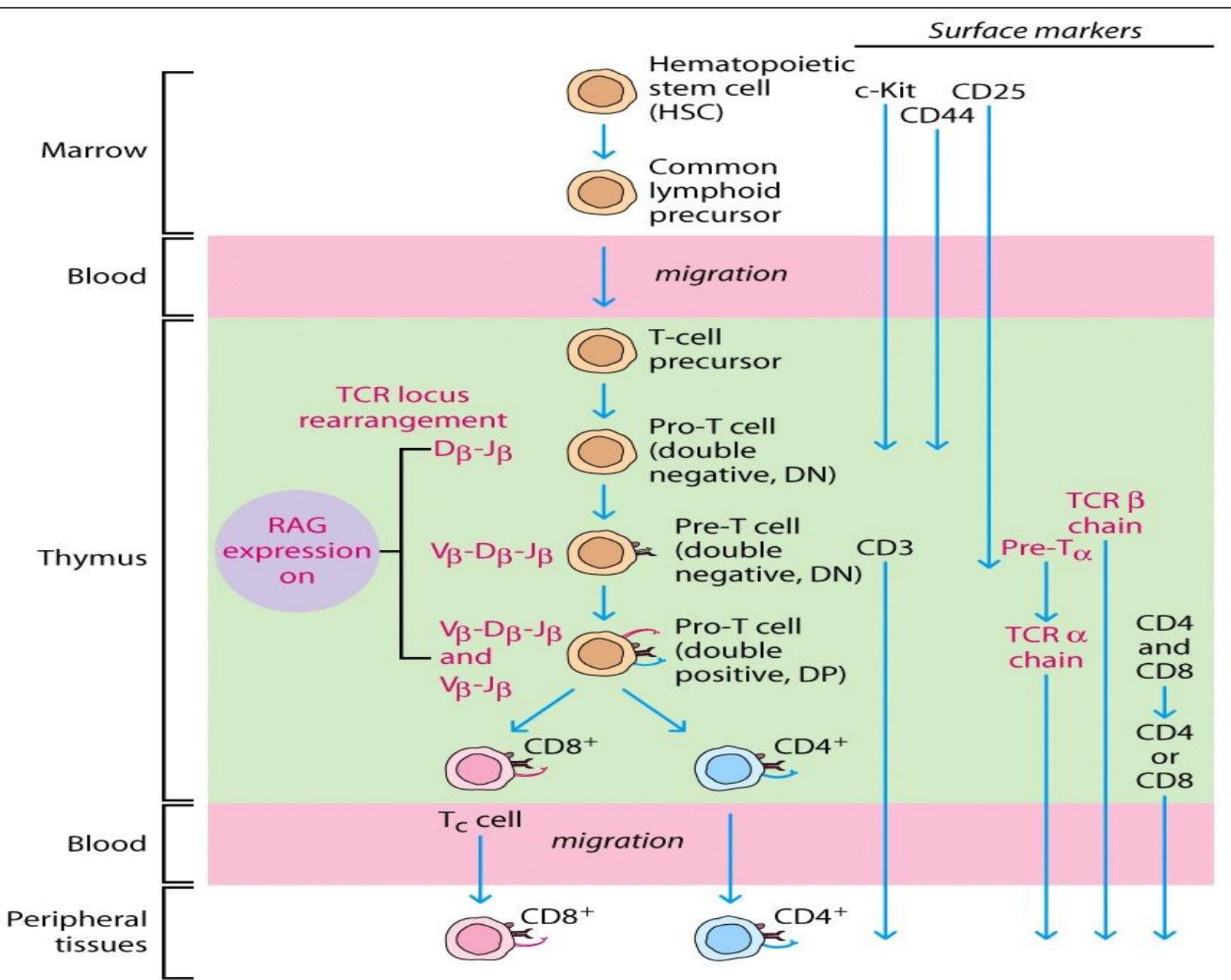
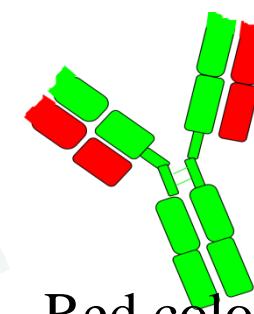
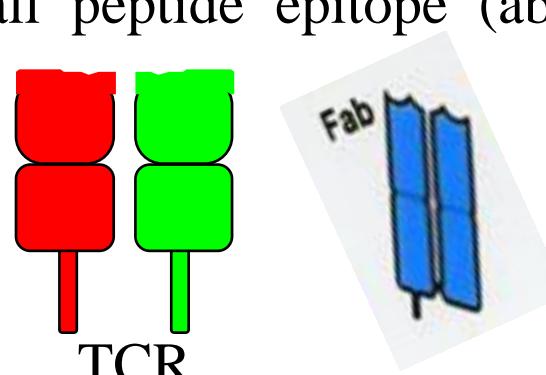


Figure: T cell development and maturation

Structure of the T cell receptor (TCR)

- During its maturation within the thymus, the T cell express a TCR on its cytoplasm membrane.
 - similar in structure to **antibody** (a single F_{ab} fragment).
 - composed of **two** glycoprotein chains (α/β or γ/δ).
 - has a **constant** region and a **variable** region, similar to an antibody light chain.
 - is produced through **genetic recombination**
 - recognizes **one** small peptide epitope (about 8-13 amino acids) displayed on MHC.



Red color = light chain

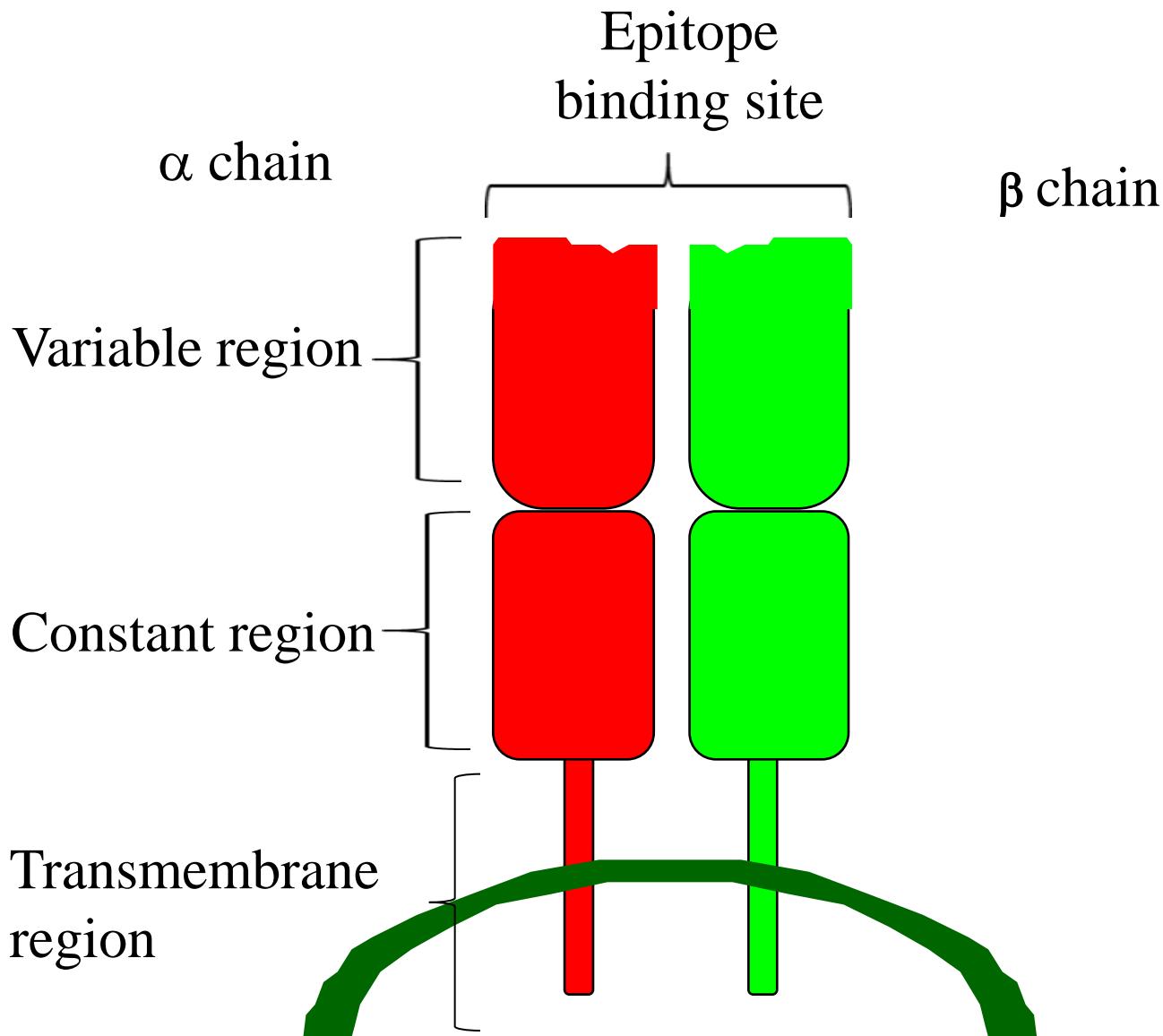
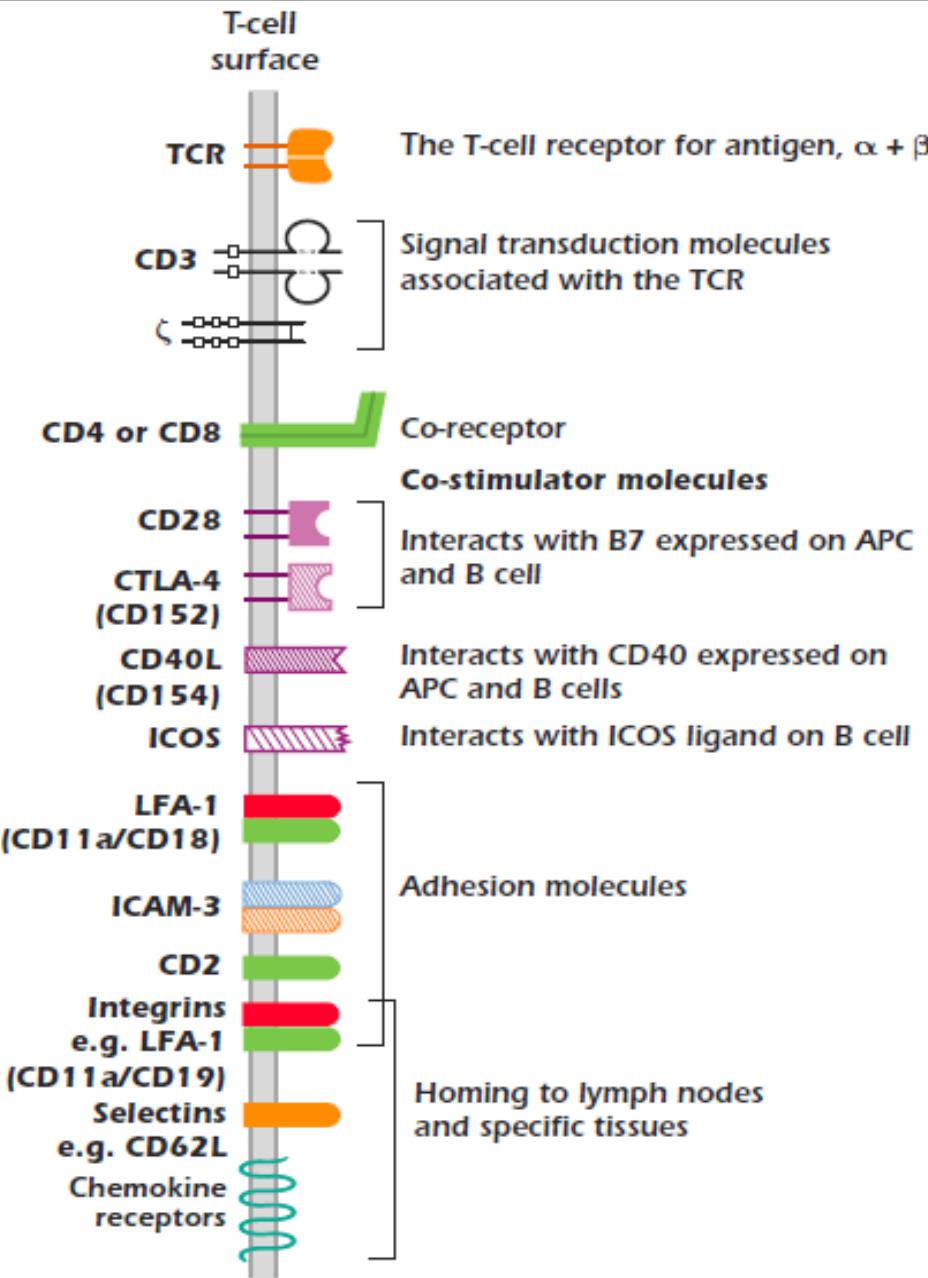


Figure: Structure of the T cell receptor

- Specificity of the T cell receptor (TCR)
 - T cell receptor does **not react** with **free antigen**
 - **Antigen** must be processed by **antigen-presenting cells (APCs)** or **proteasome** into **peptide**
 - TCRs only bind epitopes associated with a **MHC protein**
 - Class I MHC---proteasome
 - class II MHC---APC

- The affinity of T cell binding to antigen helped by
 - ✓ **Co-receptor** (CD4 and CD8)
 - ✓ **Signal transduction** (CD3)
 - ✓ Receptor (**CD28**) for co-stimulatory B7
 - ✓ **Accessory molecules**
 - LFA-1 or CD2: provide **adhesion** at the cell contact site. strengthening the interaction between the T cell and APC
 - CD40 ligand (CD40 L)---**stimulates** APC through binding to CD40, leading to the up-regulation of CD80 (B7-1) and CD86 (B7-2) on APC

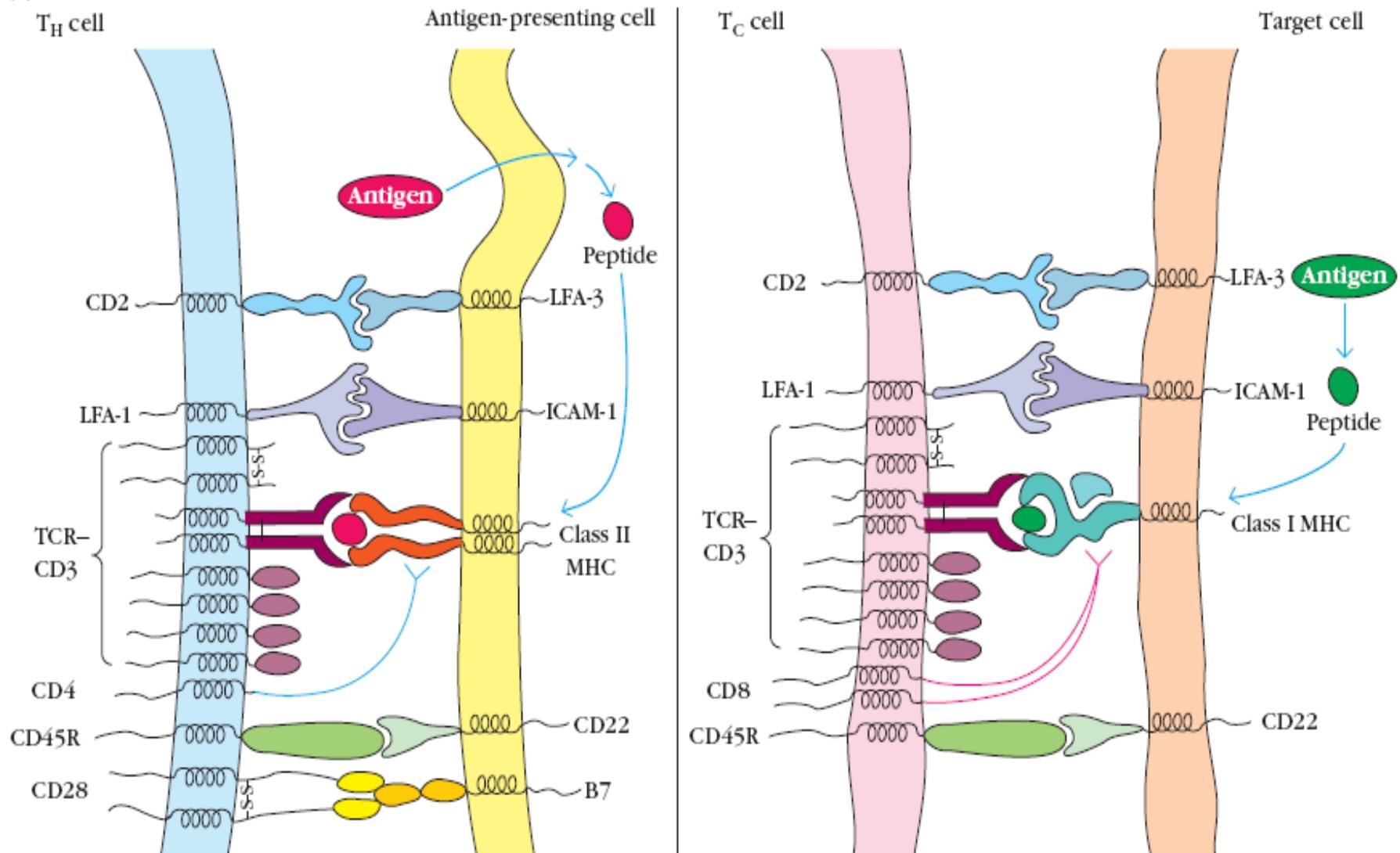


LFA-1: leukocyte function antigen-1
 Intercellular adhesion molecule-1 (ICAM-1)

Table: T-cell surface molecules

Surface molecules	Function
The T-cell receptor complex	
TCR	Antigen-specific receptor (most T cells utilize $\alpha\beta$ dimers; some use $\gamma\delta$ dimers)
CD3 (γ , δ , ϵ , and ζ chains)	Signaling complex associated with the TCR: mediates T-cell activation on binding of TCR to MHC-peptide complexes
Subset markers	
CD4 (on helper and regulatory T cells)	Binds to MHC class II molecules and restricts Th cells to recognizing only peptides presented on MHC class II
CD8 (on cytotoxic T cells)	Binds to MHC class I molecules and restricts Tc cells to recognizing only peptides presented on MHC class I
Co-stimulatory molecules	
CD28	Binds to CD80/CD86 on B cells and APCs and positively regulates T-cell activation
CTLA4	Binds to CD80/CD86 on B cells and APCs and downregulates T-cell activation (present on regulatory cells)
CD154 (CD40L): on activated Th cells	Binds to CD40 on B cells and APCs: triggers activation of APC and activation and antibody class switching of B cells
Adhesion molecules	
LFA-1	Binds to ICAM-1 and facilitates interactions with other cells, including B cells, APCs, and target cells
CD2 (LFA-2)	Binds to LFA-3 and facilitates interactions with other cells, including B cells, APCs, and target cells
CD45RA (on naive T cells)	Involved in signal transduction
CD45RO (on activated/memory T cells)	Involved in signal transduction

(b)



LFA-1: leukocyte function antigen-1

Intercellular adhesion molecule-1 (ICAM-1)

CD designation*	Function	B cell	T CELL		
			T _H	T _C	NK cell
CD2	Adhesion molecule; signal transduction	–	+	+	+
CD3	Signal-transduction element of T-cell receptor	–	+	+	–
CD4	Adhesion molecule that binds to class II MHC molecules; signal transduction	–	+ (usually)	– (usually)	–
CD5	Unknown	+	+ (subset)	+	–
CD8	Adhesion molecule that binds to class I MHC molecules; signal transduction	–	– (usually)	+	+
CD16 (Fc γ RIII)	Low-affinity receptor for Fc region of IgG	–	–	–	+
CD21 (CR2)	Receptor for complement (C3d) and Epstein-Barr virus	+	–	–	–
CD28	Receptor for co-stimulatory B7 molecule on antigen-presenting cells	–	+	+	–
CD32 (Fc γ RII)	Receptor for Fc region of IgG	+	–	–	–
CD35 (CR1)	Receptor for complement (C3b)	+	–	–	–
CD40	Signal transduction	+	–	–	–
CD45	Signal transduction	+	+	+	+
CD56	Adhesion molecule	–	–	–	+

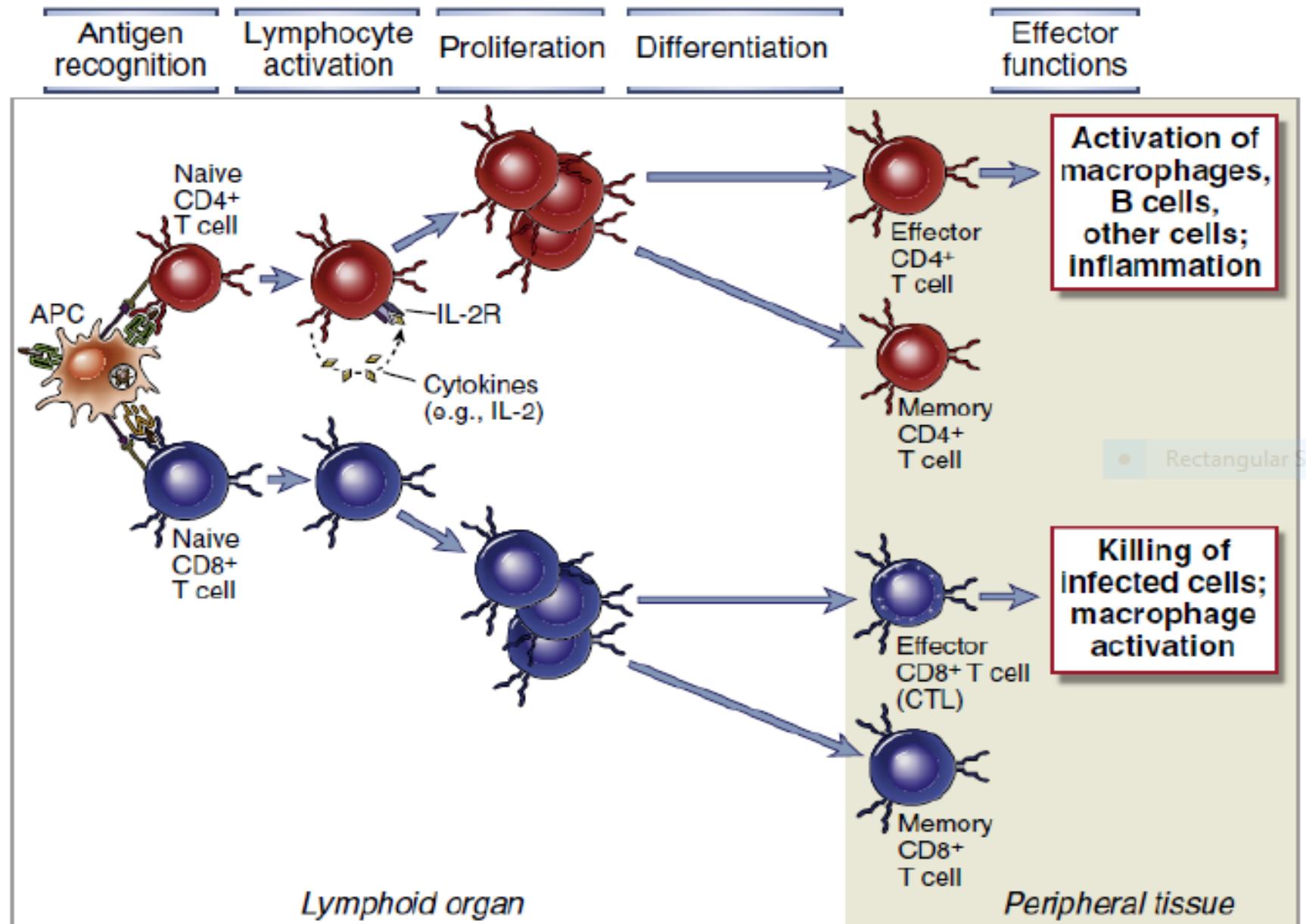
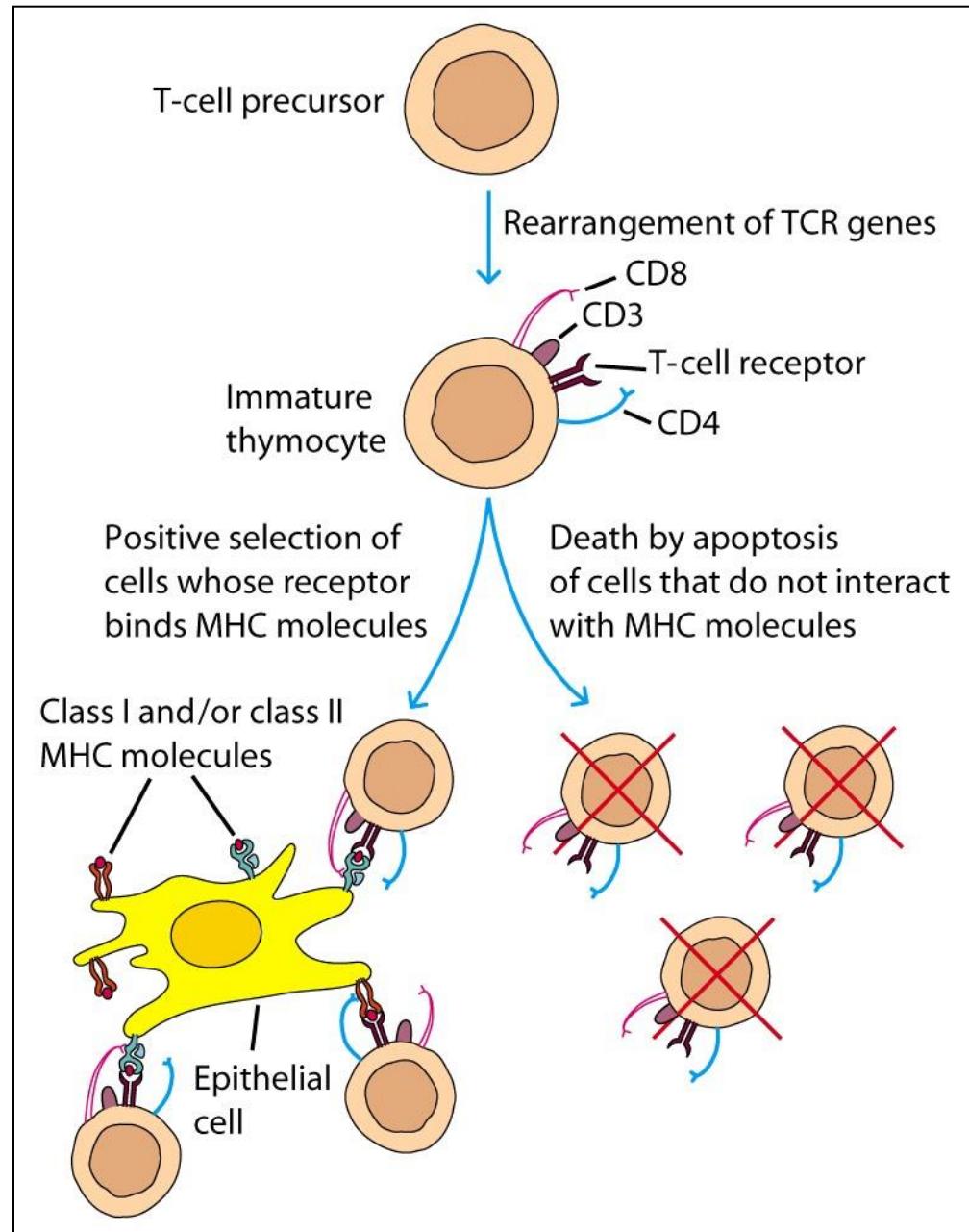
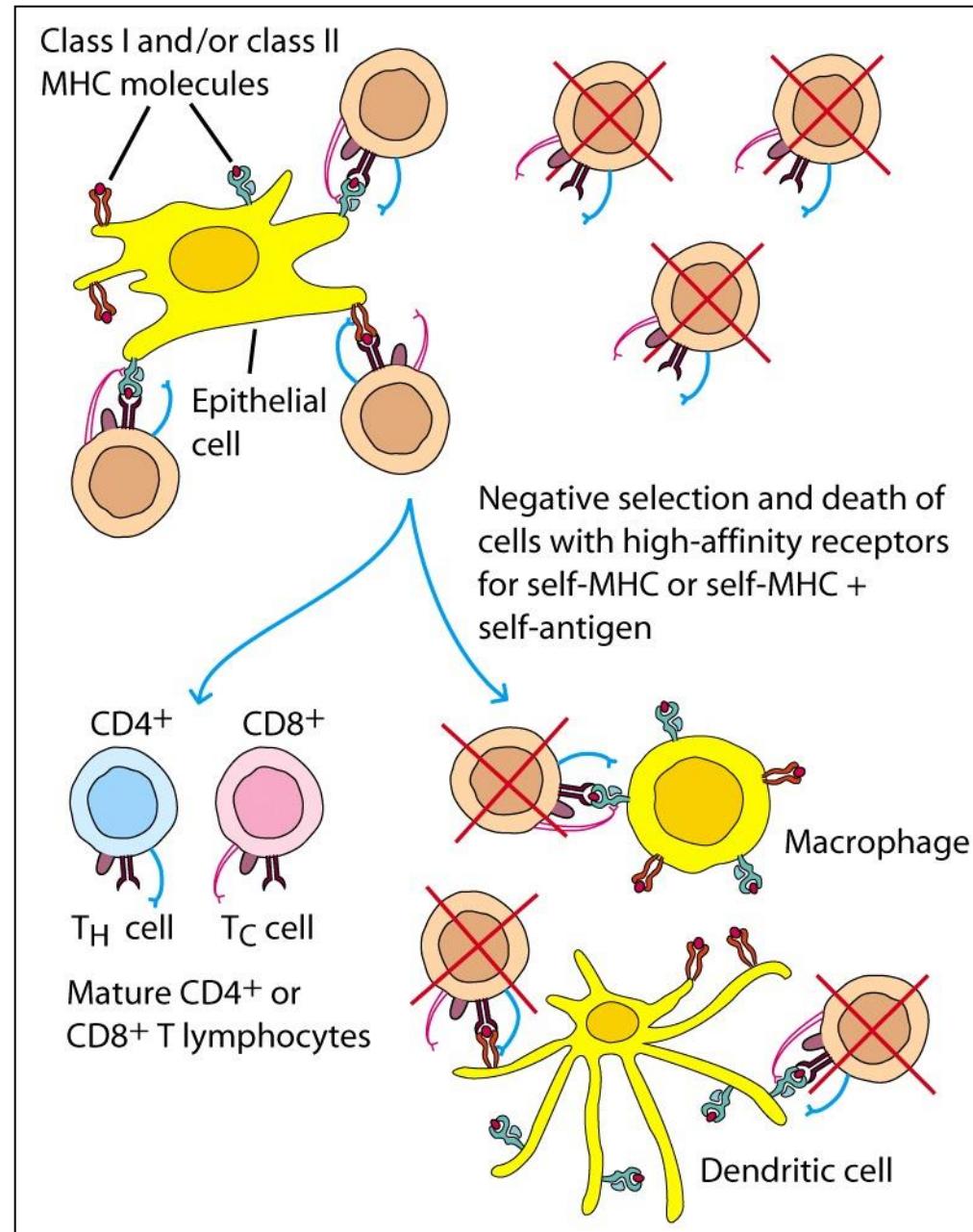


Figure: Effector and memory CD4 and CD8 T cells development

Negative and positive selection

- CD4 and CD8 T cells undergo two selection processes:
 - **positive selection:** T cell binds MHC cell I or II. It permits to survive
 - **negative selection:** T cells recognize **strongly self** MHC cell I or II + self antigen. It is killed through apoptosis





➤ Steps for effector CD4 T cell development

- ✓ antigen (AG) processing and presenting
- ✓ antigen recognition
- ✓ activation
- ✓ effector

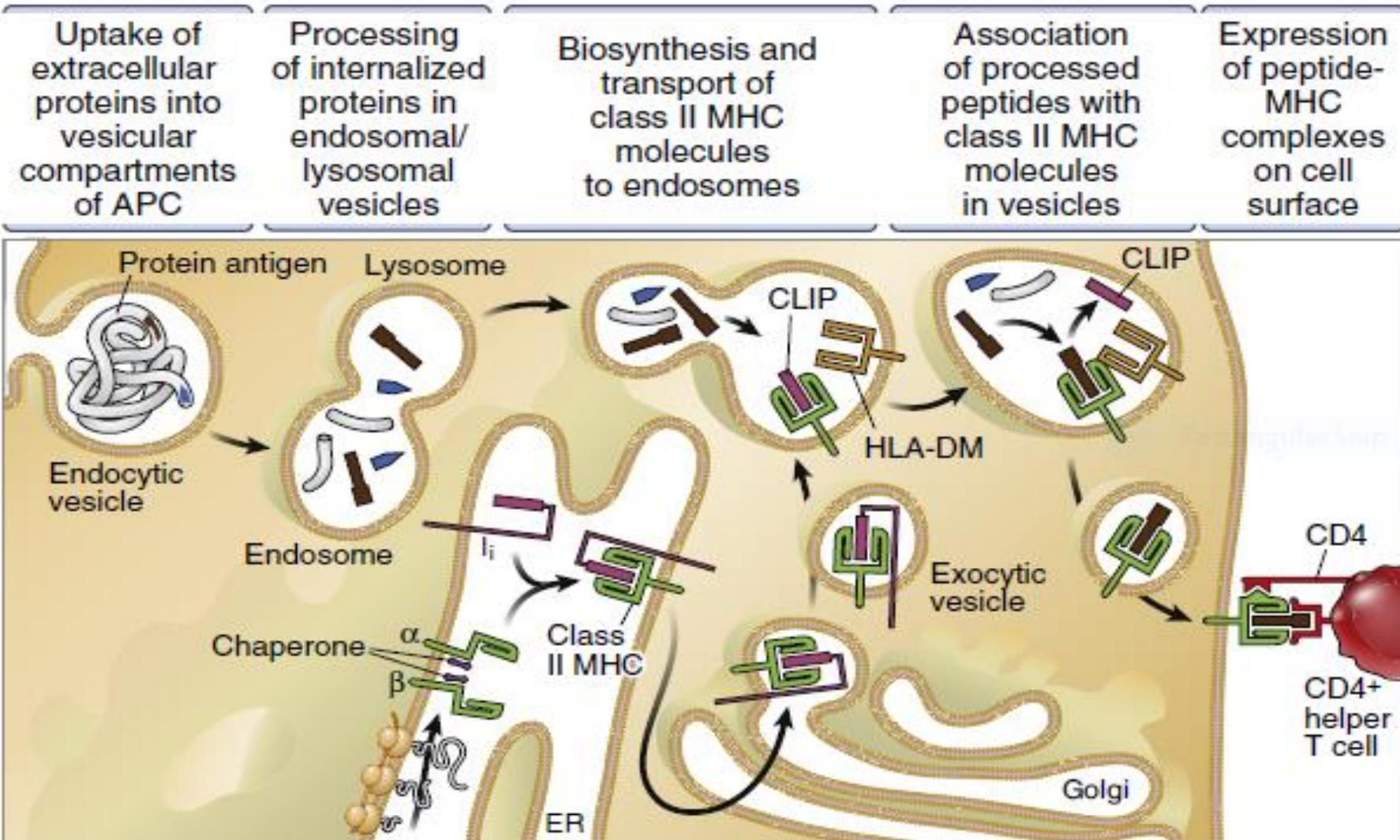
AG processing and presenting

AG processing: is a generation of peptides from intact proteins through APC

AG presenting: is a display of the AG peptides in complex with MHC molecules on the surface of the cell (CD4)

procedure

1. Antigens are ingested through APC
2. APC degrade ingested exogenous antigen into peptide fragments
3. class II MHC molecule are expressed and bind degraded antigen and present it to **CD4 Th** cells

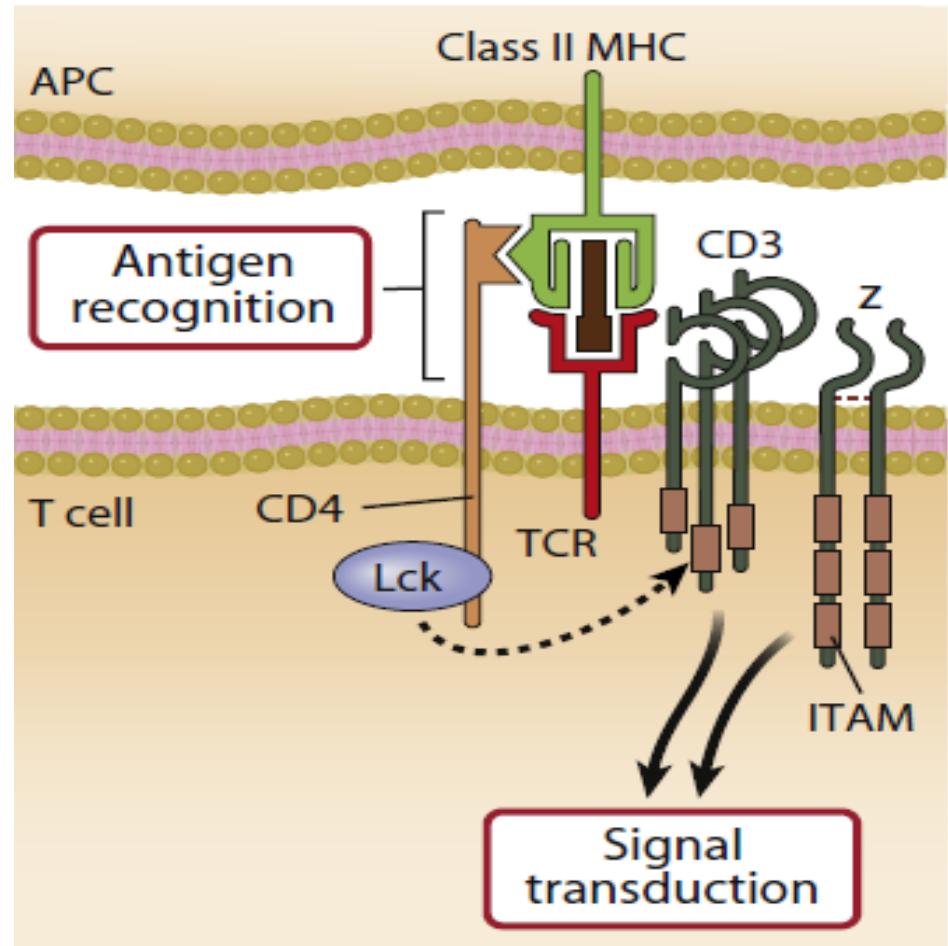


Antigen processing and presenting

class II invariant chain peptide (CLIP), i , invariant chain

Antigen recognition

- TCR recognizes antigen on the surface of APC in association with **class II MHC** molecules with the help of **CD4**



ITAM, immunoreceptor tyrosine-based activation motifs

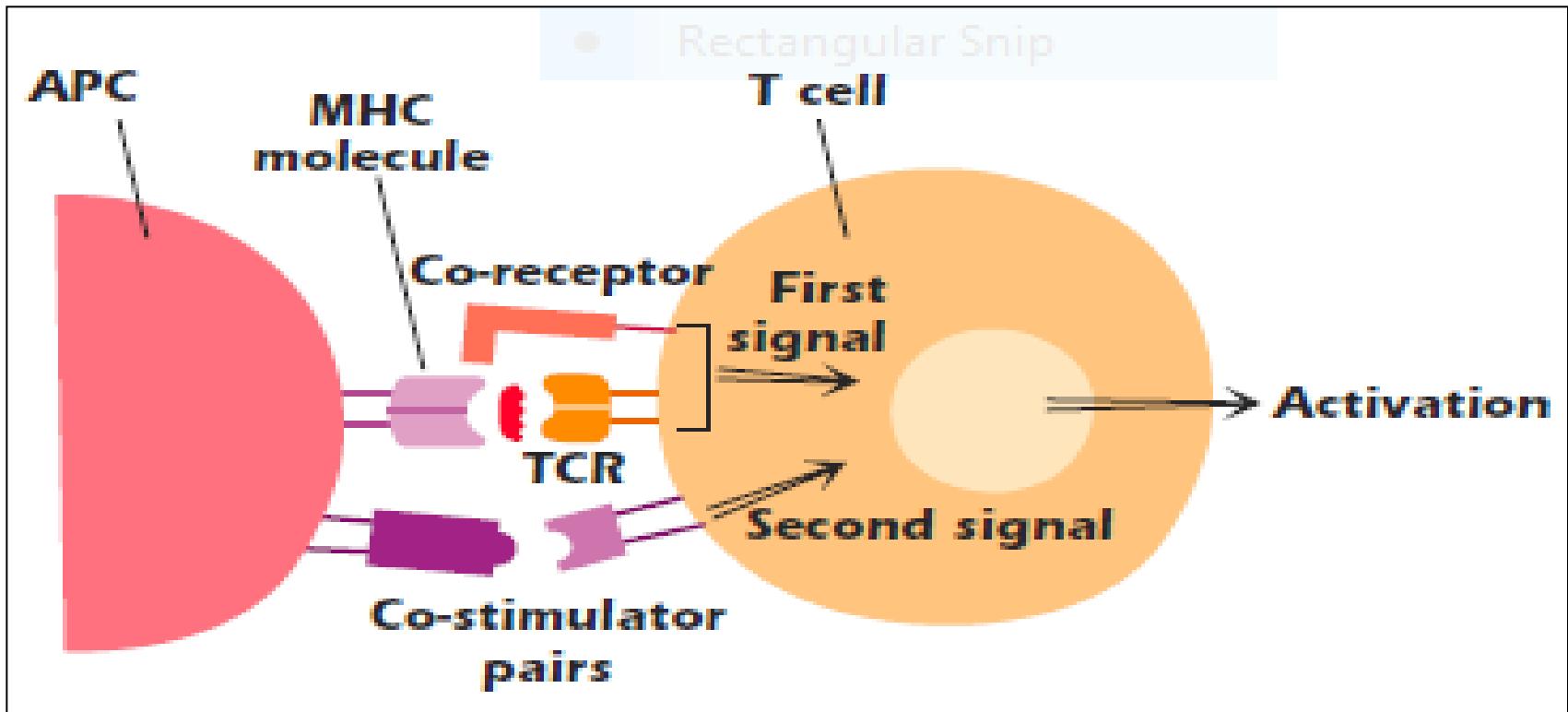
CD4 T cell activation

- T cells require two signals for its activation

Signal 1: interaction of an antigenic **peptide** with the **TCR-CD3** complex

Signal 2: co-stimulatory signal provided by interactions between **CD28** on the T cell and **B7** proteins on APC.

- The **two signals** result the activation of **CD4** T cell which cause the proliferation and differentiation of **CD4** T cell into Th cell and Treg cell
- **CD4** T cell proliferation and differentiation are controlled by **cytokines**

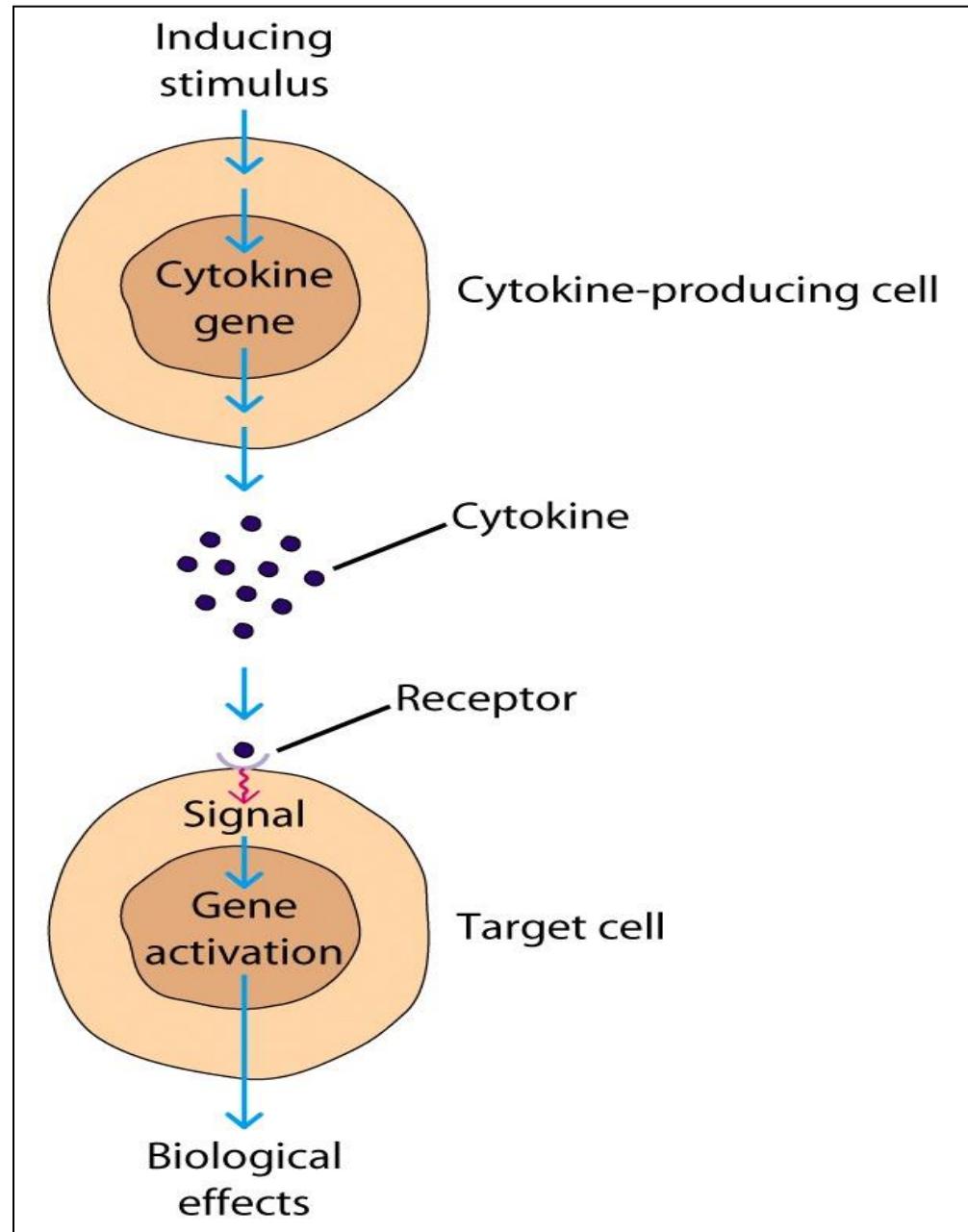


First signal = TCR with peptide + class II MHC

Second signal = co-stimulator pairs at the APC–T-cell surface

Effector:

- ✓ CD4 T cells secrete a variety of cytokines and act as helper or regulatory cells by interacting with other cell types.



Subsets of CD4 T cell

- When **CD4** T cell encounters with antigen combined with a MHC molecule on a cell, it proliferates and differentiates into
 - **TH1**: produces cytokines and involved in **cellular immunity** and **delayed type hypersensitivity**
 - **TH2**: produces various cytokines and involved in **humoral immunity**, **activates macrophage**, and helps for host defense against helminthes by **activating eosinophils**
 - **TH17**: stimulates many cells of the innate immune system (in particular, recruiting and activating neutrophils to sites of **inflammation**)
 - **Regulatory T cells (Treg)**: **Represses** adaptive immune responses at the end of reaction as well as prevent autoimmune response

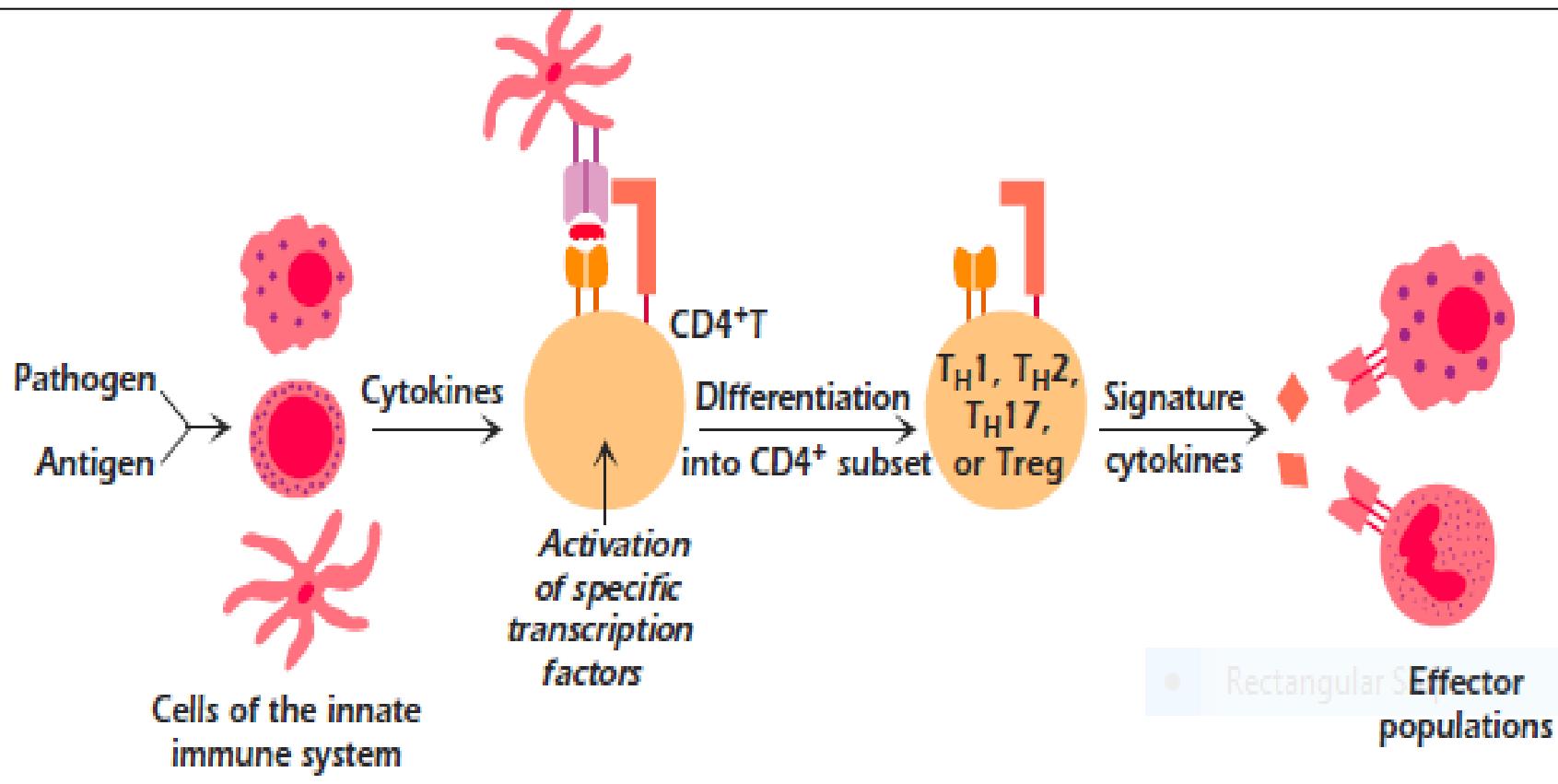
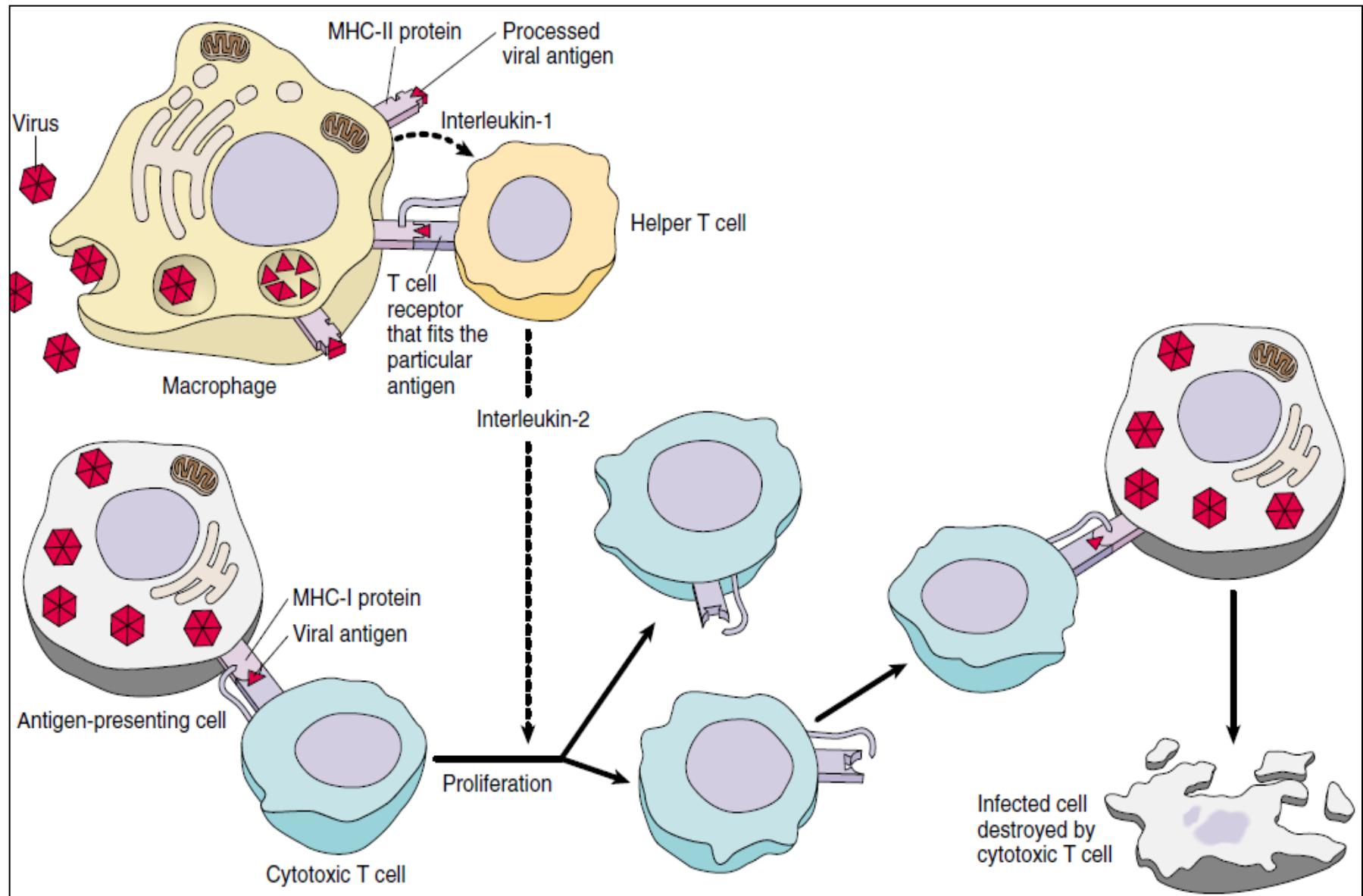


Figure: Development of subsets of CD4 T cell

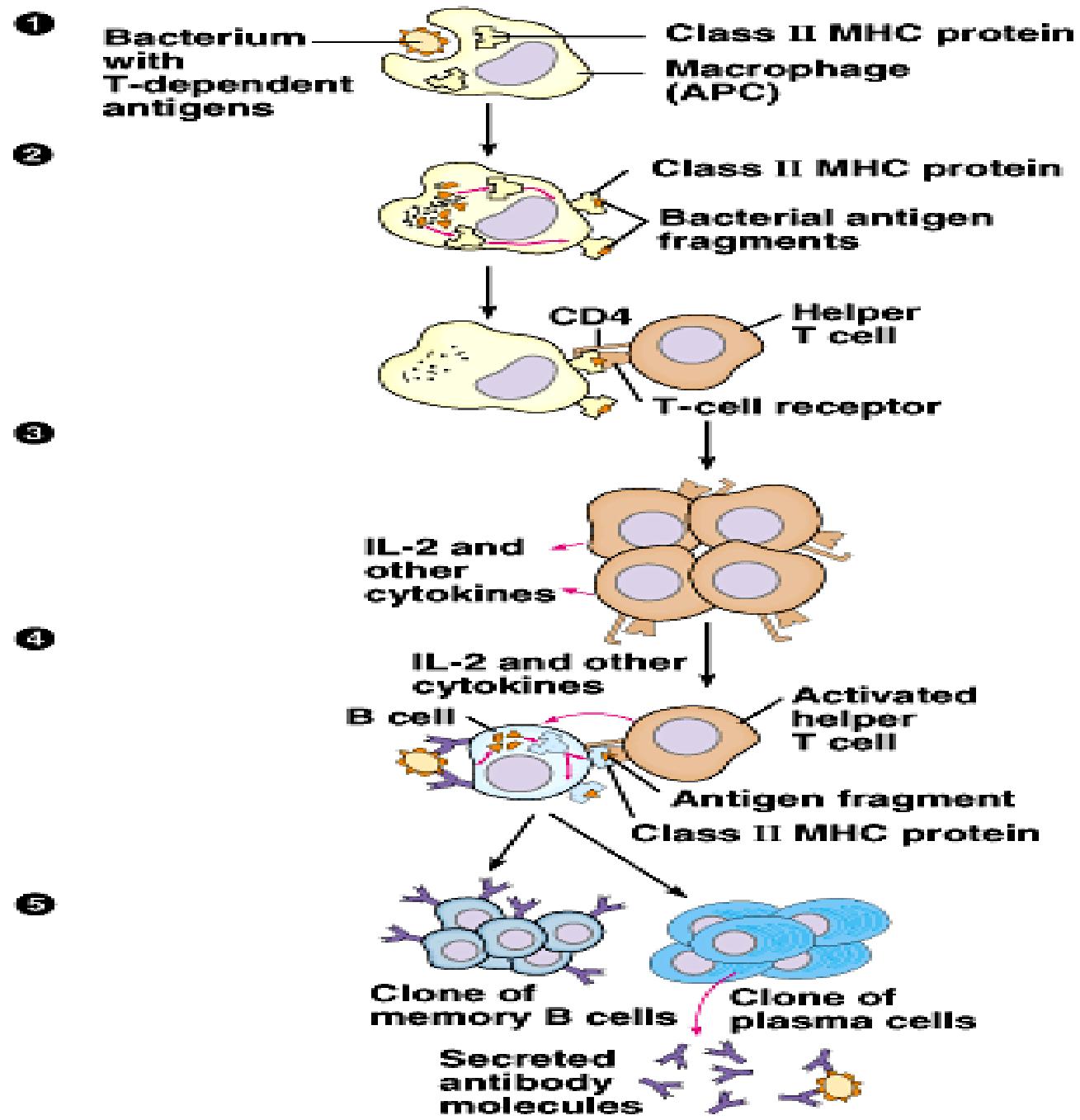


Role of T_{H1} cell in cytotoxic T cell (cellular immunity)

Role of Th2 cells in B cell activation

- If Th cell encounters with B cell bearing peptide: MHC class II complex, T_h cell secrete **cytokines**
- Th cell secreted cytokines activate and differentiate B cells into:
 - ✓ **plasma cells**: class switching of antibodies
 - ✓ **memory B cells**

Role of Th2 cell in humoral immunity

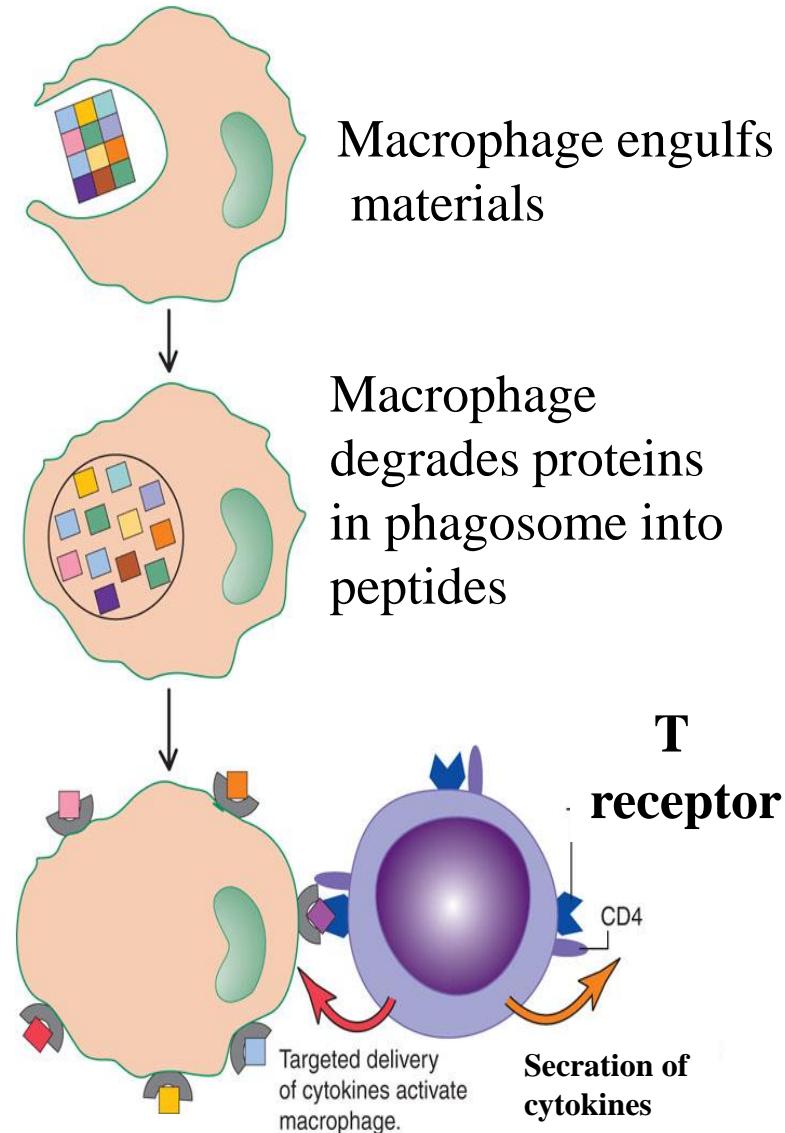


□ Role of Th2 cells in macrophage activation

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- Macrophages routinely engulf invading microbes **resistant to lysosomal killing**
- Th cells recognize macrophage with engulfed microbes resistant to killing
- Th cells activate macrophages by **delivering cytokines** that induce **more potent destructive mechanisms**

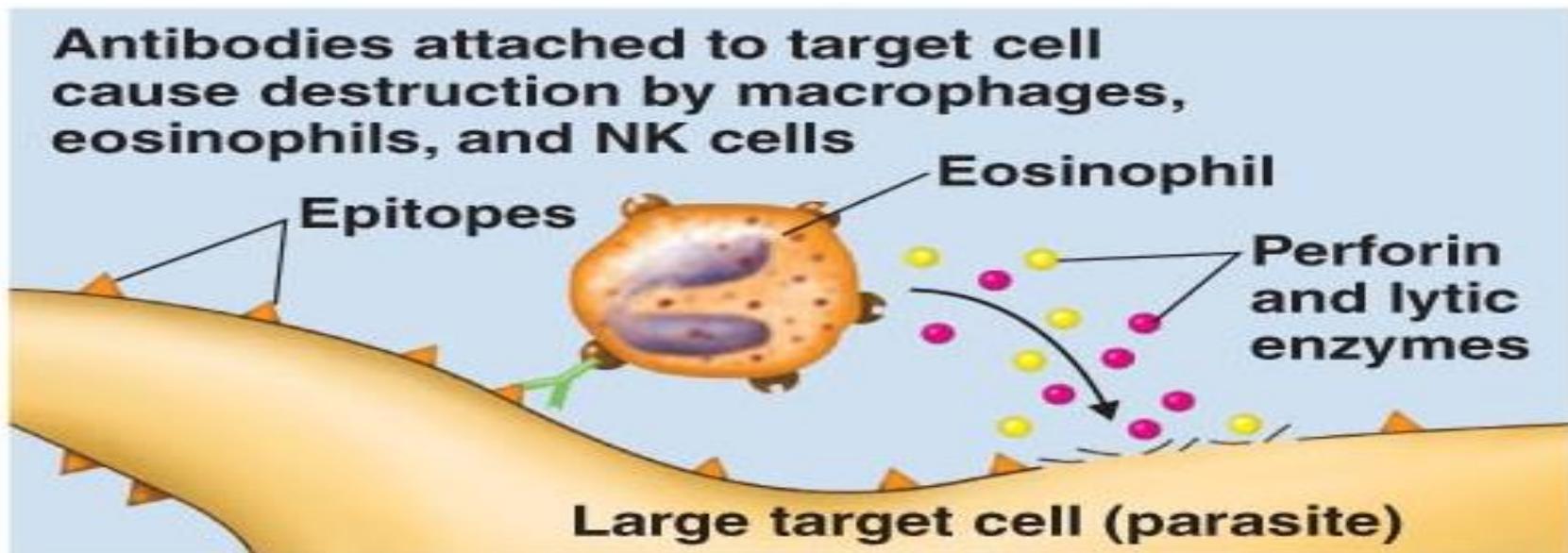
Peptide fragments from engulfed material are presented by MHC class II molecules



Th cell recognizes a peptide being presented by the macrophage and responds by activating the macrophage

Role of Th2 cell in activation Eosinophils

- Helps to against helminthic parasites
- Th2 cells secrete cytokines which stimulate the production of IgE and finally, activation of eosinophils.



➤ **B cells**

- ✓ are lymphocytes
 - ✓ arise and mature in the bone marrow (**primary lymphoid organs**)
 - ✓ activation, proliferation and differentiation occur in **secondary lymphoid organs**
- When a naive B cell encounters with antigen, the B cell activated proliferated and differentiated into
- **plasma** cells----antibodies
 - **memory** B cells.

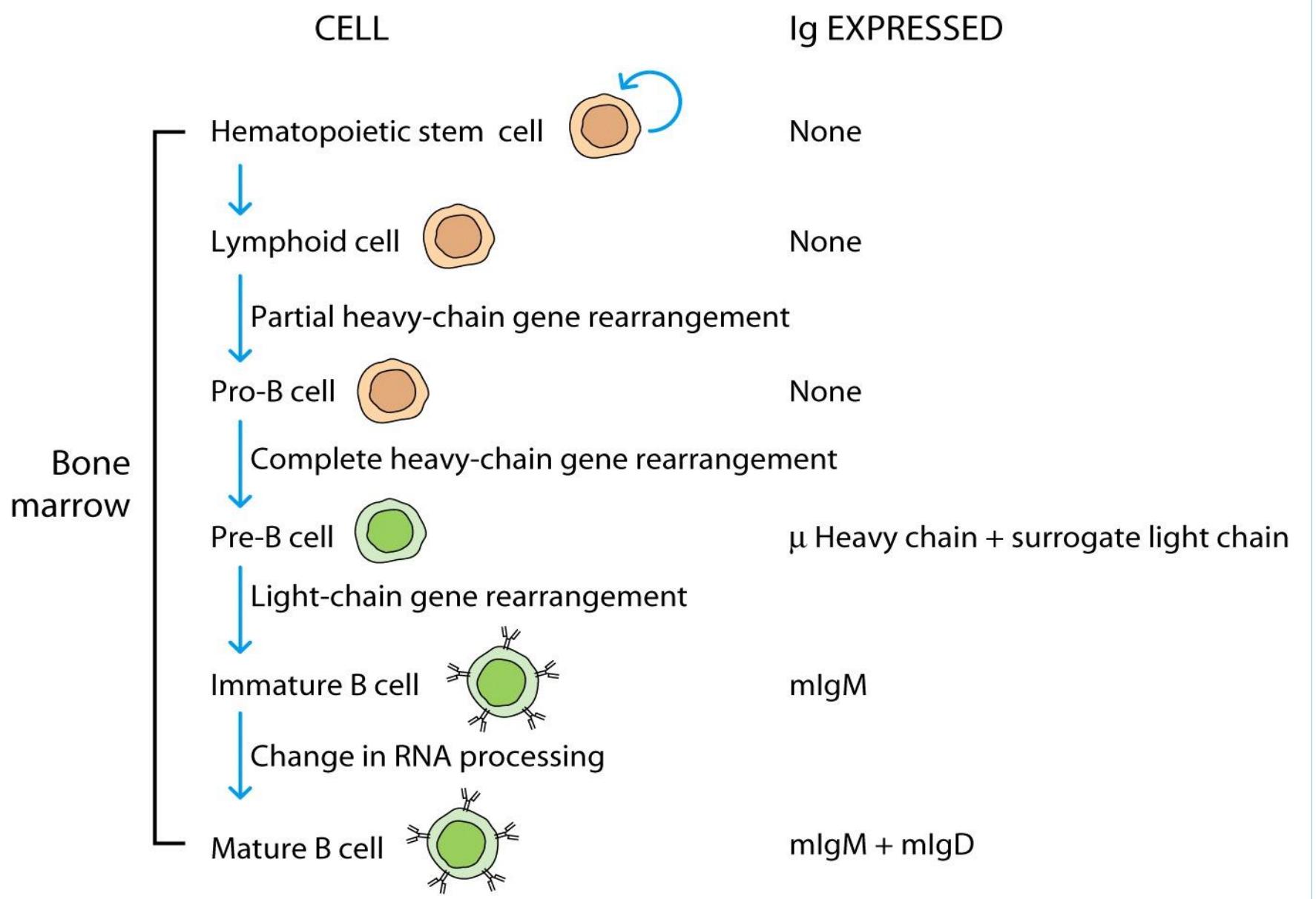


Figure: B cell development and maturation in bone marrow

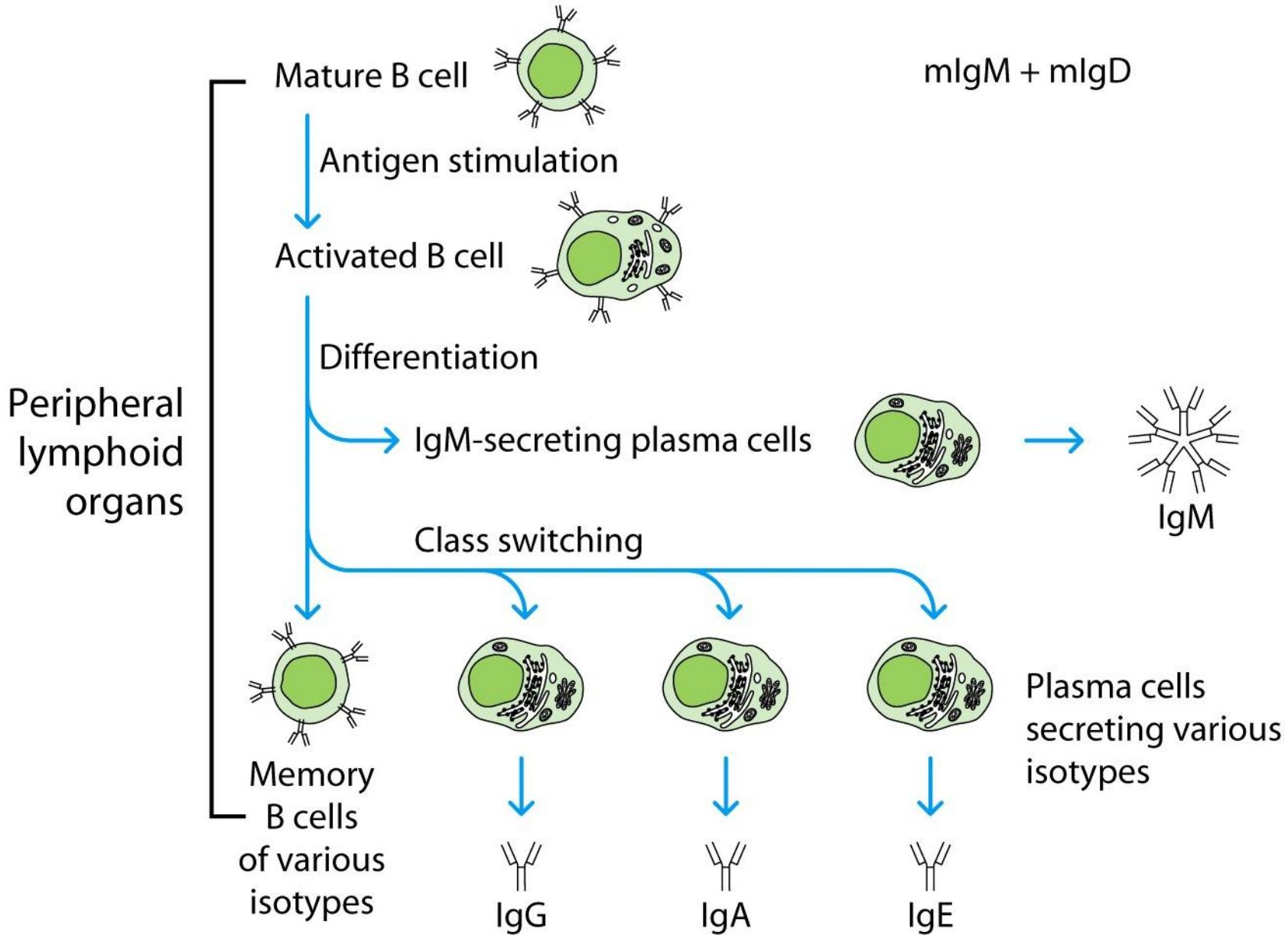
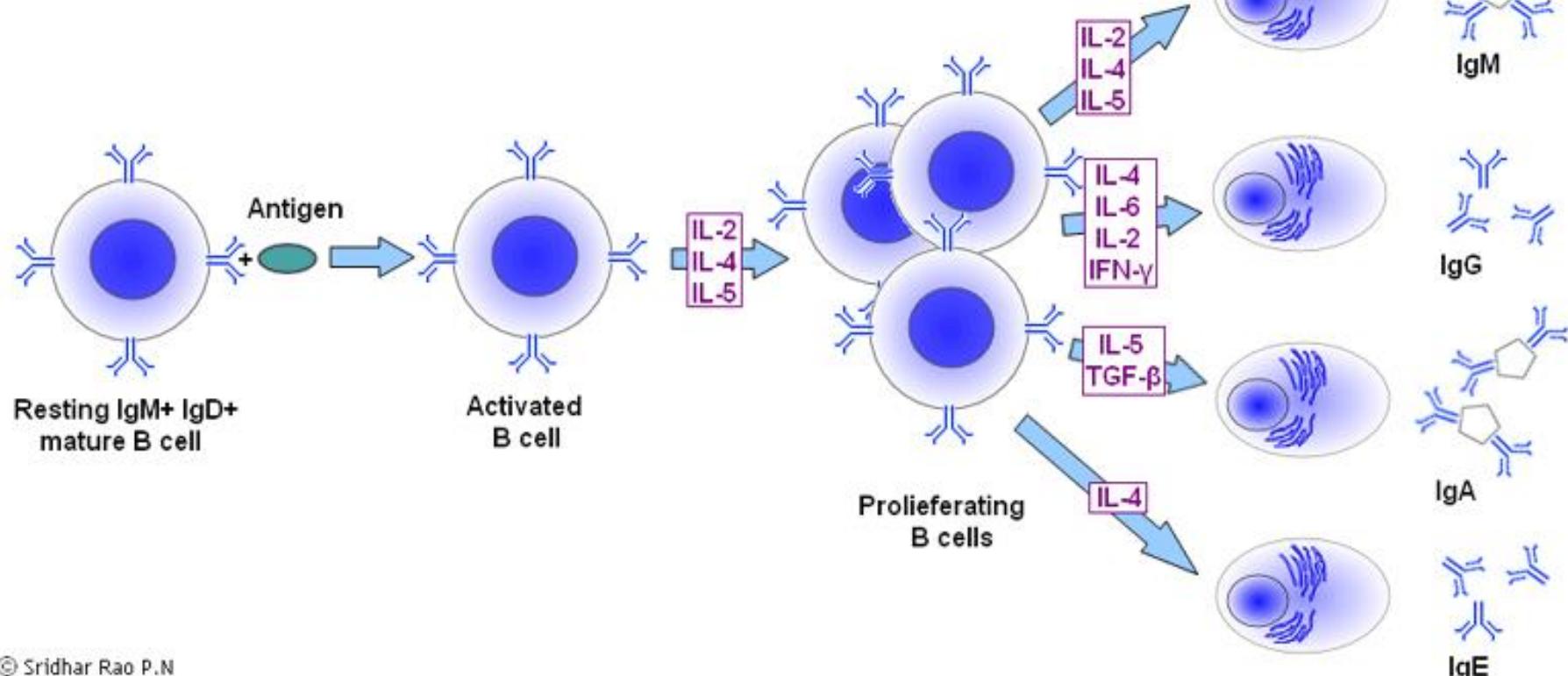


Figure: Effector and memory B cells development

Antigen recognition

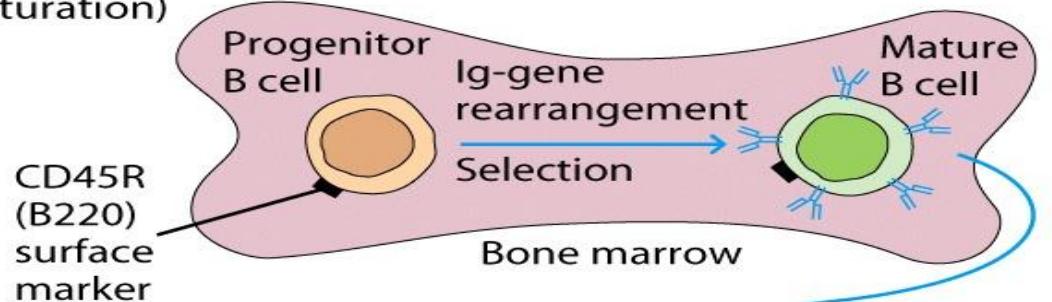


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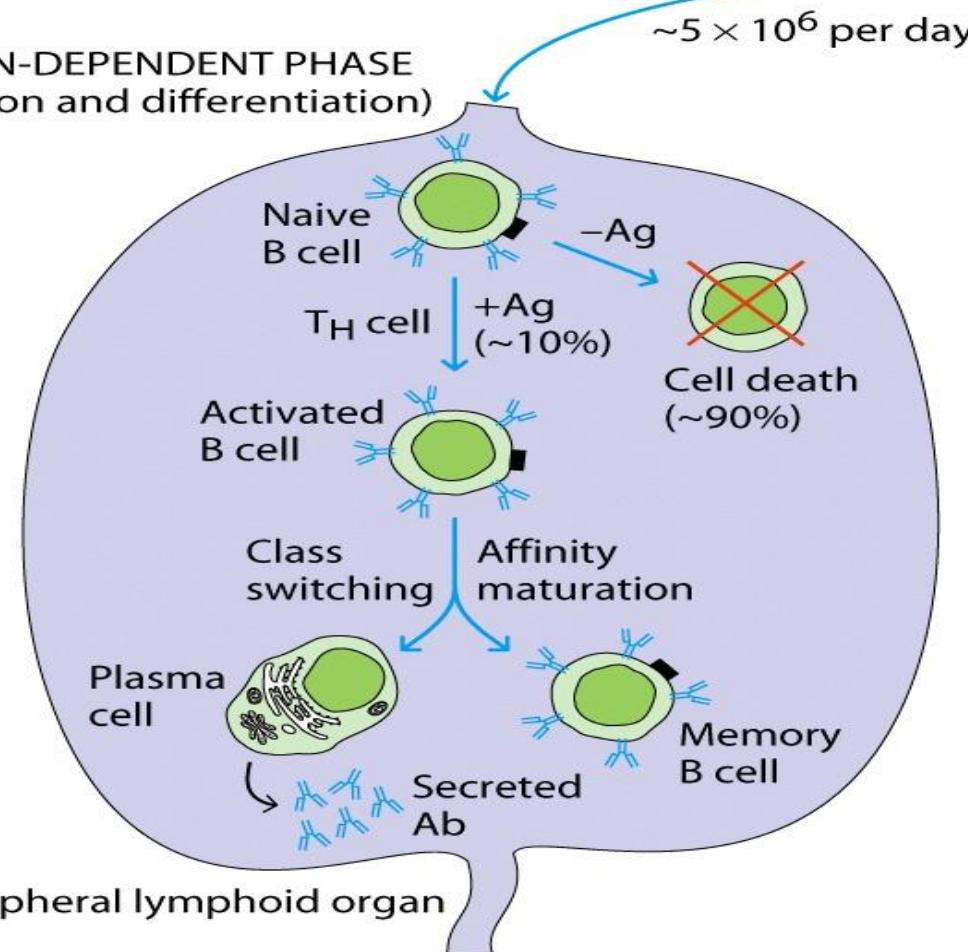
Class switching---is the process of conversion of one antibody (membrane bound = IgM or IgD) into another (secreted form = IgG, IgE, IgA) . This conversion is controlled by cytokines. For example: IL-4: stimulate IgE antibody production

Note: an individual plasma cell will secrete only one class of antibody.

ANTIGEN-INDEPENDENT PHASE
(maturation)



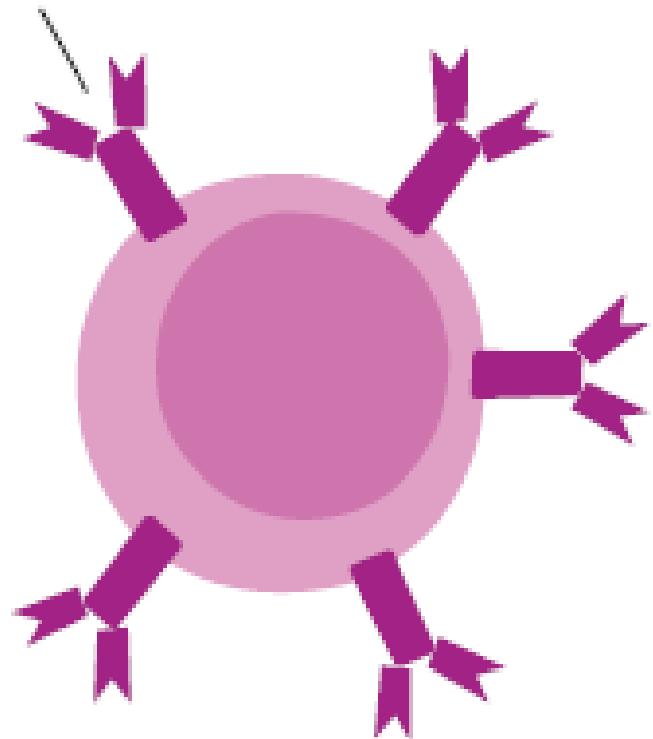
ANTIGEN-DEPENDENT PHASE
(activation and differentiation)



B cell receptor (BCR) structure

- recognize complementary parts of foreign antigens
- Recognition of antigen by the BCR is important for B cell activation, proliferation and differentiation
- The affinity of B cell binding to antigen helped by
 - ✓ Co-receptor: four molecules—CD19, CD21, CD81 and CD225
 - ✓ Signal transduction: CD79a, CD79b
 - ✓ Receptor for co-stimulatory, B7
 - ✓ Adhesion molecules: LFA_1 and ICAM_1

membrane-bound form



secreted form

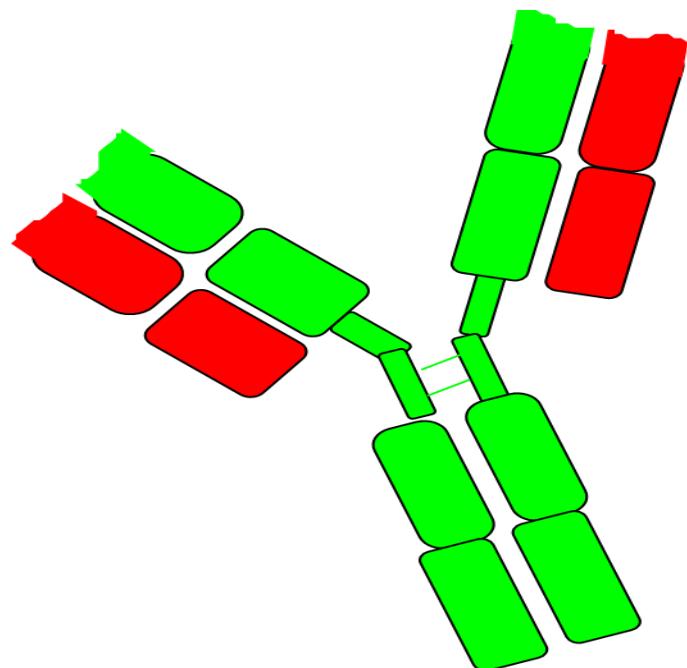
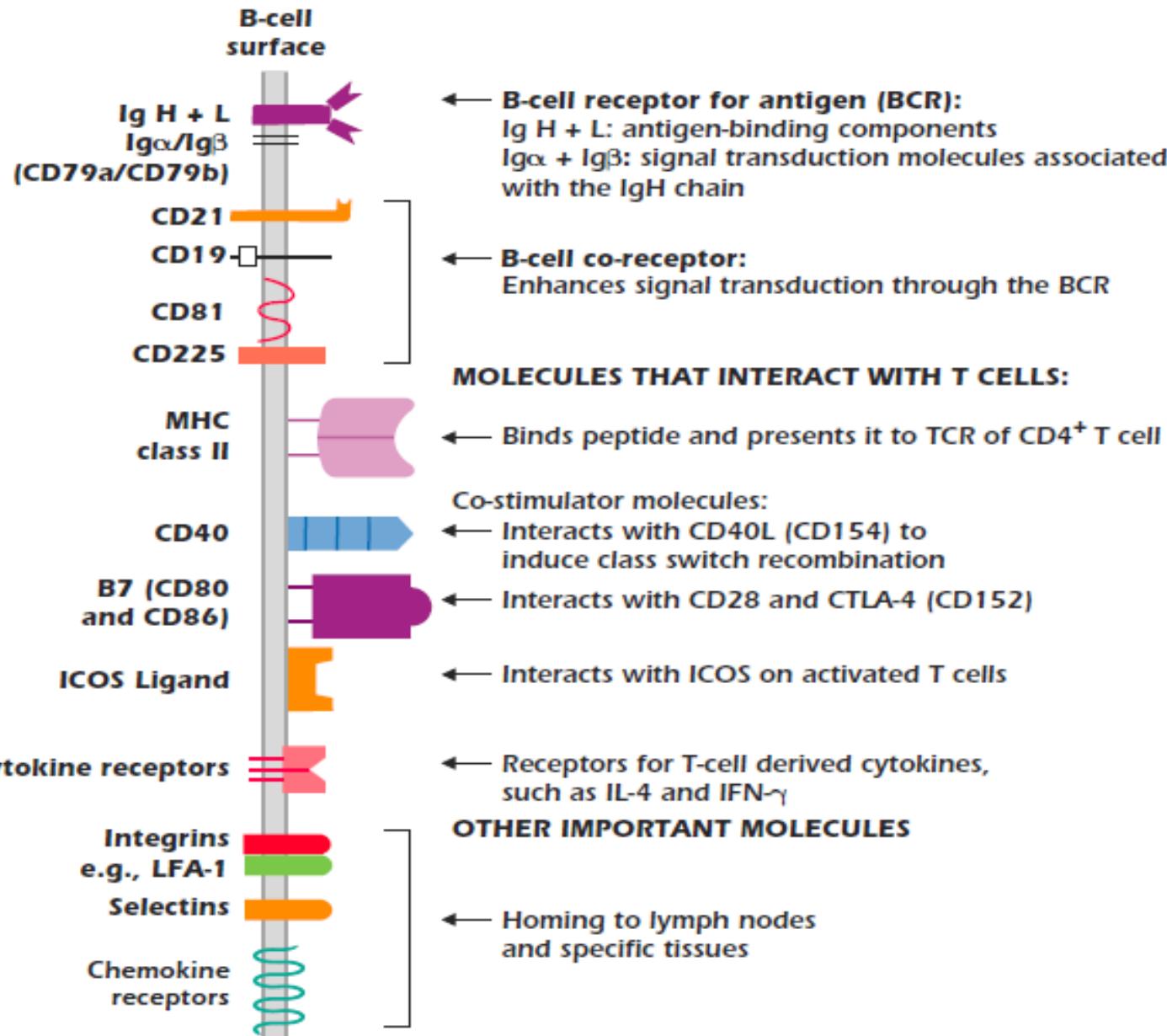


Figure: B cell receptor structure



Important molecules express at the surface of B cells.
 Inducible costimulatory(ICOS) ligand (ICOSL)

B-cell surface molecules

Surface molecules	Function
The B-cell receptor complex	
Antibody (IgM and IgD on mature B cells)	B-cell receptor (BCR) for antigen
CD79a/CD79b (Igα/Igβ) heterodimer	Mediates cellular activation on binding of BCR to antigen
Co-receptors	All these molecules modulate B-cell activation
CD19	Influences B-cell activation
CD20	Ca ²⁺ channel
CD21 (complement receptor CR2)	Binds to C3d, C3bi
CD32 (FcγRII: Fc receptor for IgG)	Binds to IgG complexed to antigen
CD40	Signals B-cell activation and antibody class switching after engagement of CD40 ligand (CD154) on activated T cells
Molecules required for T-cell activation	
MHC class II molecules	Present peptides to Th cells
CD80/CD86 (also called B7.1, B7.2)	Bind to CD28 on T cells to trigger their activation
Adhesion molecules	
ICAM-1	Binds to LFA-1 and facilitates interaction with T cells
LFA-3	Binds to CD2 and facilitates interaction with T cells

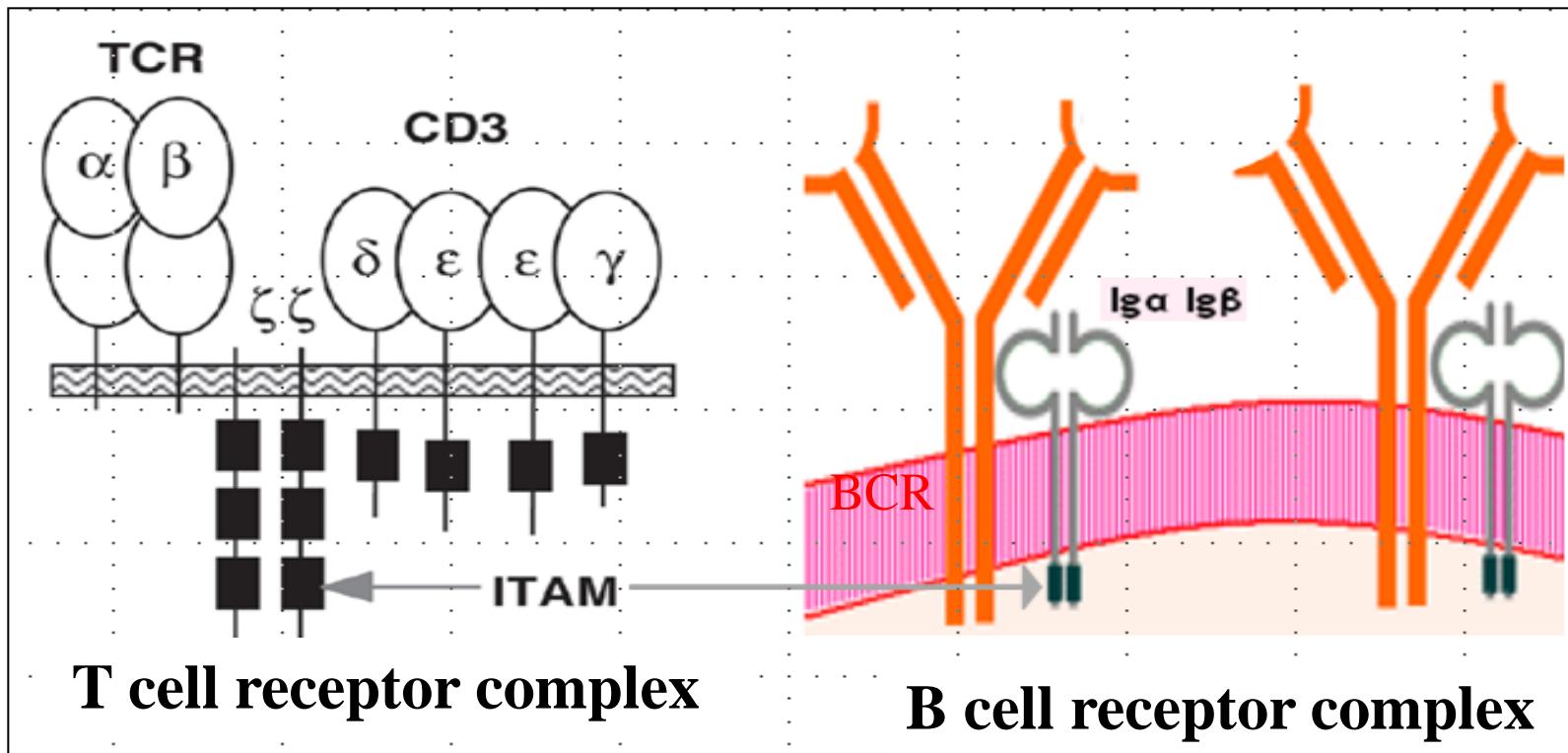
Comparison of TCR and B CR

Similarities

- ✓ Both have **specific antigen-binding** region created by the variable regions of two polypeptide chains.
- ✓ One T cell or B cell expresses **only one** specific type of TCR/BCR.
- ✓ Both display great potential for diversity via **genetic recombination** at the genome level

Differences

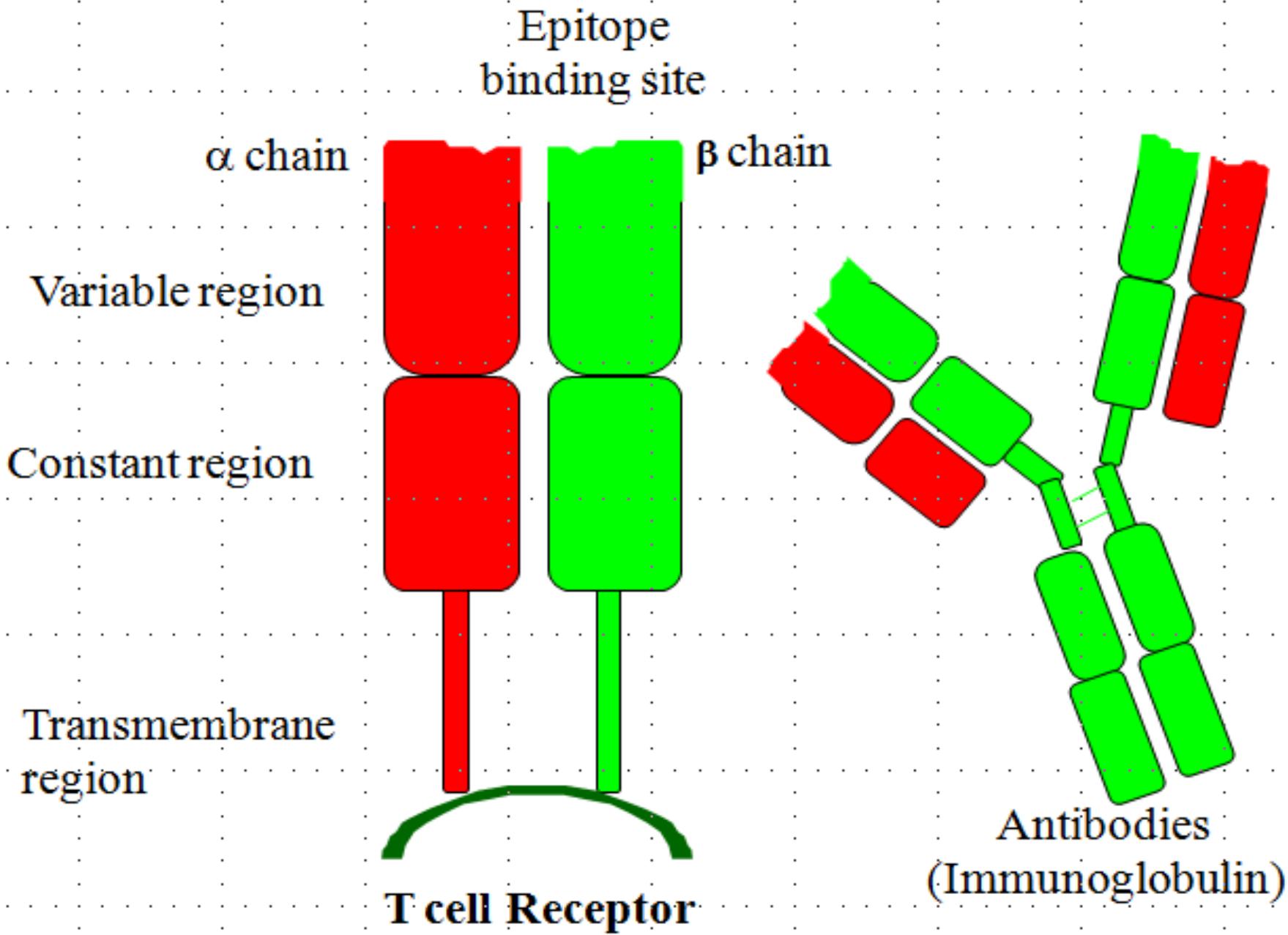
1. TCR is **monovalent** (has one binding site) while an Ig is **bivalent**.
2. The TCR is **membrane-bound** while Ig is both **membrane-bound** and **secreted form**
3. The TCR recognizes **processed antigen** while BCR recognizes **unprocessed antigen**
4. There is class switching in **BCR** but **not in TCR**



T cell receptor complex

B cell receptor complex

immunoreceptor tyrosine-based activation motifs (ITAMs).



Antibodies (Ab)

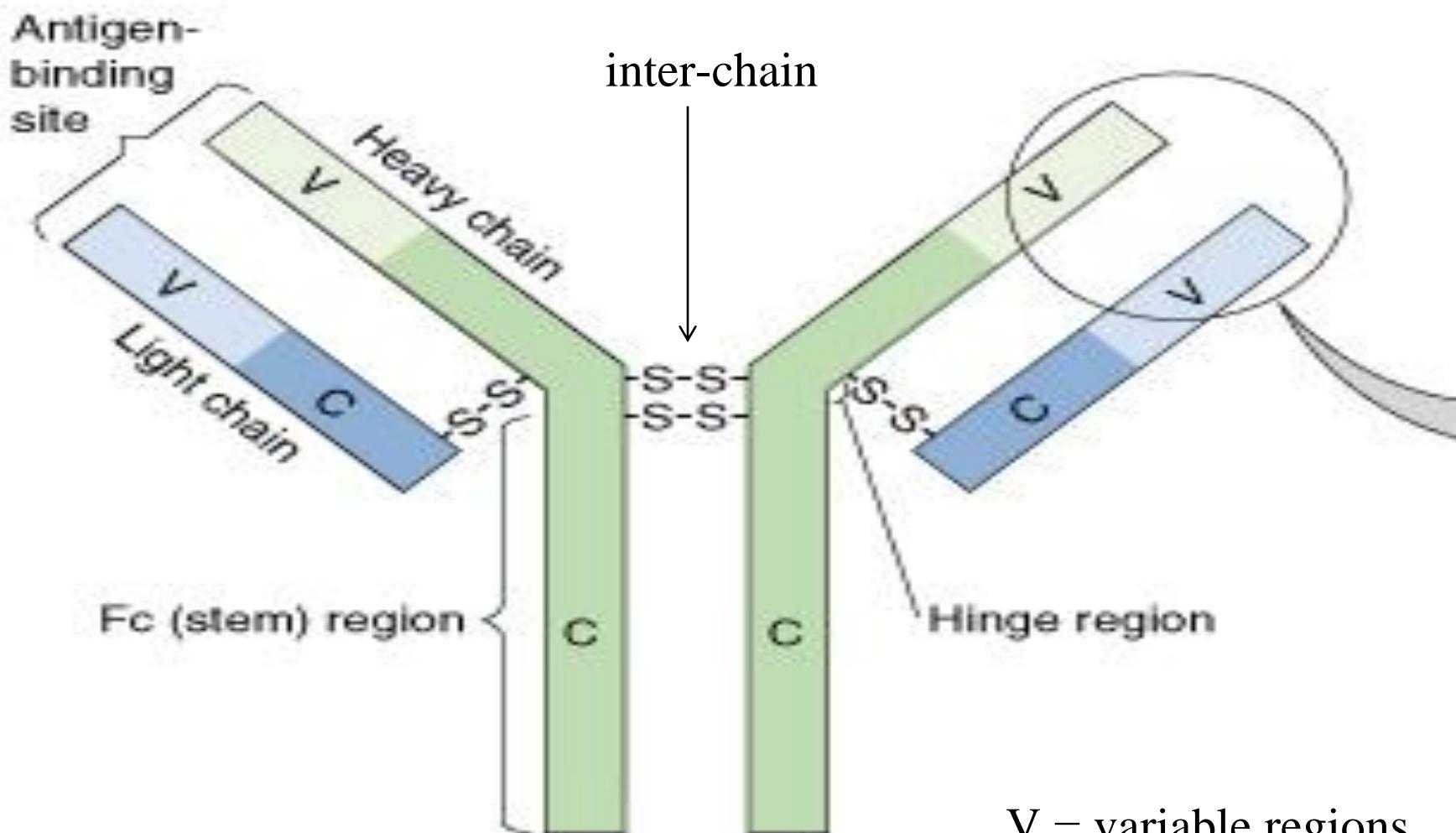
- are antigen- binding **proteins** present on the B-cell membrane and secreted by plasma cells

Immunoglobulin(Ig):

- It refers to all globulins that **possess the activity** of Ab or show a similar structure to Ab
- Therefore, **all Abs** are IgS, **but not all IgS** possess the functions of Abs

Structure of antibodies

- Antibodies
 - ✓ composed of two **heavy chains** and two **light chains** joined by S-S bonds
 - ✓ has **inter-chain** and **intra-chain** disulfide bonds (S-S).
These chains bind together to make a “T” or “Y” shape antibody
 - ✓ consists **variable** region, **hinge** region and **constant** region



(a) Antibody molecule

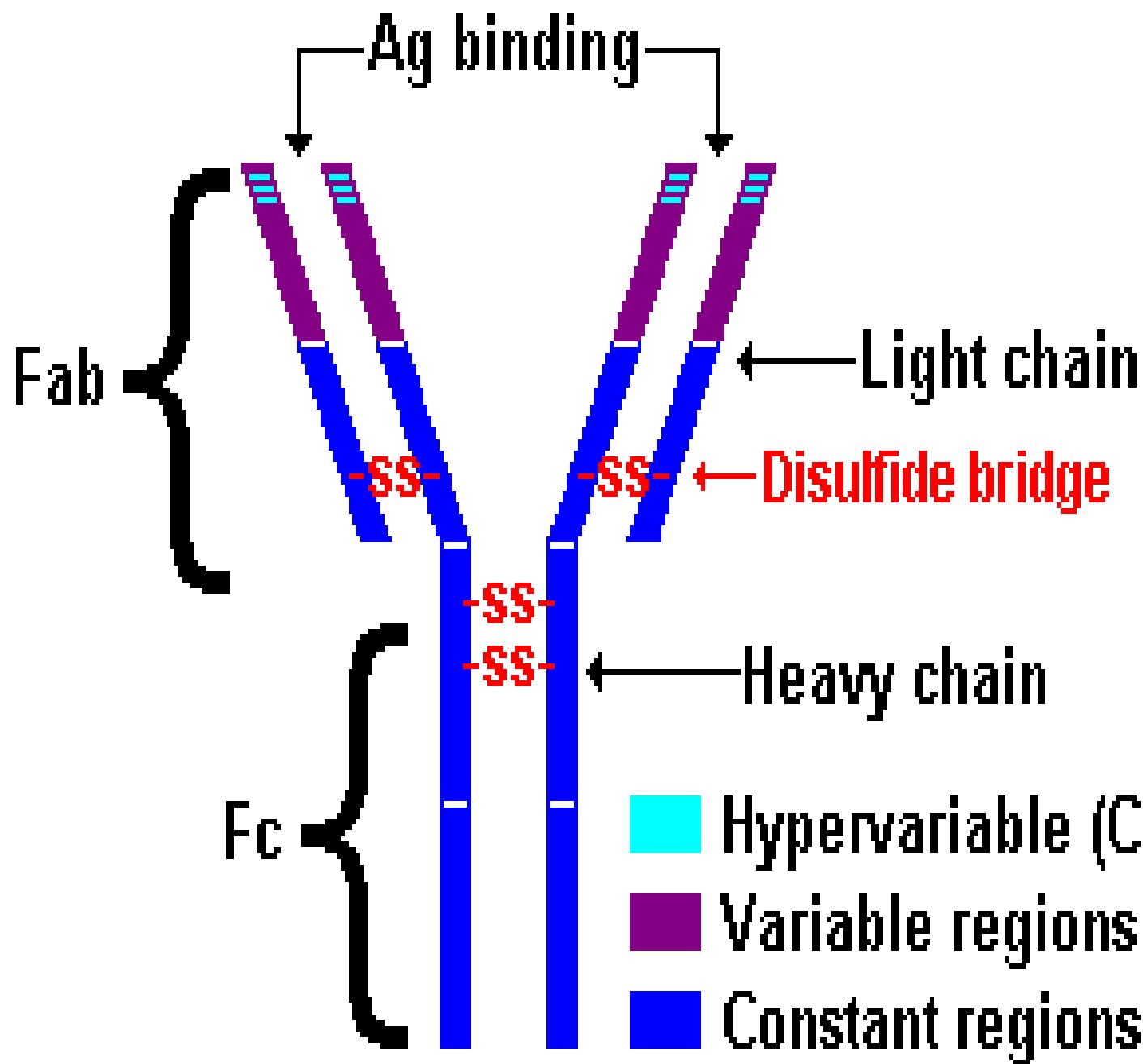
V = variable regions
C = constant region

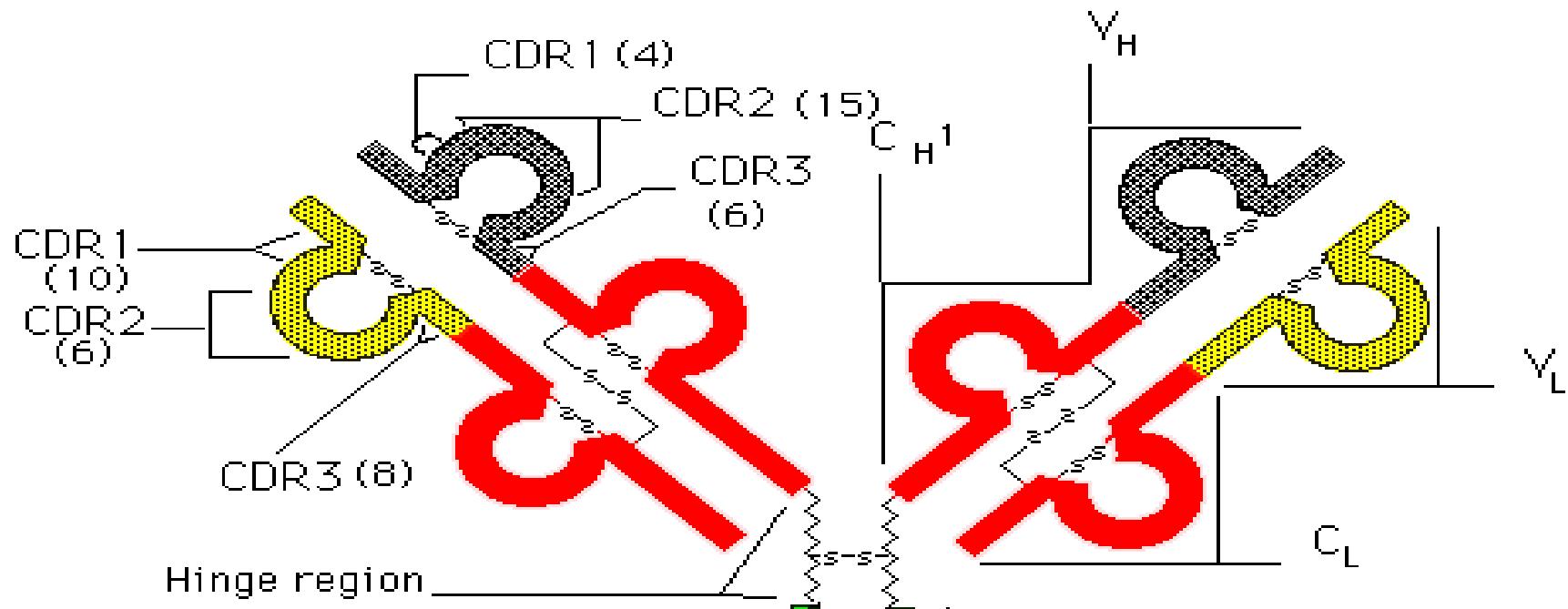
Variable region (V)

- is the **arms** of antibody
- is **antigen binding site**
- has **hypervariable regions** (highly variable amino acid sequences of light and heavy chains) and **framework regions** (similar amino acid sequences)
- **differs** from antibody to antibody
- has **N-terminal** end

➤ Hypervariable region

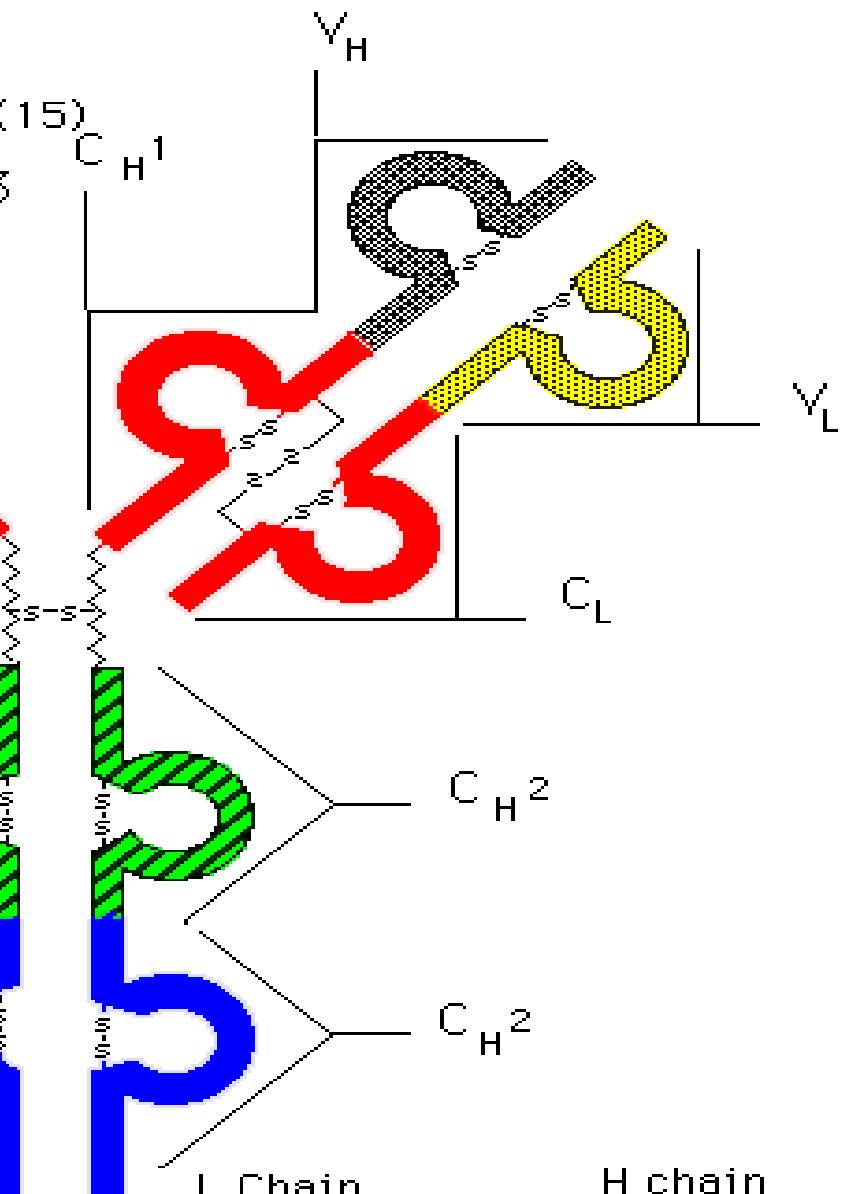
- ✓ forms the **antigen binding site** of variable region
- ✓ is structure that is **complementary** to the structure of epitope
- ✓ is called complementarity determining region (CDR)





H Chain 440 amino acids
L Chain 220 amino acids
H chain 4 or 5 domains
L Chain 2 domains
Each domain has intra-disulfide bridge of 60 amino acids.

There are 5 classes of H chain (IgG, IgM, IgA, IgD, and IgE)
There are two class of L chains (Lambda and Kappa)



L Chain
CD1 24-34 (10)
CD2 50-56 (6)
CD3 89-97 (8)

H chain
CD1 31-35 (4)
CD2 50-65 (15)
CD3 96-102 (6)

Constant (C)

- ✓ The **stem** and lower part of the antibody.
- ✓ The **same** for all antibodies in the same class
- ✓ The **different** for all antibodies in the **different class** in terms of protein sequences, carbohydrate content, and size
- ✓ has **C-terminal** end
- ✓ is a structure where it **binds other immune cell**

Hinge region

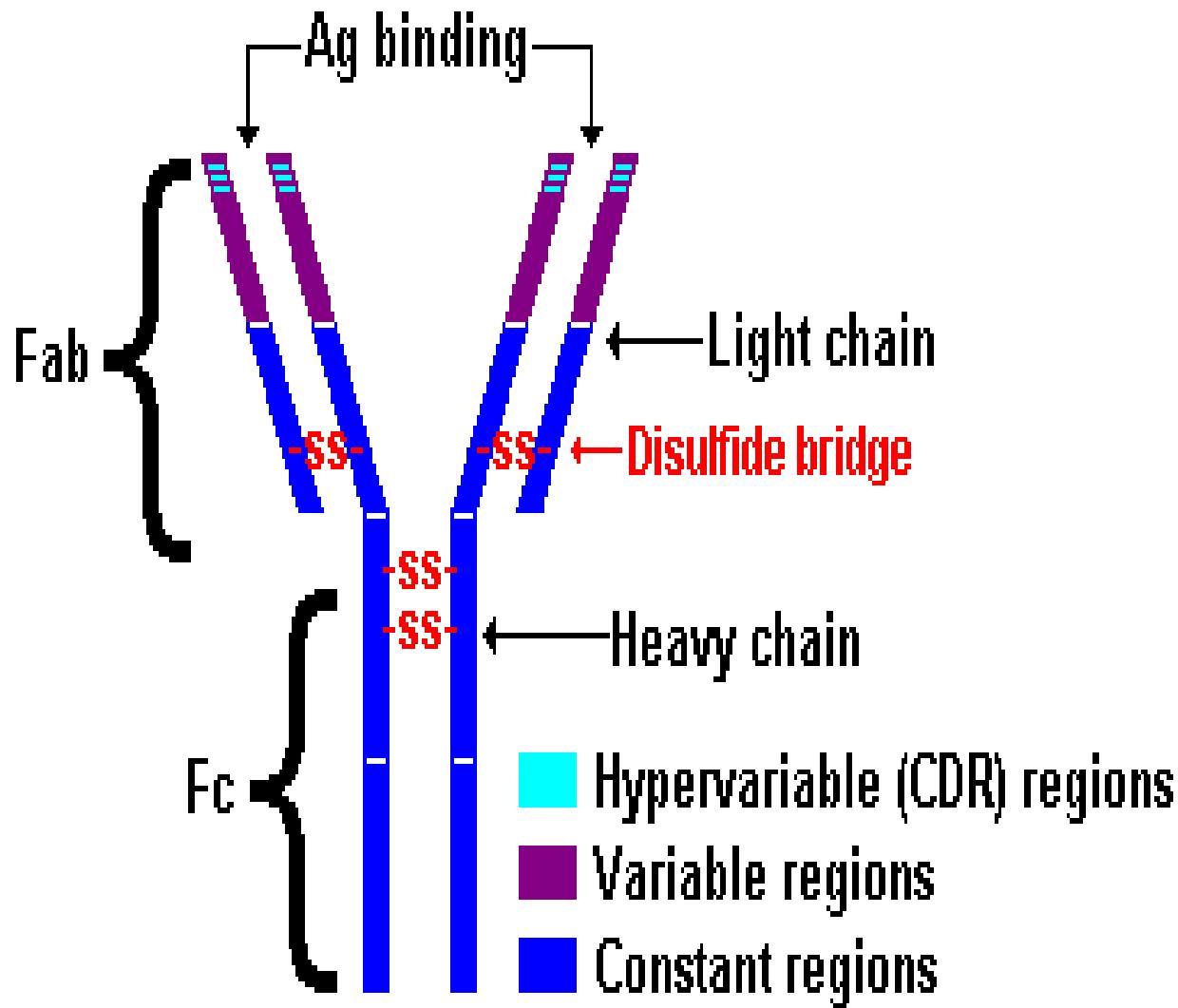
- is a **cite** where the **arm** and stem of antibody is joined together
- is **flexible** and suitable for CDR of Ig binding to antigenic determinants.
- it allows the **two Fab arms** to open and close to accommodate binding to two identical antigenic epitopes,
- presents only in **IgG, IgD, and IgA** while absent in **IgM and IgE**.
- sensitive **to proteolytic enzyme**

➤ **Fab (fragment, Ag binding) region:**

- ✓ **Ab arms** that contain two specific foreign Ag binding sites.
- ✓ Ag binding sites

➤ **Fc (Fragment, crystallizable) region:**

- ✓ Is **site of binding other immune molecules** (such as complement proteins, phagocytic and killer cells) in order to ensures that each Ab generates an appropriate immune response for a given Ag.
- ✓ performs **biologic functions**



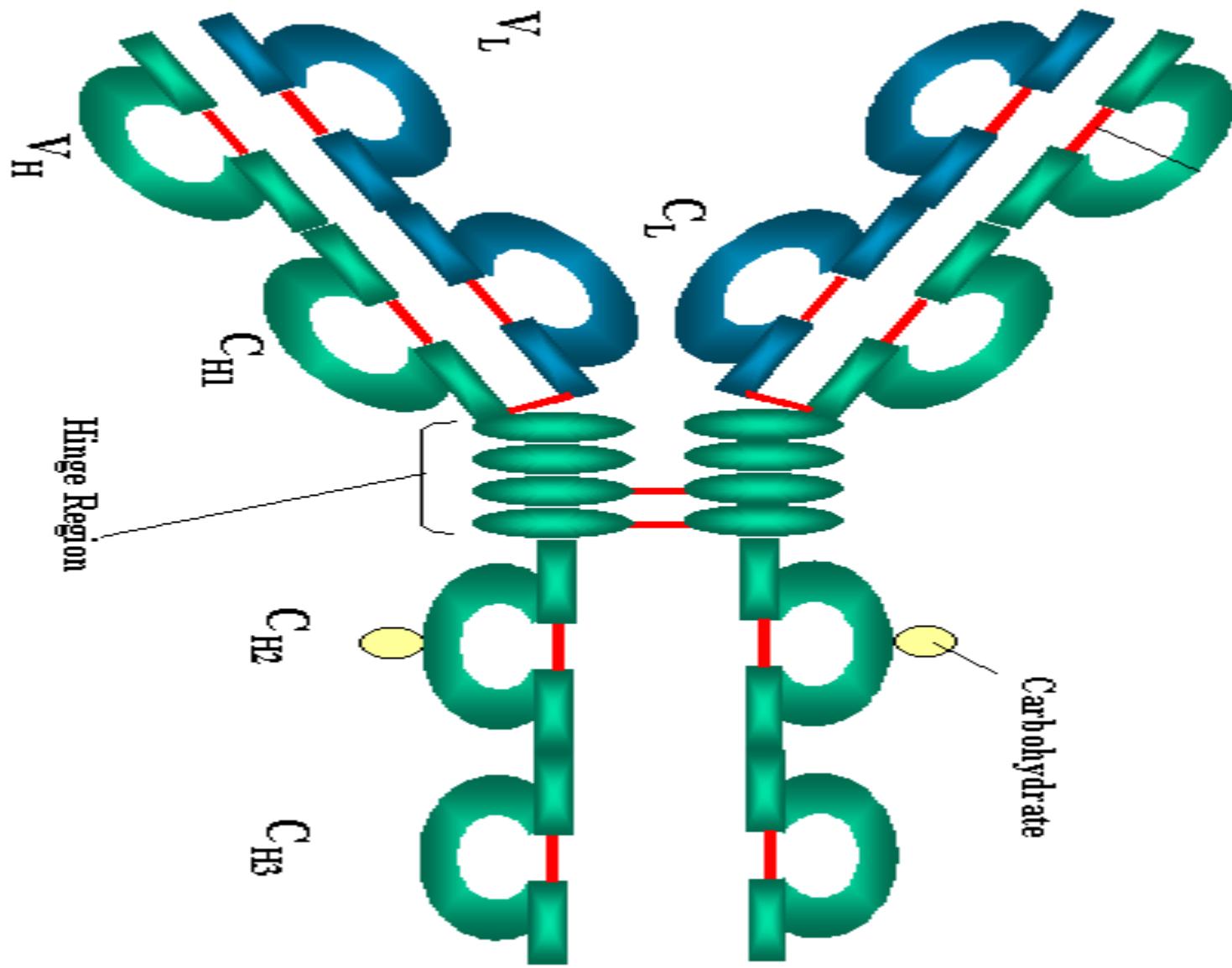
Domains of antibody and their function

➤ globular regions of antibody is called domains

- Polypeptide chains of Ig are folded into a globular structure by intra chain s-s bond within each 110aa region

➤ Function

- VH, VL: antigen-binding site
- CH1, CL: allogeneic marker
- CH2: complement-fixing site
- CH3
- CH4 (in IgM, IgE) → cell-binding site



- Ig can be digested by papain and pepsin

1. Digested by papain

Position: near the S-S bonds of H inter-chains from the N end

Fragments:

2Fab :fragments: antigen-bindings

1Fc :fragment: crystallizable

Function:

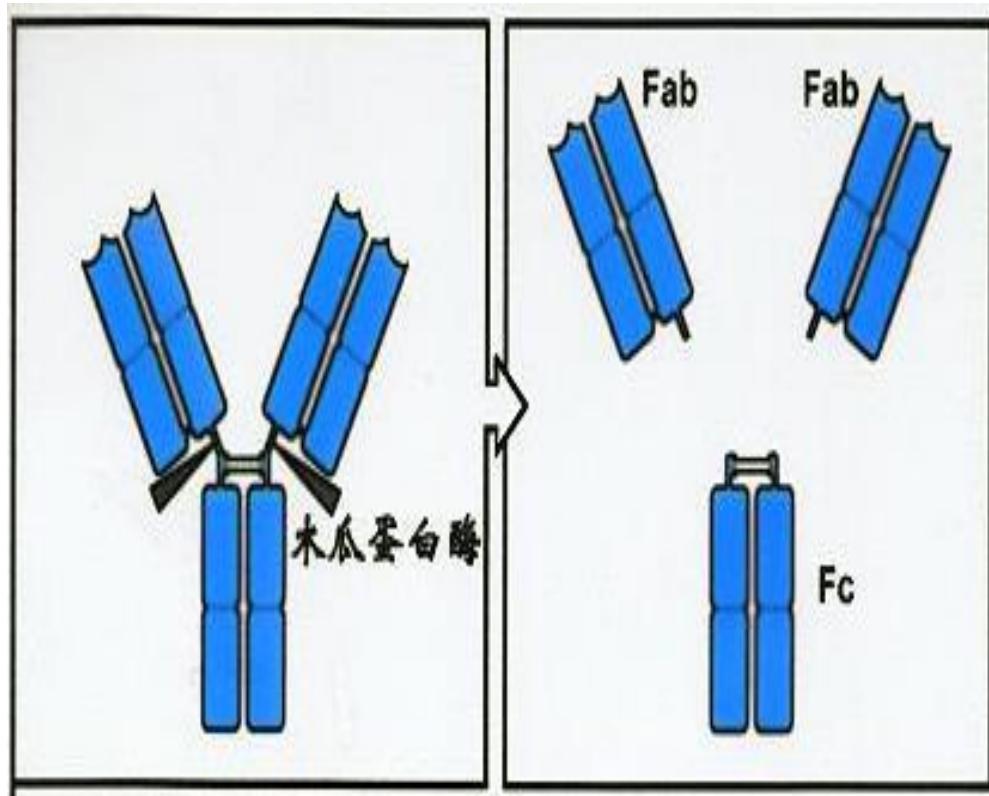
Fab: recognize and bind Ag

Fc:

(1) fix complement

(2) crossing the placenta

(3) bind to FcR in different cells



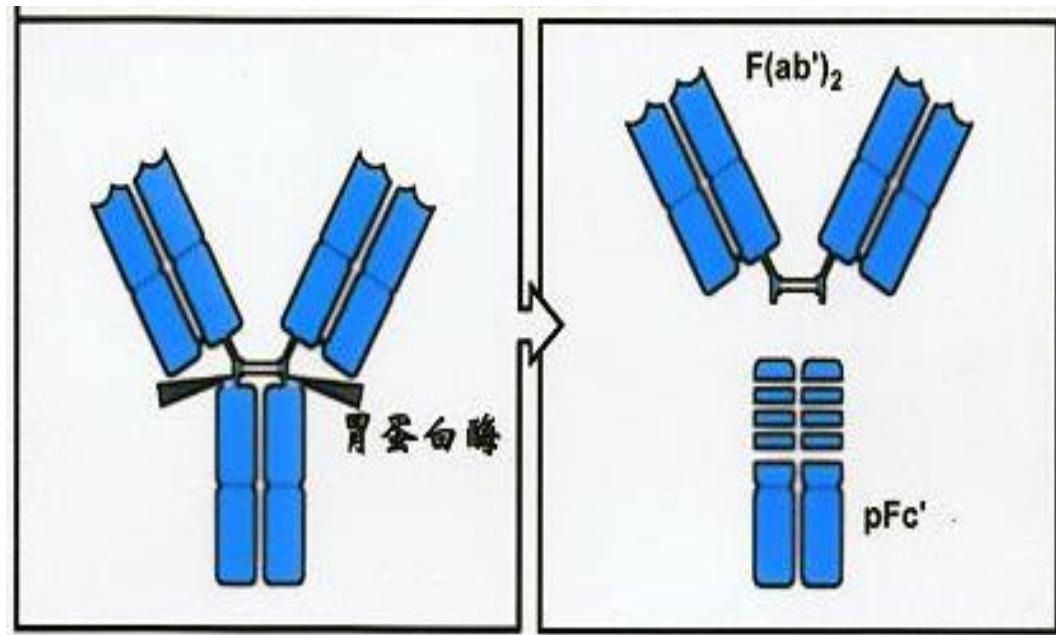
2. Digested by pepsin

Position:

- near the S-S bond of H inter-chains from the C end

Fragments and function :

- $F(ab')_2$: bind antigen(2 valence)
- pFc' : no function



Significance

- Elucidating the **relationships** between the structure and function of Ig's
- Decrease the **immunogenicity** of Ig for clinical treatment

Questions

1. List the functions of antibody?
2. What is cytokine?

Immunoglobulin classes (isotypes)

- The immunoglobulins are divided into 5 classes, based on the amino acid sequences **difference on constant region of the heavy chains**

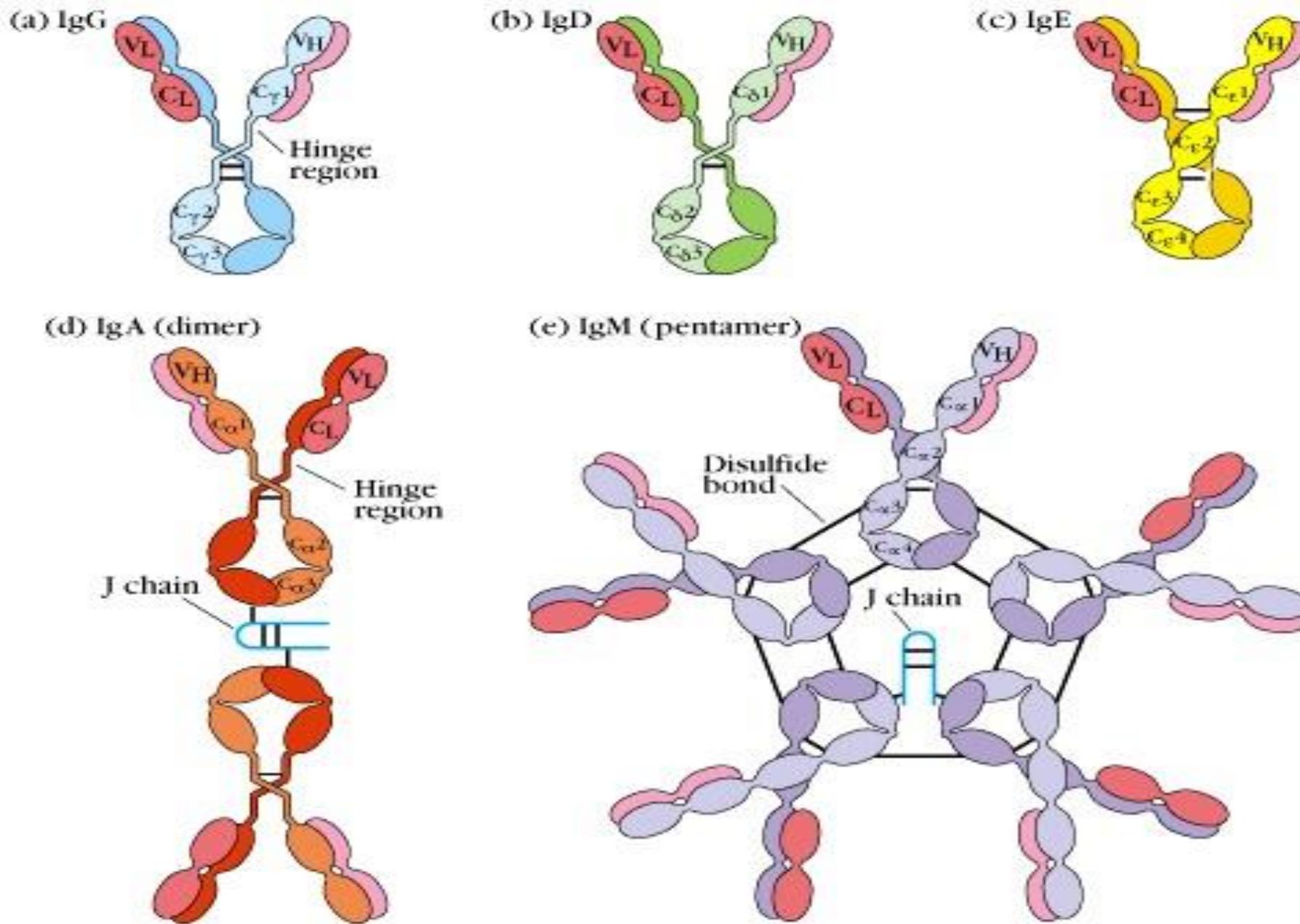
IgA - Alpha heavy chains (α)

IgE - Epsilon heavy chains (ϵ)

IgM - Mu heavy chains (μ)

IgD - Delta heavy chains (δ)

IgG - Gamma heavy chains (γ)



Immunoglobulin subclasses

- The classes of immunoglobulins can be further divided into **subclasses** based on **small differences** in the amino acid sequences of the constant region of the heavy chains (CH region).
- Only **IgG** and **IgA** immunoglobulins have subclasses.

IgG Subclasses

IgG1 - Gamma 1 heavy chains

IgG2 - Gamma 2 heavy chains

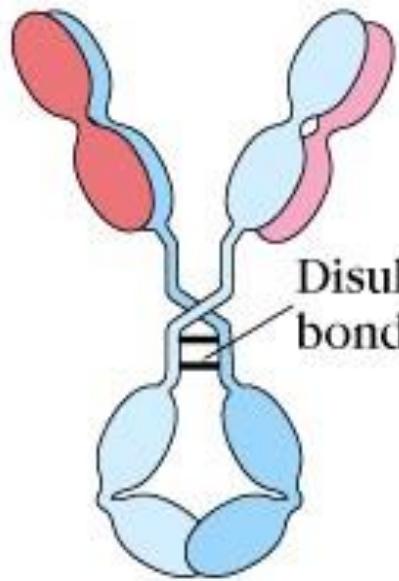
IgG3 - Gamma 3 heavy chains

IgG4 - Gamma 4 heavy chains

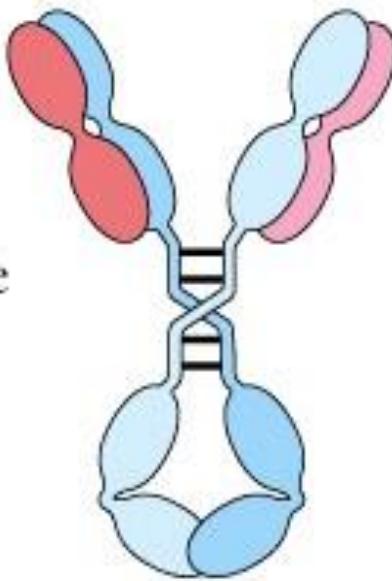
IgA Subclasses

- IgA1 - Alpha 1 heavy chains
- IgA2 - Alpha 2 heavy chains

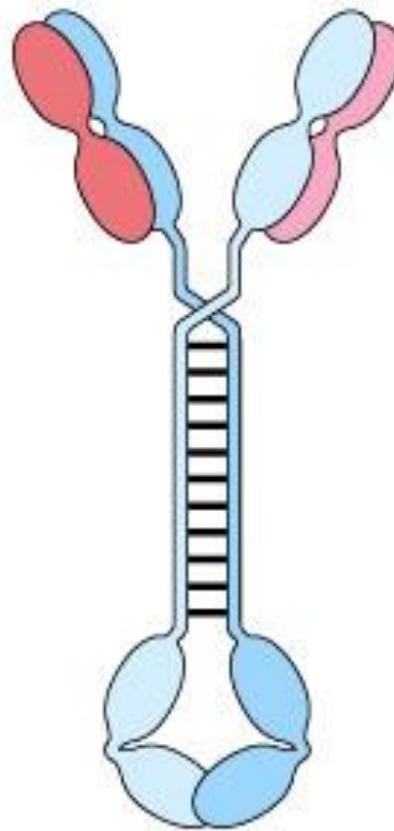
IgG1



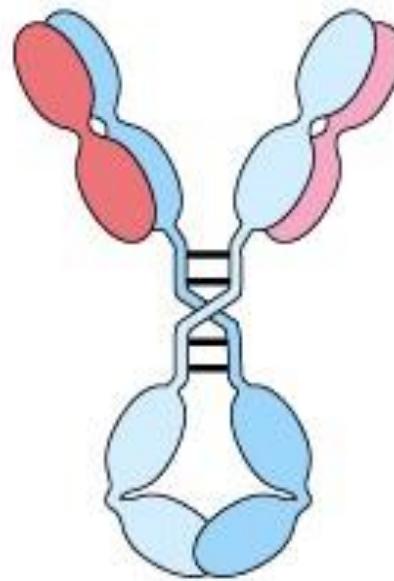
IgG2



IgG3



IgG4



Immunoglobulin Types

- Immunoglobulins are classified into **two types** based on amino acid sequence difference on **constant region of the light chain**:
 - **kappa** light chains (κ) and
 - **lambda** light chains (λ).

Immunoglobulin Subtypes

- Based on **small differences** in the amino acid sequences in the **constant region of the light chain**, the Lambda (λ) type is divided into four subtypes
 - $\lambda 1, \lambda 2, \lambda 3, \lambda 4$.

Table. Characteristics of immunoglobulins

Characters	Classes of immunoglobulin				
	IgG	IgM	IgA	IgD	IgE
Concentration in serum (%)	80	5-10	10-15	0.2	0.002
Form	monomer	pentamer	Mono/other	monomer	monomer
Half life (days)	21	5	6	3	2
Heavy chain	γ	μ	α	δ	ϵ
Light chain	κ or λ	κ or λ	κ or λ	κ or λ	κ or λ
No.of antigen site	2	10	4	2	2
Cross placenta	Yes	No	No	No	No
Present on mature B cell membrane	No	Yes	No	Yes	No

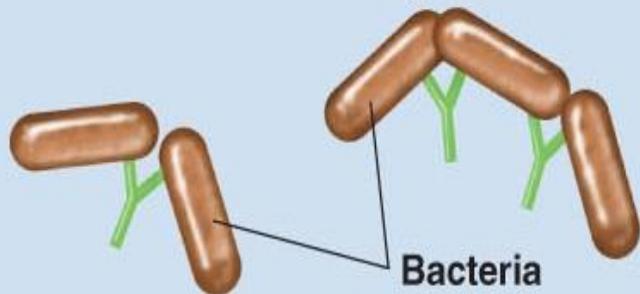
➤ Functions of antibody

1. Agglutination: Reduce number of infectious agents
2. Opsonization: Facilitate phagocytic cells to lysis pathogens
3. Neutralization
 - Prevent entering into the cell (blocking active sites)
 - Used to neutralize virus, toxins and bacteria
4. Antibody-dependent cell-mediated cytotoxicity (ADCC):
 - Activate ADCC
 - Used to destroy large organisms (e.g.: worms).
5. Activate complement system

Table. Summary of characteristics and functions of antibodies

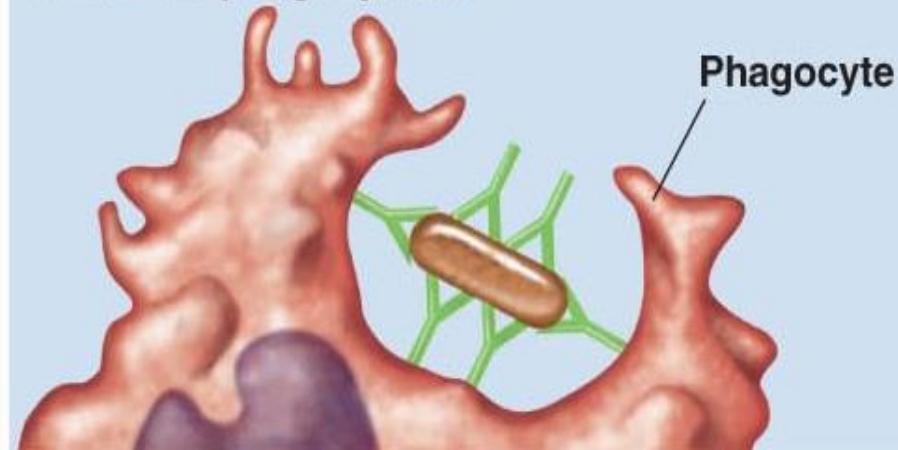
Antibodies	Character	Function
IgG	✓ predominant ✓ longest half life	<ul style="list-style-type: none">– Providing immunity for the new borne– Activate complement system– Opsonization– Neutralizing toxins and virus– agglutination– Participate in type II and III hypersensitivity
IgM	macroglobulin	<ul style="list-style-type: none">– Complement fixation– Agglutination– Opsonization– Toxin neutralization– Participate in type II and III hypersensitivity
IgA	mucosal antibody	<ul style="list-style-type: none">– Prevents microbial colonizing in mucosal surface– Neutralizing toxins
IgD	short half life	<ul style="list-style-type: none">– Regulate B cells maturation and differentiation
IgE	✓ least abundant ✓ allergic	<ul style="list-style-type: none">• Participate acute inflammatory reaction• Mainly involve against parasitic infections• Participate type I hypersensitivity

Reduces number of infectious units to be dealt with



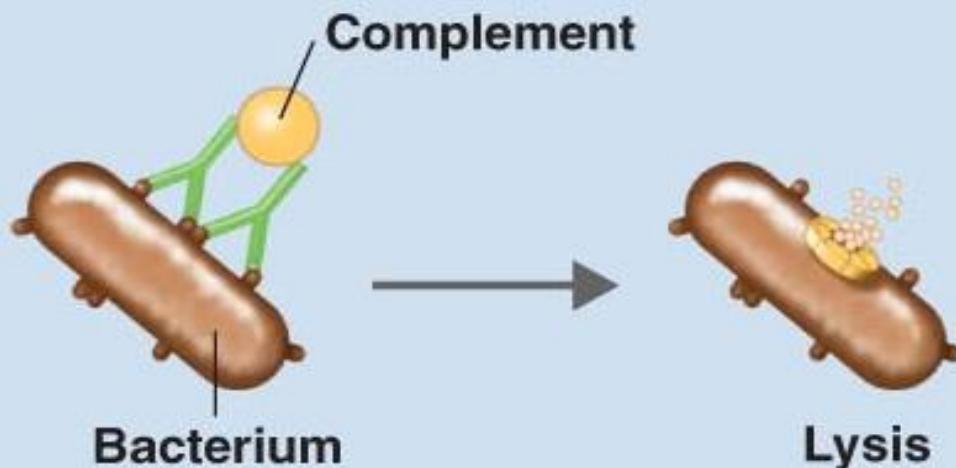
Agglutination

Coating antigen with antibody enhances phagocytosis



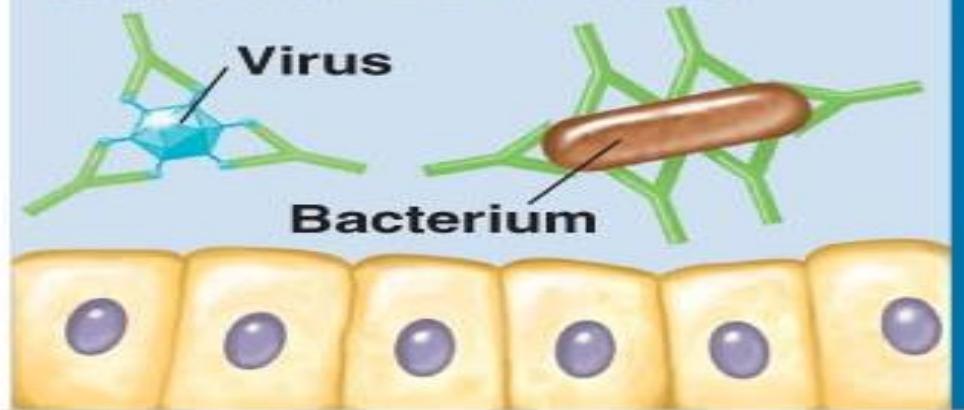
Opsonization

Causes inflammation and cell lysis

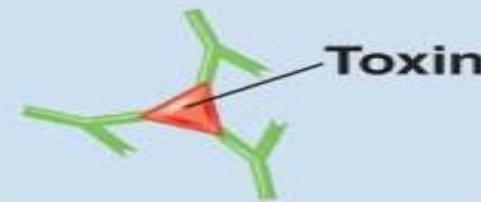


Complement activation

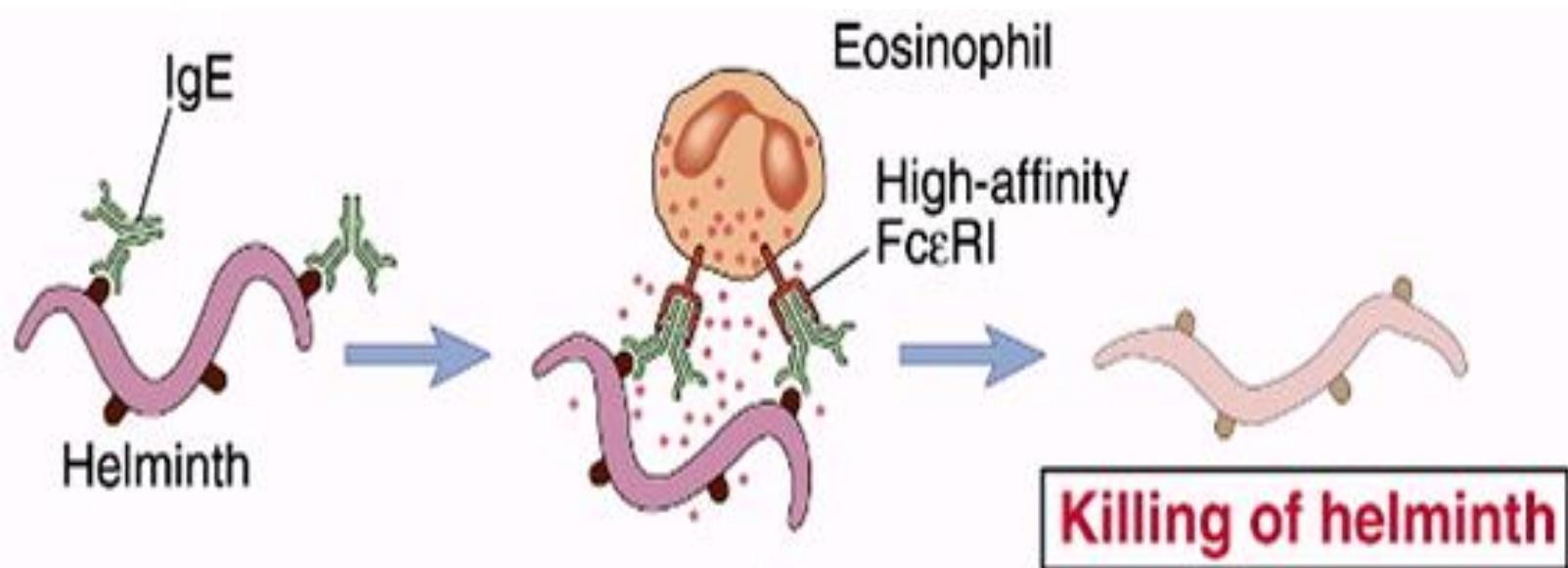
Blocks adhesion of bacteria and viruses to mucosa



Blocks attachment of toxin



Neutralization



Antibody-Dependent Cell-Mediated Immunity

Production of antibody from B cell

- The first exposure to a microbe or an antigen, either by **infection** or by **vaccination**, leads to the activation of naive B cell and result
 - **plasma** cells ---- **antibodies**
 - **memory B cells.**
- Some of the **antibody producing cells** migrate to the bone marrow
 - they live in this site for **several years**
 - they continue to **produce antibodies** even when antigen has been eliminated.

- Memory B cells
 - ✓ circulate into lymphoid and non-lymphoid compartments.
 - ✓ survive for long periods without antigenic stimulation.
 - ✓ provide rapid antibody responses
- When the same antigen enters the body again
 - the circulating antibodies provide immediate protection
 - at the same time, memory cells provides high level of protection.

T-dependent vs T-independent antibody production

T-cell dependent antibody production

- ✓ B cell responses to **protein antigens**
- ✓ B cell requires assistance from **T helper cells** for antibody production
- ✓ produce **both** antibody and memory
- ✓ primarily produce **IgG** antibodies

Procedure for T cell-dependent antibody production

1. B cell **binds antigens** through its receptors (IgM or IgD)
2. B cell **degrades** protein into peptide, express class II MHC and co-stimulatory B7
3. class **II MHC bind** the peptide and display on the surface
4. B cell **presents** antigen into CD4 Th cells and B cell express B7 and CD40
5. Antigen presentation and B7 expression **activate** CD4 Th cell which secrete **cytokines** and **CD40L**

6. B cell express receptors for cytokines

- Secretion of cytokines and ligation of CD40L result the **activation of B cells**

7. The activated B cell enter into **germinal center**

8. B cell are **proliferated** in dark zone

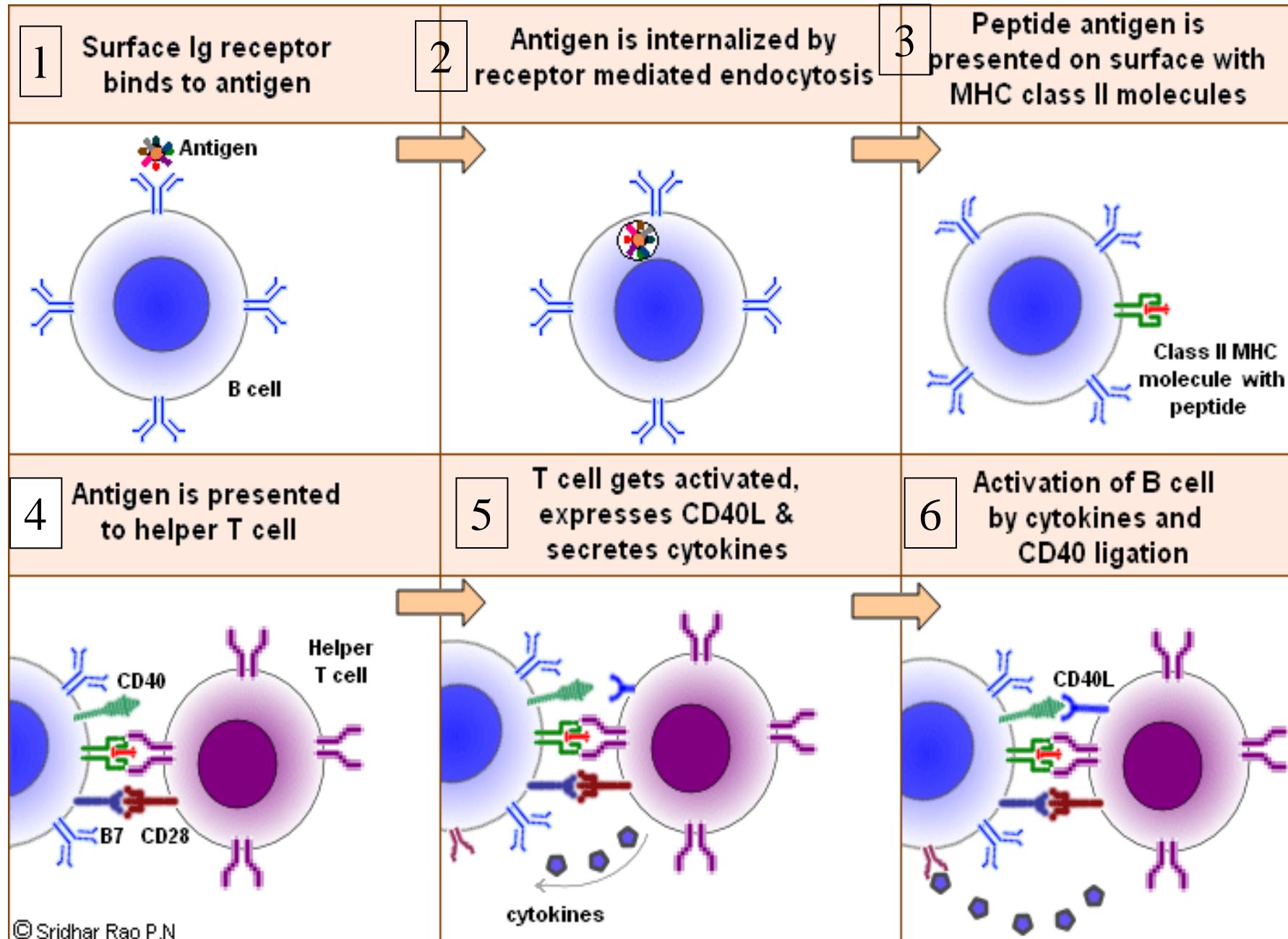
9. Somatic **hypermutation**

10. **Selection** high affinity B cells in light zone by introducing **antigens**

- Differentiate B cells either binding or non-binding antigens

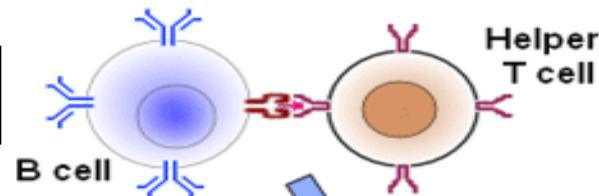
11. Apoptosis

- Non-antigen binding B cells under go **apoptosis**
- Antigen binding B cell differentiate into **plasma** and **memory cells**



Activated B cells migrate into the germinal center

7



B cell proliferation in the dark zone

8

9

10

11

B cell recognition of antigen on follicular dendritic cells and selection of high-affinity B cells

Apoptosis of B cells that do not bind to antigens

High-affinity B cells exit lymph node

Generation of antibody secreting B cells and memory B cells

Germinal center in the follicle of lymph node

Antibody secreting B cell

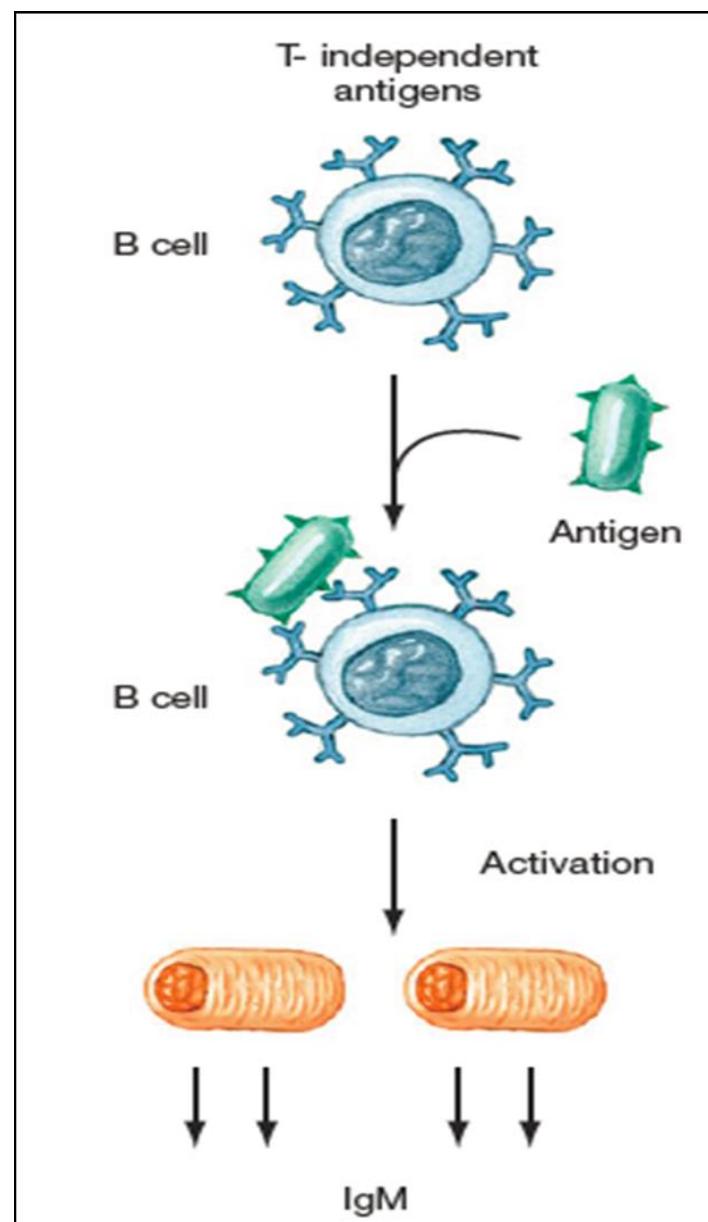
Memory B cell

➤ T cell-independent antibody production

- ✓ B cell responses to **non-protein** antigens: example:
polysaccharides or **lipopolysaccharide** with repeating subunits
(bacterial capsules).
- ✓ does **not** require assistance from T cells
- ✓ produces only **antibodies**
- ✓ **Lacks** production of memory
- ✓ produces primarily **IgM** antibodies

Procedure for T cell independent antibody production

1. B cell receptor binds antigen
2. B cell is activated
3. B cell activation leads its proliferation and differentiation into plasma cell
4. plasma cells secrete antibodies



Clonal selection and deletion

- **Clonal selection:** When different B cell receptors encounter an antigen, **only one B cell receptor** that binds the antigen is stimulated, proliferated and differentiated into **plasma cells and memory B cells**
- **Clonal deletion:** B cells are destroyed through **apoptosis** when they
 - ✓ are not bind foreign antigen and
 - ✓ bind self antigen

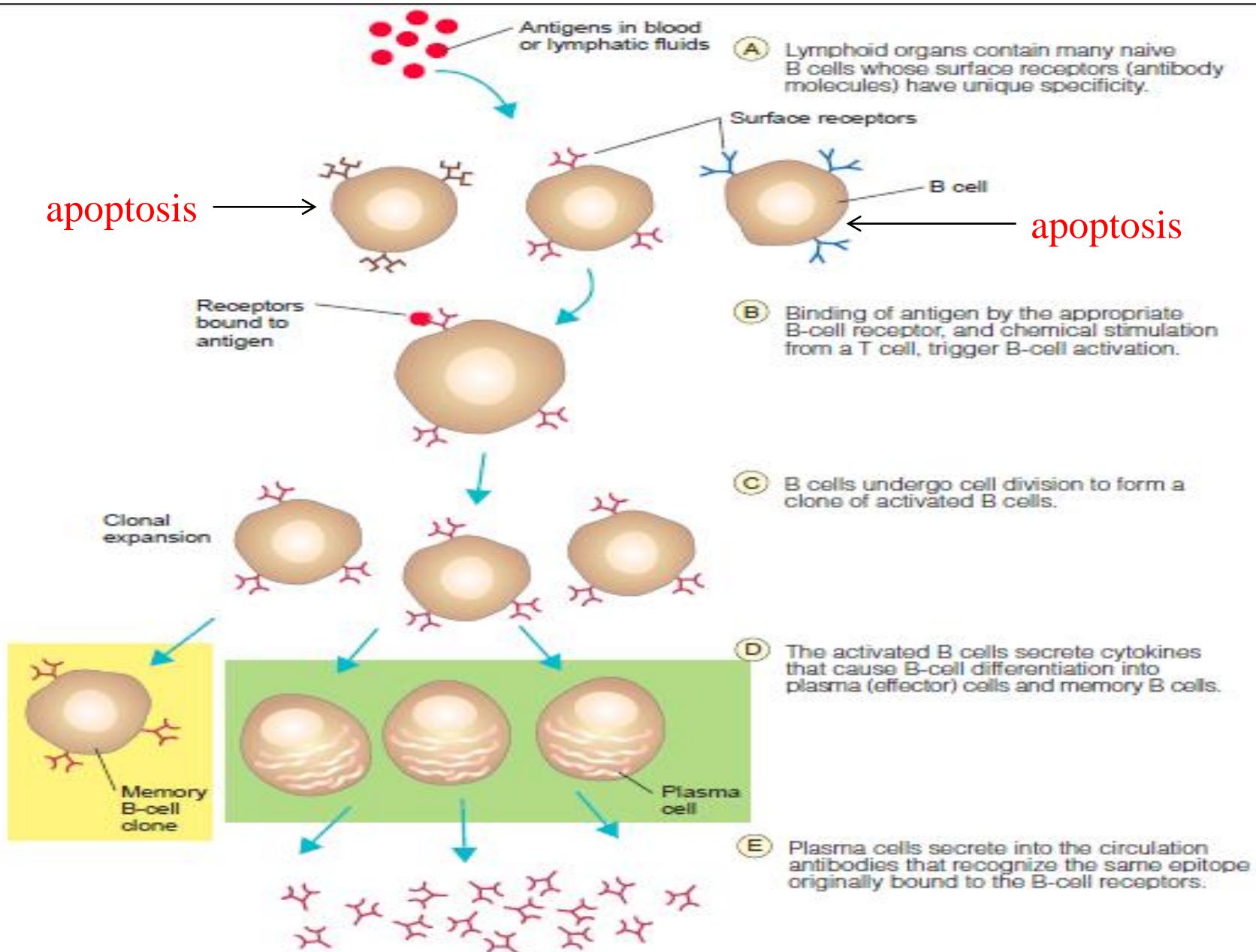


Figure: Clonal selection and deletion

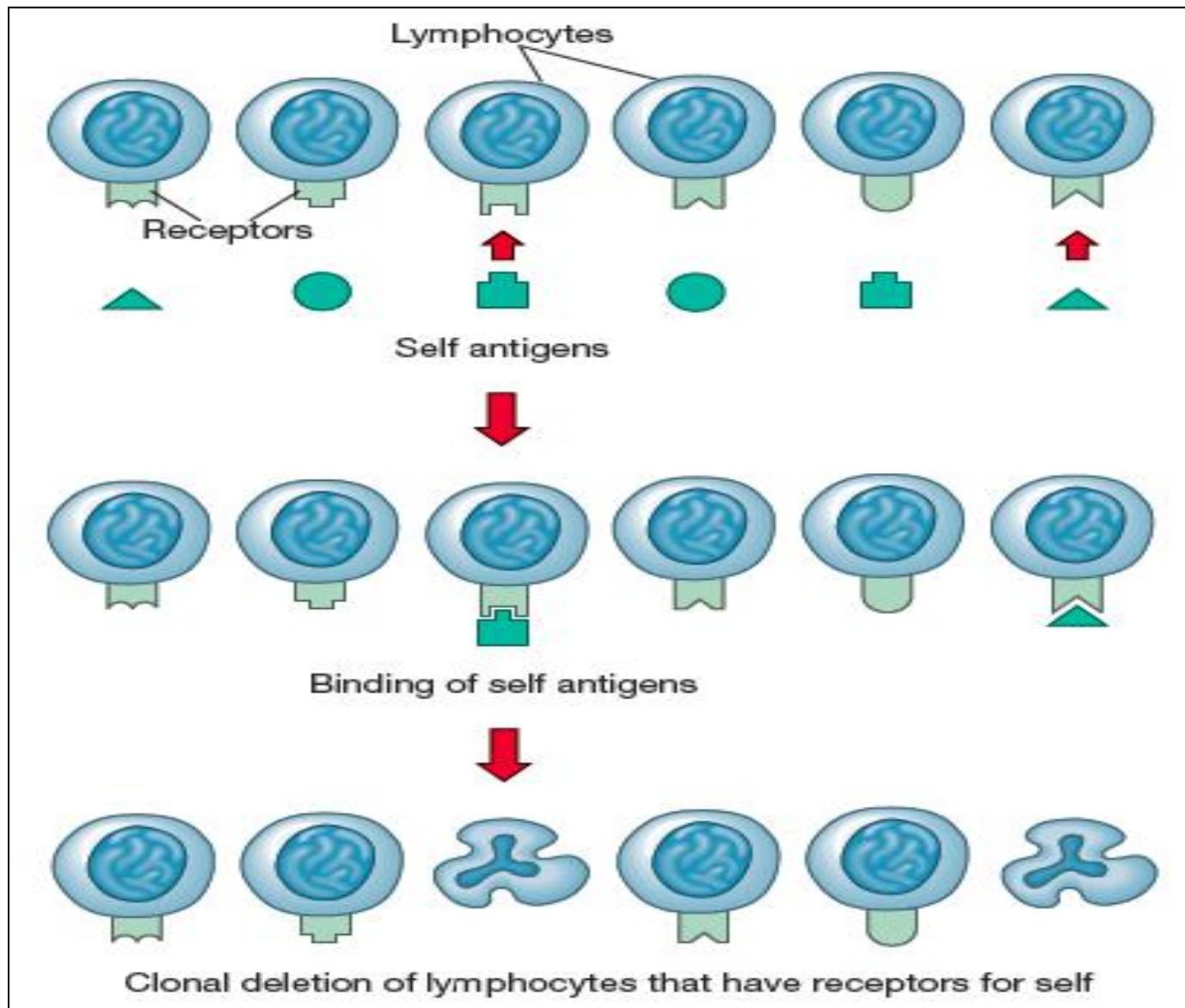


Figure: Clonal deletion

Apoptosis

- ✓ programmed cell death
- ✓ is occurred due to
 - To remove non-antigen binding B cell
 - To remove self binding B cells
 - To remove old cells
 - To maintain production of cells in the body
 - To remove infected cells (e.g. virus infected cells)
 - To remove abnormal cells

Apoptosis

Programmed cell death as result of
oldness of the cell or homeostasis

Proteolytic, lytic enzymes, cationic proteins,& oxidase molecules are
not released in to the surrounding tissue

Does **not induce a local inflammatory** response

Have role in maintaining proper number of cells (i.e. homeostasis)

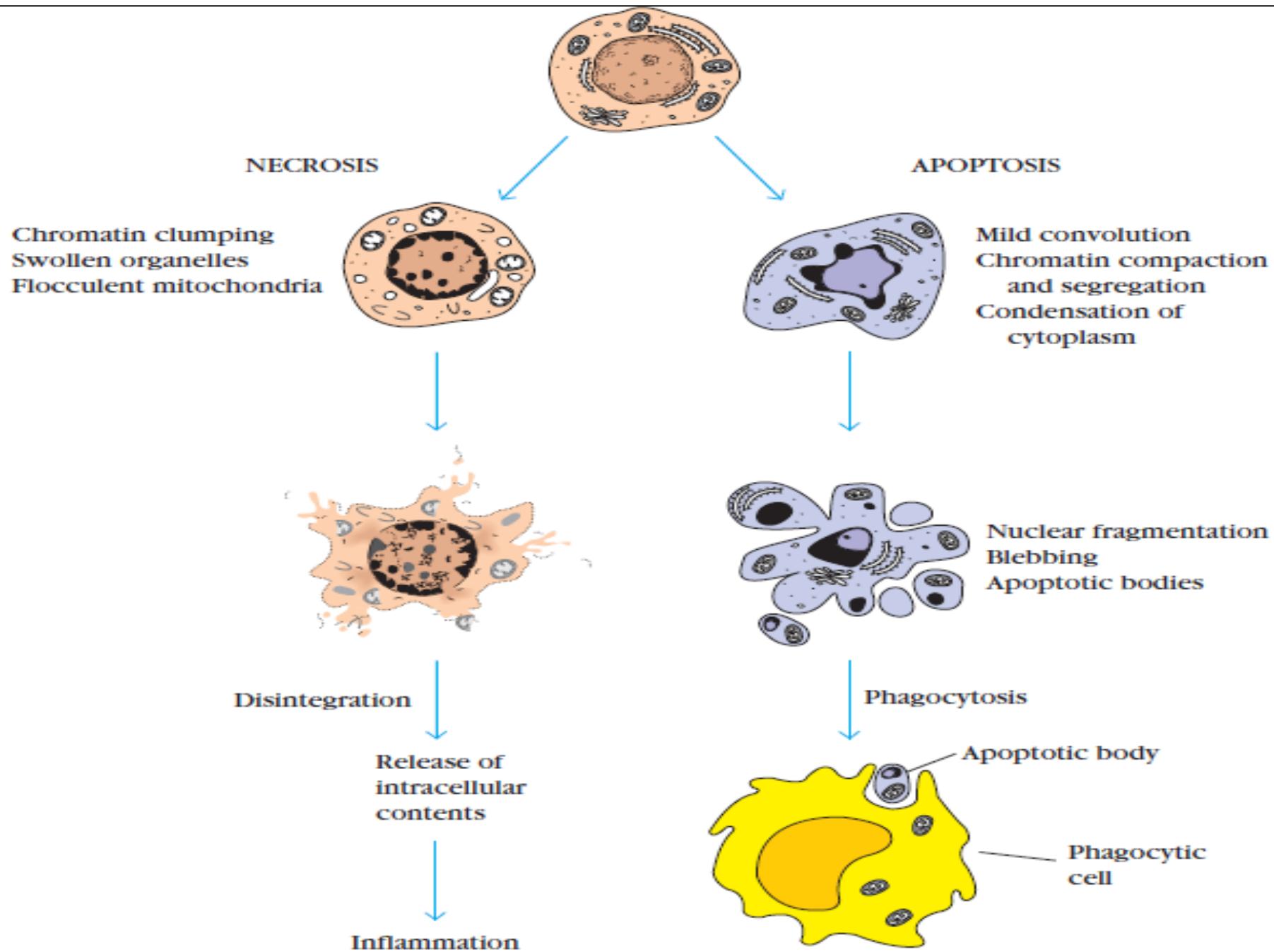
Important in immunological process “ Tolerance”

Necrosis

Cell death arising from
injury

Injured cell swells and bursts which **are released** its contents

Induce inflammatory response



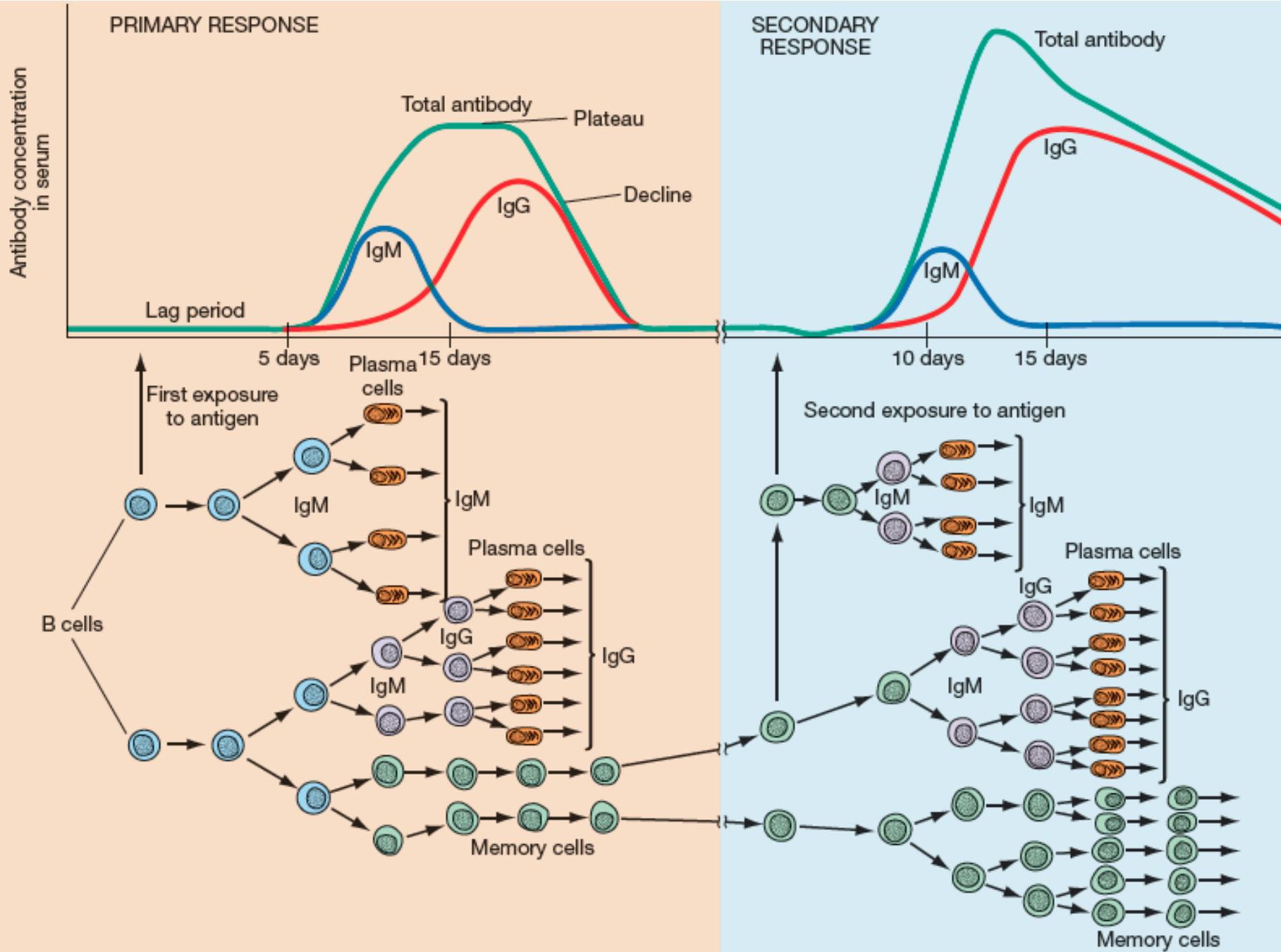
Pattern of Antibody Levels During Infection

➤ Primary Response:

- ✓ Stimulate **naïve B cells** (1st exposure)
- ✓ Require **long time** for production antibody
- ✓ Most B cells become **plasma cells**
- ✓ Produce **less numbers** of antibodies
- ✓ A gradual increase in **IgM** and then of **IgG** is observed

➤ Secondary Response

- ✓ Response by **memory** B cells
- ✓ Occur after **second exposure** to the same Ag
- ✓ Require **short time** for antibody production
- ✓ Produce **high number** of antibodies
- ✓ Display a **faster** response



❖ Phases of primary antibody response

1. Latent or lag phase: antibody is detectable after 1 to 2 weeks
2. Log (Exponential) phase: the concentration of antibody increases exponentially
3. Plateau (Steady state): production and degradation of antibody are balanced
4. Declining (decay) phase: antibody degradation exceeds that of antibody synthesis. Finally, the level of antibody may reach base line levels.

Table: Comparison of primary and secondary responses

Property	Primary response	Secondary response
Responding B cell	Naïve (virgin) B cell	Memory B cell
Lag period following antigen administration	Generally 4-7 days	Generally 1-3 days
Time of peak antibody response	7-10 days	3-5 days
Magnitude of peak antibody response	Varies depending on antigen	Generally 100-1000times higher than primary response
Isotype produced	IgM predominates early in the response	IgG predominates
Antigen	Thymus dependent and independent	Thymus dependent
Antibody affinity	Lower	Higher

Antigen (Ag)

- ✓ is a **foreign molecule**
- ✓ causes the body to **produce specific** antibodies or sensitized T cells
- ✓ Coined from Antibody generator (Antigen means **antibody generation**)
- ✓ is **mostly proteins** or large polysaccharides
- Proteins and polysaccharides induce strong response
- Proteins are highly antigenic –**size and structural complexity**
- Lipids and nucleic acids **are haptens**

➤ Antigens may be

✓ **Microbes:**

- Capsules, cell walls, toxins, viral capsids, flagella, etc.

✓ **Non-microbes:**

- Pollen, egg white, red blood cell surface molecules, serum proteins, and surface molecules from transplanted tissue.

➤ Based on their origins:

- Exogenous antigens, endogenous antigens and autoantigens

➤ Exogenous antigens

- ✓ have been **entered** the body from the outside
- ✓ for example: **inhalation, ingestion, or injection.**

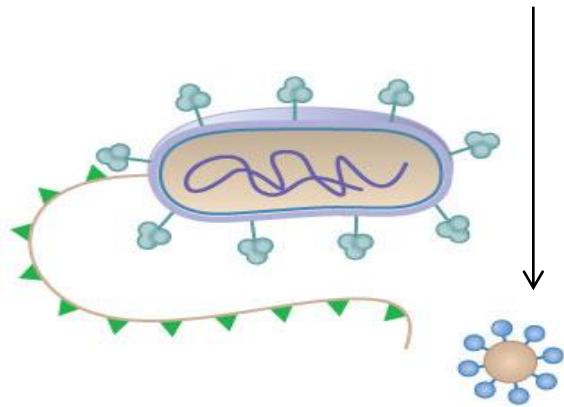
➤ Endogenous antigens

- ✓ have been **generated** within cells
- ✓ for example: **viral proteins, cancer cells**

➤ Autoantigens

- ✓ **self antigen:** usually normal proteins
- ✓ under **normal** conditions, **not be targeted** of the immune system
- ✓ but the **lose** of immune tolerance, be **targeted**
- ✓ **lose** of tolerance due to **genetic** and **environmental** factors

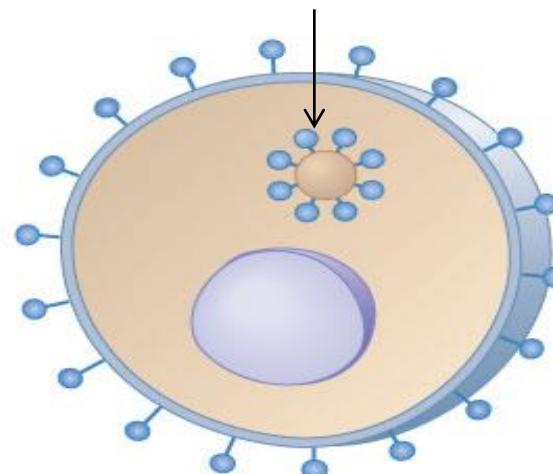
Extracellular microbes



(b)

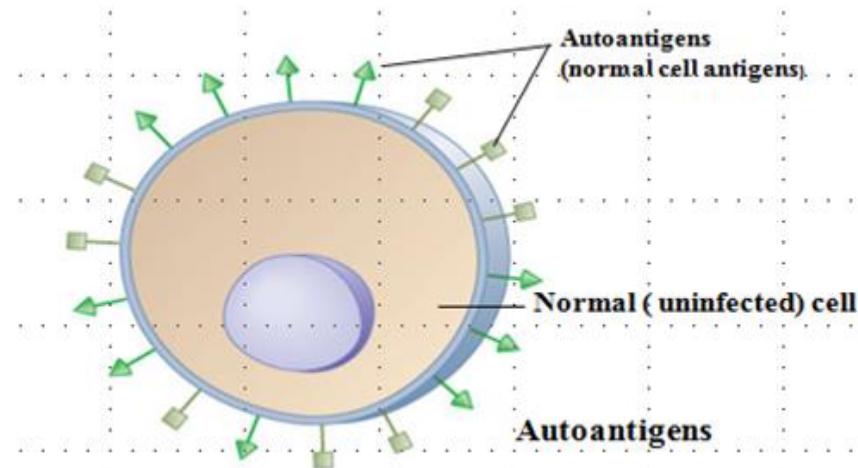
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Intracellular microbes



(c)

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(d)

➤ Antigen-Presenting Cells (APC)

- ✓ Known as professional antigen-presenting cells
- ✓ Have two unique properties:
 - (1) they express **class II MHC** molecules
 - (2) they serve as **co-stimulatory** signal B7 (Th cell activation)

➤ There are three types APCs :

1. dendritic (**DC**) cells

- constitutively express **MHC I, MHC II and B7**
- ingest antigens by **pinocytosis**
- can present antigens with class I MHC (**cross presentation**)

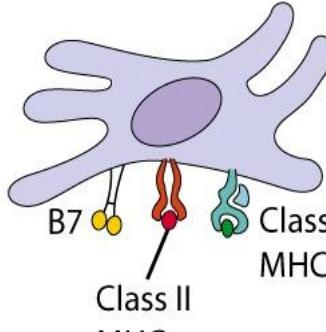
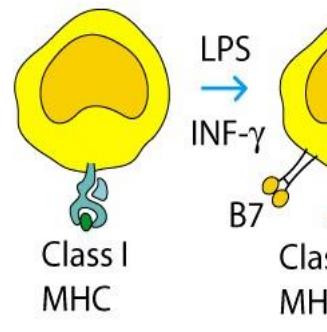
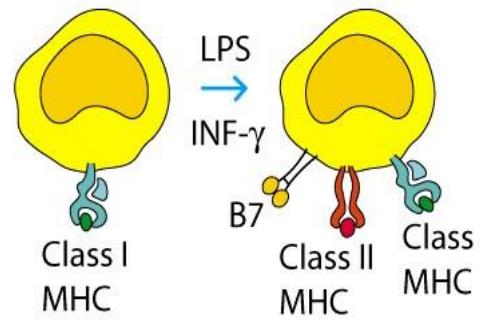
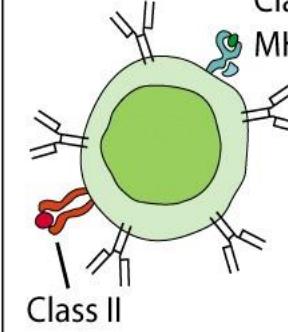
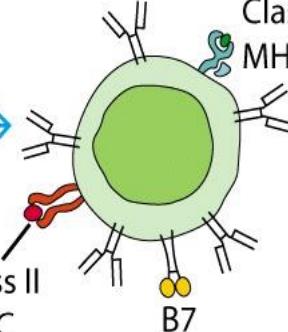
2. Macrophages

- ✓ express **little** MHC II or B7 (**resting**)
- ✓ express **high levels** of B7 and MHC II (**activated**)
- ✓ ingest Ag by **phagocytosis**

3. B cells

- ✓ express high levels of **MHC II**, but not B7.
- ✓ microbial **cell wall components** can induce **B7** expression
- ✓ can take up **antigen** through their Ig receptors (**endocytosis**)

Table: Comparison among antigen-presenting cells

	Dendritic cell	Macrophage		B Lymphocyte	
					
Antigen uptake	Endocytosis phagocytosis (by Langerhans cells)	Phagocytosis	Phagocytosis	Receptor-mediated endocytosis	Receptor-mediated endocytosis
Class II MHC expression	Constitutive (+++)	Inducible (-)	Inducible (++)	Constitutive (++)	Constitutive (+++)
Co-stimulatory activity	Constitutive B7 (+++)	Inducible B7 (-)	Inducible B7 (++)	Inducible B7 (-)	Inducible B7 (++)
T-cell activation	Naive T cells Effector T cells Memory T cells	(-)	Effector T cells Memory T cells	Effector T cells Memory T cells	Naive T cells Effector T cells Memory T cells

Major histocompatibility complex (MHC)

➤ **characteristics of MHC**

- ✓ are **glycoproteins**
- ✓ **cluster of genes**
- ✓ are **polygenic** (each cell has many MHC genes)
- ✓ are **polymorphic** (there are many alleles for each locus)
- ✓ have almost **identical 3-D structure.**
- ✓ play role in **discriminating** self/non-self
- ✓ found in the membranes of most cells of **vertebrate animals**
- ✓ participant in **both** humoral and cell-mediated immunity

➤ Type of MHC

Class I MHC

- ✓ important in cytotoxic responses (**CD8+** Tc cells)

class II MHC

- ✓ important in humoral responses (**CD4+** Th cells)

Note:

- MHC genes do not undergo recombination.
- Human MHC are called human leukocyte antigen (HLA)

➤ Location of MHC

- Class I MHC found on **all nucleated cells**
- Class II MHC found only on antigen presenting cells (**APC**)

➤ Function:

✓ class I MHC:

- Displays peptides derived from antigen originating **inside the cell** to **CD8⁺** (cytotoxic responses).

✓ Class II MHC:

- Displays antigen derived from ingested antigens (**exogenous antigen**) to **CD4⁺** (humoral immune responses)

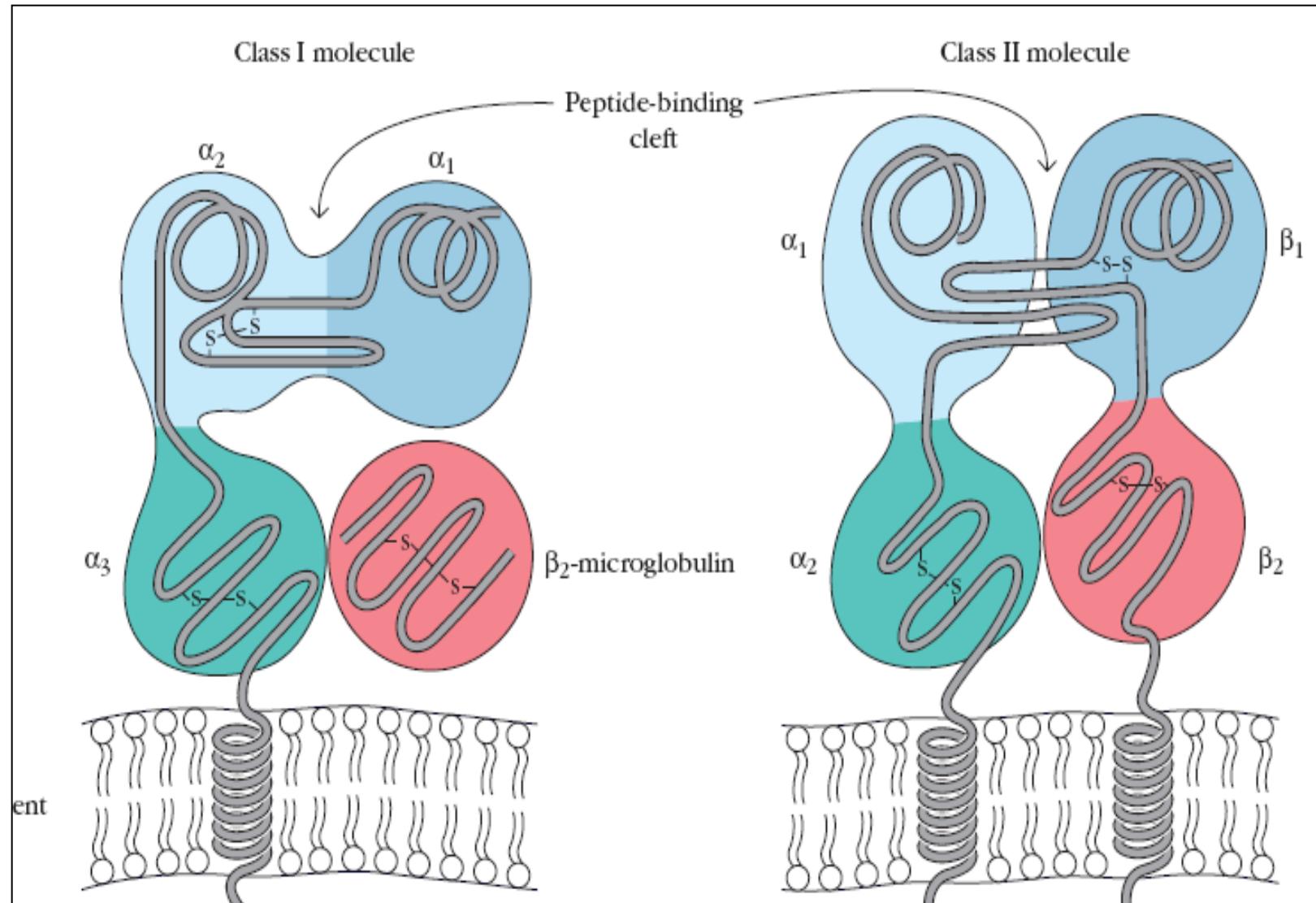


Figure: Schematic diagrams of a class I and a class II MHC molecule

Cytokines (CKs)

➤ Characteristics of CKs

- ✓ low molecular weight proteins/ glycoproteins
- ✓ are primarily produced by immune system but many other organs (liver, brain, endocrine glands) make CKs to influence immune response.
 - The biggest producers: macrophage (MΦ) and T helper cells
- ✓ are involved in both the innate and adaptive immune response
- ✓ regulates the immune system (via cell to cell communication)
- ✓ can induce both protective and damaging responses

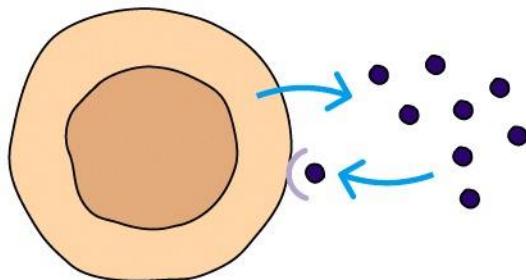
Classification of cytokines

- CKs are classified accordingly to the **cells** produced them
 - **Lymphokines:** secreted by **lymphocytes**
 - **Monokines:** secreted by **monocytes and macrophages**
 - **Chemokines:** chemotactic activities
 - **Interleukins:** produce by leukocytes and acts on other leukocytes

- Depending on **their function**, CKs can be grouped as
 - Pro- inflammatory, TNF, IL-1, IL-6 and chemokines
 - Anti-inflammatory:- IL-10, TGF β
 - Inhibition of viral replication:- INF α , IFN β
 - Macrophage activating:- IFN γ
 - B- Cell activating:- IL-4, IL-5, IL-6, IL-21
 - T- Cell activating: IL-2, IL-4, IL-12, INF γ
 - Eosinophil- and / or mast cell activating:- IL-3, IL-4, IL-13, IL-5

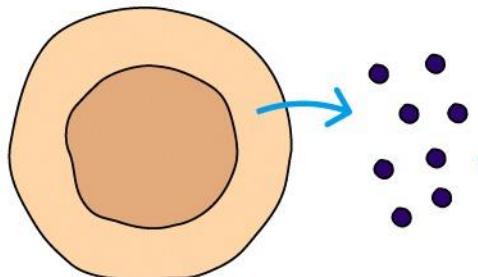
➤ Action of Cytokines

- autocrine: the **same** cell that secreted cytokine
- paracrine: a **nearby** cell
- endocrine: a **distant** cell reached through the circulation



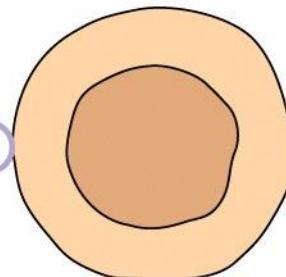
IL-2 for
T-cell activation

Autocrine action

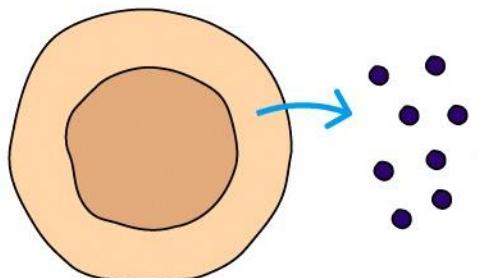


T-cell help
for B cells

Paracrine action

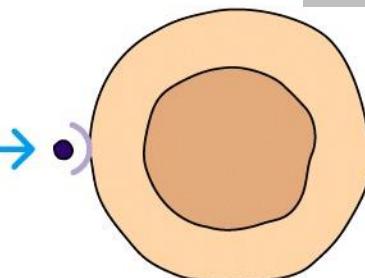


Nearby cell



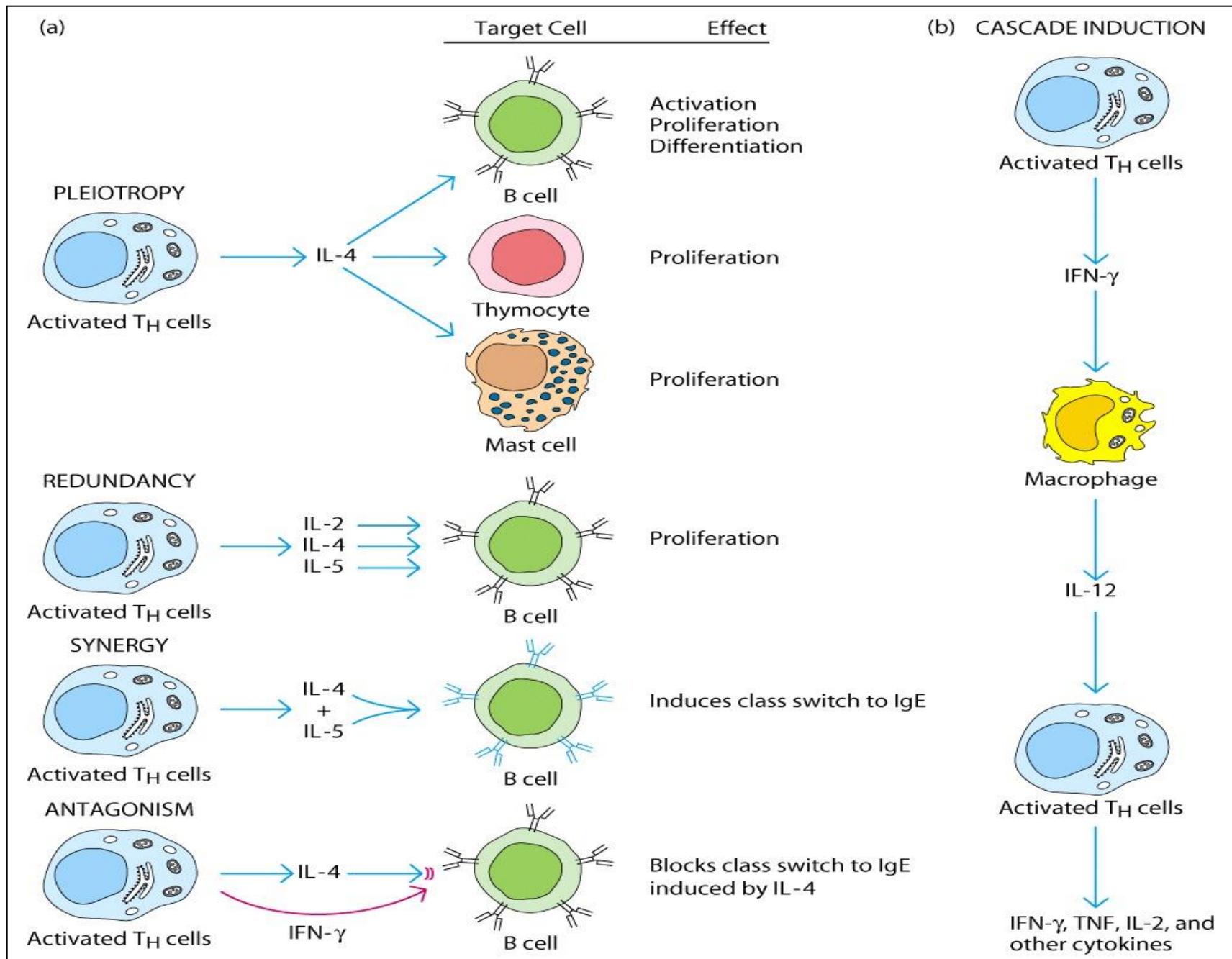
Inflammatory
cytokines

Endocrine action



Distant cell

- Cytokines exhibit the following attributes or properties
 - a) **pleiotropic** ... one cytokine can have different effects on different cells.
 - b) **redundant** ... different cytokines can have the same effects.
 - c) **synergy**... Combined effect of two cytokine on cellular activity is greater than the additive effects of the individual cytokine
 - d) **antagonism**... The effects of one cytokine inhibit or offset the effects of another cytokine
 - e) **cascade effect**...CKs stimulate cells to produce other cytokines.



Biological Functions of cytokines

- Cytokines are involved in a staggeringly broad array of biological activities including
 - innate immunity, adaptive immunity, inflammation, and hematopoiesis.
- Cytokines regulate the intensity and duration of the immune response by **stimulating or inhibiting**
 - activation, proliferation, and/ or differentiation of various cells and
 - the secretion of antibodies or other cytokines.

Cytokines in Hematopoiesis

- cytokines regulate hematopoiesis differentiations and developments of into a particular lineages. E. g.
 - IL-3, IL6 --- induce formation of **myeloid cells**
 - IL-7 - induces the differentiation of **lymphoid** progenitor
 - M-CSF and G-CSF ---- **monocytes** and **granulocytic** cells, respectively.
 - IL-4 - stimulates **B** progenitors, **mast** progenitors, and **basophil** progenitors
 - IL-5 - stimulates **eosinophil** progenitor
 - IL-8 - stimulates the **neutrophil** progenitor
 - IL-9 - stimulates **mast cell** growth

Cytokines in development of T cell subsets

- The development of TH and Treg subsets is determined by:
 - IL-12 and IFN- γ --- activate **Th1** cell production
 - IL-4 --- **Th2** cell
 - IL-6 and TGF- β ---- **Th17** cells
 - IL-2 and TGF- β --- **Treg** cells.
- Th cells determine adaptive immune response through cytokines production
 - **T_h1 cells**
 - produce IL-2, IFN- γ , TNF- β cytokines
 - activate cell mediated immunity
 - **T_h2 cells**
 - Produce IL-4, IL-5, IL-6 cytokines
 - activate humoral immunity

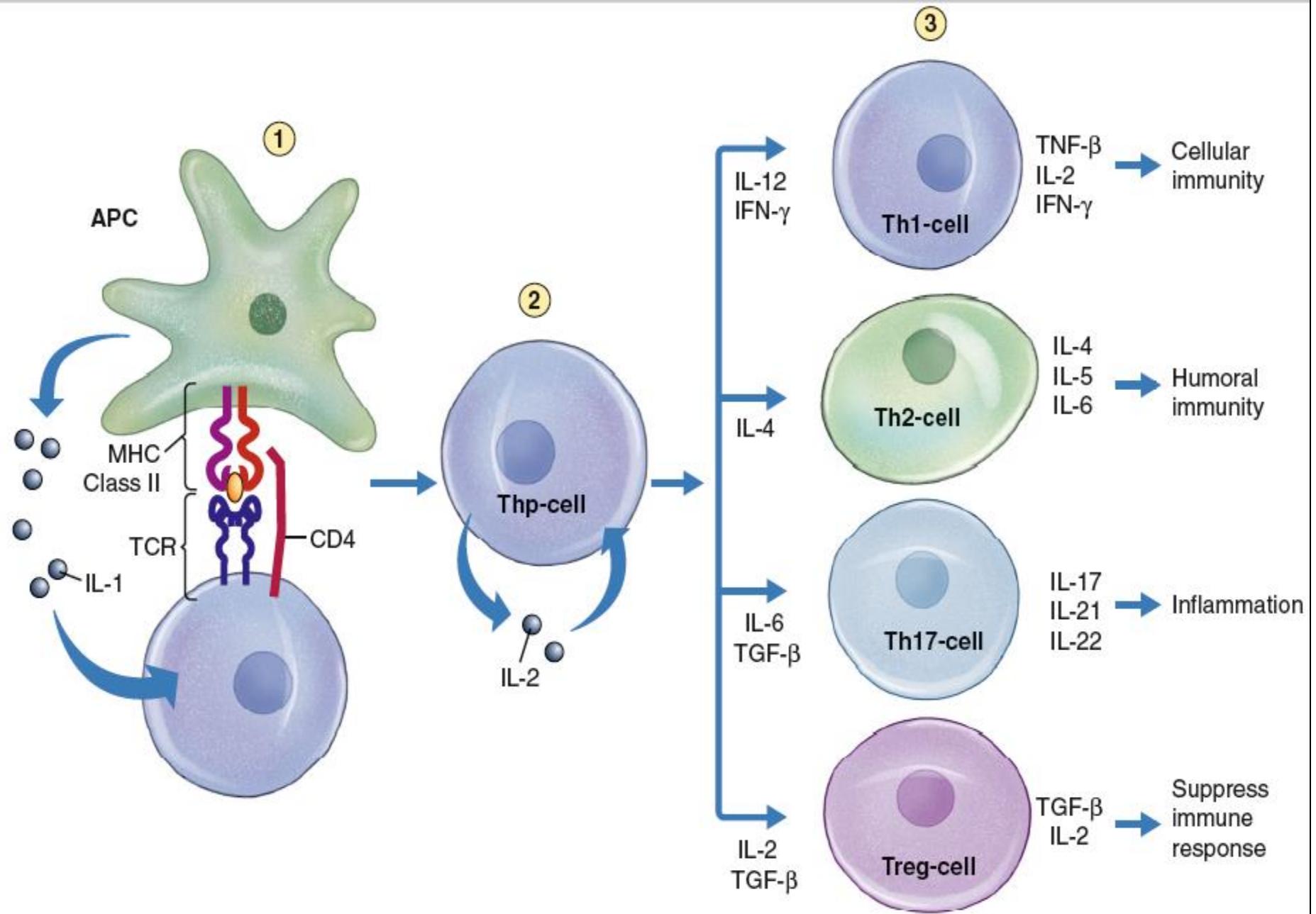


Figure: Development of T Cell Subsets

➤ cytokines produced by TH1 and TH2 subsets have two characteristic effects on subset development.

A. Cascade effect

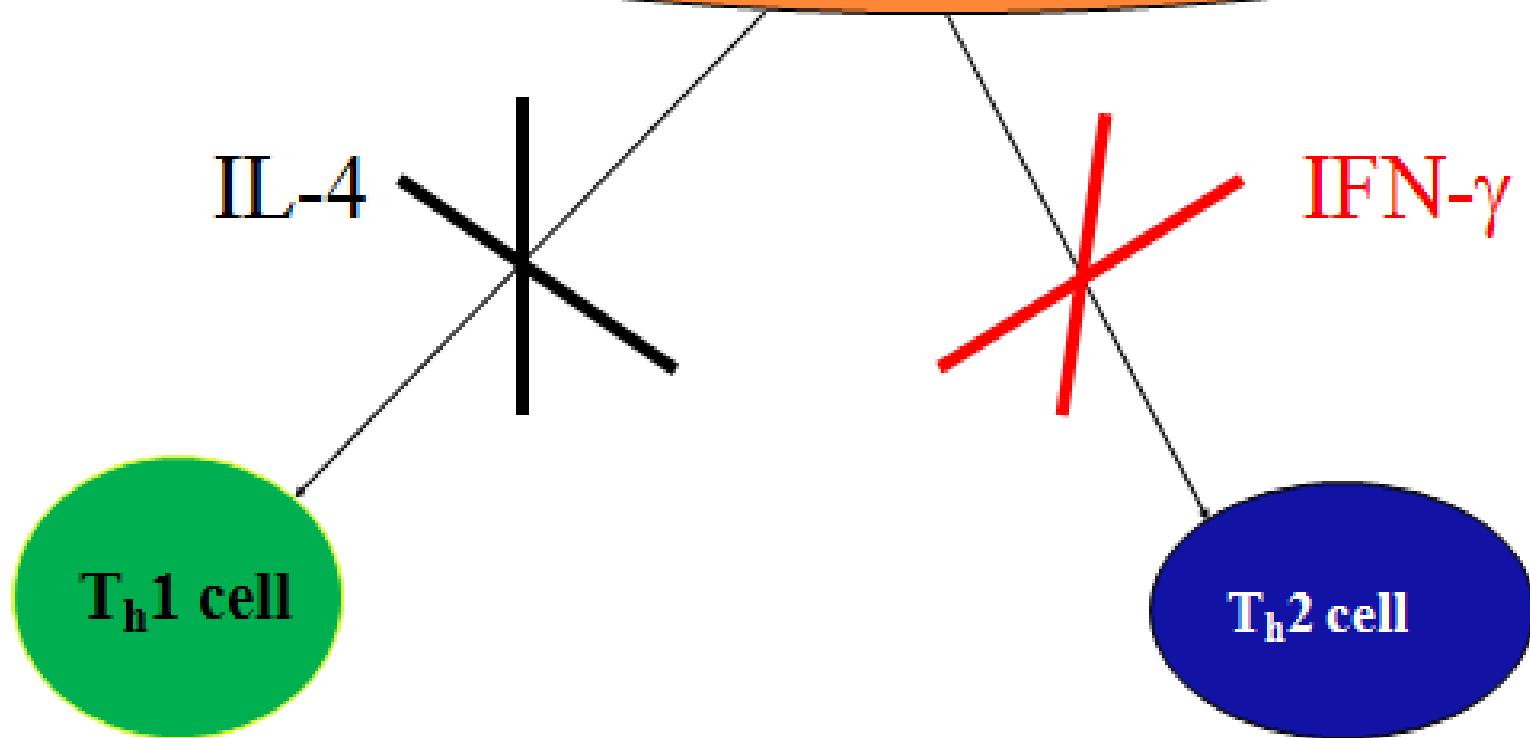
- ✓ Promote the growth of the subset that produces them

B. cross-regulation/ antagonism

- ✓ Inhibit the development and activity of the opposite subset cytokine. E. g.

- IFN- γ (secreted by the Th1 subset)
 - inhibits proliferation of the Th2 subset
- IL-4 and IL-10 (secreted by the Th2 subset)
 - inhibit Th1 differentiation

naive T_h0 IL-2, IFN- γ , TNF- β and IL-4, IL-6, IL-10



Cell-mediated immunity (CMI) response

- ✓ protect the body by activating **CTLs, macrophages and NK cells**
- ✓ stimulate cytokines secretion
- ✓ participates in defending **intracellular microbes** (fungi, protozoans, bacteria), cancers, and transplant rejection.
- ✓ **kill cells** that have been infected by viruses

➤ Cytotoxic T Cells (Tc cells)

- ✓ known as killer T cell or cytotoxic T lymphocyte (CTL)
- ✓ is also Known as cellular immunity
- ✓ involve the production of CD8 T cell
- ✓ recognize Ag-MHC I complex
- ✓ target cells are self carrying endogenous antigens
- ✓ kill the target through cytolysis or induce apoptosis
- ✓ destroy intracellular microbes, cancer cells, transplanted tissue and cells infected with virus

➤ **Phases of CMI:**

1. Antigen processing and presentation
2. Antigen recognition
3. Activation of Tc cell
4. Effector function
5. Decay

Antigen (AG) processing and presenting

AG processing

- is a generation of peptides from proteins

AG presenting

- is a display of the AG peptides in complex with MHC I molecules on the surface of the cell, **CD8 Tc cell**

Procedure

1. antigens are degraded into peptide fragments using proteasome
2. class I MHC molecule bind degraded peptide fragments and present to CD8+, cytotoxic T cells
3. cytotoxic T cells kill the presented antigens

(7) Peptide is presented on MHC class I to CD 8+ cytotoxic T cell

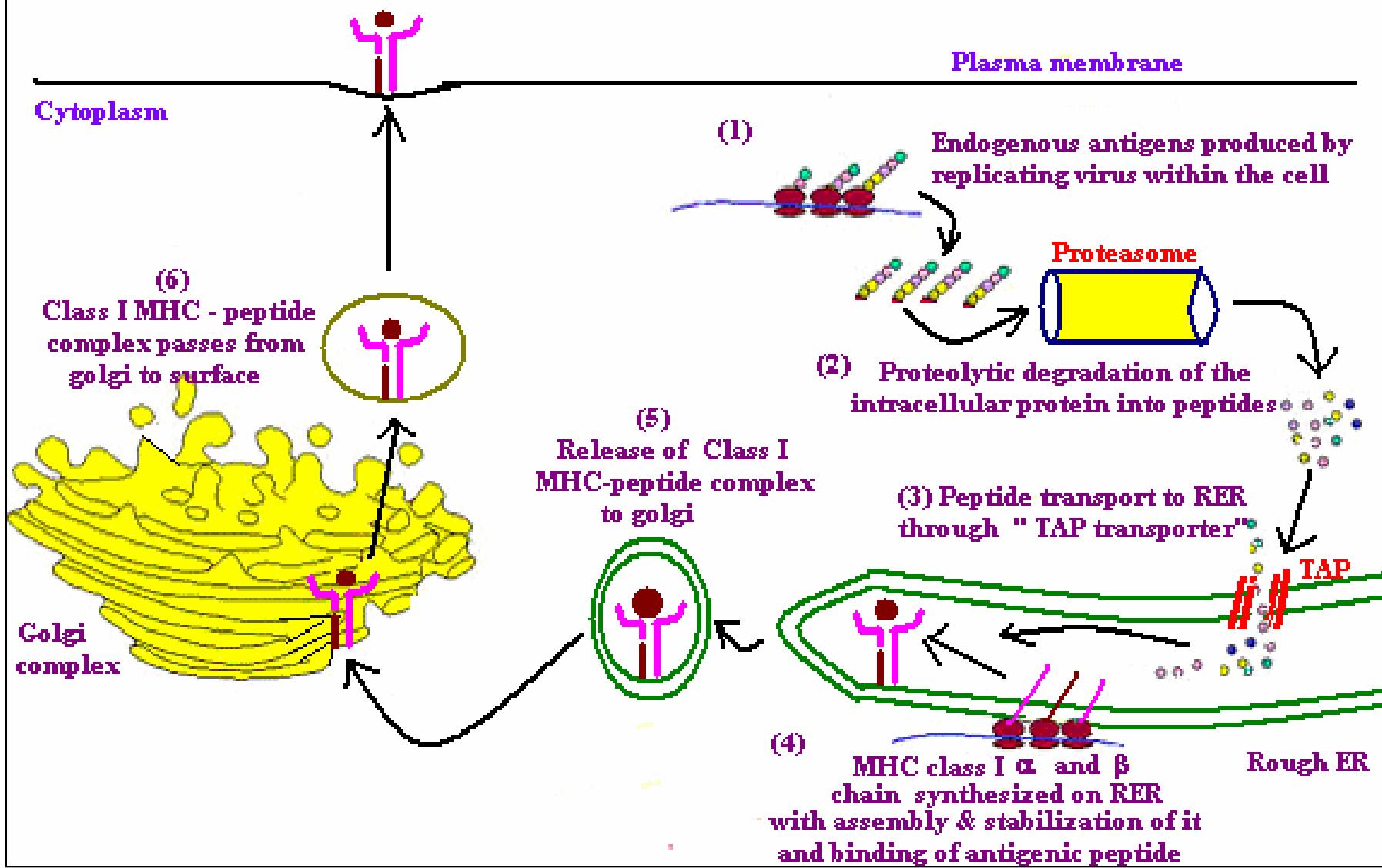
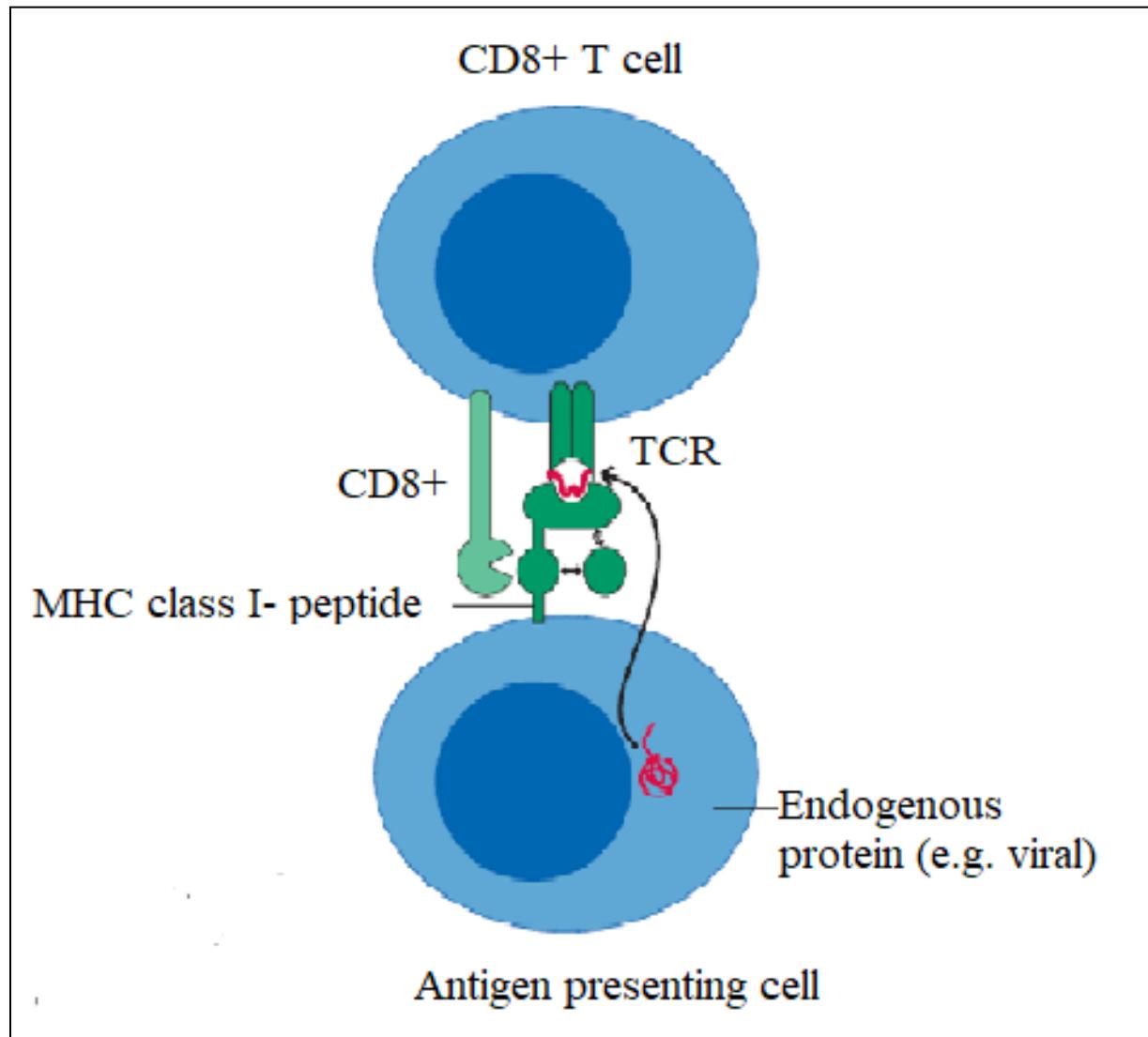


Figure: Antigen processing and presenting

Antigen recognition

- T cell display CD8+ for recognizing antigen peptide fragment with MHC I.



Activation of Tc cell

- Tc cell activation requires cytokine production (Interleukin-2, IL-2)
- Production of cytokine is initiated by
 - ✓ TCR + antigen with APC or
 - ✓ TCR + antigen with APC + Th cells

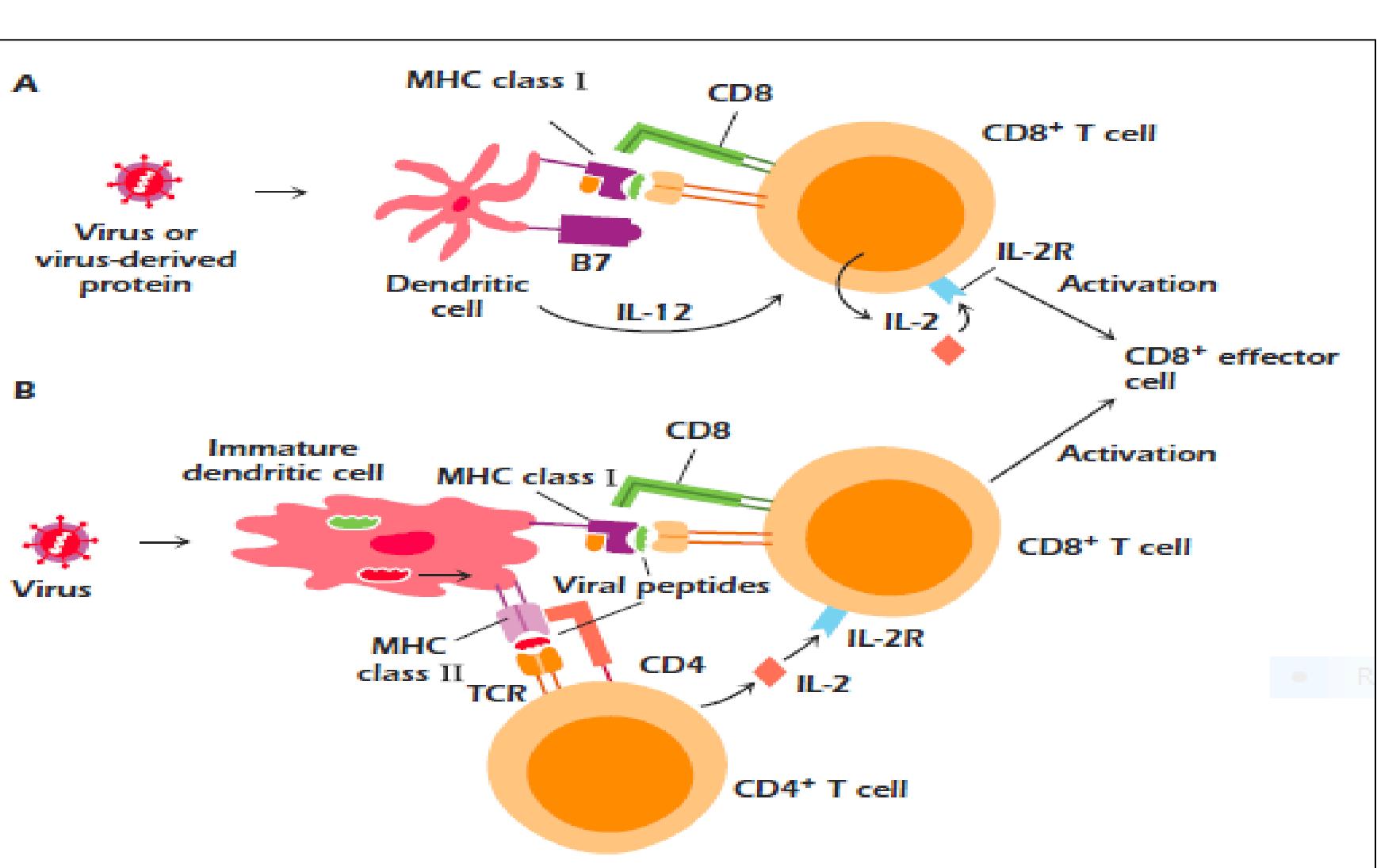


Figure: Generation of virus-specific CD8+ effector T cells. (A) Dendritic cells activate CD8+ T cells directly. (B) Responses to some viruses require CD4+ T cells to activate CD8+ T cells.

Effector function

- Once activated, Tc cell destroy target cells by two different mechanisms:

Granule-dependent mechanisms

- ✓ Use **perforin** and **granzymes**
- ✓ Tc cell is contacted with the target cell, Tc cell release granules through exocytosis, **channels** are formed by using **perforin**, **Granzymes** are entered the target cell and initiate **apoptosis** of the target cell

Granule-independent mechanisms

- ✓ Use **antibody** (Antibody Dependent Cell Mediated Cytotoxicity, **ADCC**)
- ✓ Tc cell expressed Fas ligand which is bind antibody coated target cell.
This interaction leads to initiate **apoptosis**

Cytotoxic T cell

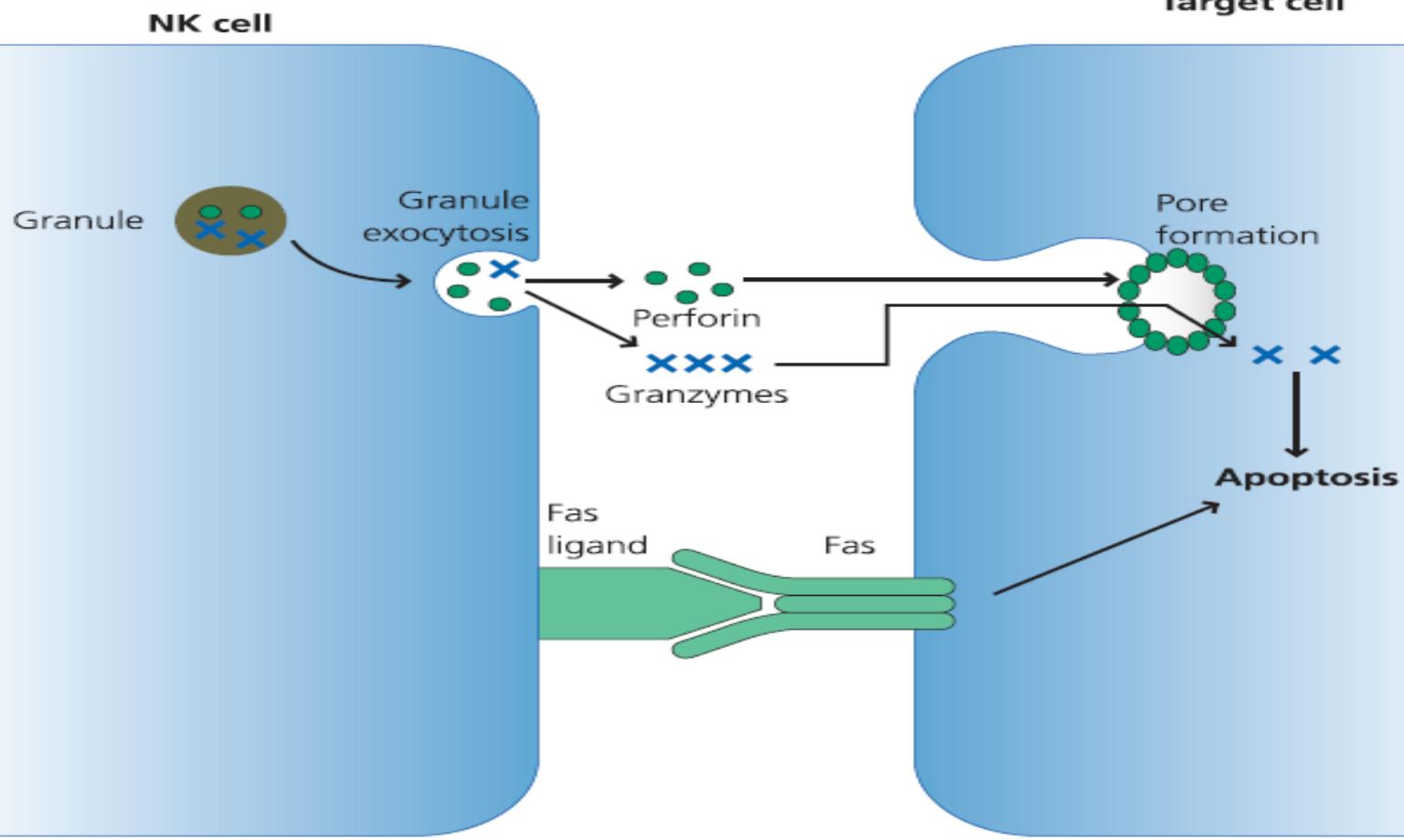


Figure: Mechanisms of cytotoxicity of Tc cell.

- **Humoral immune response**
 - ✓ Involves the production of **antibodies** from B cells
 - ✓ Defense against **extracellular** pathogens
- **B cells** have receptor proteins on their surface that can recognize
 - ✓ protein antigens --- T cell dependent
 - ✓ nonprotein antigens --- T cell independent

Table: Comparison between humoral and cell mediated immunity responses

Property	Humoral immunity response	Cell mediated immunity response
Definition	B cells secrete antibody which circulate in the blood as soluble proteins	Mediated by activated antigen specific T cells
Mediator	B cell	T cell
Action	Extracellular microbes and their toxins	Intracellular microbes (virus, bacteria, parasite) and tumor cells
Receptor	BCR markers	TCR markers
Accessory receptors	Ig α , Ig β , CD40, CD21, and Fc receptors	CD2, CD3, CD4, CD8, CD28, integrin
Recognition	Unprocessed antigens	Antigens are processed and presented by MHC complex
Secretion	B cells secrete antibody	T cells secrete cytokines
Response	rapid	delayed
Acts on tumor and transplants	No	Yes

Procedure for T cell dependent antibody production

1. Antigens are ingested through APC
2. Antigen presenting cells degrade ingested exogenous antigen into peptide fragments
3. class II MHC molecule are expressed and bind degraded antigen and present it to CD4 Th cells
4. Th cells activate B cells (humoral immune response)
5. B cells differentiate and develop memory cell and antibodies
6. Antibodies are coated antigens

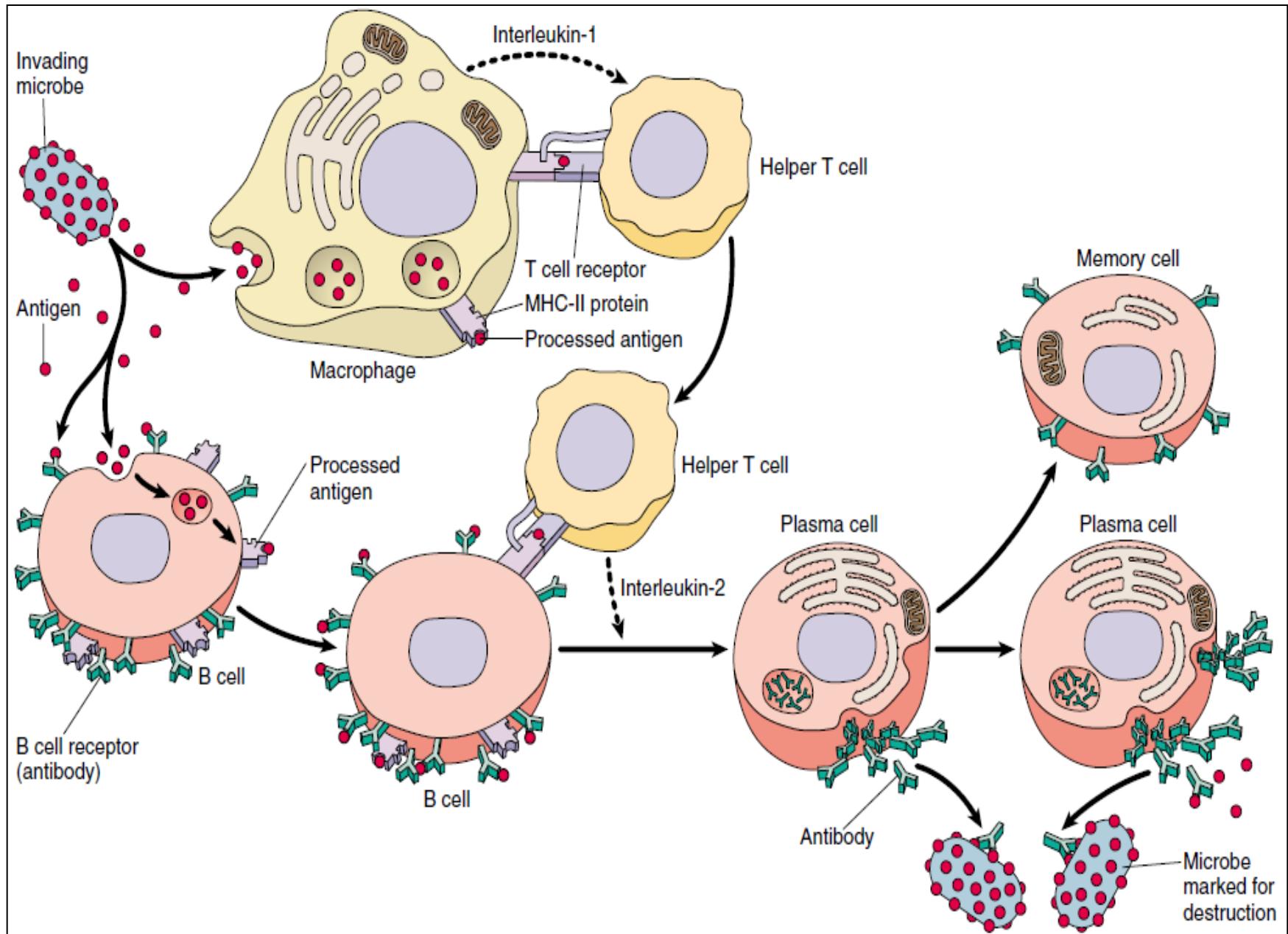
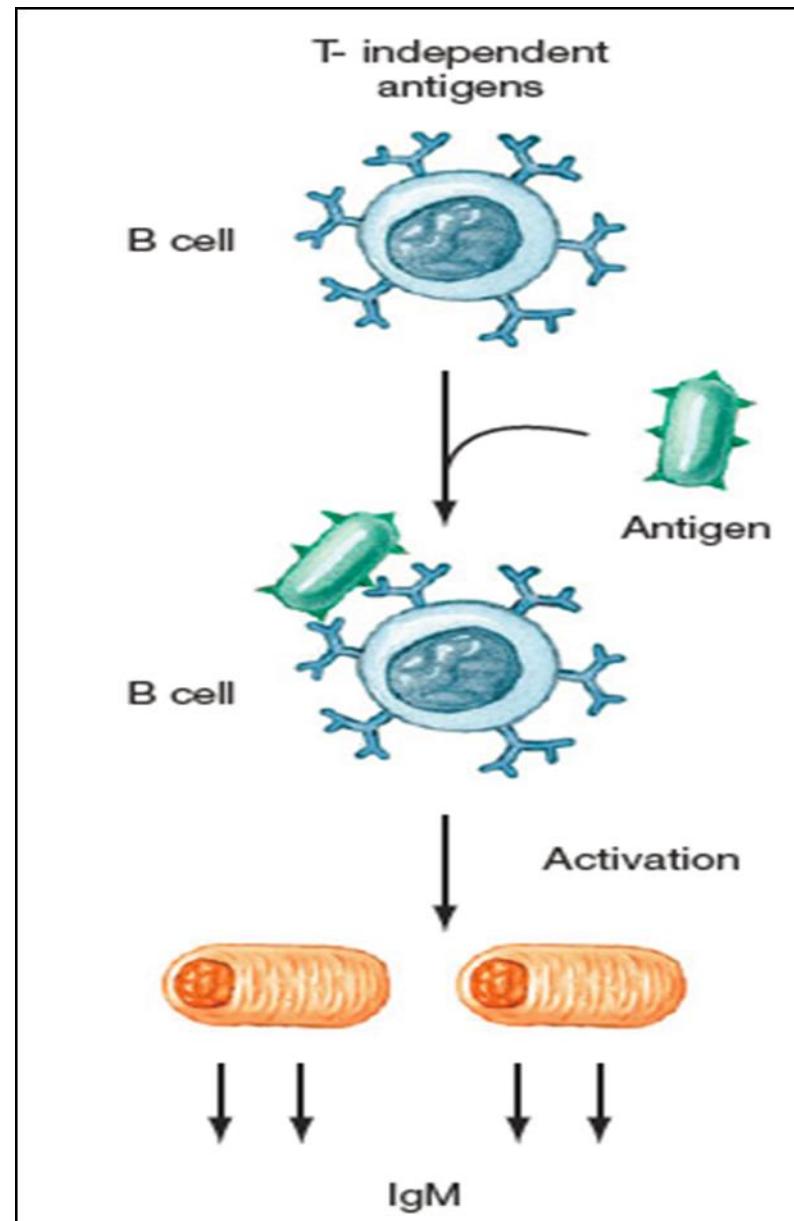


Figure: Humoral immunity response

Procedure for T cell independent antibody production

1. B cell receptor binds antigen leads to its activation
2. B cells secrete antibodies and coated pathogens



Mechanisms of B cell killing pathogens

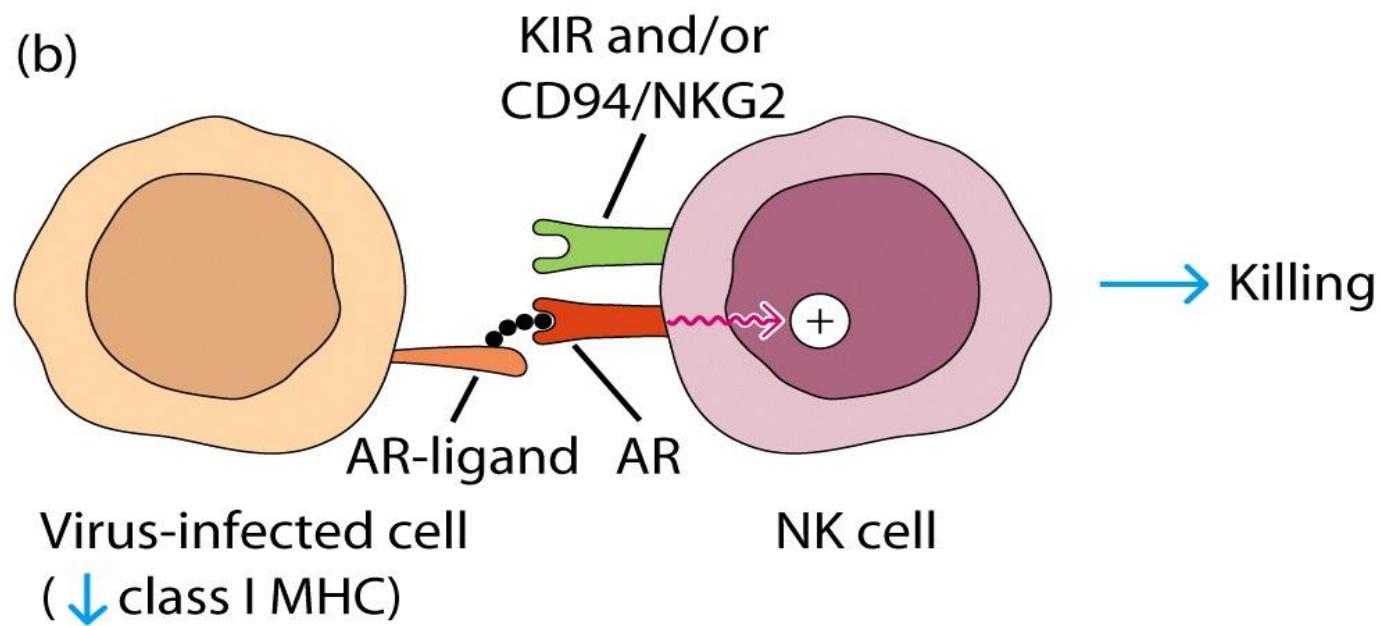
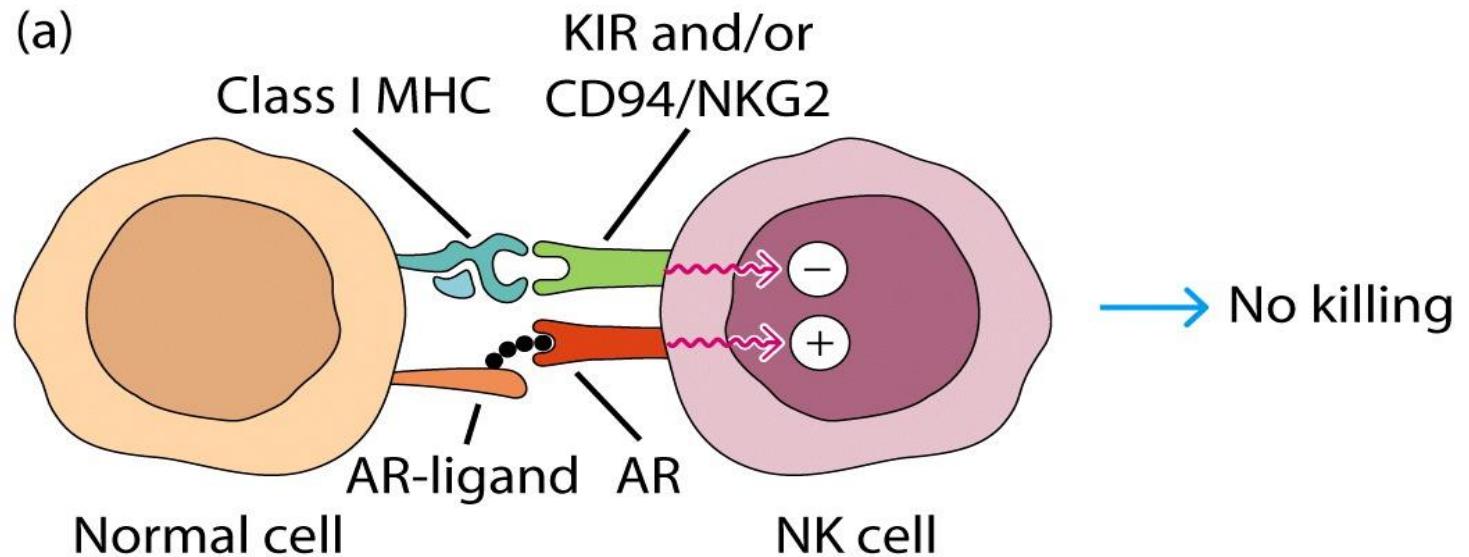
- B cells **do not go** on the attack pathogens but they mark the pathogen to destroy by
 - macrophages
 - Complement systems
 - NK cells

➤ Natural killer (NK) cells

- ✓ are a special group of lymphoid cells
 - lack antigen-specific receptors
- ✓ have not CD8, MHC restriction, and memory`
- ✓ does not directly attack invading microbes
- ✓ destroy tumor cells and cells infected with virus
- ✓ have receptors for MHC class I

Target Recognition

- NK Cells use **kill activation receptors** (KAR) and **killer inhibiter receptor** (KIR) to differentiate normal from altered cells
- The **inhibitory receptors** recognize MHC molecules on normal cells and **prevent** their killing by NK cells.
- The **inhibitory** receptors **do not** recognize the MHC molecules on cells which leading to **death** of the cells by NK cells



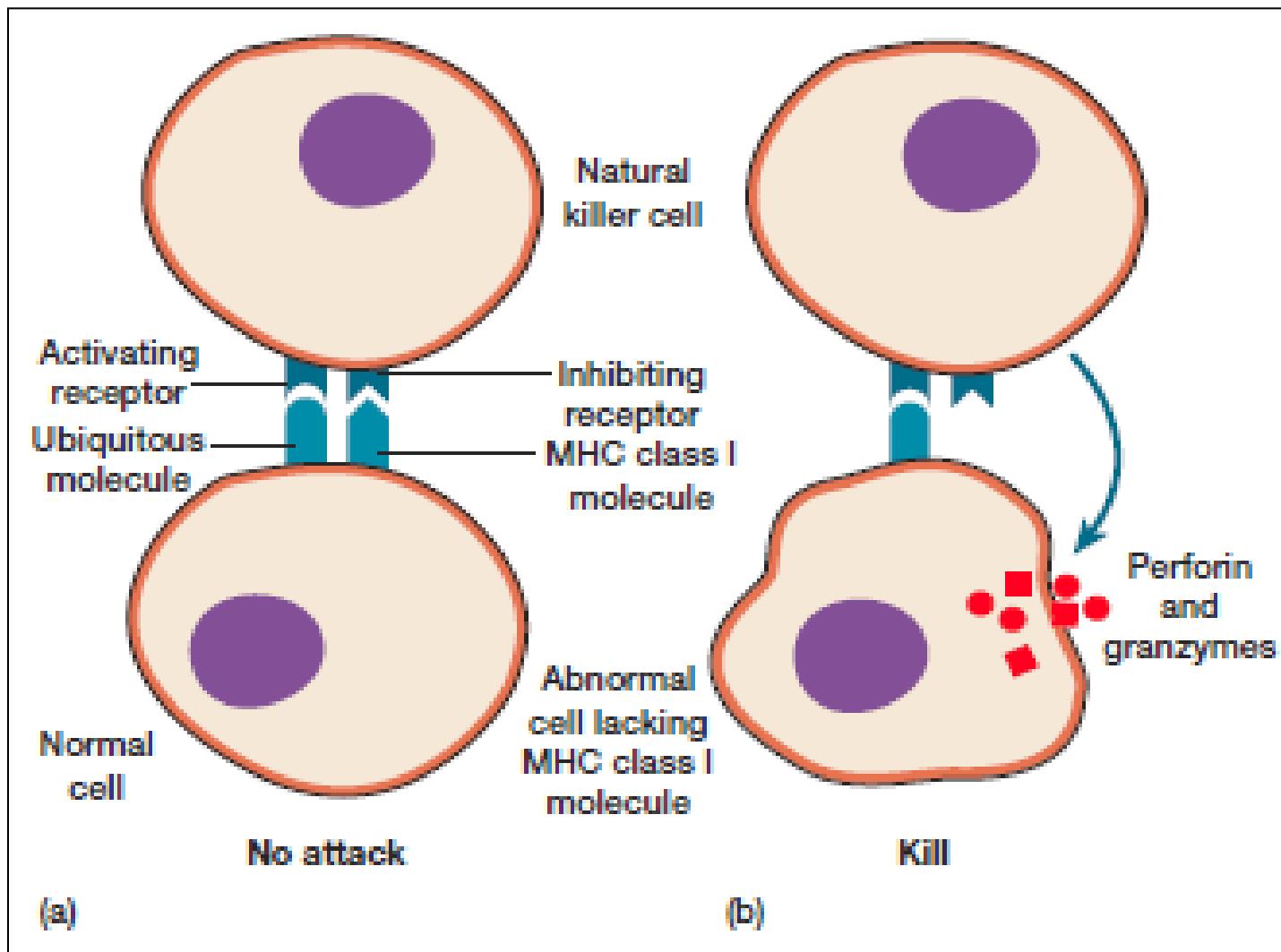


Figure. The System Used by Natural Killer Cells to Recognize Normal Cells and Abnormal Cells That Lack the Major Histocompatibility Complex Class I Surface Molecule.

➤ NK cells Kill their target cells similar way to Tc cells

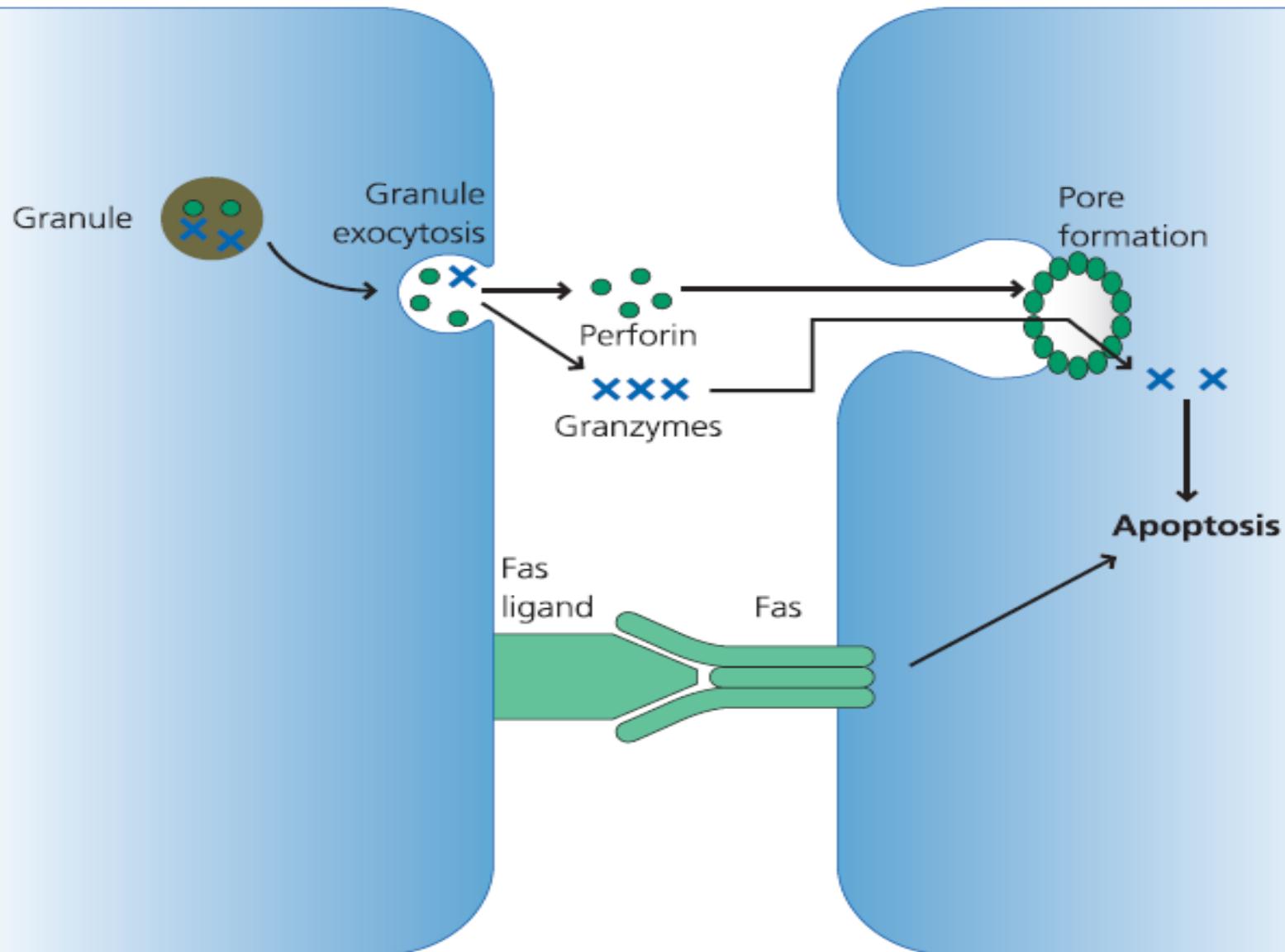
✓ **Granule-dependent mechanisms**

- Use **perforin** and **granzymes** to destroy target cells

✓ **Granule-independent mechanisms**

- Use **antibody** to destroy target cells

NK cells



Mechanism of NK cells killing target cells

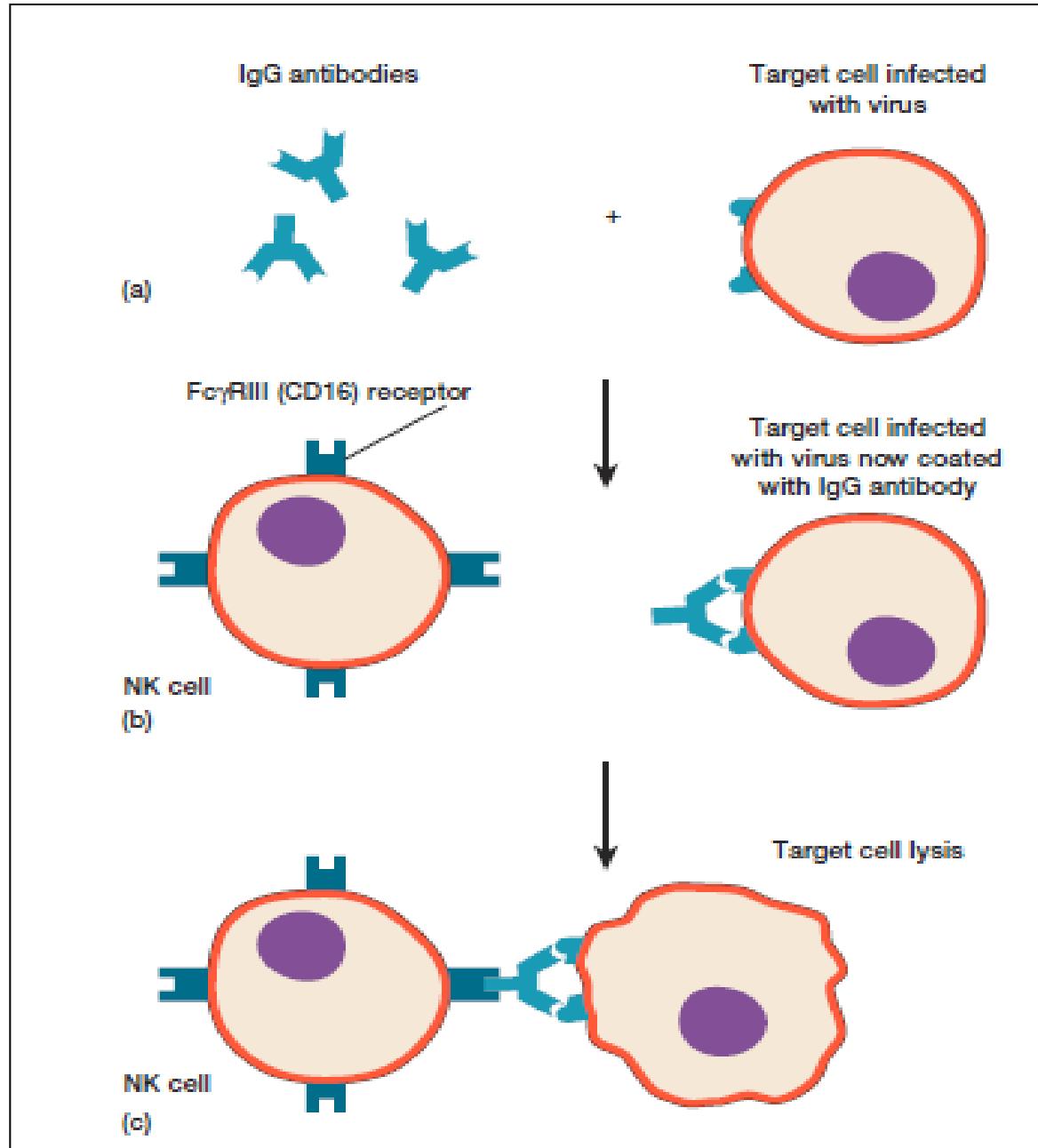


Figure: Antibody-Dependent Cell-Mediated Cytotoxicity

Questions

➤ Define the following terms:

- ✓ Hypersensitivity
- ✓ Food allergy
- ✓ Immunotherapy
- ✓ Vaccine

Hypersensitivity

- It refers to the process whereby the immune response **overreacts** to a variety of infectious and inert antigens resulting in **damage to the host tissue.**
- The disease may be caused by two types of abnormal immune responses:
 - ✓ **Uncontrolled or unregulated response** against foreign Ags, resulting in tissue injury
 - ✓ **Response against self** antigens due to **failure** of self-tolerance

Classification of hypersensitivity

- Based on **types of immune response**:
 - ✓ **Immediate** hypersensitivities: **humoral** immune response
 - ✓ **Delayed** hypersensitivities: **cellular** immune response
- Based on **mediator inducers**:
 - ✓ Type I– IgE (**Anaphylaxis**)
 - ✓ Type II– IgG/M (cytotoxic hypersensitivity)
 - ✓ Type III – Immune complex
 - ✓ Type IV– Delayed T cell hypersensitivity (DTH)

Type I hypersensitivity (Anaphylaxis)

- Anaphylaxis (against protection)
 - ✓ is an inclusive term for the reactions caused when certain **allergens** (antigens) combine with **IgE antibodies**
 - ✓ is mediated by **IgE**
 - ✓ is commonly called **allergic** or **immediate** reaction
 - ✓ is initiated by **allergen** molecules

➤ Allergens

- ✓ are **soluble** and **low molecular** weight, low dose, that enter the body through mucosa or the skin
- ✓ induce production of specific IgE include:
 - **Inhalants**: dust, mite faeces , tree or pollens, mould spore.
 - **Ingestants**: milk, egg, fish, chocolate
 - **Contactants**: wool, nylon, animal fur
 - **Drugs**: penicillin, salicylates , anesthetics
 - **Insect venom**: insect stings

- **Effector cells:** mast cells, basophils, and eosinophils
- IgE-mediated diseases in humans include:
 - ✓ Anaphylactic shock—decrease **blood** pressure
 - ✓ Asthma—affect **lungs**
 - ✓ Hay fever (allergic rhinitis)-- **mucous membranes** of the eyes and nose are inflamed
 - ✓ Allergic conjunctivitis—swelling of **eye**
 - ✓ Food allergies—gastrointestinal tract (**GIT**)

➤ **Phases of type I reaction:**

1. First exposure to allergen
 - stimulates **formation** of antibody (IgE)
2. Second exposure to the same allergen
 - **activation and degranulation** of mast cells and **release** of mediators

➤ Released mediators cause:

✓ Smooth muscle contraction,

✓ Mucous secretion

✓ vasodilatation

✓ Vascular permeability

✓ edema

✓ chemotaxis



Toxic enzymes
are released

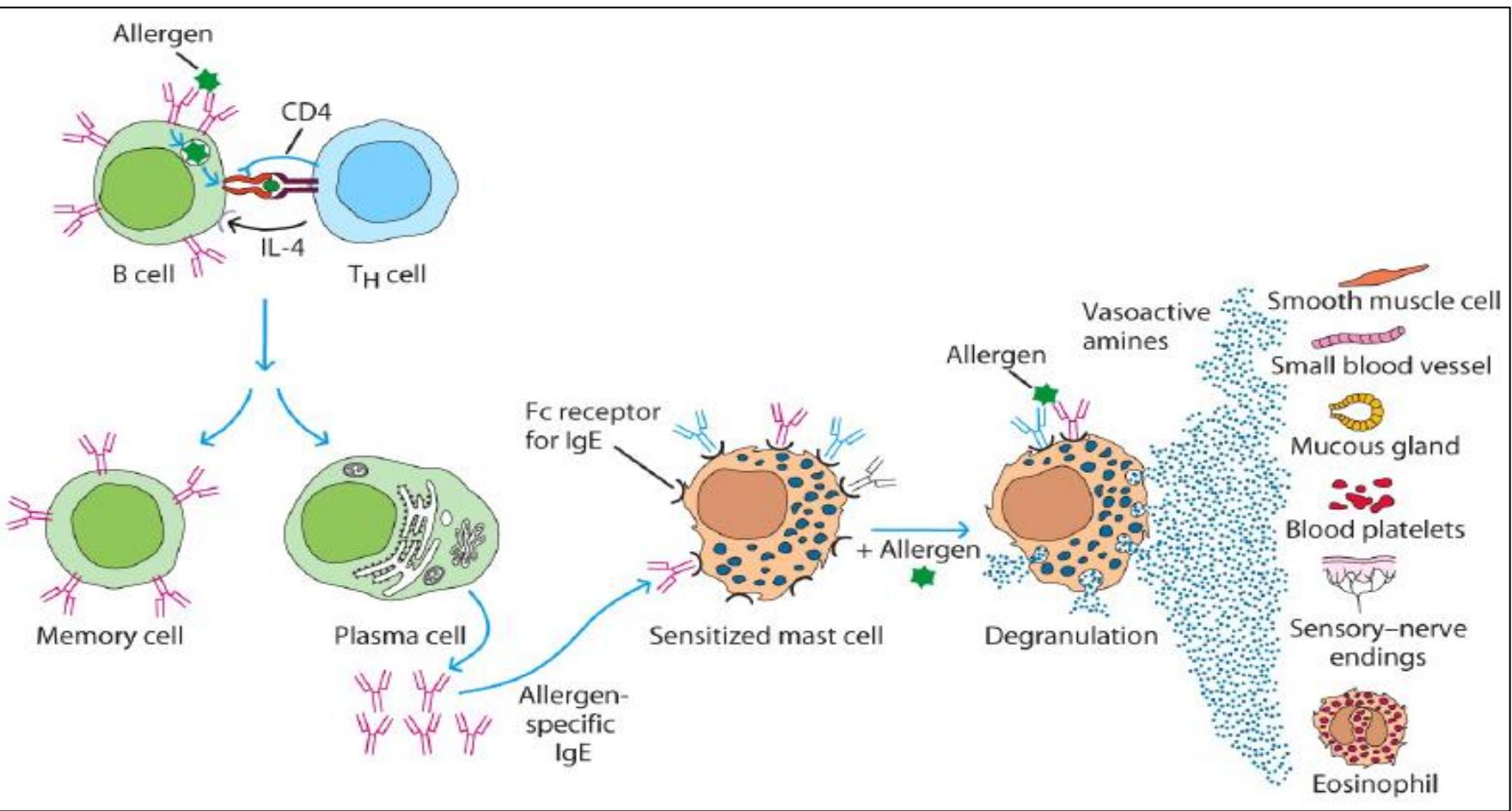
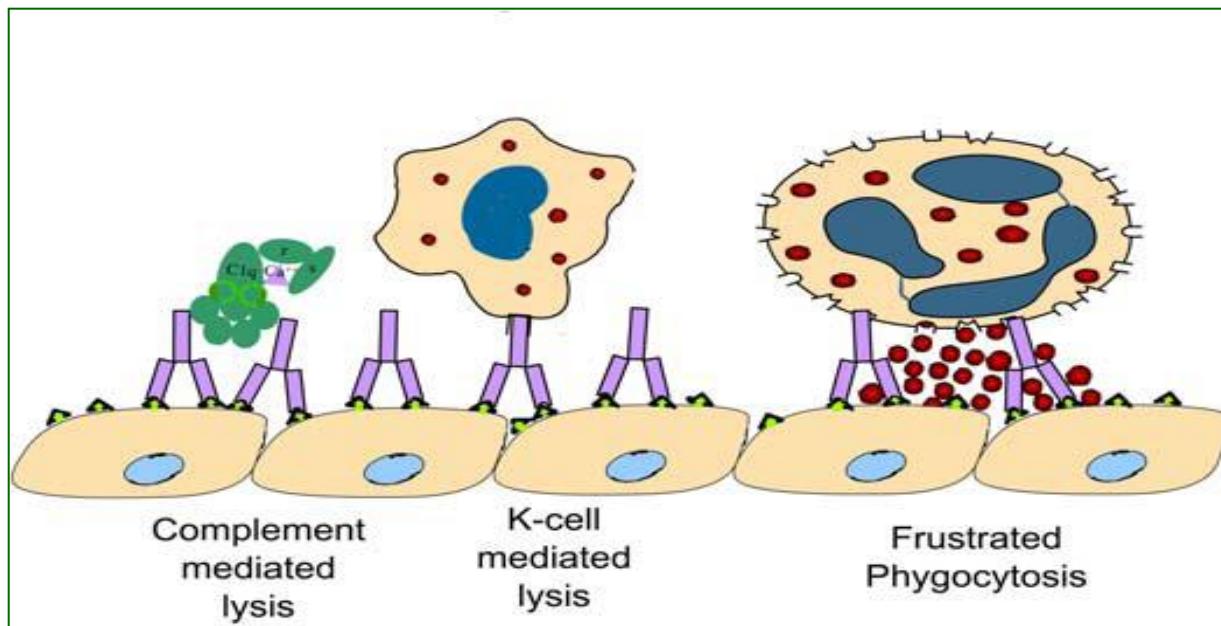


Figure: Type I hypersensitivity

➤ Type II hypersensitivity

- ✓ activation of complement by IgG or IgM antibodies
- ✓ antibody-mediated destruction of cells
- ✓ This activation results lyse the affected cell by:
 - complement, phagocytes (Opsonization) and NK cells
- ✓ The most familiar **Type II** hypersensitivity reactions are **blood transfusion** reactions, **erythroblastosis fetalis** and **drug** induced cytotoxic reactions



Blood transfusion reactions

E. g. incompatible blood transfusion

- red blood cells are destroyed

➤ Procedure

1. type **B blood** is transfused into a person with type **A blood**
2. the **antigens** on the type B blood cells will react with **anti-B antibodies**
3. this antigen- antibody reaction **activates complement**, which in turn causes **lysis** of the donor's RBCs

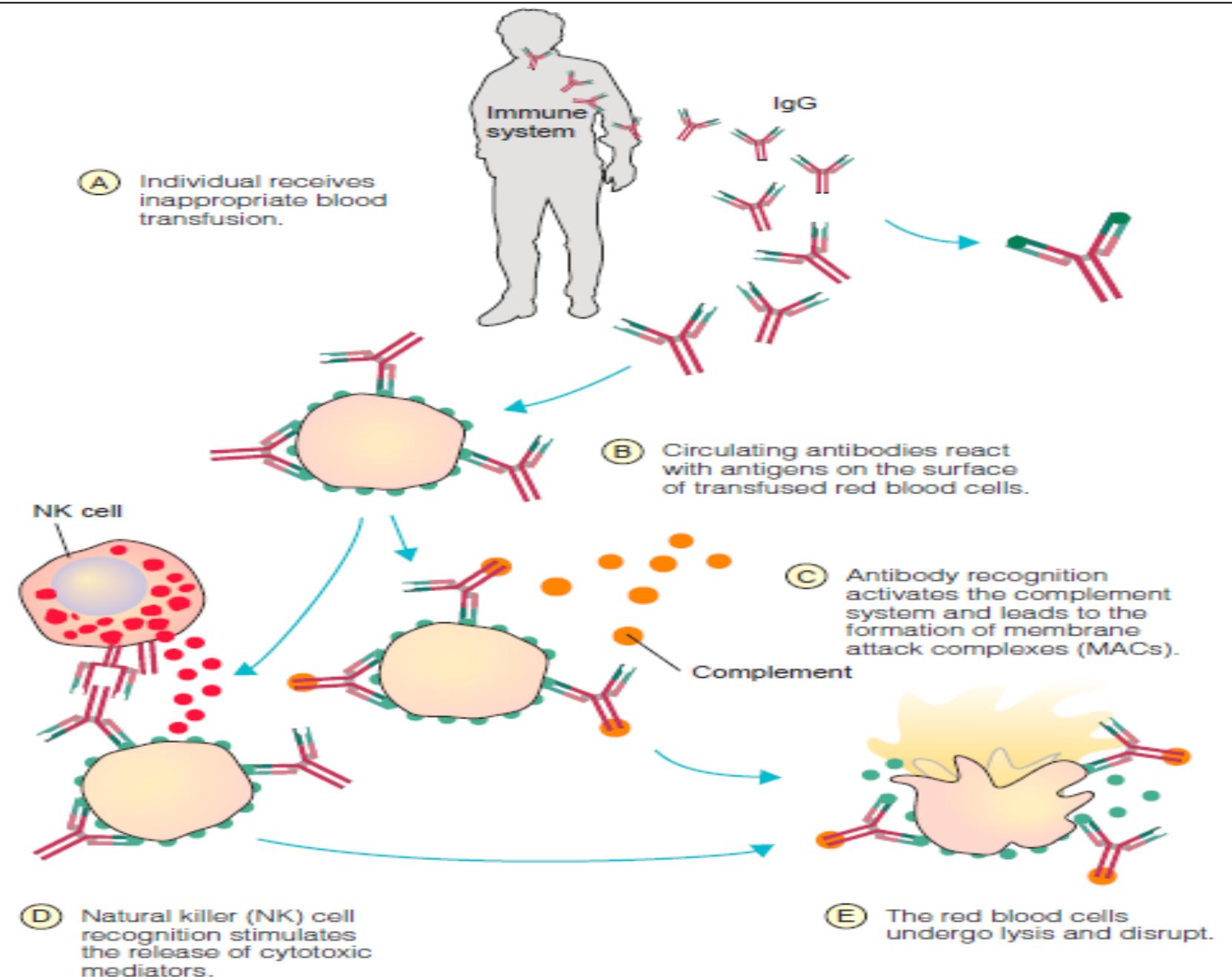


Figure: Lysis of red blood cells due to incorrect blood cell typing

Table: Blood groups

Blood group	RBC antigen	Antibody in plasma	Blood receive
A	A	Anti-B	A, O
B	B	Anti-A	B, O
AB	A, B	none	All blood group
O	none	Anti-B and Anti-A	O

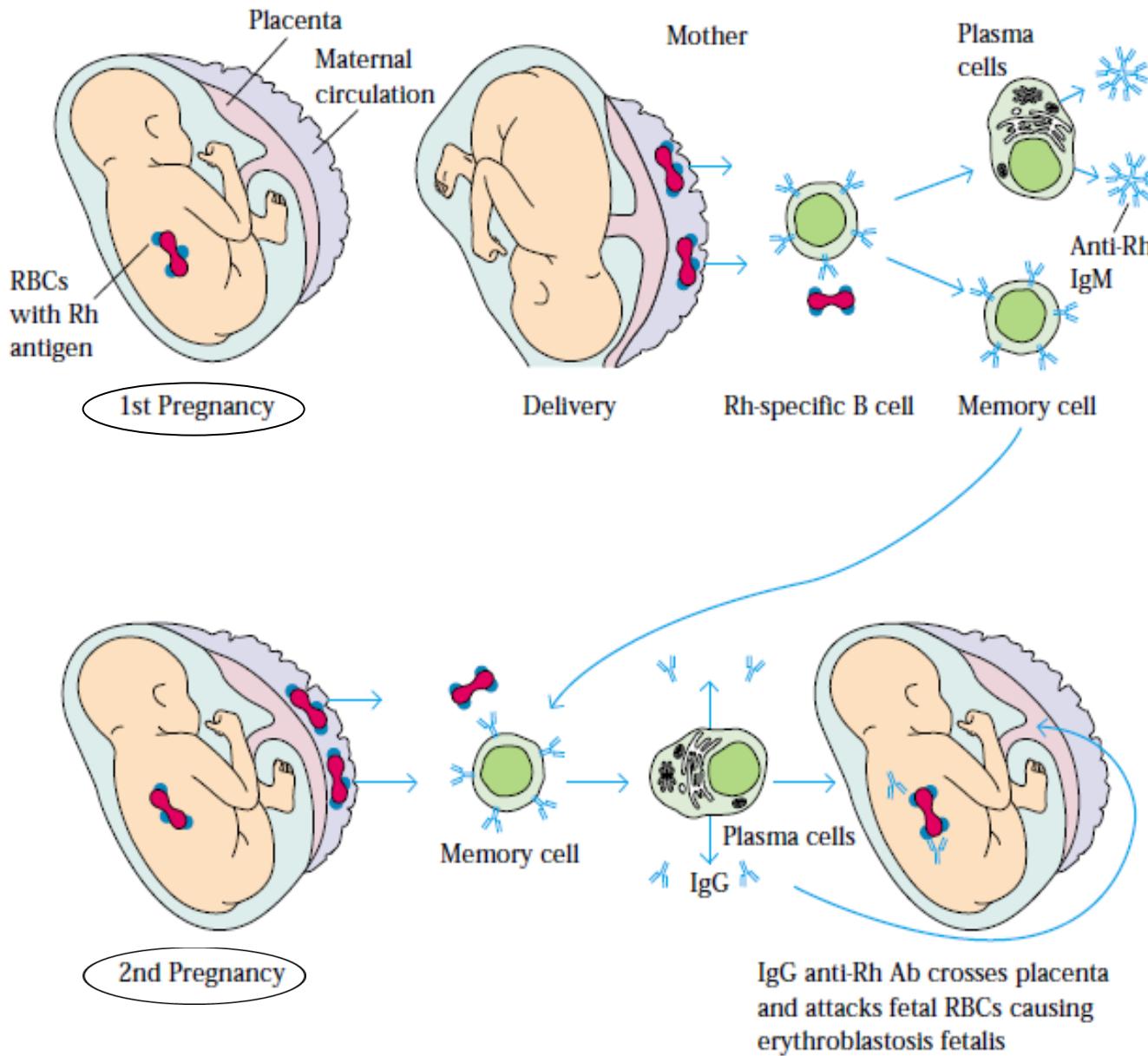
Table: Rh factors

man	woman	Children death
Rh-	Rh-	no
Rh-	Rh+	no
Rh+	Rh-	yes
Rh+	Rh+	no

Erythroblastosis fetalis

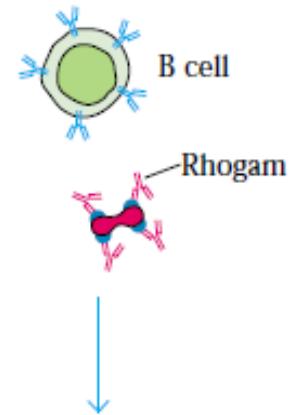
- When a Rh - woman and a Rh + man produce a child, there is a 50% chance that the child will be Rh +.
- If the child is Rh +:
 - fetal Rh positive (Rh +) RBCs secrete antigens (erythroblasts)
 - mother's body produce anti-Rh antibodies
 - The reaction of fetal Rh+ and mother anti-Rh- activate complement that result loss of fetal RBCs--- hemolytic disease of the newborn (HDNB).
- HDNB is usually prevented by immunization of the Rh – mother with Rhogam.

DEVELOPMENT OF ERYTHROBLASTOSIS FETALIS (WITHOUT RHOGAM)



PREVENTION (WITH RHOGAM)

Mother (treated with Rhogam)



Prevents B-cell activation and memory cell formation

Figure: Erythroblastosis fetalis development

Drug-Induced Cytotoxic Reactions

- The drug molecules are usually **hapten** because they are too small to be immunogenic by themselves
- but, **blood** has become coated with molecules of a **drug** and the combination is immunogenic.
- The combination of **drug** and **blood** activates **complement** that cause
 - thrombocytopenic purpura----loss of **platelets**
 - agranulocytosis --- loss of agranulocytic **white cells**
 - hemolytic anemia --- loss of **red blood cells**

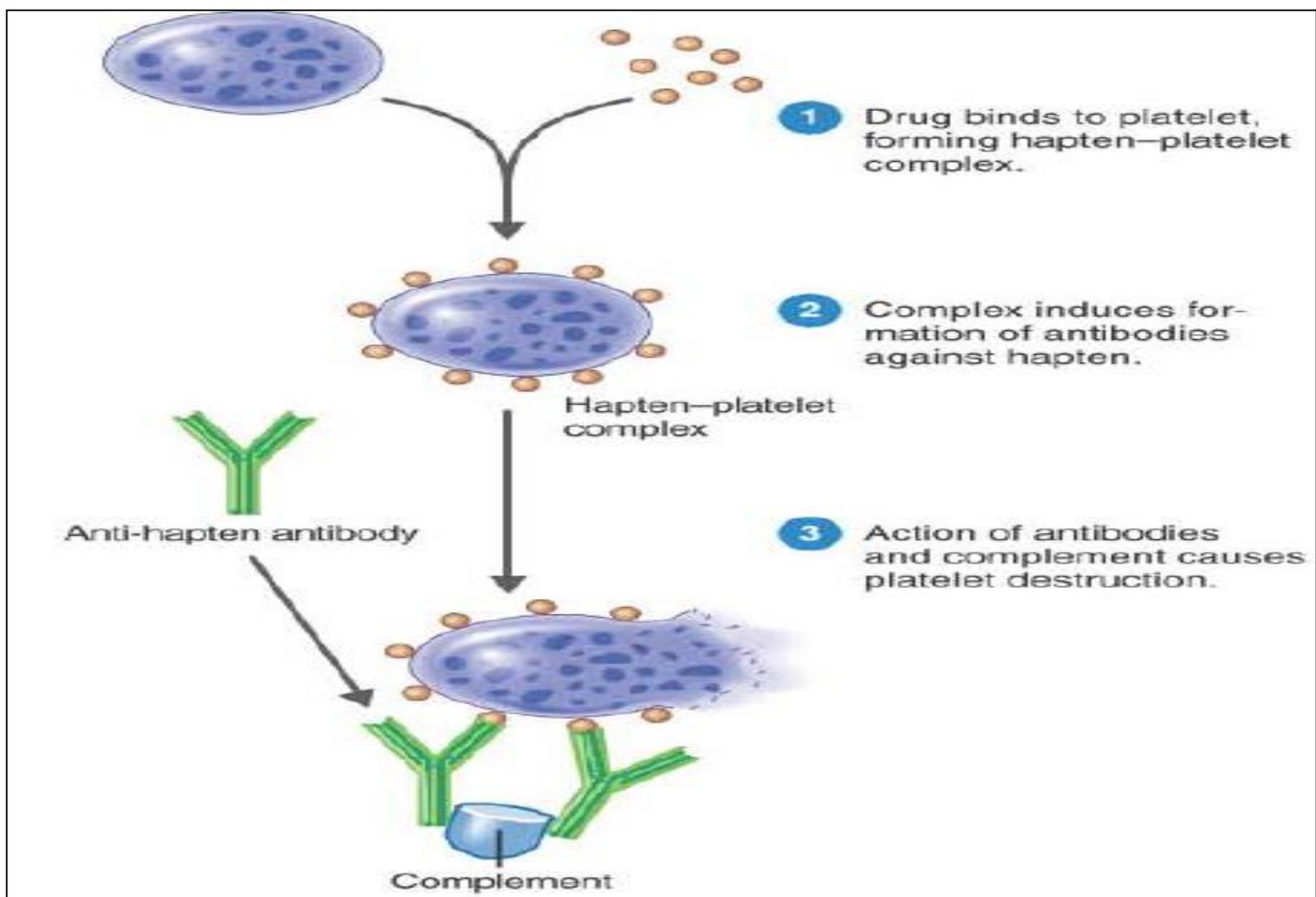
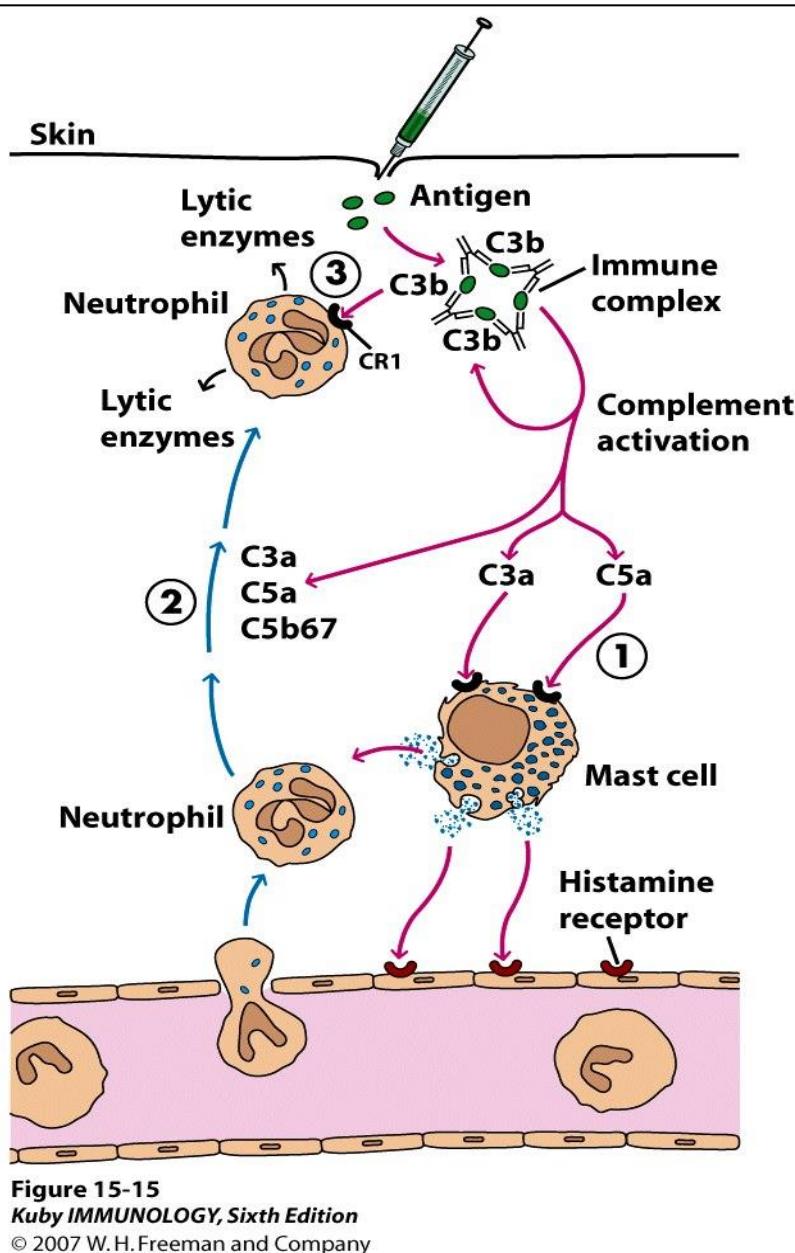


Figure: Drug-induced thrombocytopenic purpura

Type III hypersensitivity

- This is known as immune complex hypersensitivity.
- When antigens bind antibodies, immune complexes of different sizes are formed:
 - large complexes can be cleared by macrophages
 - but small complexes can not phagocytized
- These small immune complexes insert themselves into blood vessels, joints, and kidney, causing inflammatory and complement fixing. E. g.
 - Aspergillosis ---- lung
 - Arthritis ---- joints
 - Glomerulonephritis --- kidneys
 - Serum sickness --- blood stream



1. Complement initiates mast cell degranulation
2. Neutrophils are chemotactically attracted to the site
3. Neutrophils release lytic enzyme after failed attempts to endocytose the immune complex

Figure: Type III hypersensitivity reaction (**Inflammatory** response)

Figure 15-15
Kuby IMMUNOLOGY, Sixth Edition
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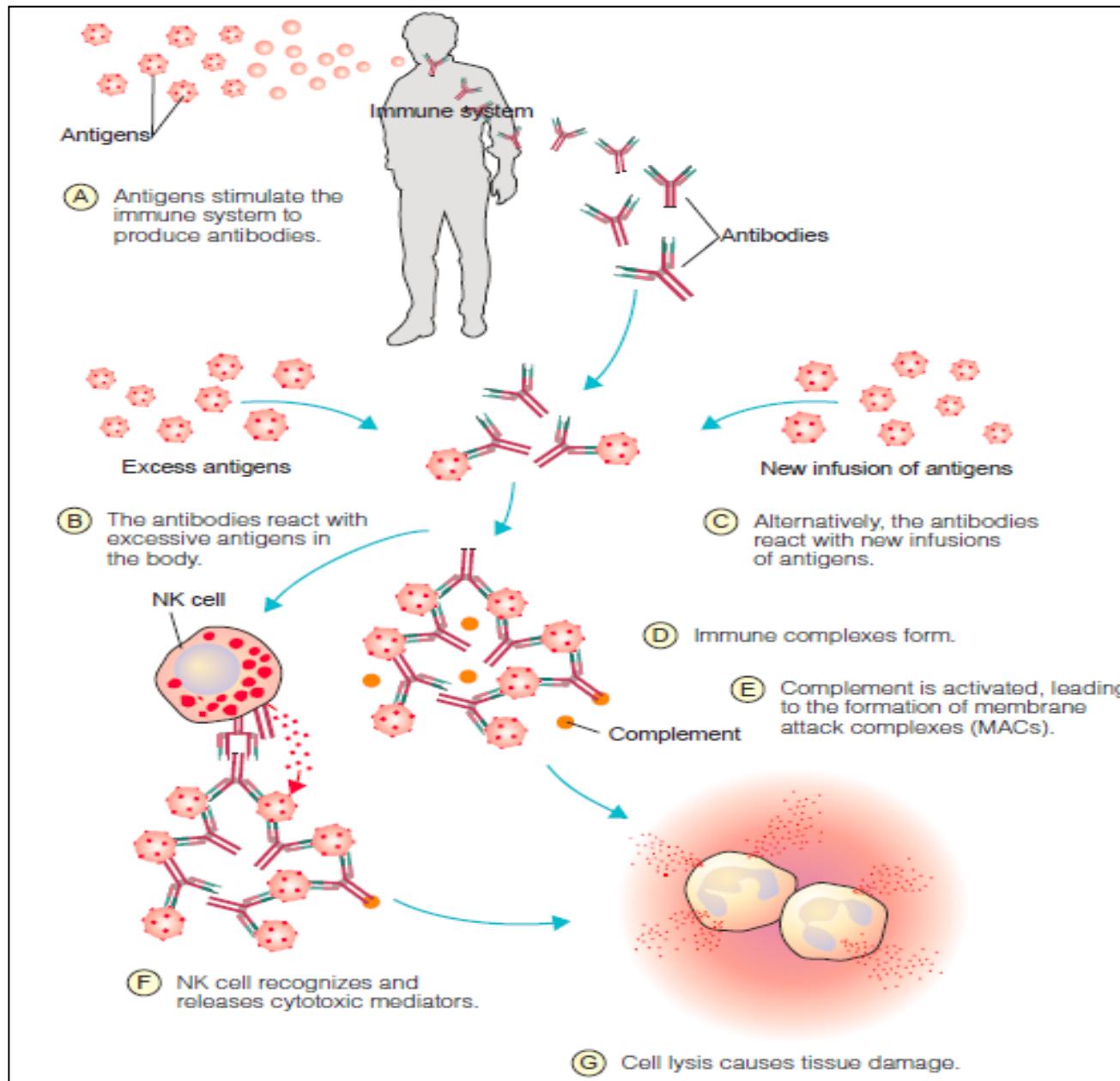


Figure: Type III hypersensitivity reaction (**NK cell and complement**)

Type IV hypersensitivity reactions

- They involve **cell-mediated immune** responses and are caused mainly by **T cells.**
- When certain foreign antigens are phagocytized and presented to T cell, causes proliferation of T cells into memory T cells, Th cells and Tc cells. Then, If the antigen is
 - ✓ removed --- **not develop** delayed type hypersensitivity (DTH)
 - ✓ **not removed** and **re-exposed** to the same antigen --- **develop** DTH

➤ Examples of DTH

- contact dermatitis -- skin
- Transplant rejection --destroys the transplanted tissue
- Tuberculosis --- lung
- Leprosy --- skin, mucous membranes, and nerves
- Blastomycosis --- skin or internal organs
- histoplasmosis --- lung and others
- leshimaniasis --- blood and tissue
- etc

How T cell damage the host tissue?

- There are two mechanisms for destruction of non specific host tissues

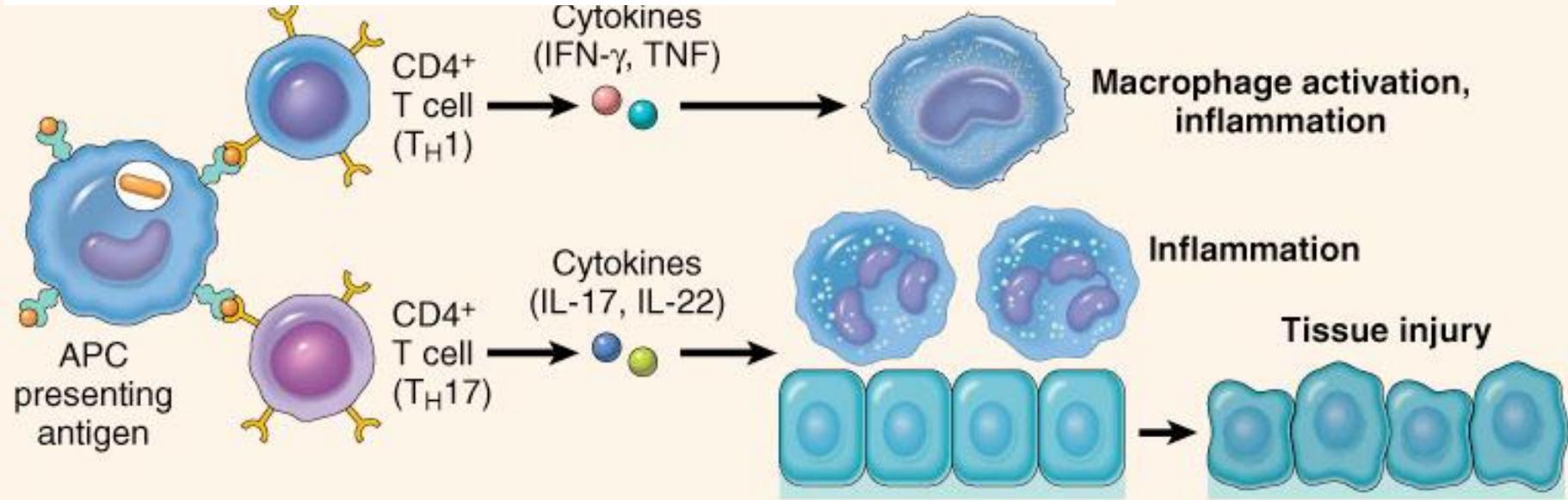
A) Macrophage and neutrophill activation

- ✓ Indirect killing
 - Th1 and CD4+
 - Th17 and CD4+

B) Tc cell activation

- ✓ Direct killing host tissues

a) Activation of macrophage and neutrophill



B) Activate Tc cell

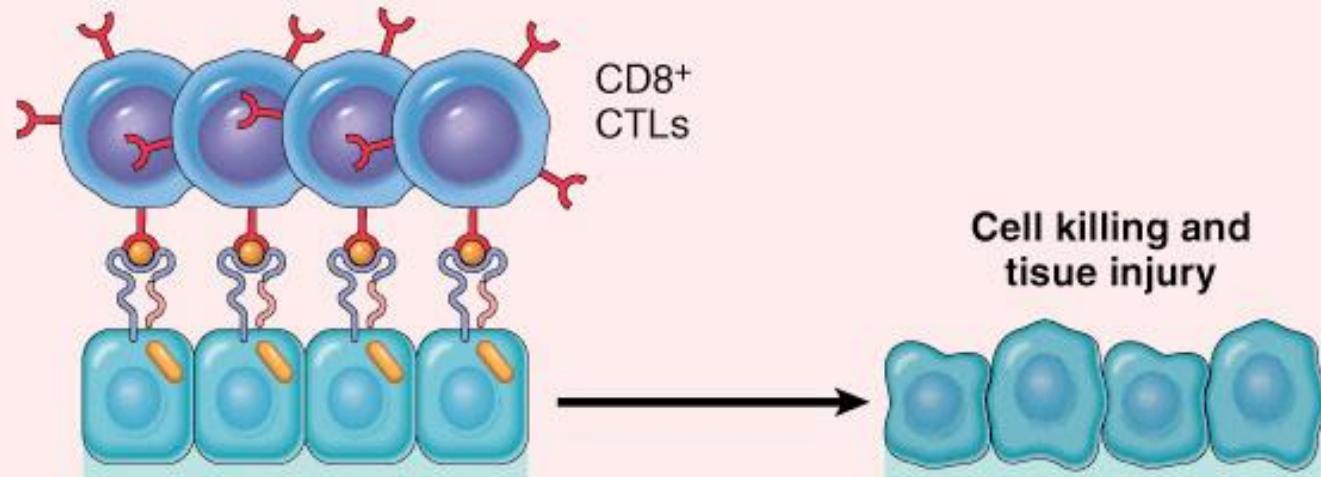


Table: Summary of the different groups of hypersensitivity

character	Type I	Type II	Type III	Type IV
Common name	Anaphylaxis Or immediate	cytotoxic	Immune complex	Cell mediated or DTH
Main mediator	IgE	IgG or IgM	IgG or IgM	Th1
	Antibody	Antibody	Antibody	T cell
Causative agent	allergens	-incompatible blood transfusion -drug, Rh factor	Small immune complex	Delayed T cell response to the antigens
Effector mechanisms	Degranulation of mast cell	Complement or ADCC	Mast cell degranulation, neutrophils and macrophage activation	Machrophages and Tc cells activation
Reaction time	15-30 minutes	Minutes to hours	3-8 hours	48-72 hours

Application of Immunology

1. Vaccine
2. Diagnostic tests
3. Immunotherapy

Vaccines

➤ Definition

- ✓ is suspension of organisms or fraction of organisms used to induce immunity

➤ Properties of ideal vaccine:

- ✓ Provide **long** lasting immunity
- ✓ Should induce both **humoral** and **cellular** immunity
- ✓ Should **not induce** hypersensitivity
- ✓ Should be **inexpensive** to produce, **easy** to store and administer
- ✓ Should be **safe**

➤ Examples of vaccine:

- Attenuated vaccine
- Killed vaccine
- Subunit vaccine
- DNA vaccine
- Toxoid vaccine

Table. Some microbial diseases and their vaccine

Vaccine	Attenuated	Killed	DNA	Subunit	Toxoid
characters	Weakened	Killed	Antigen coding DNA	Microbial components	Bacterial toxin
preparation	<ul style="list-style-type: none"> • growth unsuitable condition • Select low virulence strain 	<ul style="list-style-type: none"> – heat or – chemicals 	Prepared DNA sequence	purified microbial components	detoxifying toxins with formalin
usage	Adenovirus, Measles, mumps, rubella, polio, yellow fever	Typhoid, Cholera, flu, hepatitis A, Japanese encephalitis, plague, polio, rabies	Hepatitis B, Rabies, Influenza	Influenza, Hepatitis B, pertussis, pneumonia	Diphtheria, tetanus

Diagnostic Immunology

- The immunological binding reaction between **antibodies** and **antigens**, is termed **immunodiagnosis**
- The **assay** for measuring these antibodies or antigens, is called **immunoassay**.
- How to measure immunoassay?
 - A) Use known **antibodies**---to detect specific antigen
 - B) Use known **antigens**---to detect specific antibodies
- The **need** for immunological test is particularly in detecting **infections**

❖ Common types of immunodiagnosis techniques

1. Precipitation reaction
2. Agglutination reaction
3. Agglutination inhibition
4. Fluorescent-antibody (FA) technique
5. Enzyme Linked ImmunoSorbant Assay(ELISA)
6. Western bloating analysis

Precipitation Reactions

- ✓ In aqueous solution, antibody and soluble antigen form visible precipitate.

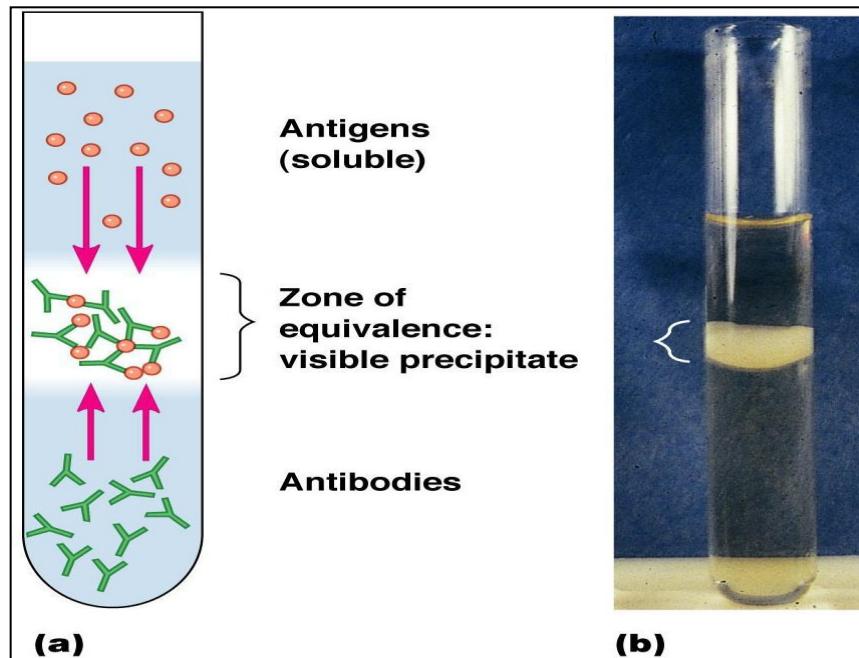


Figure: Precipitation Reactions

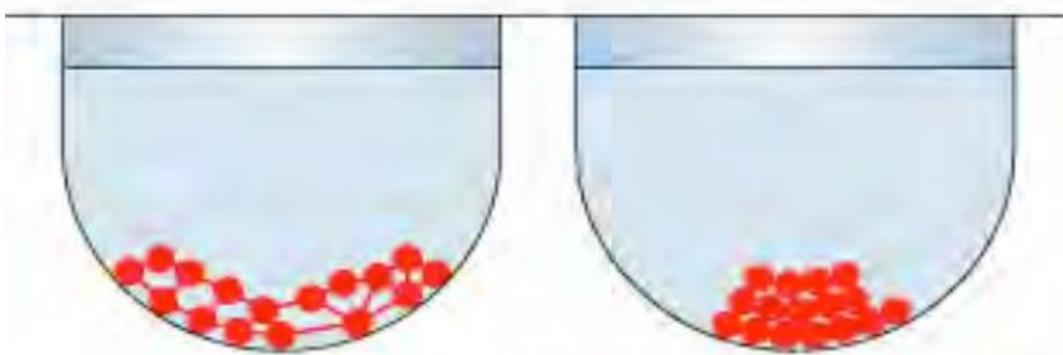
Agglutination Reactions

- It is used to detect **antigens** and **antibodies** from different samples (i.e. urine, blood, saliva, and cerebrospinal fluid).
- Two types of Agglutination Reactions
 - ✓ Direct agglutination test
 - use **free antigen** to detect **antibodies**
 - ✓ Indirect agglutination test
 - use **latex bead coated antibodies** and **antigen** to detect **antigen and antibodies** respectively

Top view of wells



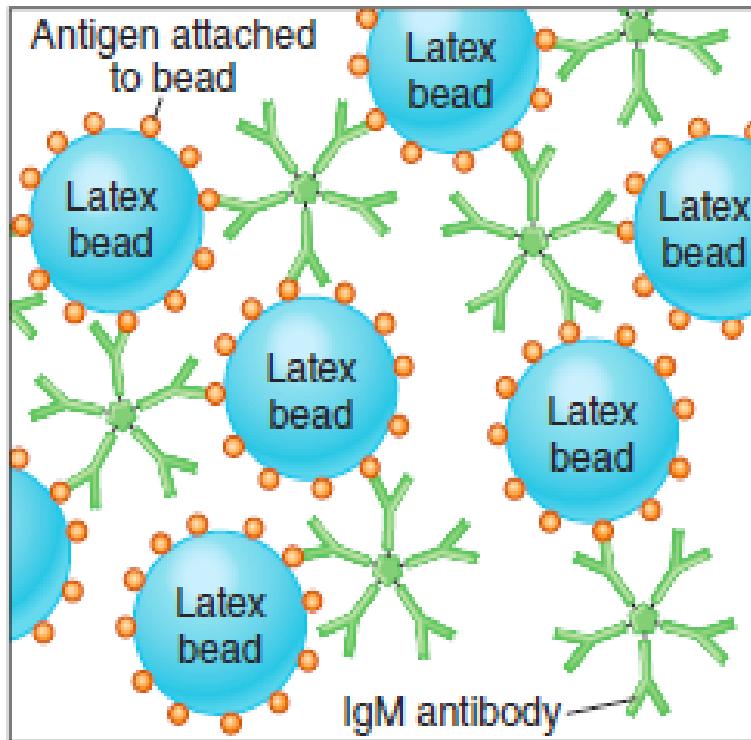
Side view of wells



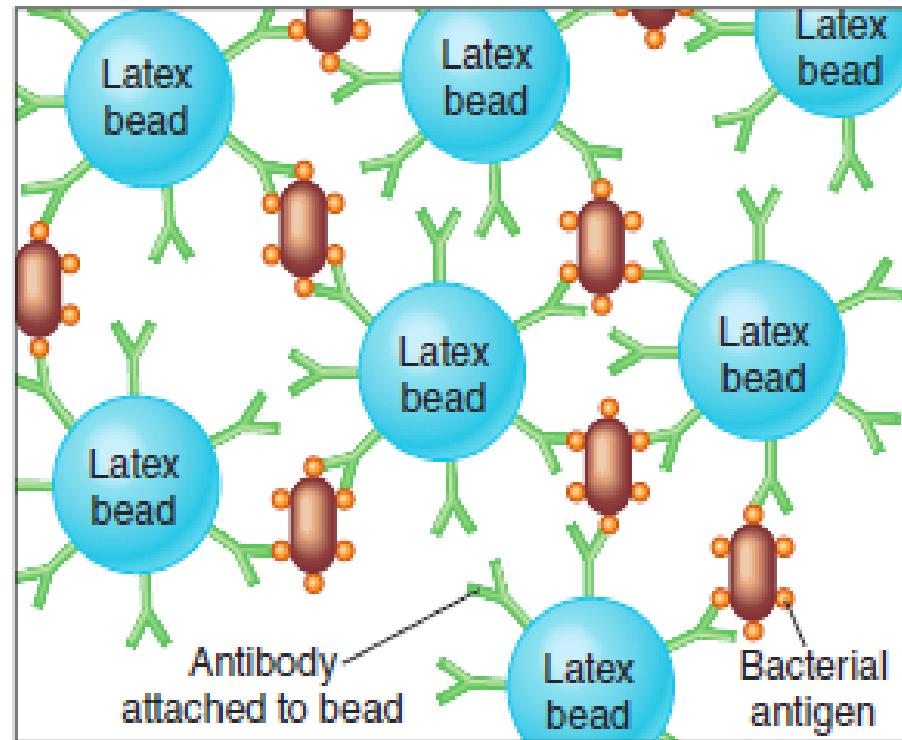
(A) Agglutinated

(B) Non-agglutinated

Figure: Agglutination method (direct)



(A) detect antibodies by using latex bead coated antigen

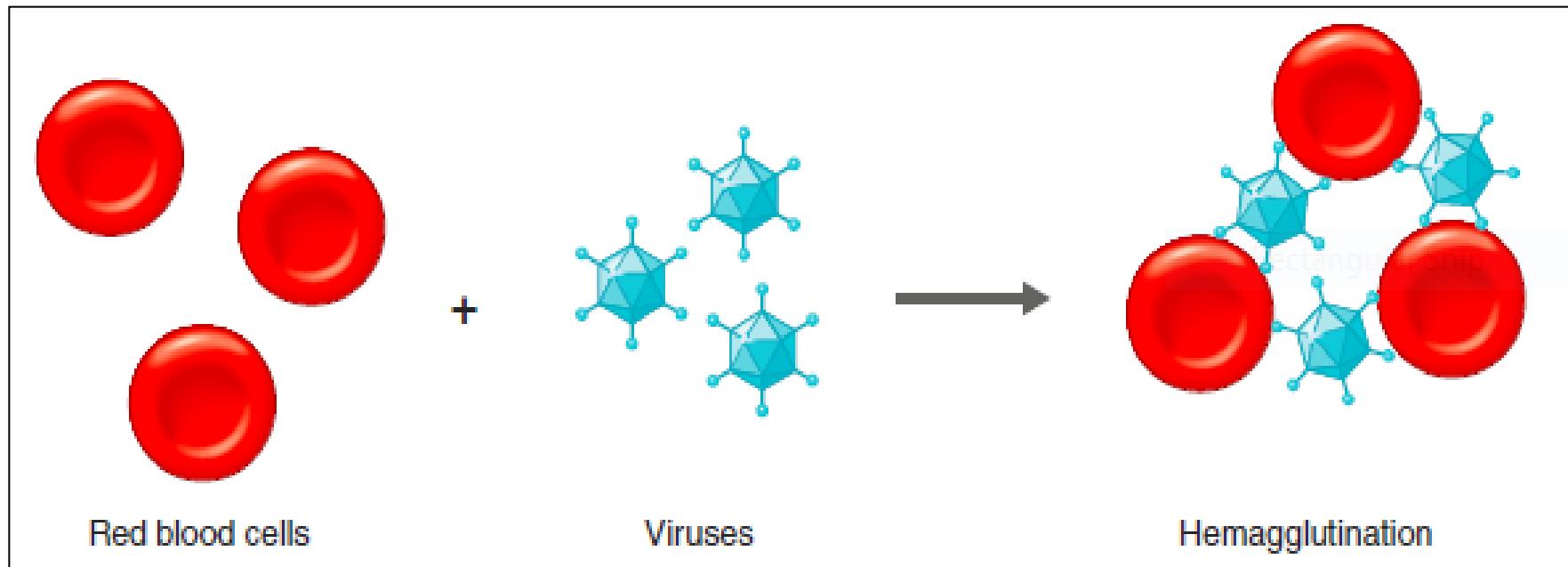


(B) detect antigen by using latex bead coated antibody

Figure: Agglutination method (indirect)

Hemagglutination

- Certain viruses, such as those causing mumps, measles, and influenza, have the ability to agglutinate red blood cells without an antigen–antibody reaction; this process is called viral hemagglutination



Agglutination Inhibition

- It is a modification of the agglutination reaction
 - Absence of agglutination
 - use antibody
 - E. g. In pregnant test, illegal drug test, exposure of individual to virus
- Procedure for pregnancy test
 1. Add known anti-HCG antibody (HCG = human chorionic gonadotrophin = hormone)
 2. Add urine
 - ✓ If visible clumping is formed = no pregnancy
 - ✓ If no visible clumping = there is pregnancy

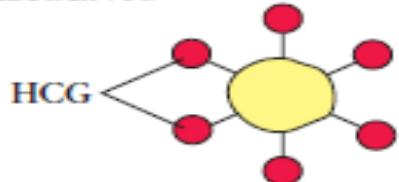
➤ **Procedure for testing individuals who exposed to virus**

1. Mix RBC with antiviral antibodies
2. Add sample
 - ✓ If no agglutination=individual is infected with virus

➤ **Procedure for illegal drugs users** (cocaine or heroin).

1. A blood sample is first incubated with antibody specific for the suspected drug.
2. Then red blood cells coated with the drug are added.
 - ✓ If the RBCs **are not agglutinated** --- the individual **used drug**.
 - ✓ If the RBCs **are agglutinated** --- the individual **not used drug**.

KIT REAGENTS



and



Haptens carrier-conjugate

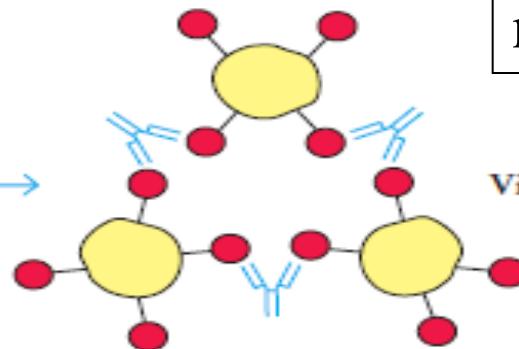
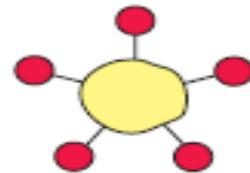
Anti-HCG antibody

TEST PROCEDURE

Urine + Anti-HCG $\xrightarrow{\text{Incubate}}$ + HCG carrier conjugate \longrightarrow Observe for visible clumping

POSSIBLE REACTIONS

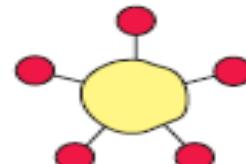
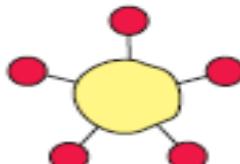
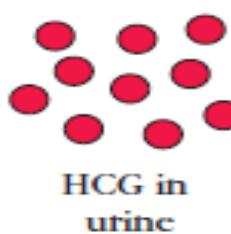
(-) reaction: not pregnant



no pregnancy

Visible clumping

(+) reaction: pregnant



No visible clumping \leftarrow pregnancy

Figure: Procedure for pregnancy test

- Reaction between antibody and virus in which cause viruses are not bind RBC is called **viral hemagglutination inhibition test**

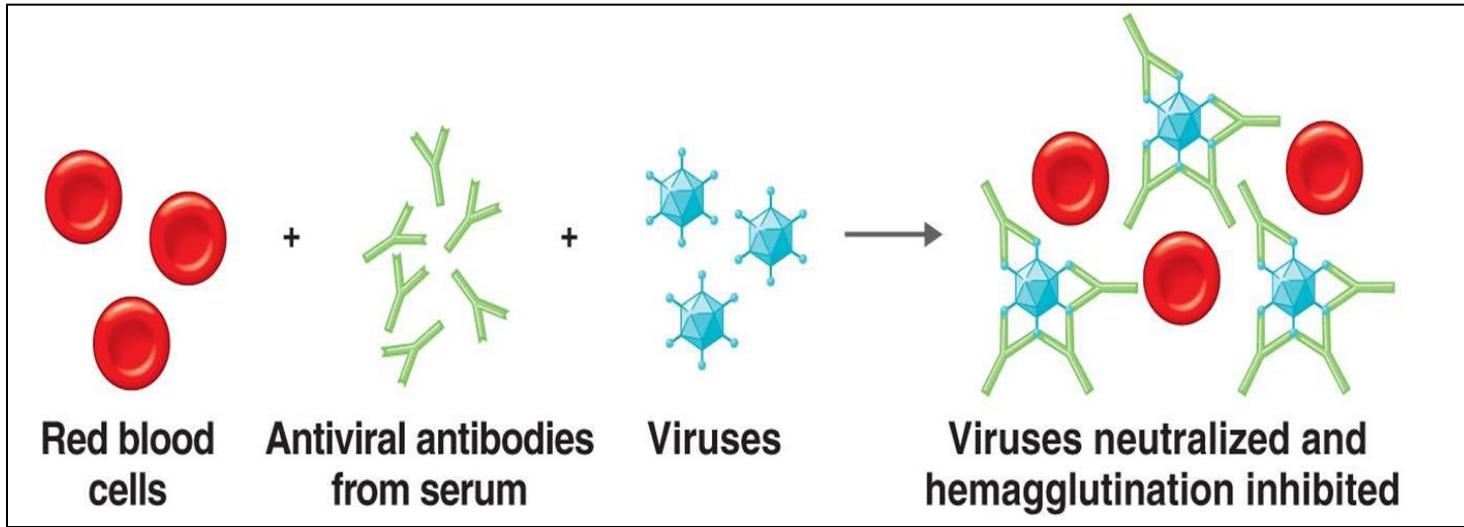
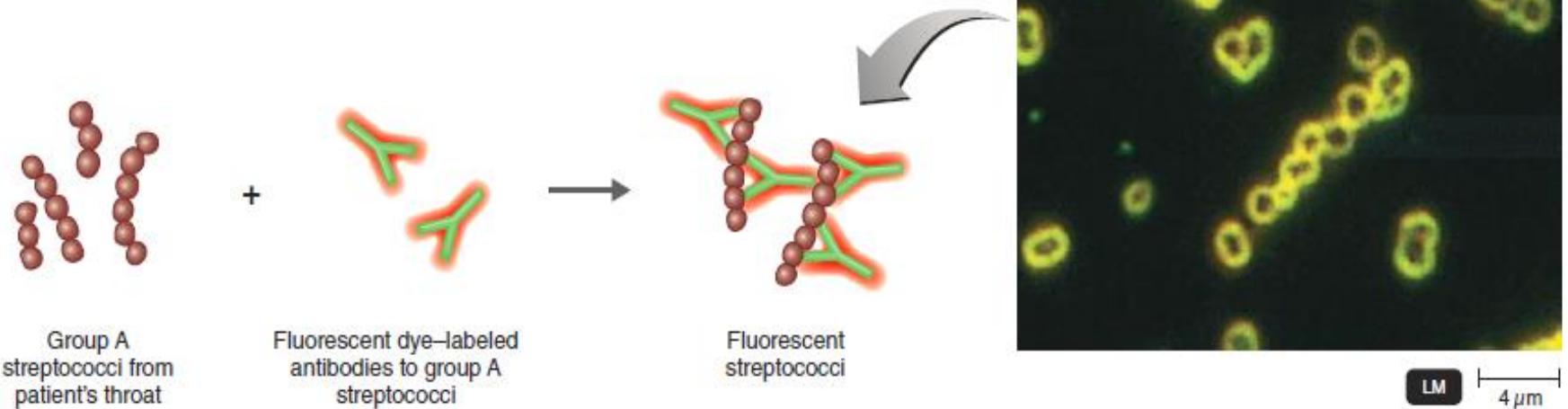


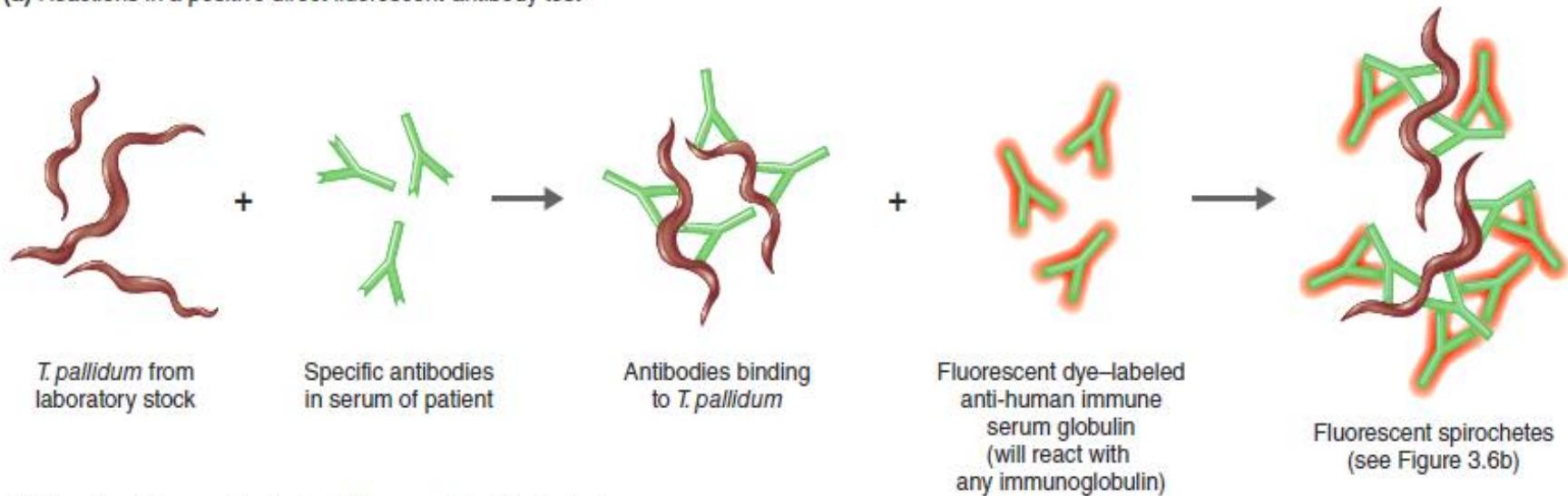
Figure: Viral hemagglutination inhibition test

Fluorescent-antibody (FA) technique

- FA technique is used to detect the presence of a **specific antigen**.
- two types of FA techniques:
 - **direct:** Use Fluorescein-labeled **antibodies**
 - **indirect:** Use fluorescein-labeled anti-human immune serum globulin (**anti-HISG**)
- anti-HISG: is **an antibody** that reacts specifically with any human antibody



(a) Reactions in a positive direct fluorescent-antibody test



(b) Reactions in a positive indirect fluorescent-antibody test

Figure: Fluorescent-antibody

T. pallidum = *Treponema pallidum*

Enzyme Linked ImmunoSorbant Assay(ELISA)

- It is used to detect either **antibodies or antigens** in patient sample
- Two types of ELISA:
 - ✓ **Direct**: detect antigen
 - ✓ **Indirect**: detect antibody

➤ Procedure

1. The specific **antibody or antigen** is attached to a microtitre plate well.
2. The **sample** is added
3. **Removing** the unbound sample
4. Another **antibody/antigen** labeled with a specific enzyme is added
5. **Wash** the unbound enzyme-linked
6. Adding a chromogenic **substrate** specific for the enzyme

Result: Positive reaction produces **color change**

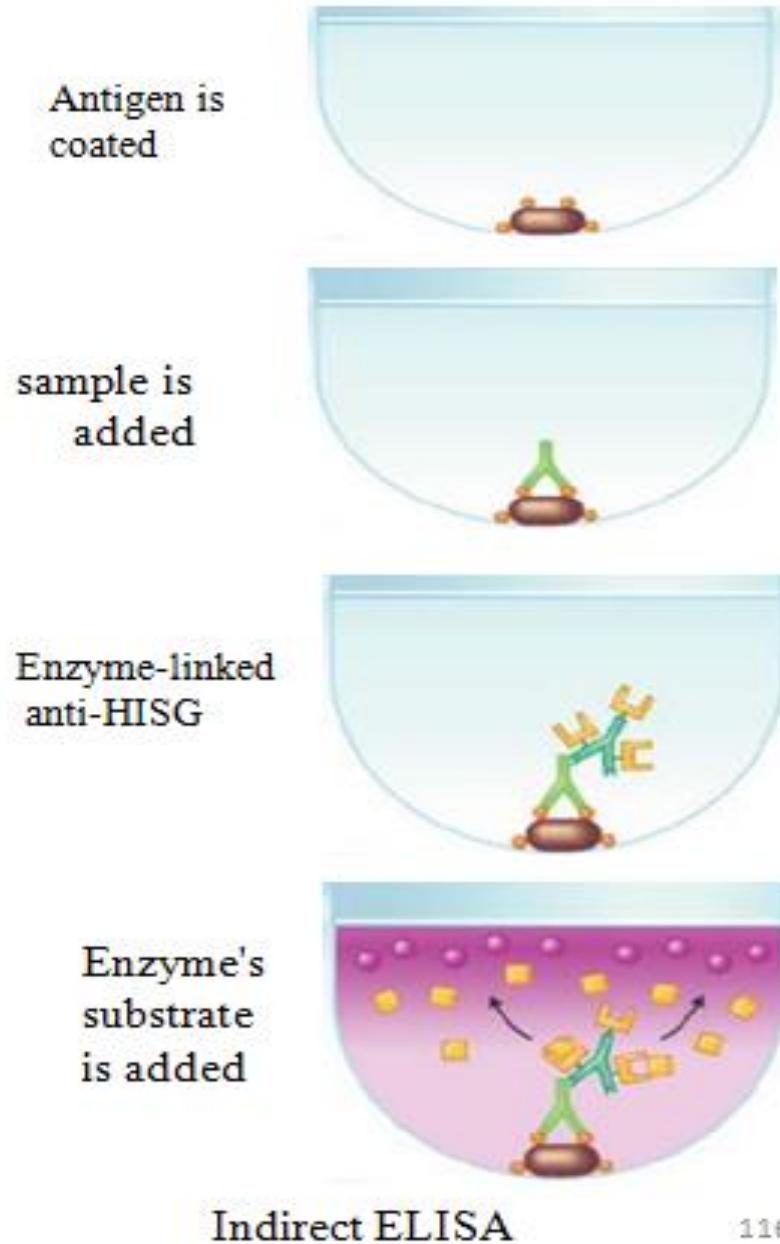
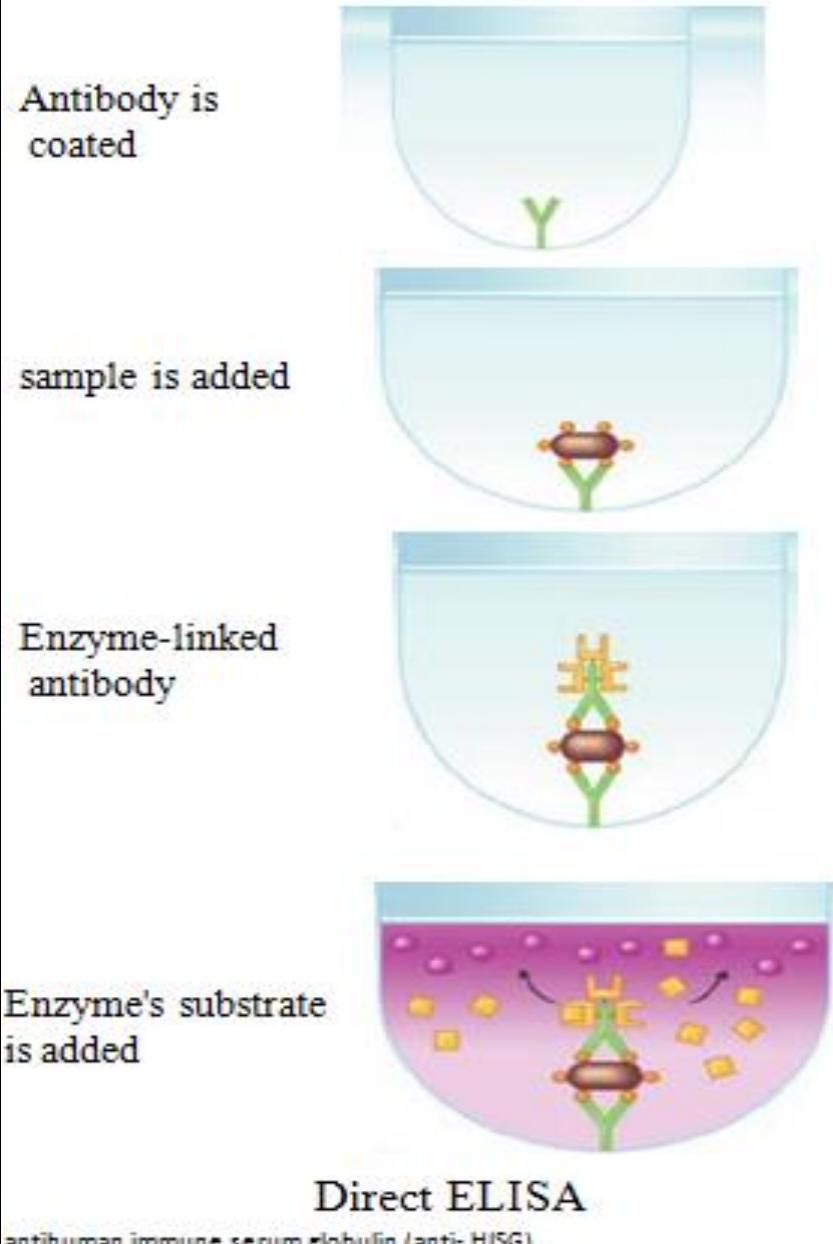
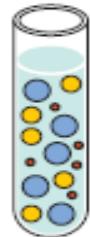


Figure: Enzyme Linked ImmunoSorbant Assay(ELISA) test

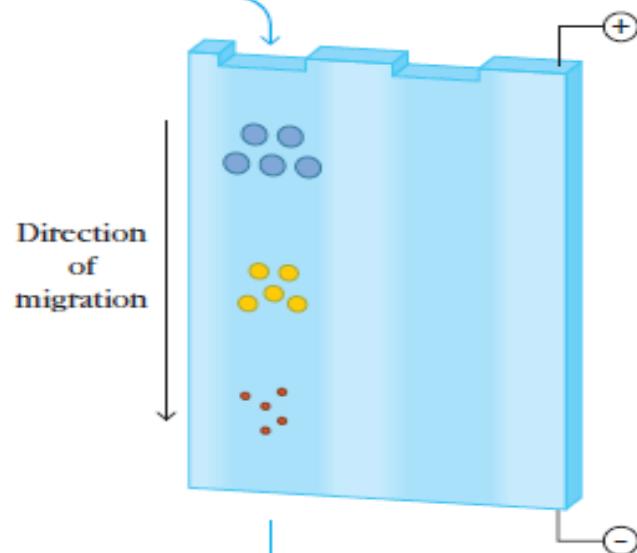
Western Blotting

- It is used to detect **specific protein** in a complex **mixture** of proteins
- **Procedure**
 1. Protein mixture is treated **sodium dodecyl sulfate** (SDS)
 2. SDS treated protein is transferred to **SDS-polyacrylamide gel (SDS-PAGE)**
 3. The individual protein bands are transferred to **nitrocellulose membrane**
 4. Add **radiolabeled** or **enzyme linked** antibody specific for the protein of interest
 5. Expose radiolabeled antibody to x-ray film, and enzyme linked antibody to chromogenic substrate

(a) Add SDS-treated protein mixture to well of gel

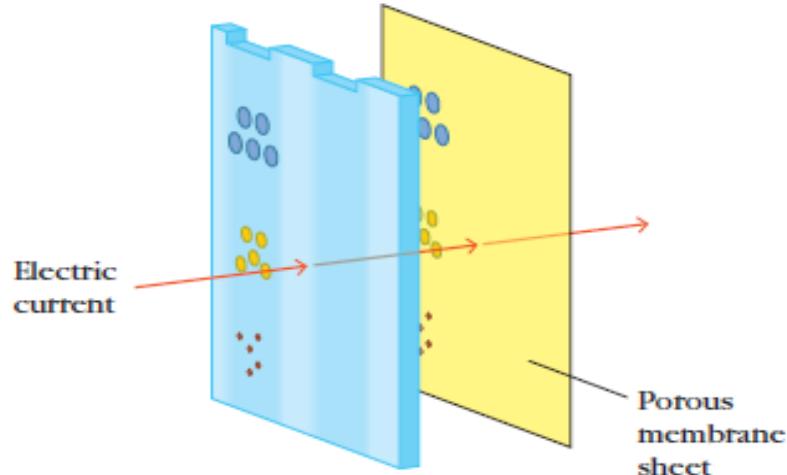


(b) Electrophoresis in SDS-polyacrylamide gel

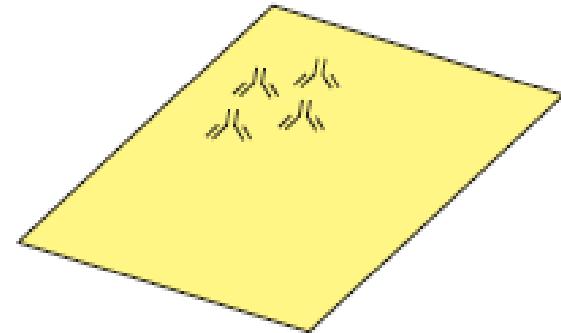


Protein antigens denatured in SDS

(c) Remove gel and perform electrotransfer



(d) Bind antigen of interest with enzyme-linked antibodies



(e) Add substrate to activate color reaction

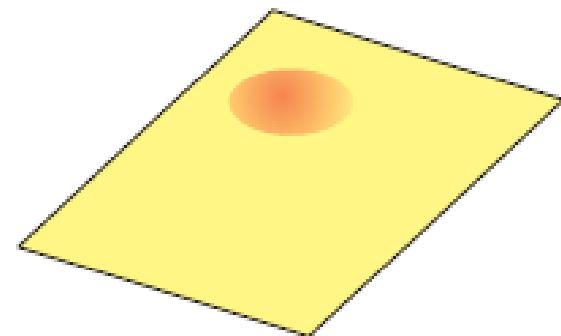
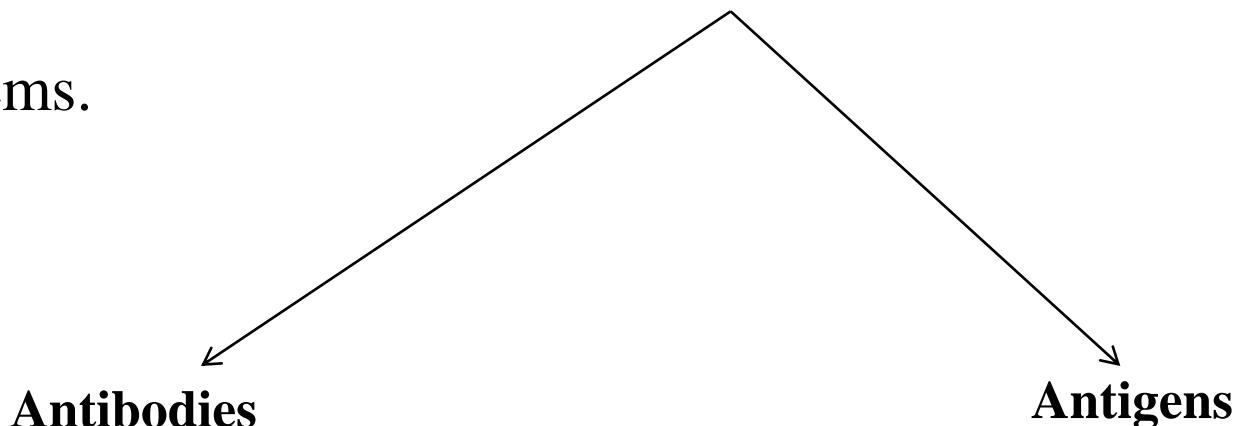


Figure: Western Blotting analysis

Immunotherapy

- It is treatment of diseases with **substances** which stimulate immune systems.



Antibodies

- ✓ Monoclonal antibodies
- ✓ Polyclonal antibodies

Antigens

- Vaccine

- **Monoclonal antibodies:** are **homogeneous population** of immunoglobulins directed against **a single epitope**
- **Polyclonal antibody:** are a **heterogeneous mixture** of antibodies directed against **various epitopes** of the same antigen

Thank you very much!!