ADDIS ABABA UNIVERSITY

CVMA

Department of Veterinary microbiology, immunology public health (MIVP)

Diagnostic veterinary immunology

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VLT immunology

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1. introduction

Immunology :-Study of the **components** and function of the immune system.

- **The immune system** is defense system ,which able to generate an **enormous variety of cells and molecules** capable of specifically **recognizing and eliminating** an apparently limitless variety of **foreign invaders**.
  - **Molecules, cells, tissues and organs** which provide **non-specific** and **specific protection against**.
    - ✓**Microorganisms**
    - ✓**Microbial toxins, Tumor cells**

- These **cells and molecules** act together in a dynamic network whose complexity rivals in a similar way as of nervous system.

Function

- two related activities—**recognition** and **response**.
  - able to differentiate between **foreign molecules** and the **body's own cells** and **proteins**.

- Once a **foreign organism** has been recognized, **recruits a variety of cells and molecules** to mount an appropriate **response,** called an **effector response,** to eliminate or neutralize the organism.

- **Later exposure** to the same foreign organism induces a **memory response,**
  - characterized by a more rapid and heightened immune reaction.

- In some circumstances, the **immune system** fails to act as protector because of some **deficiency** in its components; at other times,
  - it becomes an aggressor and turns its awesome powers against its own host.

Historical back ground of immunology

- Immunology is young , even the word “immunology “did not known **until,1910.**
- **It had been noticed for centuries that although many people died after exposure to a particular diseases,** some survived and did not get sick **when exposed the second time.**

I. Thucydides (historian ,concept of immunology)

- **Thucydides described a plague (*Yersinia pestis*) in Athens, he wrote in 430 BC** that only those who had recovered from the plague could nurse the sick because they would not **contract the disease a second time.**

- Although early societies recognized the **phenomenon of immunity**, **almost two thousand years passed** before the concept was successfully converted into **medically effective practice.**

II. Chinese and Turkeys

- The **first recorded attempts** to induce immunity **deliberately** were performed by the **Chinese** and Turkeys in the 15th century.
- Various reports suggest that the **dried crusts** derived from **smallpox pustules** were either;
  - **inhaled into the nostrils** or
  - **inserted into small cuts in the skin which** a technique called ***variolation***).

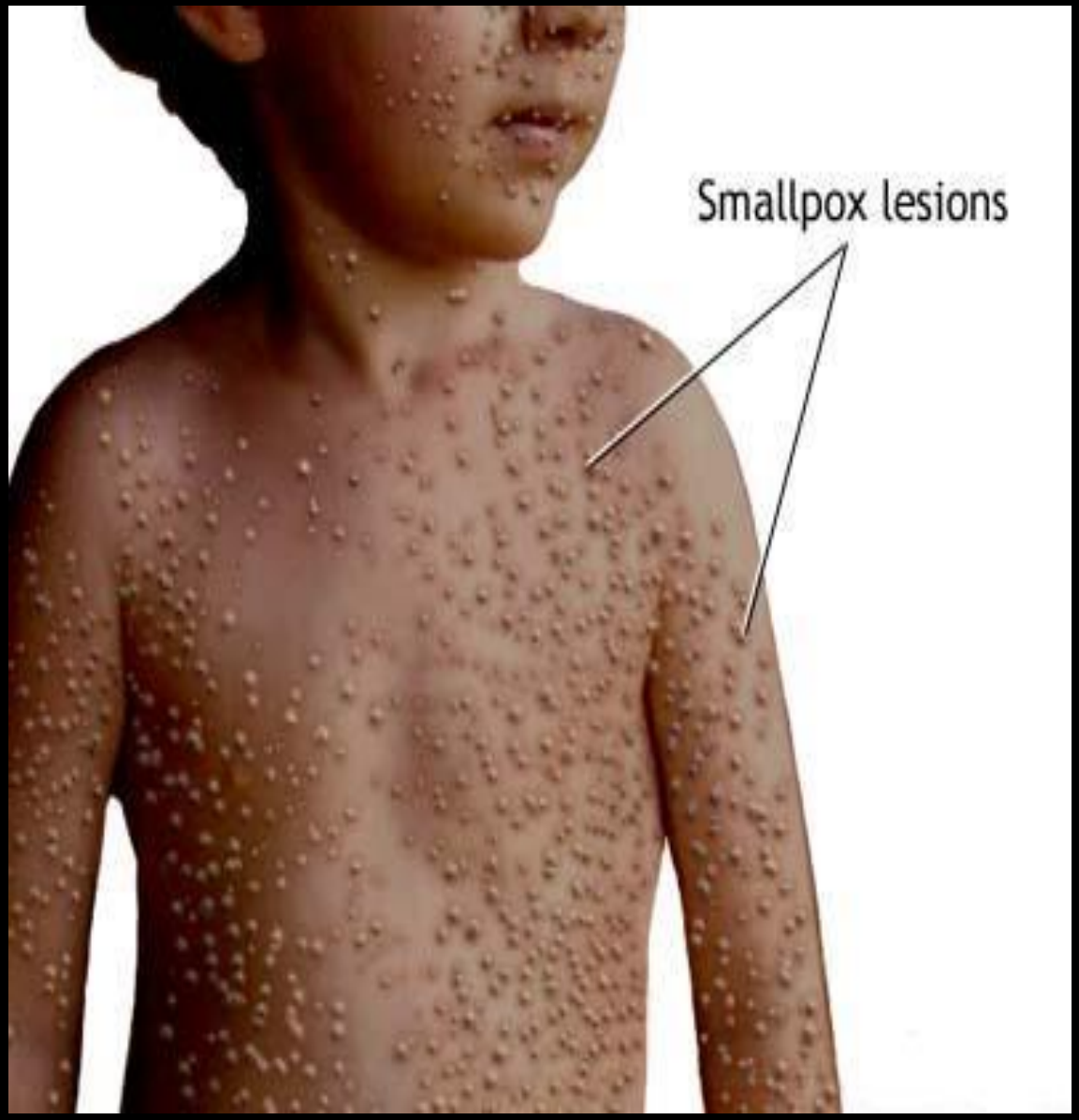
Variolation vrs vaccination

- Small pox was one of the most feared diseases of human **kind, killing huge numbers of people.**
- **At 11th century, the Chinese determined that persons who survived on attack of small pox would not get the diseases for the second time.**
- **There fore, in ancient china young children were infected with small pox using scabs from the pocks on the skin of an infected person.**

- The process of "v**ariolation**" involved exposing healthy people to material from the lesions caused by the disease, either by **putting it under the skin**, or,
  - more often, **inserting powdered scabs from smallpox pustules into the nose.**
- Variolation was known and practiced frequently in the **Ottoman Empire around 1670.**

III. Lady Mary Wortley Montague

- **Variolation** later became popular in **England**, mainly due to the efforts of **Lady Mary Wortley Montague** and the practice of variolation spread rapidly throughout **England in the 1740s and then to the American colonies.**


Smallpox lesions

IV. Edward Jenner

- **Edward Jenner** an England physician had observed that d**airymaids** and **farmers** were "**fair skinned**" and **lacked that pock marked complexions of their fellow citizens.**
- This observation led Jenner to conclude that the cattle workers had some how **immune to small pox**.
- In 1796, **Jenner** inoculated **James Phipps** with material obtained from a **cowpox lesion** that appeared on the **hand of a dairymaid** .

- **Six weeks later, he** inoculated the experimental subject with smallpox without producing disease.
  - Although this experiment justifiably lacked an appropriate control, further studies by Jenner established the **efficacy of his vaccination procedure**.
- **Jenner's approach to small pox prevention was subsequently published and was quickly adopted in through out Europe**.

V. Robert koch (after 88years from Jenner)

- in 1884 Robert koch proposed the **germ theory of diseases** which was the base of identification of infectious causes.

- **Robert Koch**, inoculated the ear of a rabbit with the blood of an animal that had died of anthrax.
- Collected isolated infected lymph nodes from the died rabbit. Inoculated to health one .

VI. Louis Pasteur

- Quite independently, **Louis Pasteur** "father of immunology" began his studies of the "**chicken cholera bacillus**."
- In his discovery, Pasteur accidentally left a flask of the bacillus on the bench over the summer and inoculated 8 chickens with this "**old but viable**" stock of chicken cholera bacillus.

- He found that not only did the chickens not die, but they did not even appear ill, Pasteur said that the **virulent chicken cholera bacillus** had become **attenuated** by sitting on the bench over the summer months.
- The similarity between these results and those of **Jenne**r using **vaccinia virus** was immediately apparent to him.
- In honor of Jenner, Pasteur called his treatment **vaccination**.
- Pasteur later worked on anthrax and rabies and developed the first viable vaccine for anthrax and rabies.

7. **Metchnikoff** was the first to recognize the contribution of phagocytosis to the generation of immunity.
8. **Paul Ehrlich** predicted the existence of immune bodies (antibodies) and **side-chains** from which they arise (receptors).
   - **Ehrlich surmised** that **erythrocytes would not** have this capacity and speculated that this immune function might be a specialized characteristic or "haemopoietic tissue."

9. The **first Nobel prize in Physiology** or Medicine was awarded to **"von Behring** "for his work on **serum therapy**, especially its application against diphtheria, by which he has opened a new road in the domain of medical science and thereby placed in the hands of the physician a victorious weapon against illness and deaths."

- Metchnikoff and Ehrlich shared the Nobel prize in 1**908** "in recognition of their work on immunity."

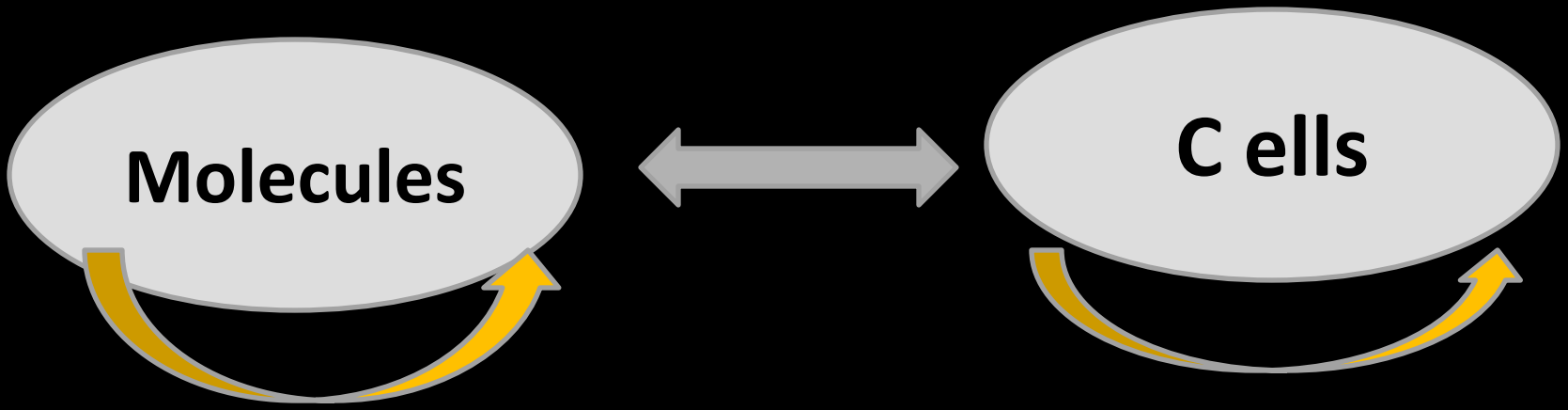
Summary of the state of immunology at the end of the 19th century

1. 1796-Edward Jenner (smallpox)
2. 1881-Loius Pasteur (vaccines)
3. 1884-Elie Metchnikoff (phagocytes)
4. 1890-Emil von Behring* (antibodies)
5. 1895-Jules Bordet* (complement)
6. 1959-Rodney Porter and Gerald Edelman* (antibodies)
7. 1960-F McFarlane Burnet* (tolerance)
8. 1975-Cesar Milstein*(monoclonal Ab)
9. 1987-Susumu Tonegawa* (genetics)
10. 1996-Peter Doherty and Rolf Zinkernagel* (MHC)

Overview of the immune system

Immune response /immunity has two components :-

- **cells and molecules**
- **They communicate with each other;**
- **Also communicate between their own kinds.**



- Immunity is classified in to two major groups.
  - Non specific immunity
  - Specific immunity

	Cells	Molecules
Specific	-B-lymphocytes	-antibodies
	- T-lymphocytes	-T-cell receptors (TCR)
Non-specific	-phagocytes	-complements
	-Natural killer cells	-cytokines
	-mast cells	-pharmacological Cpds

Types of the immunity /immune response

1. Natural /innate/non-clonal/non-adaptive immunity

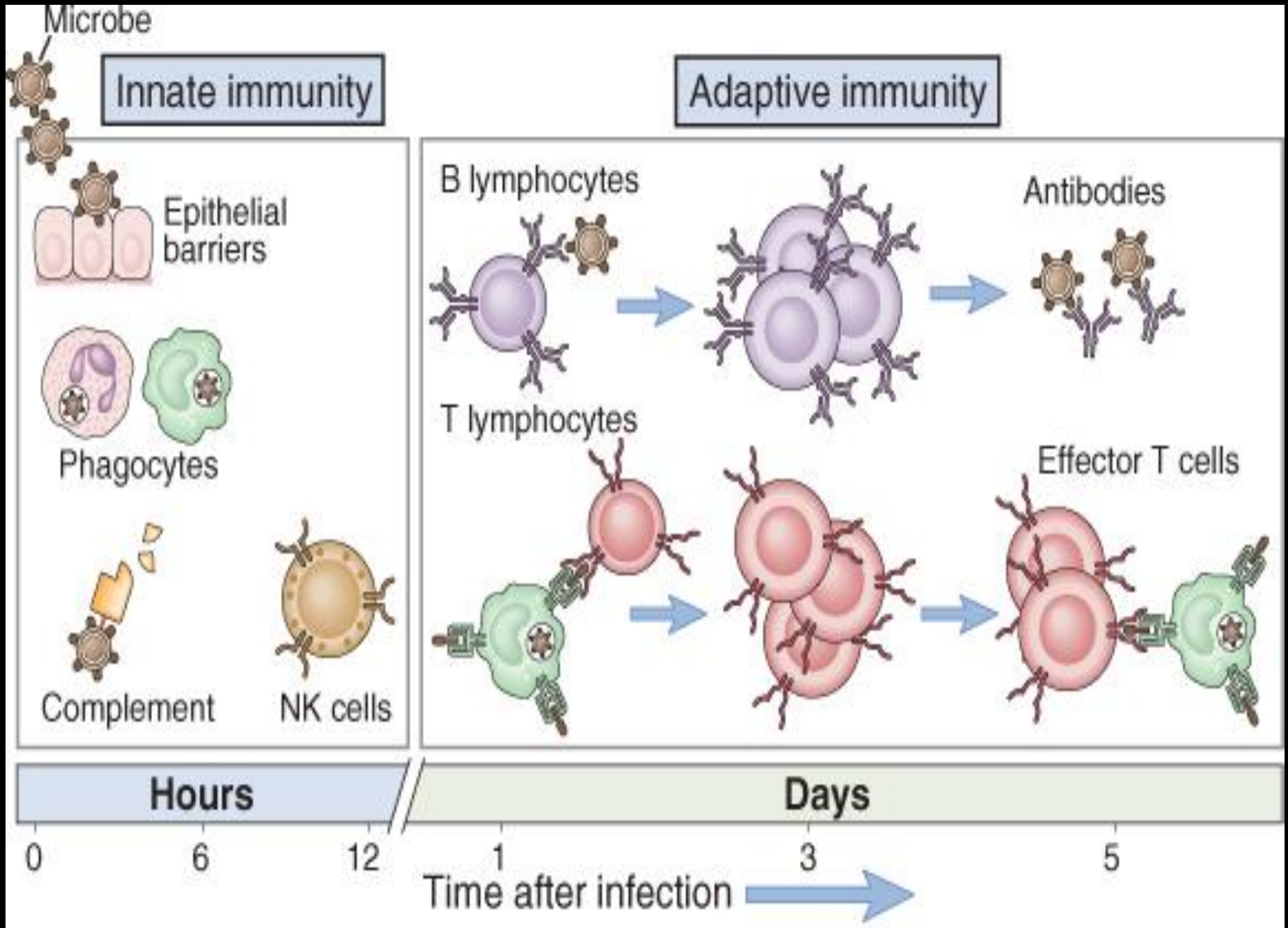
- **Present in a constituent way**
- **Aseptic**
- **Has no memory**

2.Acquired /clonal/adaptive immunity

- **Developed after the birth of the animal**
- **Specific**
- **Has memory**

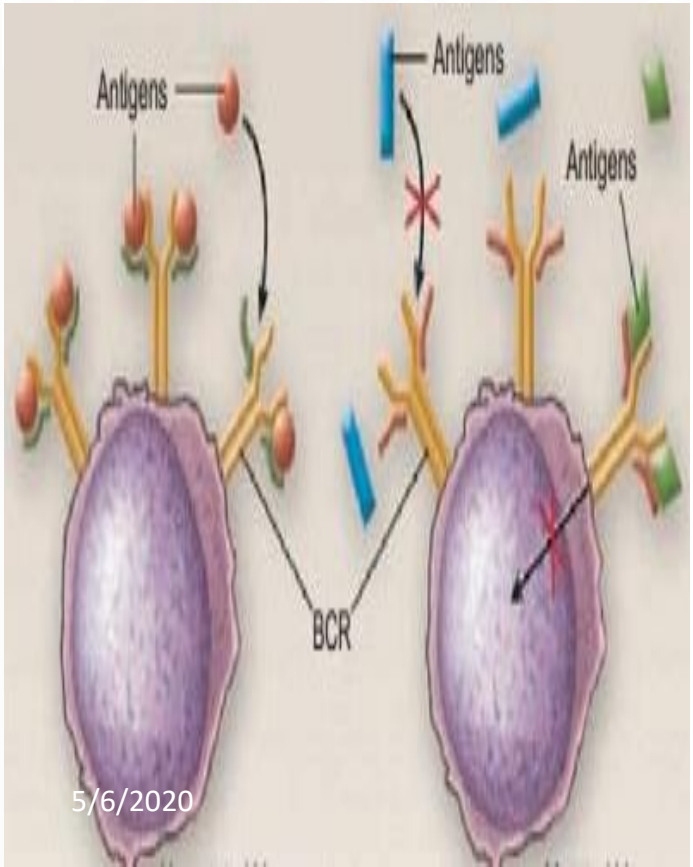
– Natural immune system starts from body's physical barriers (skin, mucous membrane, enzymes etc.)

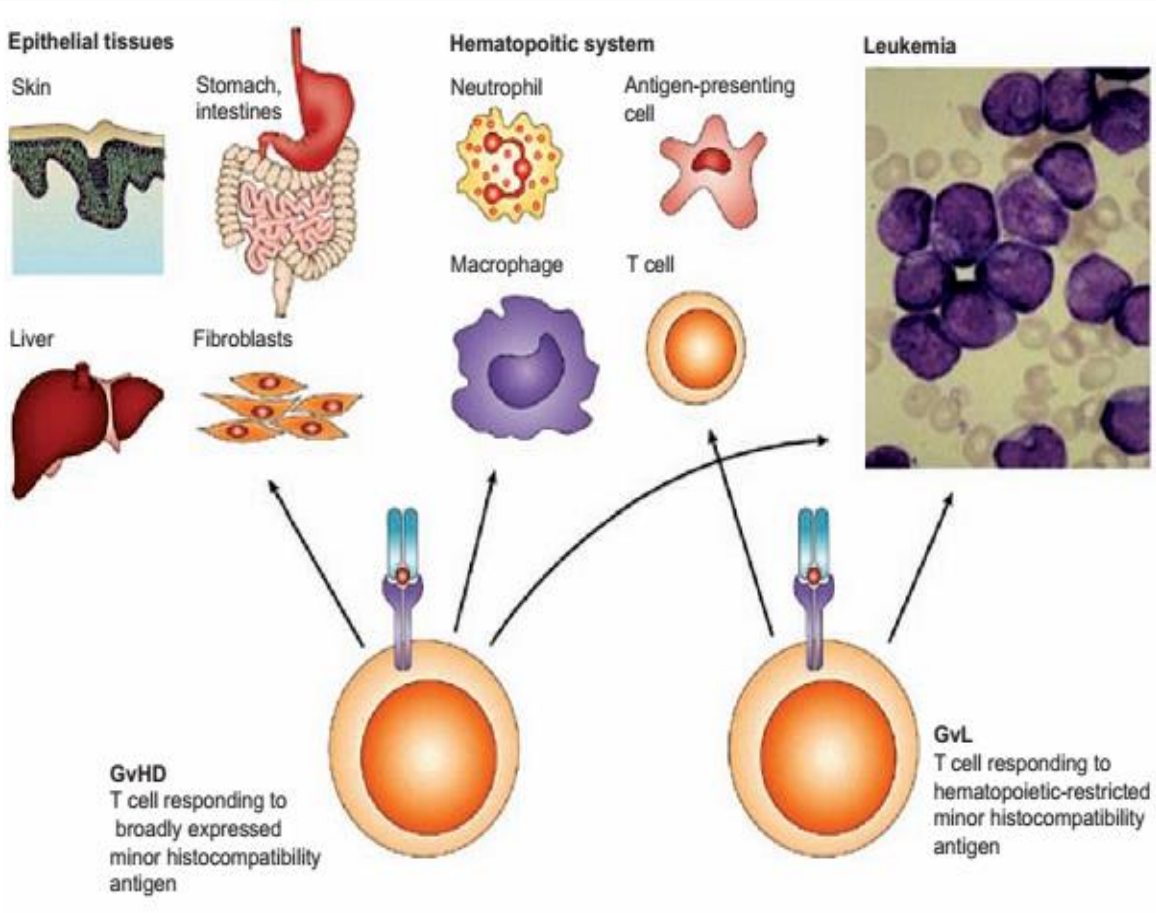
– Cells of the innate immunity.



Chapter two

Cells and Organs of the Immune System





2.1 Cells and Organs of the Immune System

The immune system consists of many different **organs and tissues** that are found throughout the body.

- These organs can be classified functionally into **two main groups**.
  - ***primary lymphoid organs*** ; **development and maturation of lymphocytes; (training and preparation site)**
  - **secondary lymphoid organs =sites where mature lymphocytes interact with antigen**.(battle field)

- **blood vessels and lymphatic systems** connect these organs, uniting them (logistics)

- The cells of the immune system circulate through the **body via lymph and blood**.

- The cells of the immune system spend much of **their time in lymphoid organs**.

- Thus, they develop (arise) in **primary lymphoid organs**, and **they interact with antigens** in secondary lymphoid organs.

2.1 Hematopoiesis

- **All blood cells** arise from a type of cell called the **hematopoietic stem cell (HSC) and process is called hematopoiesis.**

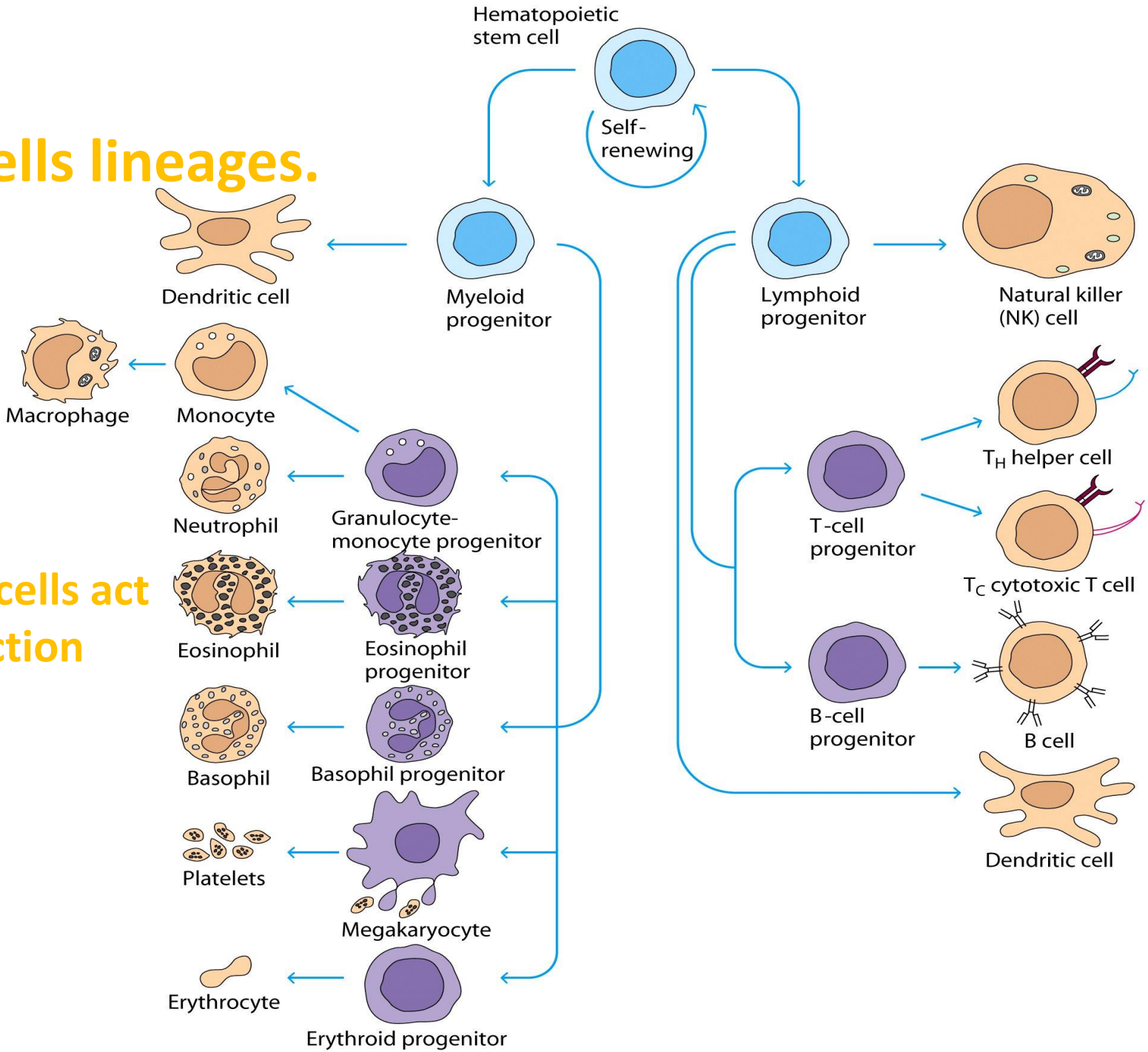
- **Stem cells** are cells that can differentiate into **other cell types**.
  - **they are** self-renewing.
  - they maintain their population level by **cell division**.

- **Early in hematopoiesis**, a **multipotent stem cell** differentiates along one of two pathways, giving rise to either;
  - ✓ **lymphoid progenitor/ancestor cell** or
  - ✓ **myeloid progenitor cell.**

- Sites of **hematopoiesis;-**
  - **Early embryogenesis :-in the yolk sack**
  - **Fetus:- liver , spleen and bone marrow**
  - **Adult:-marrow of spinal column, femur and humerus, ileum, ribs and sternum.**

Blood cells lineages.

Most blood cells act to fight infection



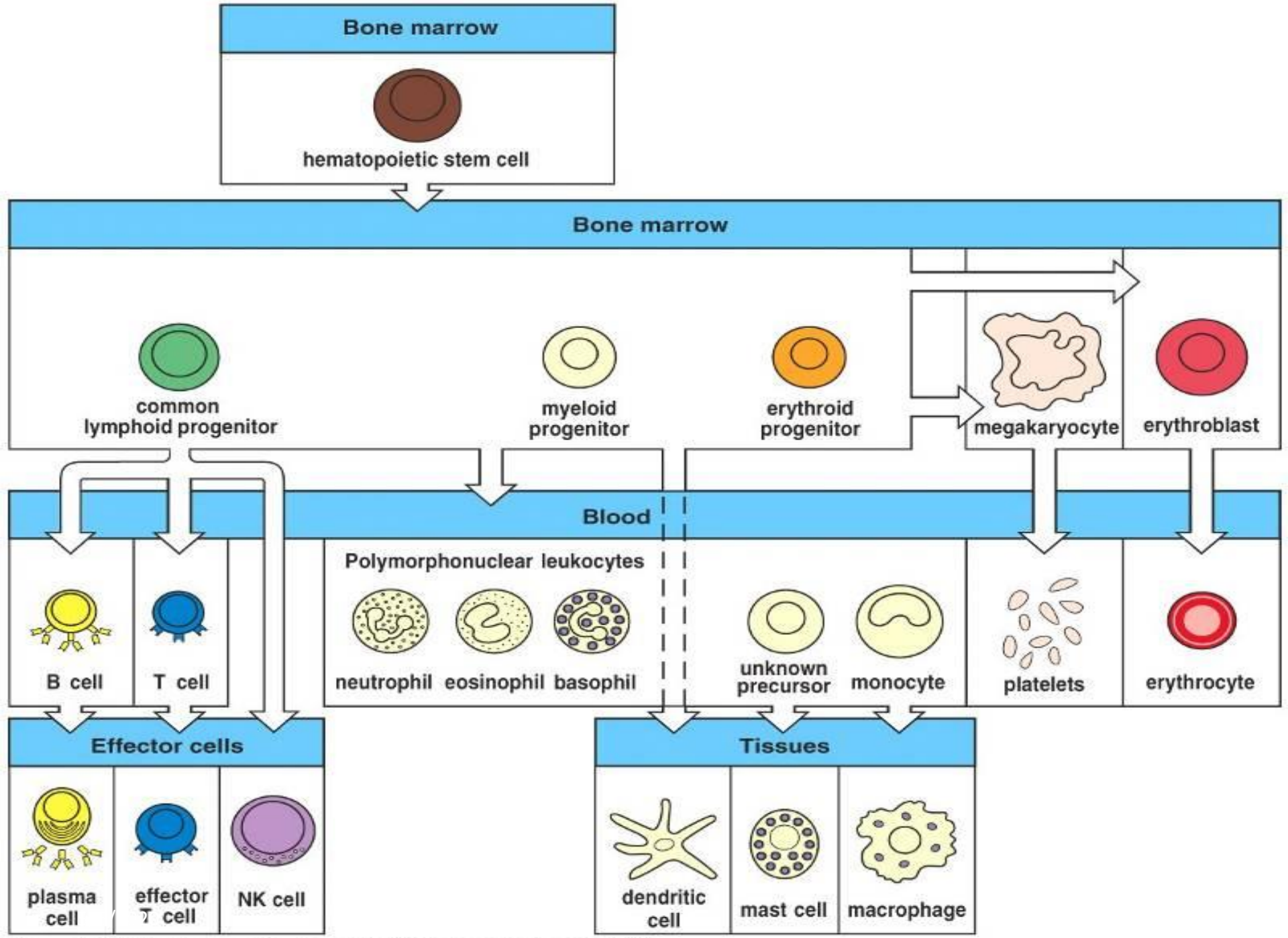


Figure 1-11 The Immune System, 2/e (© Garland Science 2005)

2.1.1cells of myeloid lineage

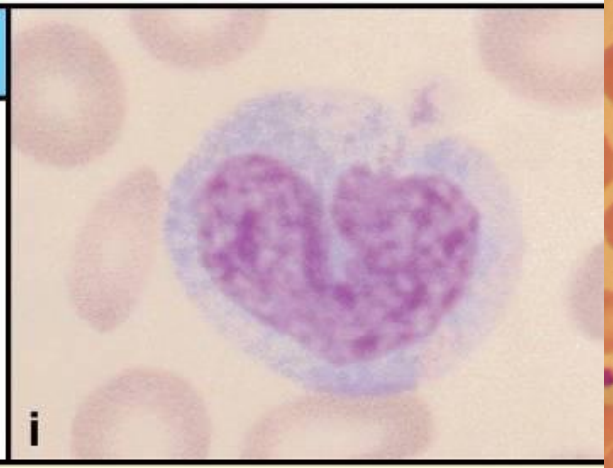
1. Monocytes :-

- Among the **largest blood cells** in the circulation.
- Have **cytoplasmic lysosomes**
- They **circulate in the blood** at low density and 3-5% of all blood leukocytes .
- In the tissues they mature further to become macrophages.

2. Macrophages

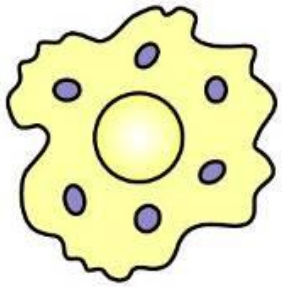
- Derived from the greek word "**big eaters**".
- Large, powerful phagocytes that function primarily in the tissues, tirelessly engulfing and digesting not only foreign entities, but also spent host cells and cellular debris.
- They are the '**garbage collectors** " of our tissues.

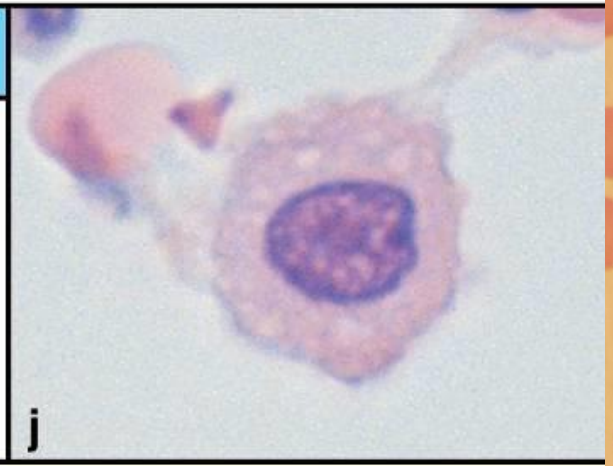
Monocyte



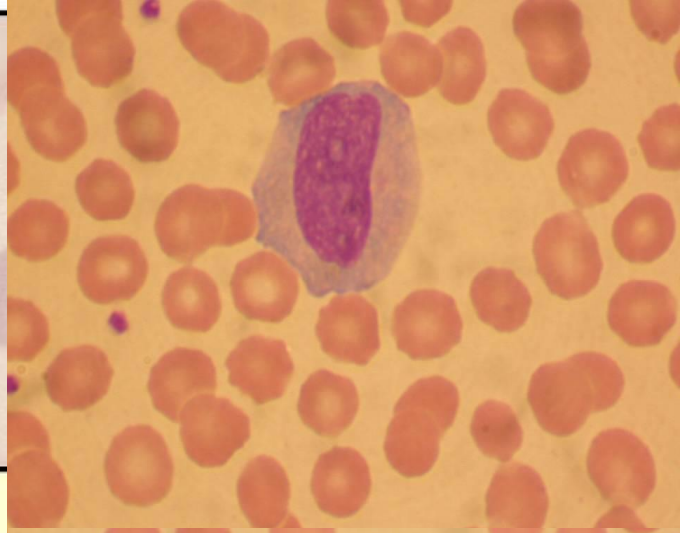
Circulating precursor cell to macrophage

Macrophage





Phagocytosis and killing of microorganisms.
Activation of T cells and initiation of immune responses



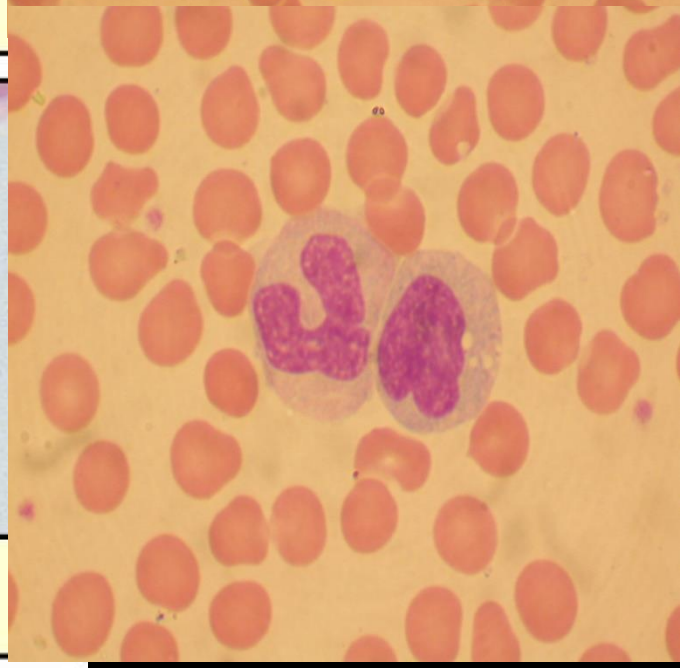
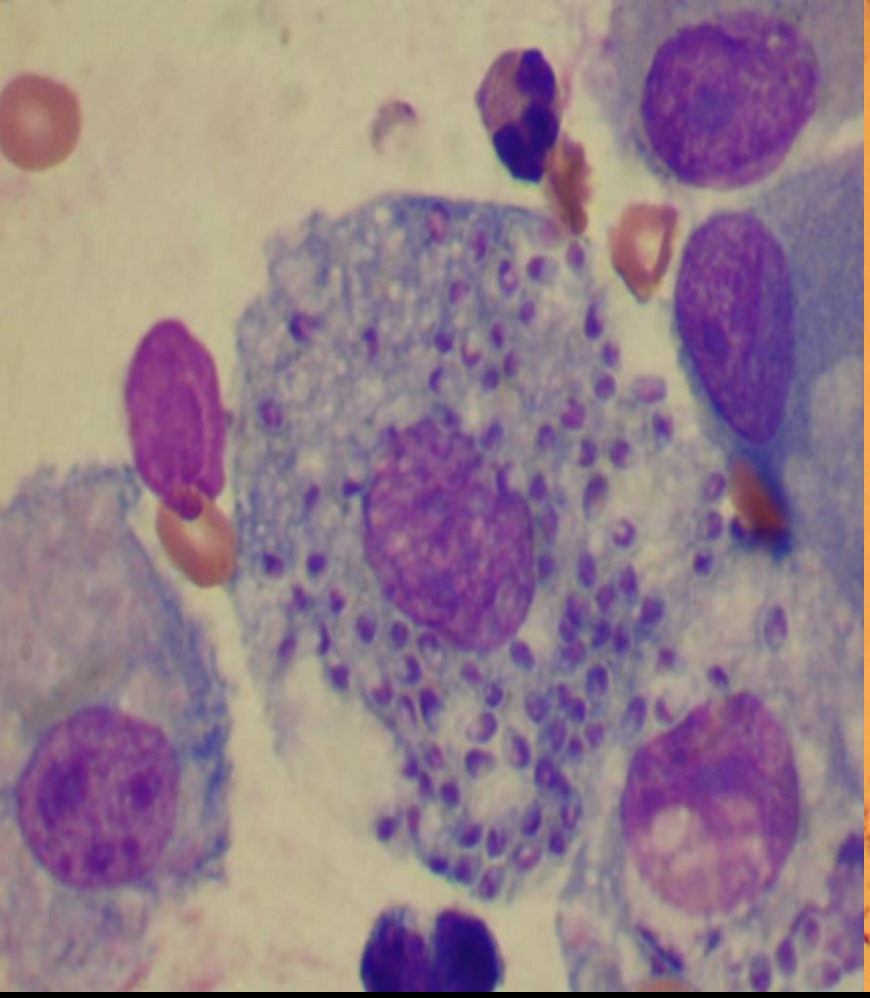
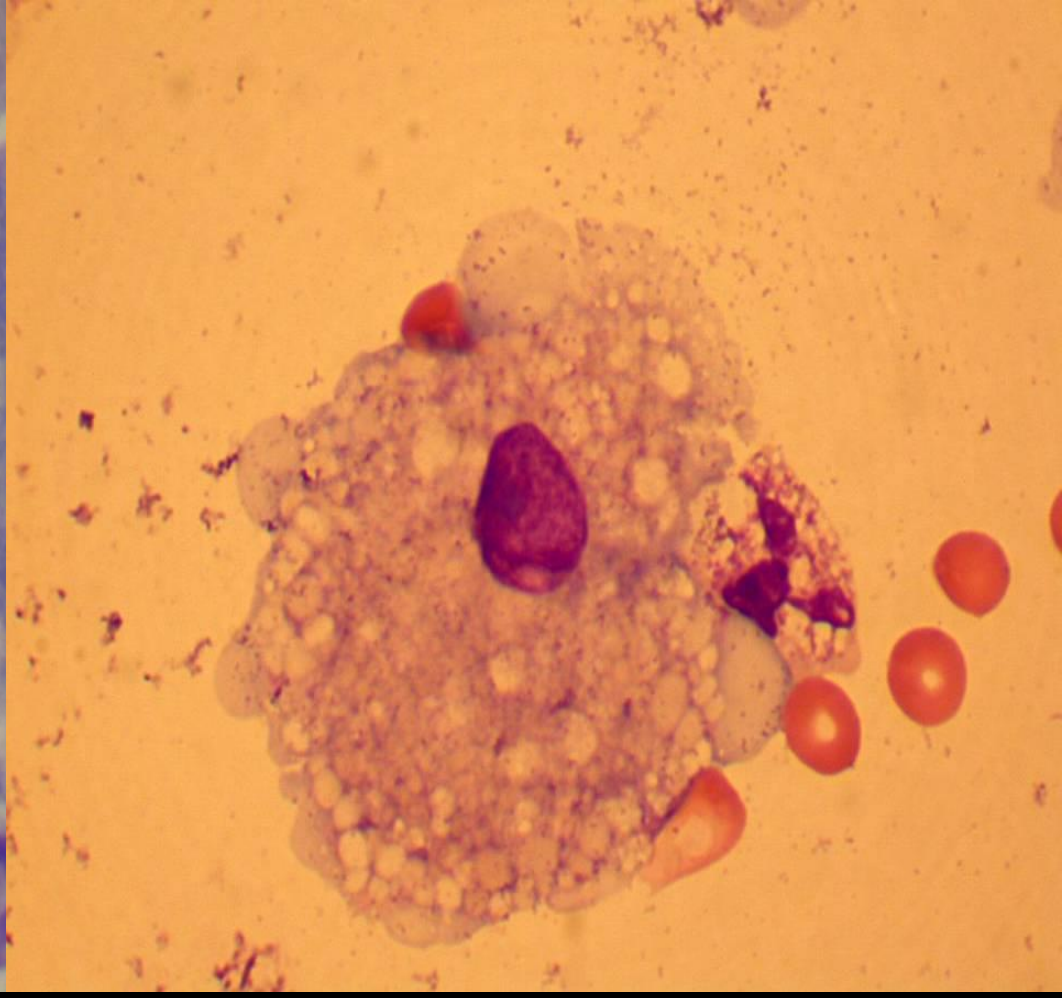


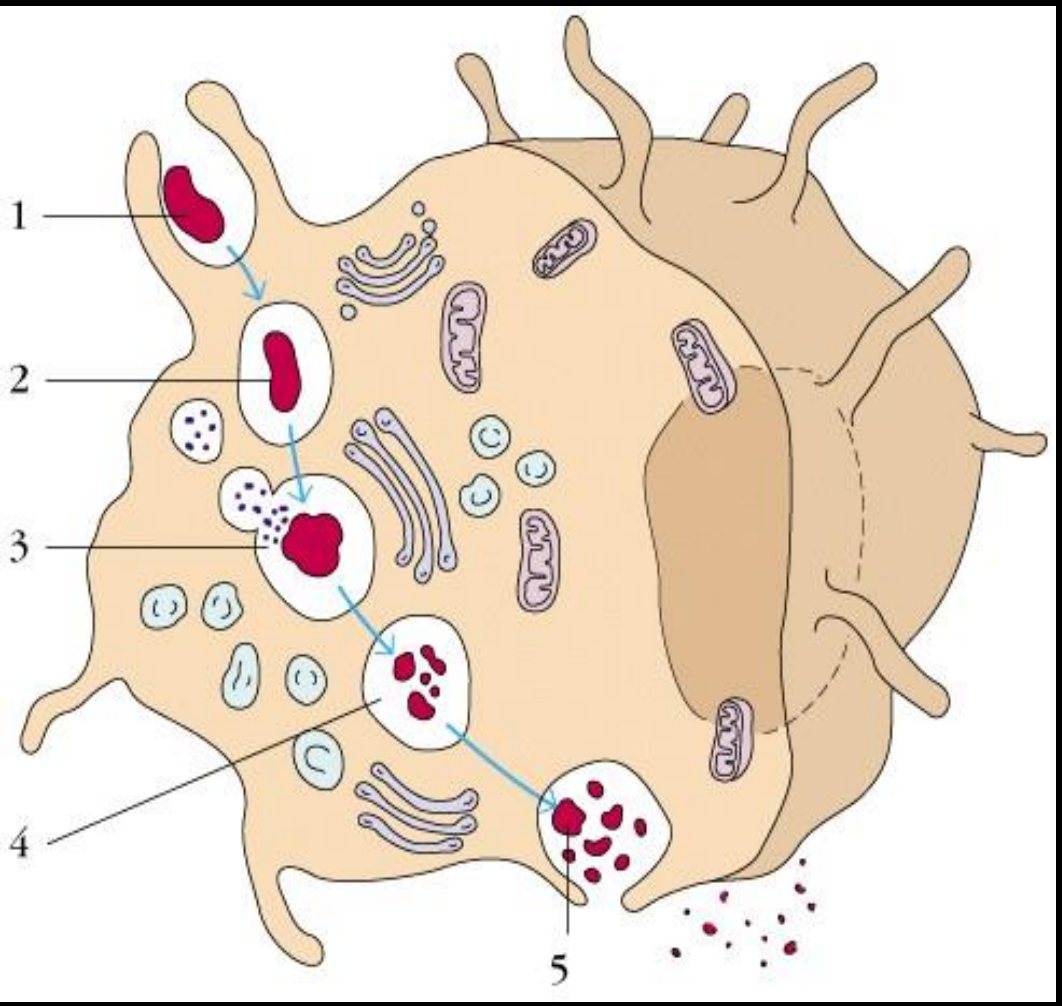
Figure 1-9 part 5 of 6 The Immune System, 2/e (© Garland Science 2005)





Tissue Macrophages

- They are **long-lived mononuclear** phagocytic cells found in all organs and tissues(2-4 months).
- Tissue macrophages in different locations in the body have different names, often reflecting where they reside in the body.
  - **Brain = microglial cells**
  - **Connective tissues= histocytes**
  - **Kidney=mesangial cells**
  - **Liver =kupffer cells**
  - **Lung=alveolar macrophages**.
- They do have also different receptors
  - **Transport receptors**
  - **Cytokine receptors**
  - **Complement receptors**
  - **Antibody receptors**



1. Bacterium attaches to membrane

2. Bacterium is ingested, forming phagosome,

3. Phagosome fuses with lysosome.

4. Lysosomal enzymes digest the bacteria.

5. Digested material is released from cell.

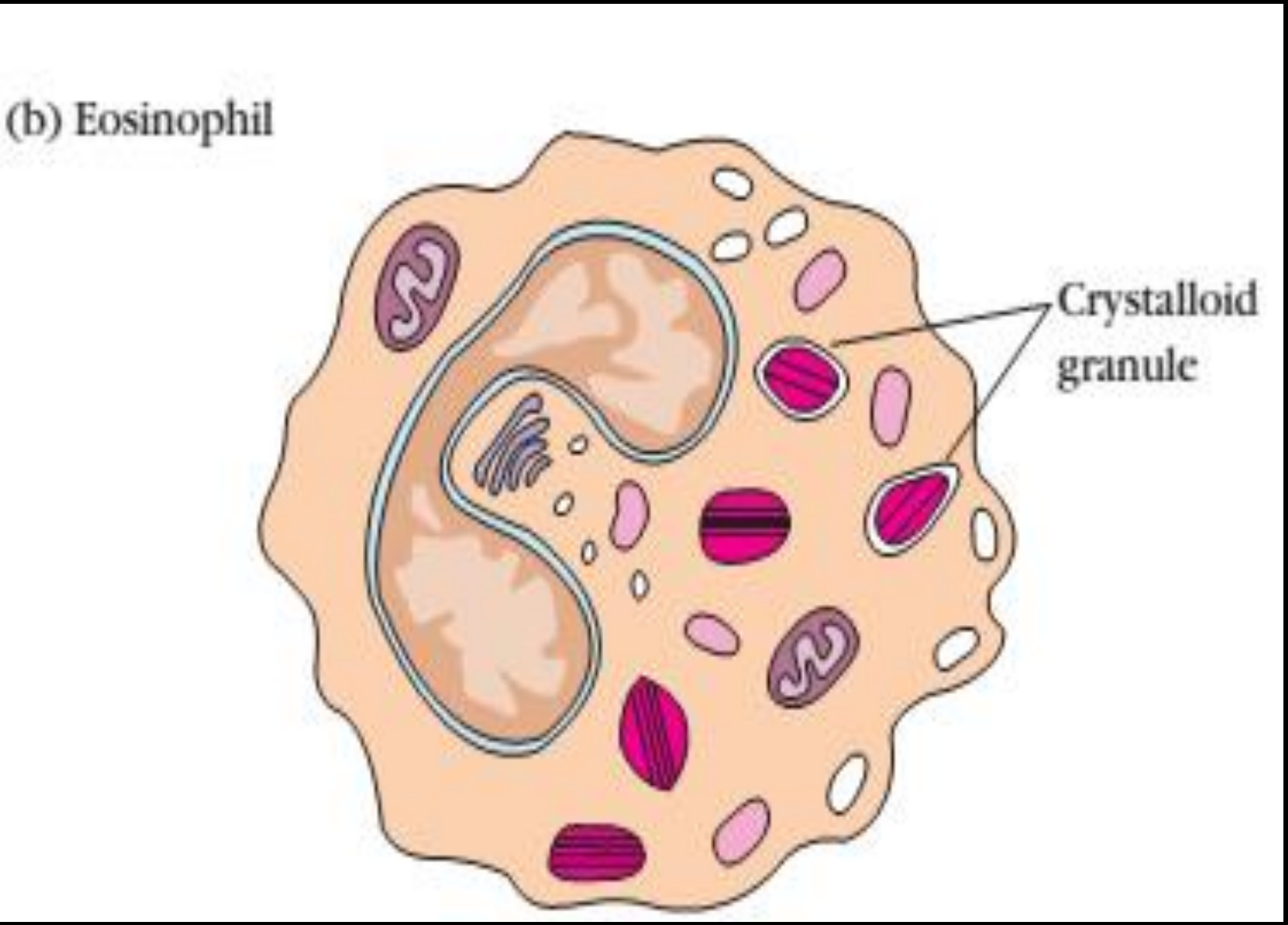
Phagocytes: -macrophage, neutrophils, dendritic cells

3. Granulocytes

- It comprises **neutrophiles**, **basophiles** and **eosinophiles.**

A. Eosinophils :-

- Leukocytes with **bilobed nuclie** and large **cytoplasmic granules (crystalloid granules)**
- They stain reddish with certain acidic (such as eosin).
- Their primary function is the removal of parasites.
- 99% of mature eosinophils reside in the **connective tissues** and so constitutes less than 4% of all leukocytes in blood.
- Also have a role in **allerge**

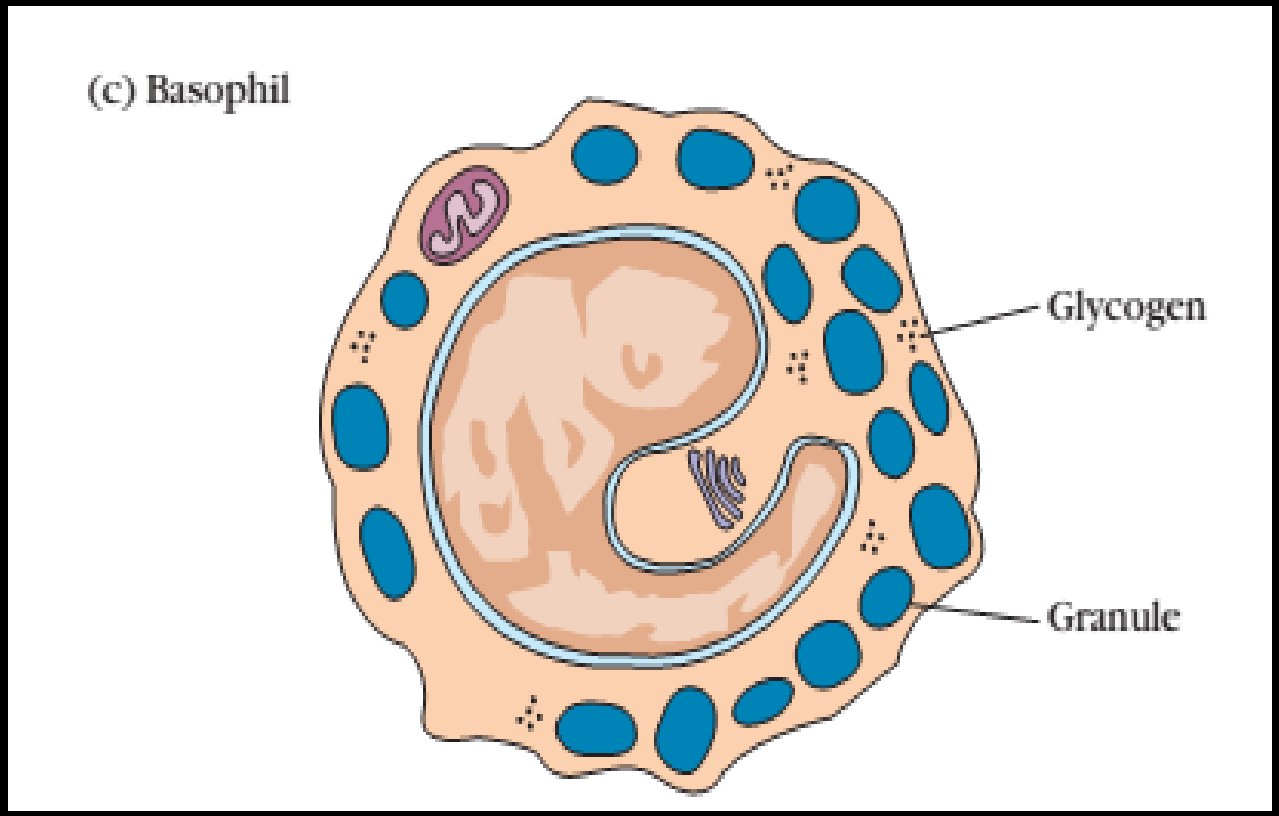

(b) Eosinophil
Crystalloid granule

4.Basophiles

- Are circulating leukocytes with **irregular shaped nuclei**.
- Cytoplasmic granules that react with basic dyes such as **hematoxylin**, thus staining a **dark blue color**.

- Their granules contain **Heparin**, **vasoactive amines** and **many enzymes.**
- **Are important for inflammation.**

- They **reside primarily in the blood** until they move into the tissues during an **inflammatory response**.



5. Mast cells

Cytoplasmic granules stain in a way similar to basophiles and also contain **Heparin and histamines.**

- However, **mast cells** are derived from separate cell linage.
  - **Nuclei not lobed**
  - **More granules .**
  - **Smaller size compared to basophiles**.
- Unlike basophiles rarely found in blood.
  - Resides to live connective tissues and gastrointestinal mucosa.

Tissues injury → degranulation →

-release of histamine

-dozens of cytokines

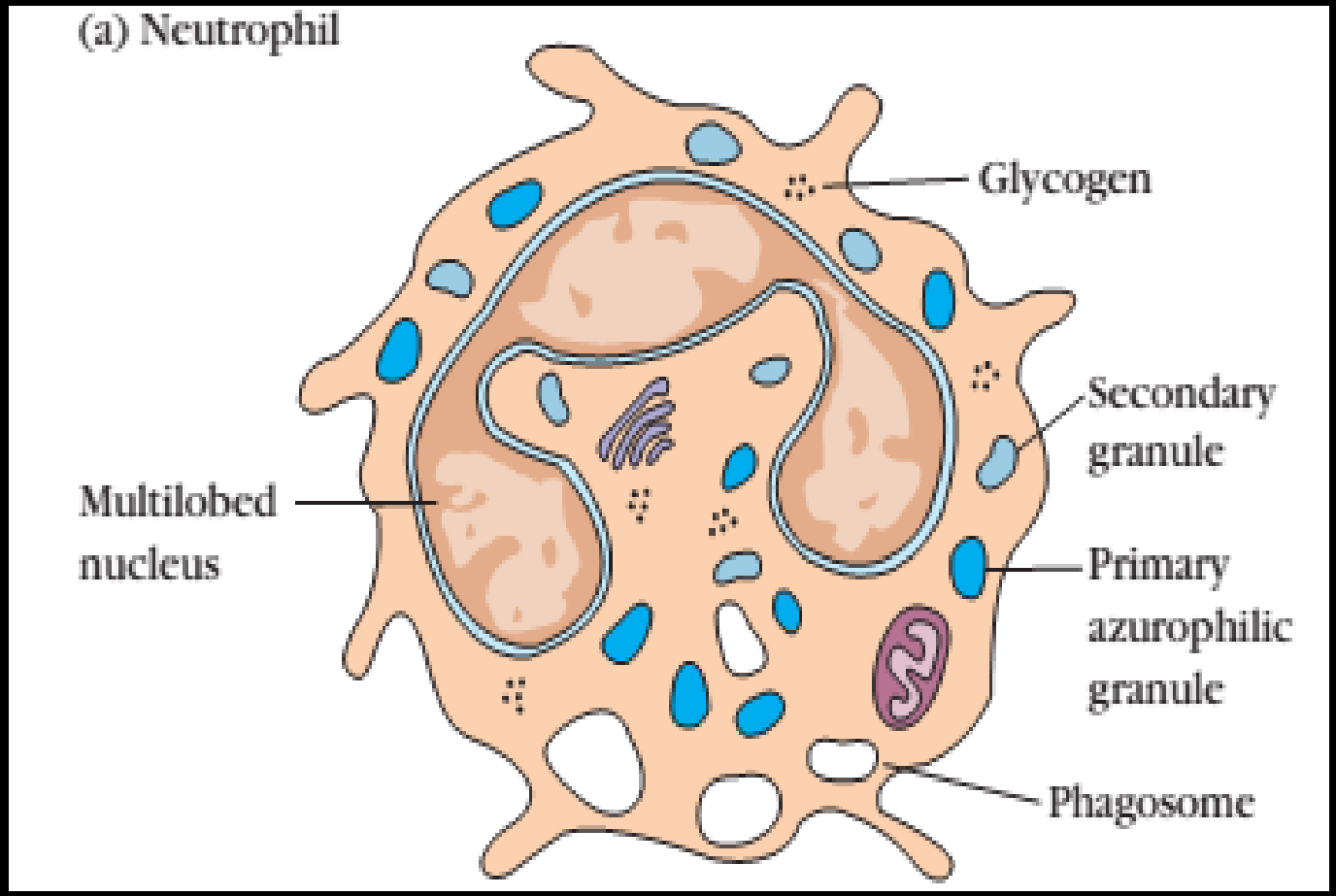
-growth factors

Inflammatory response

  - Occasionally hypersensitivity and allergy

6. Neutrophiles

- The most common white cells in the body.
- **Immediate response in tissue injury**.
- Are **phagocytes capable** of engulfing macromolecules and particles.
- Irregular shaped, **multilobed nuclei.**
- Stain neutrally with contain dyes.
- Grow fully mature in **bone marrow**.
- Mature neutrophiles then enter the **blood circulation in greater no.**
- those large number necessary because each **neutrophiles has a life span of only 1-2 days.**
- Eg. Adult human 50 billion neutrophiles in circulation at any given time.



Azurophilic and specific granules also contain anti-microbial proteins and peptides that are the cornerstone of innate immunity

7. Dendritic cells

- Cells with dendriform (star shaped) morphology.
- Interdigitating reticular cells (synonym)
- Capture and present antigens to T lymphocytes.

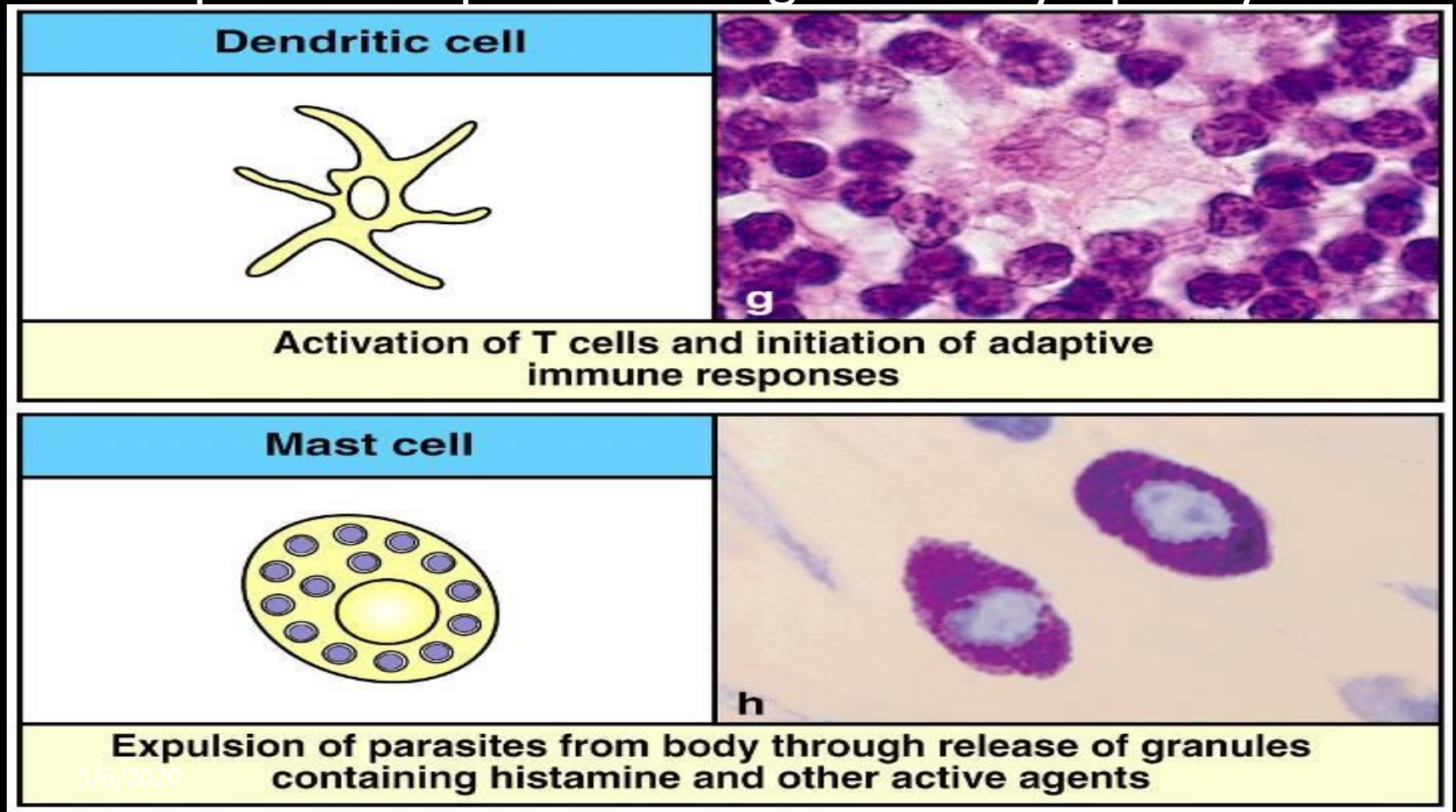


Figure 1-9 part 4 of 6 The Immune System, 2/e (© Garland Science 2005)

2.1.2 Cells of lymphoid lineage

- **Cells of lymphoid lineage divided into three** based on **function and cell-membrane components**.
  - T-lymphocytes
  - B- lymphocytes and
  - Natural killer cells.
- Lymphocytes constitute **20%–40%** of the body's white blood cells and **99% of the cells in the lymph**.
- **Lymphocytes responsible** for **adaptive immunity** and the immunologic attributes of **diversity, specificity, memory, and self/nonself recognition.**
  - **But the other types of white blood cells play important roles, engulfing and destroying microorganisms, presenting antigens, and secreting cytokines.**

They continually circulate in the **blood and lymph**

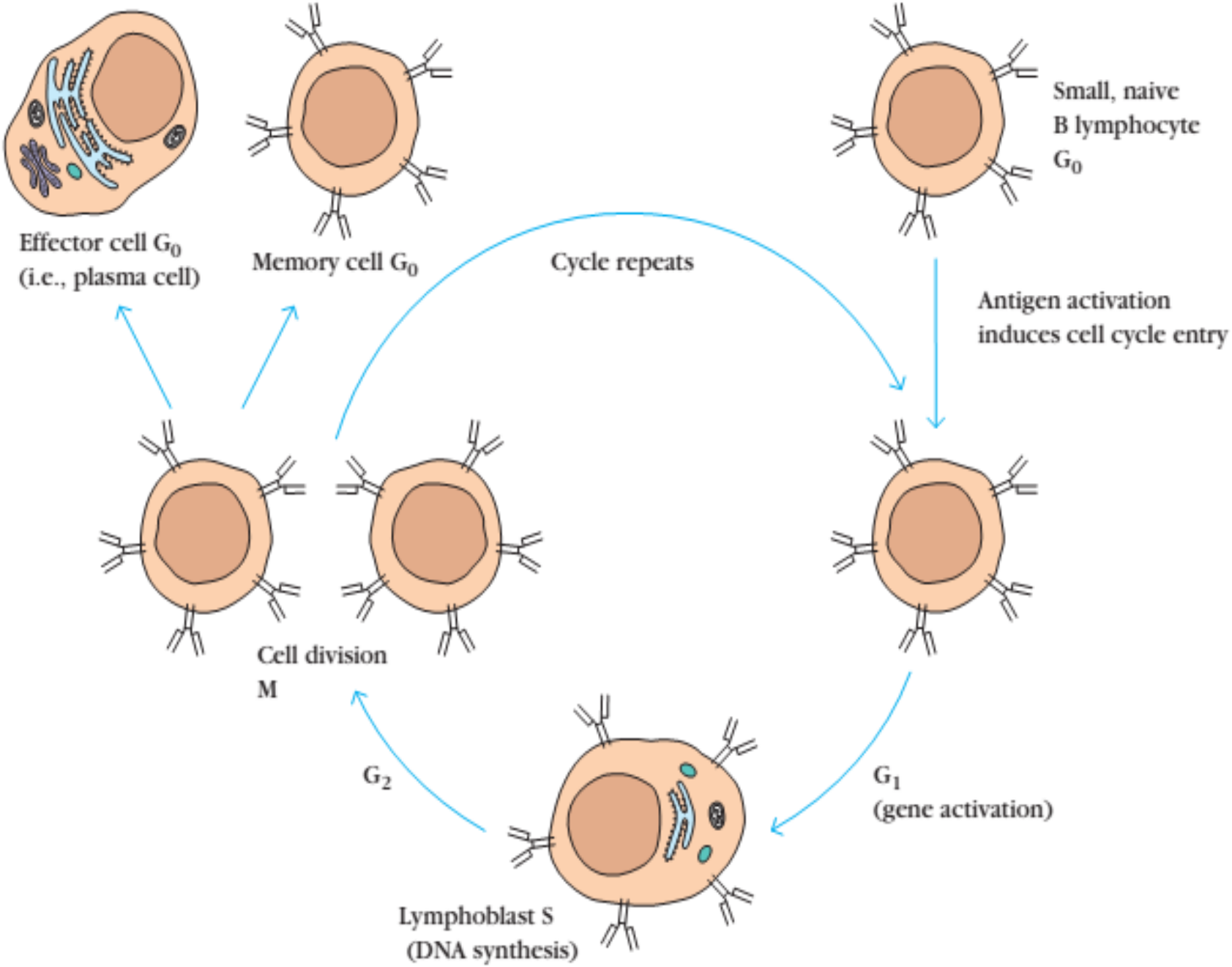
- **Also** are capable of migrating into the tissue spaces and lymphoid organs,
  - ✓thereby integrating the immune system to a high degree.

1. B-lymphocytes: its site of maturation ***b*ursa of Fabricius/ lymphoid tissue associated with the gut:/** in birds but **bone marrow** is its major site of maturation in a number of mammalian species, including humans .

2. T lymphocytes derive their name from their site of maturation in the *t*hymus.

3. Natural killer cells (NK cells) are **large, granular lymphocytes t**hat do not express the set of surface markers **typical of B or T cells .**

- **Resting B and T lymphocytes** are ***small,*** **motile**, **non-phagocytic cells,** which cannot be distinguished morphologically.
- **B and T lymphocytes** that have not interacted with antigen— referred to as naive, or unprimed—are resting cells in the G0 phase of the cell cycle.
- **The naive lymphocyte** is generally thought to have **a short life span.**
- **Interaction of small lymphocytes with antigen**, in the presence of certain **cytokines** → induces these cells to enter the cell cycle → **lymphoblasts.**
- **Lymphoblasts** proliferate and eventually differentiate into **effector cells** or into **memory cells.**
- **Plasma cells**—the antibody-secreting effector cells of the B-cell lineage.

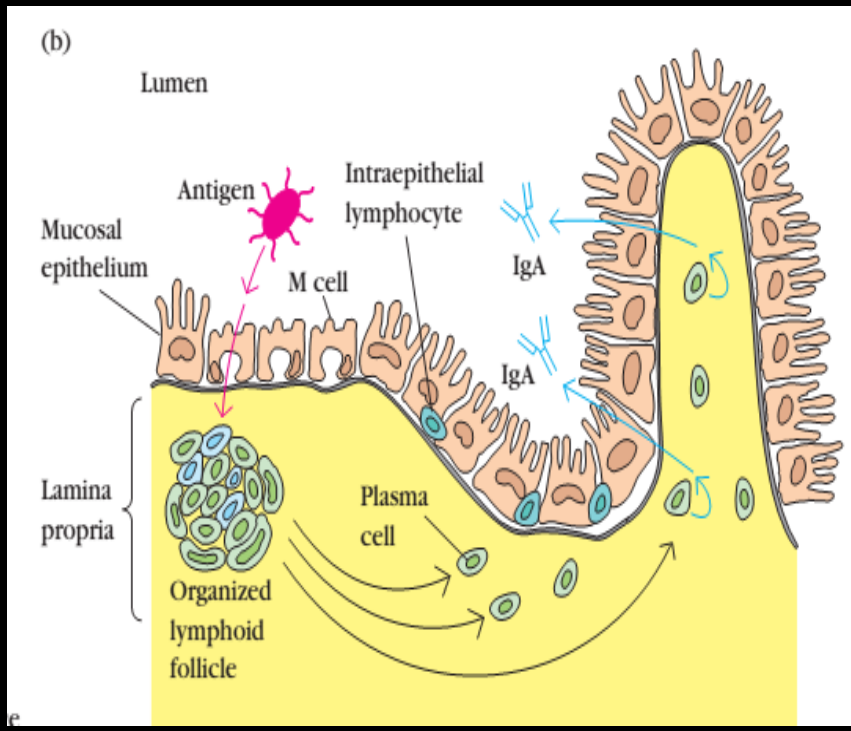

Effector cell G_0
(i.e., plasma cell)
Memory cell G_0
Small, naive
B lymphocyte
G_0
Cycle repeats
Antigen activation
induces cell cycle entry
Cell division
M
G_2
G_1
(gene activation)
Lymphoblast S
(DNA synthesis)

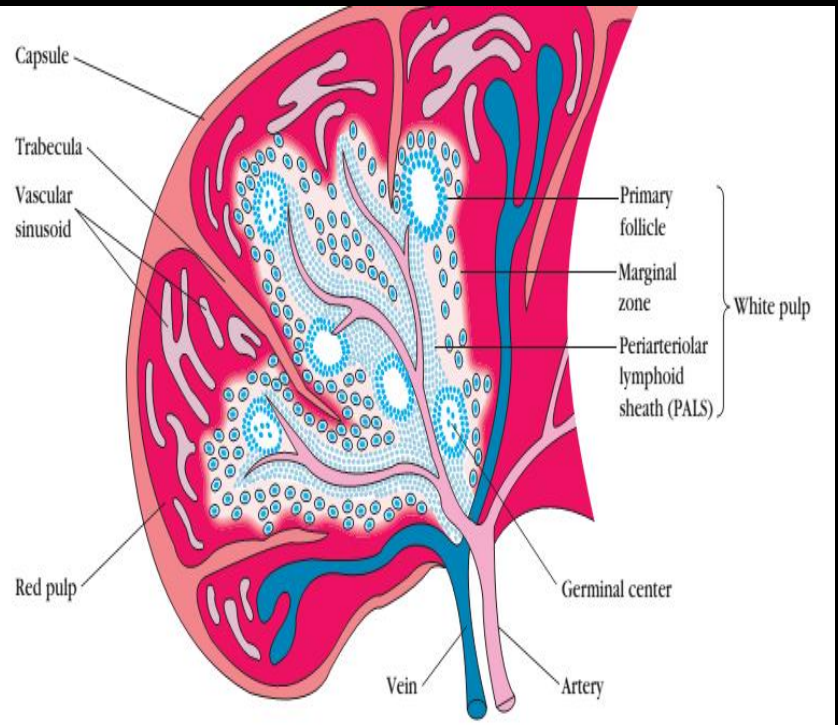
- The **effector cells of the T-cell lineage include the**
  - **cytokine-secreting T helper cell** (TH cell)
  - **T cytotoxic lymphocyte (TC cell)**

cluster of differentiation (CD) :-***CD marker***

- Although the CD nomenclature was originally developed for the membrane molecules of human leukocytes but also found in all animal leukocytes.
- **More 200 CD markers that have been described.**

lymphoid organs (Tissues)





2.2. lymphoid organs (Tissues)

- The immune system is not a discrete organ, like liver or a kidney.
- It is an integrated partnership; with contribution by :-
  - The circulatory system
  - Lymphatic system
  - Various lymphoid organs and tissues
  - The specialized hematopoietic cells moving among these.
- **A lymphoid tissue**:-is simply a tissue in which **lymphocytes** are found.
- **Lymphoid follicles**:- are organized **cylindrical clusters of** lymphocytes that ,when gathered into groups are called **lymphoid patches**.

Lymphoid organs

1. **Primary Lymphoid organs ;-** thymus, bursa, bone marrow and payer's patches.

2. **Secondary Lymphoid organs :-**spleen ,lymph node, some PPs, bone marrow ,the mucosal-associated lymphoid tissues (MALT),the skin-associated lymphoid tissues.

2.2.1 Primary Lymphoid organs

A. Thymus and bone marrow

- **The thymus** and **bone marrow** are referred to a primary lymphoid organ,
  - are essential for **initial production of lymphocytes from progenitor cells.**

Thymus

Progenitor cells that leave the **bone marrow** → **thymus** → **proliferation and differentiation**.

- This process is facilitated by a hormone, **thymosin.**

1. Bone marrow

- **Bone marrow** is the source of progenitor cells.
  - These cells can differentiate in to **lymphocytes, granulocytes, erythrocytes, etc.**
  - differentiation of progenitor cells into **B-lymphocytes** and functions as the bursa equivalent in human.

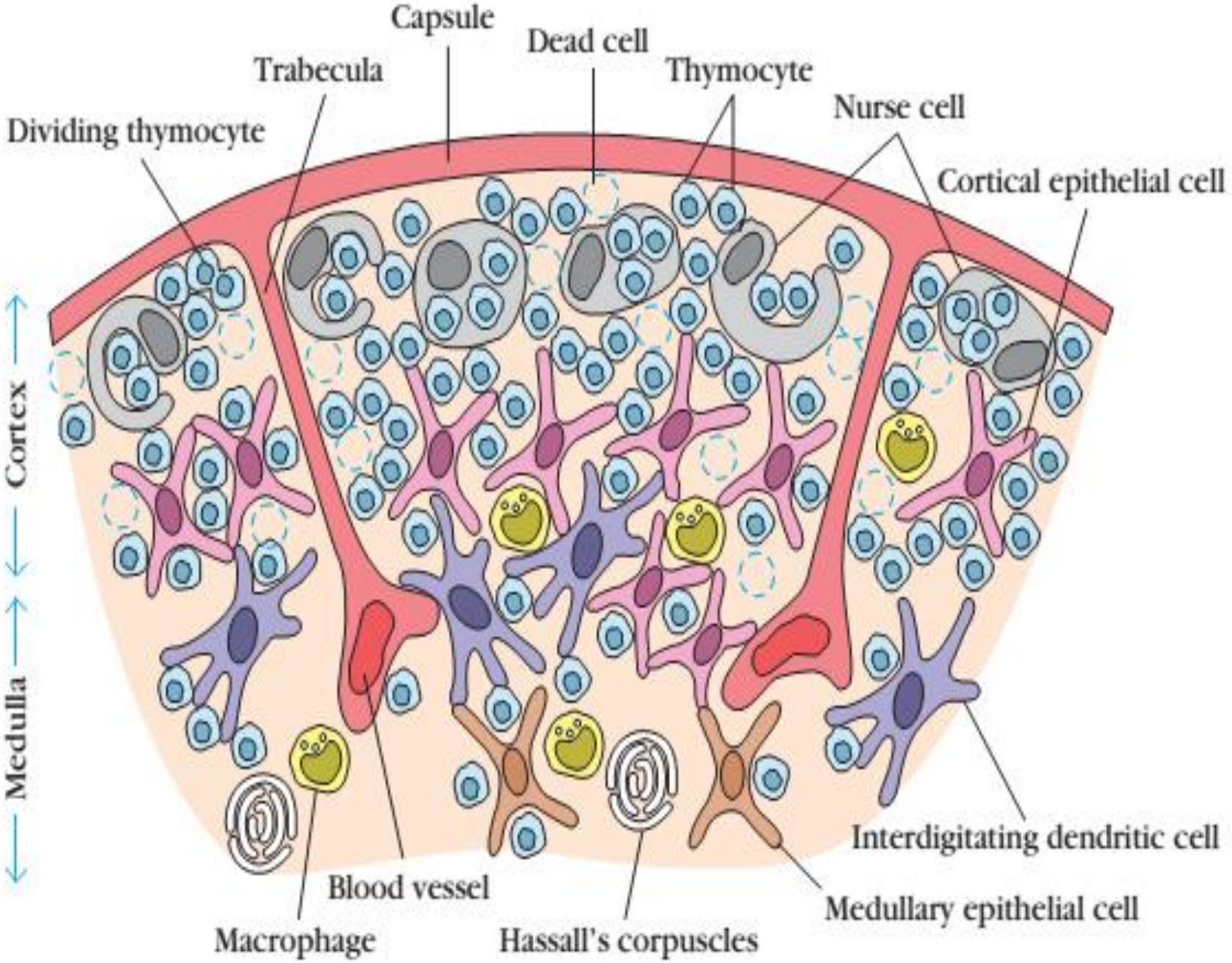
2. THYMUS

- The **thymus** is the site of **T-cell development** and **maturation.**
- It is a **flat, bilobed** organ situated above the heart,

- **Composed of stromal-cell** network composed of :-
  - **epithelial cells,**
  - **dendritic cells,**
  - **and macrophages**

3. Bursa of Fabricius

- **Bone marrow** is not the site of B-cell development in all species.
  - In **birds**, a lymphoid organ called the **bursa of Fabricius**, "a **lymphoid tissue associated with the gut**", is the **primary site of B-cell maturation**


Capsule
Dead cell
Trabecula
Thymocyte
Nurse cell
Dividing thymocyte
Cortical epithelial cell
Cortex
Medulla
Interdigitating dendritic cell
Blood vessel
Medullary epithelial cell
Macrophage
Hassall's corpuscles

4. Peyer's patch

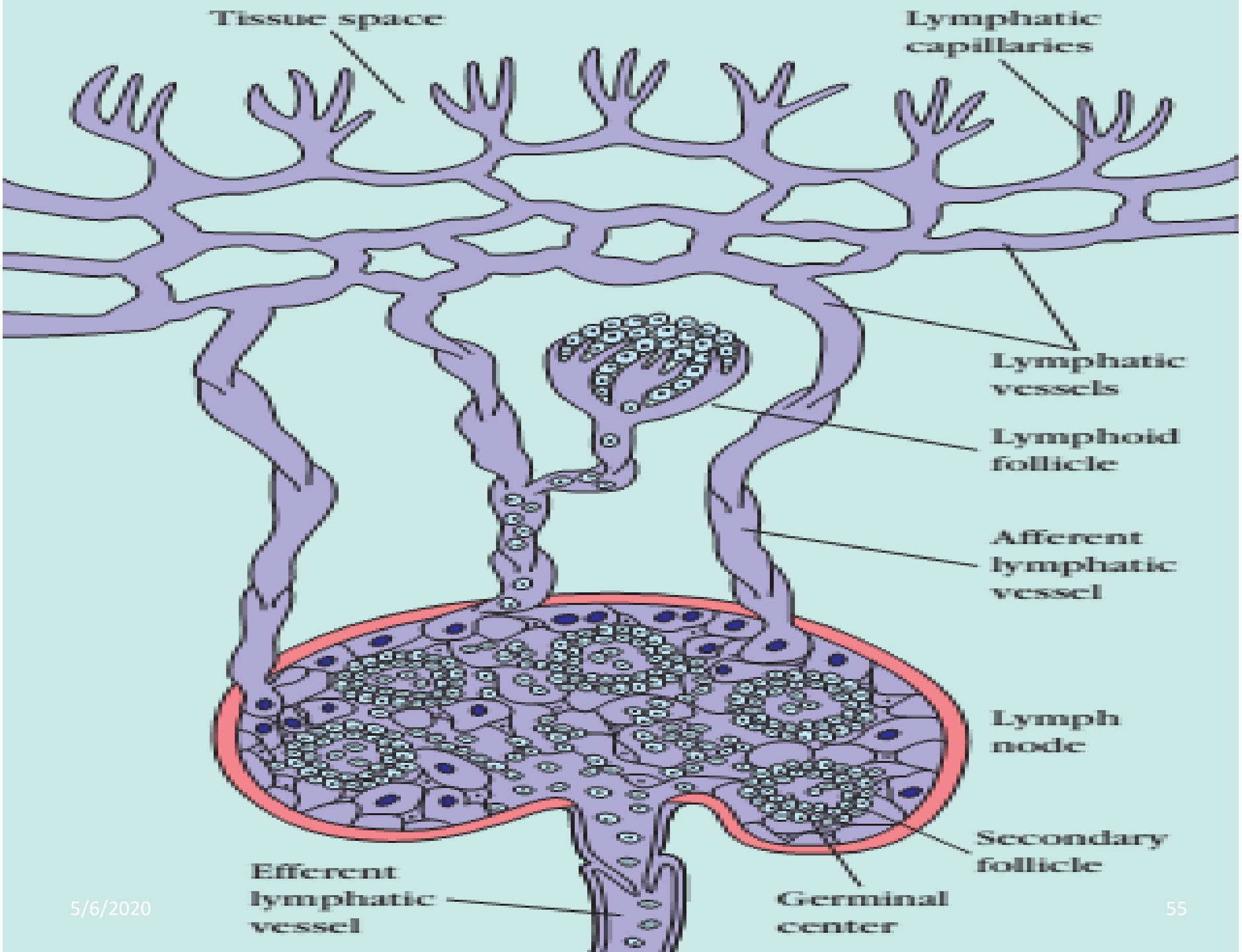
- In **cattle and sheep**, **fetal spleen is** the **primary lymphoid tissu**e hosting the maturation, proliferation, and diversification of **B cells early in gestation.**

- **Later in gestation**, this function is assumed by a **patch of tissue embedded** in the wall of the intestine called the **ileal Peyer's patch**, which contains a **large number of B cells.**

2.2.2 Secondary Lymphoid organs

Introduction

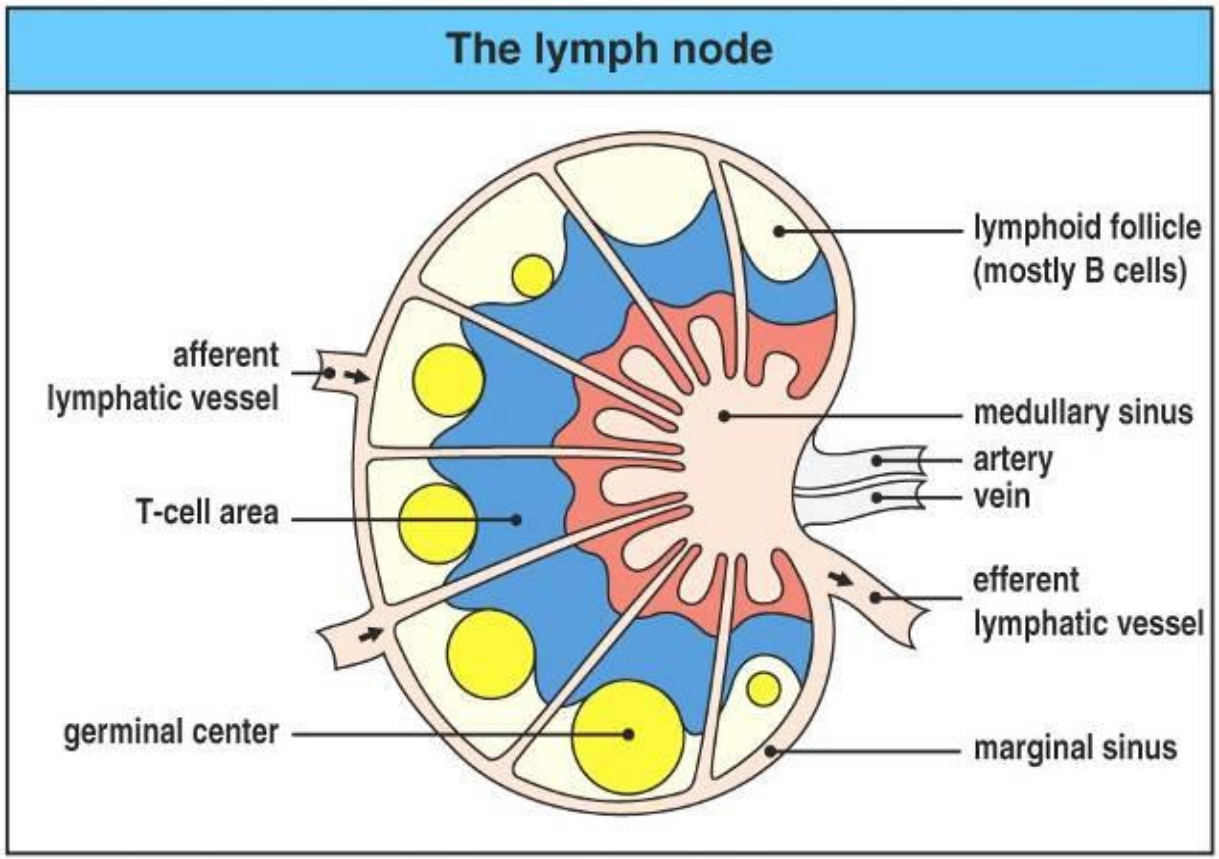
- The **lymphatic system** also serves as a means of **transporting lymphocytes and antigen** from the **connective tissues** to organized **lymphoid tissues which** Includes:-
  - **spleen** ,lymphnode, some PPs, bone marrow ,the **mucosal-associated lymphoid tissues (MALT),**the **skin-associated lymphoid tissues.**
  - Their **function** is to **maximize encounters between lymphocytes and foreign substances .**


Tissue space
Lymphatic capillaries
Lymphatic vessels
Lymphoid follicle
Afferent lymphatic vessel
Lymph node
Secondary follicle
Efferent lymphatic vessel
Germinal center

A. Lymph nodes

- Filled with dense aggregates of lymphocytes and macrophages.
- From outer(cortex) ⟶ to center (Medulla)

B-cells ⟹ T-cells ⟶ macrophages



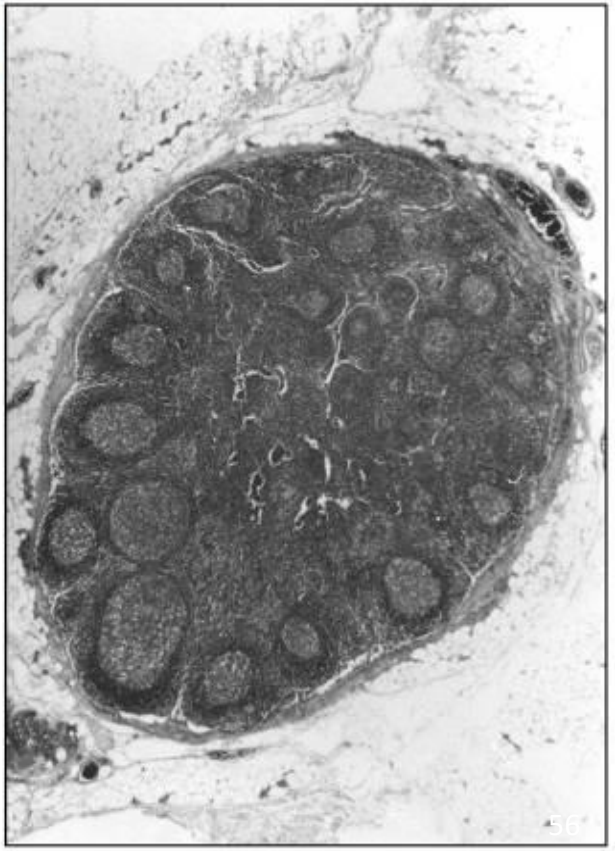
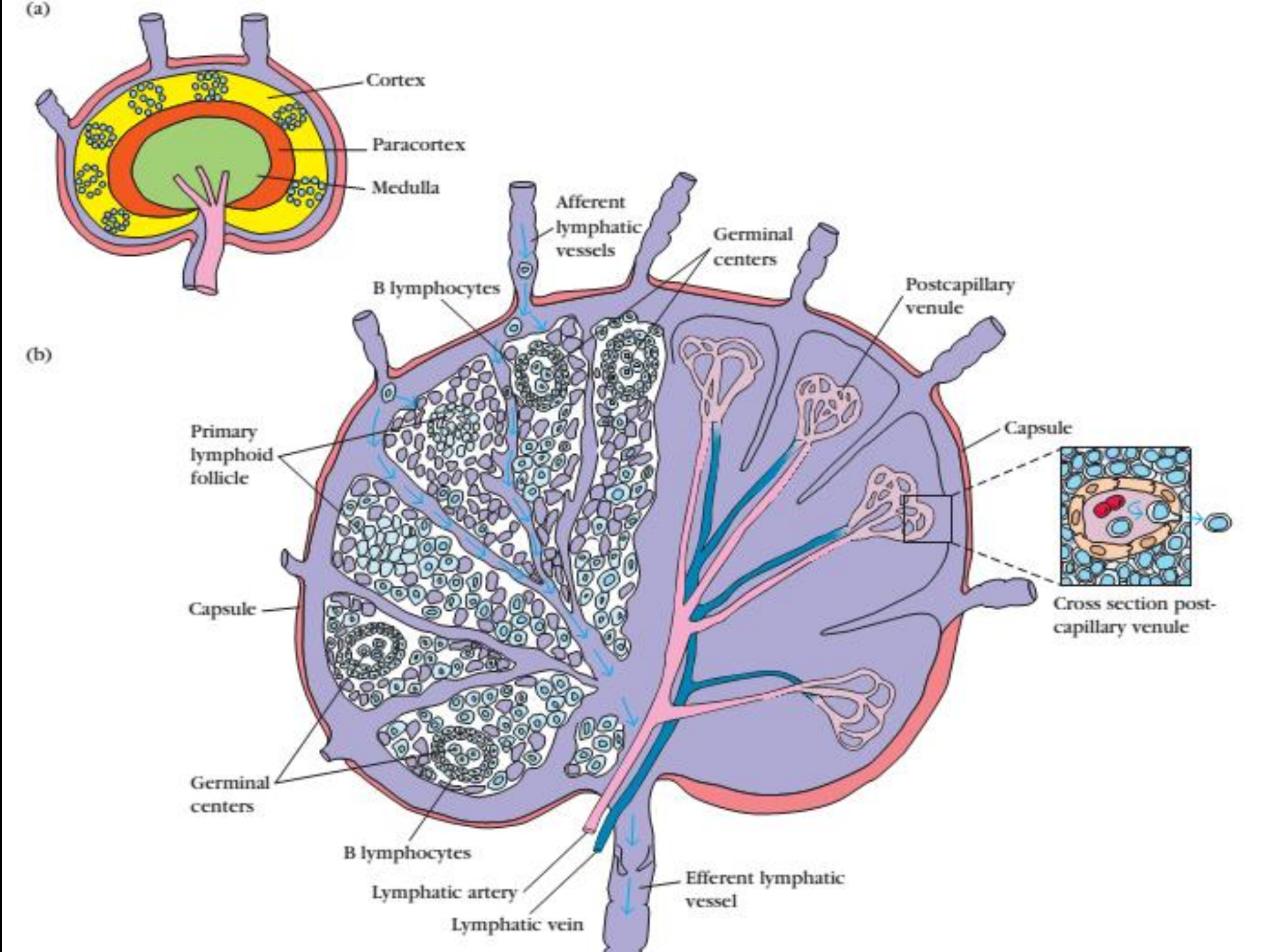


Figure 1-17 The Immune System, 2/e (© Garland Science 2005)


(a)
Cortex
Paracortex
Medulla
(b)
Afferent lymphatic vessels
Germinal centers
B lymphocytes
Postcapillary venule
Primary lymphoid follicle
Capsule
Capsule
Cross section post-capillary venule
Germinal centers
B lymphocytes
Lymphatic artery
Lymphatic vein
Efferent lymphatic vessel

- **Lymph nodes** act like **lymphoid filters** in the lymphatic system.
  - It **responds to antigens** introduced distantly and routed to them by afferent lymphatic.
- They are **encapsulated bean** shaped packed with
  - **lymphocytes, macrophages, and dendritic cells**.

1. The cortex, contains lymphocytes (mostly B cells),

- few **macro-phages**, and **follicular dendritic cells** arranged in primary follicles.

2. The paracortex, populated largely by **T lymphocytes,**

- also contains **interdigitating dendritic cells** thought to have migrated from tissues to the node.

3. The medulla, is more populated with are **plasma cells actively secreting antibody molecules**.

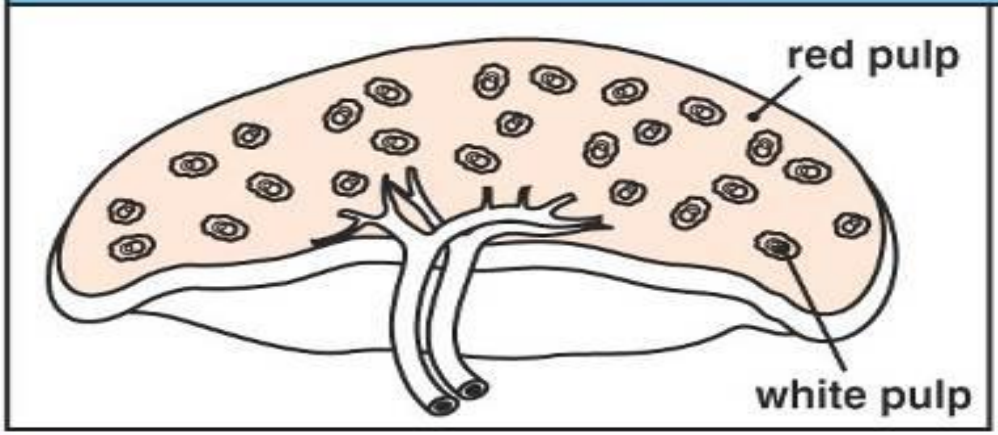
B. Spleen

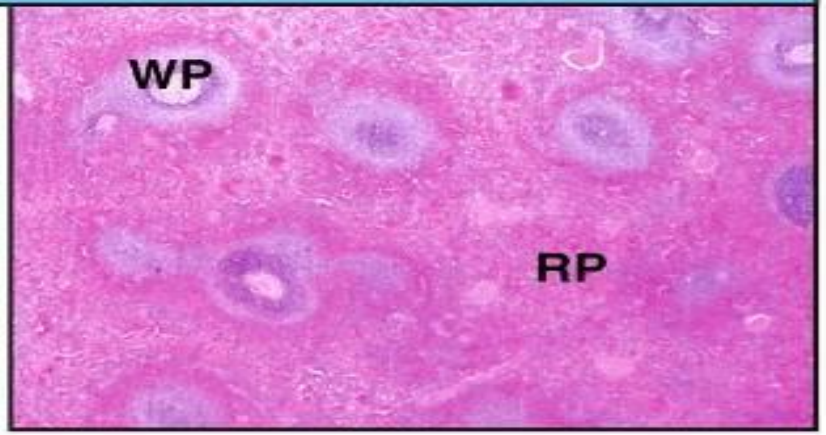
- The spleen act like a **lymphatic filter** with **in the blood vascular tree.**
  - It is an **important site of antibody production** in response to **intravenous particulate antigen** (e.g. bacterial).

- The spleen is also a **major organ for the clearance of particles.**

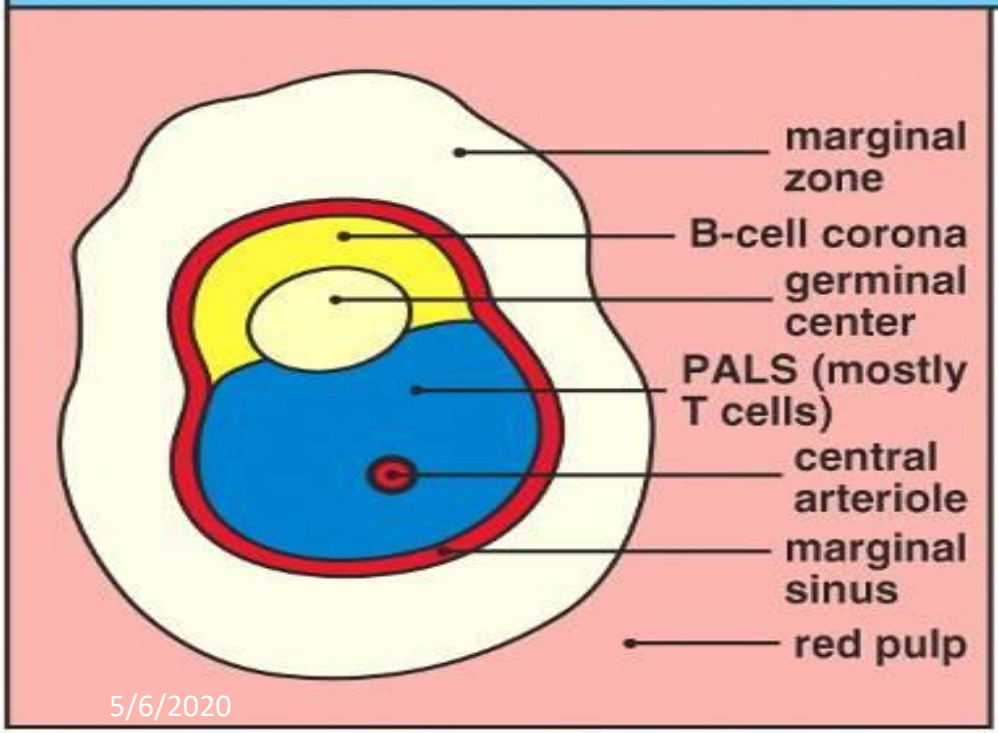
- The **spleen, It is specialized for trapping blood-borne antigens.**

The spleen





Transverse section of white pulp of spleen



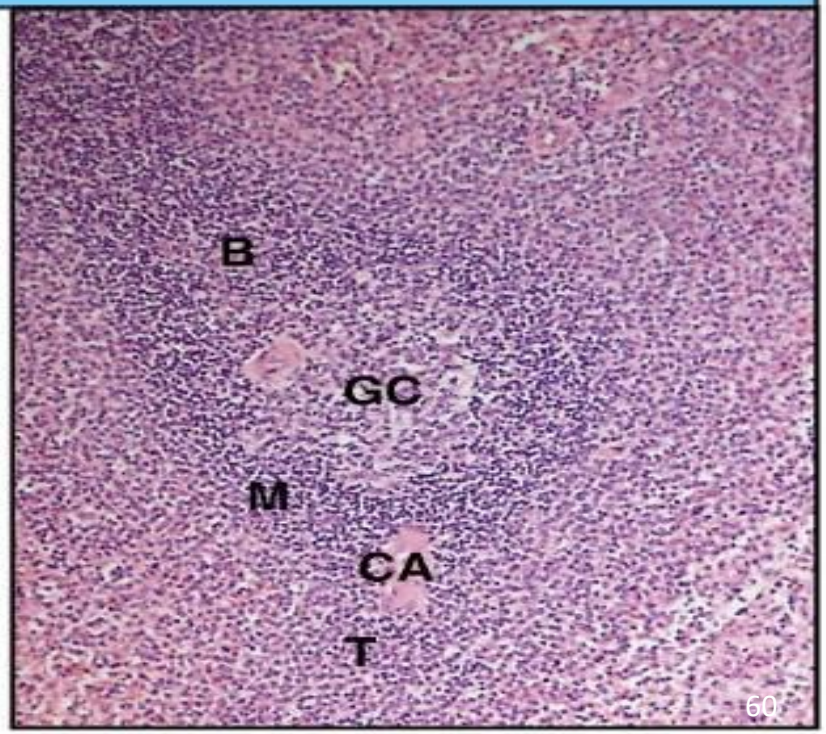


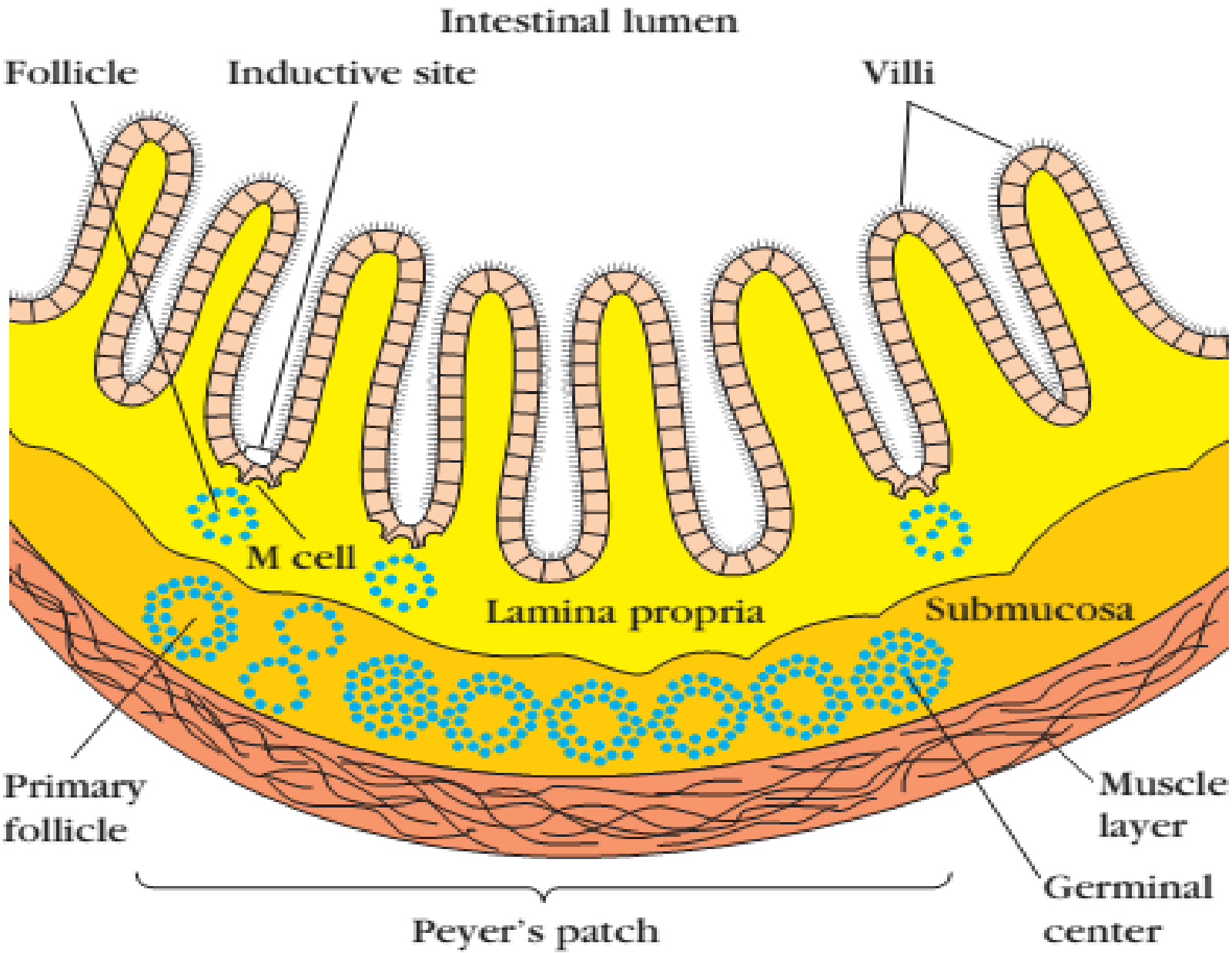
Figure 1-19 The Immune System, 2/e (© Garland Science 2005)

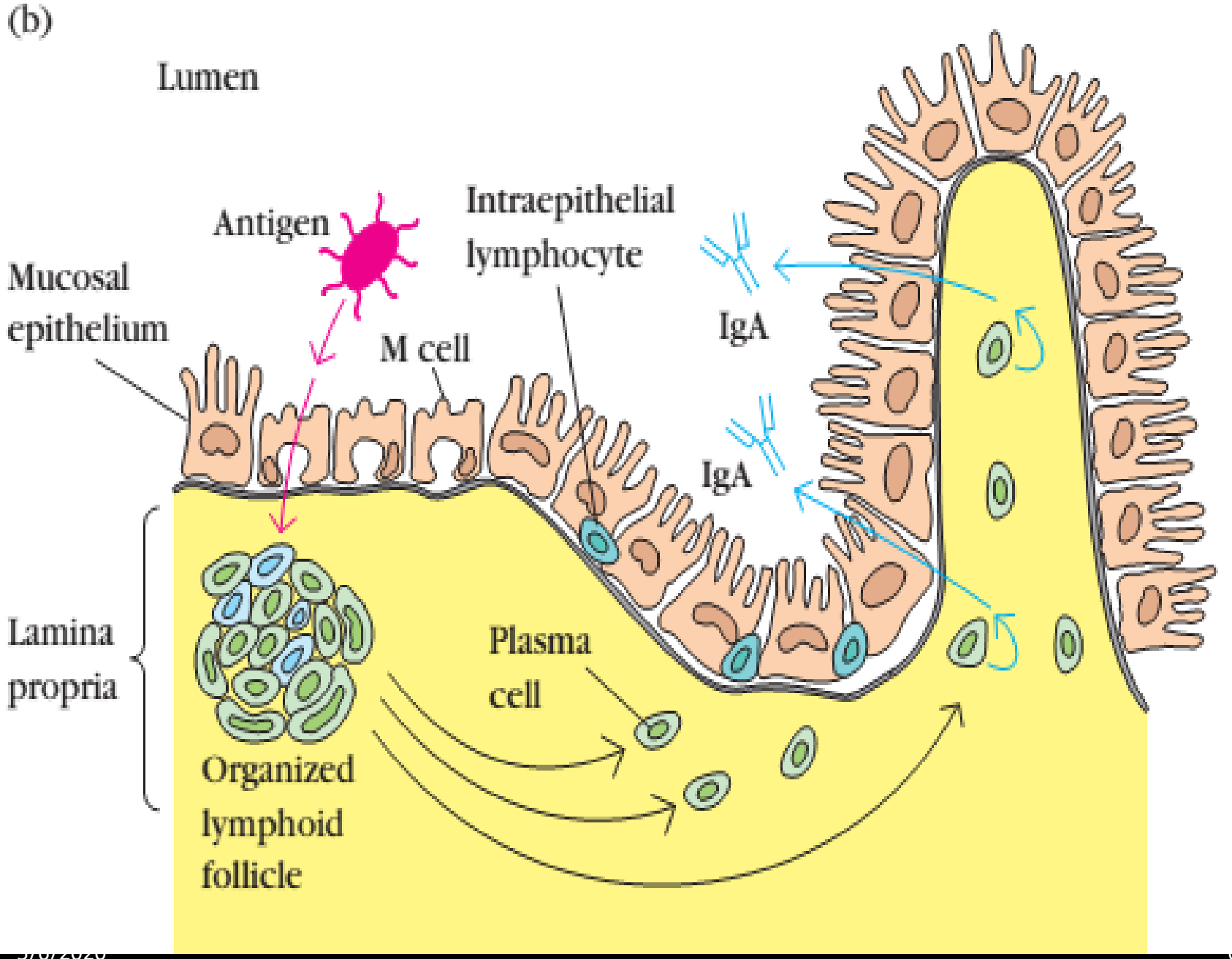
C. Gut – associated lymphoid tissue (GALT)

- **Gut-associated lymphoid tissue** includes lymphoid tissue in **the intestines (payer's patches) and the liver**.

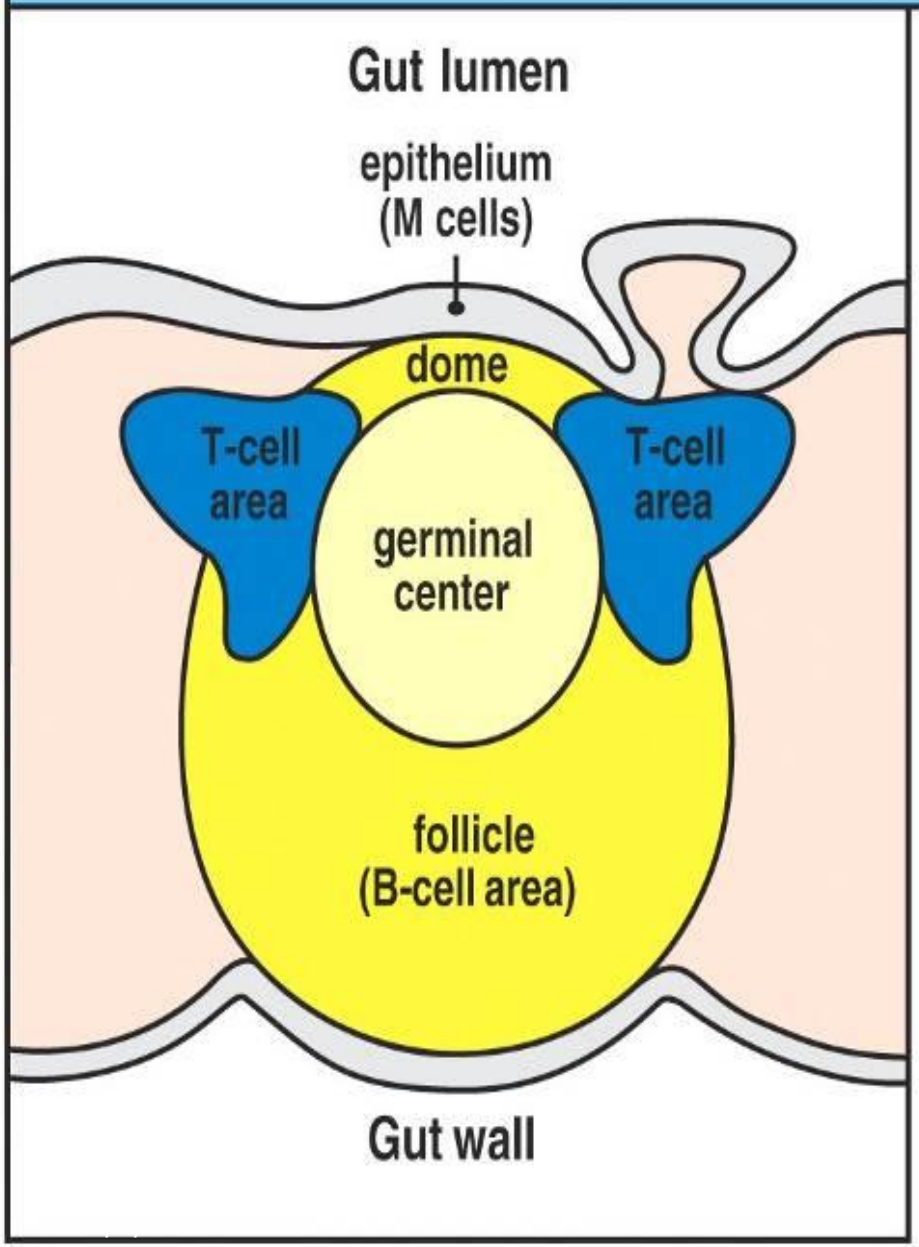
- Gut associated lymphoid tissue is involved **in lymphocyte circulation,**

  – i.e. **pre-B cells develop in payer's patches** and after meeting antigen from the gut, they enter to the general circulation and then return back to the gut.


Intestinal lumen
Follicle
Inductive site
Villi
M cell
Lamina propria
Submucosa
Primary follicle
Muscle layer
Germinal center
Peyer's patch


(b)
Lumen
Antigen
Intraepithelial lymphocyte
Mucosal epithelium
M cell
IgA
IgA
Lamina propria
Plasma cell
Organized lymphoid follicle

Gut-associated lymphoid tissue



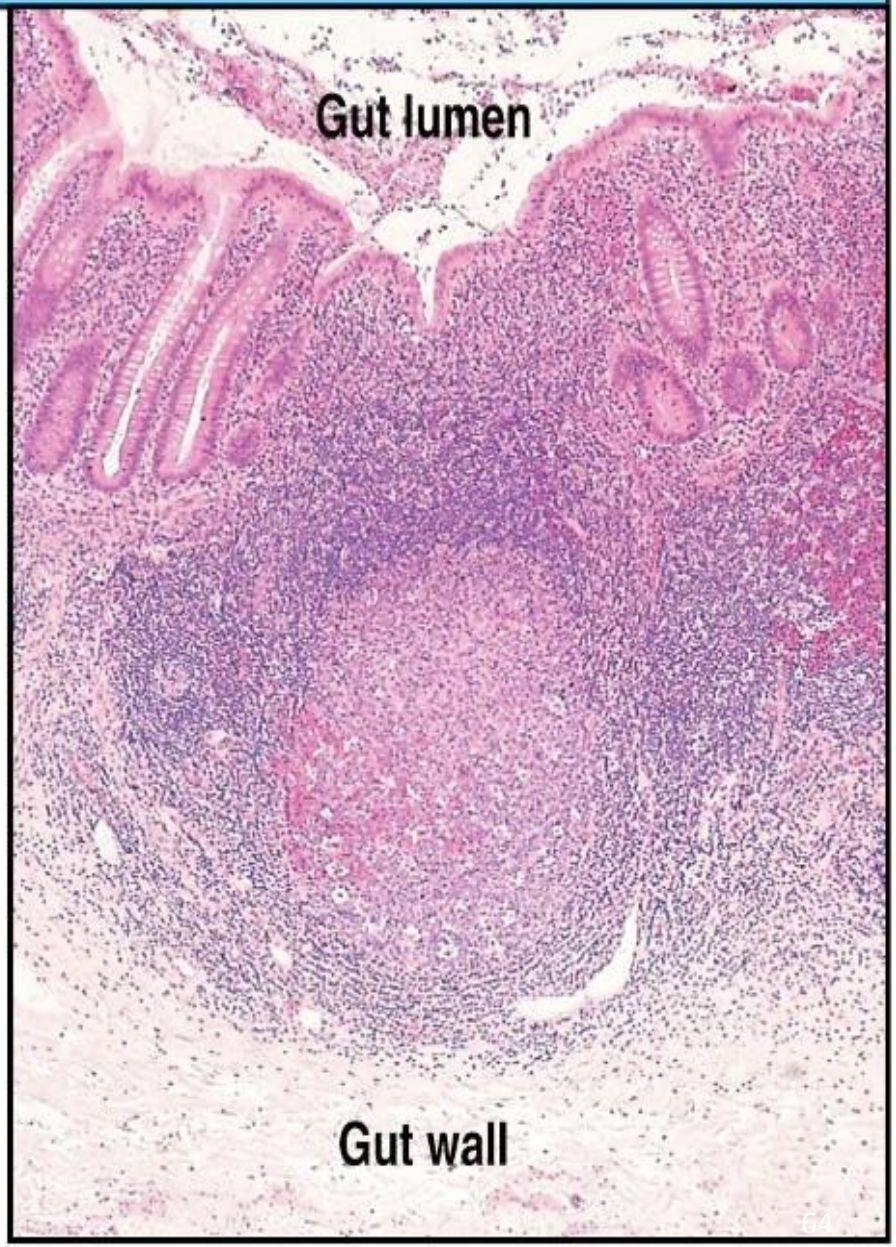


Figure 1-20 The Immune System, 2/e (© Garland Science 2005)

D. Tonsils

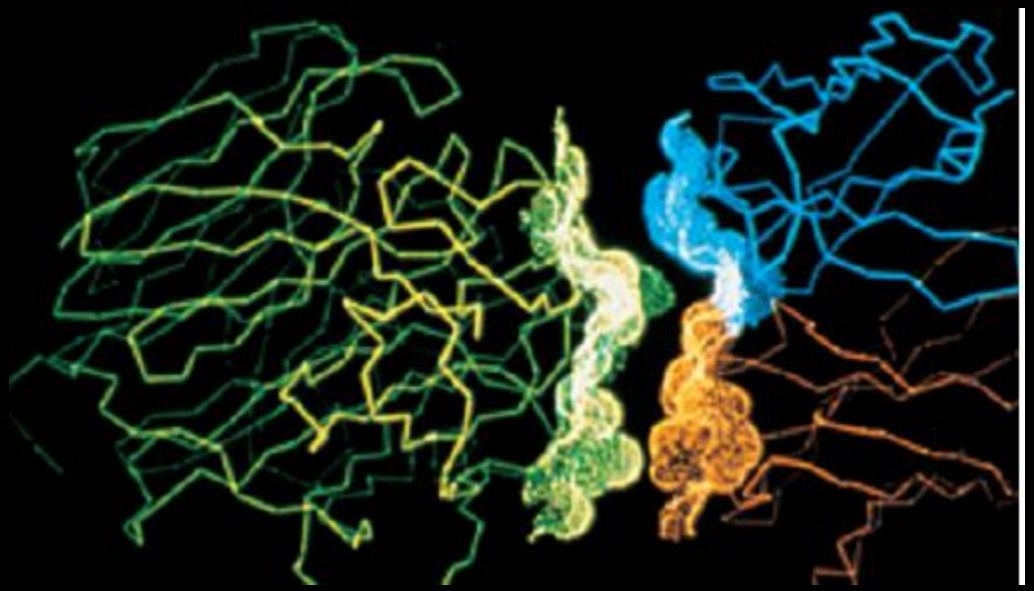
- Tonsils are **nodular aggregates of lymphoid** tissues, their function is to detect and respond to pathogens in the respiratory secretion.

E. Blood

- The blood is an important lymphoid organ and immunologic effector tissue. Circulating blood has enough mature T-cells to produce graft- versus-host reaction.

Chapter three

Antigens and antibodies



Introduction

Definition : -

1. An **antigen** means :- a substance which can be **recognized** by :-
   - immunoglobulin receptor of B cells,
   - or by the T- cell receptor when complexes with MHC.

- The **molecular properties of antigens** and the way in which these properties ultimately contribute to immune activation are central to our understanding of the immune system.

3.1 Immunogenicity Versus Antigenicity

- **Immunogenicity and antigenicity** are related but distinct immunologic properties that sometimes are confused.

1. Immunogenicity is the ability to induce a humoral and/or cell mediated immune response:

- **B -cells + antigen** → **effector B cells + memory B cells**

  ↓

  (plasma cells)

- **T- cells + antigen** → effector T cells + memory T cells

  ↓

  (**e.g., CTLs, THs**)

2. Antigenicity is the **ability to combine specifically** with the **final products** of the above responses (i.e., **antibodies and/or cell-surface receptors).**

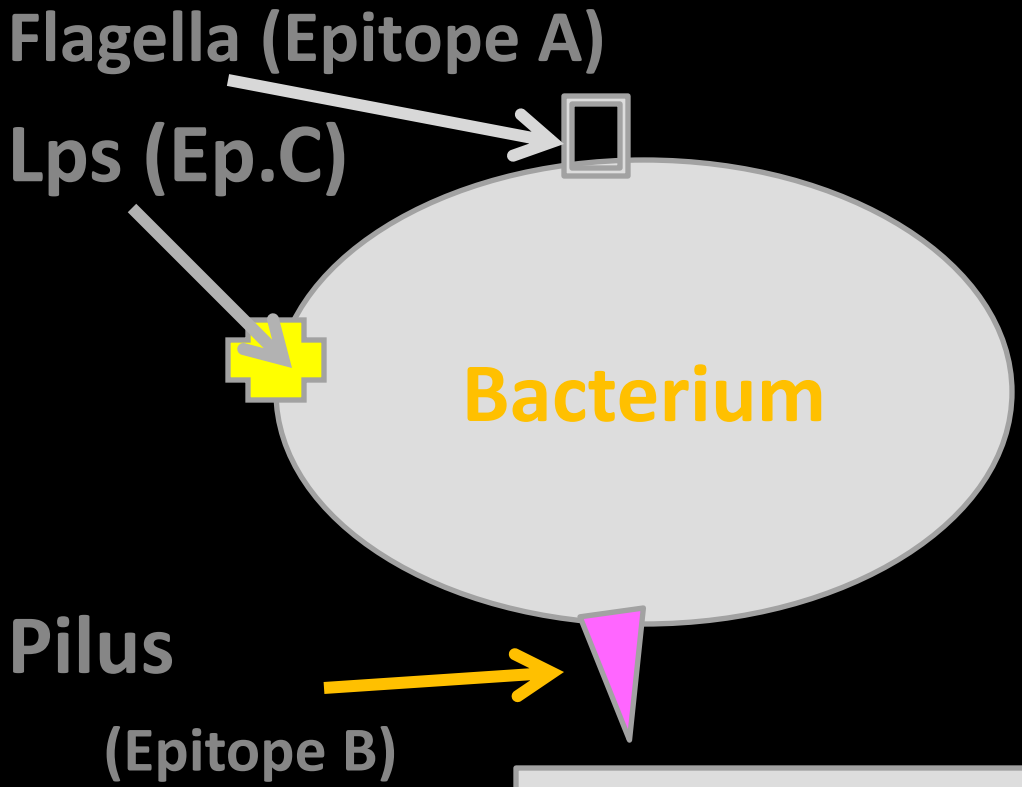
- Although all molecules that have the property of i**mmunogenicity** also have the property of **antigenicity**, the reverse is not true. Eg. ***haptens***
-

- **Proteins** are the most potent immunogens, with **polysaccharide**s ranking second.
- In contrast, **lipids and nucleic acids** of an infectious agent generally do not serve as **immunogens unless** they are complexed with **proteins or polysaccharides**.
- For **cell-mediated immunity**, only proteins and some **lipids** and g**lycolipids** serve as immunogens
- **Immunogenicity** is determined by four properties of the immunogen:
  - **its foreignness,**
  - **molecular size,**
  - **Chemical composition and complexity,**
  - **and ability to be processed and presented with an MHC molecule on the surface of an antigen-presenting cell or altered self-cell**.

3.2 Epitopes

- immune cells do not interact with, or recognize, an **entire immunogen molecule**;
  - instead, lymphocytes recognize **discrete sites on the macromolecule** called **epitopes, or antigenic determinants.**
- **Epitopes** are the **immunologically active regions** of an immunogen that bind to **antigen-specific** membrane receptors on lymphocytes or to secreted antibodies or ,
- **Small parts of a protein** that contain **specific aminoacid** sequences,or certain confirmationof the protiens is called **Epitope** .

- Complexity of Antigens :-
  - E.g b acteria: bacterial protein + polysaccharides +lipids(bacteria=∑antigens).

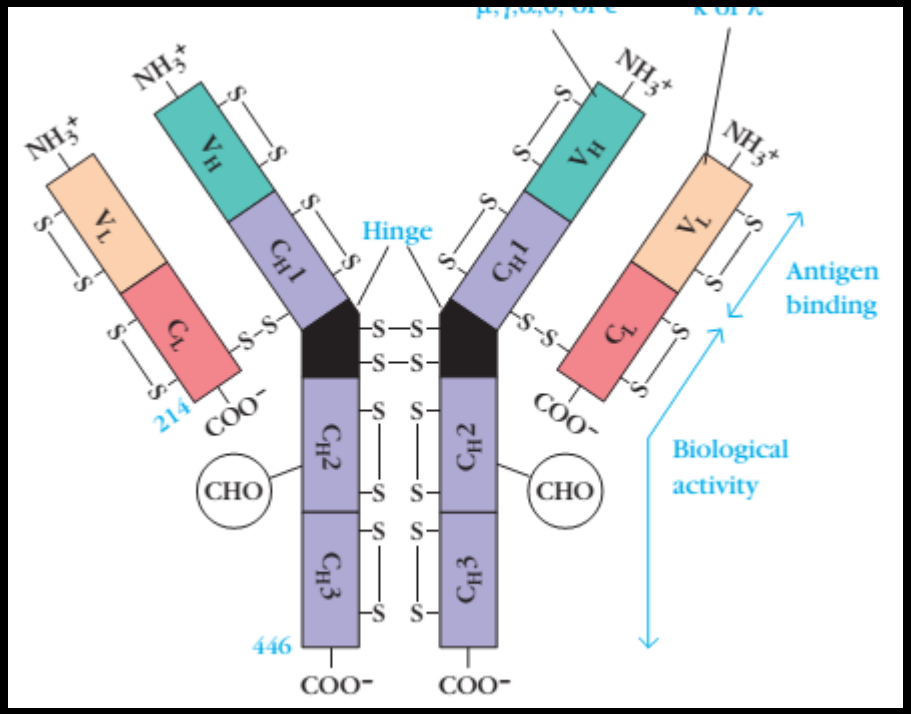


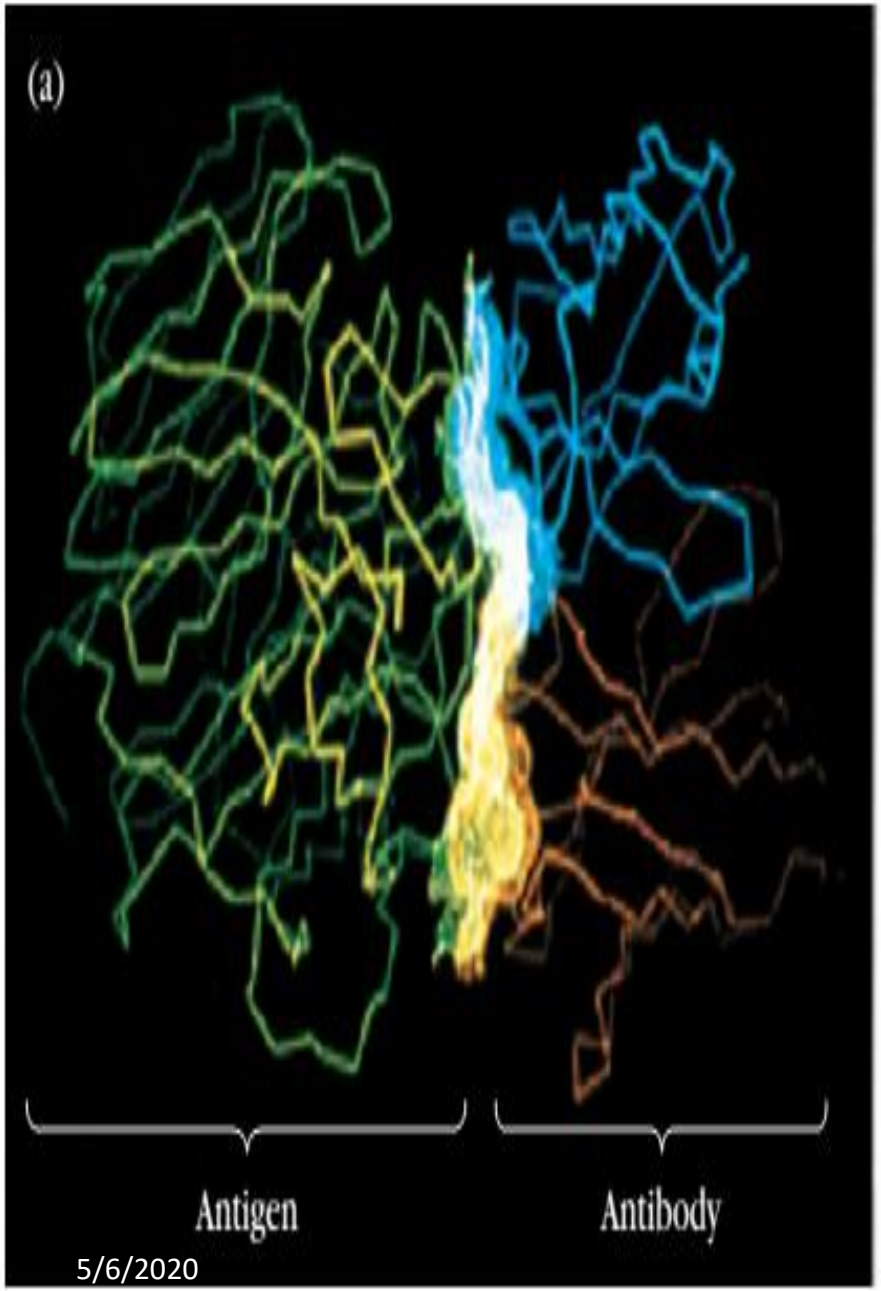
Antigens= ∑epitopes

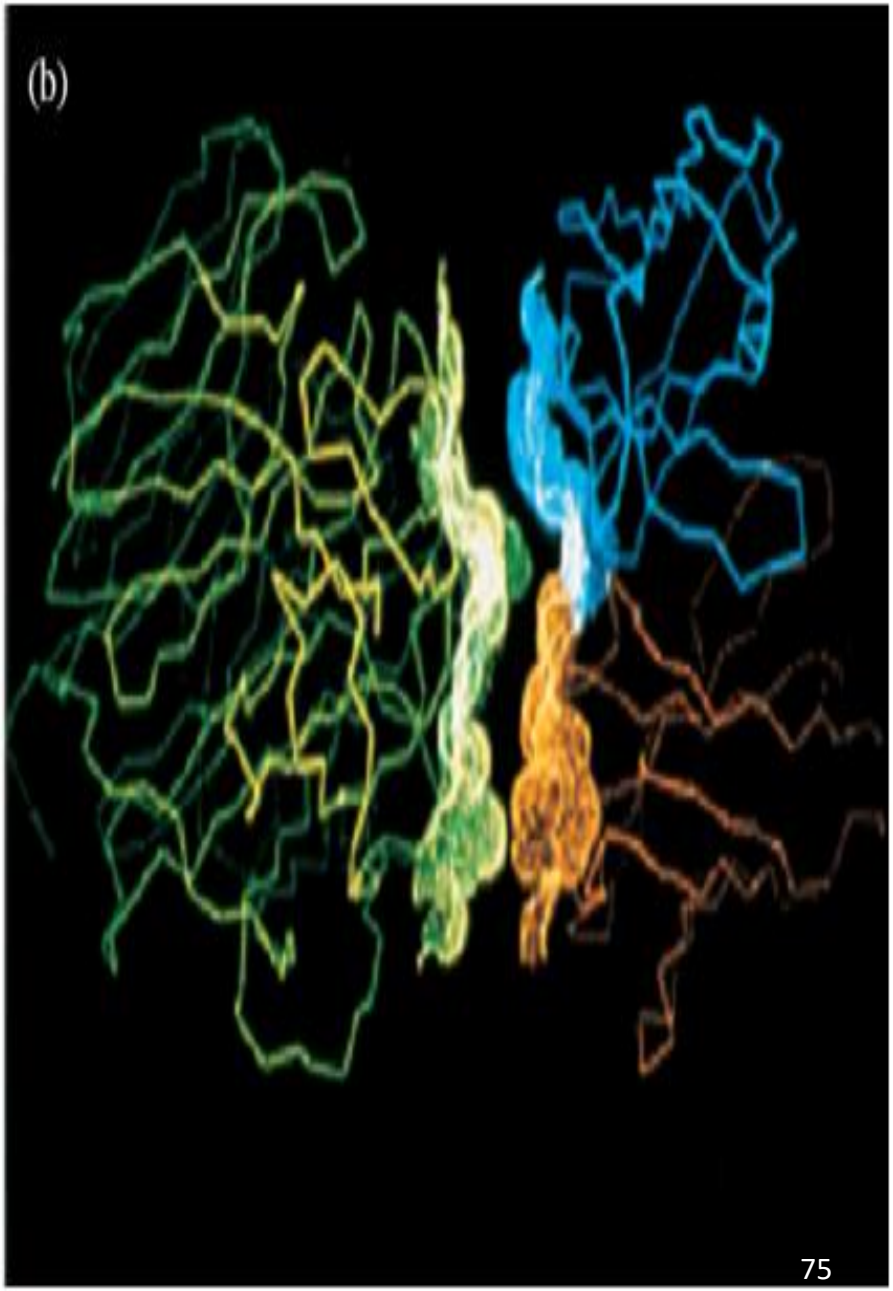
1. Properties of B-Cell Epitopes:-

- The **antibody's binding site and the epitope** must have complementary shapes that place the interacting groups near each other.
  - The **size of the epitope recognized by a B cell** can be no larger than the size of the antibody's binding site
  - **Smaller ligands** such as **carbohydrates**, **small oligonucleotides**, **peptides**, and **haptens** often bind within a deep pocket of an antibody.

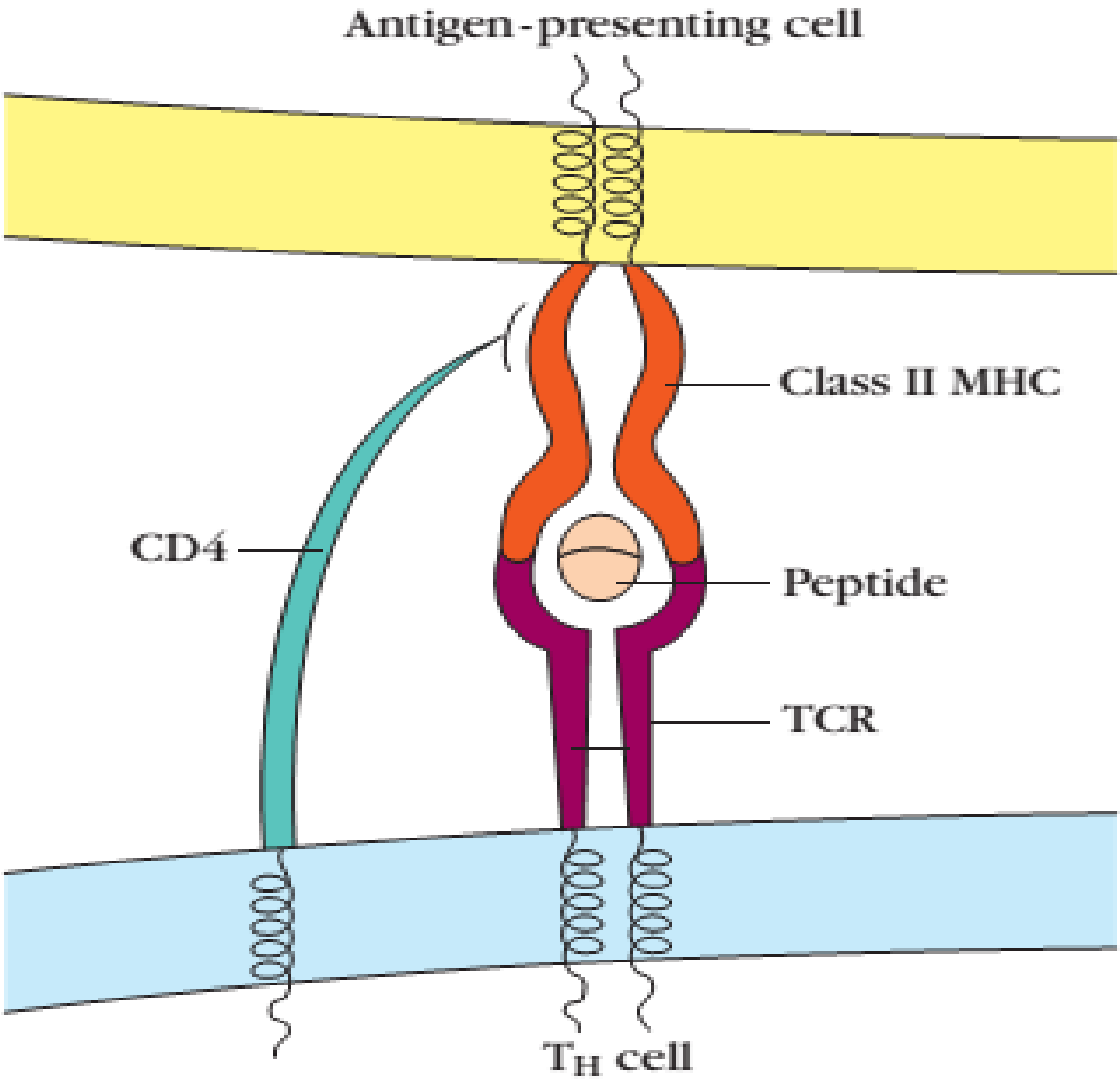
Antibody structure




(a)
Antigen
Antibody


(b)

- **Antigen-Derived Peptides** are the key elements of **T-Cell Epitopes**.
- **T-cell epitopes must include :-**
  - **antigen-presenting cells**
  - **or target cells that can display the peptides bound to an MHC molecule.**


Antigen-presenting cell
Class II MHC
CD4
Peptide
TCR
TH cell

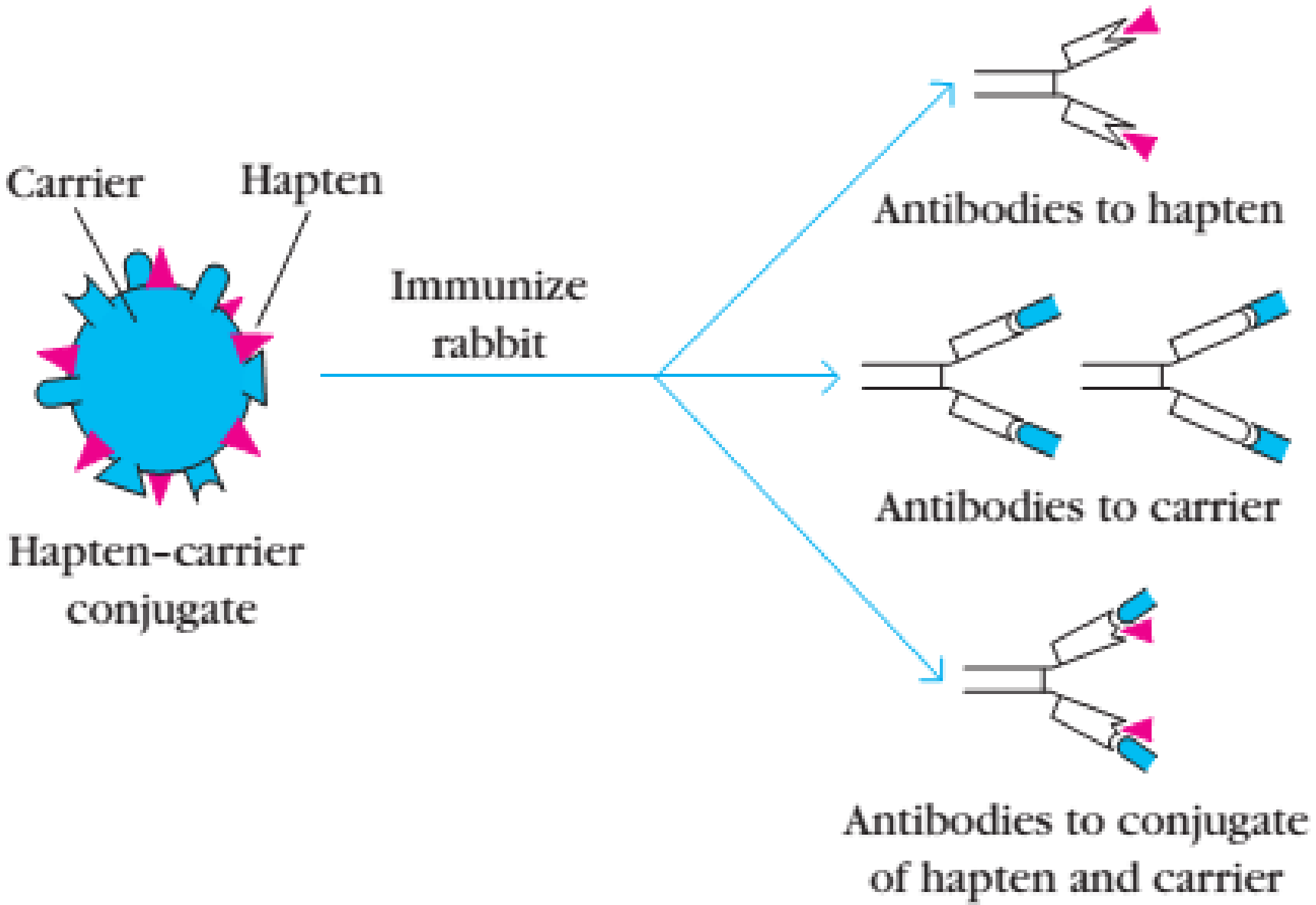
3.3 Haptens and the Study of Antigenicity

- **Haptens** are **small molecules**, which are **antigenic** but incapable, by themselves, of inducing a specific immune response.
  - In other words, they **lack immunogenicity**.
- Chemical coupling of a hapten to a large protein, called a **carrier**,
  - yields an immunogenic **hapten carrier conjugate.**
- Animals immunized with such a **conjugate produce antibodies** specific for:-

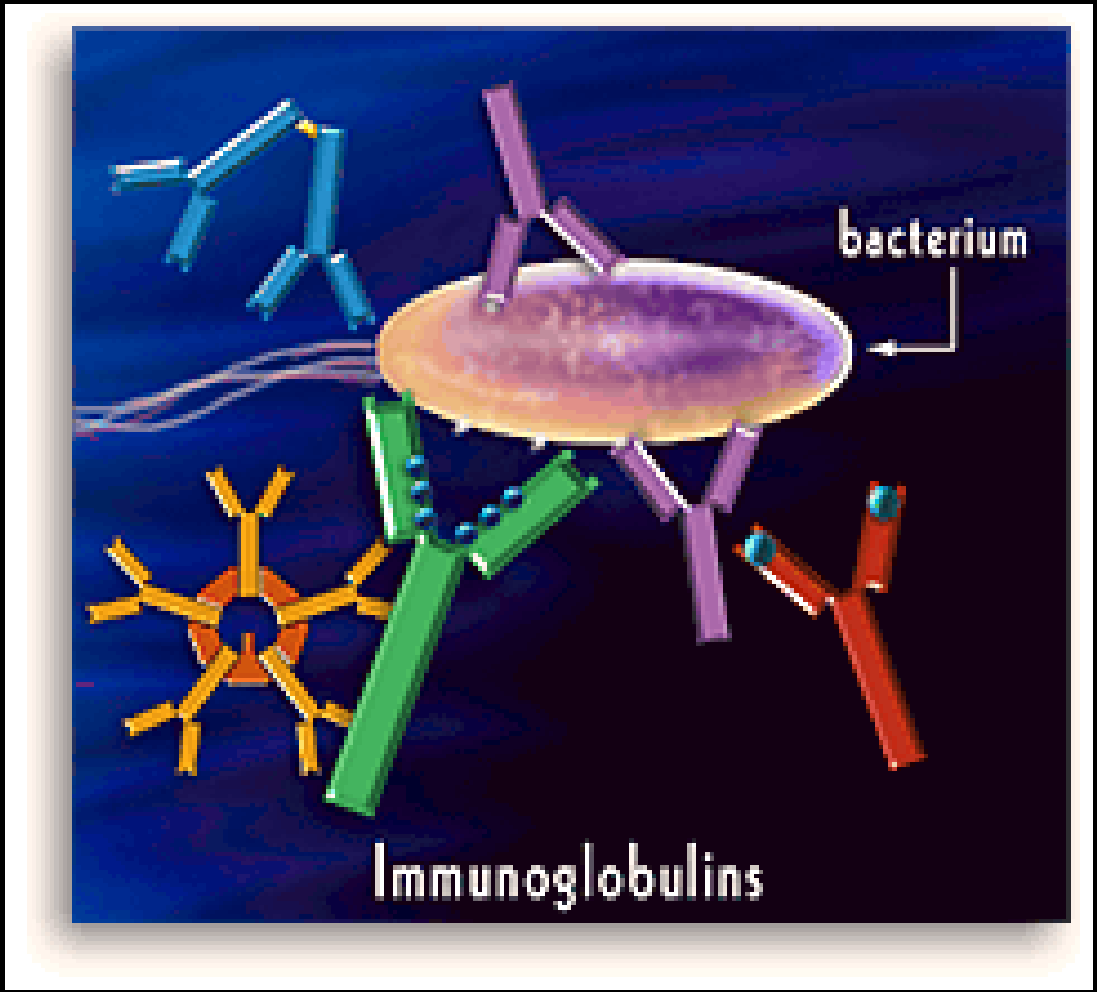
  (1) the hapten determinant,

  (2) unaltered epitopes on the carrier protein, and

  (3) new epitopes formed by combined parts of both the hapten and carrier


Carrier
Hapten
Hapten–carrier conjugate
Immunize rabbit
Antibodies to hapten
Antibodies to carrier
Antibodies to conjugate of hapten and carrier

3.5. Antibodies



- **Anybodies** are the **antigen binding proteins** present on the **B-cell membrane** and secreted by plasma cells.
- Or they **proteins that appear in the blood serum** following exposure of an animal to certain foreign substances.
- All **antibodies** share :-
  - **structural features**,
  - **bind to antigen**,
  - **have** effector functions.

- The **antibodies** produced in response to a particular antigen are **heterogeneous**.

Basic Structure of Antibodies

- **Plasma/fluidy part of blood/** contains all of the **soluble small molecules** and **macromolecules of blood**,

- If **the blood or plasma** is allowed to clot, the fluid phase that remains is called **serum** **and,**
  - **antibodies reside in the serum.**

- the **γ-globulin Serum** was identified as containing **serum antibodies**, which were called **immunoglobulins.**

- **Immunoglobulins /soluble antibodies/** are **glycoproteins made up of:**

  - **Four polypeptide chains (IgG):**

    a- **Two light (L) polypeptide chains**

    b- **Two heavy (H) polypeptide chains**

- The **four chains** are **linked by disulfide** bonds **.**
- **And each chain is with a variable region (V) & constant region (C).**

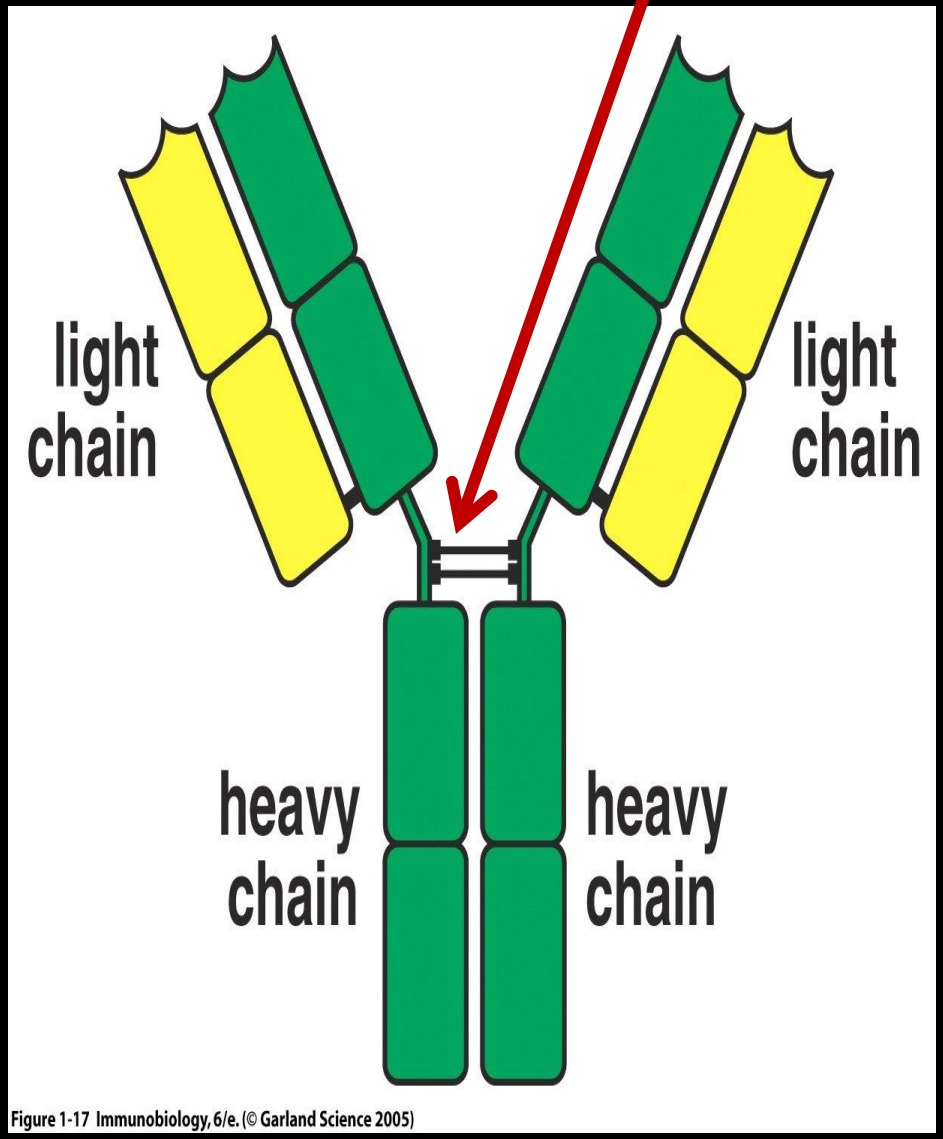


Figure 1-17 Immunobiology, 6/e. (© Garland Science 2005)

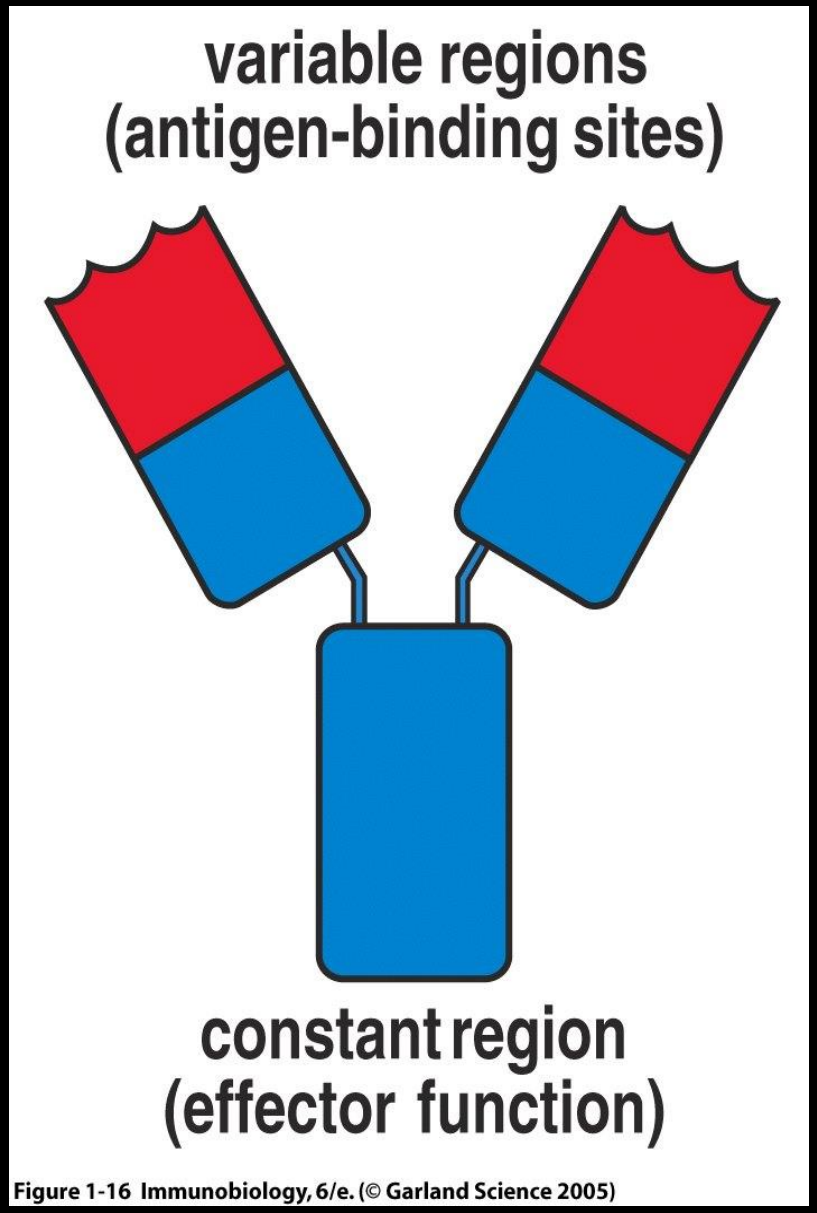


Figure 1-16 Immunobiology, 6/e. (© Garland Science 2005)

- The **primary structure**, "the amino acid sequence",
  - accounts for the **variable** and **constant regions**
    - For both **heavy and light chains**.

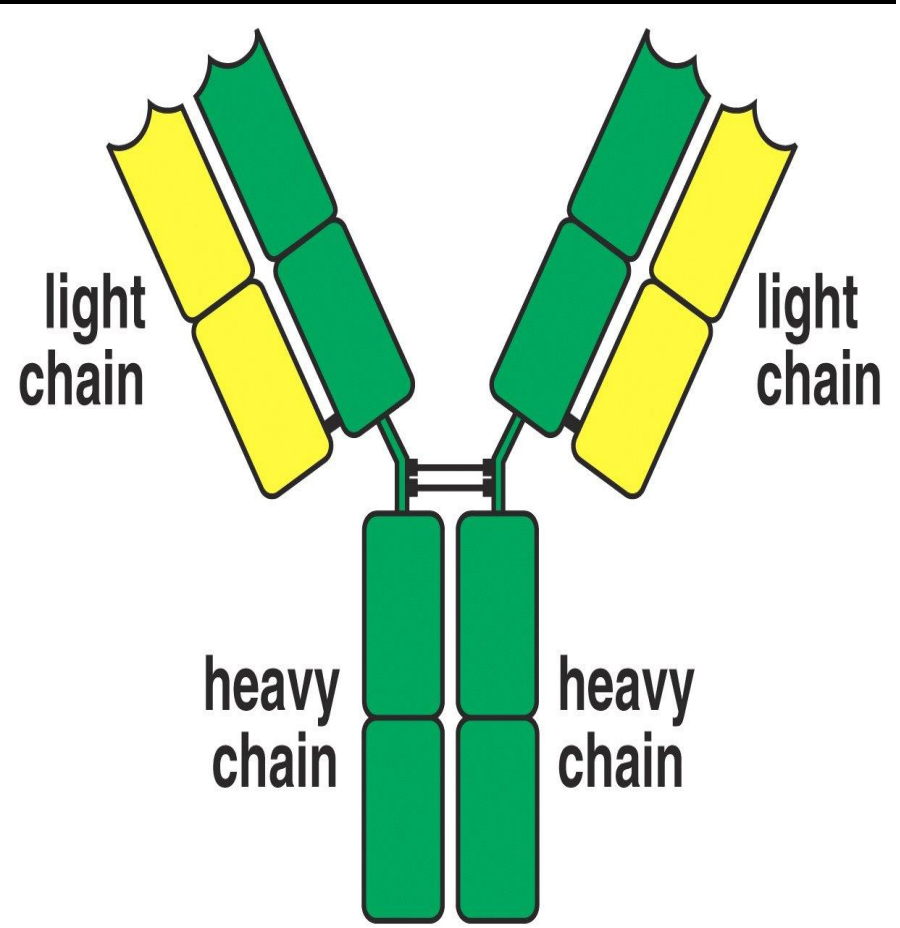
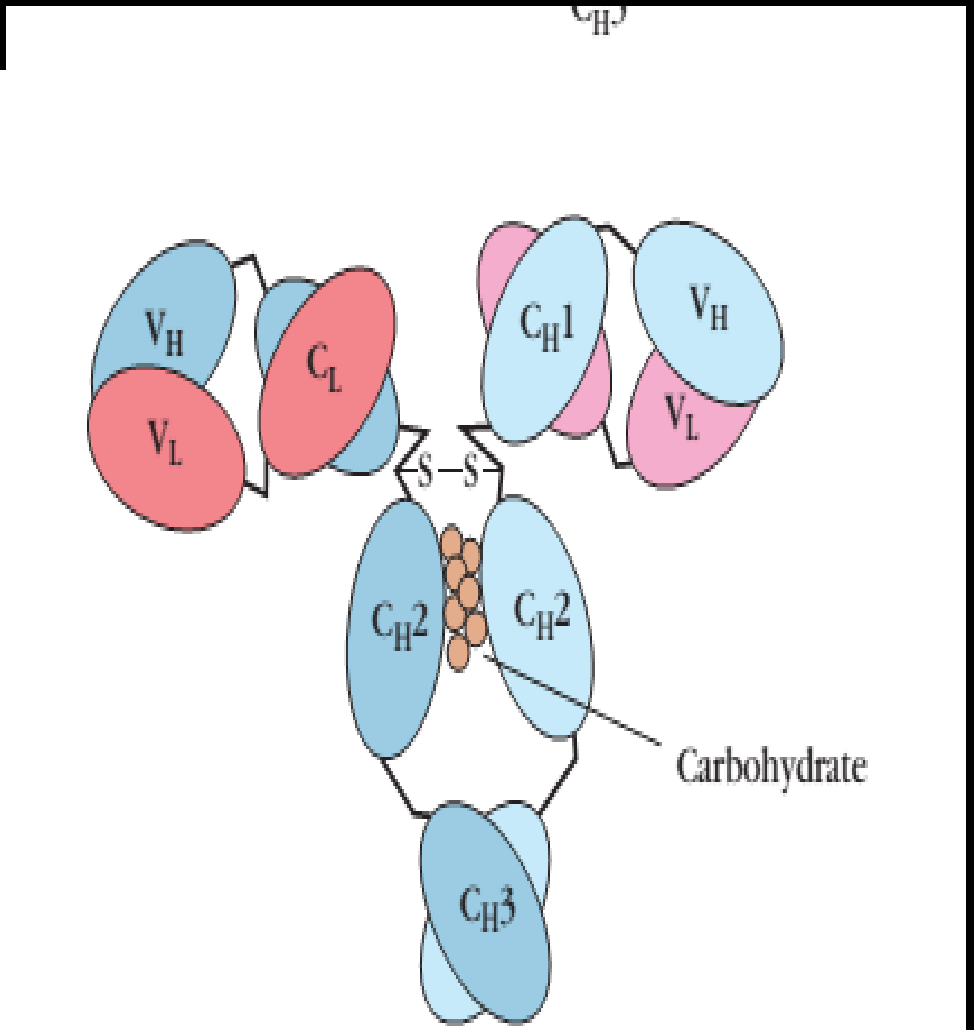
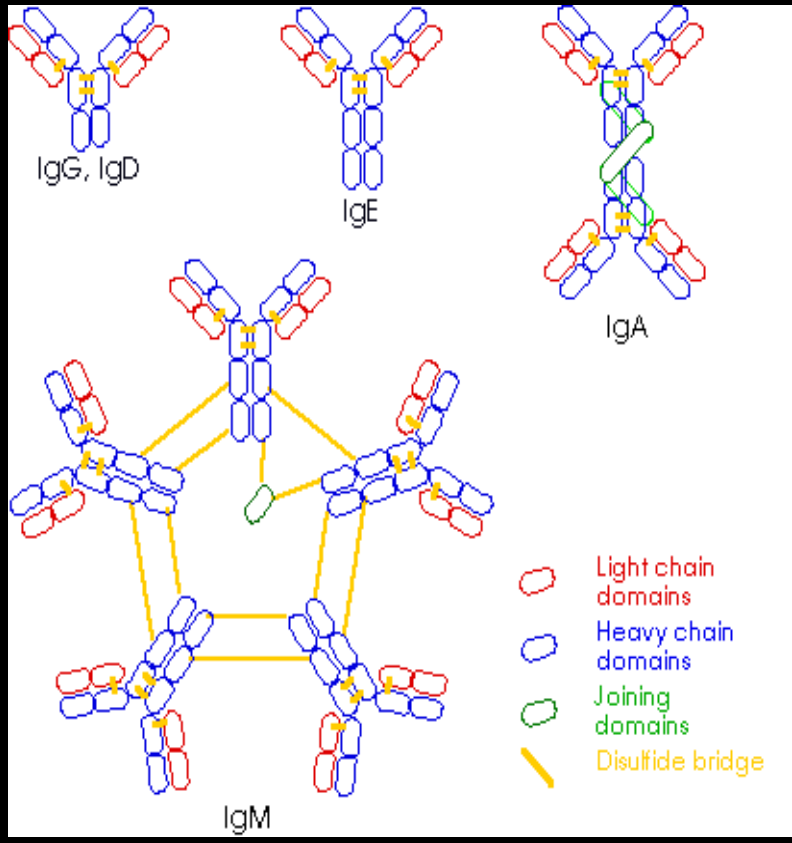


Figure 1-17 Immunobiology, 6/e. (© Garland Science 2005)



Heavy chains

- The **heavy chains** of a given antibody molecule determine the **class of that antibody**:



Five classes of Antibodies:

- IgG =monomer(80%)
  - subclass IgG1, IgG2, IgG3

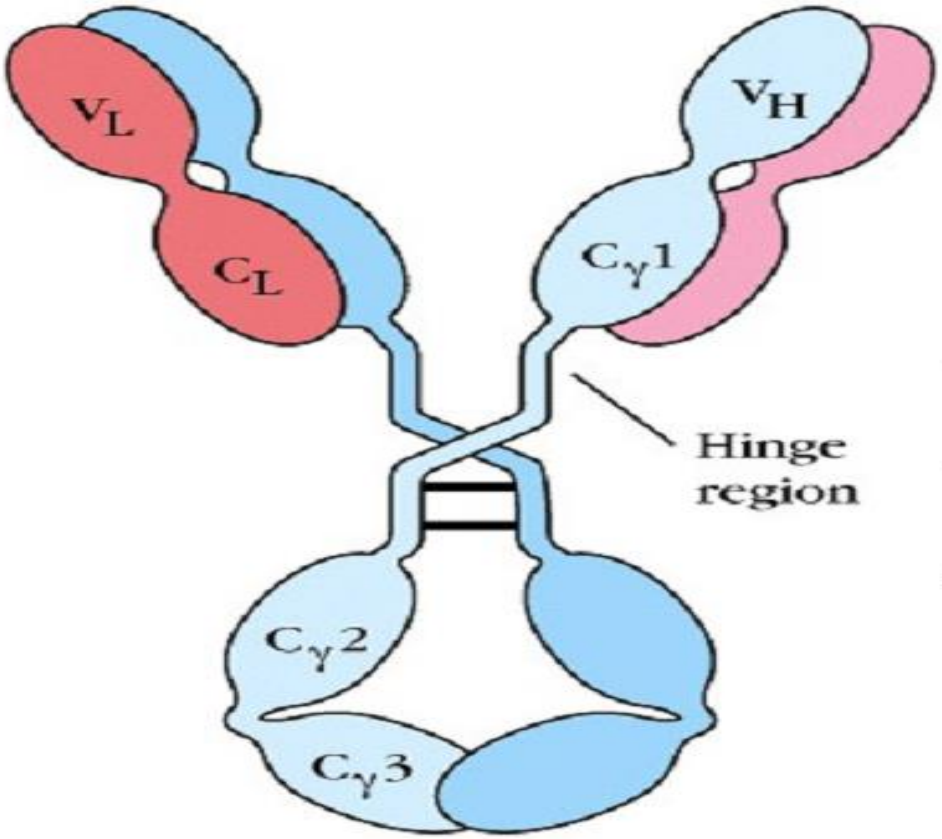
, IgG4.

- IgM=pentamer (5-10%)
- IgA=dimer (5-15%)
  - subclass IgA1, IgA2
- IgD=monomer(0.2%)
- IgE=monomer(0.002%)

It contains

- one variable domain (VH)),
- **three or four constant** domains (CH1, CH2, CH3, and CH4), depending on the antibody class.

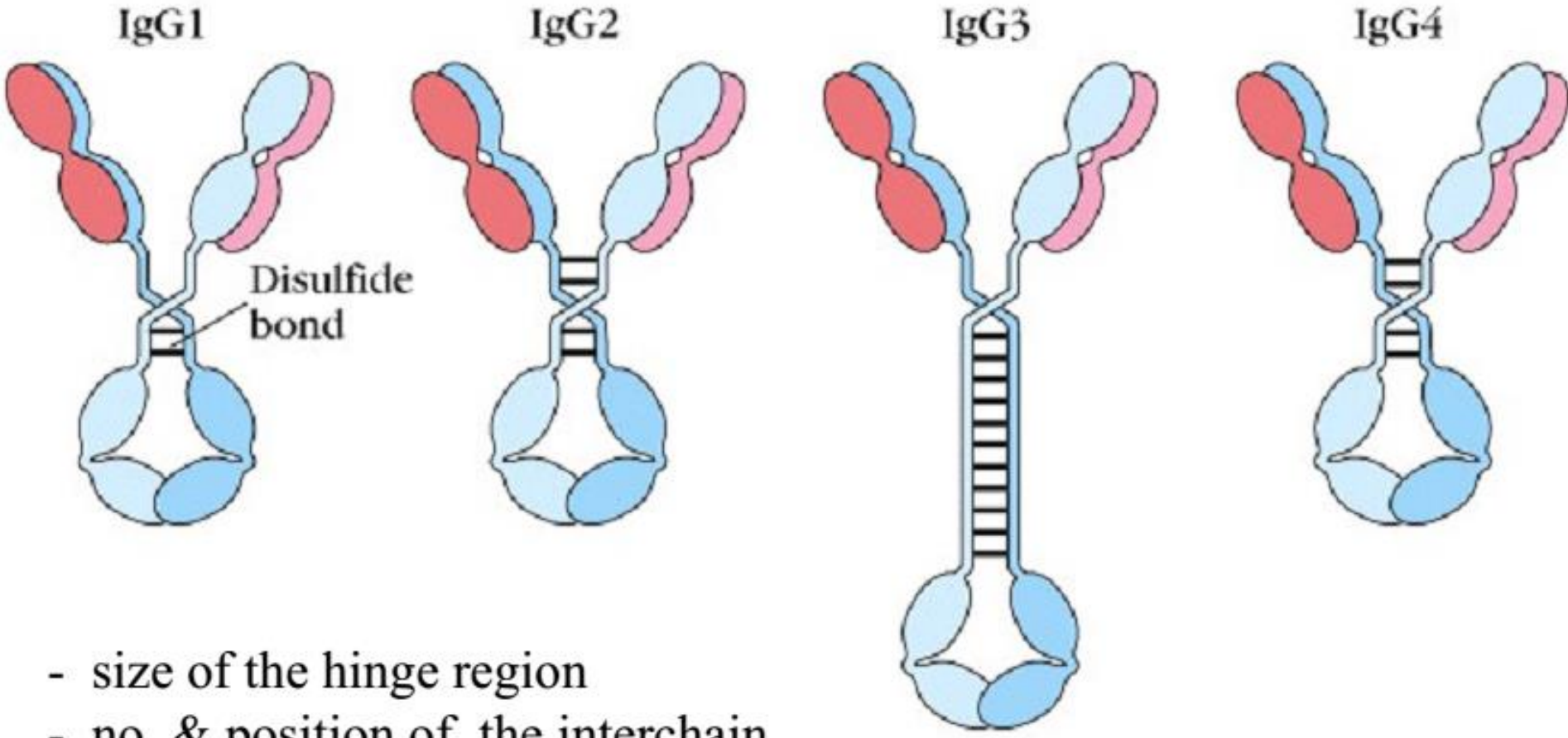
IgG



- most abundant in serum
- 80% of total serum Ig
- 4 IgG subclasses

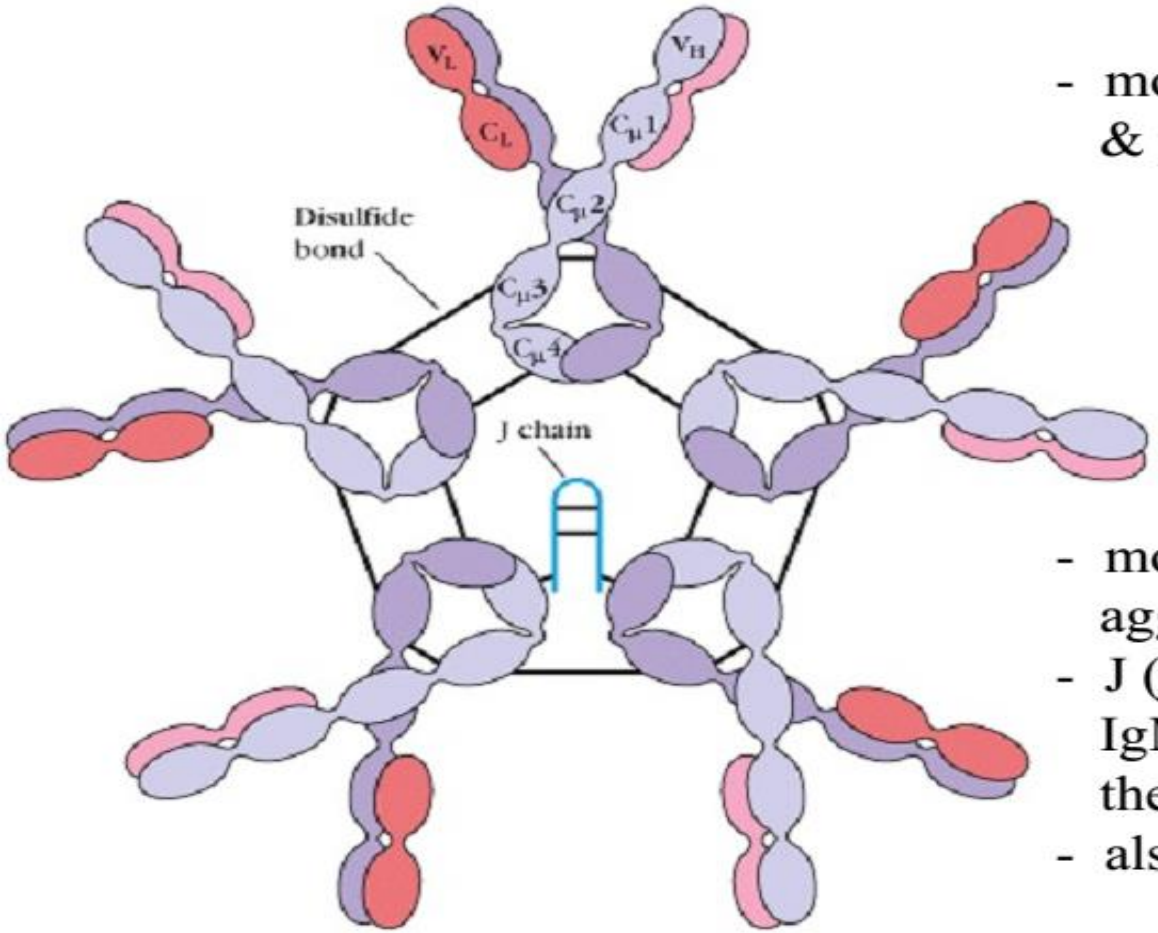
- **IgG** =bind to **antigen of Fab** and bind to **phagocytic white blood cells** on the other end (Fc) .
- it is the **smallest of all molecules which easily migrate to all tissues and only 45% found in the blood.**

4 subclasses of human IgG



- size of the hinge region
- no. & position of the interchain -S-S- bond
- IgG1>IgG2>IgG3>IgG4 in serum conc.
- 90% - 95% homologous in DNA sequences

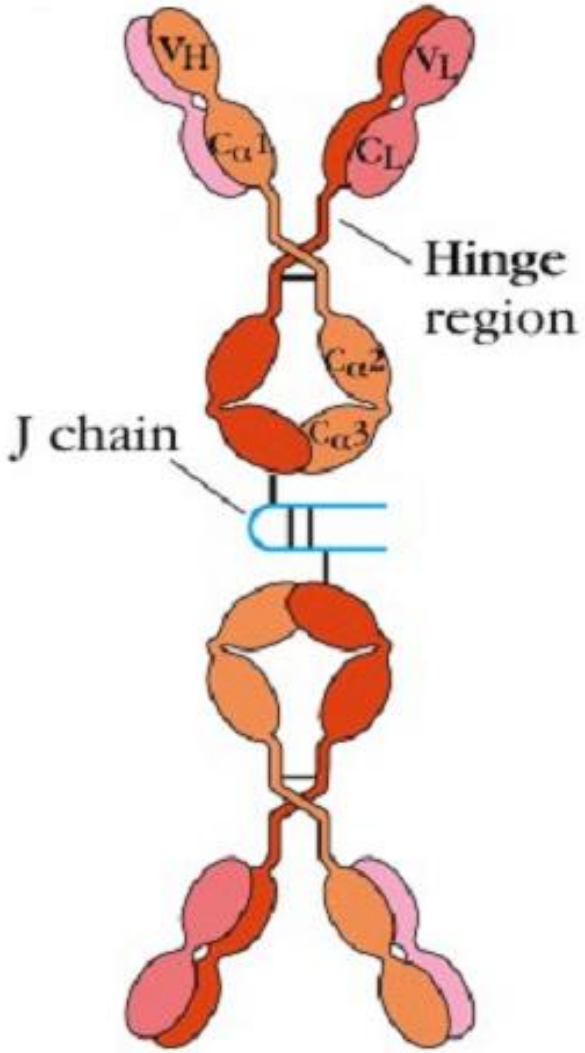
IgM Pentamer



- monomer on the membrane & pentamer in secretion
  - 5% - 10% serum Ig
  - 1st Ab in neonates
  - 1st Ab in primary response
- more efficient in agglutination & C fixation
- J (joining) chain allows IgM to transport across the epithelium.
- also present in secretions

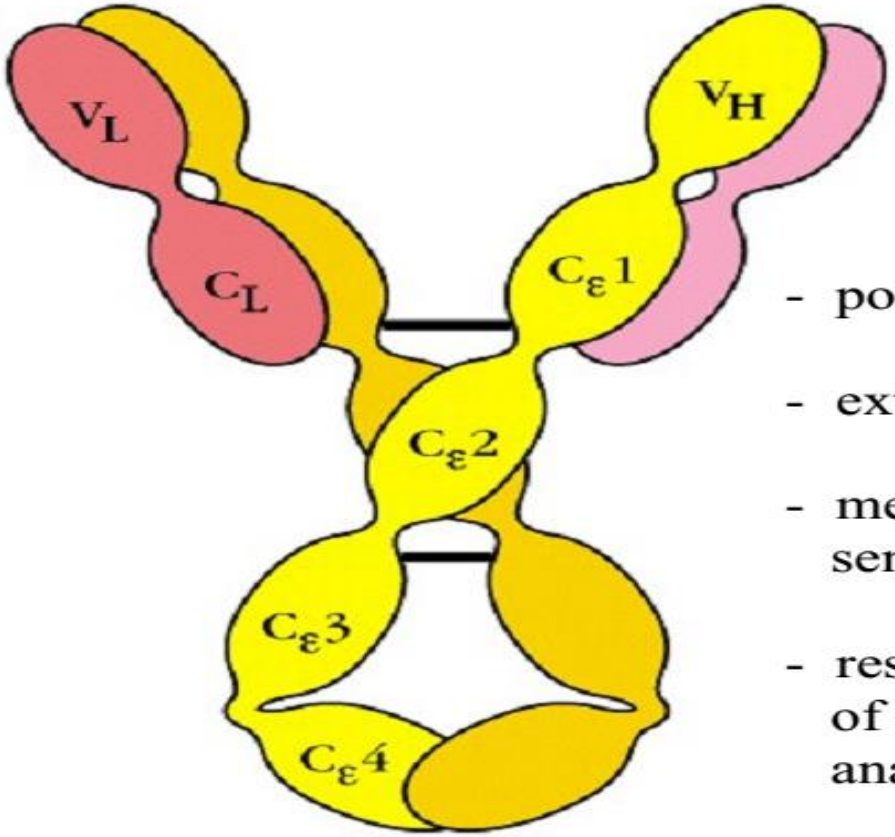
- **IgM= responds early during the attack before the amount of IgG sufficient.**
- **These are called Macroglobulin C/Z of their large size.**

IgA Dimer



- 10% - 15% of total serum Ig
- monomers, dimers, trimers and tetramers in serum
- predominant in external secretions, e.g., breast milk, saliva, tears, and mucus of the bronchial, genitourinary, and digestive tracts

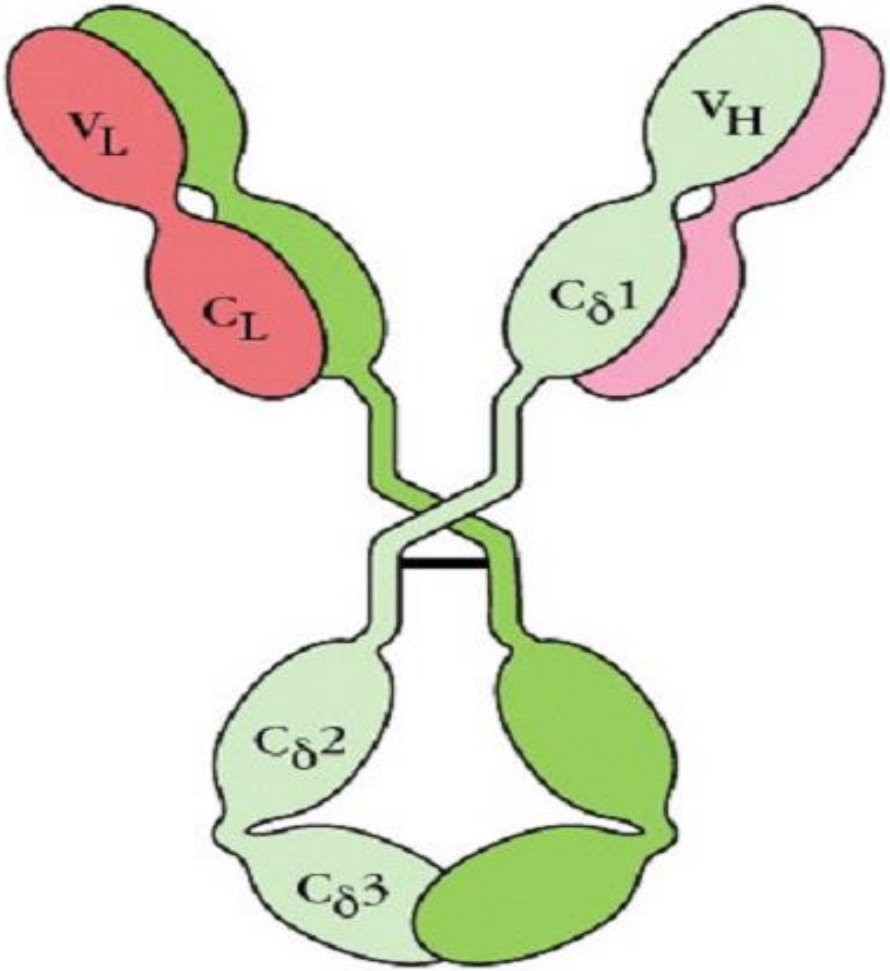
IgE



- potent biological activity
- extremely low conc. in serum
- mediates the immediate hyper-sensitivity reactions
- responsible for the symptoms of hay fever, asthma, hives, and anaphylactic shock

- IgE activates **mast cells** and **basophiles** which respond to parasitic worms and allergens such as pollen.
- **Mast cell** and **basophiles release Histamine molecules as an inflammatory reaction.**

IgD

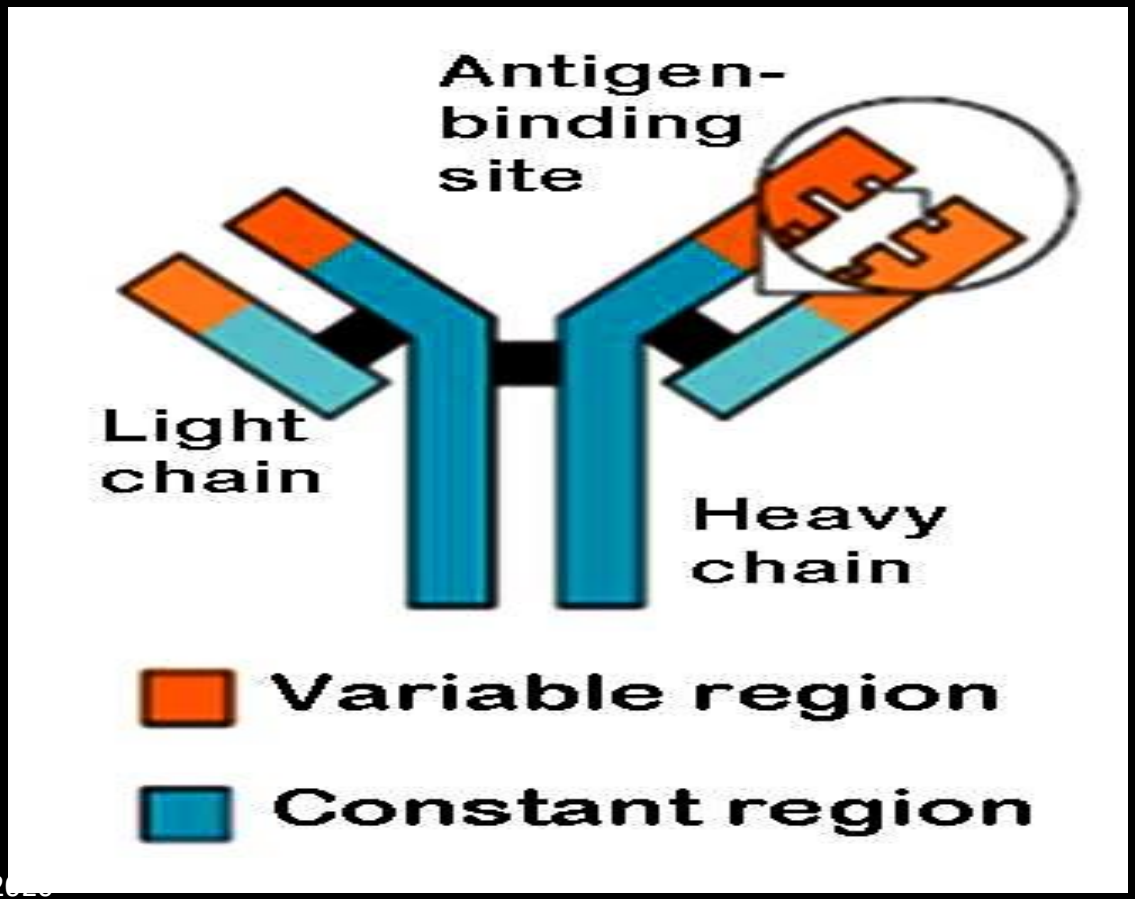


- 0.2% of total serum Ig
- together with IgM, is the major membrane-bound Ig on mature B cells
- thought to function in the activation of B cells
- no biological effector function has been identified

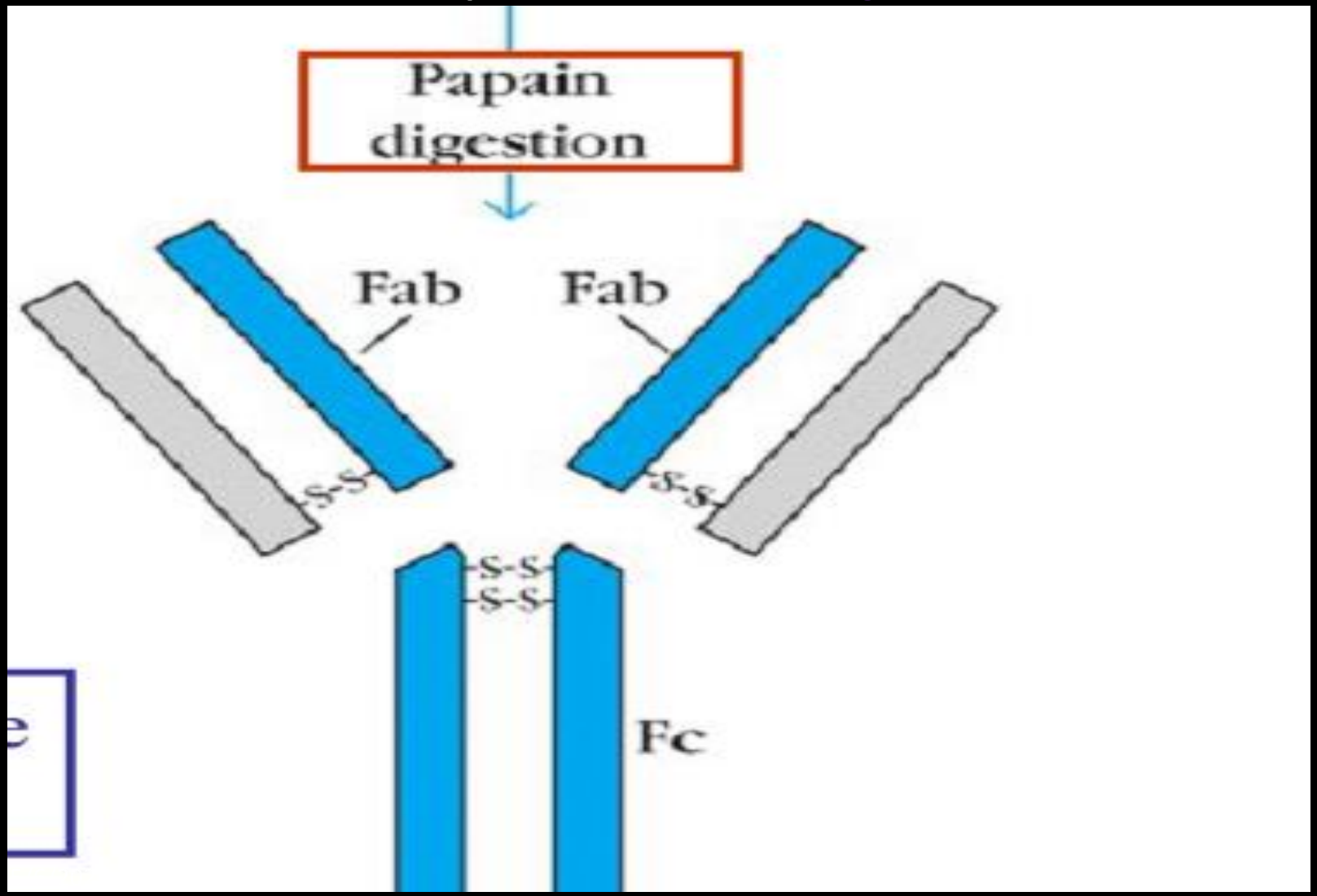
IgD activates **mast cells** and **basophiles** which respond to parasitic worms and allergens .

Light chains

- **Light chains** contain:-
  - **one variable domain (VL)**,
  - **one constant domain (CL);**



- **Fab** =consists of **portions** of an **H** and a **L cha**in.
- **Fc**= contains only **H chain components**.



Function of immunoglobulines

- **Antibodies:** generally **do not kill** or **remove pathogens** solely by **binding to them**.
- While **V regions(Fab)** bind to **Ag**,

- **The FC region** is responsible for a **variety of collaborative interactions** with other **proteins**, **cells**, and tissues that result in the effector functions of the humoral responses.

Ig-Mediated Effector Functions

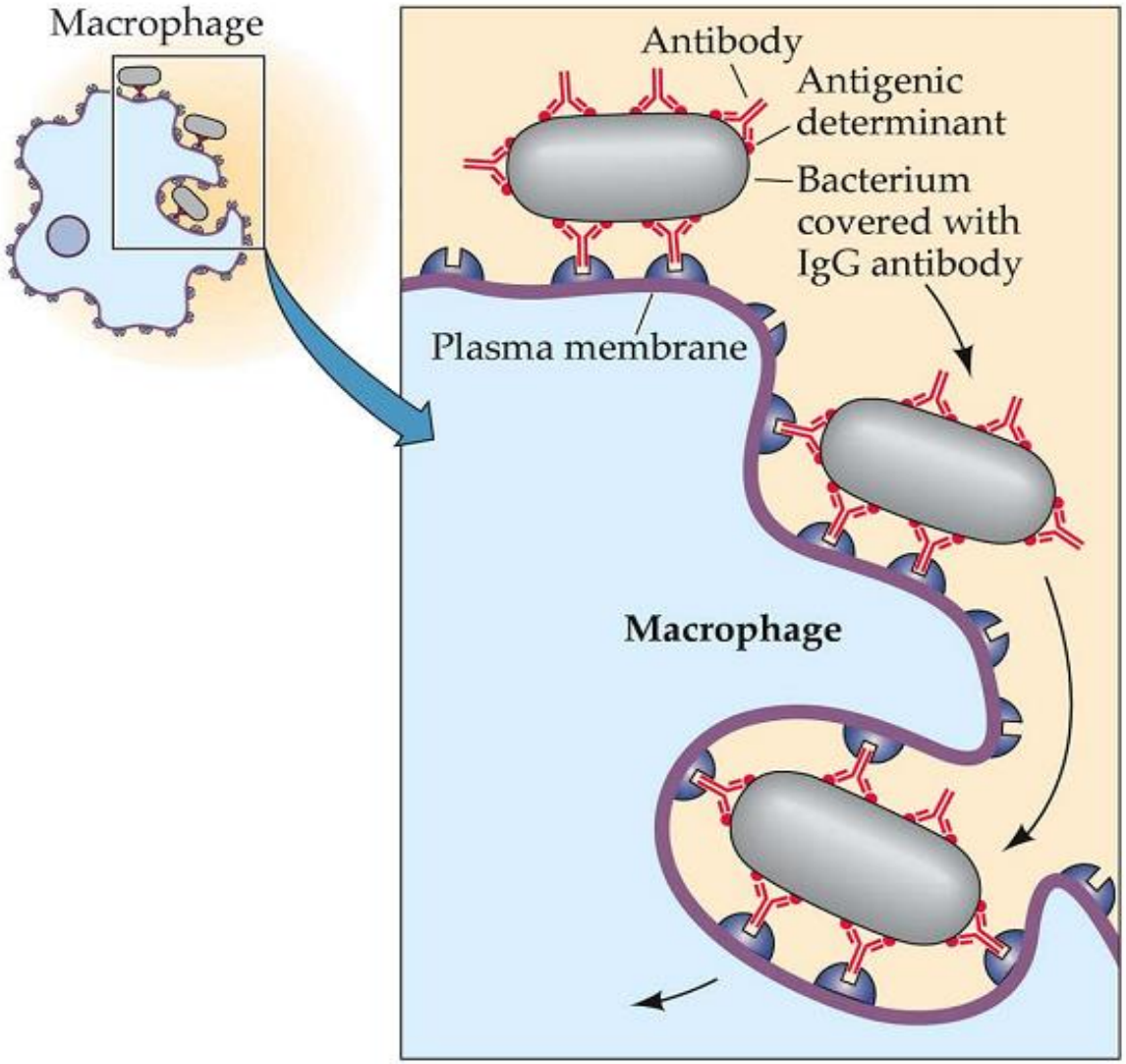
- neutralization
- Opsonization
- Activation of complement .
- Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)
- Ab transport through epithelium or placenta
- Activation of mast cells, eosinophils and basophiles by IgE

A. Neutralization

- Antigen neutralization is carried out by **secreted or secretory antibodies.**
- Certain viruses, **bacterial toxins** and **venom of insects** or **snakes** cause disease by binding to proteins on the host cell surface and using them to enter host cells.

- A neutralization antibodies can recognize and bind to the **virus, toxin or venom** can physically prevent it from binding, thereby protecting the cell.

B. Opsonization:-The promotion of **phagocytosis** of **Ags** by Macrophages and **neutrophiles (**FcR).



LIFE: THE SCIENCE OF BIOLOGY, Seventh Edition, Figure 18.11 IgG Antibodies Promote Phagocytosis

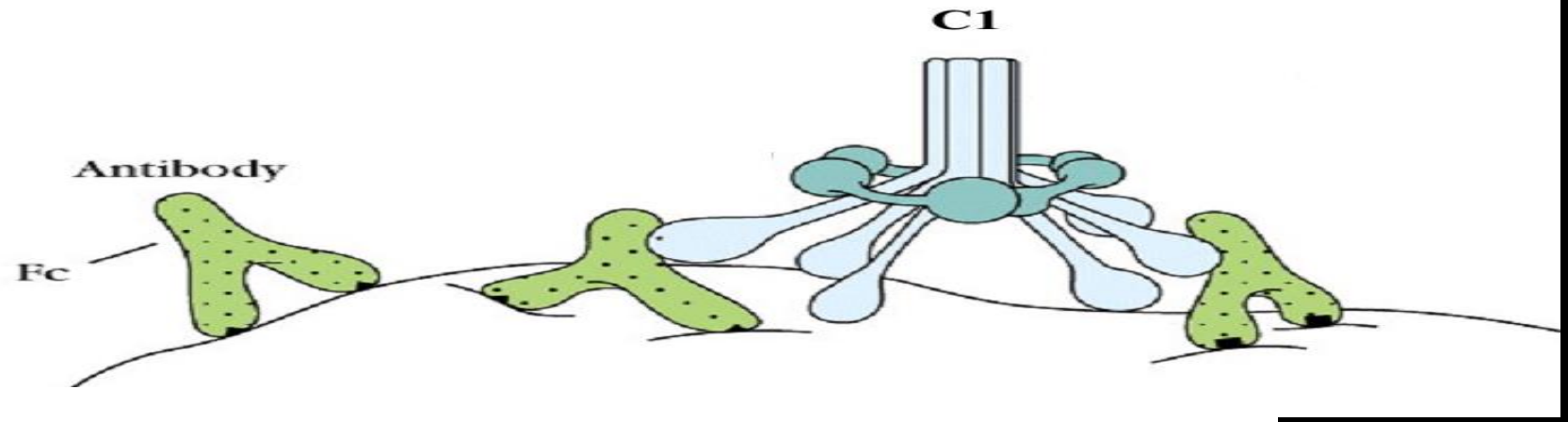
C. Activation of Complement

- **Complements** are a group of **serum proteins** found in **inactive form.**
  - **Activate inflammation**
  - **Cell lysis (destroy abnormal cells)**
  - **Participate in opsonization.**

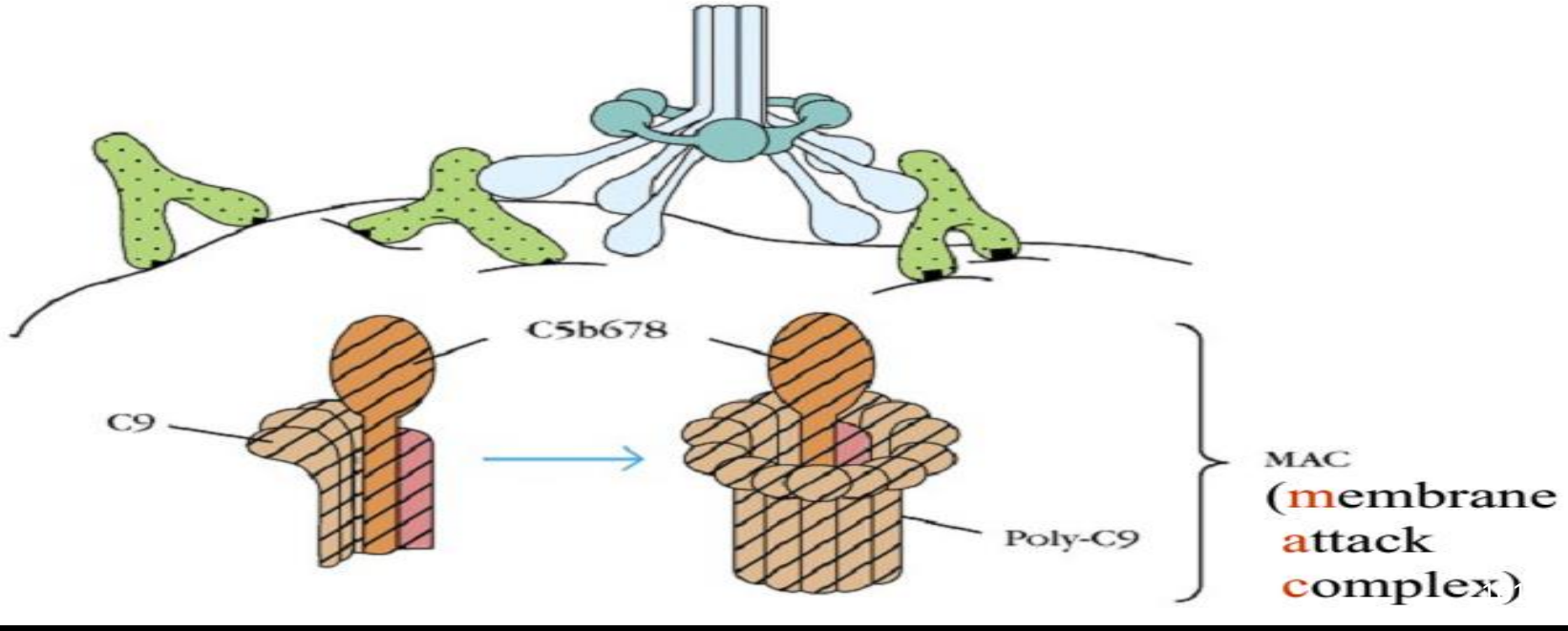
Classical pathway

- **Complements are activated by different foreign molecules.**
- **The formation of immunocomplex (Ag+Ab)= leads to comformational change on Fc region of the Igs(IgG,IgM)**
- **Activated C1 cleaves C2 →C2a+C2b and C4 =C4a+C4b.**
- **Then C2b+C4b combined to form C3 convertase enzyme (protease).**
- **C3 convertase cleaves C3→C3a+C3b.**

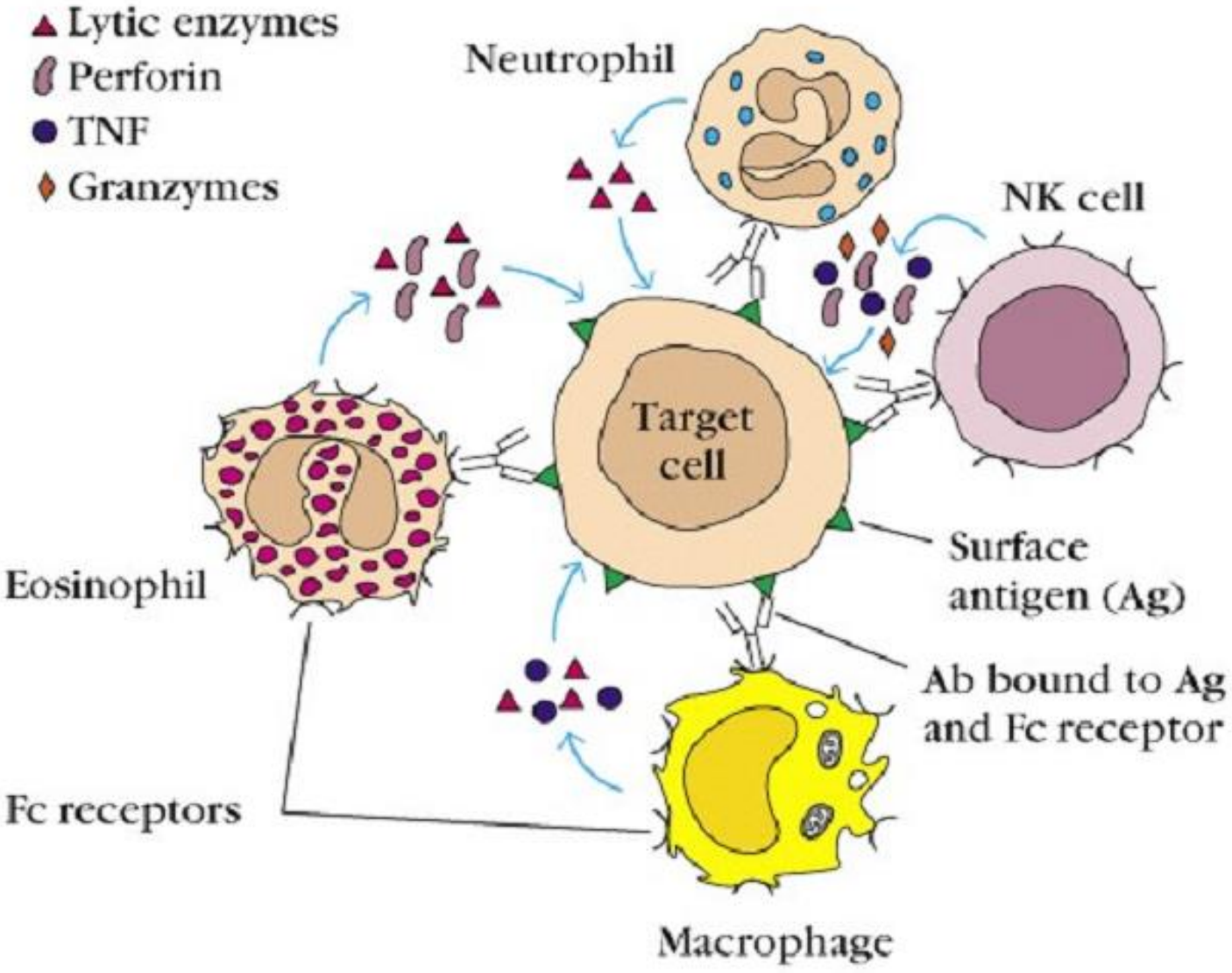
- C3a activates inflammation.
- C3b reacts with factor D and factor B ➡ C3 convertase.
- C3b also attaches with Ags ➡ activates opsonization.
- C3 convertase +properdin →C5 convertase → breaks C5=C5a+C5b.
- Again C5a enhances inflammation.
- C5b+C6C7C8C9 is a lytic complex similar with perforin of NKs is called membrane attack complex (MAC).
- MAC forms a hole in the cell membrane → leakage of lots of substances out of the cell → death of the cell (antigen).


C1
Antibody
Fc

C1 ⇒ ⇒ ⇒ ⇒ ⇒ ⇒ ⇒ C9


C5b678
C9
Poly-C9
MAC
(membrane
attack
complex)

Antibody-dependent Cell-mediated Cytotoxicity (ADCC)



Clonal selection

- **How Do B Cells Produce Antibodies?**
- **First of it is the Ag which selects the lymphocytes which makes antibodies.**
- **Antibodies are formed before Ag is ever seen→ then they are selected by the antigen based on exact fit.**
- **Each B-lymphocyte subset is programmed to make one, and only one, antibodies and it places this Ab on its outer surface to act as areceptor.**
- **Each lymphocyte has of the order of 10^5 identical Ab molecules on its surface.**

Clonal selection theory

- Lymphoid stem cells differentiated randomely to produce clones of lymphocytes.

- **A lymphocytes clone**:- all of the progeny cells derived from **any single virgin lymphocyte**.
- **Each clone** is committed to respond to **a single epitope.**

- **All the Ig proteins expressed by B-cells in a given cone are identical.**

- clone : A **group of identical cells** derived from a **single cell.**

- **B cells** exist as clones.

Mature B-cell +Ag +stimuli from TH cells

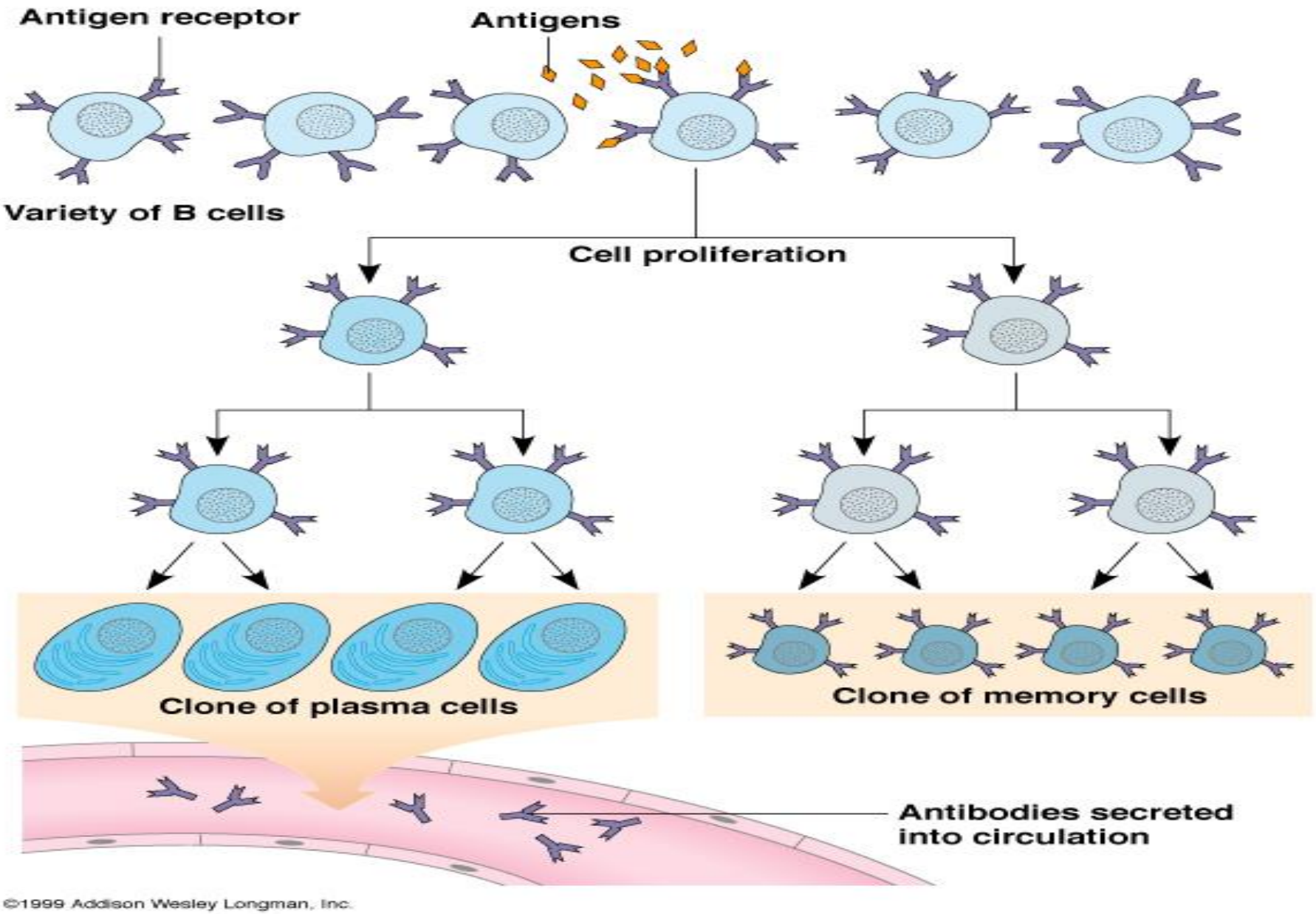
↓

B-cell division and differentiation

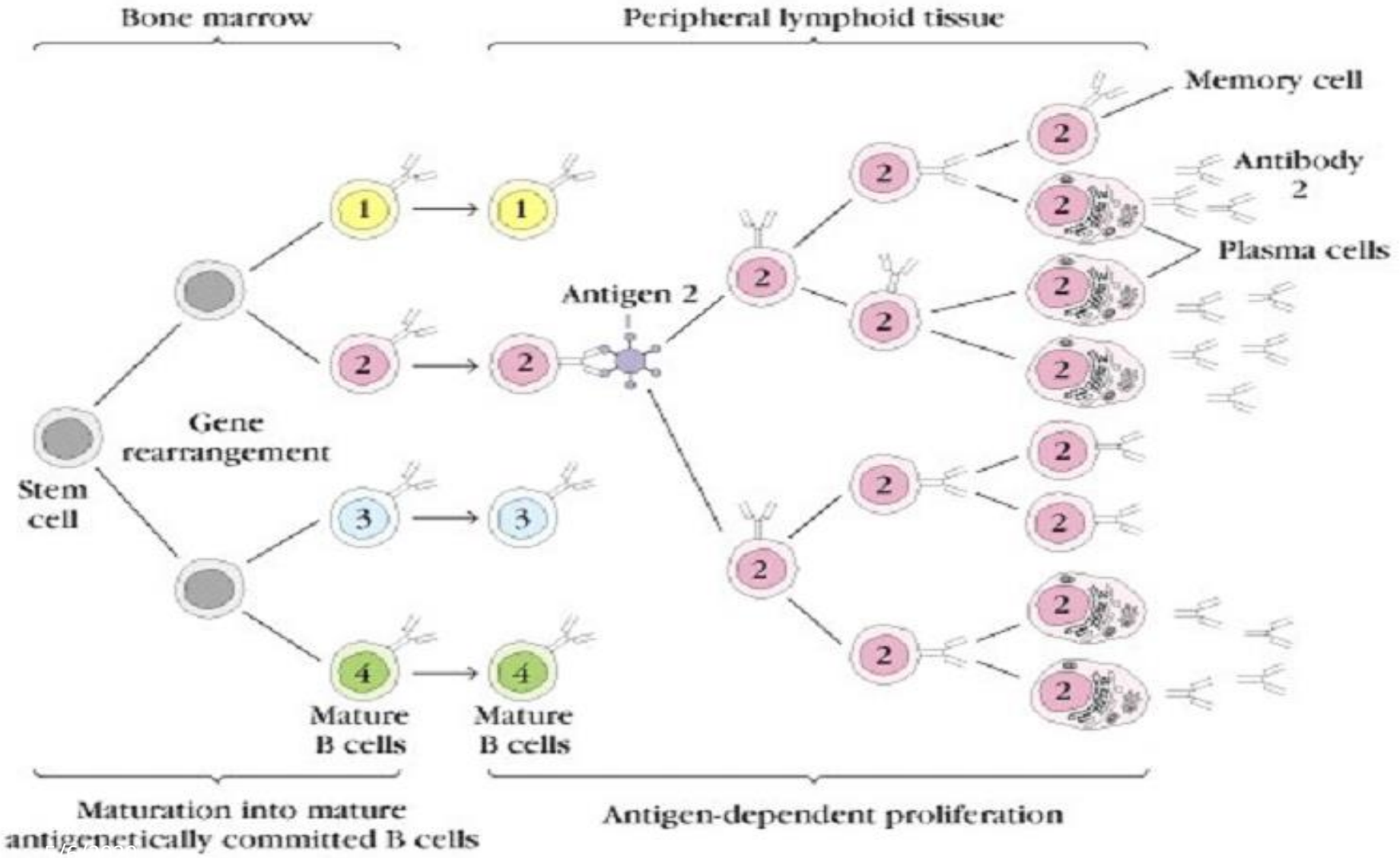
- Upon encountering its specific antigen, **a single B cell**, or a **clone of cells** with **shared specificity,** divides to **produce many B cells**.
-
- Most of such B cells differentiate into **plasma cells** that **secrete antibodies into blood** that bind the **same epitope** that elicited proliferation in the first place.
- A **small minority** survives as **memory cells** that can **recognize only the same epitope**.

- However, with each cycle, the **number of surviving memory cells increases.**

Clonal Selection of B Cells is Caused by Antigenic Stimulation



Clonal Selection of B Lymphocytes



Chapter 5

5. Innate immunity (Cellular immunity)

Introduction

- **Innate immunity:- is working by interaction of certain cells, cell surface receptors and soluble molecules.**
- **Innate immunity** can be seen to comprise **four types of defensive barriers**:
  - **anatomic and physiologic**
  - **Phagocytic (cellular internalization)**
  - **inflammatory.**

- **Innate immunity**→ the first line immunologic defense,which is always **active or nearly active.**

- Innate immunity is prorammed to:
  - **To recognize** broad range of molecules asscociated with pathogens.
  - To destroy the invaders rapidly: **Activation of the phagocytic system** and devoloping an inflmmatory response.

5.1.Types and mechanisms of protection

I. Defensive barriers

A. Skin and the Mucosal Surfaces

The ***Skin and the Mucosal Surfaces*** Provide **Protective Barriers** Against Infection.

- **Physical and anatomic barriers** prevent the entry of pathogens .
  - **first line of defense against infection**.
- The **skin** consists of two a **thinner outer layer:-**
  - **the epidermis**
  - **thicker layer** (**dermis**).
- The **epidermis** contains several layers of **tightly packed epithelial cells.**

A. The **outer epidermal** layer consists of dead cells and is filled with a waterproofing protein called **keratin**.

B. The **dermis**, which is composed of :-

- **connective tissue,**
- **hair follicles, sweat glands**
- **sebaceous glands (sebum consists lactic acid** and **fatty acids ,**

B. Physiologic Barriers to Infection

- **waving of nasal cilia or tears and mucous that work to reinforce the anatomical barriers.**

- The physiologic barriers that contribute to **innate immunity** include
  - **temperature**
  - **pH (gastric acidity is an innate physiologic barrier to infection).**

II. Innate immune cells ;phagocytes ,dendritcs, NK……..

- **What is detected by innate immunity?**
  - **highly conserved and essential components of microbes "Pathogen-associated molecular patterns" (PAMPs).**
- The **molecules of the microbes** targeted by th **immune system** are -→**pathogen asscociated molecular patterns** (**PAMPs**).

- The **receptors of the innate immunity** for these molecules are "pattern recognition receptors" (**PRRs**)

- **4 key families of cellular receptors:**
  - **Toll-like receptors (TLRs; transmembrane receptors)**
  - **C-type lectin receptors (CLRs; transmembrane receptors)**
  - **Rig I-like receptors (RLRs; cytoplasmic RNA helicases)**
  - **NOD-like receptors (NLRs; cytoplasmic sensors).**

A. Toll like receptors (TLR)

- **TLR activates :-"transcrition factors"**, which **stimulate the expression of genes encoding:**
  - **cytokines,**
  - **enzymes**
  - **other proteins (endotheliala dhesion factor).**

- Most important **transcription factors** are:
  - **Nuclear factor-kappaB (NF-kappaB) and**
  - **interferon response factor -3 (IRF-3).**

1. **NF-kappaB**: promotes **expression of cytokines** and **endotheliala dhesion factor.**

2. **IRF-3** :-stimulates **type I interferons** and **cytokines** with antiviral activity.

Different mammalian TLRs are specific for different classes of microbial products.

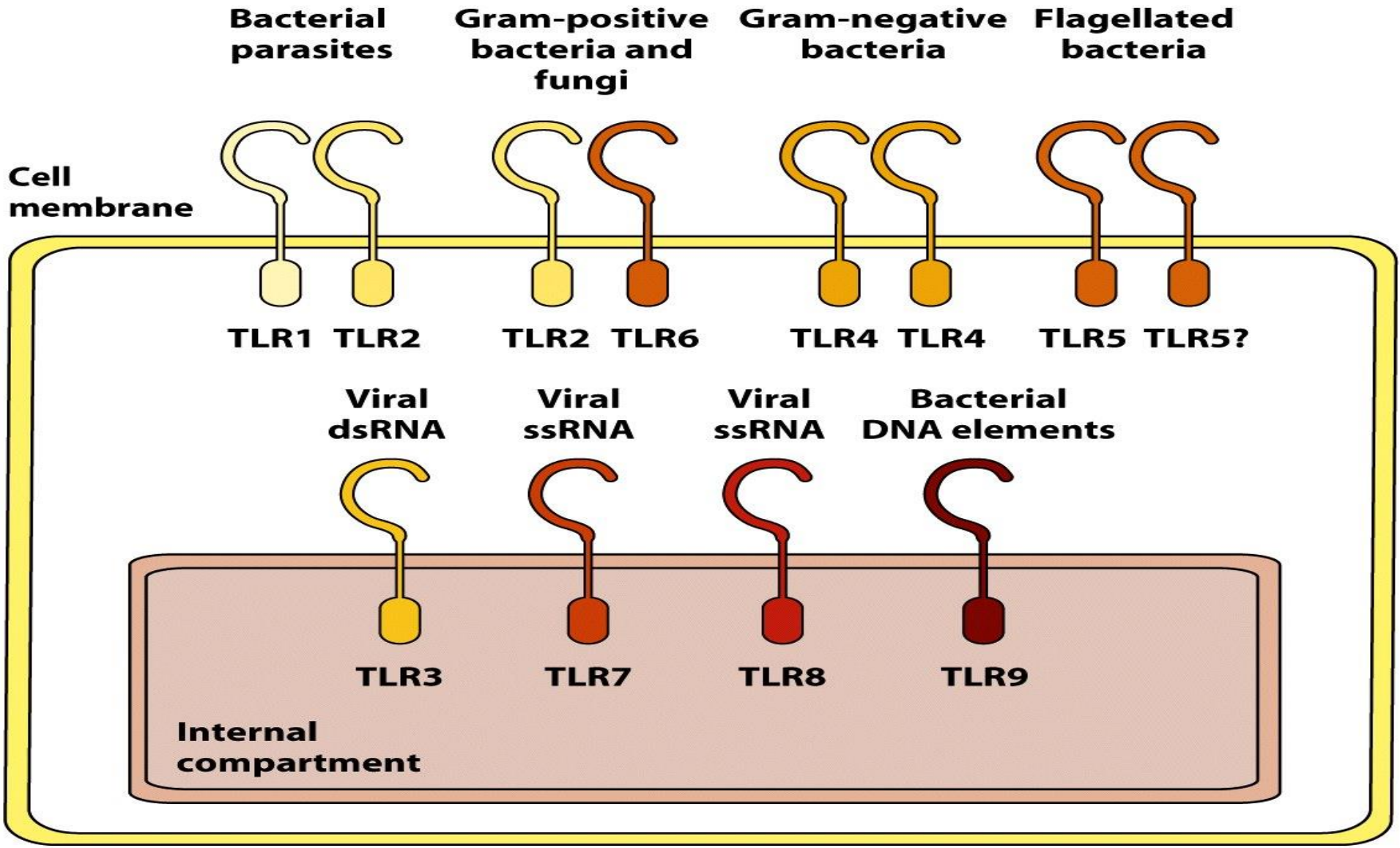
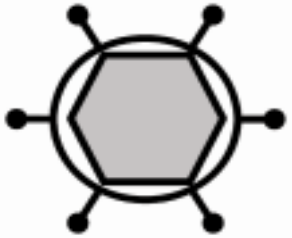
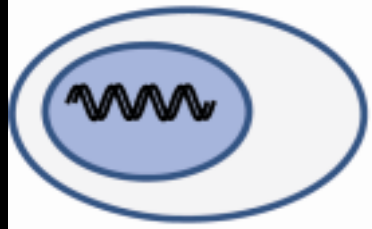
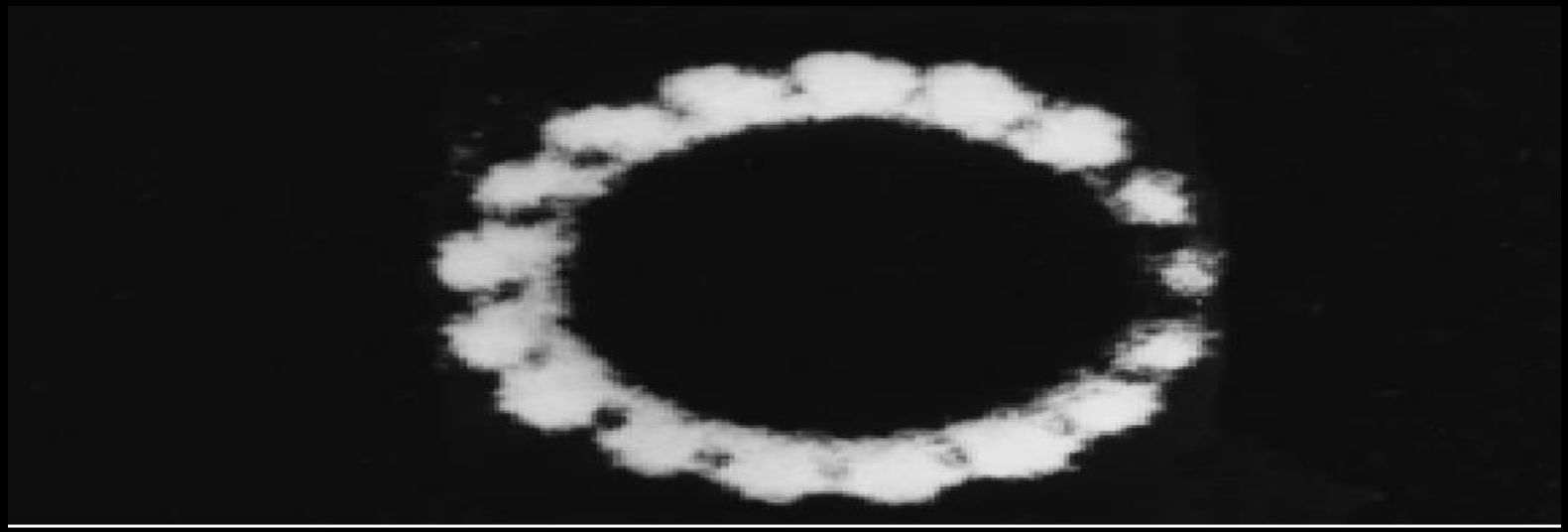


Figure 3-10 part 1
Kuby IMMUNOLOGY, Sixth Edition

Microbe	PAMP	PRR	Site of recognition
 Virus	Glycoproteins	TLR2, TLR4	Cell surface
	5'PPP RNA	RIG-I	Cytoplasm
	ss RNA	TLR7, TLR8	Endosomes
	dsRNA	TLR3, MDA5, RIG-I	Endosomes/cytoplasm
	Genomic DNA	TLR9, DAI	Endosomes/cytoplasm
 Bacteria	Lipopeptides	TLR2	Cell surface
	Lipoteichoid acid	TLR2	Cell surface
	Peptidoglycan	TLR2	Cell surface
	LPS	TLR4	Cell surface
	Flagellin	TLR5	Cell surface
	CpG DNA	TLR9	Endosome
	B-form DNA	DAI	Cytoplasm
	Diaminopimelic acid	NOD1	Cytoplasm
	Muramyl dipeptide	NOD2	Cytoplasm
 Protozoa	GPI anchors	TLR2, TLR4	Cell surface
	dsRNA	TLR3	Endosomes
	CPG DNA	TLR9	Endosomes
	Haemozoin	TLR9	Endosomes
	Profilin-like protein	TLR11	Cell surface

III. Complements



Poly-C9 Complex

- **Serum complement proteins** and **membrane-bound complement** receptors partake in a number of immune activities **:-**
  - **Lysis of** cells, bacteria, and viruses.
  - **Opsonization**, which promotes phagocytosis of particulate antigens.
  - Activates i**nflammation**, and secretion of immunoregulatory molecules

Complement Activation

- Complement activation has **two** pathways.
  - **classical pathway,**
  - **the alternative pathway, or the lectin pathway**.

- The final steps that lead to a **membrane attack** are the same in all pathways.

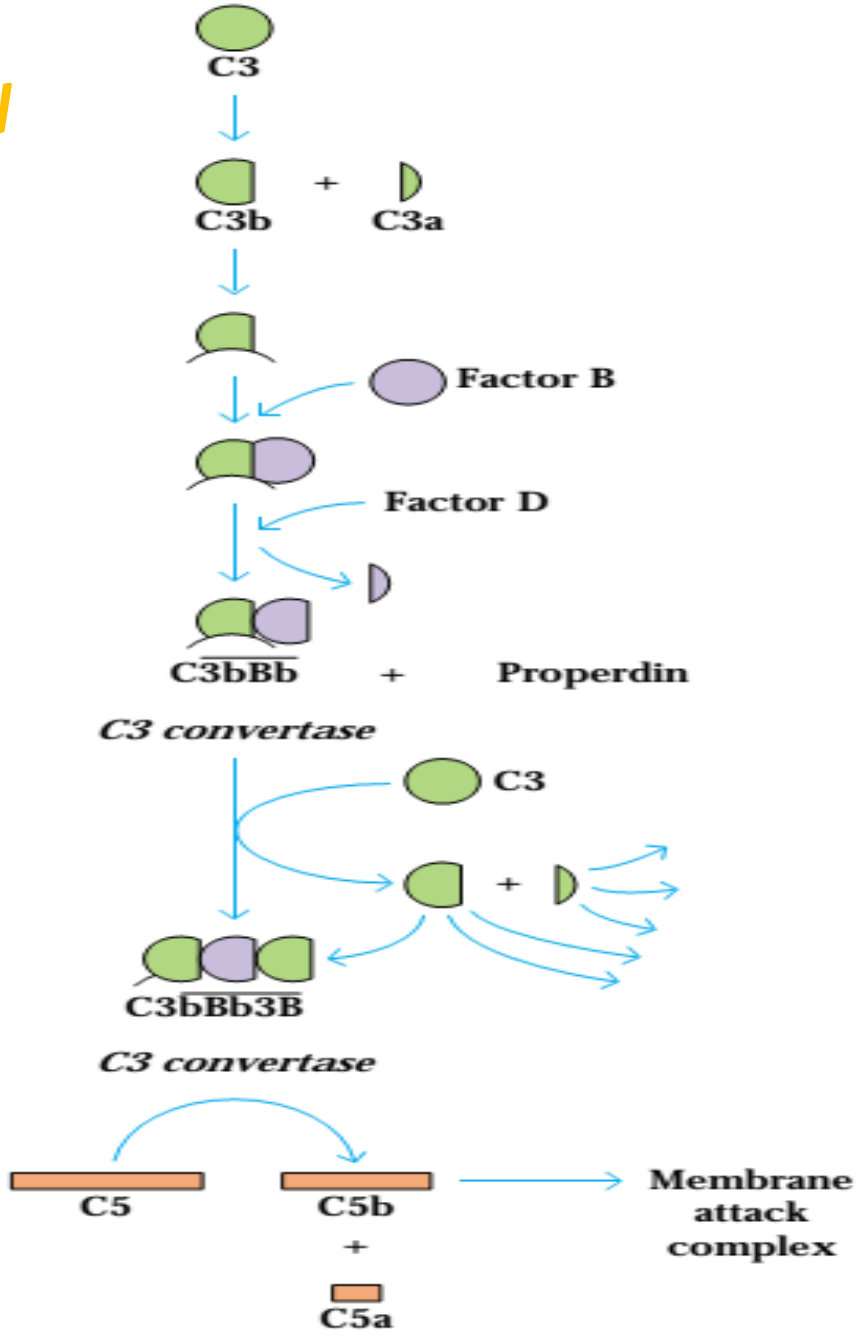
the alternative pathway

1. C3 hydrolyzes spontaneously, C3b fragment attaches to foreign surface

2. Factor B binds C3a, exposes site acted on by Factor D. Cleavage generates C3bBb, which has C3 convertase activity

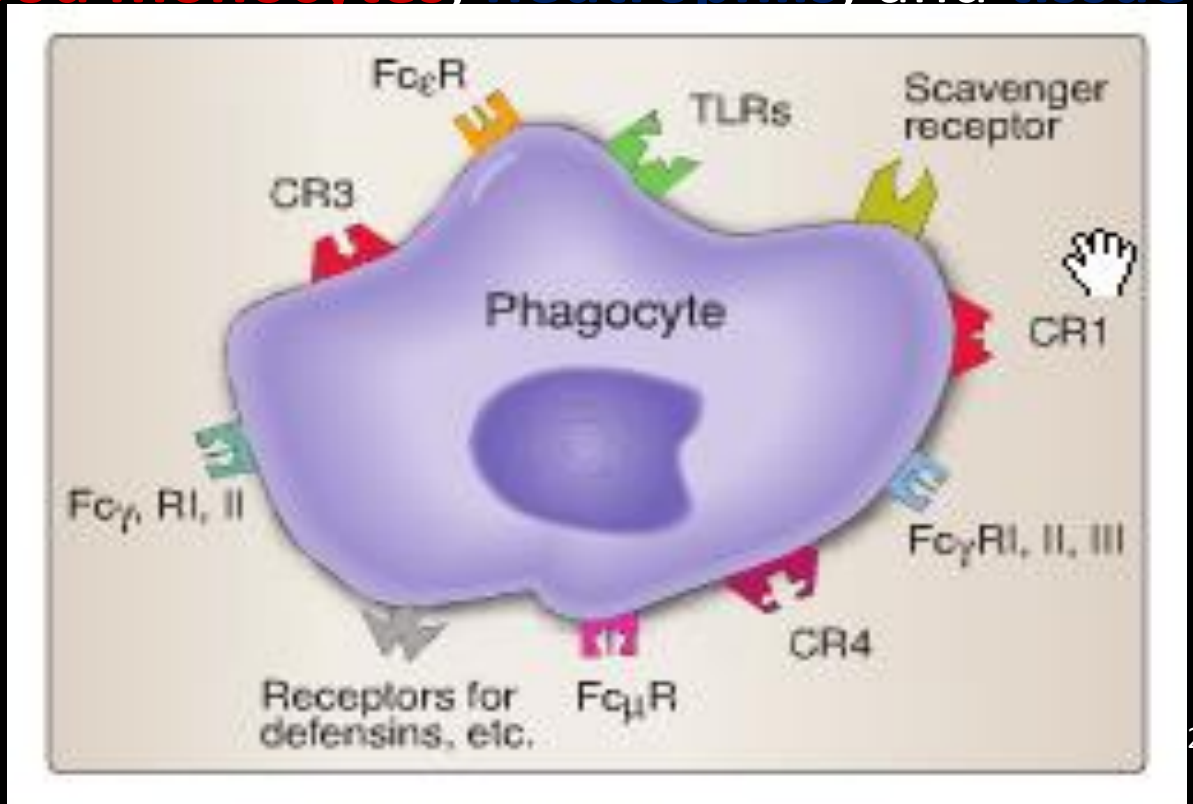
3. Binding of properdin stabilizes convertase

4. Convertase generates C3b; some binds to C3 convertase activating C5' convertase. C5b binds to antigenic surface

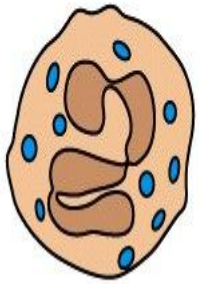


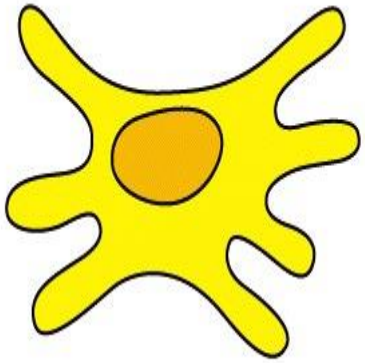
IV. Phagocytic Barriers

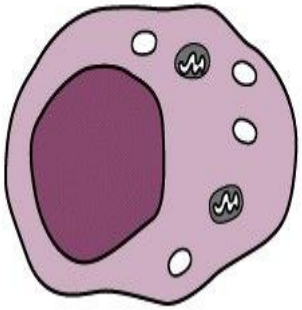
- Another important **innate defense mechanism** is the ingestion of extracellular particulate material by **phagocytosis.**
- Most **phagocytosis** is conducted by specialized cells, such as **blood monocytes**, **neutrophils**, and **tissue macrophages.**



Cell types of innate immunity



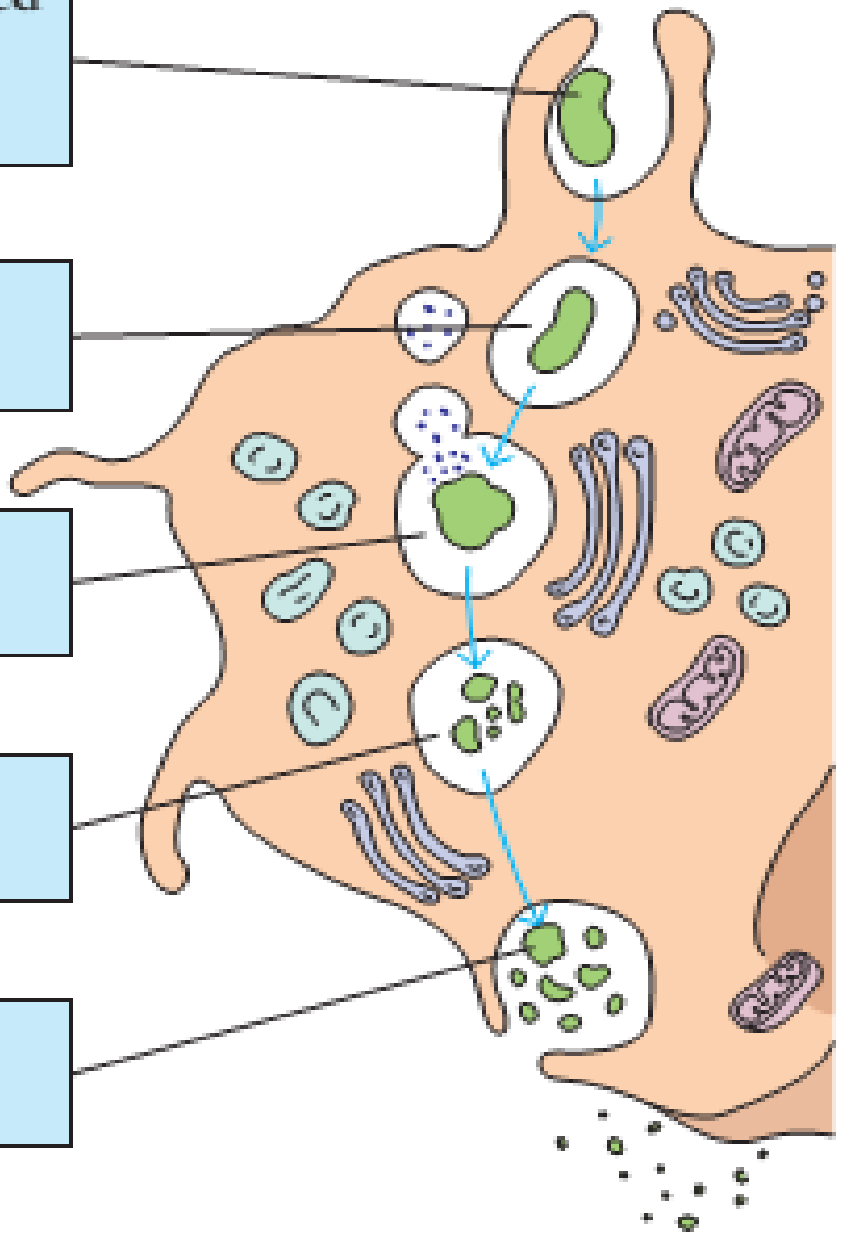




Cell type	Neutrophils	Macrophages	Dendritic cells	Natural killer cells
Function	Phagocytosis Reactive oxygen and nitrogen species Antimicrobial peptides	Phagocytosis Inflammatory mediators Antigen presentation Reactive oxygen and nitrogen species Cytokines Complement proteins	Antigen presentation Costimulatory signals Reactive oxygen species Interferon Cytokines	Lysis of viral-infected cells Interferon Macrophage activation

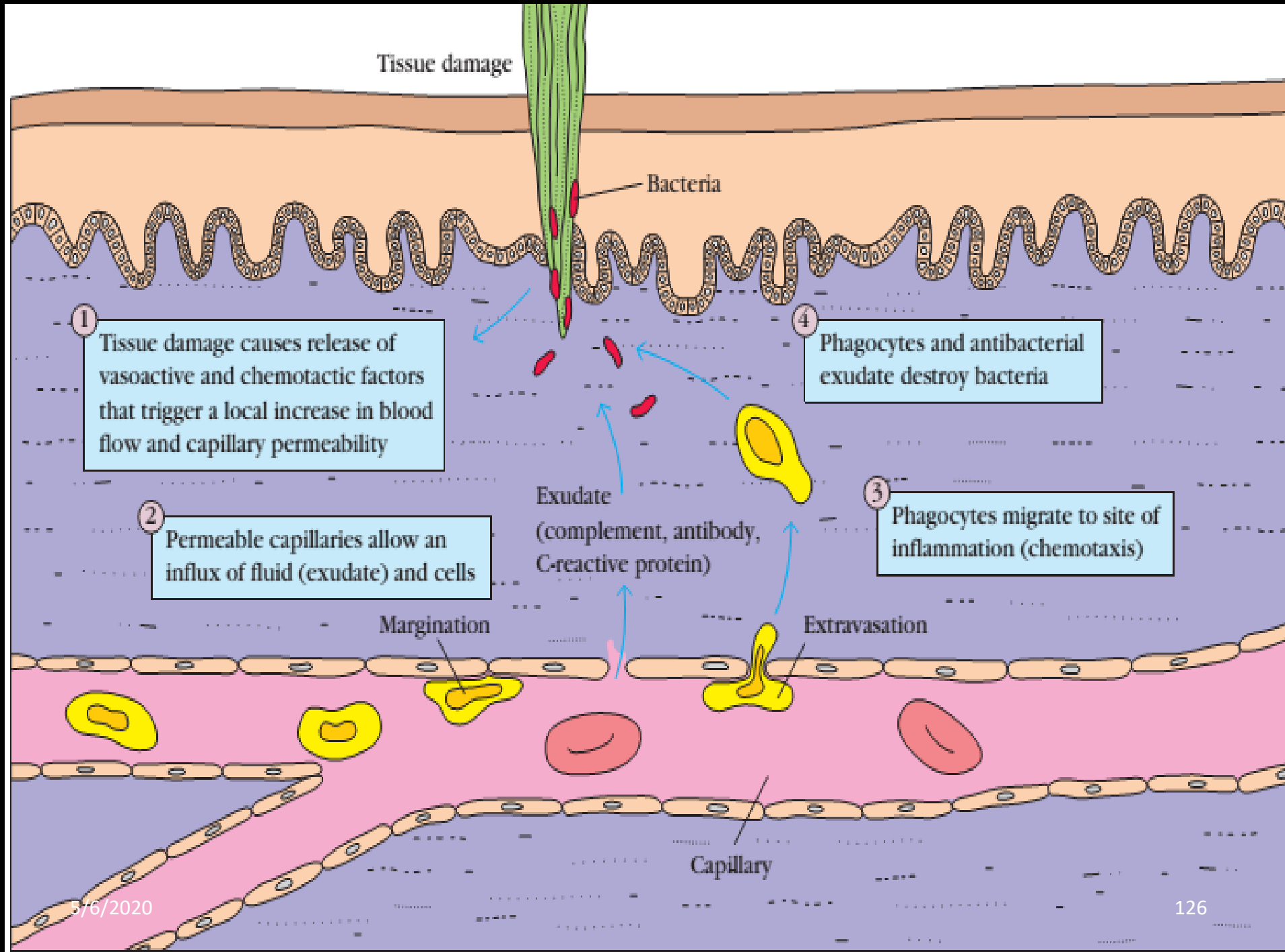
Figure 3-12
Kuby IMMUNOLOGY, Sixth Edition

1. Bacterium becomes attached to membrane evaginations called pseudopodia
2. Bacterium is ingested, forming phagosome
3. Phagosome fuses with lysosome
4. Lysosomal enzymes digest captured material
5. Digestion products are released from cell



5.2. Principles of Inflammation

- **The main goals of the innate immunity**
  - **To induce inflammation**
  - **To achieve first line of defensive against pathogen.**
- **Inflammation= Innate immunity and the initial response to infection.**
- **Tissue damage** caused by a wound or by an invading pathogenic microorganism induces a complex sequence of events collectively known as the **inflammatory response.**
- **Inflammation induced by immune recognition** of infection or tissue damage (usually good)

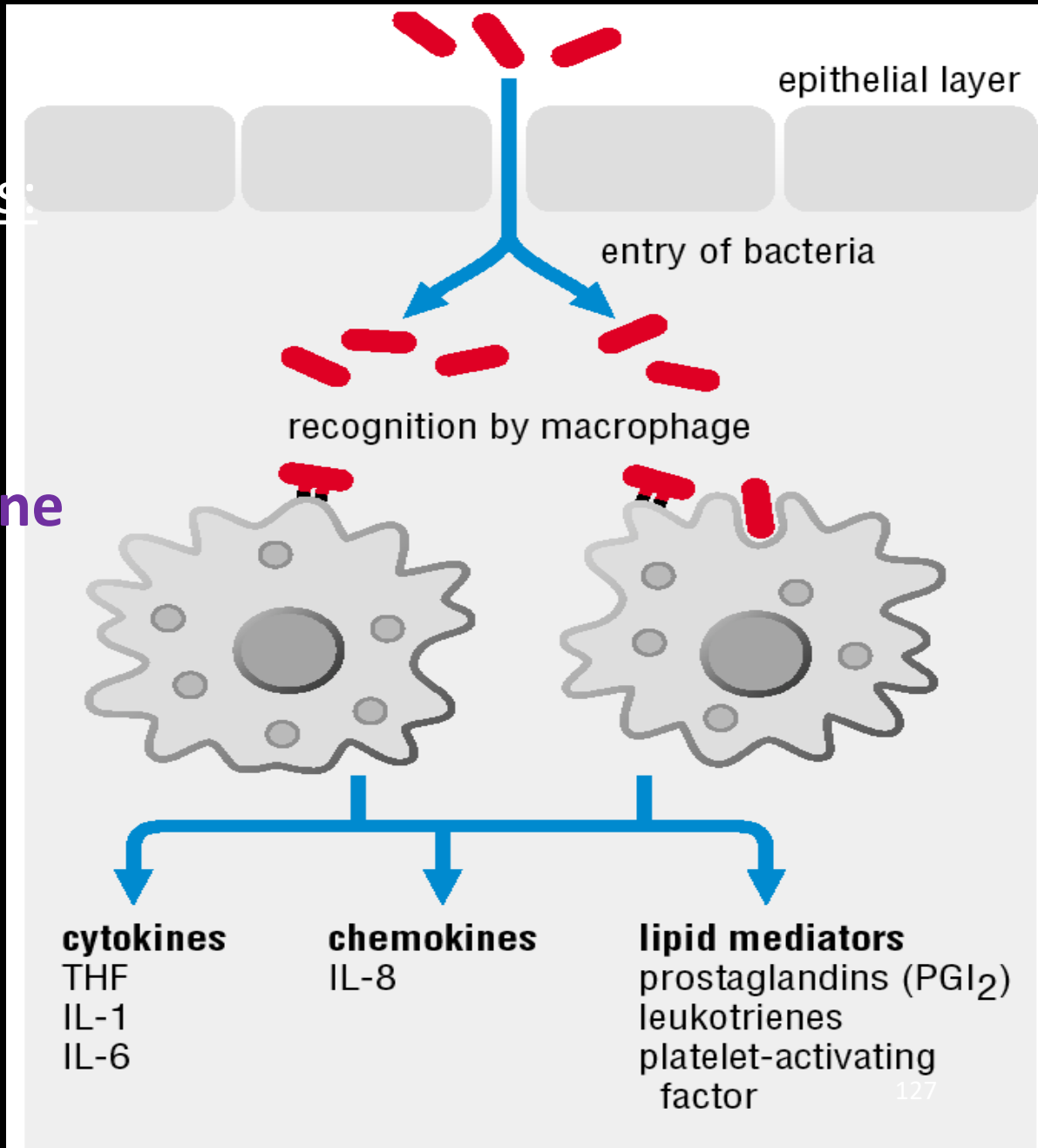

Tissue damage
Bacteria
1
Tissue damage causes release of vasoactive and chemotactic factors that trigger a local increase in blood flow and capillary permeability
2
Permeable capillaries allow an influx of fluid (exudate) and cells
Exudate (complement, antibody, C-reactive protein)
3
Phagocytes migrate to site of inflammation (chemotaxis)
4
Phagocytes and antibacterial exudate destroy bacteria
Margination
Extravasation
Capillary

- **Infection** leads to production of inducers of inflammation.

Inflammatory mediators:

Complex and many, but include:

- **Lipids and Proteins** (**cytokines/chemokines**).

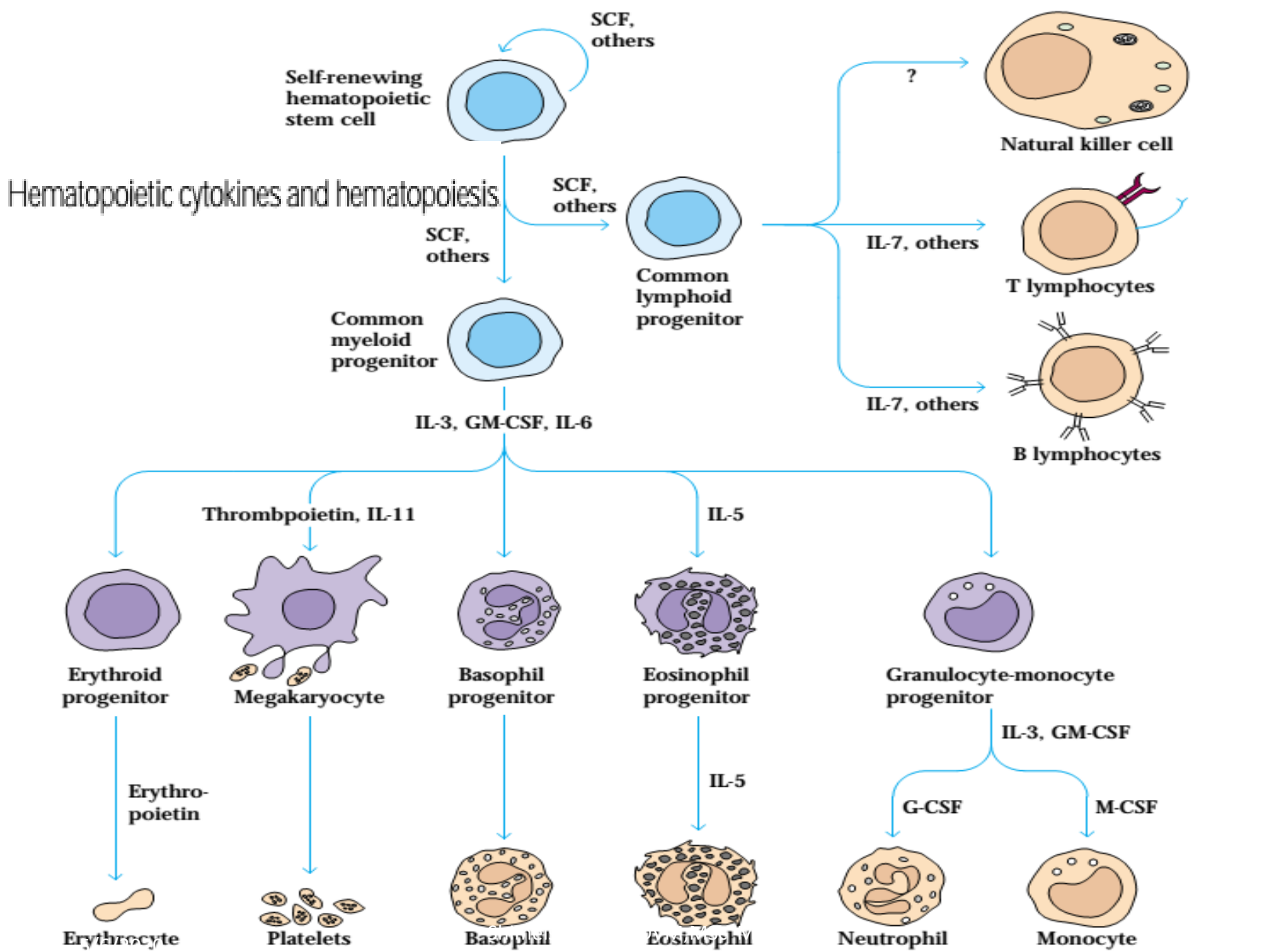


A. Cytokines

- "**Cytokines**" are **soluble protein mediators** secreted by immune cells.
  - (mostly) that **act on other cells to regulate their activity**;
  - many are called "**interleukins" (IL-1, IL-2, etc.)** (note: sometimes exist in **cell-bound forms**).

"Chemokines"

- **A subfamily of cytokines** primarily functions:-
  - " **in directing migration of cells"**, these are called "**chemotactic cytokines**" or "**chemokines**".

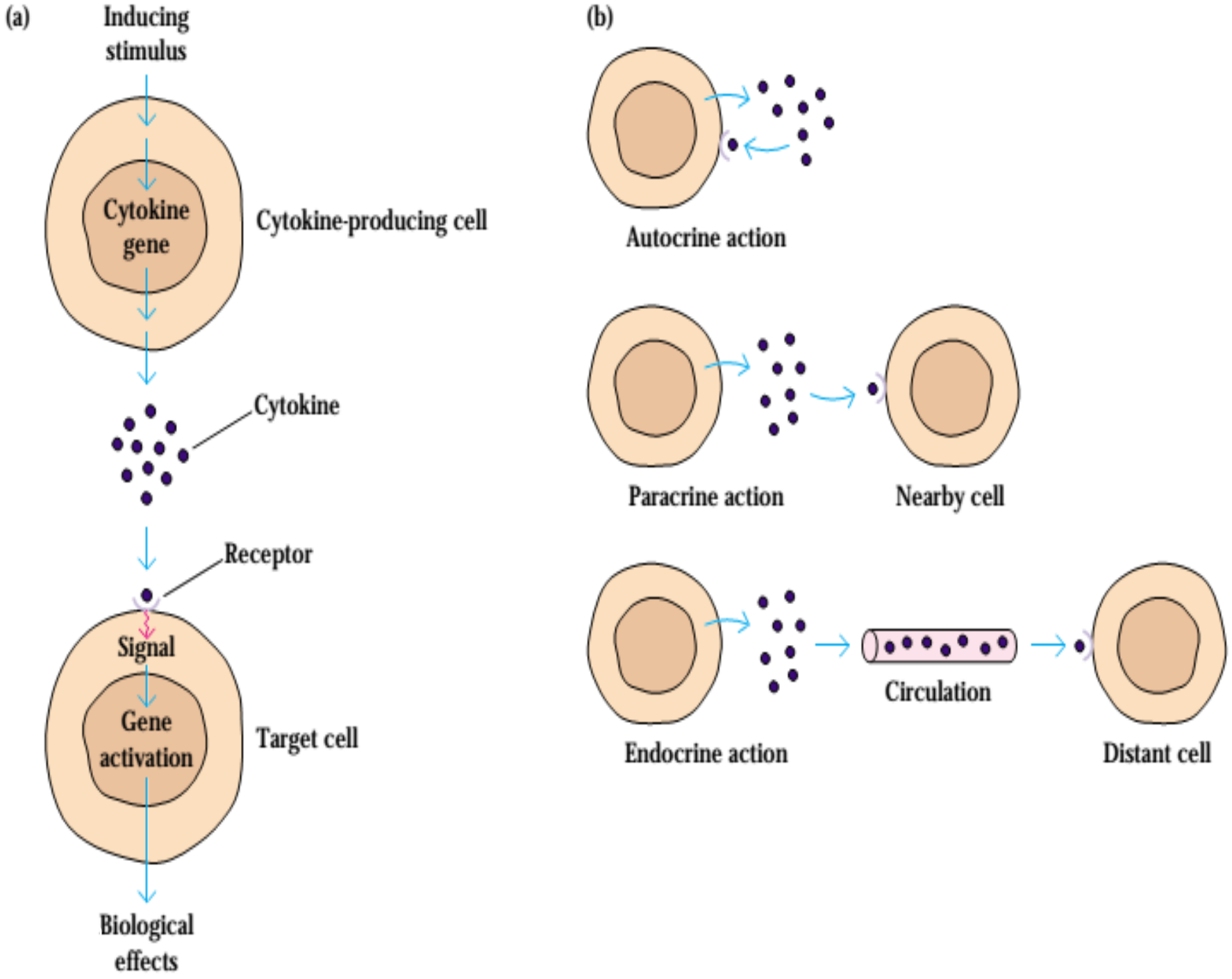

Hematopoietic cytokines and hematopoiesis
SCF, others
Self-renewing hematopoietic stem cell
SCF, others
SCF, others
Common lymphoid progenitor
?
Natural killer cell
IL-7, others
T lymphocytes
IL-7, others
B lymphocytes
Common myeloid progenitor
IL-3, GM-CSF, IL-6
Thrombpoietin, IL-11
IL-5
Erythroid progenitor
Megakaryocyte
Basophil progenitor
Eosinophil progenitor
Granulocyte-monocyte progenitor
IL-3, GM-CSF
Erythro-poietin
IL-5
G-CSF
M-CSF
Erythrocyte
Platelets
Basophil
Eosinophil
Neutrophil
Monocyte

Properties of Cytokines

- **Cytokines** are **low-molecular** weight **regulatory proteins** or **glycoproteins** secreted by **white blood cells** and **various other cells in the body** in response to a number of stimuli.

- Cytokines bind to **specific receptors** on the membrane of target cells, **triggering signal-transduction pathways** that ultimately alter **gene expression** in the target cell.

- **Cytokines** regulate the intensity and duration of the immune response.
- Most cytokines exhibit **autocrine** and/or **paracrine action**; fewer exhibit **endocrine action**.


(a)
Inducing stimulus
Cytokine gene
Cytokine-producing cell
Cytokine
Receptor
Signal
Gene activation
Target cell
Biological effects
(b)
Autocrine action
Paracrine action
Nearby cell
Endocrine action
Circulation
Distant cell

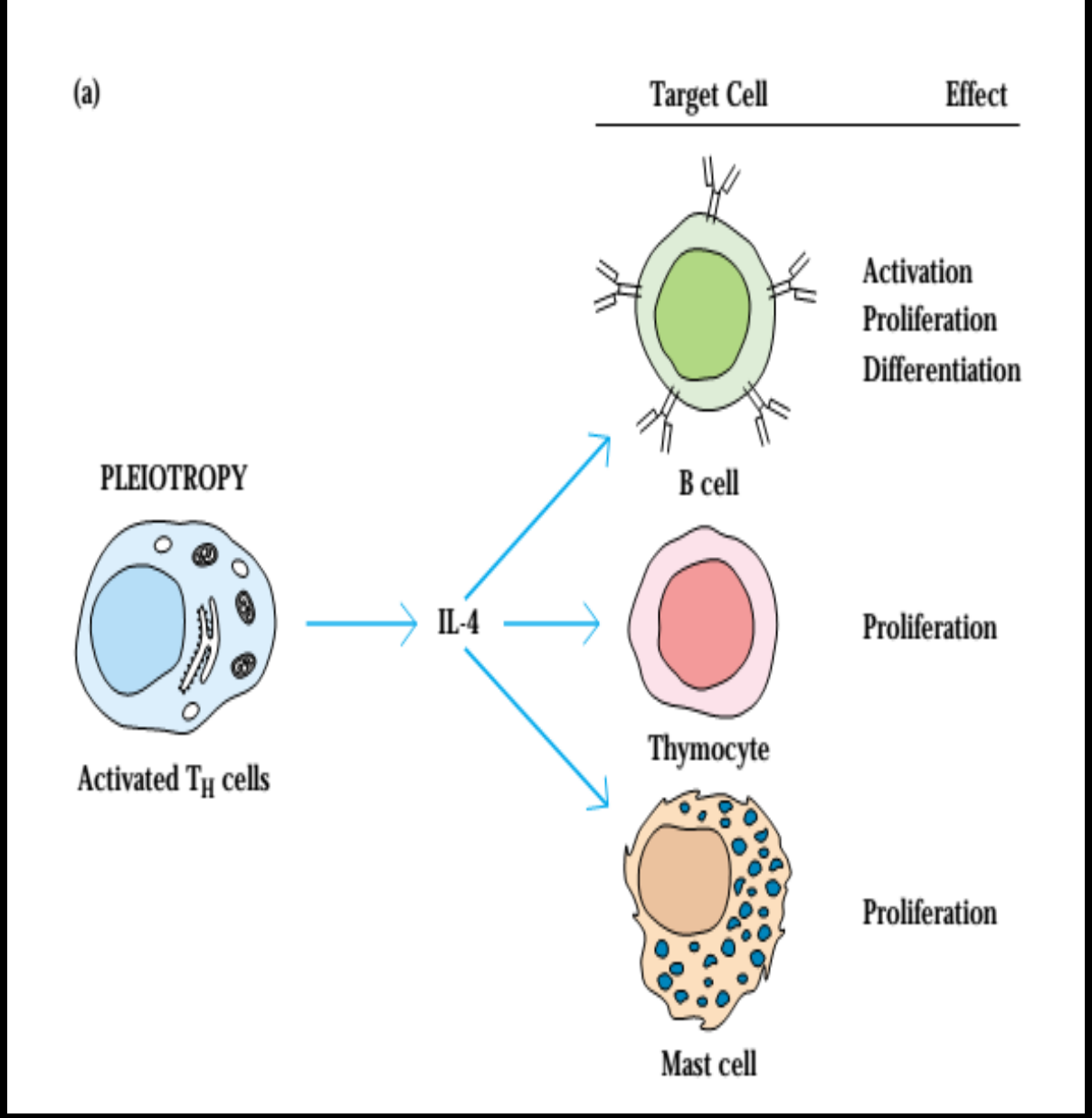
- **Many cytokines** are referred to as **inter-leukins** **"b/n leukocytes"**, secreted by **leukocytes** and act upon other leukocytes.
- **Interleukins 1–25** have been identified.
- Some cytokines are known by common names, including the **interferons** and **tumor necrosis factors.**
- **Chemokines**, a group of low-molecular weight **cytokines** that affect **chemotaxis** and other aspects of leukocyte behavior.

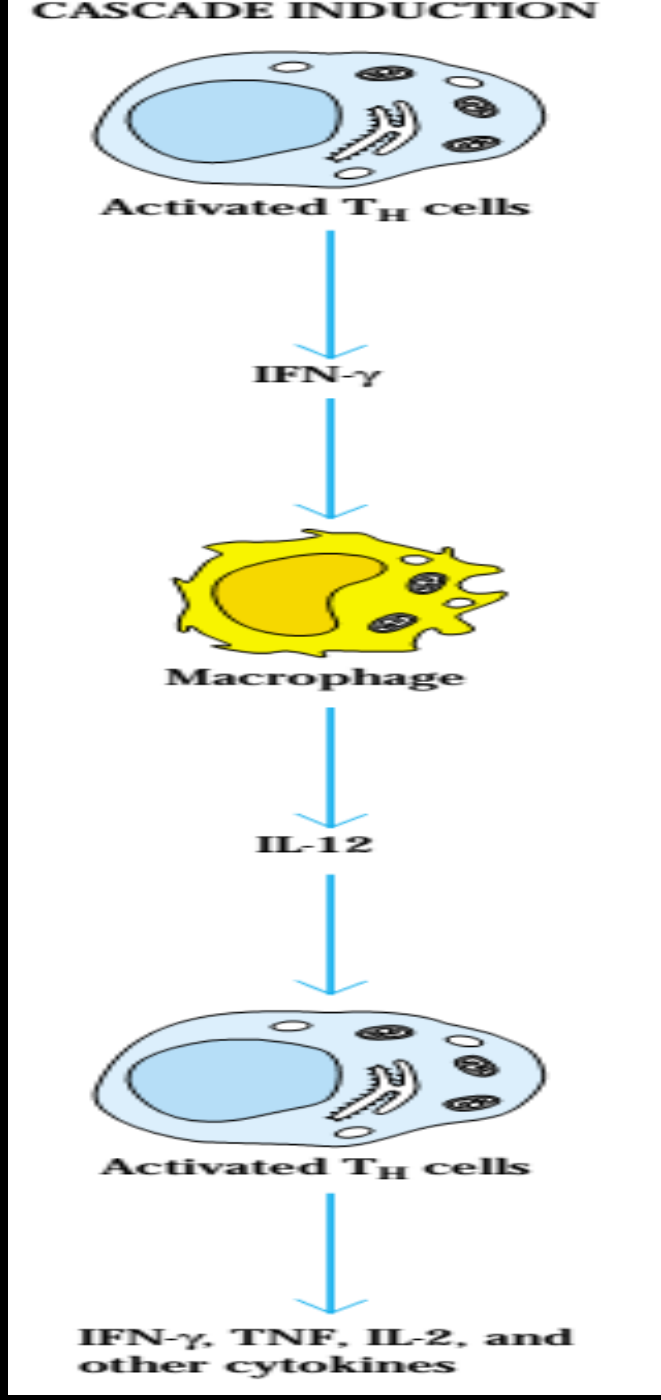
They derive from three gene family:

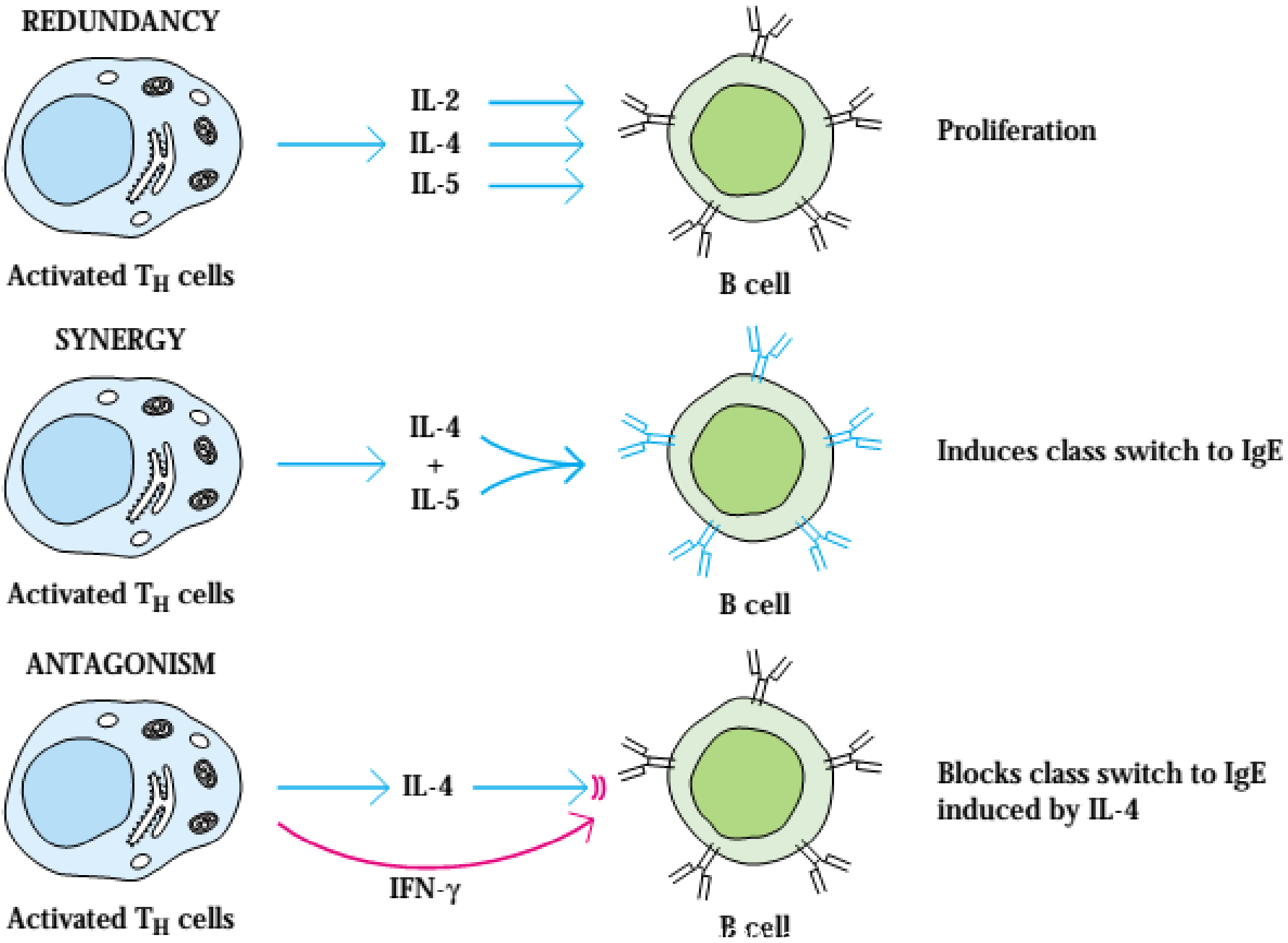
- **Hematopietin**
- **interferon**
- **tumor necrosis factor**

Function

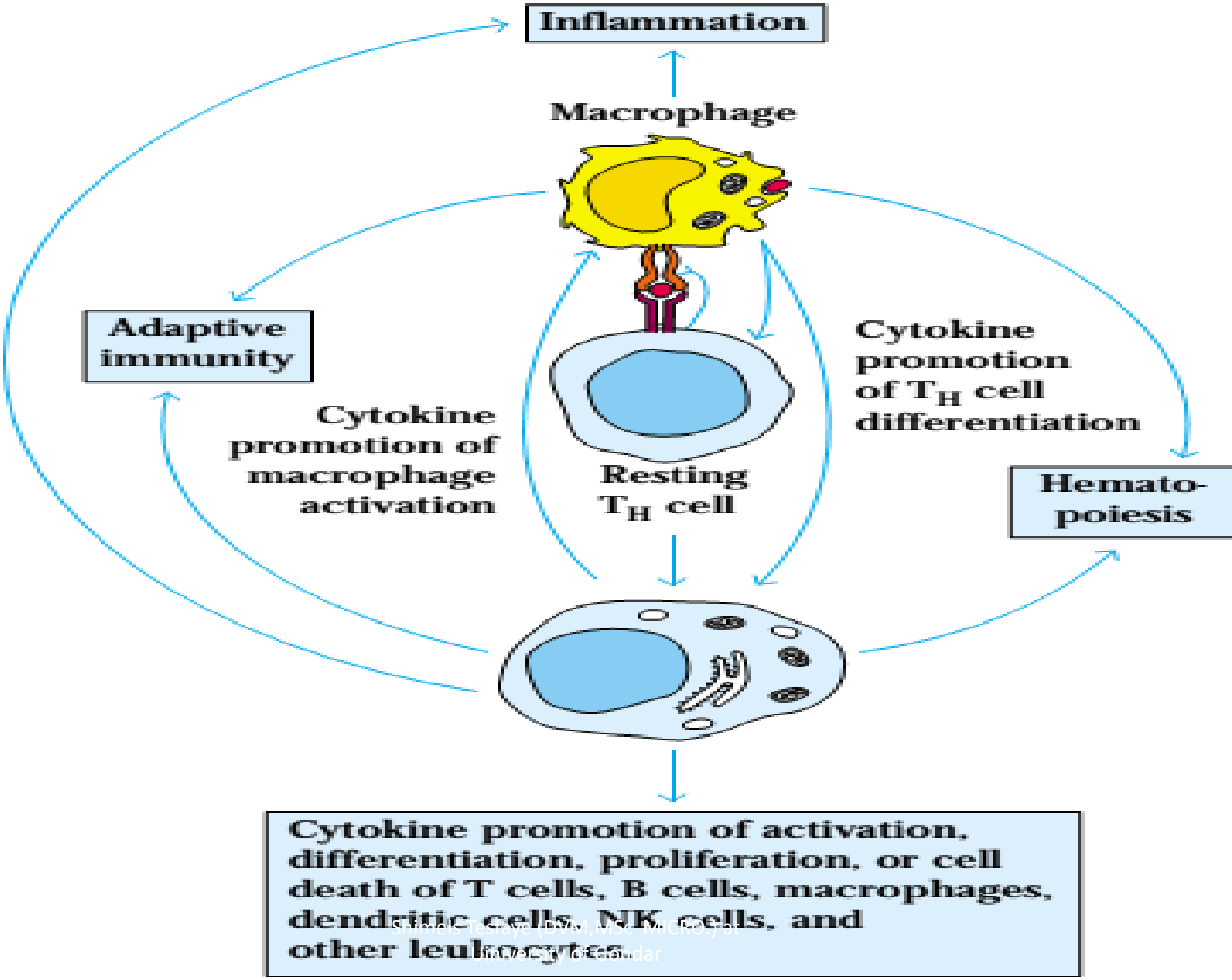
- **Pleiotrophy**:- when one cytokine function multiple jobs.
- **Redundancy** : when multiple cytokines function the same work .
- **Synergy:** when two or more cytokines work together to increase their effectiveness.
- **Antagonistic function** :-when two or more cytokines work appositively when one opposes the function of the other.


(a)
Target Cell
Effect
PLEIOTROPY
Activated T_H cells
IL-4
B cell
Activation
Proliferation
Differentiation
Thymocyte
Proliferation
Mast cell
Proliferation


CASCADE INDUCTION
Activated T_H cells
IFN-γ
Macrophage
IL-12
Activated T_H cells
IFN-γ, TNF, IL-2, and other cytokines


REDUNDANCY
IL-2
IL-4
IL-5
Activated T_H cells
B cell
Proliferation
SYNERGY
IL-4
+
IL-5
Activated T_H cells
B cell
Induces class switch to IgE
ANTAGONISM
IL-4
IFN-γ
Activated T_H cells
B cell
Blocks class switch to IgE
induced by IL-4

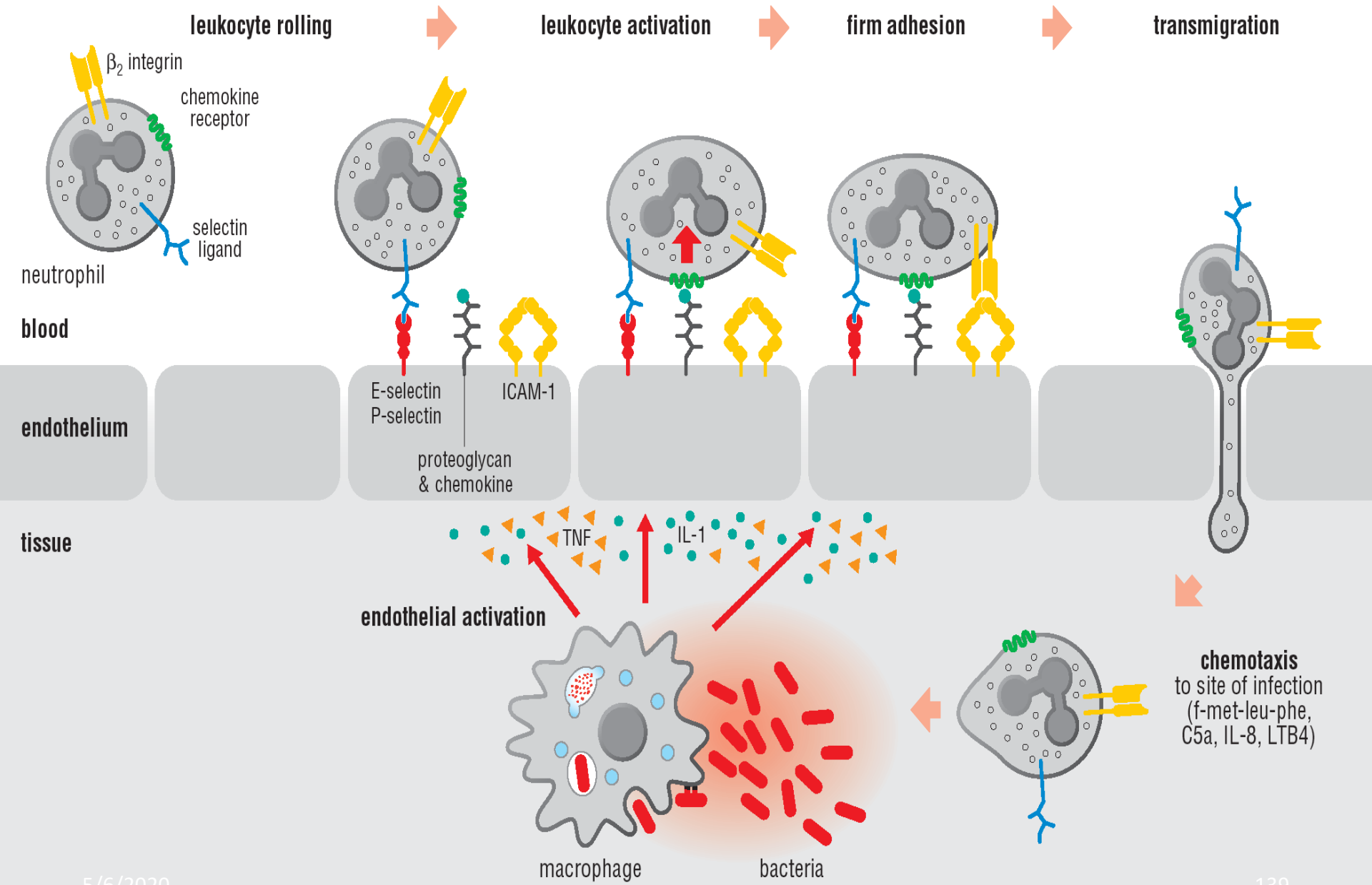
- Cytokines are involved in activities including
  - **innate immunity,**
  - **adaptive immunity,**
  - **inflammation, and**
  - **hematopoiesis**



Cytokines and Inflammation

- **Macrophages or DCs** stimulated via **innate immune receptors** make **pro-inflammatory cytokines**, especially TNF (Tumor necrosis factor), **IL-1**, and IL-6.
- **TNF and IL-1 =** signal to **endothelial cells** to make them:
  - Leaky to fluid (influx of plasma; **containing antibodies**, **complement components**, etc.)
  - Sticky for leukocytes, leading to influx of first neutrophils, later monocytes, lymphocytes
- **IL-6=** promotes **adaptive immune responses** and has systemic effects ("**acute phase response**" o**f liver**,
  - **including C-reactive protein or CRP; levels used clinically as an indication of systemic inflammation**).

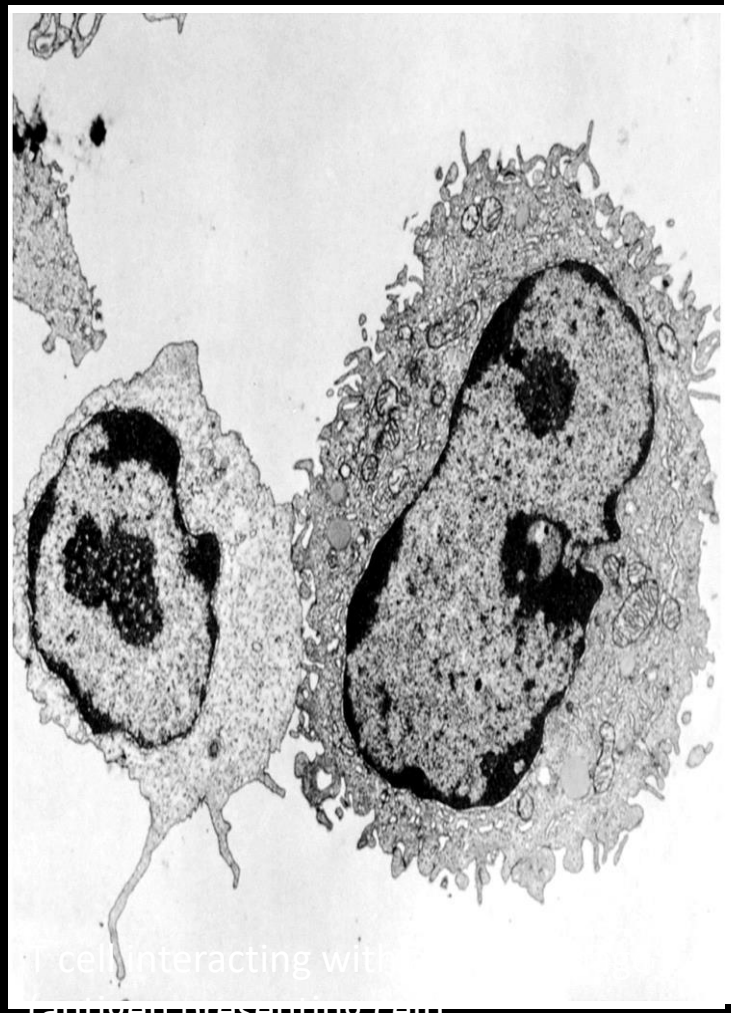
Leukocyte recruitment to sites of inflammation



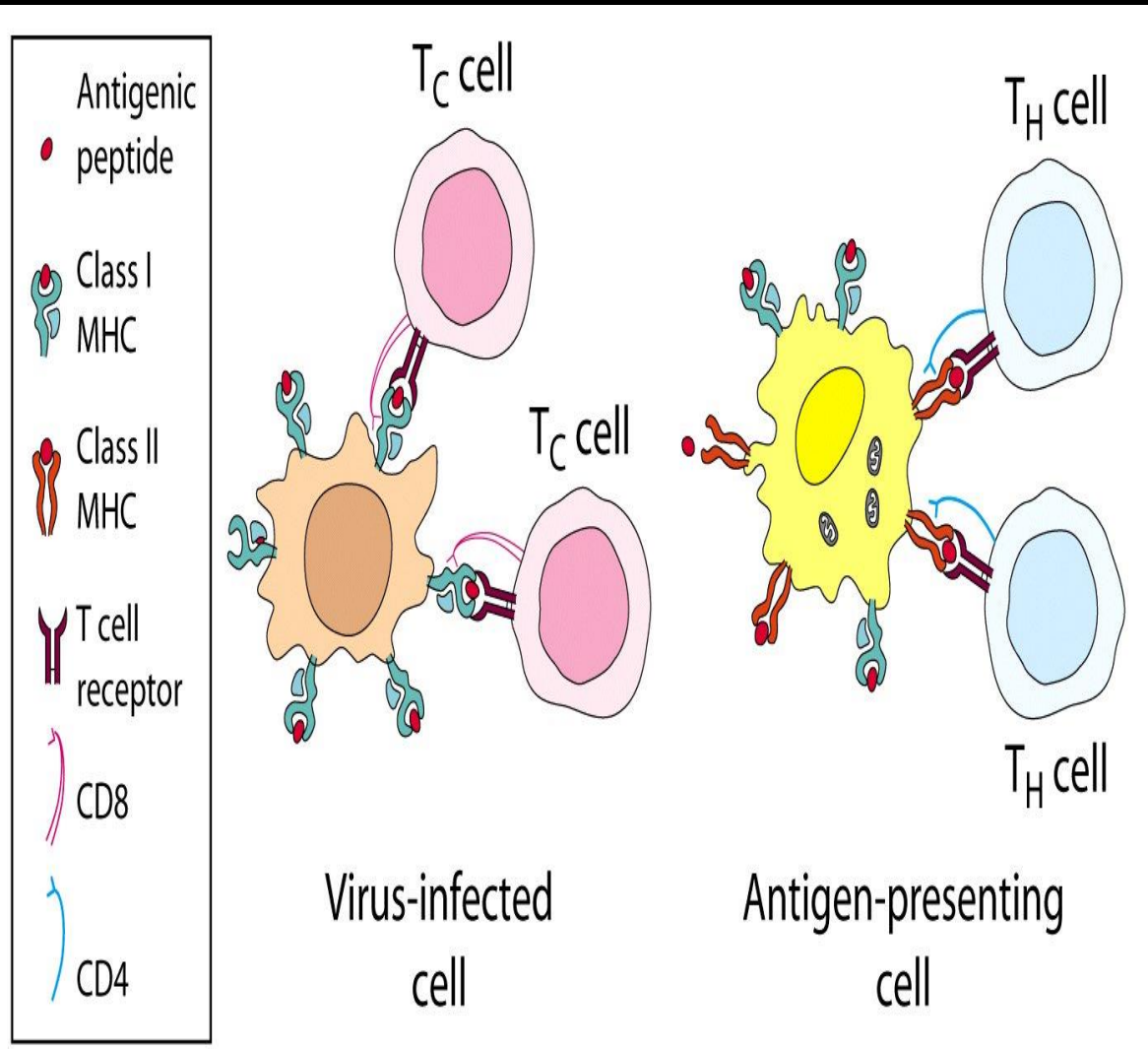
5.3 **Connections between adaptive and innate immunity**

- When innate immune signaling is insufficient to clear a pathogen, **the adaptive immune system kicks** in.
- Innate immune **signaling turns on the adaptive** immune.
- **TLR-signaling activates** innate immune cells (**macrophage and dendritic cells**) →→ Innate immune cells that have been activated by TLR-signaling are much **more effective antigen presenting cells** than **resting immune cells**.

Macrophage and dendritic cells serve as antigen-presenting cells for adaptive immune cells (T cells).



T cell interacting with
(antigen presenting cell)

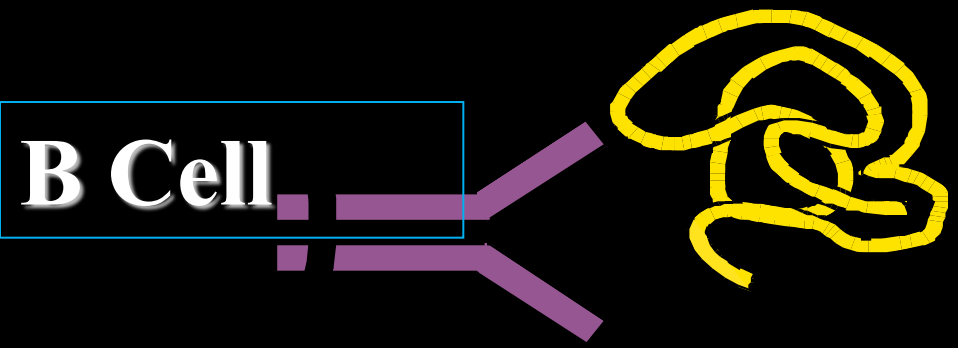


Chapter 6

Antigen processing and T-Cell mediated immunological response

Chapter 6

6.1. Antigen Recognition by B and T lymphocytes



What can you say about these pictures?

Introduction

- **Class I and class II MHC molecules** associate with **peptides** that have been processed in different **intracellular compartments**.

- **Class I MHC molecules** bind peptides derived from **endogenous antigens** that have been processed within the **cytoplasm of the cell** (e.g., normal cellular proteins, tumor proteins, or viral and bacterial proteins produced within infected cells).

- **Class II MHC molecules bind peptides** derived from **exogenous antigens** that are internalized by **phagocytosis or endocytosis** and processed within the endocytic pathway.

Intro……..

❖ Both **$CD4^+$ and $CD8^+$ T cells** can recognize **antigen only** when it is presented by a **self-MHC molecule**, an attribute called ***self-MHC restriction.***

- Nearly all nucleated cells express class I MHC molecules, virtually any nucleated cell is able to function as a target cell **presenting endogenous antigens to TC cells**.

- Most often, **target cells** are **cells that have been infected** by a **virus or some other intracellular microorganism**.

- However, a**lteredself-cells** such as **cancer cells**, **aging body cells**, or **allogeneic cells** from a graft can also serve as targets.

- Since all **cells expressing either class I or class II MHC molecules** can present **peptides to T cells,**
  - strictly speaking they all could be designated as **antigen-presenting cells.**

- however, by convention, cells that display peptides associated with **class I MHC molecules** to **CD8 $^{+}$T** cells are referred to as **target cells.**

- cells that display peptides associated with **class II MHC molecules** to **CD4^{+} TH cells** are called **antigen-presenting cells (APCs).**

Antigen presenting cells function

- **Dendritic cells** are the most effective of the antigen presenting cells.

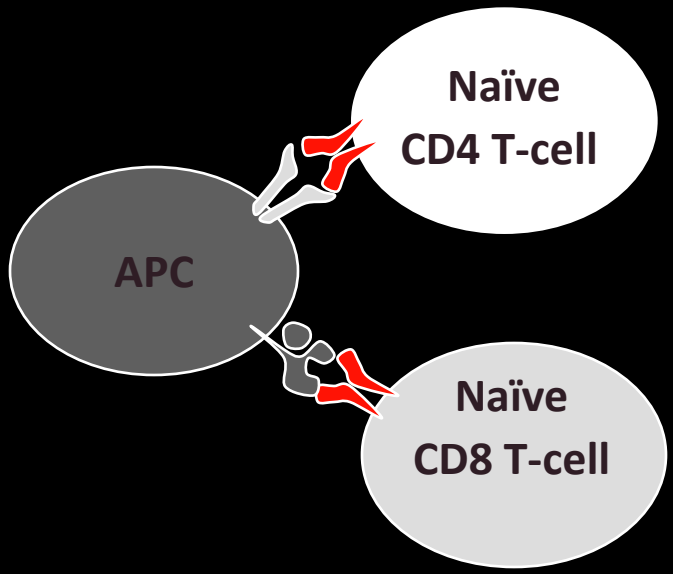
- Because these cells **constitutively express a high level of class II MHC molecules** and **costimulatory activity**, they can activate naive TH cells.

MHC Expression

Cells	MHC Class I	MHC Class II
T cells	+++	-
B cells	+++	+++
Macrophages	+++	+++
Dendritic cells	+++	+++
Thymic Epithelia	+	+++
Neutrophils	+++	-
Hepatocytes	+	-
Kidney	+	-
Muscle	+/-	-
Red blood cells	-	-

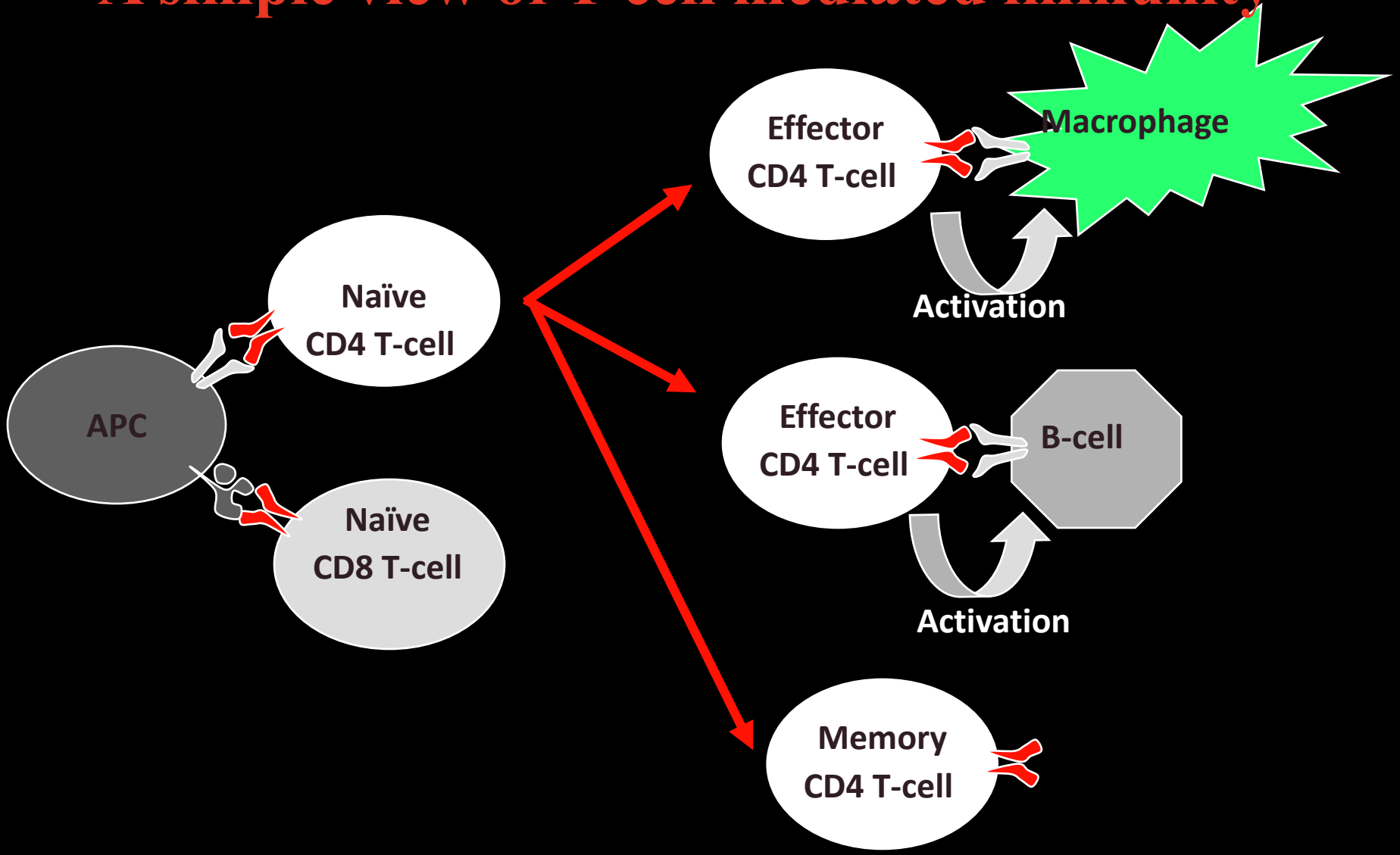
APCs

A simple view of T-cell mediated immunity



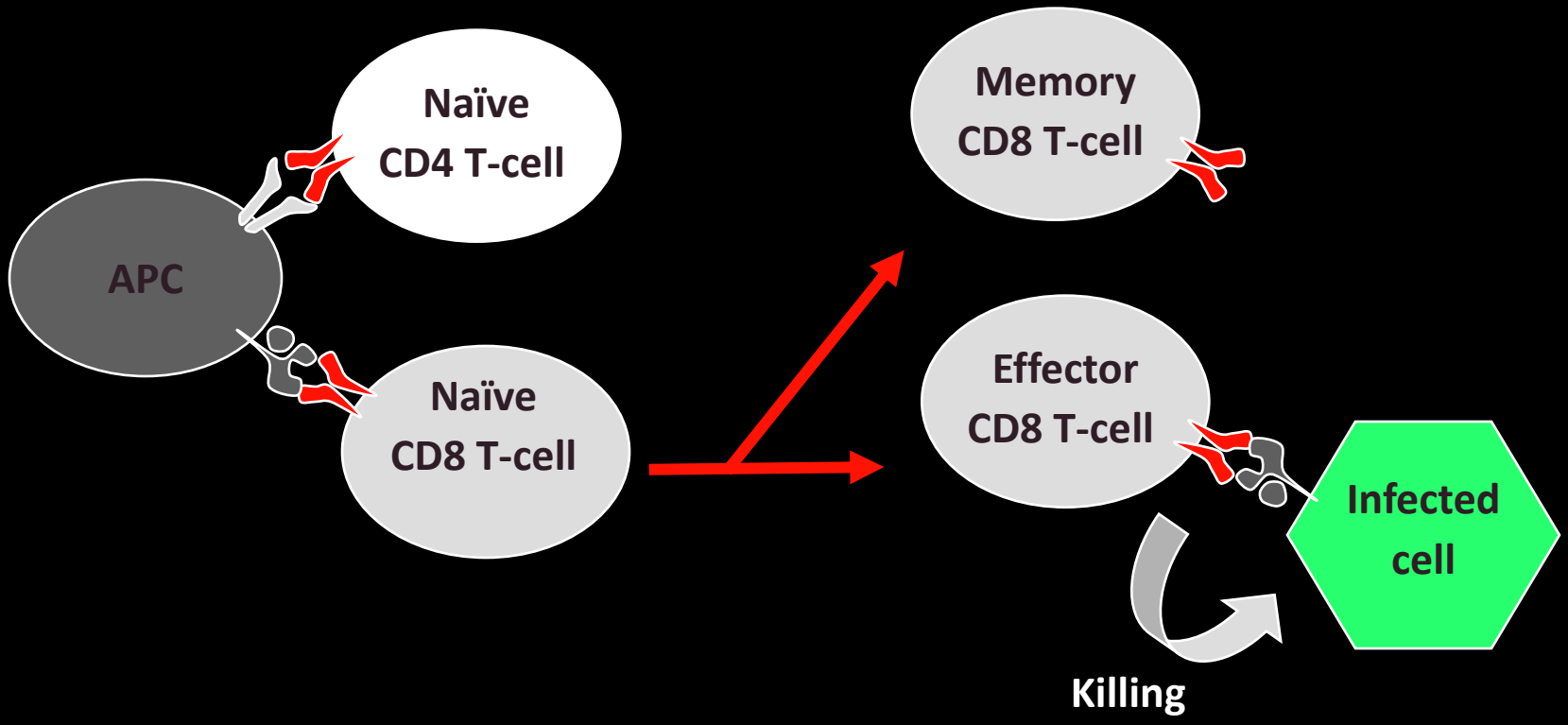
KAfter initial activation, T cells differentiate into effector cells and can be activated in lymphoid organs and periphery.

A simple view of T-cell mediated immunity



- APCs still activate effector Th cells.

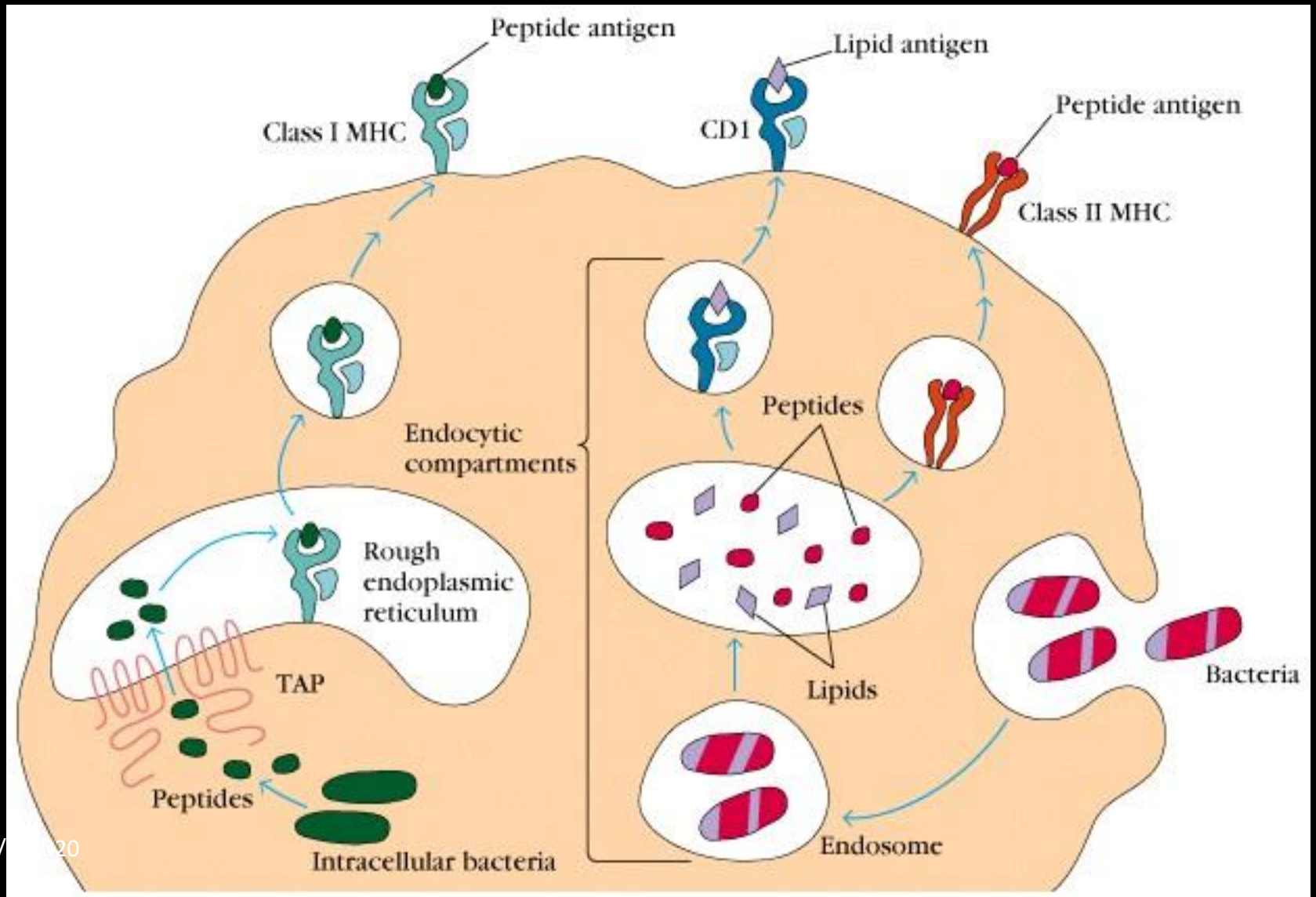
A simple view of T-cell mediated immunity



- Target cells activate effector $CD8^+$ Tc (CTLs).

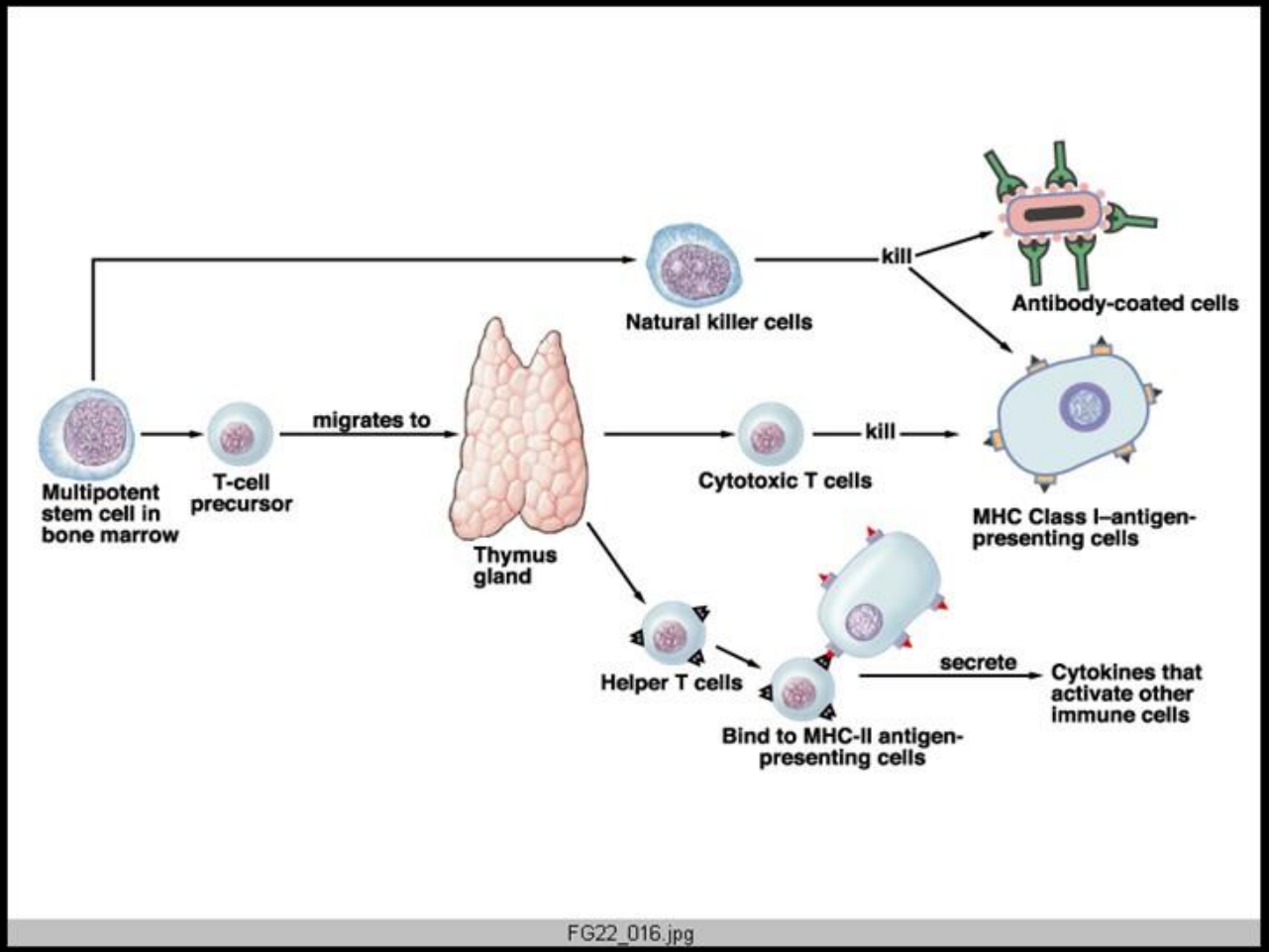
(Target cells can be any Class I MHC expressing cell.)

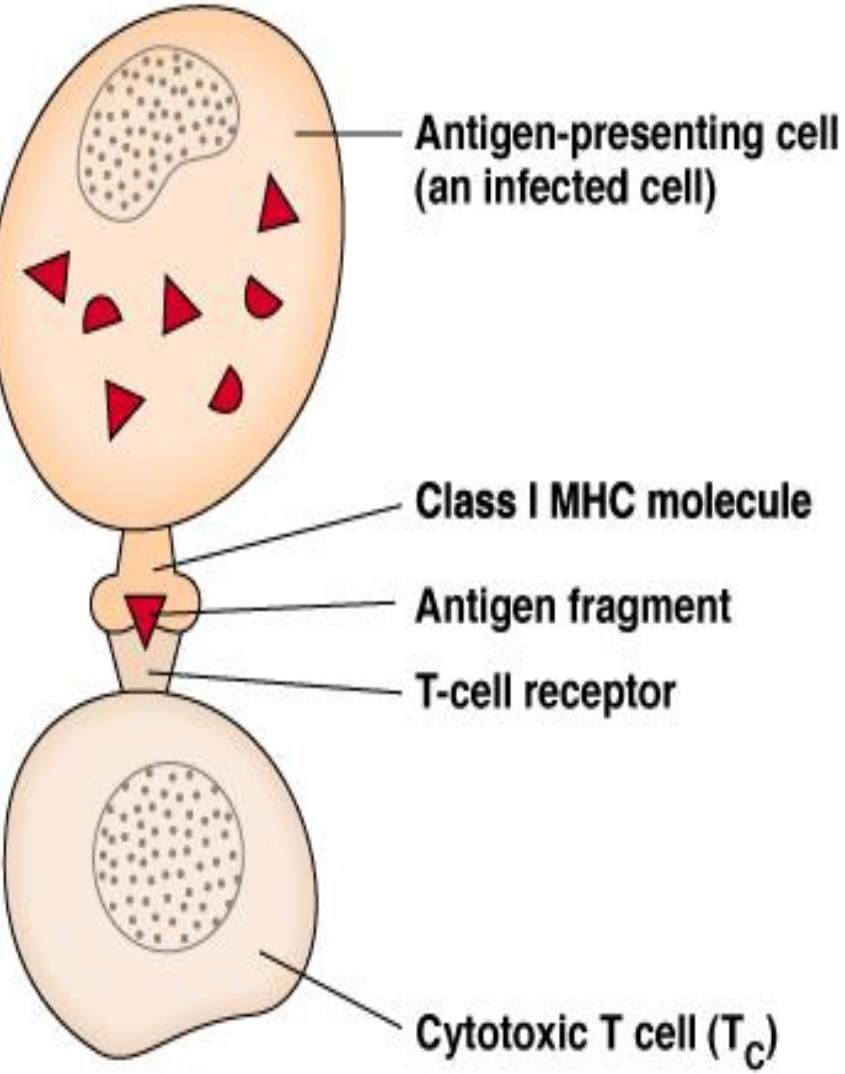
Processing of Class I and Class II vs CD1 Antigens



6.2 .T Cell Activation

- After having the Matured T-cells (CD8+ & CD4+ naïve T-cells),
- the next is Activationof those T-cells into Effector T-cells.
- To form more effectors T-cells .
- The first step is gene activation.


kill
Natural killer cells
Antibody-coated cells
migrates to
kill
Multipotent stem cell in bone marrow
T-cell precursor
Thymus gland
Cytotoxic T cells
MHC Class I–antigen-presenting cells
Helper T cells
secrete
Cytokines that activate other immune cells
Bind to MHC-II antigen-presenting cells
FG22_016.jpg

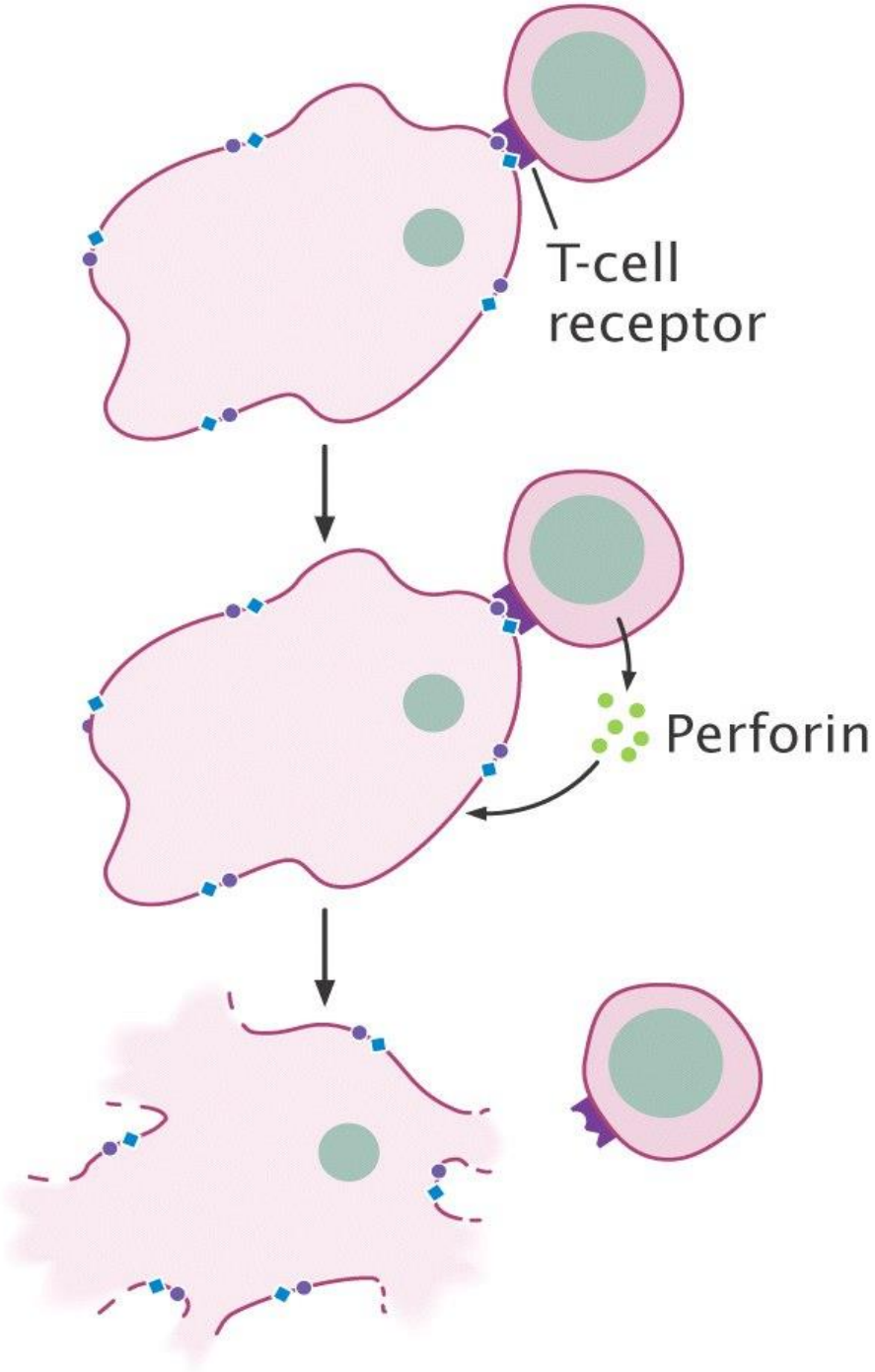


CD8+ cell

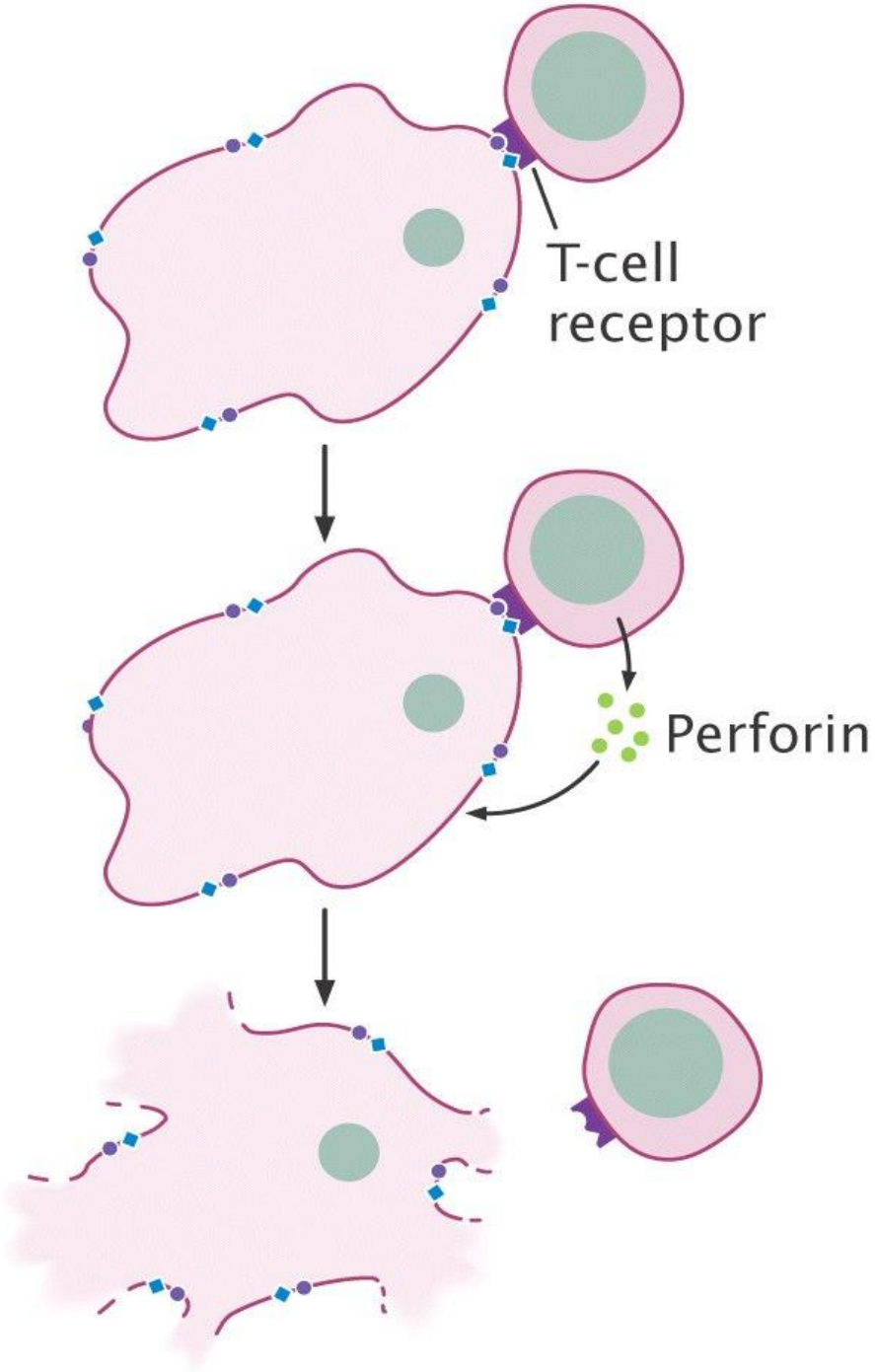
(a)

Cytotoxic T Cell and MHC I

Antigen presentation



Cytotoxic T cell= CD8+



Cytotoxic T cell= CD8+

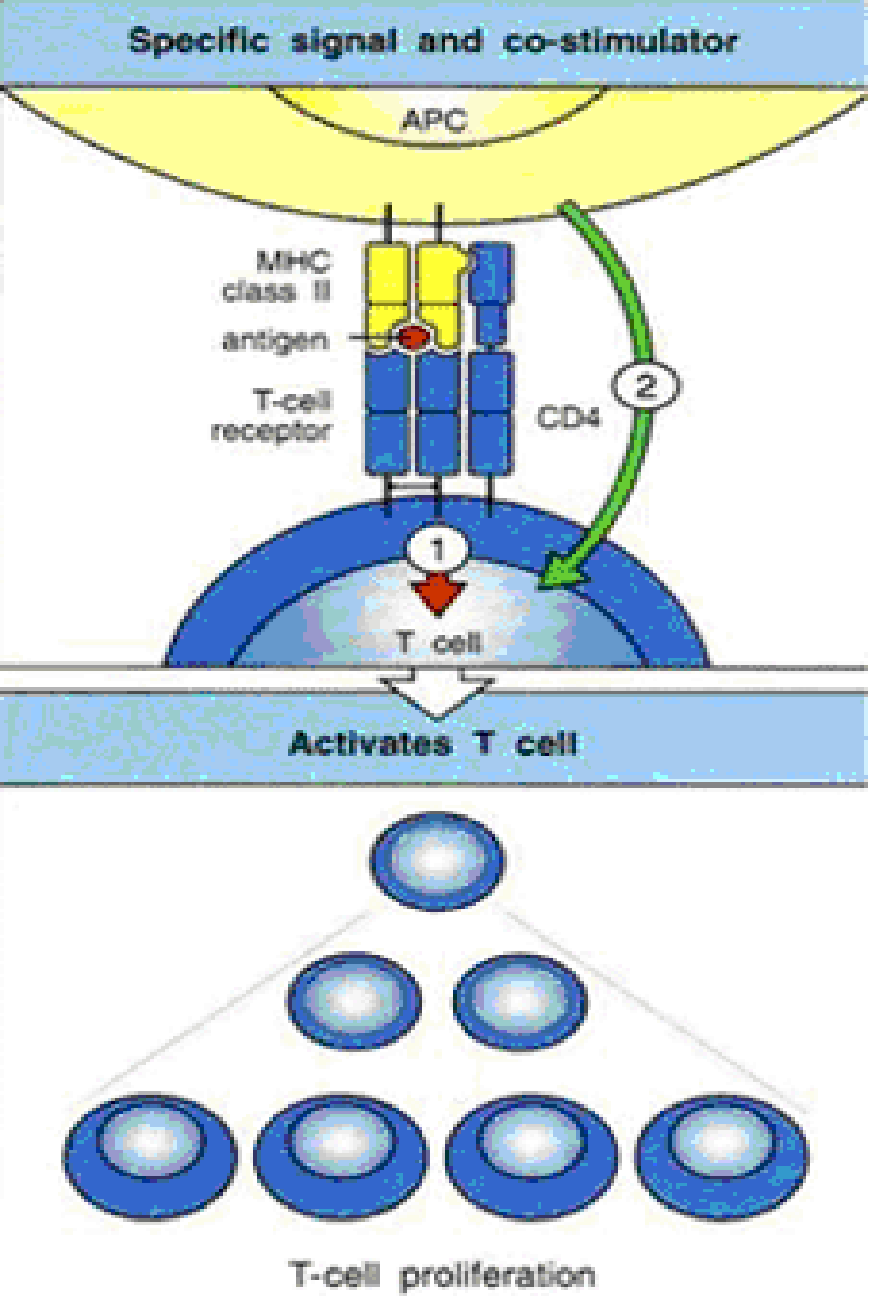
- The central event in the **generation of both humoral and cellmediated** immune responses is :
  - **the activation and Clonal expansion of TH cells**.

- Activation **of TC cells,** is generally similar to TH-cell activation.

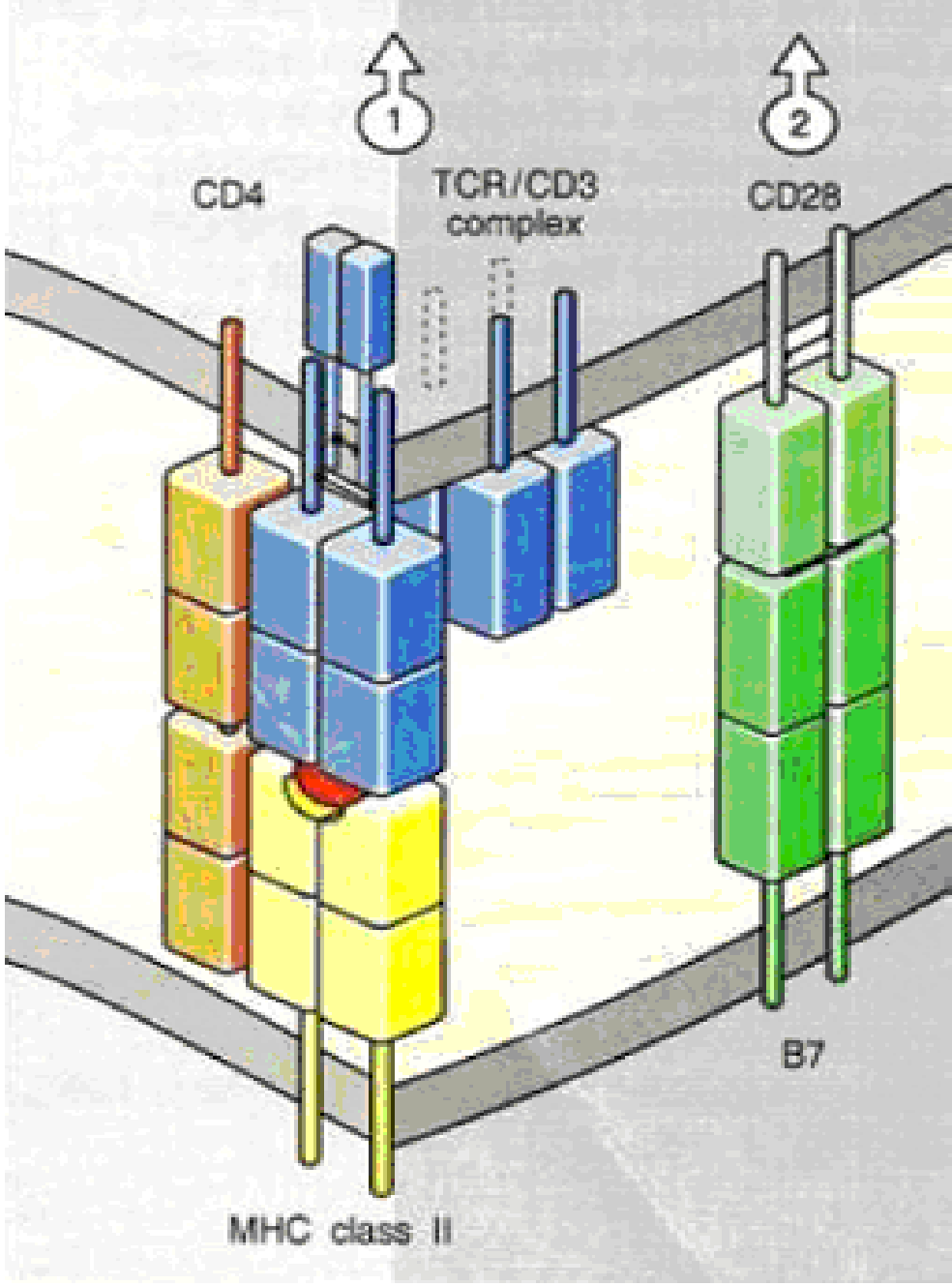
TH cell activation is initiated by :

- **interaction of the TCR-CD3 complex +antigenic peptide bound to a class II MHC molecule** on the surface of an antigen-presenting cell.
- an additional **requirement for activation** is a **co-stimulatory signal,** as provided by the binding of the T cell CD28 receptor to the APC B7 molecule.

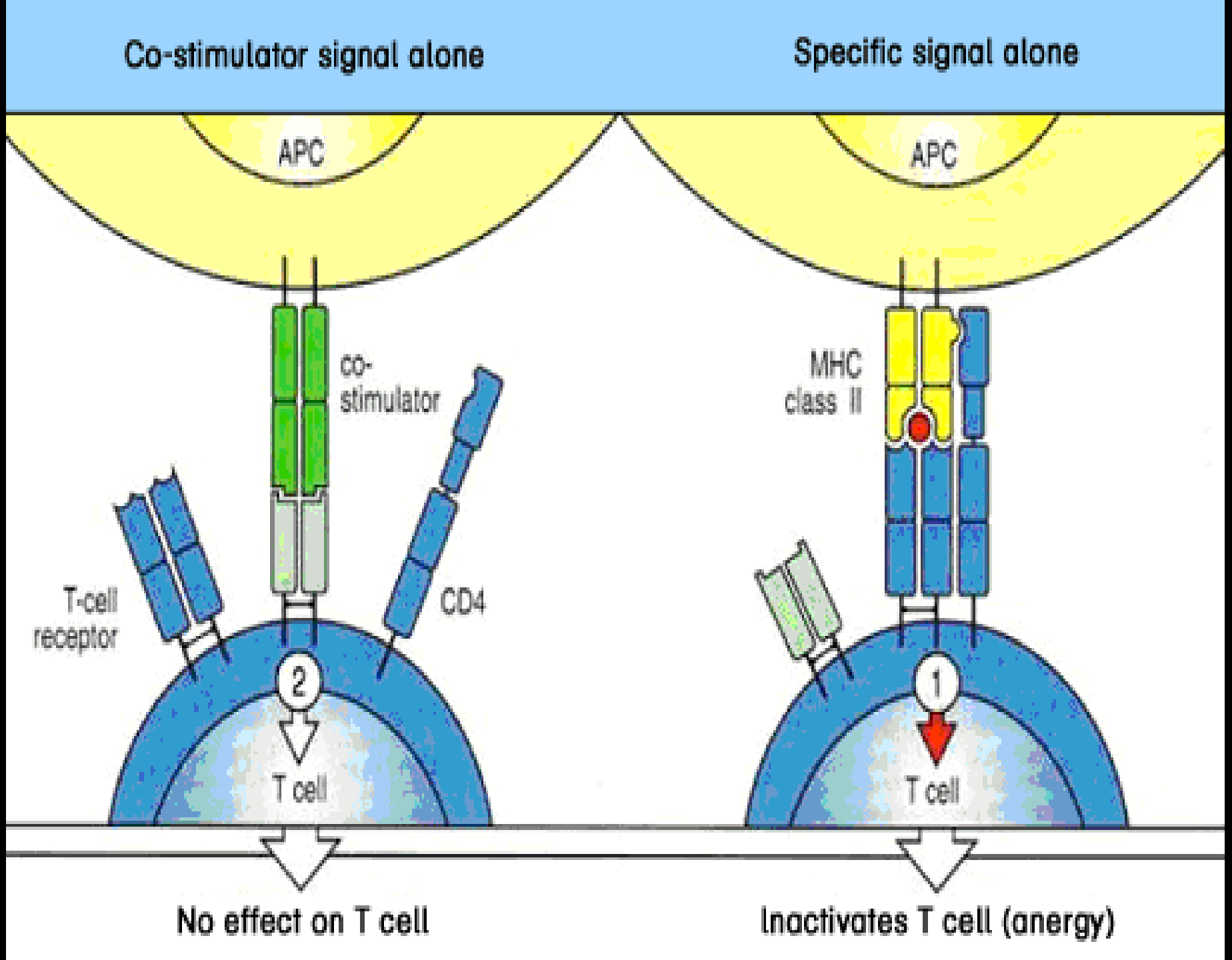
- **The interaction summarized as:-**
  - **TCR-CD3/MHC peptide complex interact**(main combination).
  - Involvement **of co-receptor**
    - CD4 to MHC II
    - CD8 to MHC I
  - **Co-stimulatory signal**
    - CD 28 to B7 (**T_H Cells/APCs**)
  - Inhibitory role of CTLA-4.


Specific signal and co-stimulator
APC
MHC class II
antigen
T-cell receptor
CD4
2
1
T cell
Activates T cell
T-cell proliferation

(a)


1
2
CD4
TCR/CD3 complex
CD28
B7
MHC class II

(b)

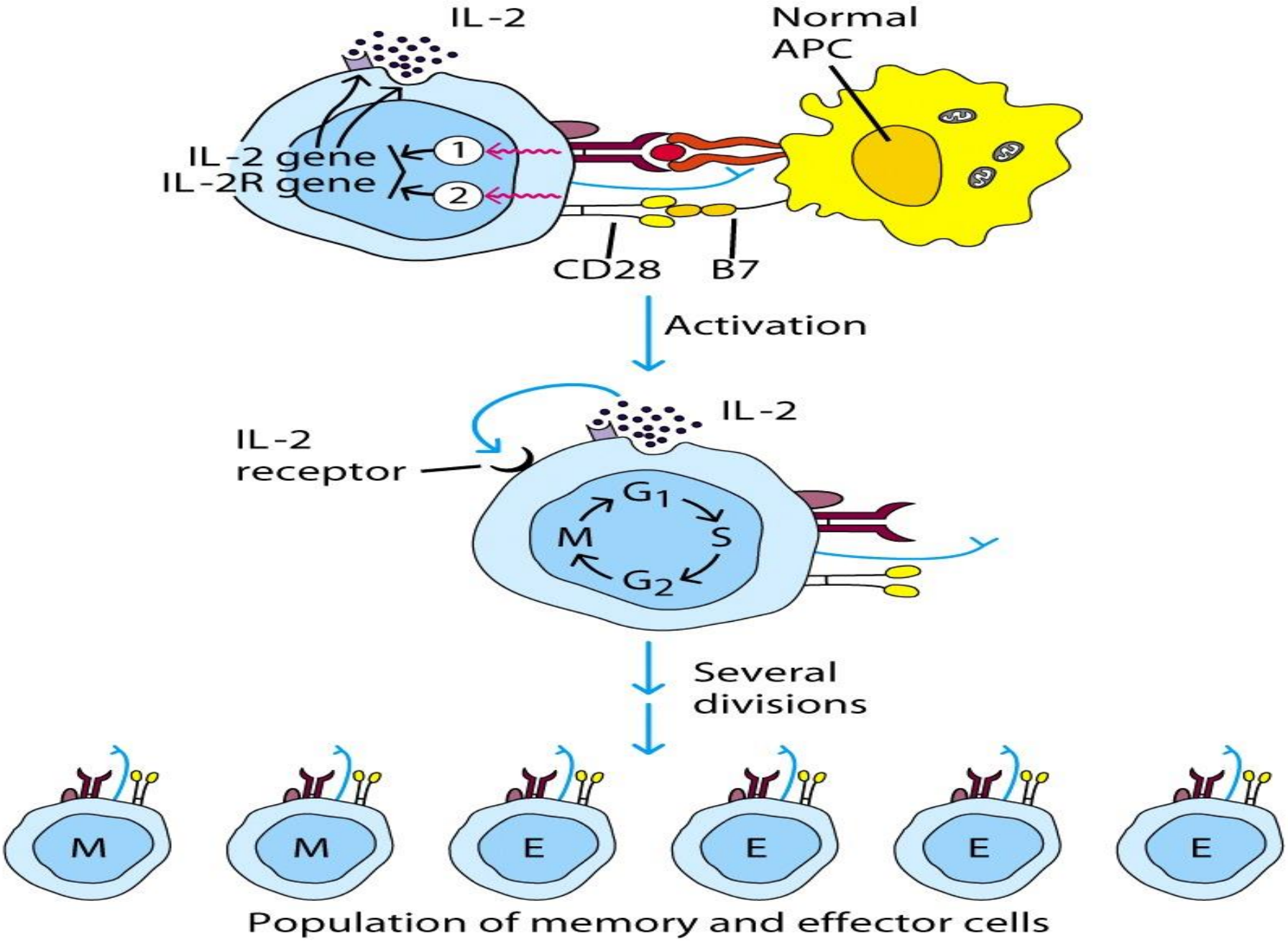

Co-stimulator signal alone
Specific signal alone
APC
APC
co-
stimulator
MHC
class II
T-cell
receptor
CD4
2
1
T cell
T cell
No effect on T cell
Inactivates T cell (anergy)

- **Post-activation Signaling** changes results of Signaling Pathways like:-

  –Gene expression changes

  –Functional change

  –Differentiation


IL-2
Normal
APC
IL-2 gene
IL-2R gene
1
2
CD28
B7
Activation
IL-2
IL-2
receptor
G1
S
G2
M
Several
divisions
M
M
E
E
E
E
Population of memory and effector cells

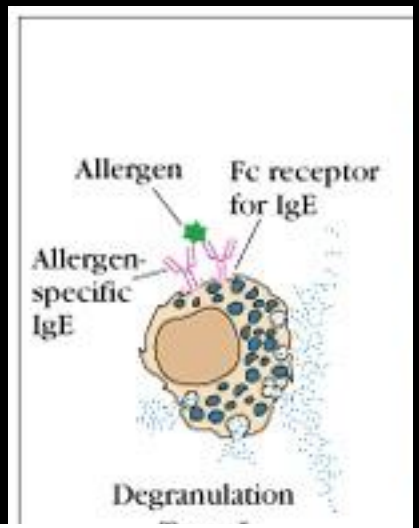
- Functions of effectors
  - B cell helper
  - Cytotoxicity

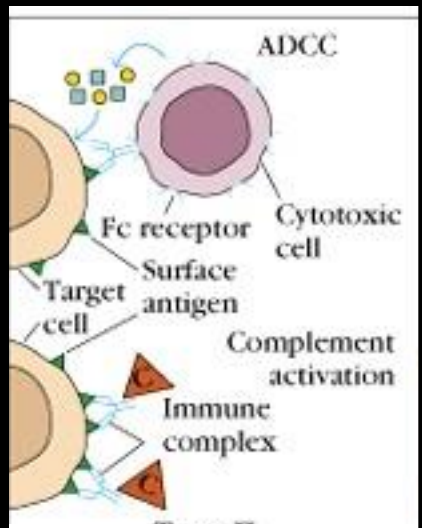
Characteristics of memory cells

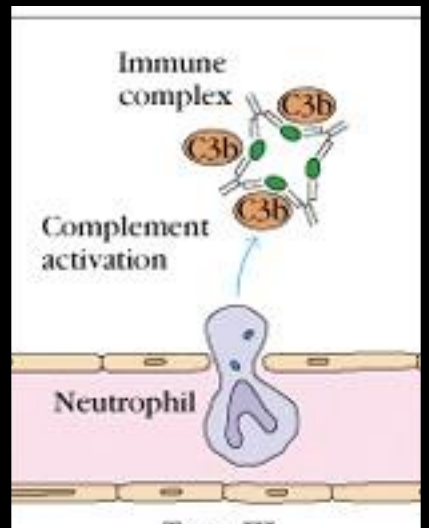
  - Last months to years vs. effector cells that last days to weeks. Memory cells more easily activated by all APCs then naïve T cells

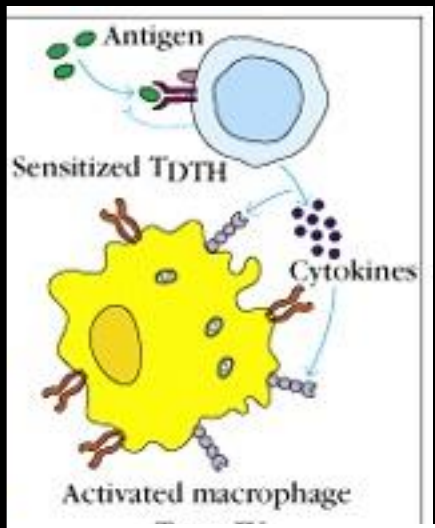
Chapter 7
Hypersensitivity

Gel and Coombs classification of hypersensitivities.









Type I	Type II	Type III	Type IV
IgE Mediated	IgG/IgM Mediated	IgG Mediated	T cell
Classic Allergy	rbc lysis	Immune complex Disease	Delayed Type Hypersensitivity

TYPE I Hypersensitivity Classic allergy

- Mediated by IgE attached to Mast cells.

- The symptoms resulting from allergic responses are known as **anaphylaxis**.
  - Includes: Hay fever(cold allergy), asthma, eczema(skin allergy), bee stings, food allergies.

Allergens

- Allergens are nonparasite antigens that can stimulate a type I hypersensitivity response.

- Allergens bind to IgE and trigger degranulation of chemical mediators.

Allergens

TABLE 16-1 COMMON ALLERGENS ASSOCIATED WITH TYPE I HYPERSENSITIVITY

Proteins
- Foreign serum
- Vaccines

Plant pollens
- Rye grass
- Ragweed
- Timothy grass
- Birch trees

Drugs
- Penicillin
- Sulfonamides
- Local anesthetics
- Salicylates

Foods
- Nuts
- Seafood
- Eggs
- Peas, beans
- Milk

Insect products
- Bee venom
- Wasp venom
- Ant venom
- Cockroach calyx
- Dust mites

Mold spores

Animal hair and dander

Characteristics of allergens

- Small 15-40,000 molecular weight proteins.
- Specific protein components
  - Often enzymes.
- Low dose of allergen
- Mucosal exposure.
- Most allergens promote a Th2 immune (antibody dependentor humoral immunity).

Allergens

Dermatophagoides pteronyssinus
(common dust mite)

Example: Der P1

Der P1 is an enzyme allergen
from the fecal pellets
of the dust mite.

Der P1 Allergen

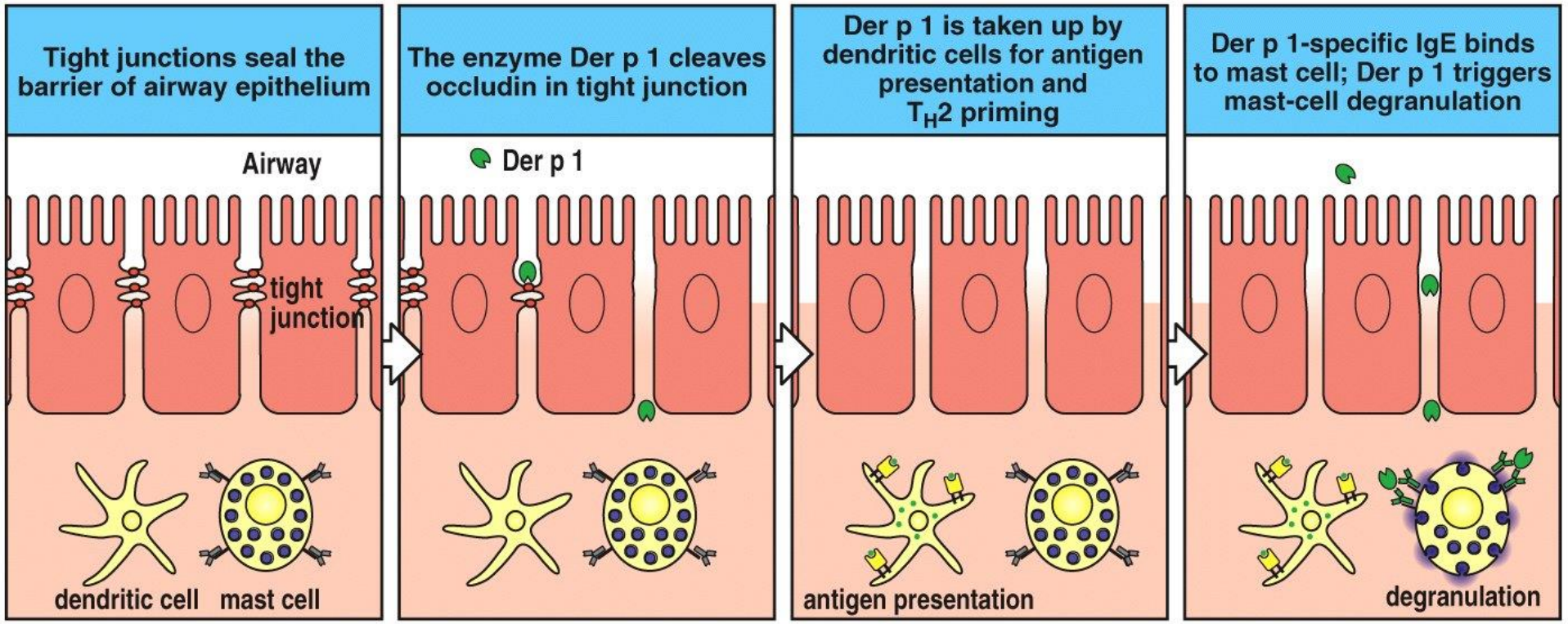


Figure 12-5 Immunobiology, 6/e. (© Garland Science 2005)

Allergen is easily aerosolized and inhaled.
Der P1 breaks down components of tight junctions which helps it to cross mucosa.

Atopy

- **Atopy** is the term for the genetic trait to have a predisposition for localized anaphylaxis.

- Atopic individuals have higher levels of IgE and eosinophils.

Genetic Predisposition

Type I hypersensitivity

- Candidate polymorphic genes include:
  - IL-4 Receptor.
  - IL-4 cytokine (promoter region).
  - FcεRI. High affinity IgE receptor.
  - Class II MHC
    (present peptides promoting Th2 response).
  - Inflammation genes.

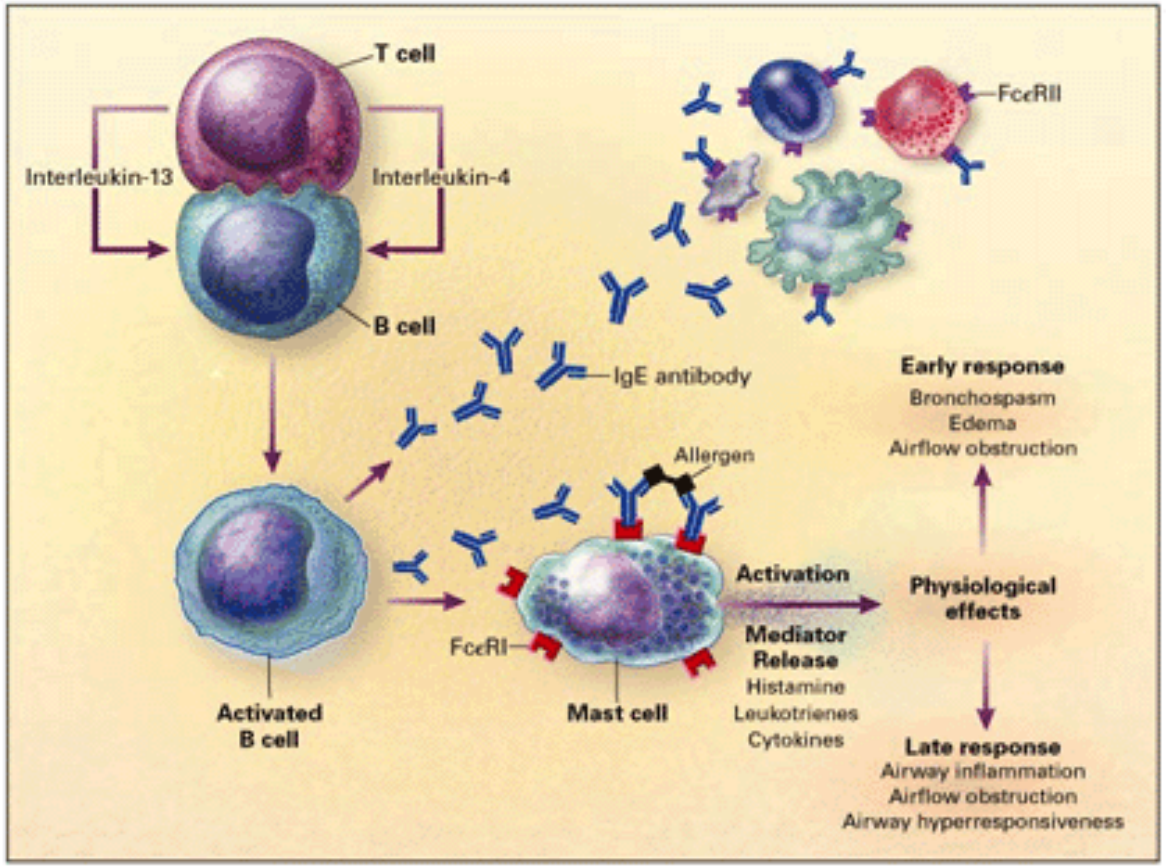
Mechanisms of allergic response

Sensitization

Repeated exposure to allergens initiates immune response that generates IgE isotype.

Th2 cells required to provide the IL-4 required to get isotype switching to IgE.

Mechanisms of allergic response
Sensitization
Th2/B cell interaction



IL-4

IL-4R

CD40

Drive B cell Activation and IgE isotype switch.

Busse and Lemanske NEJM Feb 2001. 344:350

Mechanisms of allergic response

Sensitization

- The IgE can attach to Mast cells by Fc receptor, which increases the life span of the IgE.
- Half-life of IgE in serum is days whereas attached to FcεR it is increased to months.

Mechanisms of allergic response

Fc ε receptors (FcεR)

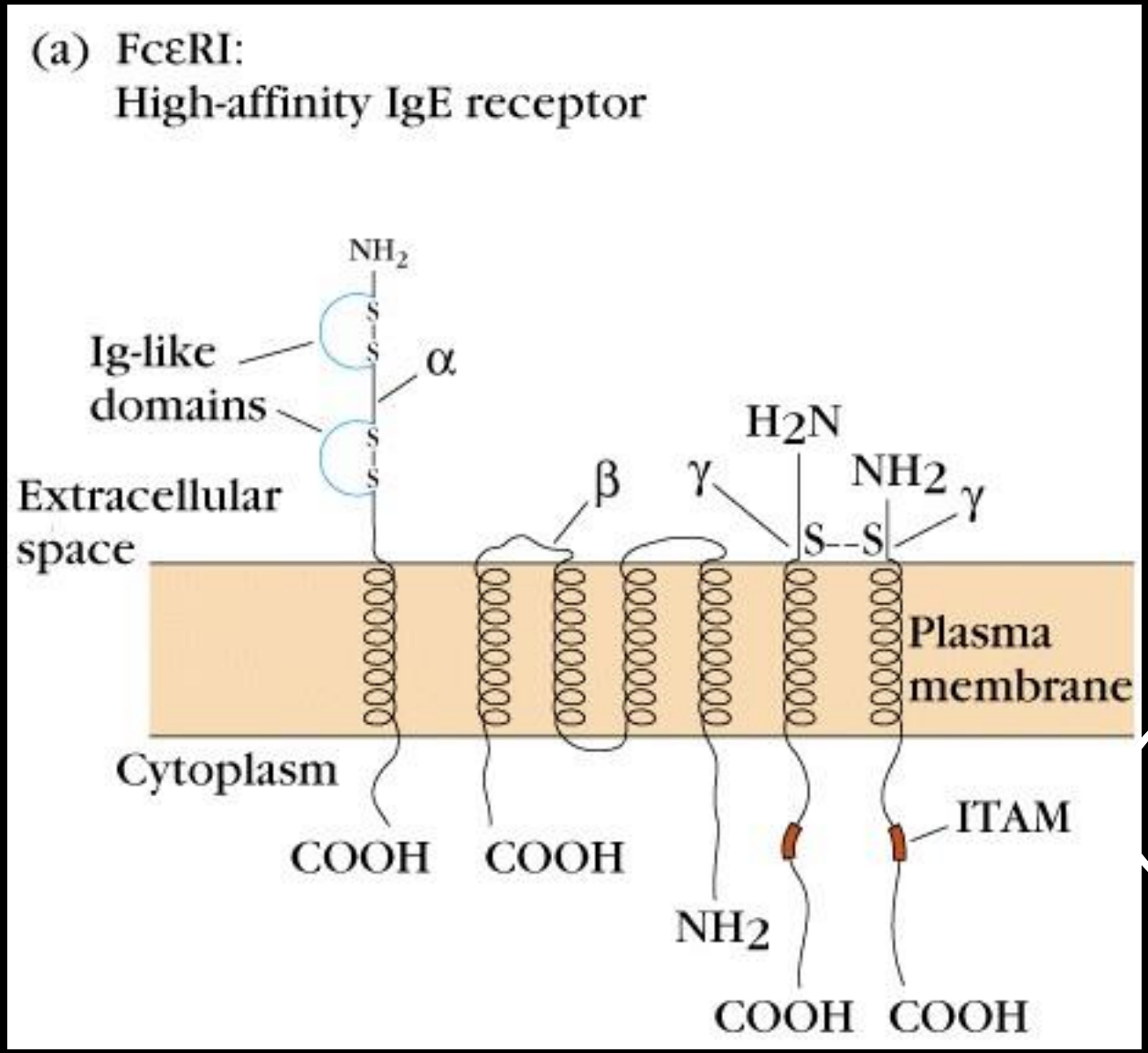
FcεR1

- high affinity IgE receptor found on
  - mast cells/basophils/activated eosinophils.

- Allergen binding to IgE attached to FcεR1 triggers release of granules from cell.

Mechanisms of allergic response

FcεRI



High affinity IgE Fc Receptor

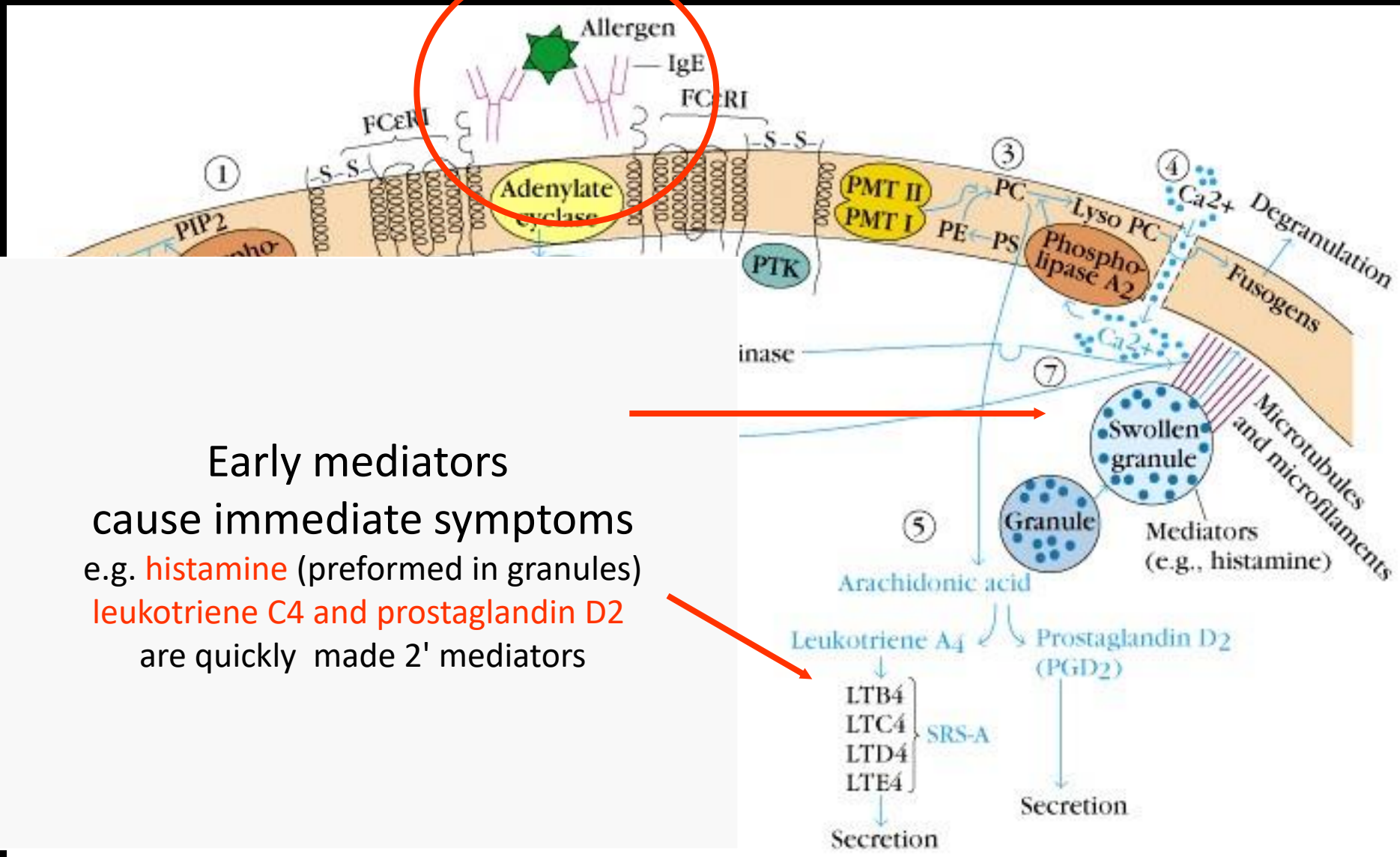
Has ITAM motifs

Mechanisms of allergic response
Effector Stage of Hypersensitivity

Secondary exposure to allergen

- Mast cells are primed with IgE on surface.
- Allergen binds IgE and cross-links to activate signal with tyrosine phosphorylation, Ca++ influx, degranulation and release of mediators.

FcεRI Triggers Release of Mediators



Early mediators
cause immediate symptoms
e.g. histamine (preformed in granules)
leukotriene C4 and prostaglandin D2
are quickly made 2' mediators

Mediators of Type I Hypersensitivity
Immediate effects

- Histamine
  - Constriction of smooth muscles. Bronchiole constriction = wheezing. Constriction of intestine = cramps-diarrhea.
  - Vasodilation with increased fluid into tissues causing increased swelling or fluid in mucosa.
  - Activates enzymes for tissue breakdown.
- Leukotrienes =Bronchial smooth muscles contract
- Prostaglandins

Mediators of Type I Hypersensitivity

Primary Mediators

Pre-formed mediators in granules

- Histamine
- Cytokines TNF-α, IL-1, IL-6.
- Chemoattractants for Neutrophils and Eosinophils.
- Enzymes
  - tryptase, chymase, cathepsin.
  - Changes in connective tissue matrix, tissue breakdown.

Type I Hypersensitivity

Secondary mediators

Mediators formed after activation

- Leukotrienes=Bronchial smooth muscles contract
- Prostaglandins=Inhibit the secretion of histamine(high conc.)but promote histamine release (low con.)
- Th2 cytokines- IL-4, IL-5, IL-13, GM-CSF

Continuation of sensitization cycle

- Mast cells control the immediate response.
- Eosinophils and neutrophils drive late or chronic response.

- More IgE production further driven by activated Mast cells, basophils, **eosinophils.**

Continuation of sensitization cycle

Eosinophils

- Eosinophils play key role in late phase reaction.
- Eosinophils make
  - enzymes,
  - cytokines (IL-3, IL-5, GM-CSF),
  - Lipid mediators (LTC4, LTD4, PAF)
- Eosinophils can provide CD40L and IL-4 for B cell activation.

Localized anaphylaxis

- Digestive tract contact results in vomiting, cramping, diarrhea.
- Skin sensitivity usually reddened inflamed area resulting in itching.
- Airway sensitivity results in sneezing and rhinitis OR wheezing and asthma.

Target organ responds to direct contact with allergen.

Systemic anaphylaxis

- Systemic vasodilation and smooth muscle contraction leading to severe bronchiole constriction, edema, and shock.

- Similar to systemic inflammation.

Treatment for Type I

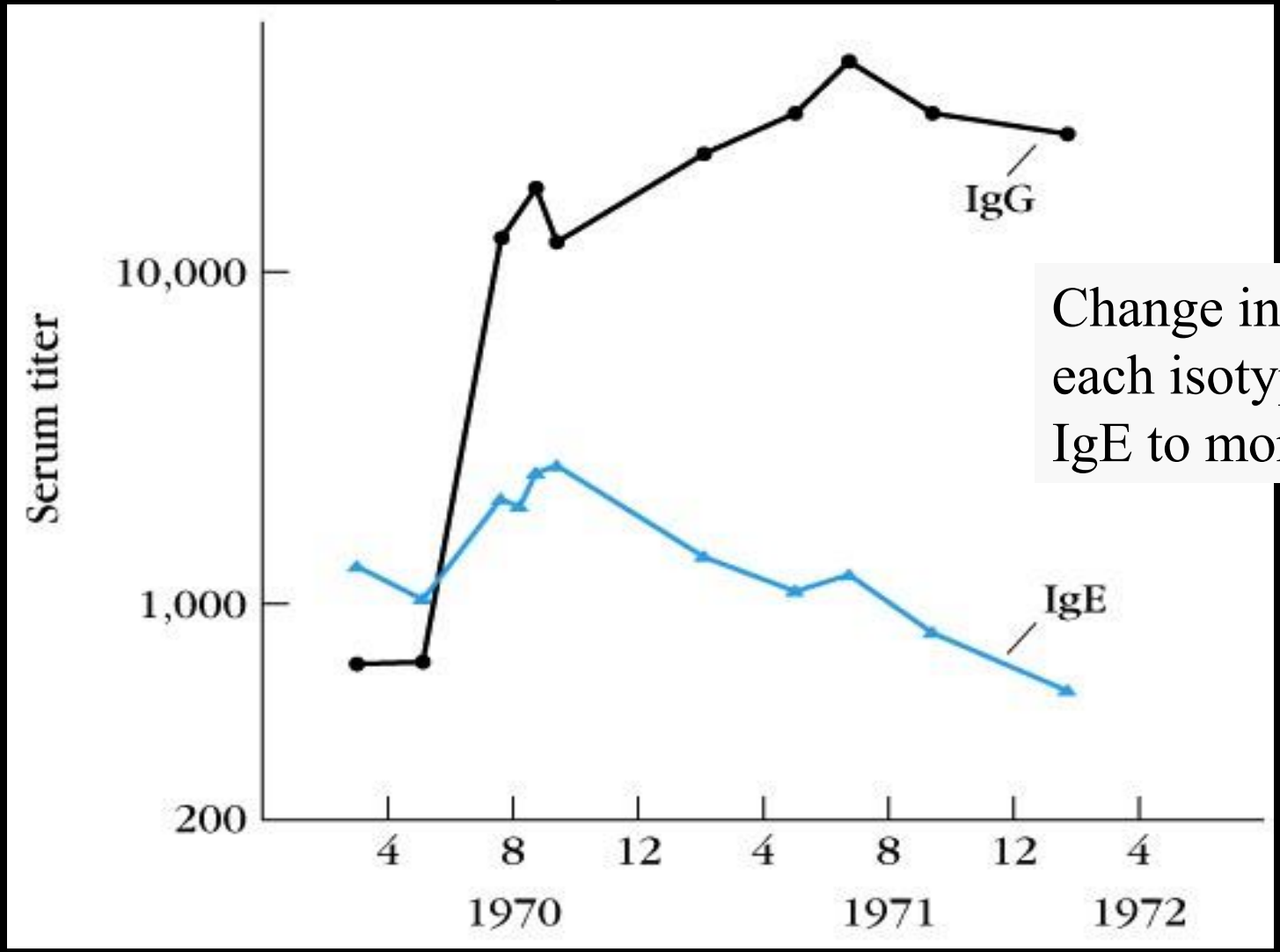
Pharmacotherapy

- Drugs.
  - Non-steroidal anti-inflammatories
  - Antihistamines block histamine receptors.
  - Steroids
  - Theophylline OR epinephrine -prolongs or increases cAMP levels in mast cells which inhibits degranulation.

Treatment for Type I

- Immunotherapy
  - Desensitization (hyposensitization)
    also known as allergy shots.
  - Repeated injections of allergen to reduce the IgE on Mast cells and produce IgG.

Treatment for Type I
Effect of allergy shots
Allergen Specific Antibodies

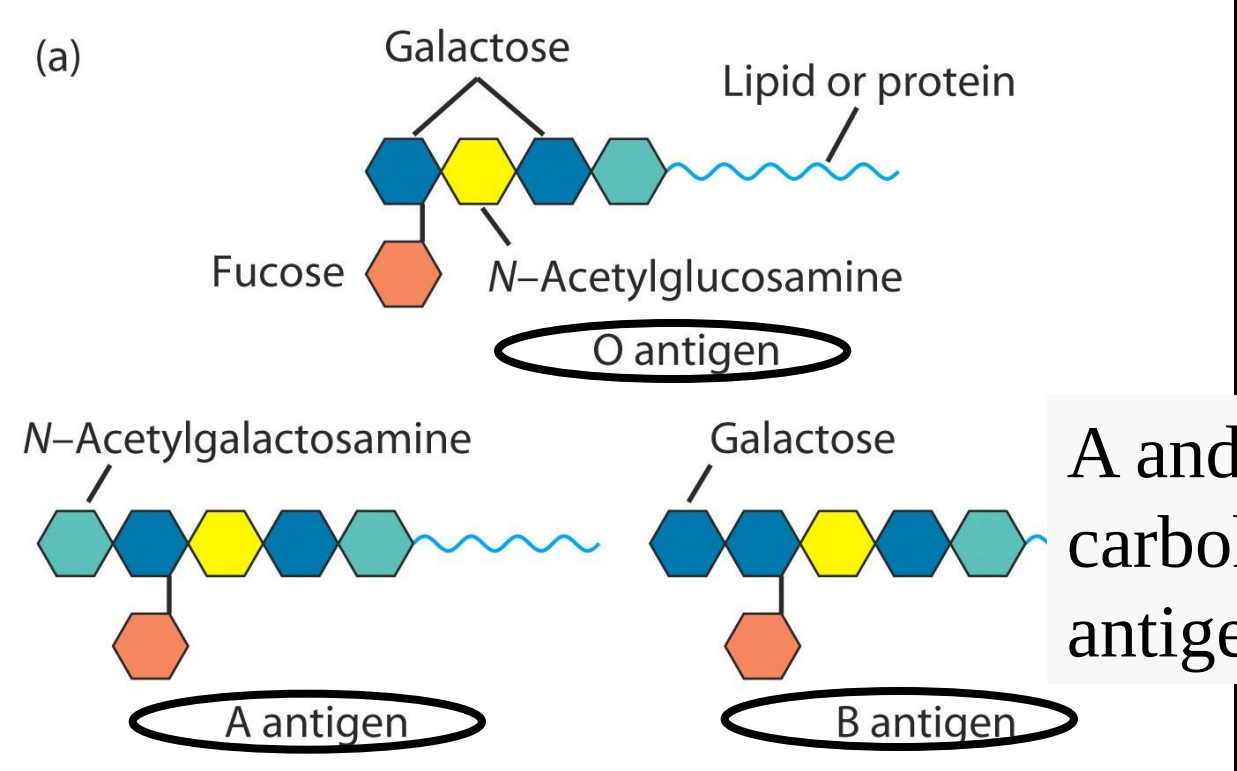


Change in amount of each isotype from more IgE to more IgG.

TYPE II Hypersensitivity
Antibody mediated cytotoxicity
Blood Transfusion reactions

Innocuous antigens on red blood cells.

EXAMPLE: ABO blood group antigens



A and B carbohydrate antigens

- **Type II hypersensitive reactions** involve antibody-mediate destruction of cells.

- **Antibody can** activate the **complement system,** creating pores in the membrane of a foreign cell
  - or it can **mediate cell destruction** by anti-bodydependent cell-mediated cytotoxicity (ADCC).

A. Transfusion Reactions Are Type II Reactions

- A large number of **proteins** and **glycoproteins** on the membrane of **red blood cells.**
  - Which are **encoded by different genes**, each of which has a number of **alternative alleles**.

- An **individual possessing** **one allelic form** of a blood-group antigen can **recognize other allelic forms** on transfused blood as foreign and mount an antibody response.

- **Antibodies** to the **A, B, and O antigens**, called iso-hemagglutinins, are usually of the **IgM class**.

ABO Blood Groups

Genotype	Blood–group phenotype	Antigens on erythrocytes (*agglutinins*)	Serum antibodies (*isohemagglutinins*)
AA or AO	A	A	Anti–B
BB or BO	B	B	Anti–A
AB	AB	A and B	None
OO	O	None	Anti–A and anti–B

Antibody against rbc antigen binds and mediates killing of rbcs via C'or ADCC causes systemic inflammation.

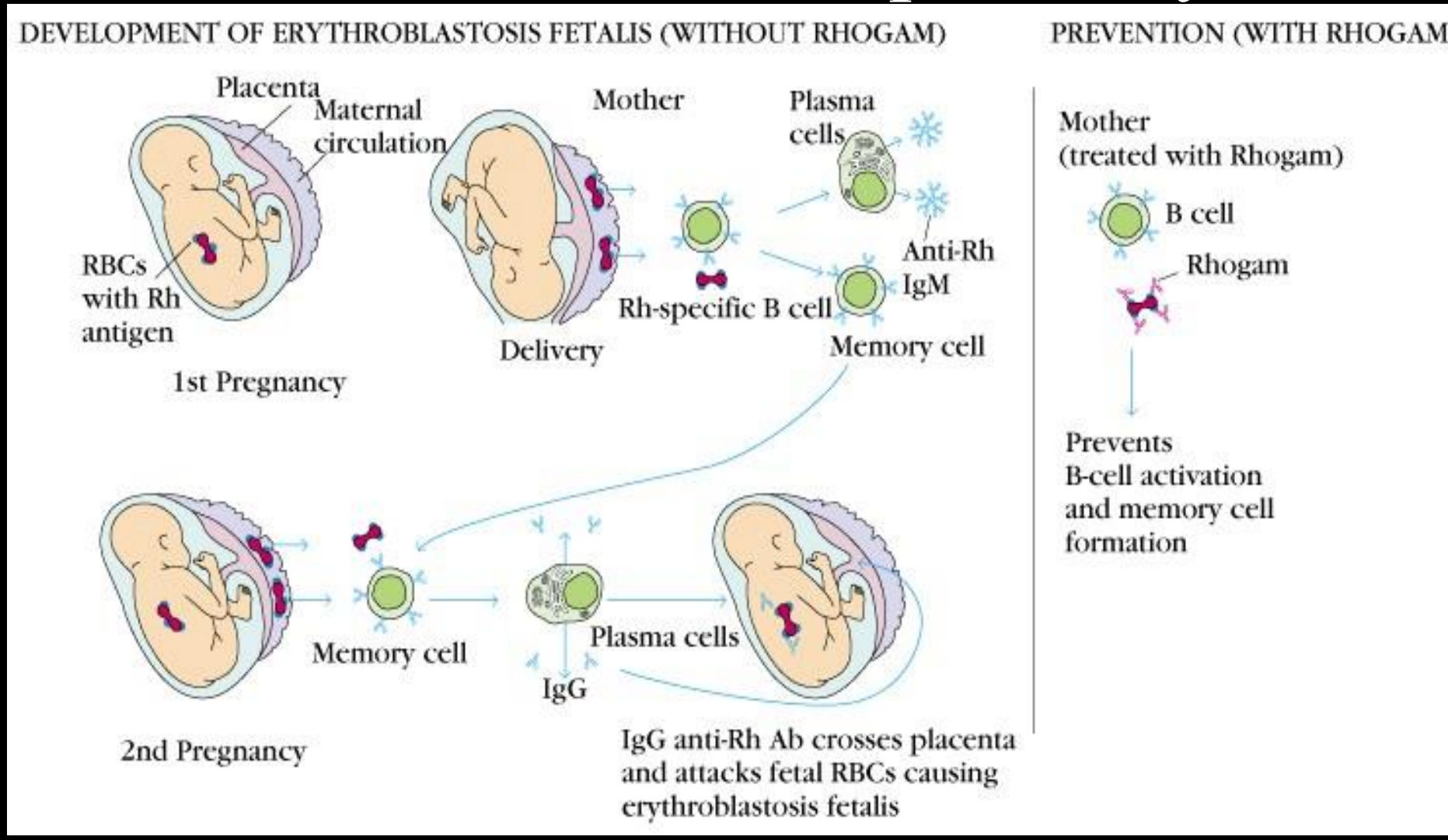
Quex: Why do we have antibodies to these innocuous antigens even before we get blood transfusion?

B. Hemolytic disease of newborn

Rh factor incompatibility

- **IgG abs to Rh** an innocuous rbc antigen.
  - Rh^+ baby born to Rh^- mother first time fine.
  - 2^{nd} time can have abs to Rh from 1st pregnancy.
  - Ab crosses placenta and baby kills its own rbcs.
  - Treat mother with ab to Rh antigen right after birth and mother never makes its own immune response.

TYPE II
Rh factor incompatibility



C. drug-Induced Hemolytic Anemia Is a Type II Response

- Certain antibiotics (e.g., **penicillin, cephalosporin, and streptomycin**) can adsorb non-specifically to **proteins on RBC membranes,** →→**forming a complex** similar to a hapten-carrier complex.

In some patients, such **drug-protein complexes** induce formation of **antibodies,**

- which then bind to the adsorbed **drug on red blood cells**, inducing **complement mediated lysis** →→ progressive anemia.

- When the drug is withdrawn, the hemolytic anemia disappears.

- **Penicillin** is notable in that it can induce all four types of hypersensitivity with various clinical manifestation.

TYPE III
Antigen antibody immune complexes (IgG) mediated
Immune Complex Disease

- Large amount of antigen and antibodies form complexes in blood.

- If not eliminated can **deposit in capillaries or joints** and **trigger inflammation**.

Neutrophiles and macrophages bind to immune complexes via FcR and **phagocytize the complexes**.

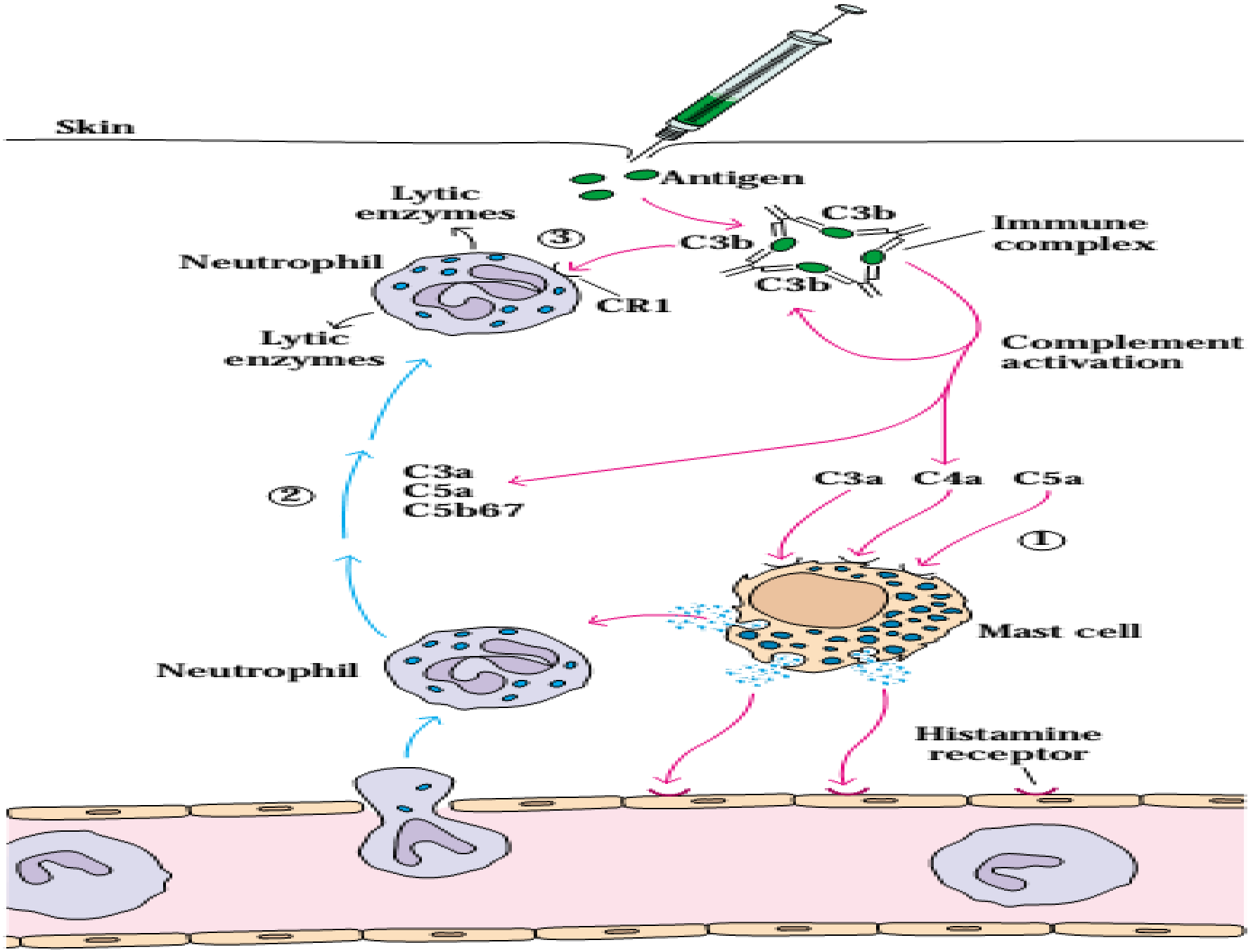
BUT

- If **unable to phagocytize** the immune complexes can cause inflammation via C' activation ---> C3a C4a, C5a and "frustrated phagocytes".

- As explained in the C3a, C4a, and C5a complement split products are **anaphyla-toxins.**
  - **that cause localized mast-cell degranulation and consequent increase in local vascular permeability.**

- **C3a, C5a, and C5b67** are also **chemotactic** factors for **neutrophils**, which can accumulate in large numbers at the **site of immune-complex deposition**.

- If **neutrophils** and **macrophages** are unable to phagocytize the immune complexes →→ (cells) **degranulate in the area of immune complex →→deposition and trigger inflammation**.
- **Unable to eat -------try to digest outside cell.**
- **Much of the tissue damage in type III reactions** stems from **release of lytic enzymes by neutrophils** as they attempt to phagocytose immune complexes.


Skin
Antigen
Lytic enzymes
③
Neutrophil
CR1
Lytic enzymes
C3b
C3b
C3b
Immune complex
Complement activation
②
C3a
C5a
C5b67
C3a
C4a
C5a
①
Mast cell
Neutrophil
Histamine receptor

Localized disease

1. **In addition**, the activation of complement can **induce aggregation of platelets**, →→release of clotting factors →→microthrombi.

2. **Deposited in joints causing local** inflammation = arthritis.

3. Deposited in kidneys = g**lomerulonephritis**.

4. **Serum sickness** from large amounts of antigen such as **injection of foreign serum**.

Delayed type hypersensitivity (type IV)
Th1 cells and macrophages

- **When some subpopulations of activated TH cells** encounter certain types of antigens, they **secrete cytokines** that induce a **localized inflammatory** reaction called **delayed-type hypersensitivity (DTH)**.
- **DTH response** is from:
  - Continued **macrophage activation →chronic inflammation →→** in tissue lesions, **scarring**, and **granuloma formation.**
  - Delayed is relative because DTH response arise **24-72 hours** after exposure rather than within minutes.

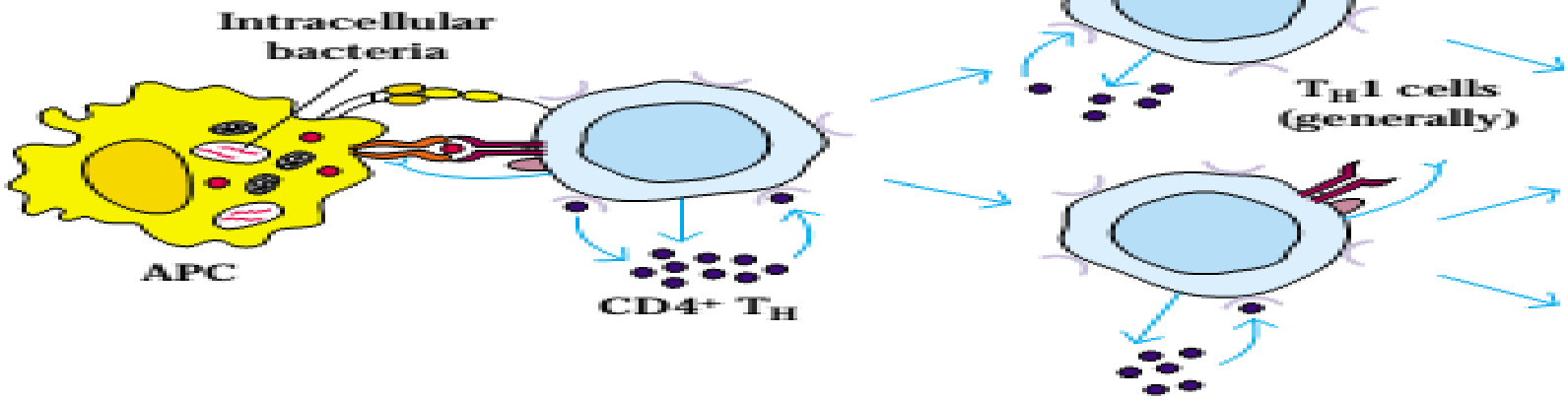
Sensitization stage

- **Memory Th1 cells** against **DTH antigens** are generated by dendritic cells during the sensitization stage.
- These **Th1 memory cells** can **activate macrophages** and **trigger inflammatory response**.

Dendritic cells+Ags → TH1 cells activation
→→**activate macrophages** **+inflammation**

Effector stage

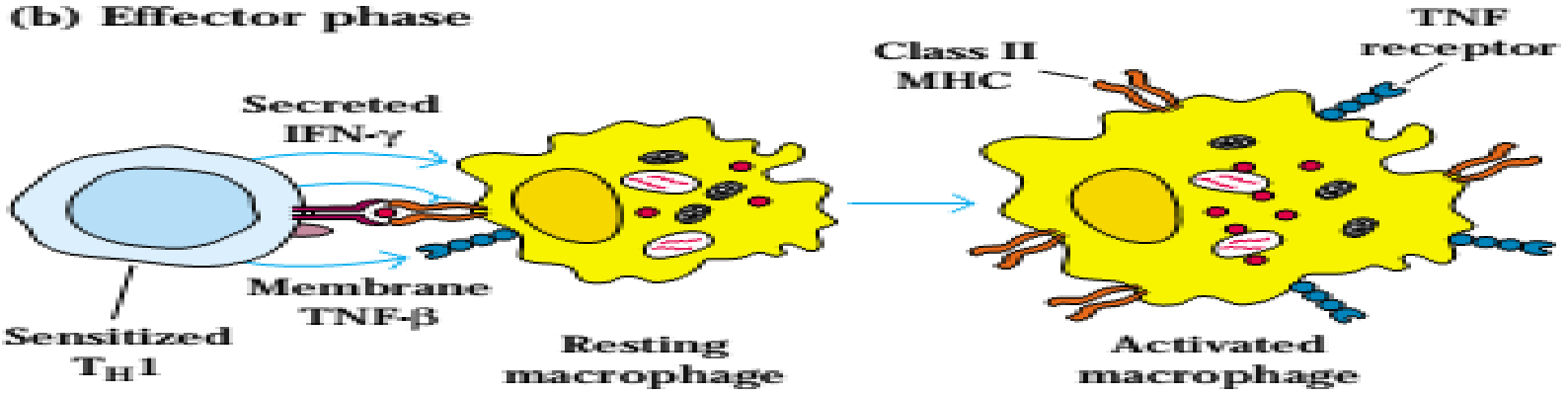
- **Th1 memory cells** are activated and produce cytokines.
  - IFN-γ, **TNF-α, and TNF-β** which cause tissue **destruction, inflammation**.
  - IL-2 that activates T cells and CTLs.
  - Chemokines- for **macrophage recruitment**.
  - IL-3, GM-CSF for increased **monocyte/macrophage.**
- Continued exposure to antigen can cause chronic inflammation and result in **granuloma formation**.



Antigen-presenting cells:
Macrophages
Langerhans cells

DTH-mediating cells:
T_H1 cells generally
CD8 cells occasionally

(b) Effector phase



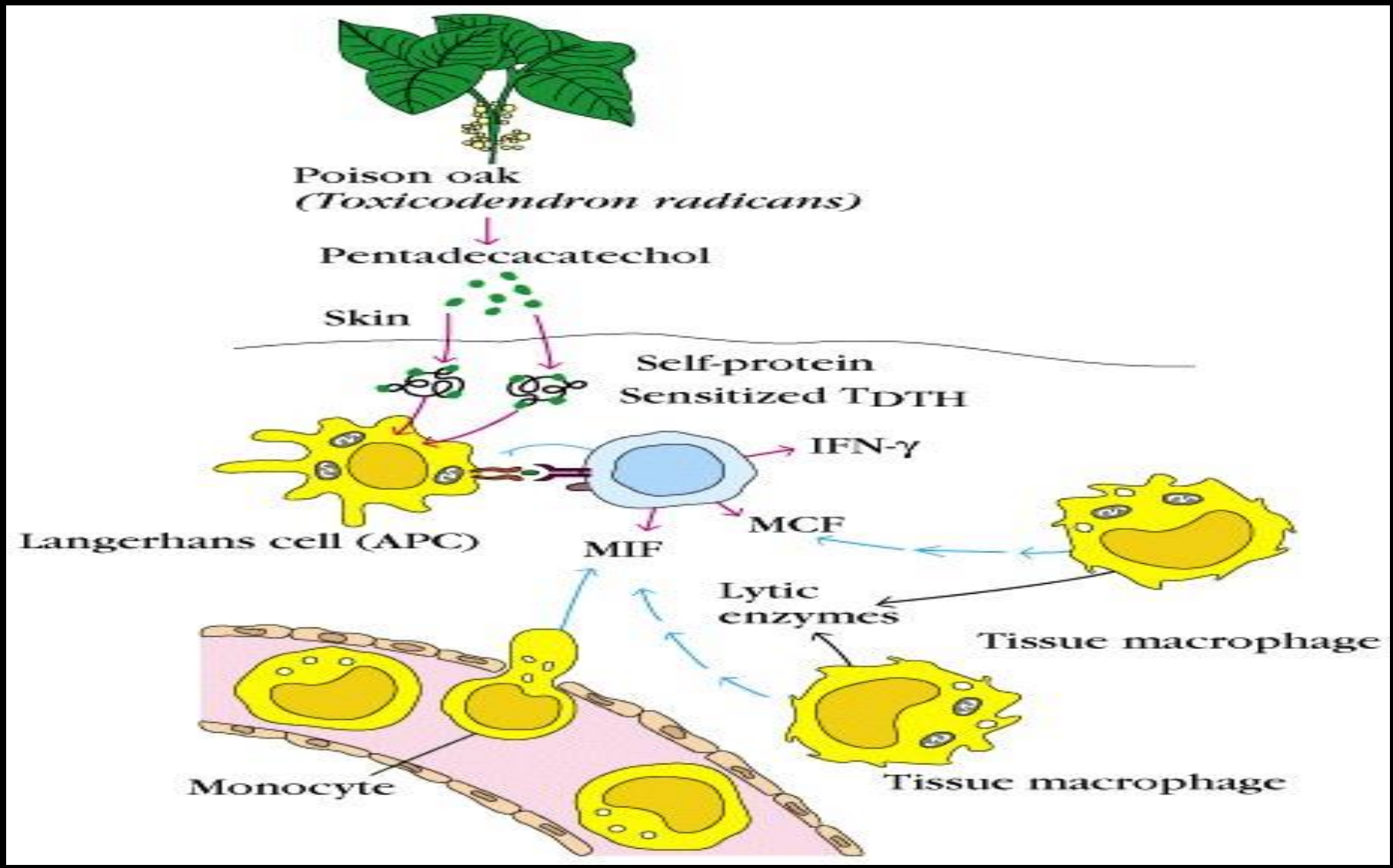
T_H1 secretions:
Cytokines: IFN-γ, TNF-β, IL-2, IL-3, GM-CSF
Chemokines: IL-8, MCAF, MIF

Effects of macrophage activation:
↑ Class II MHC molecules
↑ TNF receptors
↑ Oxygen radicals
↑ Nitric oxide

Contact dermatitis

- The response to **poison oak** is a classic Type IV.
  - Small molecules act as haptens and complex with skin proteins to be taken up by APCs and presented to Th1 cells to get sensitization.
  - During secondary exposure Th1 memory cells become activated to cause DTH.

Contact dermatitis



Delayed type hypersensitivity (DTH)

TABLE 14-3 INTRACELLULAR PATHOGENS AND CONTACT ANTIGENS THAT INDUCE DELAYED-TYPE HYPERSENSITIVITY

Intracellular bacteria
- *Mycobacterium tuberculosis*
- *Mycobacterium leprae*
- *Listeria monocytogenes*
- *Brucella abortus*

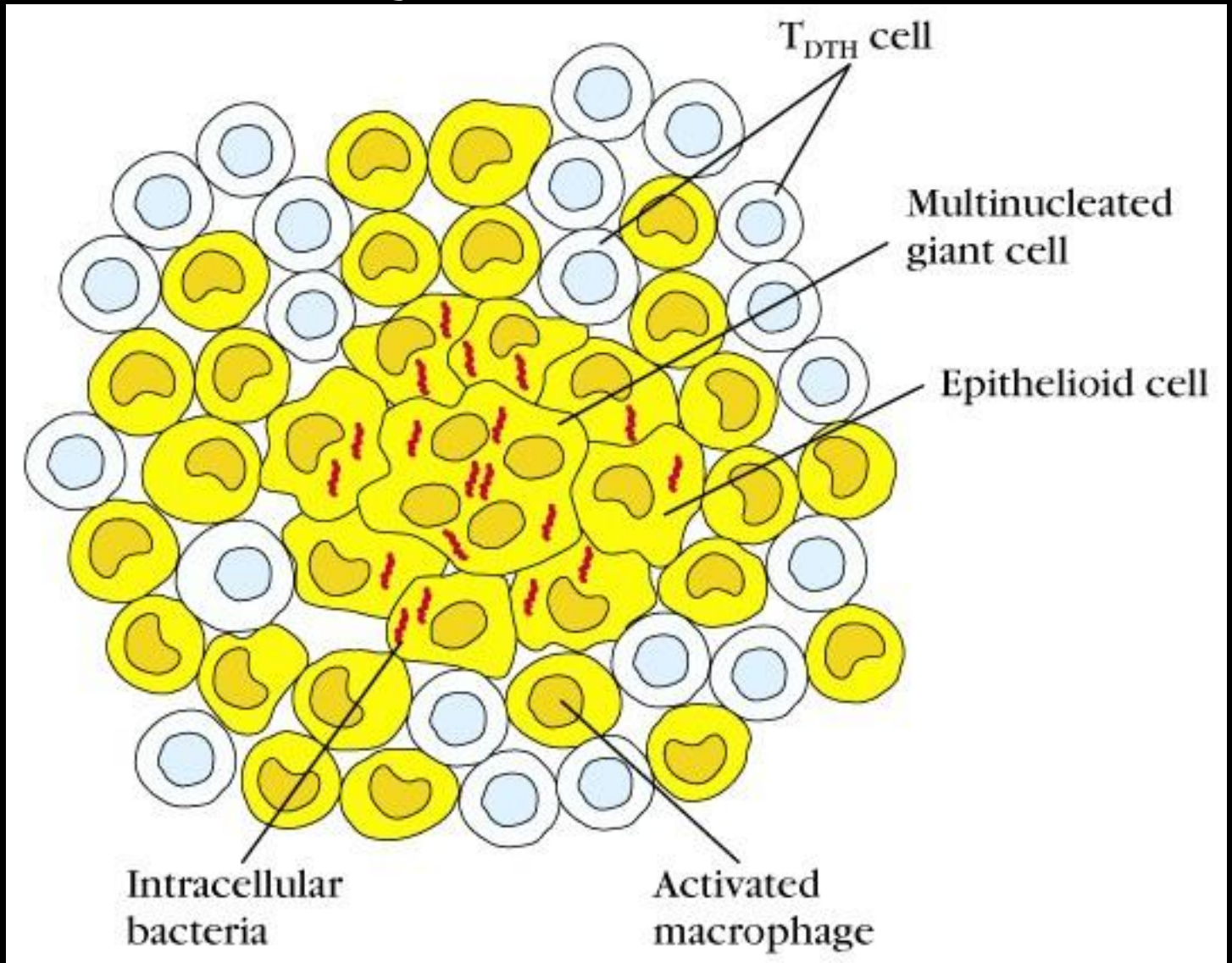
Intracellular fungi
- *Pneumocystis carinii*
- *Candida albicans*
- *Histoplasma capsulatum*
- *Cryptococcus neoformans*

Intracellular parasites
- *Leishmania* sp.

Intracellular viruses
- Herpes simplex virus
- Variola (smallpox)
- Measles virus

Contact antigens
- Picrylchloride
- Hair dyes
- Nickel salts
- Poison ivy
- Poison oak

Granuloma Formation from DTH Mediated by Chronic Inflammation



Drug reactions can be any Type of Hypersensitivity

TABLE 16-5 Penicillin-induced hypersensitive reactions

Type of reaction	Antibody or lymphocytes induced	Clinical manifestations
I	IgE	Urticaria, systemic anaphylaxis
II	IgM, IgG	Hemolytic anemia
III	IgG	Serum sickness, glomerulonephritis
IV	T_{DTH} cells	Contact dermatitis

Chapter 10

Vaccination

- Vaccine development begins with basic research.
- Recent **advances in immunology and molecular** biology have led to **effective new vaccines** and to promising strategies for finding new vaccine candidates.
- Knowledge of the differences in **epitopes** recognized by **T cells and B cells** has enabled immunologists to begin to design vaccine candidates to maximize activation of both arms of the immune system.
- As differences in antigen-processing pathways became evident, scientists began to design vaccines and to use adjuvants that maximize antigen presentation with class I or class II MHC molecules.
- Genetic engineering techniques can be used to develop vaccines to maximize the immune response to selected epitopes and to simplify delivery of the vaccines.

Type of vaccines

- Active and Passive Immunization
- Designing Vaccines for Active Immunization
- Whole-Organism Vaccines
- Purified Macromolecules as Vaccines
- Recombinant-Vector Vaccines
- DNA Vaccines
- Multivalent Subunit Vaccines

10.1. Active and Passive Immunization

- Immunity to infectious microorganisms can be achieved by **active or passive immunization**.
- In each case, immunity can be **acquired either by natural processes**
  - (usually by **transfer from mother to fetus or by previous** infection by the organism) or,
- by artificial means such as injection **of antibodies** or **vaccines**.
- The agents used for **inducing passive immunity** include antibodies from humans or animals.

- **Active immunization** is achieved by inoculation with microbial pathogens that **induce immunity**
  - but do not cause disease or with antigenic components from the pathogens.
- In history **Jenner and Pasteur** are recognized as the pioneers of vaccination, or induction of active immunity.
- **Emil von Behring** and **Hidesaburo Kitasato** for their contributions to passive immunity.
-

Passive immunization

- **Passive immunization** can also be achieved by injecting a **recipient with preformed antibodies.**
- **Passive immunization** is routinely administered to individuals exposed to botulism, tetanus, diphtheria, hepatitis, measles, and rabies.
- Passively administered antiserum is also used to provide protection from poisonous snake and insect bite.
- Produce immediate immunization.
- Because passive immunization does not activate the immune system, it generates no memory response and the protection provided is transient.
- Although passive immunization may be an effective treatment, it should be used with caution because certain risks are associated with the injection of preformed antibody.

- If the antibody was produced in another species, such as a horse, the recipient can mount a strong response to the isotypic determinants of the foreign antibody

Chapter 8

Vaccination

Introduction

- **Immunization**: a procedure designed to **increase concentrations of antibodies** and/or e**ffector T-cells** which are reactive against infection (or cancer).

- **Immunization procedure** called **vaccination a**nd the immunizing agent called **vaccine (o**r “serum” in historical references)
- When **performed before exposure** to an infectious agent (or soon after exposure in certain cases), it is called **immunoprophylaxis.**
- intended to ***prevent*** the infection.

10.1. Types of Immunization

- **Two mechanisms** by which immunization can be achieved
- **Passive immunization:**
- The agents used for **inducing passive immunity** include antibodies from humans or animals.
  - Protective Abs --> non immune recipient.
  - No immunological memory T- helper cells.
- **Active immunization:**
  - Induction of adaptive immune response, with protection and memory.
- **Passive Immunization aquarized through –**
  - **Natural maternal serum/milk**
  - **Artificial immune serum**.

Active Immunization aquared trough–

- Natural infection
- Artificial infection (vaccination)*:
- Whole-Organism Vaccines
  - ✓ Attenuated organisms (live)
  - ✓ inactivated organisms (dead)
- ✓ Purified Macromolecules as Vaccines (proteins and polysaccharides).
- ✓ Cloned genes of microbiological antigens
- ✓ Synthetic peptides.
- ✓ DNA
- ✓ Recombinant-Vector Vaccines

Passive Immunity

- **Naturally** - transplacental transfer of **maternal IgG Abs to developing fetus**; transfer of IgG + IgA Abs in milk during breast-feeding of newborn animals.
- **Passive immunization** is routinely administered to individuals exposed to **botulism, tetanus, diphtheria, hepatitis, measles, and rabies.**

- An antiserum is also used to provide protection from **poisonous snake** and **insect bite**.

- **passive immunization** does not activate the immune system, it generates **no memory response** and the protection provided is transient.

- **Artificially** - injection of Ig Performed prophylactic ally, either after diagnosis of **exposure to toxin/virus** or as a short term preventive procedure, e.g. if one is traveling to an endemic area.
- If the antibody was produced in another species, such as a **horse, the recipient** can mount a strong response to the **isotypic determinants of the foreign antibody.**

Sources of Passive Immunity

- Almost **all blood** or ***blood products***
- **homogeneous serum**: serum obtained from blood donor volunteers, have been immunized.
- **heterogeneous serum**: serum obtained from blood of animals hyper-immunized.
- Heterologous hyperimmune serum (a**ntitoxi**n)

Active Immunization

Naturally - following exposure to an infection.

Artificially - by **vaccination**: Performed either

- by i. m. injection of **killed or attenuated antigens** (often with adjuvant) or by ingestion of attenuated live organisms.

10.2. Types of Vaccines

1. Attenuated whole-agent vaccines

. Many of the common vaccines **currently in use** consist of **inactivated (killed)** or **live but attenuated (avirulent)** bacterial cells or viral particles.

- **Attenuation often** can be achieved by growing a pathogenic bacterium or virus for prolonged periods under **abnormal culture conditions**.
- E.g an **attenuated strain of Mycobacterium bovis** called **Bacillus Calmette-Guerin (BCG)** was developed by growing M. bovis on a medium **containing increasing concentrations of bile.**

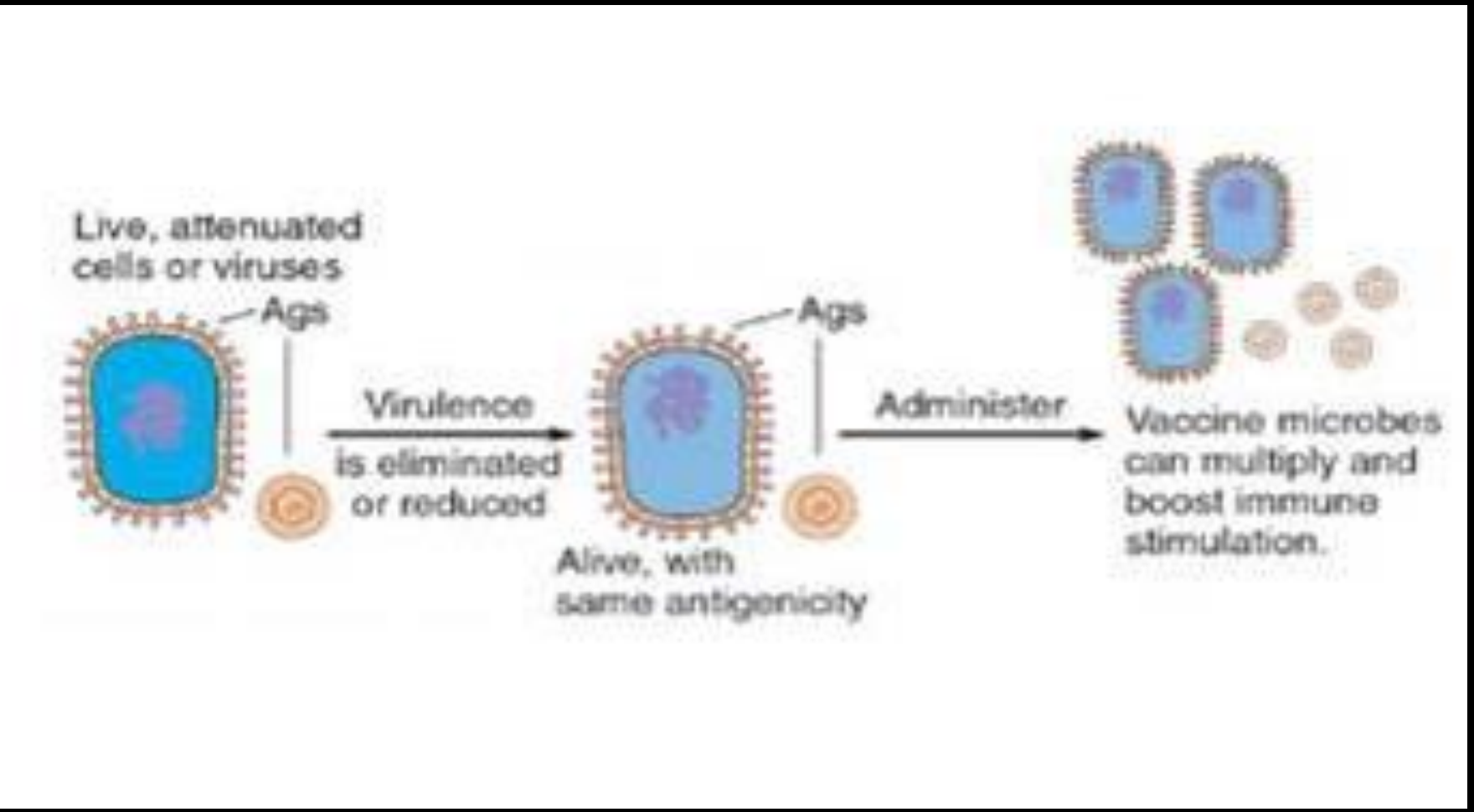
- **Eg.2 The poliovirus** used in the Sabin vaccine was attenuated by **growth in monkey kidney epithelial cells**.

- **Live vaccines** more **closely mimic an actual infection.**

- **Lifelong immunity**, especially with viruses, is often achieved **without booster immunizations**, and an effectiveness rate **of95% is common**.

- This **long-term effectiveness probably** occurs because the **attenuated viruses** replicate in the body,
  - increasing the **original dose and acting** as a series of **secondary (booster) immunizations**.
    - e.g. **Rabies virus vaccine** and **anthrax vaccine**.
    - BCG (Bacillus Calmette Guerin) vaccine for *Mycobacterium tuberculosis= human*

- A major disadvantage of attenuated vaccines is the possibility that they will revert to a virulent form



Ideal properties of a live vaccine

- **Attenuated microorganism** which replicates in the host thus **eliciting immune responses** similar to **natural infection.**

- Able to elicit **lifelong protection** using only one or two doses .
- Disease causing capacity is **virtually eliminated (attunated)**.

- Elicits both **humoral** and **cellular immunity**.

Modern attenuation mechanisms

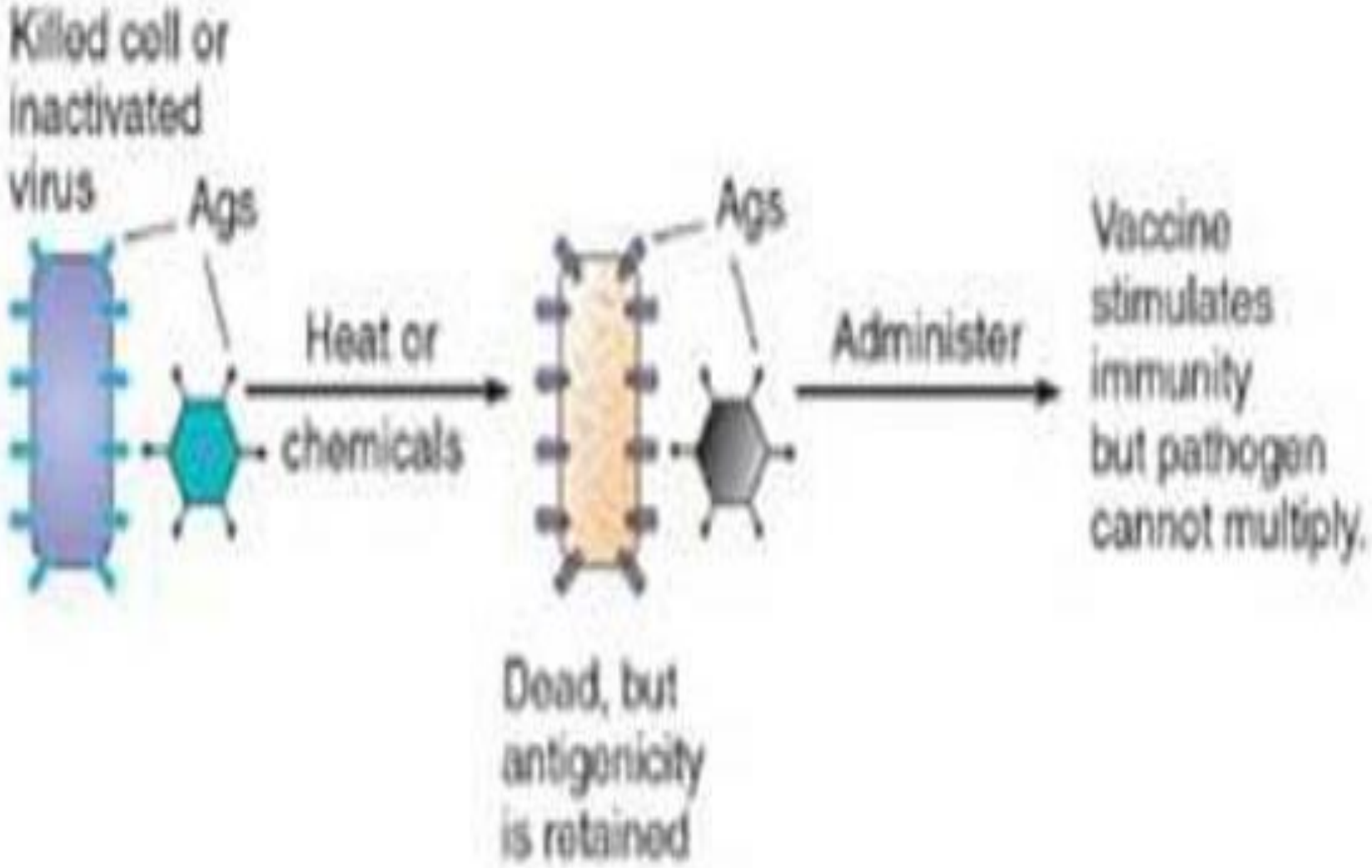
- **Genetic engineering** techniques provide a way to **attenuate a virus** irreversibly by **selectively removing gene**s that are necessary for virulence.

- More recently, a **vaccine against rotavirus**, a major cause of infant diarrhea, was **developed using genetic engineering techniques** to modify an animal rotavirus to contain antigens present on the human viruses.

2. Inactivated -whole-agent vaccines

- Another common approach in **vaccine production** is **inactivation of the pathogen** by **heat or by chemical** means so that it is **no longer capable of replication** in the host.

- **Heat inactivation** is generally unsatisfactory because it causes **extensive denaturation** of proteins;
  - thus, any e**pitopes** that depend on higher **orders of protein** structure are likely to be altered significantly.
- **Chemical inactivation** with **formaldehyde** or various **alkylating agents** has been successful.

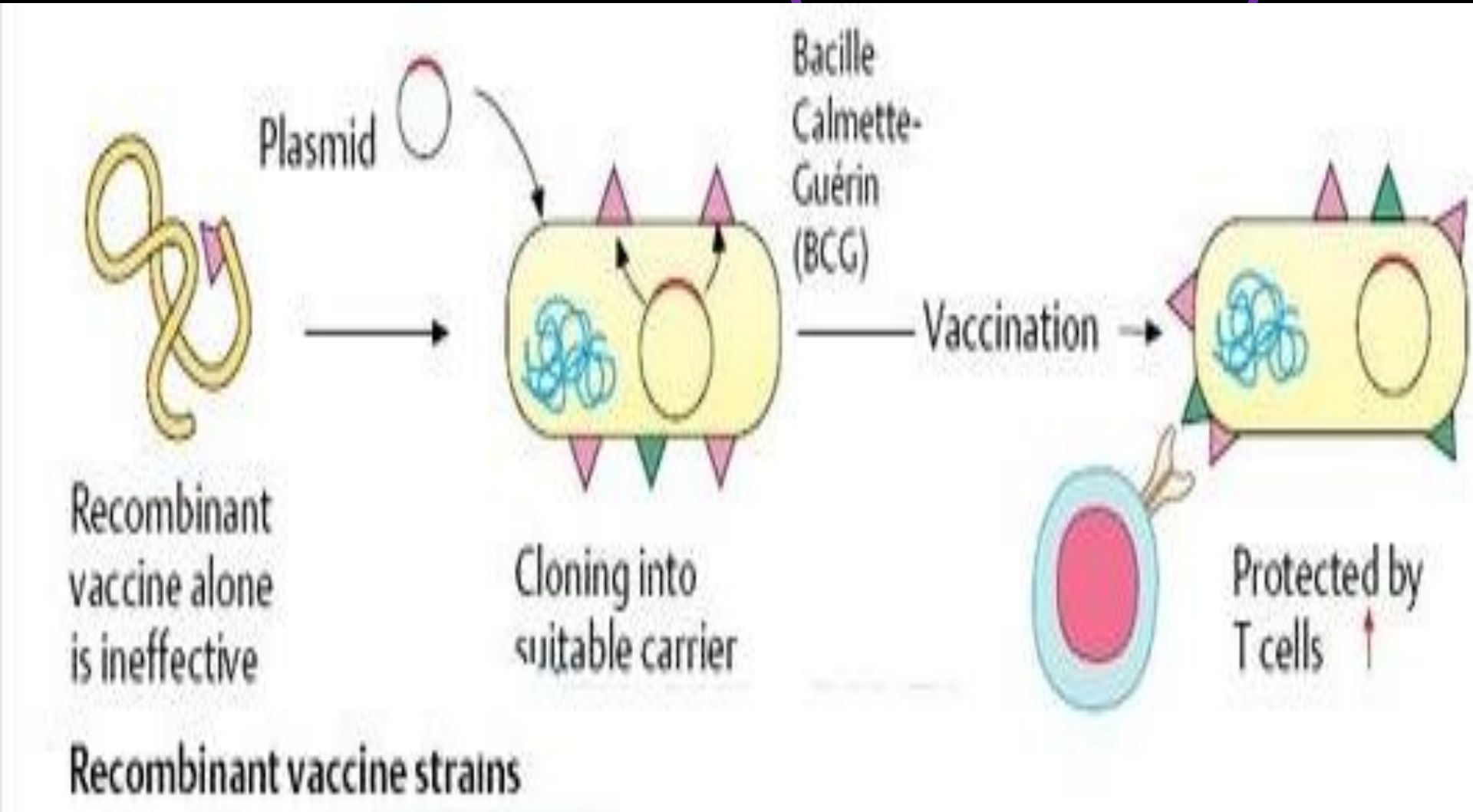

Killed cell or inactivated virus
Ags
Heat or chemicals
Ags
Dead, but antigenicity is retained
Administer
Vaccine stimulates immunity but pathogen cannot multiply.

- **Inactivated virus vaccines** used in humans include those against rabies (animals sometimes receive a live vaccine considered too hazardous for humans), influenza, and polio (the Salk poliovaccine).
- Inactivated bacterial vaccines in animals black leg diseases vaccineand pasturella vaccine.
  - **stimulates the antibody mediated response only.**
  - e.g. DPT (diphtheria, tetanus toxoids
- **Toxoids**, which are **inactivated toxins**, are vaccines directed at the toxins produced by a pathogen.
- The **tetanus** and **diphtheria toxoids** have long been part of the standard childhood immunization series.

3. Subunit vaccines:

- use only those antigenic fragments of a microorganism that best stimulate an **immune response.**
  - **Synthetic peptides**
  - **Recombinant proteins**
- **Those subunit vaccines** that are produced by **genetic modification** techniques,
  - meaning that other microbes are programmed to produce the desired antigenic fraction, are called **recombinant vaccines.**
  - For example, the vaccine against the hepatitis B virus consists of a portion of the viral protein coat that is produced by a genetically modified yeast.
  - They need **carriers** to give them called **vectors like virosomes,liposomes, beeds etc.**

Sub-unit vaccine (recombinant)



Recombinant vaccine strains

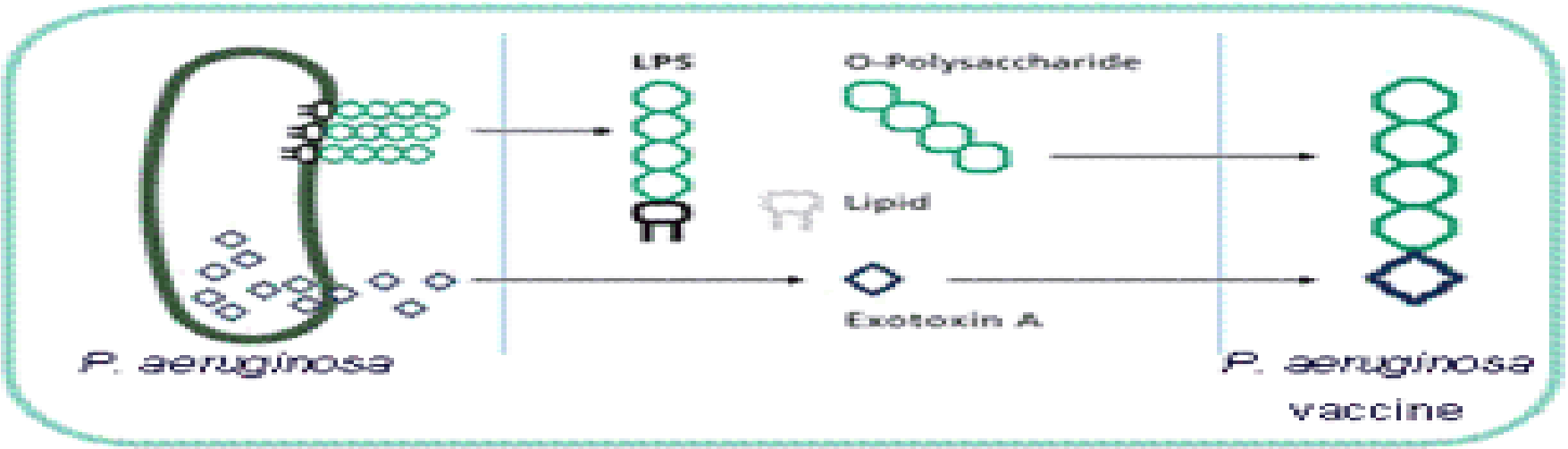
- How recombinant viruses are made
  - Hepatitis B vaccine – **purified viral coat protein**.
  - *Streptococcus pneumoniae* (PneumoShot) – **capsular polysaccharide**
  - *Hemophilus influenzae* (HiB) – **capsular polysaccharide.**
  - *Nesseria meningiditis* – **capsular polysaccharide**
- **Tuberculosis**
  - *Mycobacterium tuberculosis*
  - antibiotic resistant strains
  - use purified extracellular
- stimulates the **antibody mediated response** only.

Conjugated vaccines

- **Conjugated vaccines** have been developed in recent years to deal with the poor immune response of children to vaccines based on capsular polysaccharides.

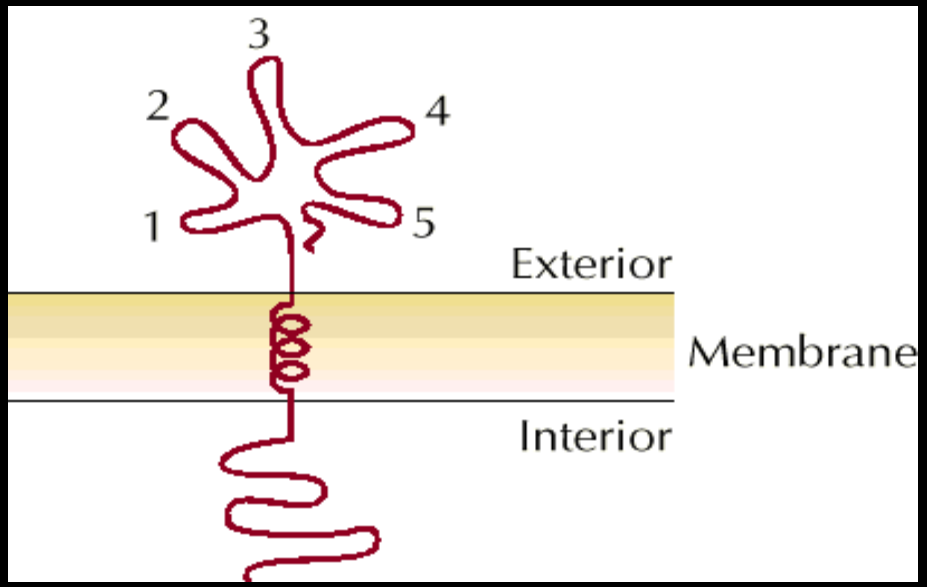
P. aeruginosa conjugate vaccine

A polyvalent conjugate vaccine combining 8 prevalent serotypes of *P.aeruginosa* with exotoxin A

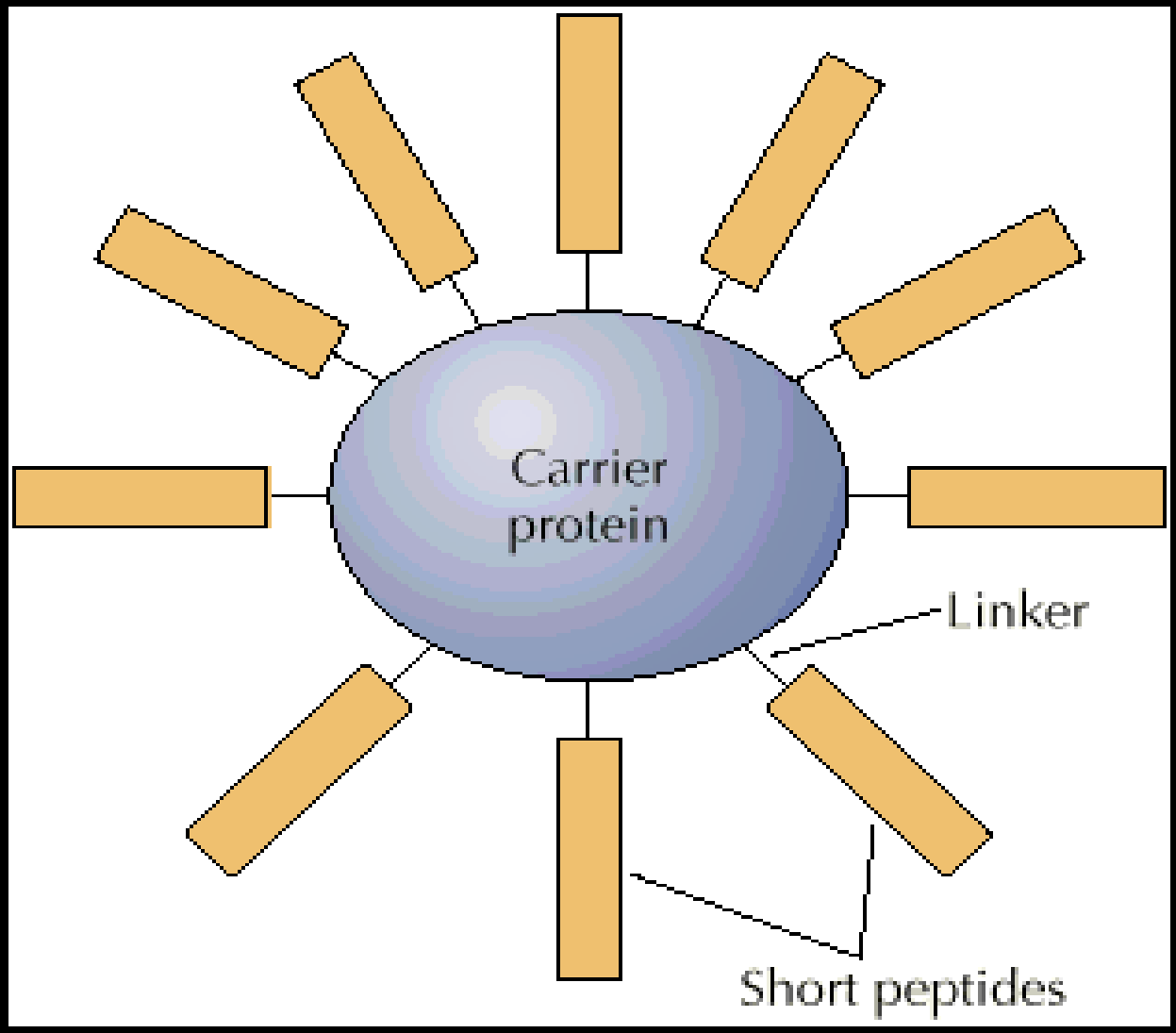


4. Peptide Vaccines

- Use discrete portion (domain) of a surface protein as Vaccine.
- These domains are ‘epitopes’
- antigenic determinants
- are recognized by antibodies



CARRIER PROTEINS – vetors



5. Genetic Immunization(DNA vaccine)

- **Delivery of a gene for the antigen** to a host organism
- **Use vector containing cDNA** from viral protein/
- eukaryotic promoter
- Inject into muscle/micr-oprojectile system
- POTENTIAL
- eliminates purification of antigen
- protein is modified post-translationally

6. Vector Vaccines

Vaccinia good candidate for a **live recombinant viral vaccine**

- **benign virus**
- replicate in cytoplasm (viral replication genes)
- easy to store

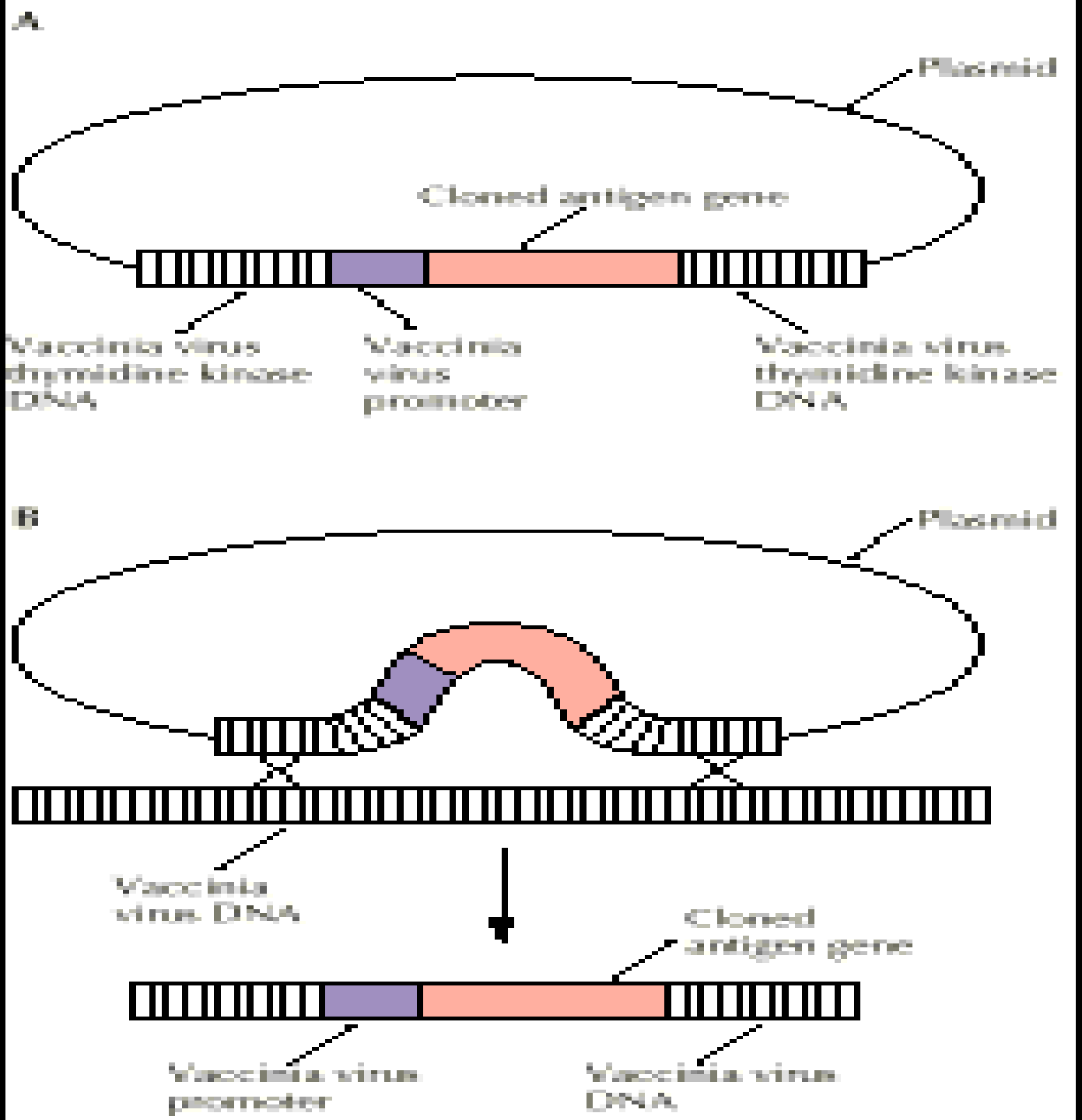
A) **Insert cloned gene encoding antigen**

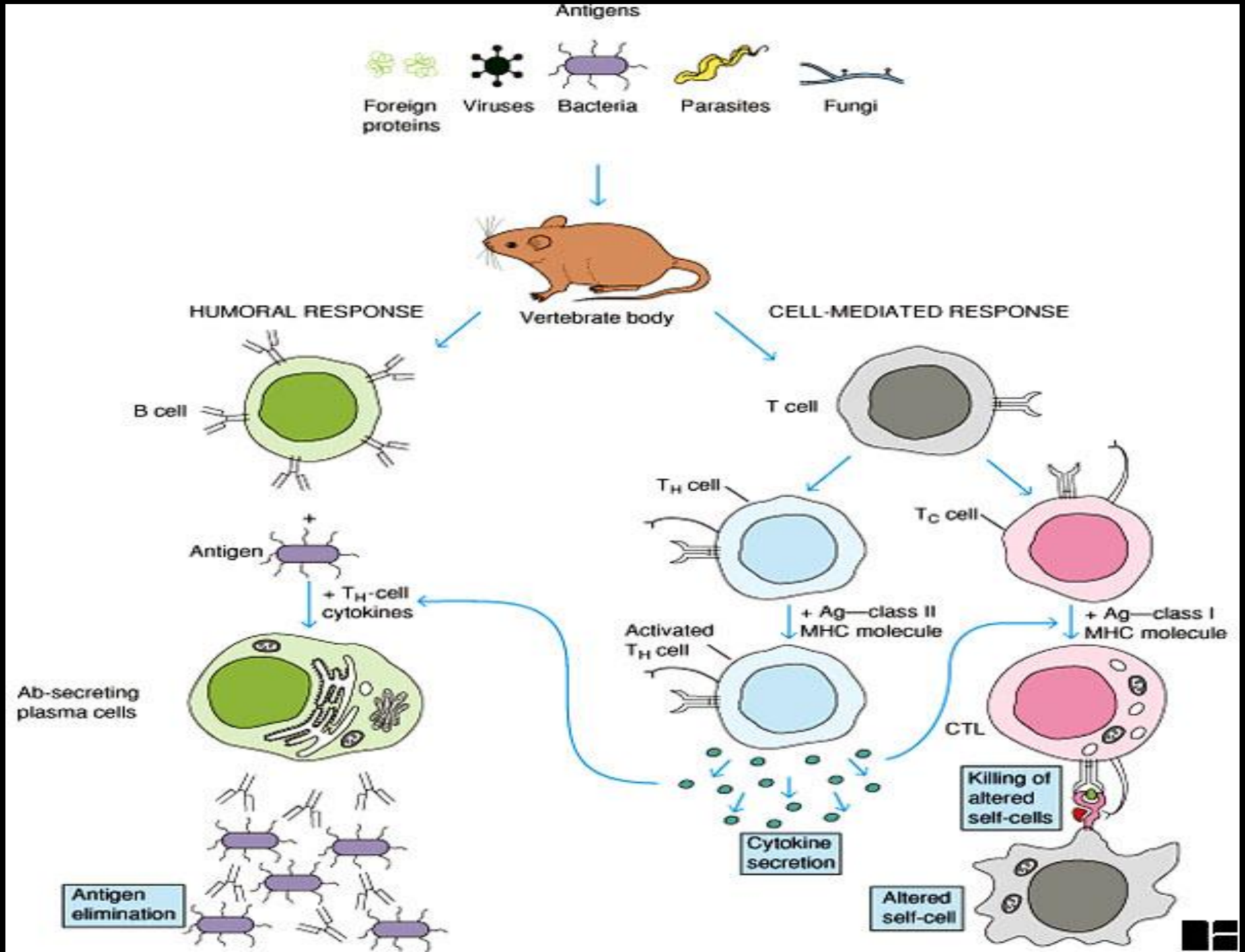
b. Infect host cell with native virus

c) Transform these **cells with recombinant plasmid**

d) HOMOLOGOUS RECOMBINATION

****MODIFIED VIRUS USED AS VACCINE****


A
Plasmid
Cloned antigen gene
Vaccinia virus thymidine kinase DNA
Vaccinia virus promoter
Vaccinia virus thymidine kinase DNA
B
Plasmid
Vaccinia virus DNA
Cloned antigen gene
Vaccinia virus promoter
Vaccinia virus DNA


Antigens
Foreign proteins
Viruses
Bacteria
Parasites
Fungi
Vertebrate body
HUMORAL RESPONSE
CELL-MEDIATED RESPONSE
B cell
T cell
T_H cell
T_C cell
Antigen
+ T_H-cell cytokines
+ Ag—class II MHC molecule
+ Ag—class I MHC molecule
Activated T_H cell
Ab-secreting plasma cells
CTL
Killing of altered self-cells
Cytokine secretion
Antigen elimination
Altered self-cell

Adjuvants

Main activities:-

- Facilitation of antigen transport, **uptake and presentation by antigen-capturing and processing cells.**
- Repeated or prolonged **release of antigen** (**depot effect).**
- Signaling of receptors activating innate immune cells to release **cytokines** which up-regulate co-stimulatory molecules.

- ‘Danger signals’ from stressed or damaged tissues activate APCs.

- Signaling **by recombinant cytokines** or **co-stimulatory molecules mimics** classical adjuvant activity

Thank you

Have a nice time