



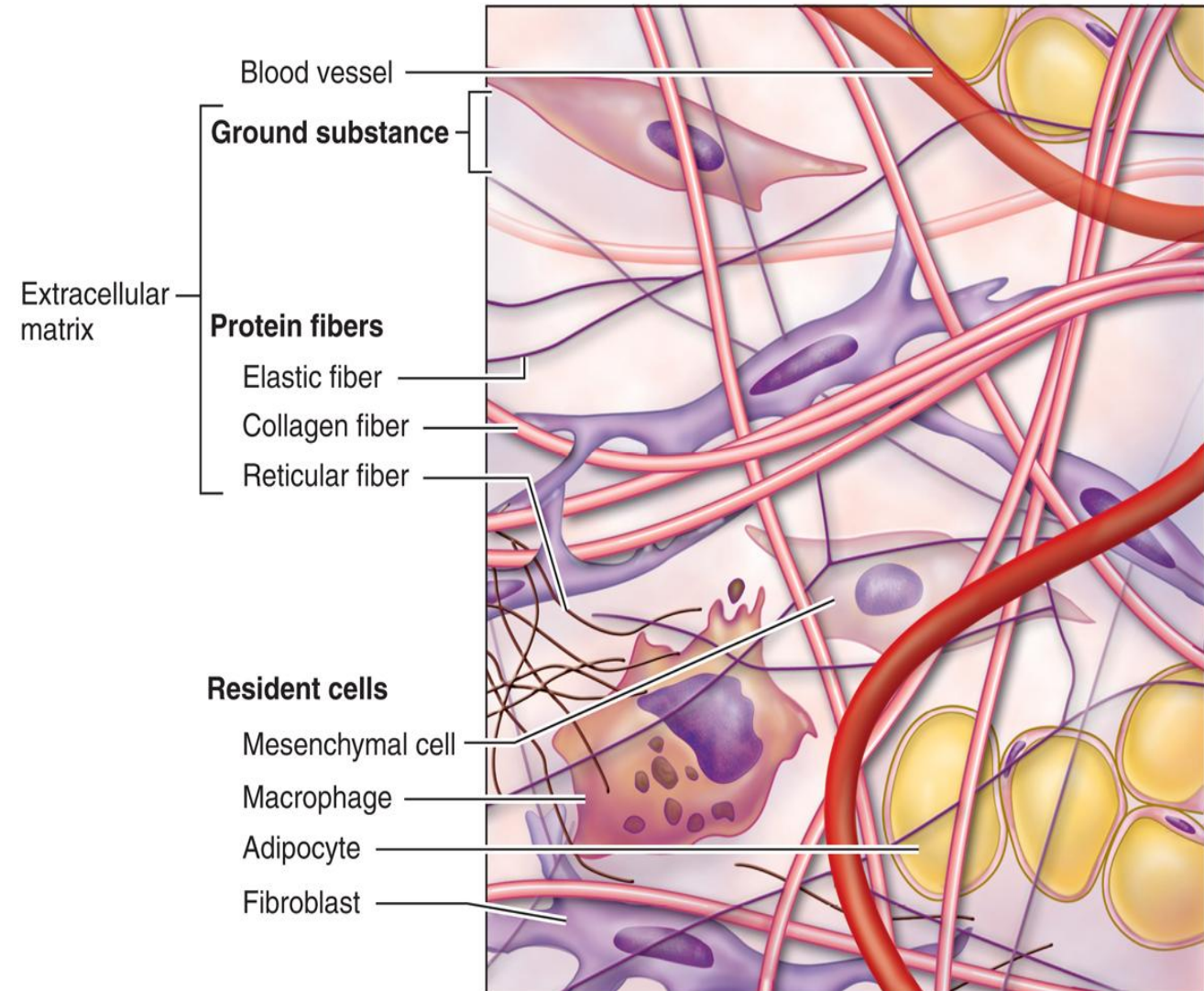
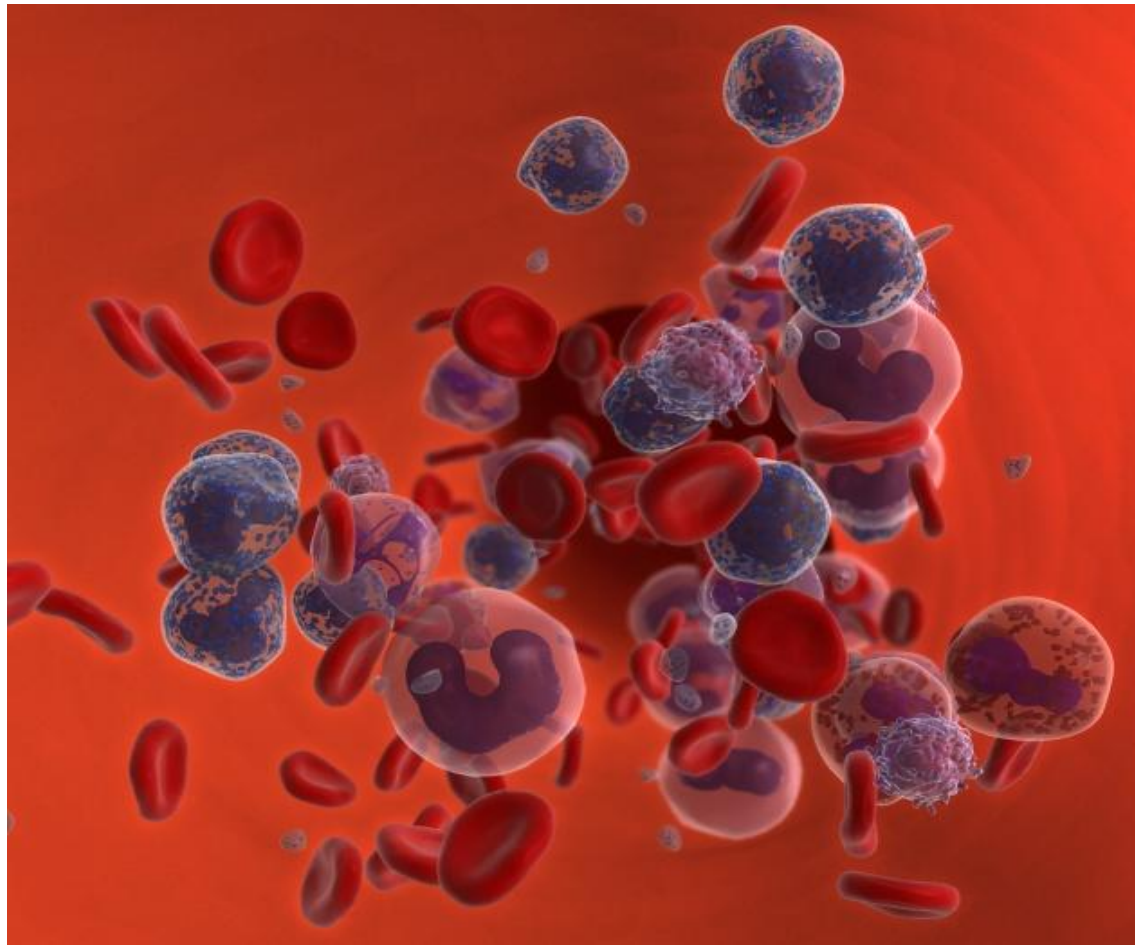
Blood Cells

Dr. Heba Kalbounch
DDS, MSc, DMD/PhD
Professor of Anatomy, Histology and Embryology

*Prepared and adapted for teaching by Prof. Dr. Heba Kalbounch.
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Blood

- Specialized form of **connective tissue**
- Components:
 - Blood cells (several types)
 - Plasma (extracellular matrix)



Functions of Blood

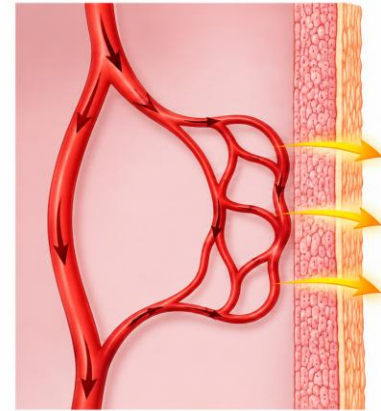
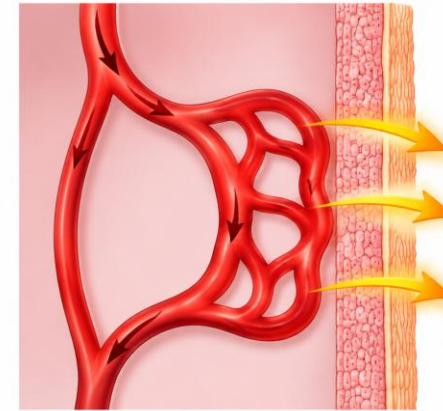
- Transports nutrients and respiratory gases.
- Transports waste products to organs and tissues where they can be recycled or eliminated.
- Transports hormones.
- Transports immune cells throughout the body.
- Helps regulate body temperature.
- Maintains acid–base and osmotic balance.

Characteristics of Blood

pH: Approximately 7.4

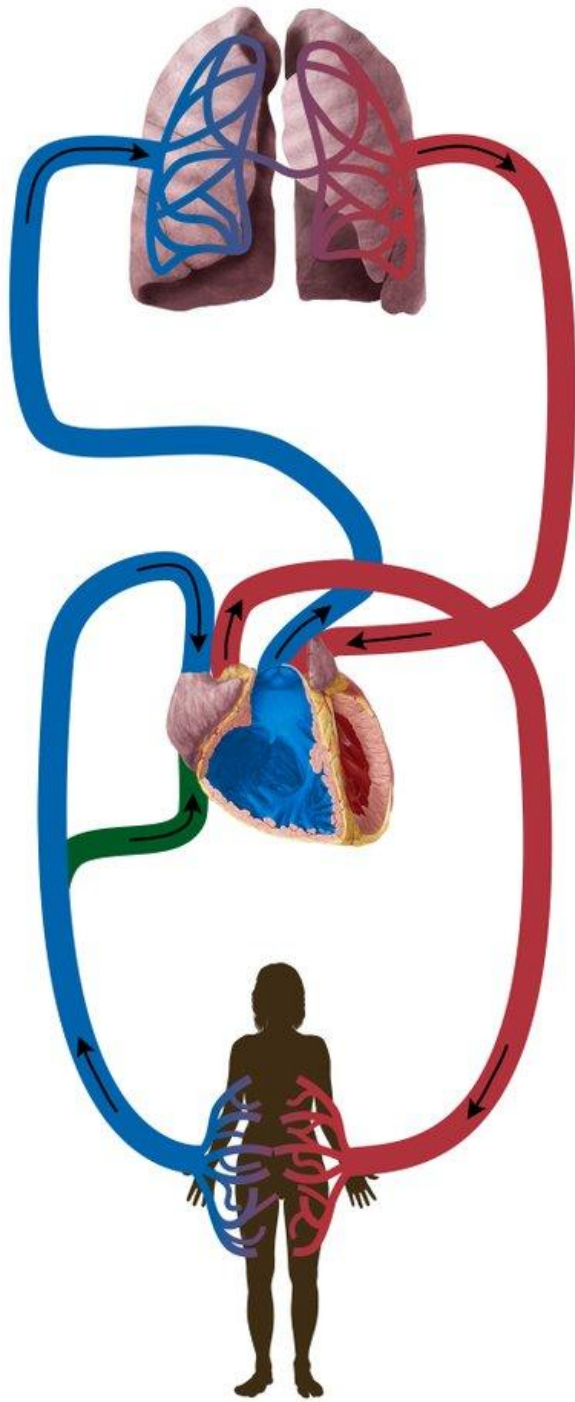
Color: Depends on the amount of oxygen:

- More oxygen → brighter red
- Less oxygen → darker/duller red



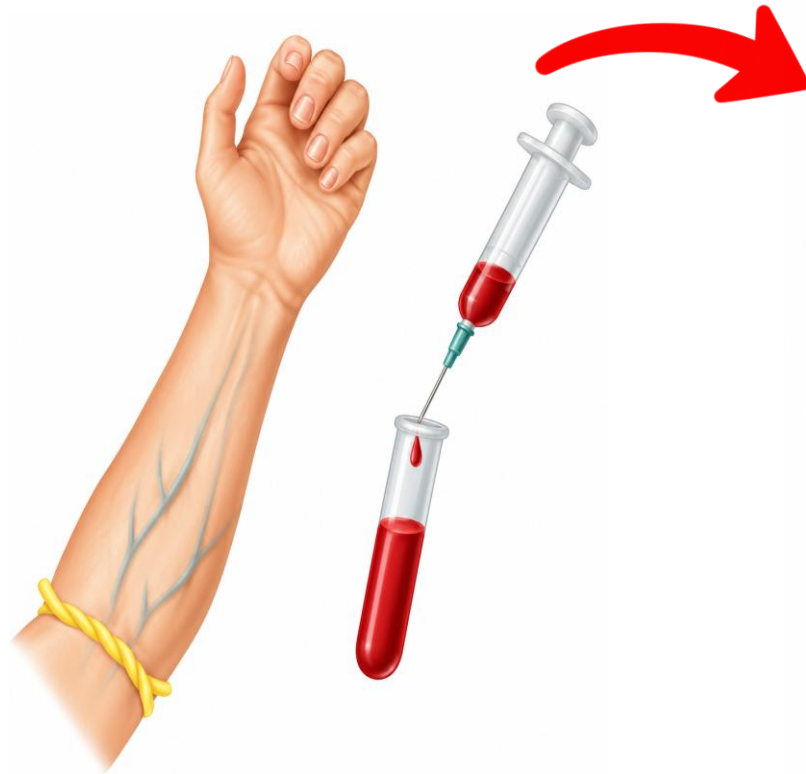
Blood Circulation

- ✓ Blood is propelled mainly by the rhythmic contractions of the heart.
- ✓ An average adult has approximately 5–6 liters of blood.
- ✓ Blood moves unidirectionally within the closed circulatory system.



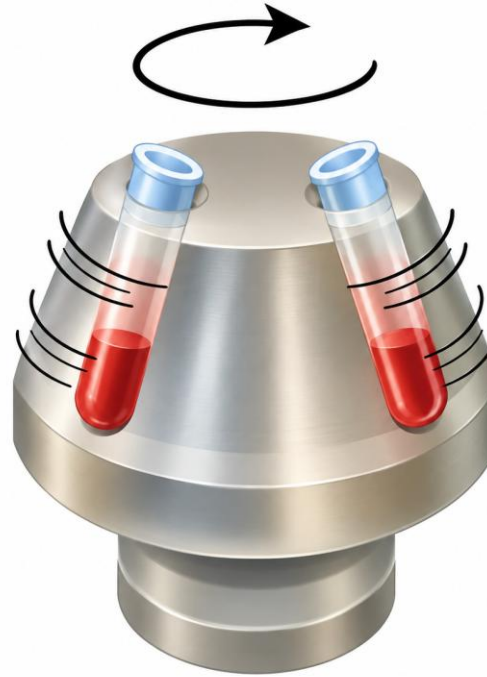
1

**Withdraw blood and
place in tube**



2

Centrifuge



**Plasma
(55% of whole blood)**

**Buffy coat:
Leukocytes and platelets
($<1\%$ of whole blood)**

**Erythrocytes
(44% of whole blood)**

**Formed
elements**

Blood collected with an anticoagulant (e.g., heparin, citrate, or EDTA) can be separated by centrifugation into distinct layers that reflect its heterogeneous composition.

Composition of Blood

Formed Elements ~45%

- **Erythrocytes (RBCs):** transport oxygen
- **Leukocytes (WBCs):** immune defense
- **Platelets (thrombocytes):** involved in blood clotting

Plasma ~55%

- Pale-yellow fluid component of blood
- ~90% water
- ~10% dissolved substances, including:
 - Plasma proteins (e.g., albumin)
 - Electrolytes
 - Nutrients
 - Metabolic waste products
 - Dissolved gases
 - Hormones

Serum = Plasma – Clotting Factors



Top layer
Plasma

Middle layer
Buffy coat

Bottom layer
RBCs
(Packed Cell Volume)

Hematocrit (Hct)

Hematocrit: The percentage of the total blood volume occupied by red blood cells (RBCs).

Example:

A hematocrit of 40% means that 40 mL of every 100 mL of whole blood consists of RBCs.

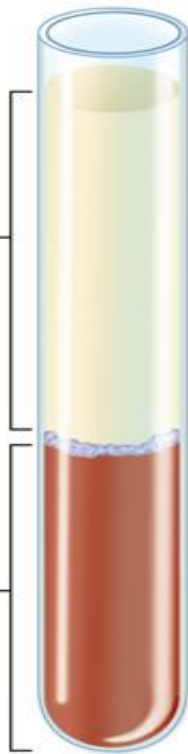
Normal hematocrit values:

- **Males: 40–53%**
- **Females: 36–48%**

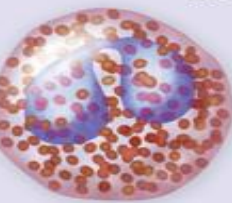
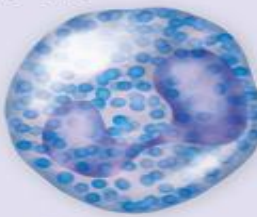
NOTE: A cubic millimeter of blood is equivalent to a microliter (μL)

Plasma		
Water 92% by weight	Proteins 7% by weight	Other solutes 1% by weight
	Albumins 58%	Electrolytes
	Globulins 37%	Nutrients
	Fibrinogen 4%	Respiratory gases
	Regulatory proteins 1%	Waste products

Erythrocytes
Erythrocytes 3.5-5.5 million/mm ³



Values are those used by the US National Board of Medical
Examiners

Buffy Coat	
Platelets 150,000-400,000/mm ³	Leukocytes 4500-11,000/mm ³
	 Neutrophils 54%-62%
	 Lymphocytes 25%-33%
	 Monocytes 3%-7%
	 Eosinophils 1%-3%
	 Basophils 0%-0.75%

Total leukocyte count (TLC): Total number of leukocytes per μL of blood.

Differential Leukocyte Count (DLC): Percentage of each leukocyte type among 100 leukocytes counted.

Albumin

- ✓ The most abundant plasma protein.
- ✓ Synthesized mainly by the liver.
- ✓ Helps maintain colloid osmotic (oncotic) pressure of the blood.
- ✓ Acts as a carrier protein, transporting substances such as: Steroid hormones and Fatty acids

Fibrinogen

- ✓ The largest major plasma protein.
- ✓ Synthesized by the liver.
- ✓ Plays an essential role in blood clotting.
- ✓ During clot formation, fibrinogen is converted into fibrin, which forms a mesh that helps stabilize the blood clot.

Fresh plasma



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Importance of Plasma Proteins

Plasma proteins help maintain the colloid osmotic (oncotic) pressure of blood, preventing excessive fluid loss from the blood vessels into the tissues.

→ help keep fluid within the blood vessels.

Globulins (α , β and or γ globulins):

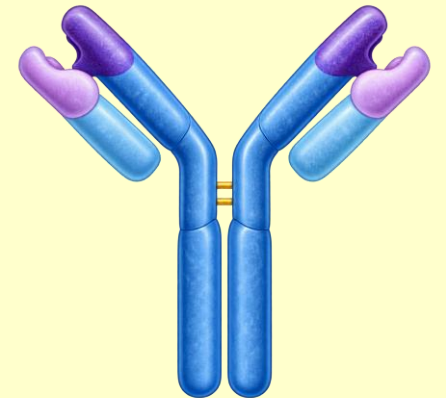
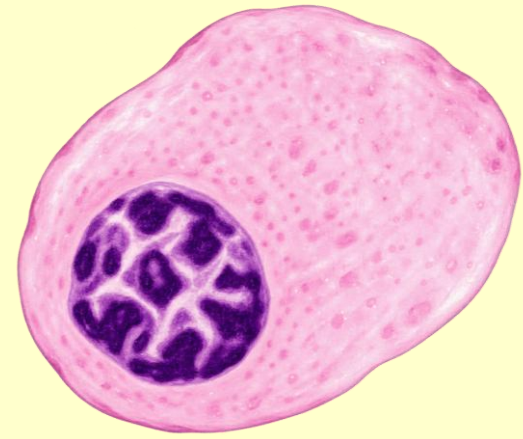
α - and β -Globulins

- ✓ Synthesized mainly by the liver.
- ✓ Function primarily as transport proteins.
- ✓ Transport substances such as: Lipids, Fat-soluble vitamins, and Iron → *Iron is transported mainly by transferrin, a β -globulin.*

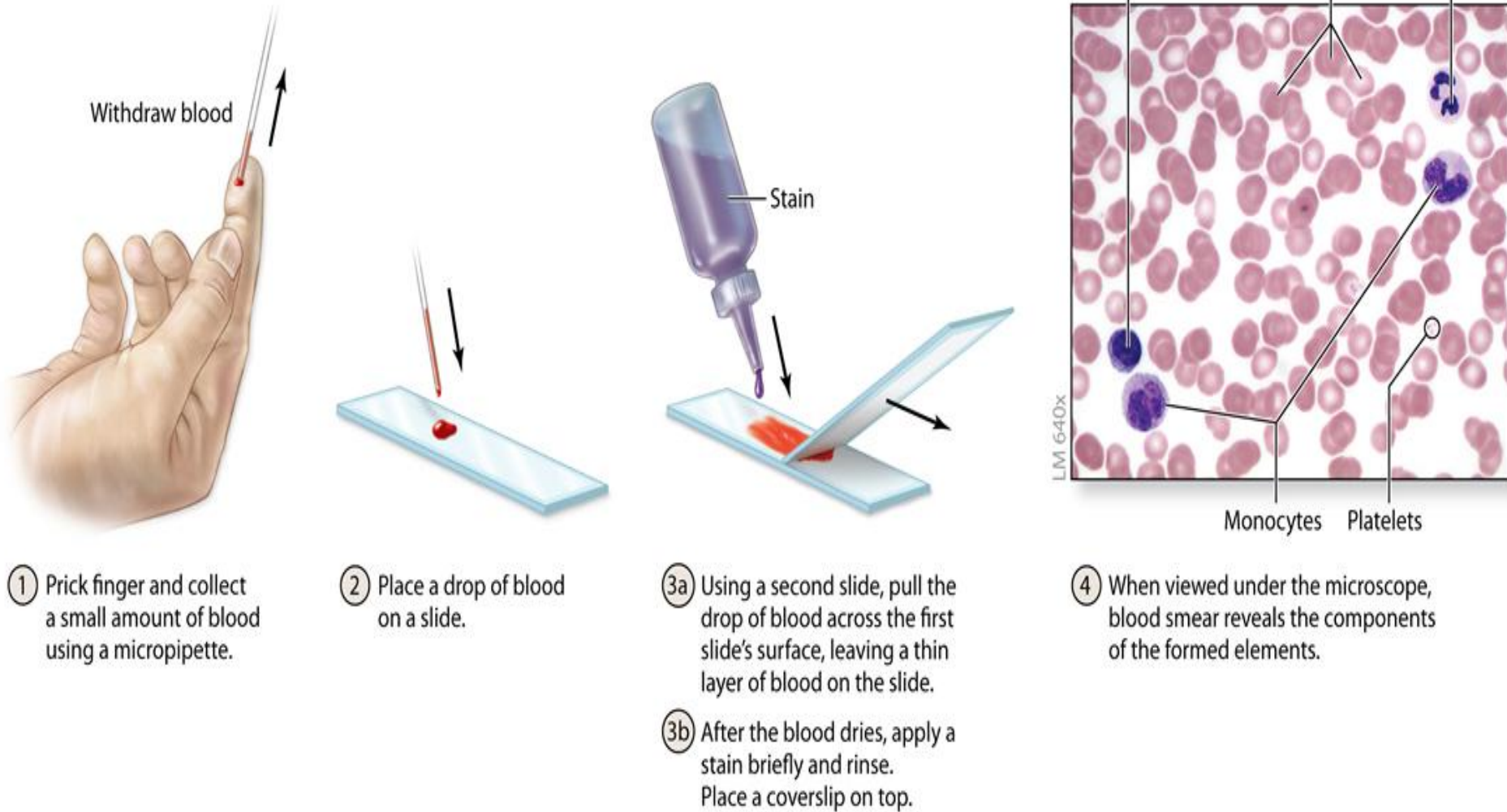
γ -Globulins (Immunoglobulins/Antibodies)

- ✓ Produced and secreted by plasma cells.
- ✓ Function as antibodies.
- ✓ Play an essential role in humoral immune defense.

Plasma cell



Preparing a blood smear



Polychromatic stains:
Giemsa
Wright
Leishman



Blood cells can be studied histologically in smears prepared by spreading a drop of blood in a thin layer on a microscope slide

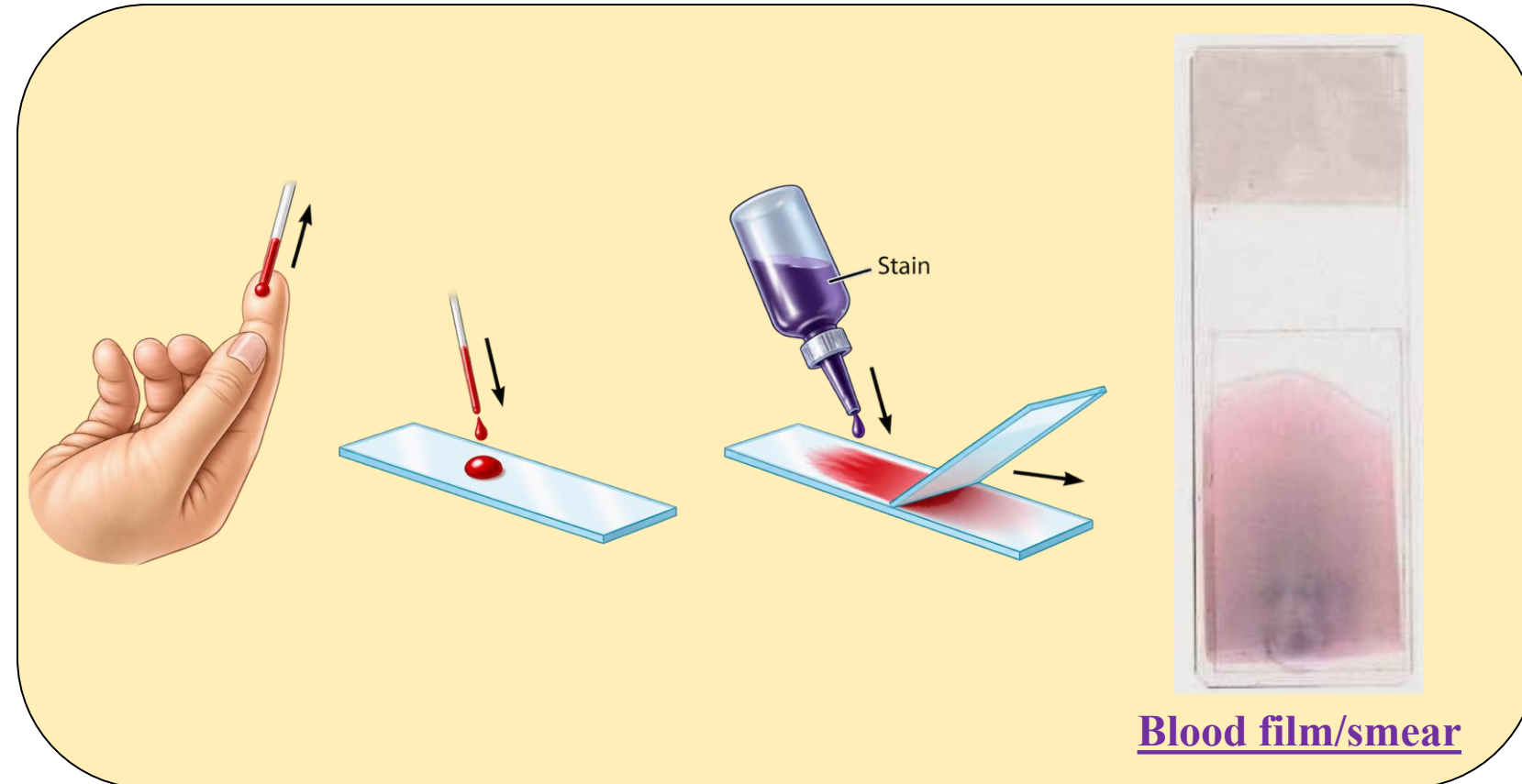
The Staining of Blood Cells

Blood film/ smear: A drop of blood is spread into a thin layer on a glass slide and allowed to air-dry.

Staining: with polychromatic stain e.g **Leishman's stain**

Leishman's stain: formed of a mixture of:

- **Eosin**, an acidic dye that stains pink to red
 - **Methylene blue**, a basic dye that stains blue to purple
- Azure dye!!
- Dissolved in methyl alcohol (fixative)



Polychromatic Stains

Commonly used for staining peripheral blood smears:

- Giemsa stain
- Wright stain
- Leishman stain

These are Romanowsky-type stains that use combinations of acidic and basic dyes to differentiate blood cells and their components.

Erythrocytes (RBCs)

- Small, biconcave discs.
- Specialized for the transport of O_2 and CO_2 .
- Cytoplasm is packed with hemoglobin.
- Mature RBCs have no nucleus or organelles.
- Pick up O_2 in the pulmonary capillaries and release it in tissue capillaries.
- Carry part of the CO_2 from tissues back to the lungs.

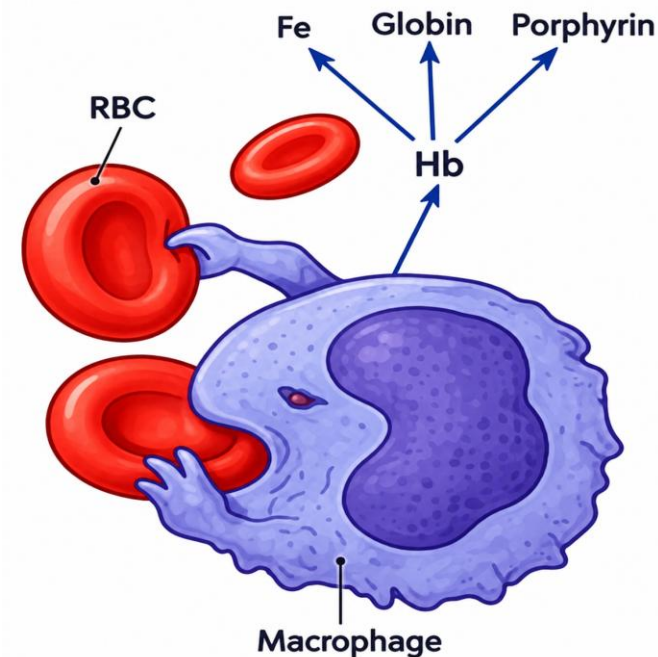
Male: 4.5-5.5 million/mm³
Female: 4-5 million/mm³



Life Span and Fate

- Circulate for approximately 120 days.
- Aged or damaged RBCs are removed by macrophages, mainly in the spleen, and also in the liver and bone marrow.
- Their components, particularly iron, are recycled.

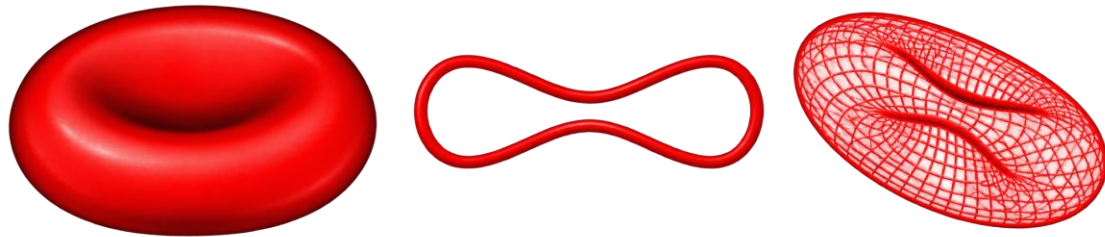
Note: During maturation, erythrocytes extrude their nuclei, progressively lose their cytoplasmic organelles and enter the bloodstream as anucleate cells.



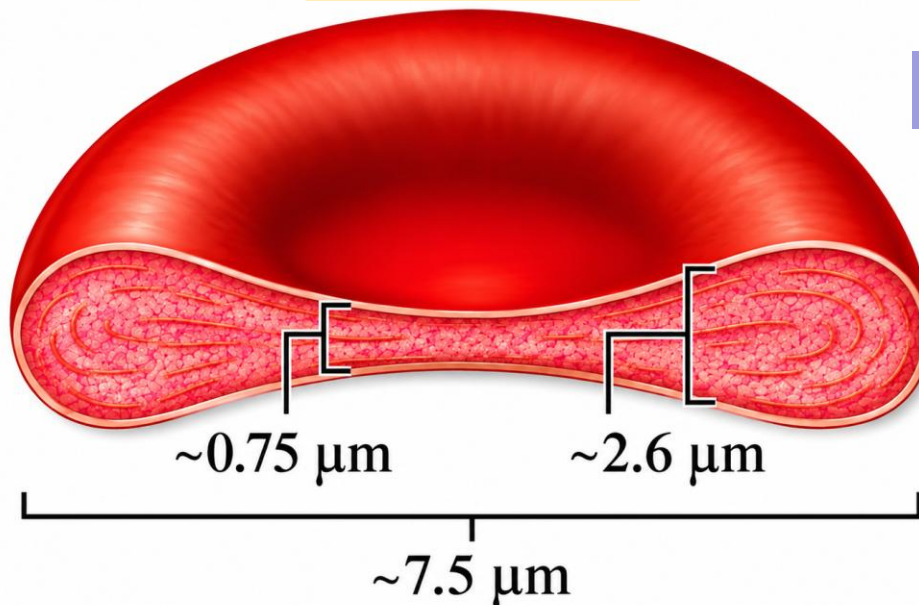
Immediately beneath the plasma membrane is a **meshwork of proteins** (Spectrin and Ankyrin) forming a cytoskeleton



This sub-membranous meshwork stabilizes the membrane, maintains the cell shape, and provides the cell elasticity required for passage through capillaries



Sectional view



Normocytic RBCs

Top view



The cell membrane is highly selective and flexible

Size

6-9 μm in diameter (7.5 μm)

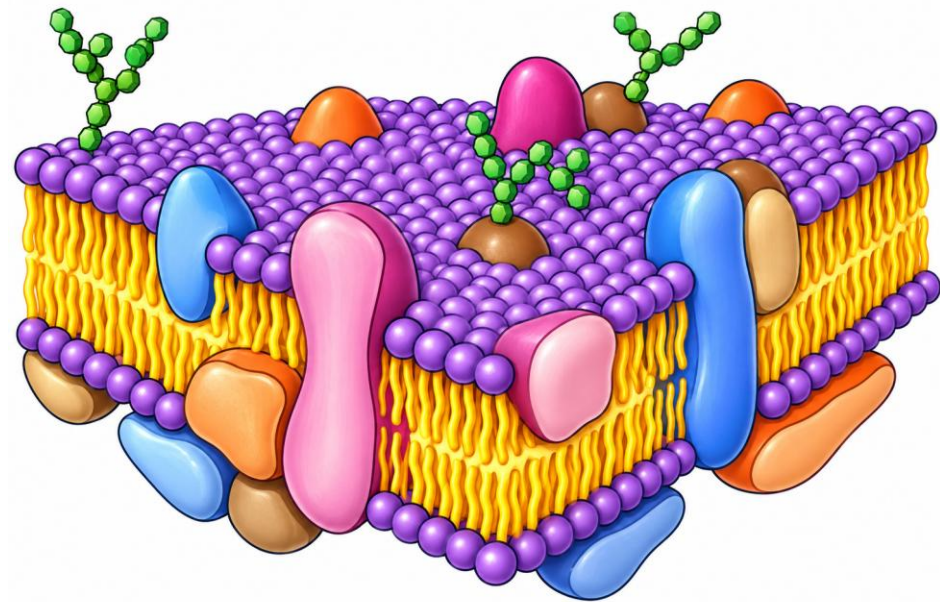
Thickness

2.6- μm thick at the rim, but only 0.75- μm thick in the center

!!!! Erythrocytes can be used as a size reference for other cell types

Biconcave shape provides 20-30% greater surface area than a sphere relative to cell volume, facilitating gas exchange

The biconcave shape, together with the flexibility of the plasma membrane and its underlying cytoskeleton, allows erythrocytes to deform and pass through narrow, tortuous capillaries.



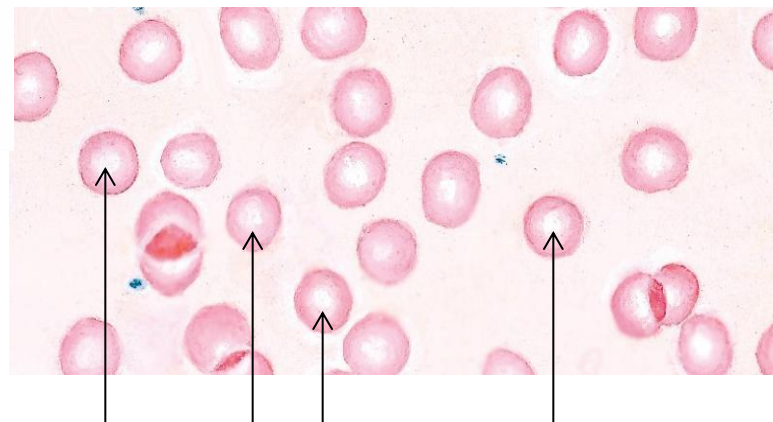
RBC membrane: approximately 50% protein, 40% lipid, and 10% carbohydrate by weight.

Lipid bilayer → provides fluidity

Erythrocyte consists of an **outer plasma membrane** enclosing hemoglobin and a **limited number of enzymes** necessary for maintenance of plasma membrane integrity and gas transport functions



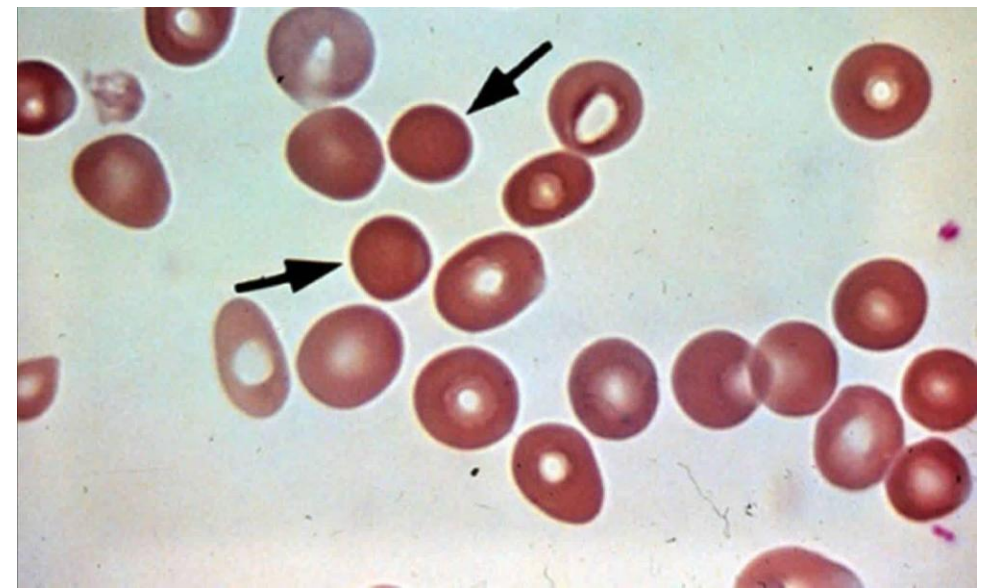
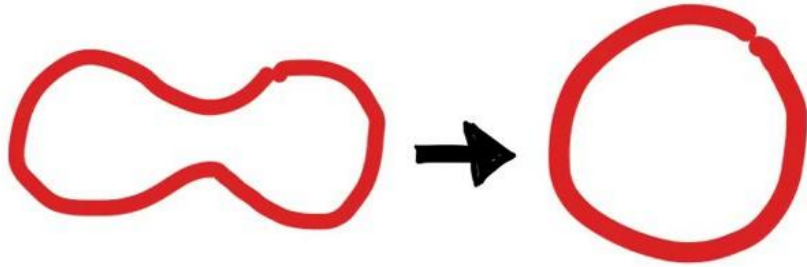
Eosinophilia/ acidophilia due to their high content of Hemoglobin (basic protein)



The pale staining of the central region is a result of its biconcave disc shape

Normochromic RBCs

Hereditary Spherocytosis



Hereditary spherocytosis: Caused by defects in RBC membrane cytoskeletal proteins (especially spectrin and ankyrin), resulting in loss of the normal biconcave shape and formation of spherical, less deformable RBCs.

In spherocytosis, membrane loss decreases the surface-area-to-volume ratio. The RBC becomes a sphere, with no central pallor

Spherocytosis → membrane loss → spherical RBC + loss of central pallor + increased splenic destruction.

BICONCAVE RBCs



SPHEROCYTES



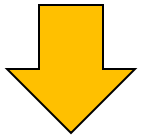
LM:

Blood film stained with Leishman:

Rounded

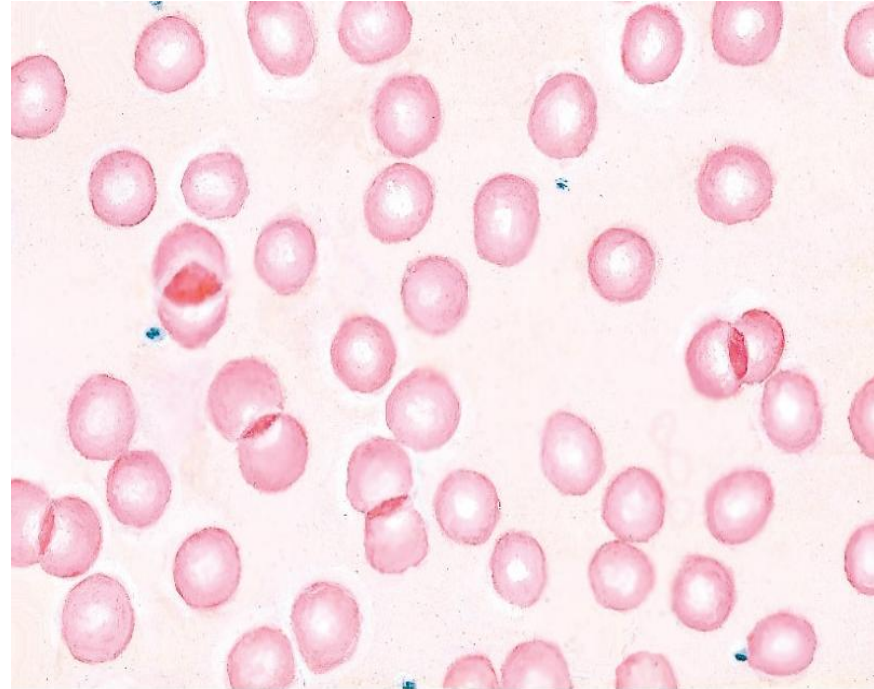
Non nucleated

Acidophilic (with pale central area)



A zone of **central pallor** is about 1/3 the size of the RBC

LM



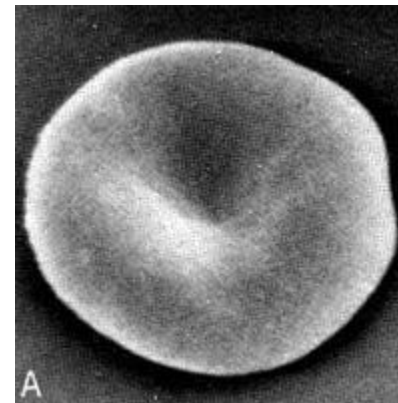
TEM Of Erythrocytes Passing Through Capillary Wall



TEM



SEM



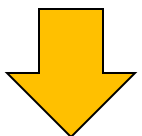
Single Erythrocyte squeezing through capillary



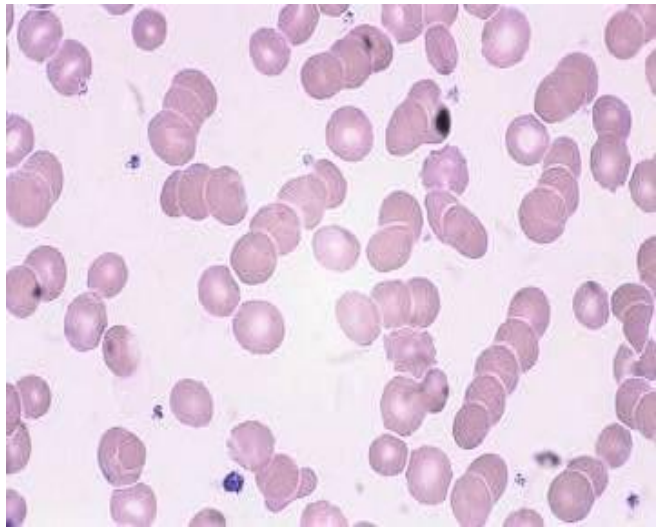
EM:

✓ Have no nucleus or organelles

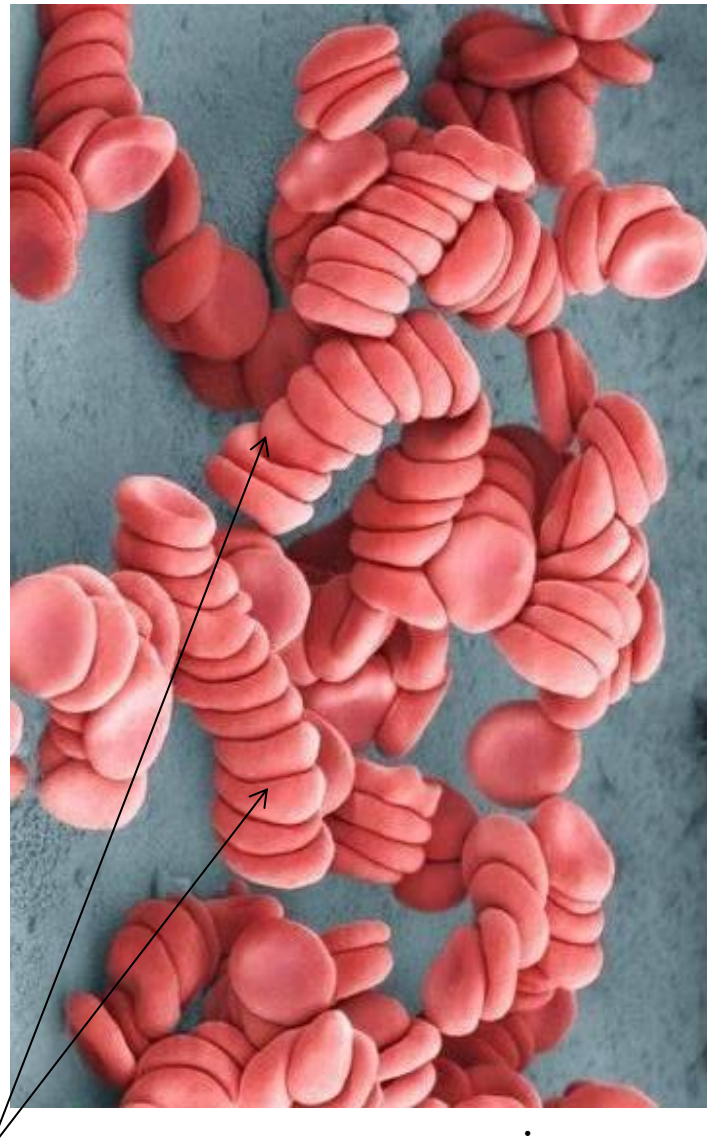
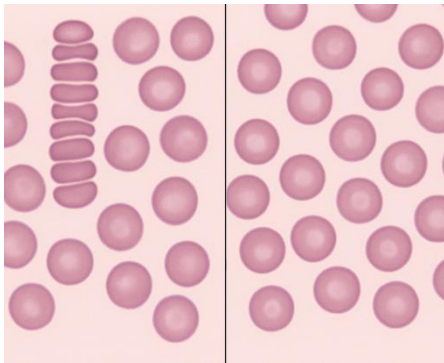
✓ Filled with hemoglobin



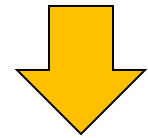
Electron dense and homogenous



Rouleaux appearance occurs to some extent in all films



Normally, RBCs tend to repel each other because their surfaces carry a negative charge (zeta potential).



When plasma proteins increase, they reduce this repulsive force, allowing RBCs to come together and form rouleaux.

Rouleaux formation: is the arrangement of red blood cells in stacks resembling a stack of coins.

✓ In slow (not in normal) circulation

Due to reduced repulsive forces (zeta potential) between erythrocytes, especially in the presence of increased plasma proteins such as fibrinogen and globulins (reversible)

Abnormalities of Erythrocytes

Change from the normal **size**, **shape** or **staining properties** of erythrocytes are **important indicator of disease**. However, some of these abnormalities may be found in healthy individuals

Abnormal sizes:

Microcytes (<6µm)

Macrocytes (>9µm)

Anisocytosis (different sizes)

Abnormal shapes:

Due to changes either in the cell membrane or Hb content

Poikilocytosis: variation in RBC shape

Abnormal staining:

Hypochromia: Decreased staining intensity of RBCs due to reduced hemoglobin, resulting in increased central pallor.

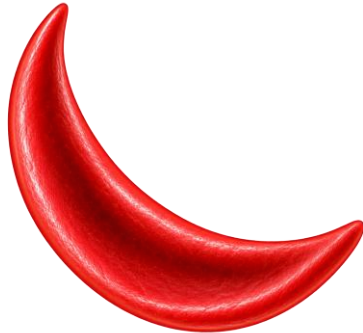
Hyperchromia: Increased staining intensity of RBCs with reduced or absent central pallor, typically due to altered RBC shape, as seen in spherocytes.

Poikilocytosis = abnormal RBC shape
Anisocytosis = abnormal RBC size

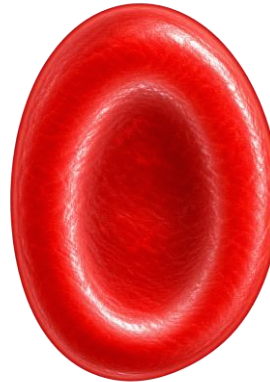
Poikilocytes (Poikilocytosis)



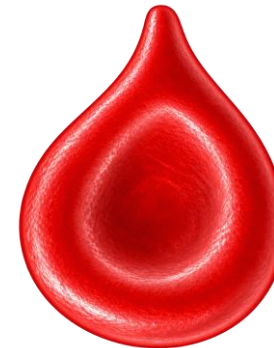
Spherocytes



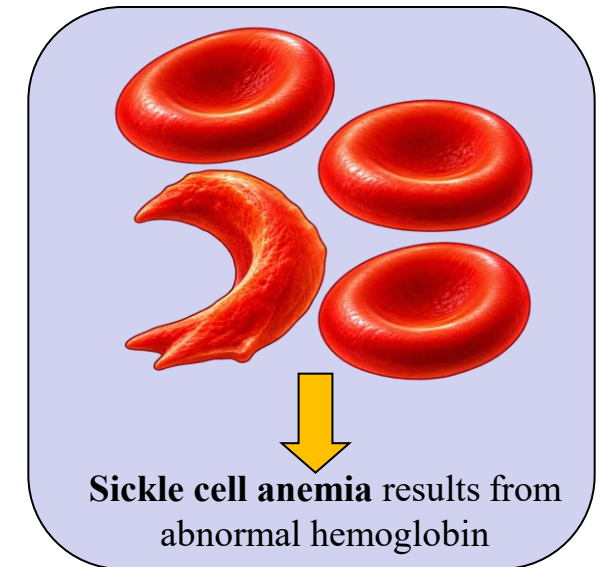
Sickle cells



Ovalocytes
Elliptocytes



Dacrocytes → Teardrop shaped cells



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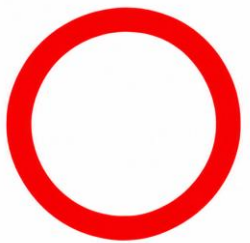
One of the most severe changes in shape occurs during SICKLING of RBCs in sickle cell anemia where erythrocytes take on the form of crescents

Based on Hemoglobin Content (color /chromasia):

Normochromic: RBCs have a normal hemoglobin concentration → exhibit normal pink staining with central pallor occupying approximately one-third of the cell diameter.

Hypochromic: RBCs have reduced hemoglobin concentration → appear paler with increased central pallor (commonly seen in iron deficiency anemia).

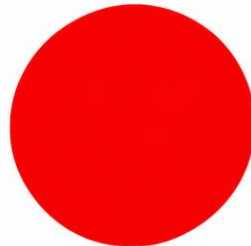
Hyperchromic: RBCs exhibit increased staining intensity with reduced or absent central pallor, often associated with increased hemoglobin concentration, as seen in hereditary spherocytosis.



Hypochromic



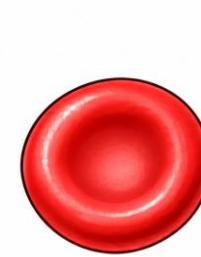
Normochromic



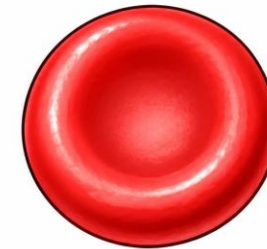
Hyperchromic

Together, these terms are used to describe and classify anemias (e.g., microcytic hypochromic anemia in iron deficiency, or macrocytic normochromic anemia in B12 deficiency).

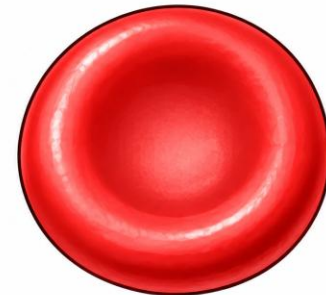
Note: Hypochromia frequently accompanies microcytosis, resulting in **hypochromic microcytic anemia**, as commonly seen in iron deficiency anemia.



Microcytic



Normocytic



Macrocytic

Based on Cell Size (cytometry):

Normocytic: RBCs are of normal size.

Microcytic: RBCs are smaller than normal (seen in iron deficiency anemia).

Macrocytic: RBCs are larger than normal (seen in vitamin B12 or folate deficiency anemia).

When RBC count or blood oxygen-carrying capacity decreases, such as during blood loss, tissue hypoxia develops.



Hypoxia is detected by the kidneys.



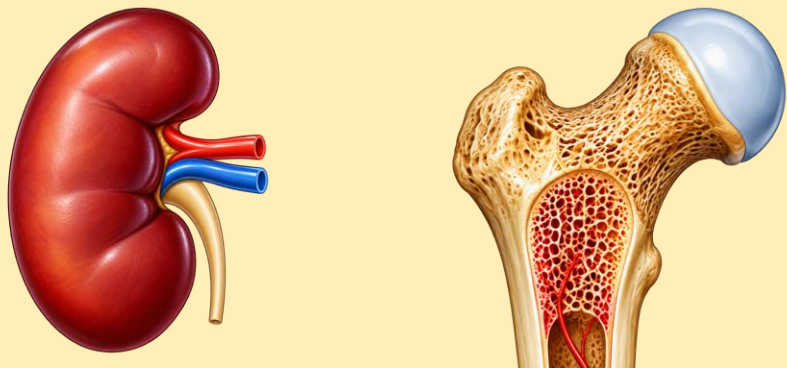
The kidneys respond by increasing the production and secretion of **erythropoietin (EPO)**.



Erythropoietin stimulates erythropoiesis in the red bone marrow.



RBC production increases, improving the oxygen-carrying capacity of the blood.



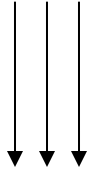
At high altitudes, lower oxygen levels stimulate erythropoietin production, leading to an increased RBC count.



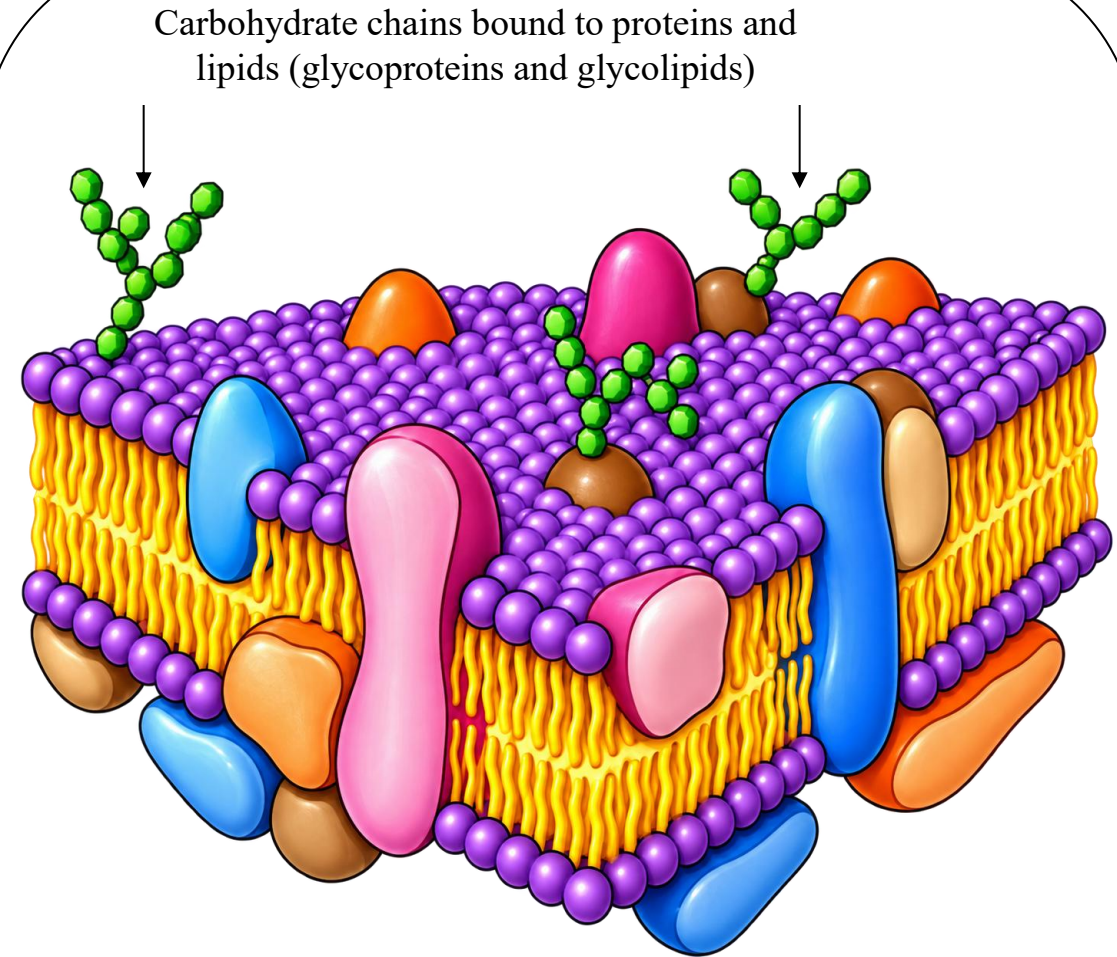
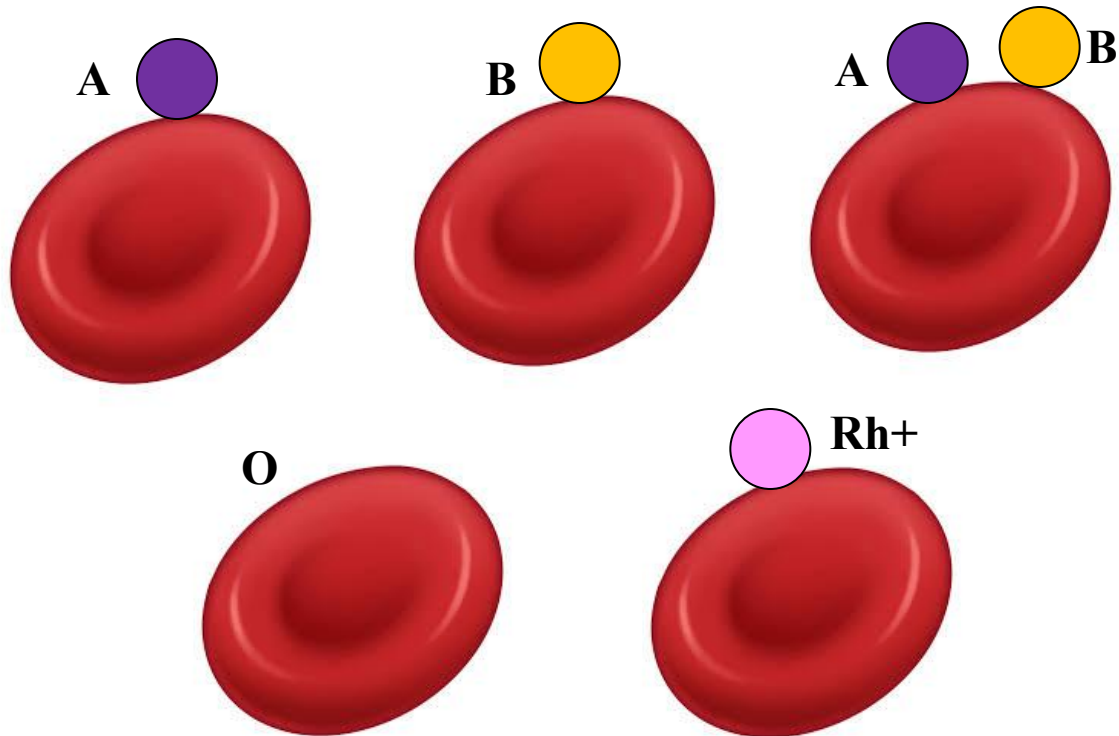
Endurance athletes may have an increased red blood cell production to support greater oxygen demands.



RBC plasma membranes carry carbohydrate antigens (A and/or B antigens) on their external surface.



These carbohydrate antigens are attached to glycoproteins and glycolipids in the RBC membrane.



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The ABO blood group system consists of four main blood groups: A, B, AB, and O. These groups are determined by the presence or absence of specific carbohydrate antigens (A and B antigens) on the surface of RBCs.

	RBCs (Erythrocytes)	WBCs (Leukocytes)
Types	1 type	5 types
Nucleus	Anucleate	Nucleated
Number	Male: ~4.5–5.5 million/mm ³ ; Female: ~4.0–5.0 million/mm ³	~4,500–11,000/mm ³
Diameter	~6–9 µm average ~7.5 µm	~7–20 µm, depending on type
Life span	~120 days	Hours/days to years, depending on type
Origin and maturation	Red bone marrow	Red bone marrow and lymphoid tissue
Shape	Biconcave discs	Variable; generally spherical in blood
Function	Transport of O ₂ and CO ₂	Defense and immunity
Motility	Non-motile	Motile
Location/ function	Function exclusively within vascular system	Function mainly OUTSIDE blood vessels in the tissues

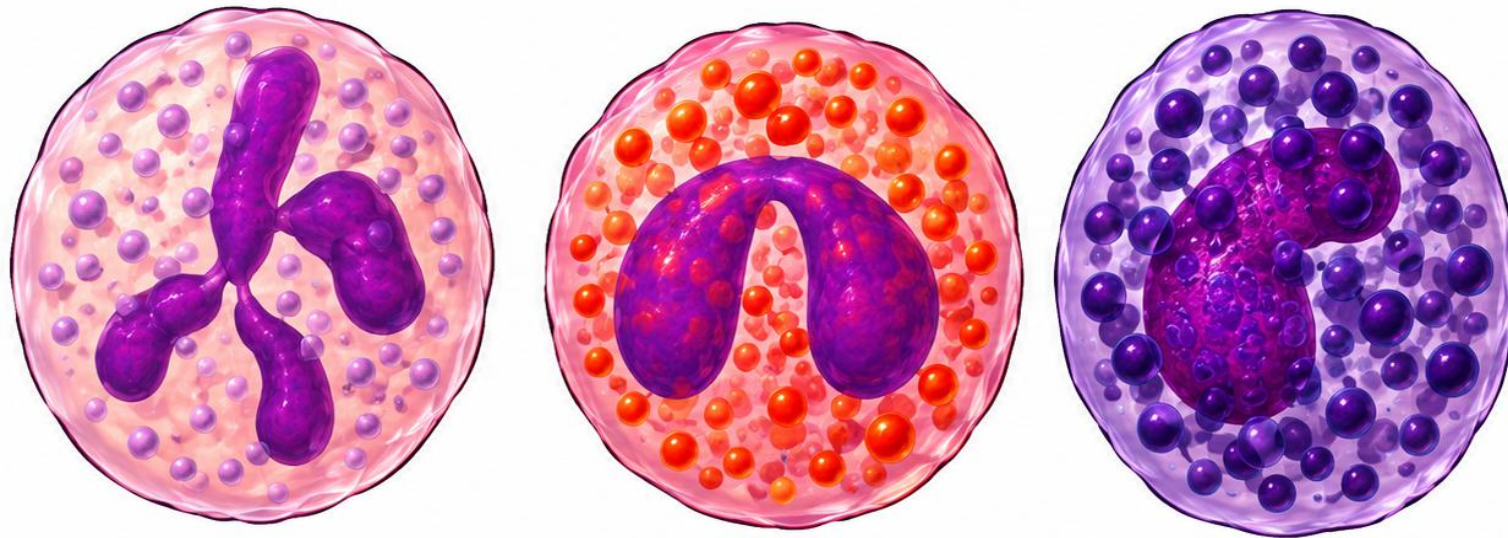
Leukocytes (White Blood Cells)

- Originate in the bone marrow and released into the blood
- Travel in bloodstream but function mainly **outside** blood vessels (in loose CT)
- Form a mobile defense system that protects the body against bacteria, viruses, parasites, toxins, and tumor cells.

Note: Agranulocytes are not literally devoid of all granules. They lack the prominent specific granules characteristic of granulocytes, although lymphocytes and monocytes can contain azurophilic granules (lysosomes).

Leukocytes, or WBCs, are nucleated and subdivided into granulocytes and agranulocytes, depending on the presence or absence of **specific** granules in their cytoplasm.

Granulocytes

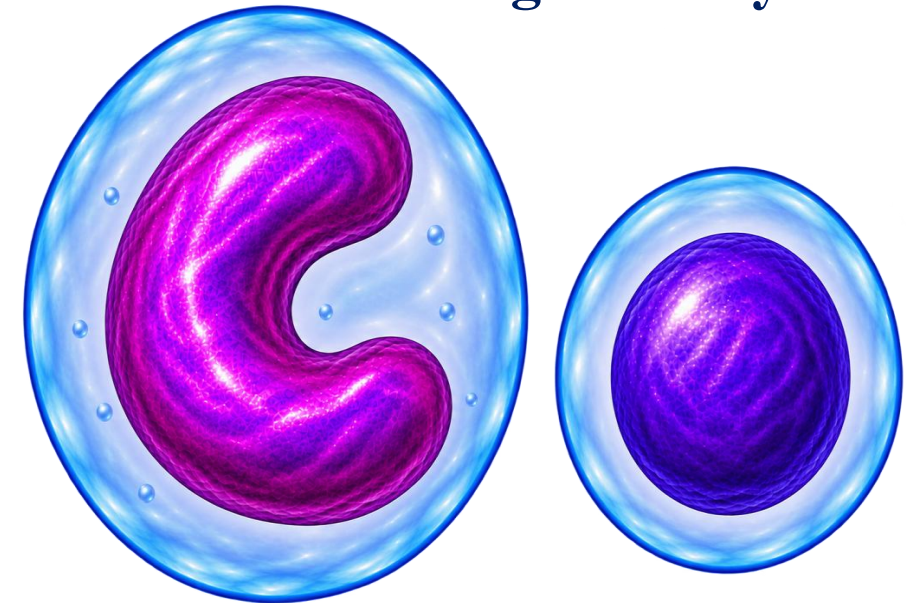


Neutrophil

Eosinophil

Basophil

Agranulocytes



Monocyte

Lymphocyte

Cytoplasmic granules

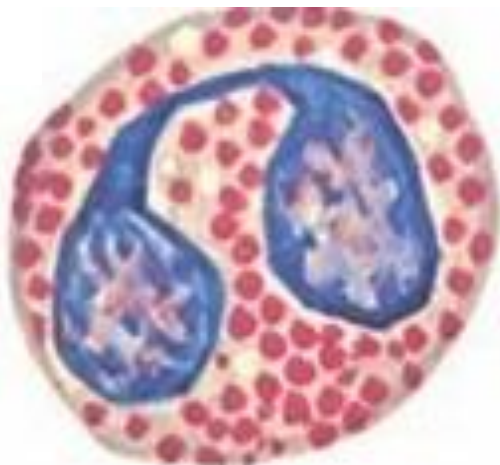
Specific granules
Secondary granules

Non-specific granules
Azurophilic granules
Lysosomes
Primary granules

Granulocytes

- Cytoplasmic granules (containing enzymes or chemicals) → makes cytoplasm look grainy
- Have a single, segmented (multilobed) nucleus.
- All are phagocytic; Phagocytic ability varies: prominent in neutrophils, limited in eosinophils, and very limited in basophils.

Large granules, Red



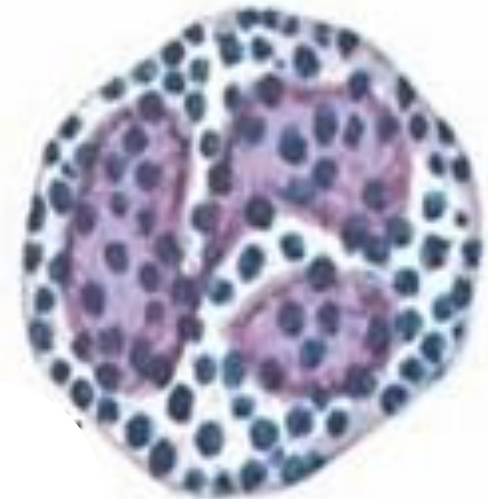
Eosinophil

**Small granules, pale pink/
salmon pink/ pale lilac**



Neutrophil

Large granules, blue



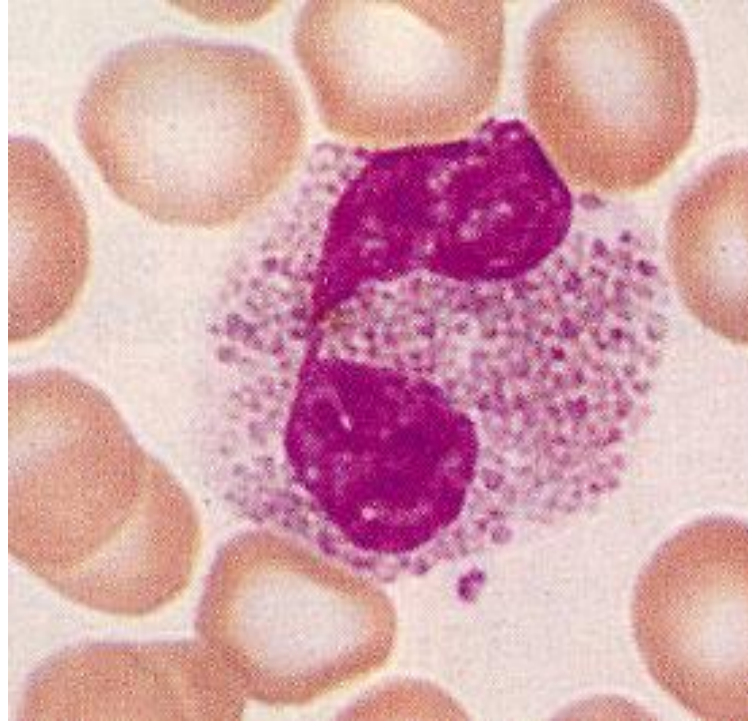
Basophil

Neutrophil



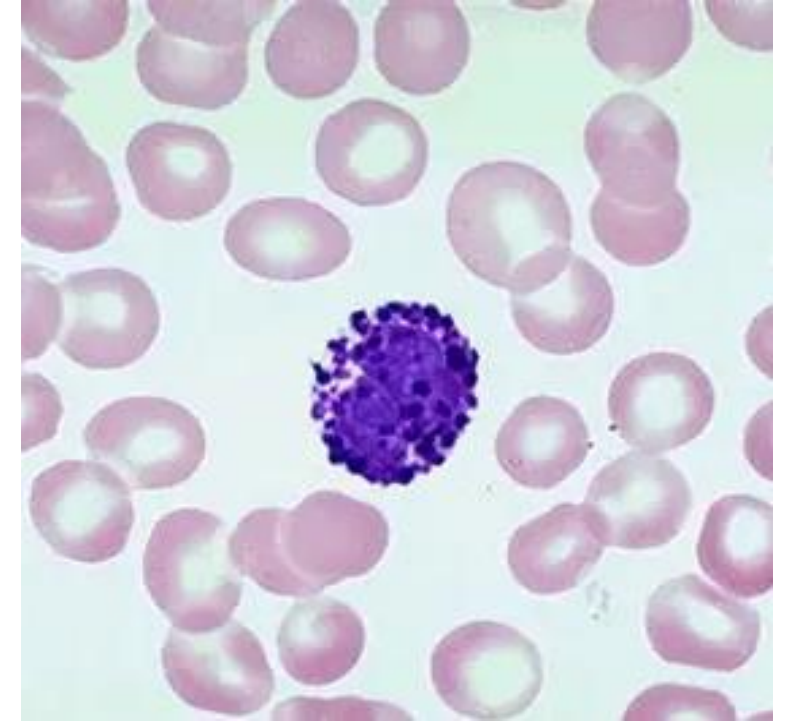
These granules are neutrophilic, meaning they show no special affinity for either acidic or basic stains but are stained mildly by both

Eosinophil



These specific granules stain red with acidic stains

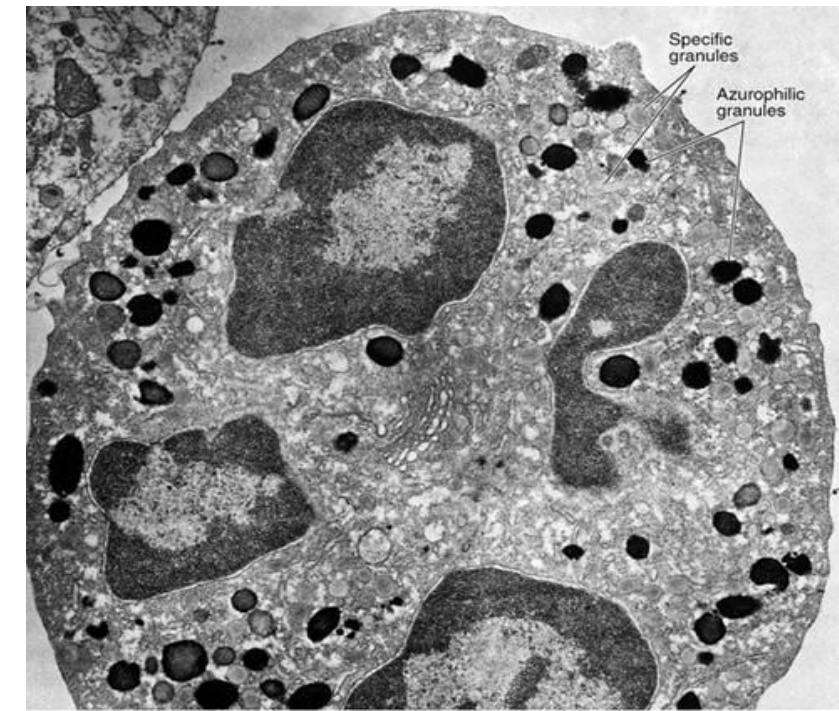
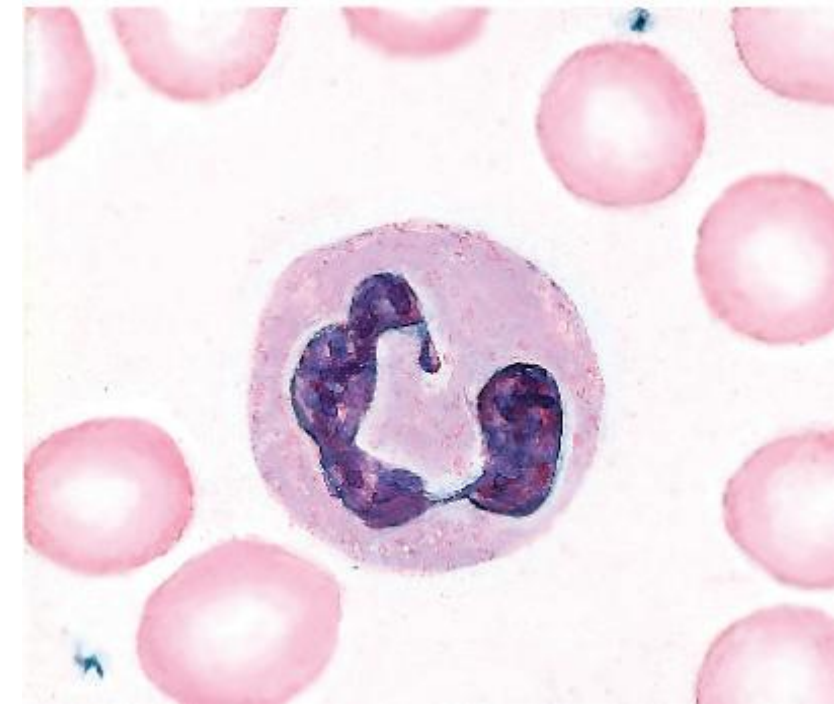
Basophil



These specific granules stain with basic stains

Neutrophils

- The most common leukocyte
- **Nucleus:** Typically consists of 2–5 lobes (most commonly 3), connected by thin strands of chromatin. Therefore, neutrophils are also called polymorphonuclear leukocytes (**PMNs or polymorphs**).
- **Cytoplasm:** Pale pink or pale lilac, containing numerous fine, specific granules that are often difficult to distinguish individually under the light microscope.
- Called neutrophils because cytoplasm takes up red (acidic) and blue (basic) stains equally
- **Function:** Specialized for rapid defense against bacterial infections, particularly acute pyogenic (pus-forming) bacterial infections, through phagocytosis and microbial killing.
- **Lifespan:** Neutrophils are relatively short-lived cells. Traditionally, their circulating half-life is estimated at approximately 6–8 hours, and they may survive for about 1–2 days in tissues. They are generally eliminated through apoptosis followed by phagocytosis by macrophages.



Specific granules (secondary)

Smaller, more numerous, formed later in development.

Lysozyme

(destroys bacterial cell walls)

Lactoferrin

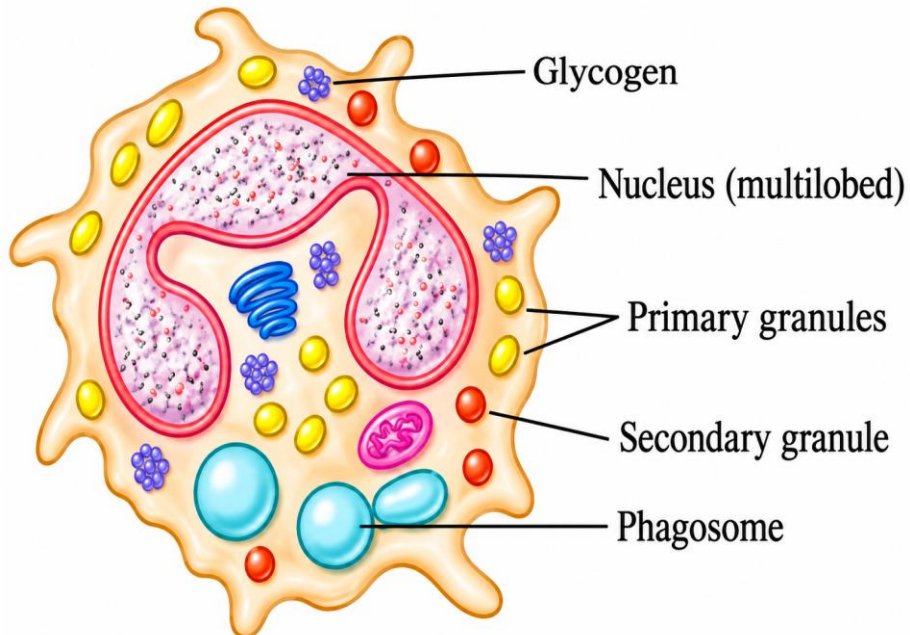
(sequesters iron and inhibits bacterial growth → bacteriostatic)

Collagenase

(degrades collagen in the ECM)

NADPH oxidase

(produces superoxide anions (O_2^-))



Azurophilic granules (primary)

Larger, formed early in neutrophil development.

Myeloperoxidase

(generates hypochlorous acid ($HOCl$) from hydrogen peroxide (H_2O_2) and chloride ions (Cl^-) → a powerful antimicrobial substance)

Acid hydrolase

(digests microbial components and cellular debris following phagocytosis)

Defensins

(disrupts microbial membranes)

Protease (eg. Elastase)

(degrades microbial proteins, degrades ECM proteins, including elastin)

Different names for neutrophils:

Polymorphs

Polymorphonuclear leukocytes (PMNs)

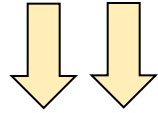
Pus cells

Microphages

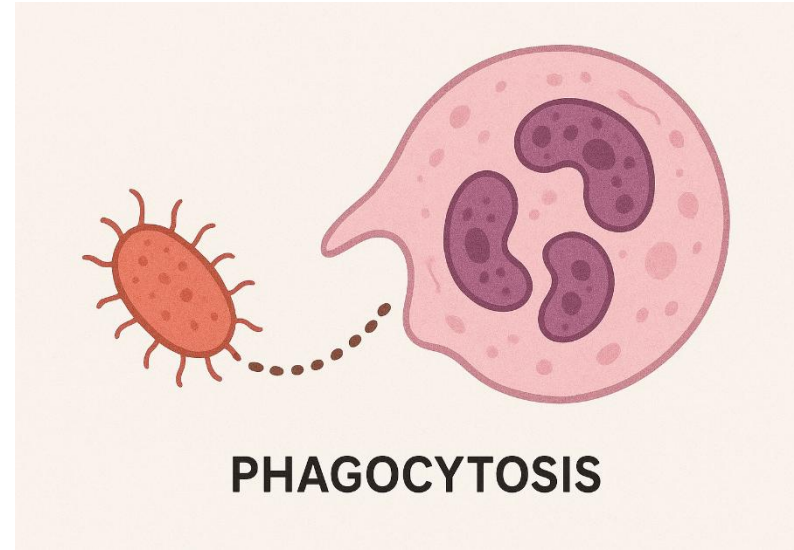
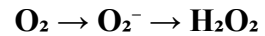
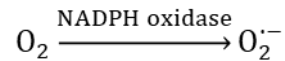
Cells of acute inflammation

Oxygen-dependent killing:

The neutrophil undergoes a ‘respiratory burst’—activating NADPH oxidase to produce *reactive oxygen species*. Hydrogen peroxide is formed, and with the help of myeloperoxidase, it generates hypochlorous acid.

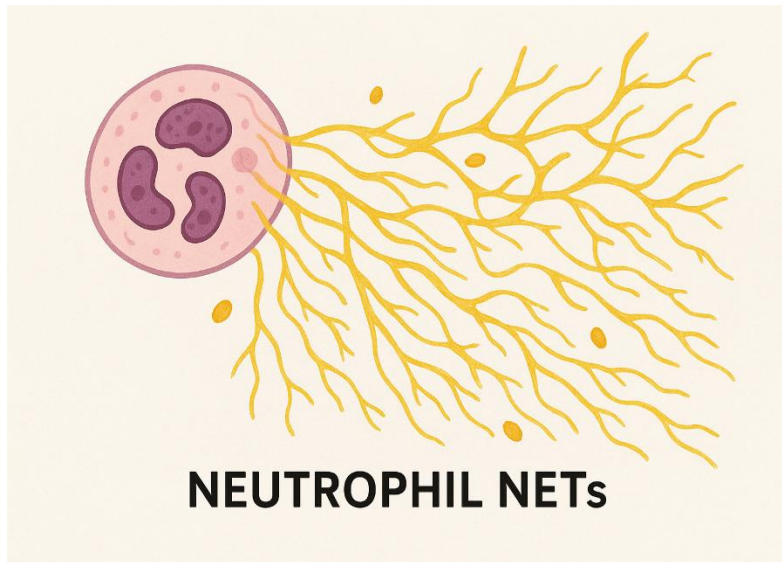


This is extremely toxic to microbes.

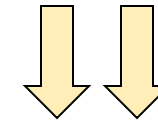


Oxygen-independent killing:

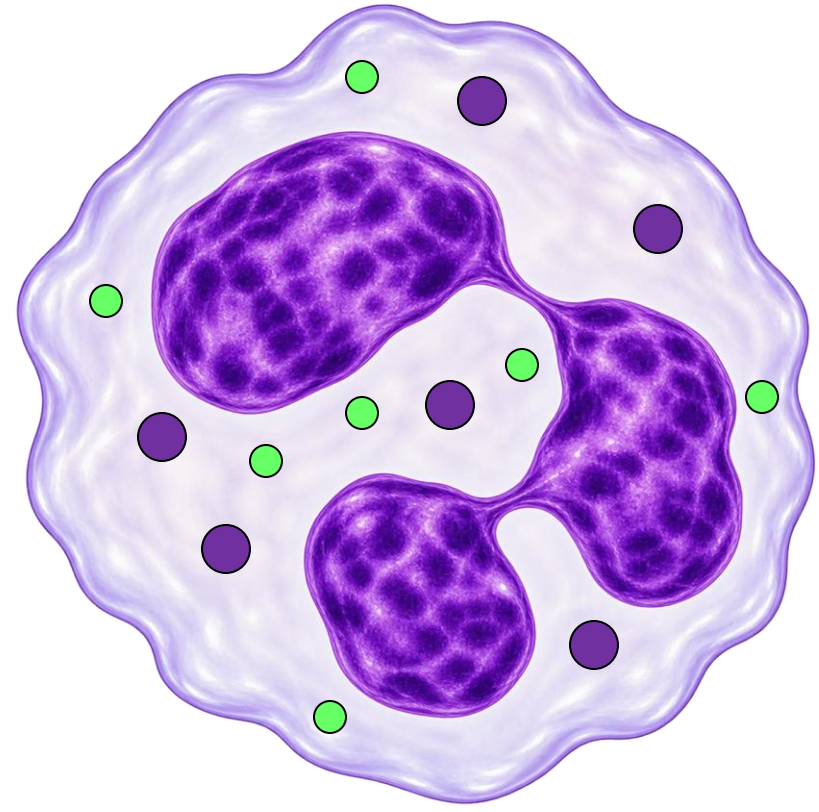
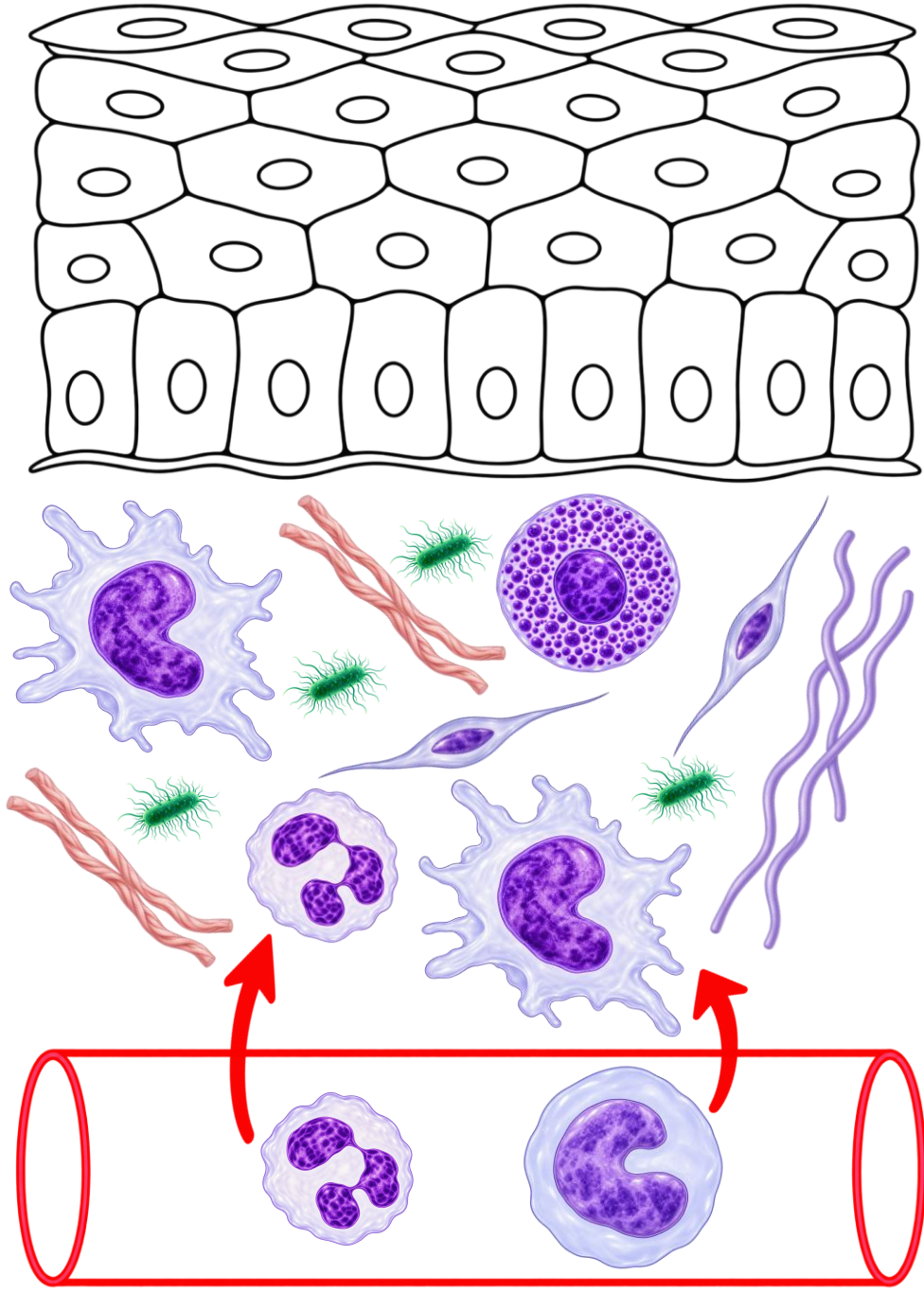
Neutrophil granules fuse with the phagosome and release antimicrobial substances that kill and degrade engulfed microorganisms without requiring reactive oxygen species.



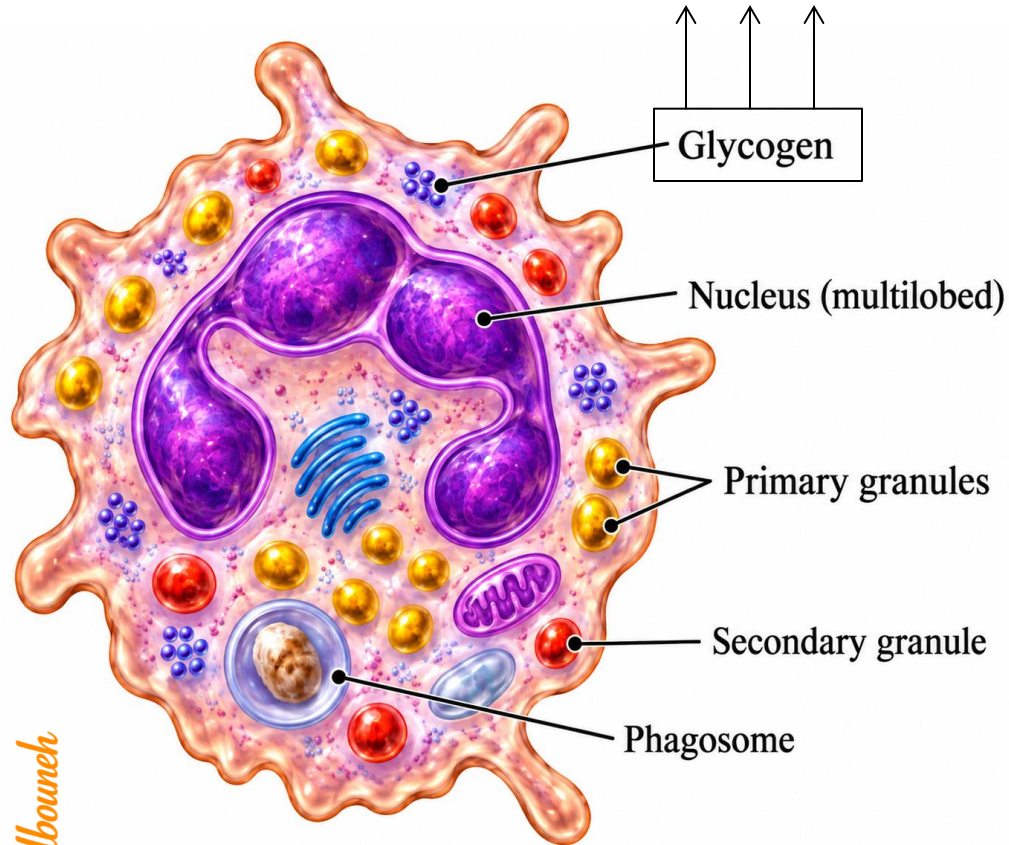
the **Neutrophil Extracellular Trap**, or **NET**. When overwhelmed, a neutrophil can undergo NETosis—a unique cell death where it releases its DNA coated with antimicrobial proteins.



This sticky web traps and kills microbes outside the cell, preventing them from spreading.



The ability of neutrophils to survive in an anaerobic environment is highly advantageous, because they can kill bacteria and help clean up debris in poorly oxygenated regions, for example, damaged or necrotic tissue lacking normal microvasculature.



Few organelles

Neutrophilia
Neutropenia

Dead neutrophils, bacteria, lysed ECM, and tissue-fluid form a viscous, usually yellow collection of fluid called **pus**.



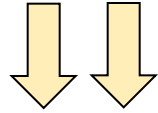
Pyogenic: Refers to infections, usually bacterial, that produce pus.

Pyrogenic: Refers to substances or processes that produce fever.

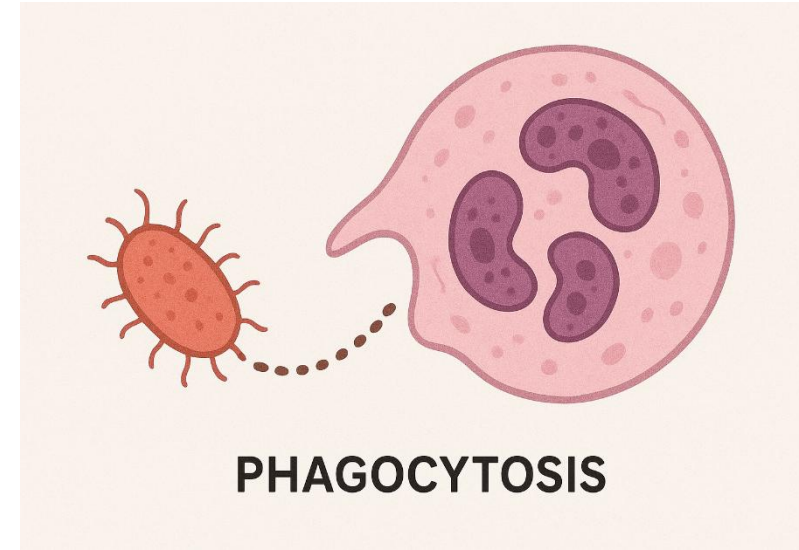
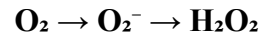
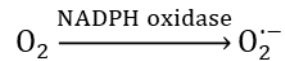
Pyo- → pus
Pyro- → fever/heat

Oxygen-dependent killing:

The neutrophil undergoes a ‘respiratory burst’—activating NADPH oxidase to produce reactive oxygen species. Hydrogen peroxide is formed, and with the help of myeloperoxidase, it generates hypochlorous acid.

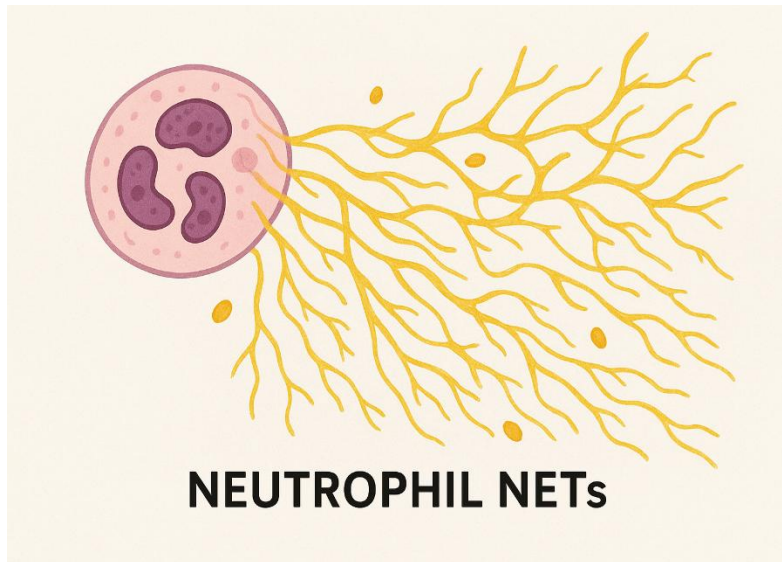


This is extremely toxic to microbes.

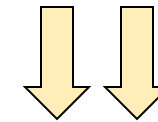


Oxygen-independent killing:

Neutrophil granules fuse with the phagosome and release antimicrobial substances that kill and degrade engulfed microorganisms without requiring reactive oxygen species.



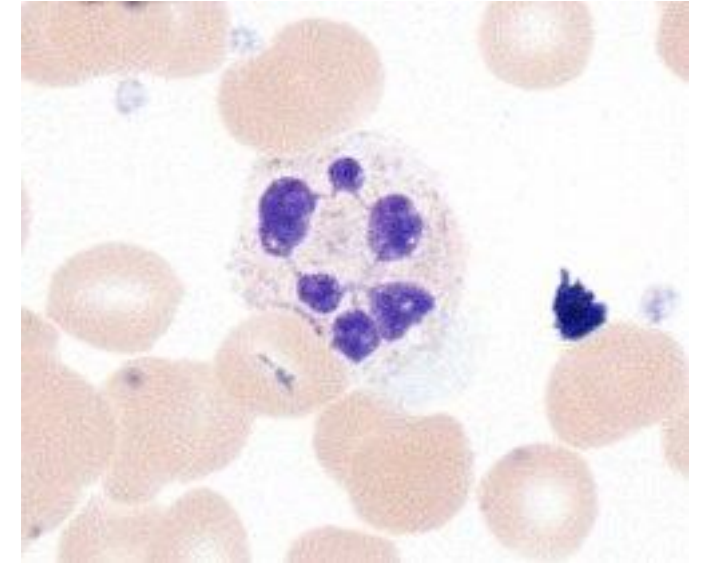
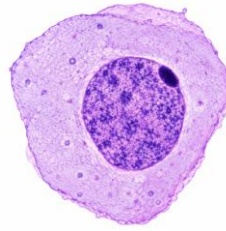
the **Neutrophil Extracellular Trap**, or **NET**. When overwhelmed, a neutrophil can undergo NETosis—a unique cell death where it releases its DNA coated with antimicrobial proteins.



This sticky web traps and kills microbes outside the cell, preventing them from spreading.



In females, the inactive X chromosome (**Barr body**) may appear as a drumstick-like appendage on one of the lobes of the nucleus (*about 3% of neutrophils in peripheral blood*)



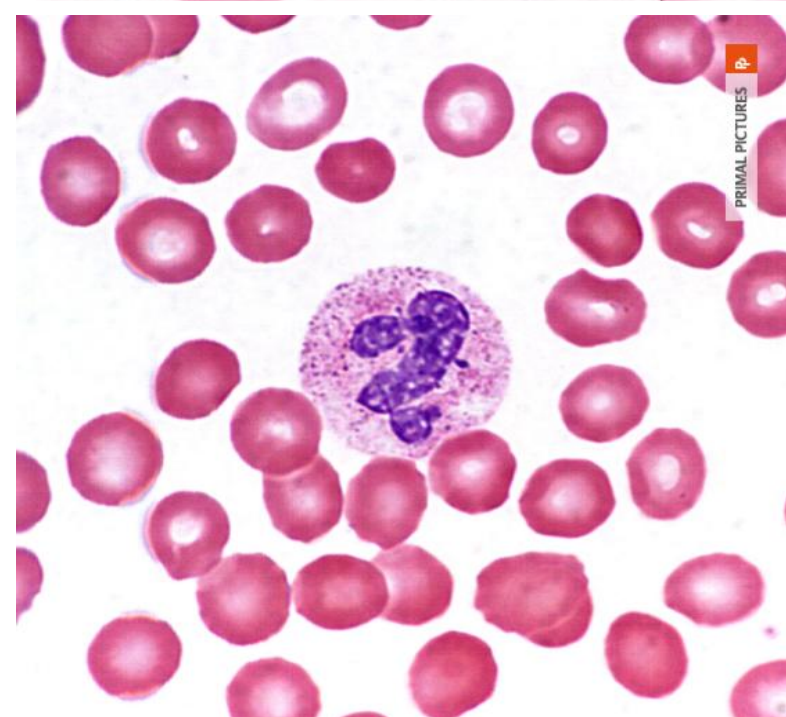
As neutrophils mature, their nuclei become increasingly segmented. A mature neutrophil typically has 2–5 nuclear lobes. Hypersegmentation (usually ≥ 6 lobes) is abnormal and may be seen in certain pathological conditions.

Neutrophils are the first WBCs that leave the blood in large numbers to reach the site of inflammation

Cells of acute inflammation

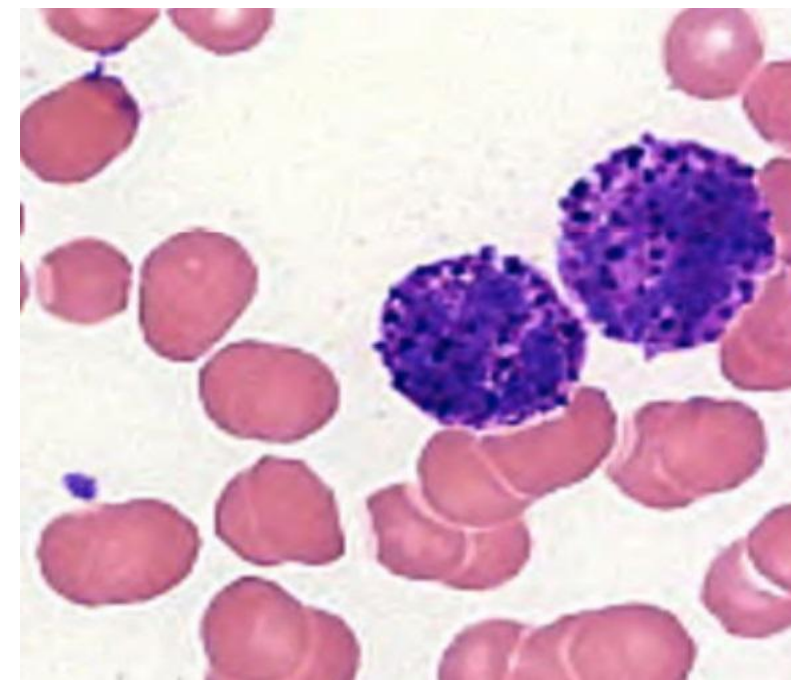
Why???

- 1- The most abundant
- 2- The most motile (migrate rapidly)
- 3- Highly responsiveness to chemotactic factors



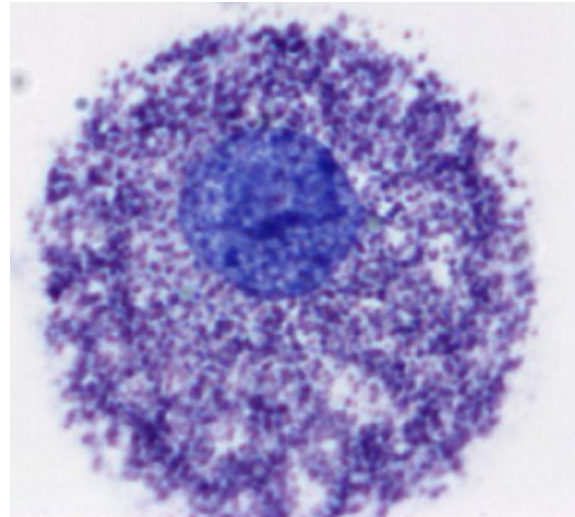
Basophils

- Rarest circulating leukocytes, accounting for approximately 0–1% of white blood cells; therefore, they may be difficult to find in routine blood smears.
- Usually have a bilobed or S-shaped nucleus, which is frequently obscured by large cytoplasmic granules.
- Contain numerous large, coarse, basophilic granules that stain dark blue to purple with basic dyes.
- Granules contain histamine, heparin, and other inflammatory mediators. Basophils can also release eosinophil-attracting chemokines and cytokines.
- Express high-affinity receptors for IgE and participate in immediate hypersensitivity reactions and immune responses to parasitic infections.



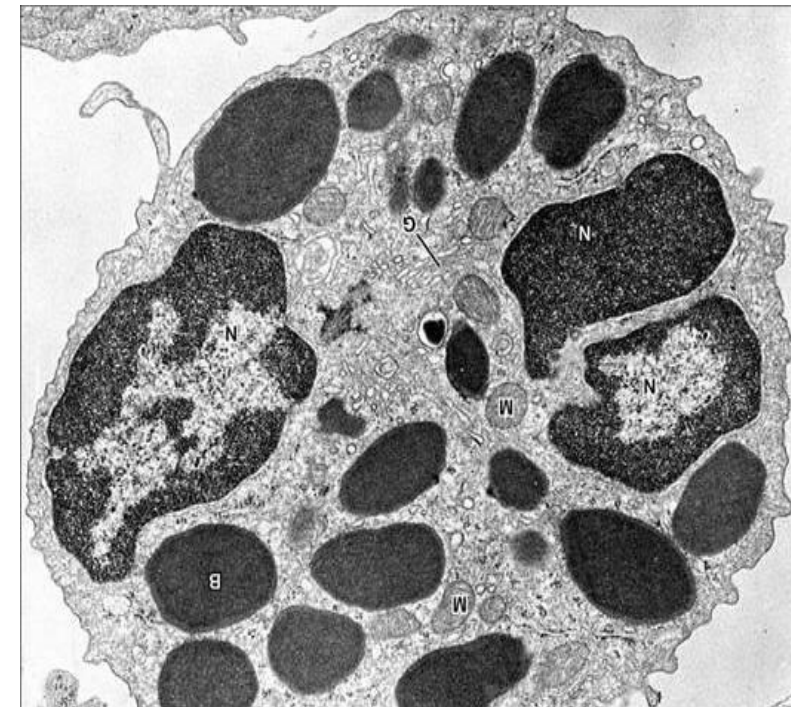
Remember: Mast cells are large immune cells found mainly in connective tissues, particularly near blood vessels and beneath epithelial surfaces.

Note: Although functionally similar to basophils, mast cells are distinct tissue-resident cells.

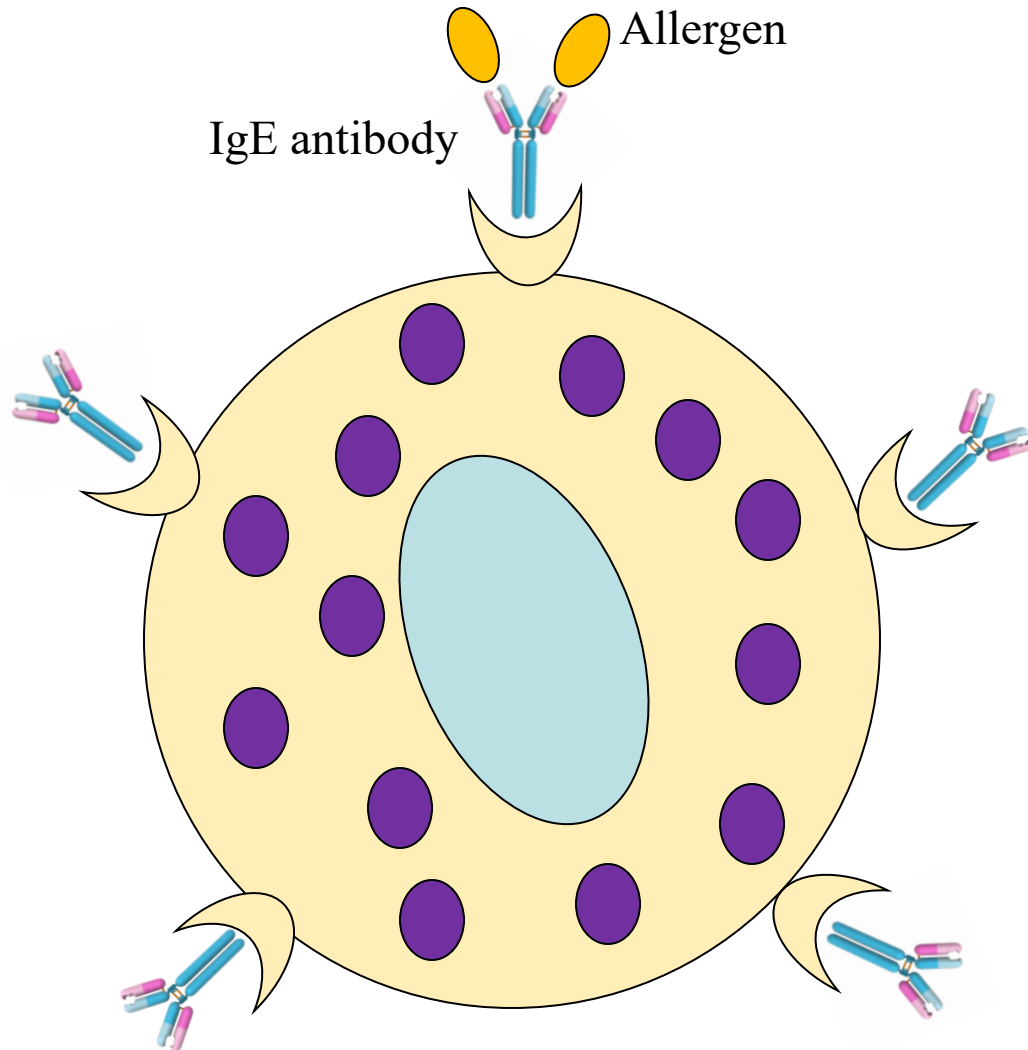


Mast cell

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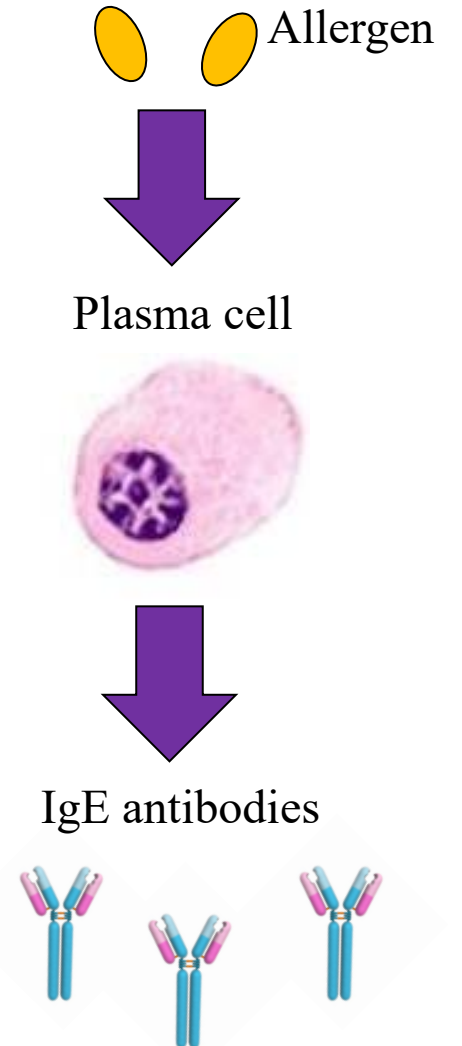


Both basophils and mast cells have surface receptors for immunoglobulin E (IgE), and secrete their granular components in response to certain antigens and allergens.



Activation causes degranulation, releasing histamine and other mediators that promote vasodilation and increased vascular permeability.

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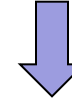
Basophils may constitute up to 15% of infiltrating inflammatory cells in allergic dermatitis and skin allograft rejection.



Exposure may be by ingestion, inhalation, injection, or direct contact

First
exposure

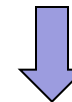
In some individuals substances such as certain pollen proteins or specific proteins in food are allergenic, that is, elicit production of specific IgE antibodies, which then bind to receptors on mast cells and immigrating basophils.



Second
exposure

Upon subsequent exposure, the allergen combines with the receptor-bound IgE molecules, triggering rapid degranulation of the cytoplasmic granules.

Release of the inflammatory mediators in this manner can result in bronchial asthma, cutaneous hives, rhinitis, conjunctivitis, or gastrointestinal allergic reactions

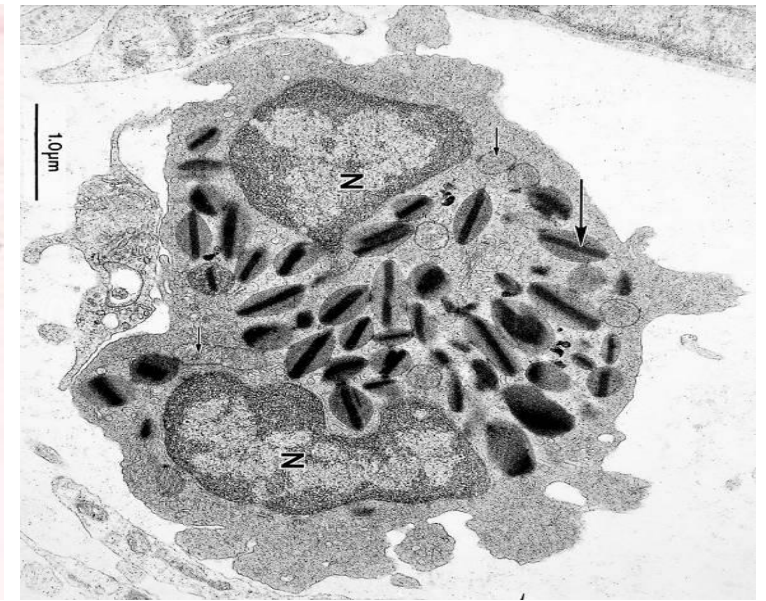
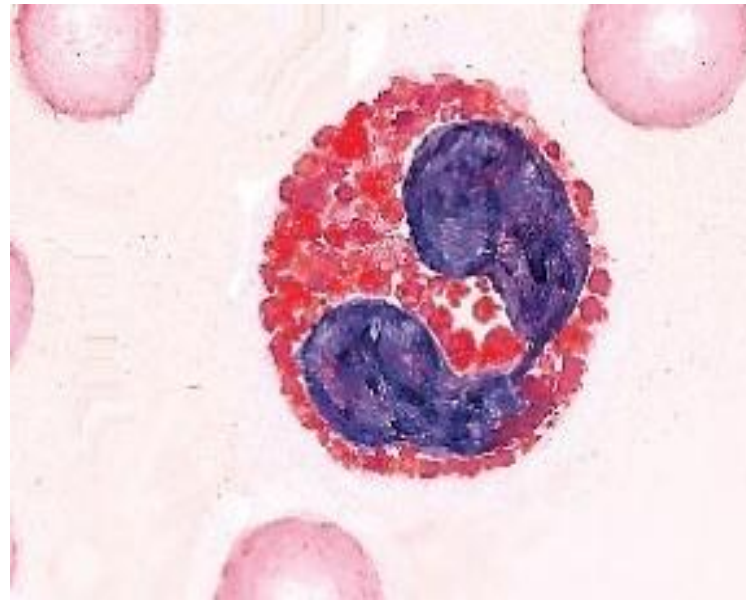


Immediate or type 1 hypersensitivity

In some individuals a second exposure to a strong allergen, such as that delivered in a bee sting, may produce an intense, adverse systemic response. Basophils and mast cells may rapidly degranulate, producing vasodilation in many organs, a sudden drop in blood pressure, and other effects comprising a potentially lethal condition called **Anaphylaxis or anaphylactic shock.**

Eosinophils

- Usually have bi-lobed nuclei connected by a short “thread” of nuclear material
- Large cytoplasmic granules, which stain red with the acidic eosin dye (*eosinophil* = eosin loving)
- Modulate allergic reactions and fight parasitic infections

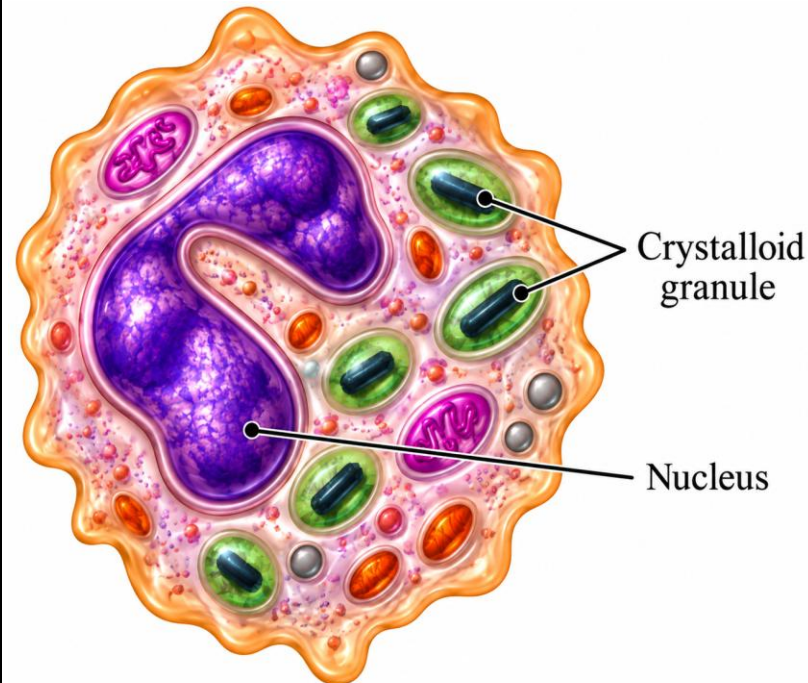
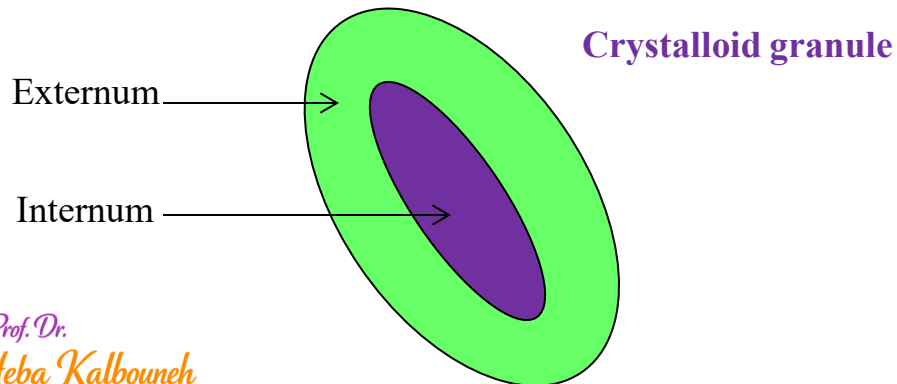


Specific granules (Crystalloid granules):

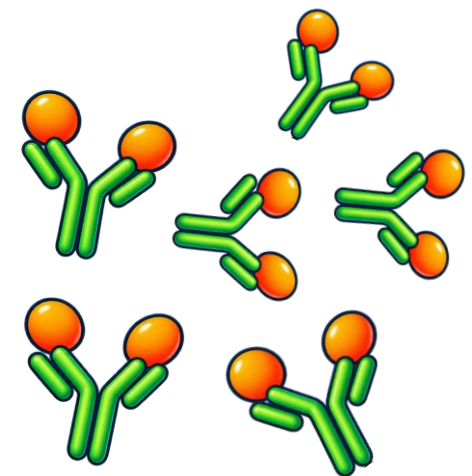
- Oval in shape, with flattened crystalloid cores
- Two parts:

Externum (pale): contains histaminase and sulfatase

Internum (dark): contains major basic protein to kill parasites

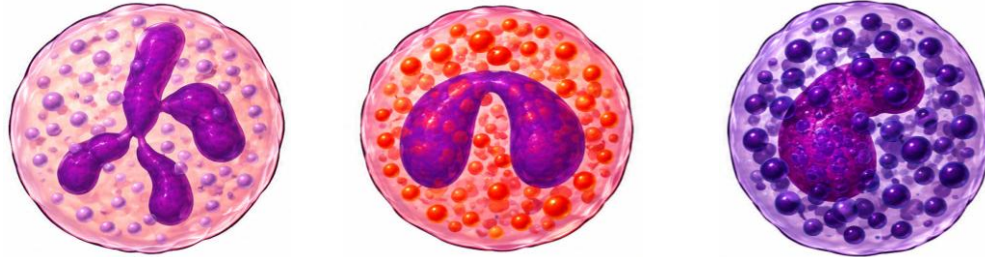


Eosinophils have a particular phagocytic affinity for **antigen-antibody complex**



General features of granulocytes

- Have lobulated/segmented nuclei.
- Spherical in blood stream, irregular in connective tissue.
- Highly motile with different shapes due to their amoeboid movement.
- Leave blood stream by migrating between the endothelial cells by a process called **diapedesis**, and move toward sites of inflammation by **chemotaxis**.
- Generally short-lived cells.



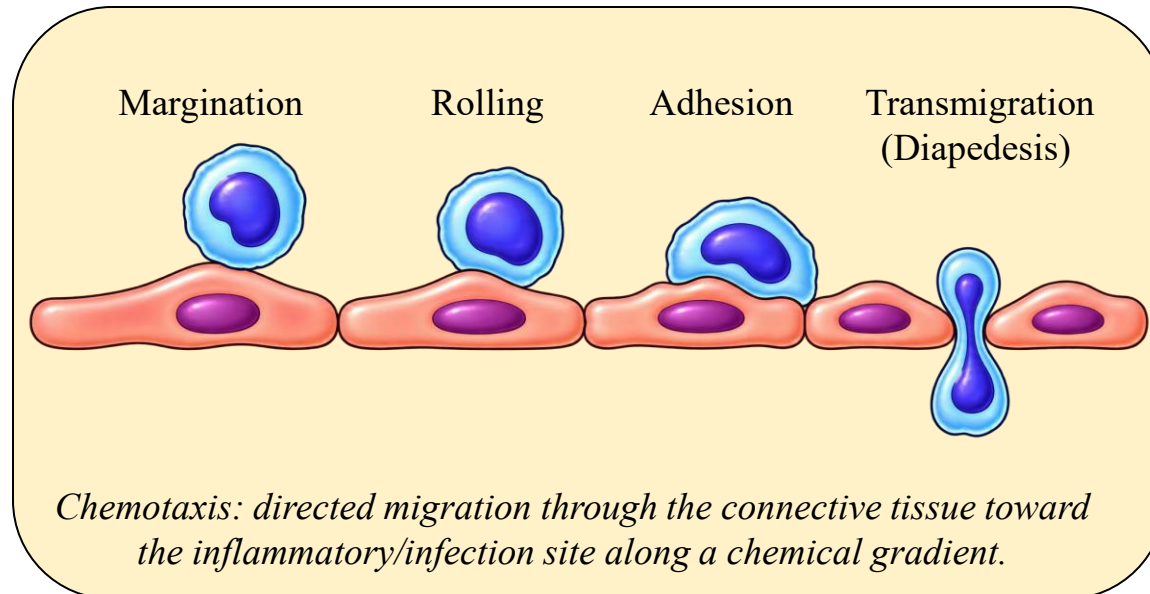
Neutrophil

Eosinophil

Basophil

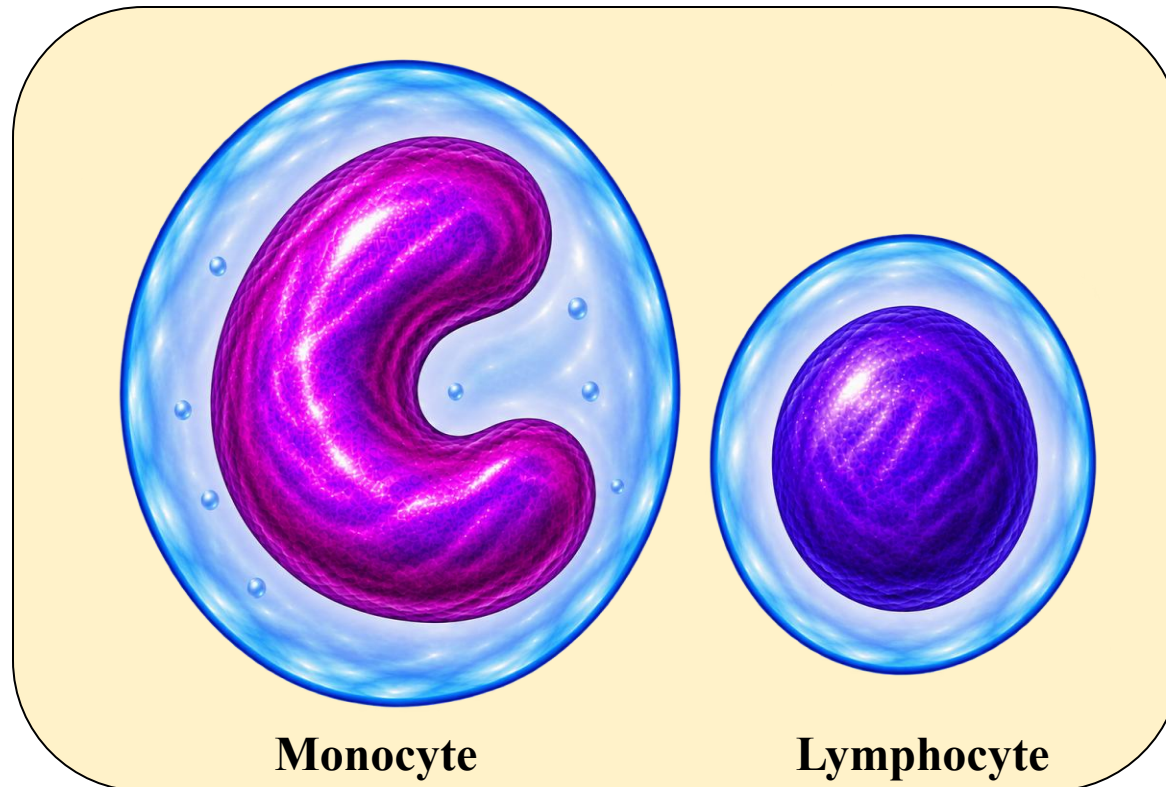
	Neutrophil	Eosinophil	Basophil
Percentage (WBCs)	Most	→ Least	
Size	12-15um	12-17um	12-15um
Life span	Hours to days	Hours to days	Hours to days
Nucleus	2-5 lobes	2 lobes	Irregular (S-shaped)
Phagocytic activity	Most	→ Least	
Motility	Most	→ Least	

Diapedesis
(Gr. dia, through + pedesis, to leap)



Agranulocytes

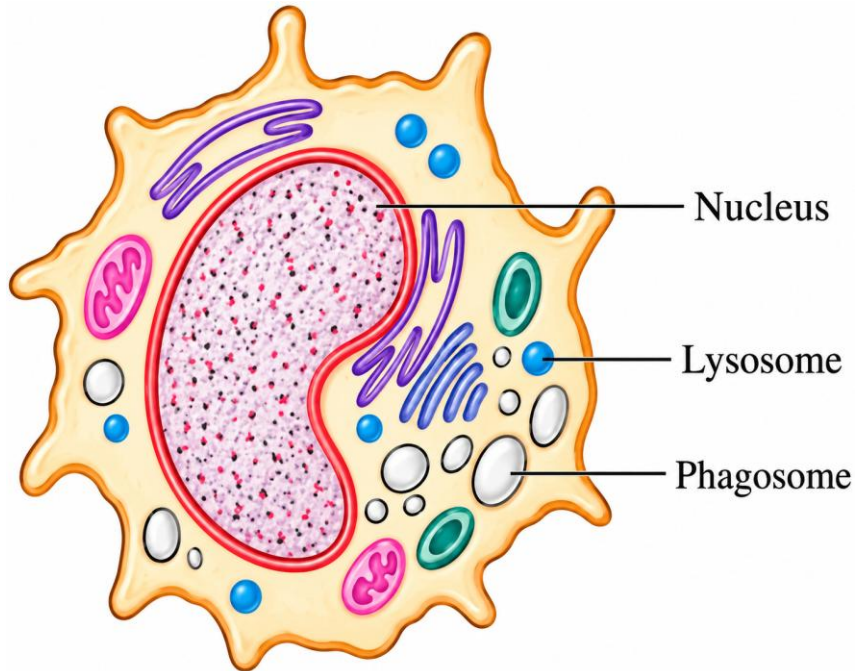
- Single, non-lobulated nucleus
- Lack specific cytoplasmic granules, but contain azurophilic (primary) granules, which are lysosomes and may be difficult to see by light microscopy
- Generally have a longer lifespan than granulocytes, ranging from days to years, depending on the cell type.
- Two types based on morphology, not cell lineage:
 - Lymphocytes
 - Monocytes



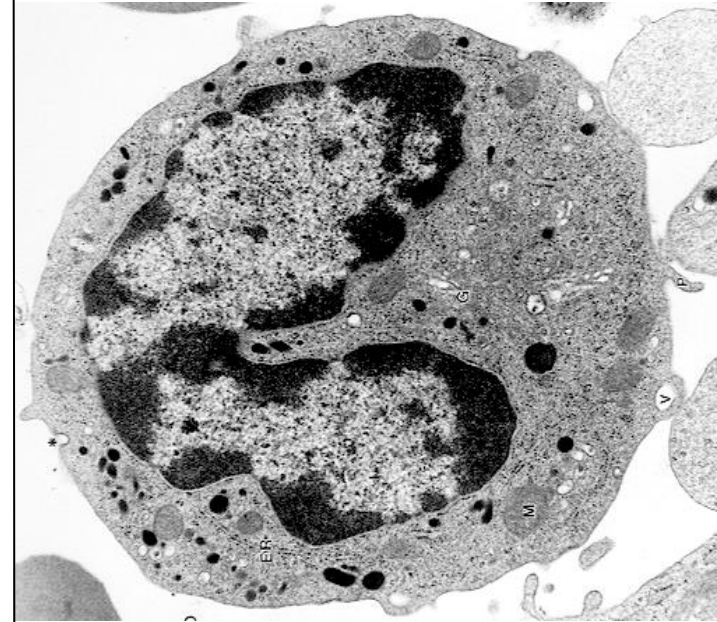
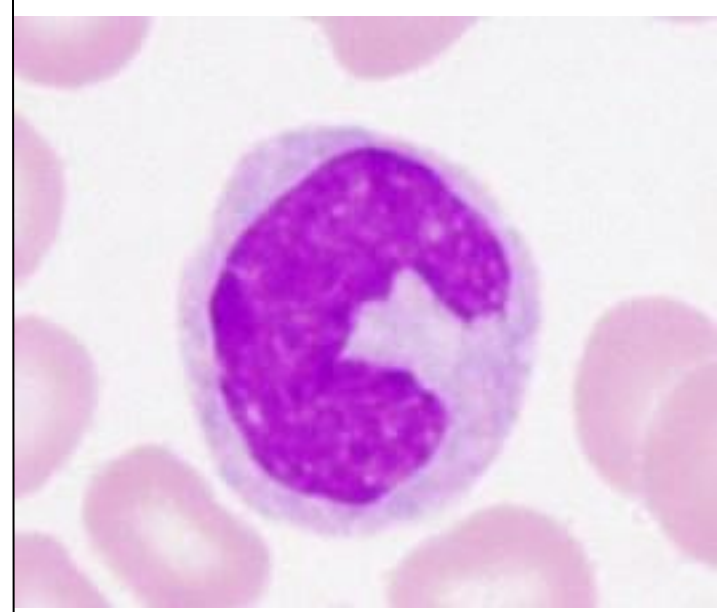
Monocytes

- Largest leukocytes
- Bluish cytoplasm (*frosted glass appearance*) & a large C-shaped nucleus
- Highly motile and phagocytic
- Travel through bloodstream to reach connective tissues, where they transform into **macrophages** (large phagocytic cells) or **dendritic cells**

Azurophilic granules
(lysosomes)

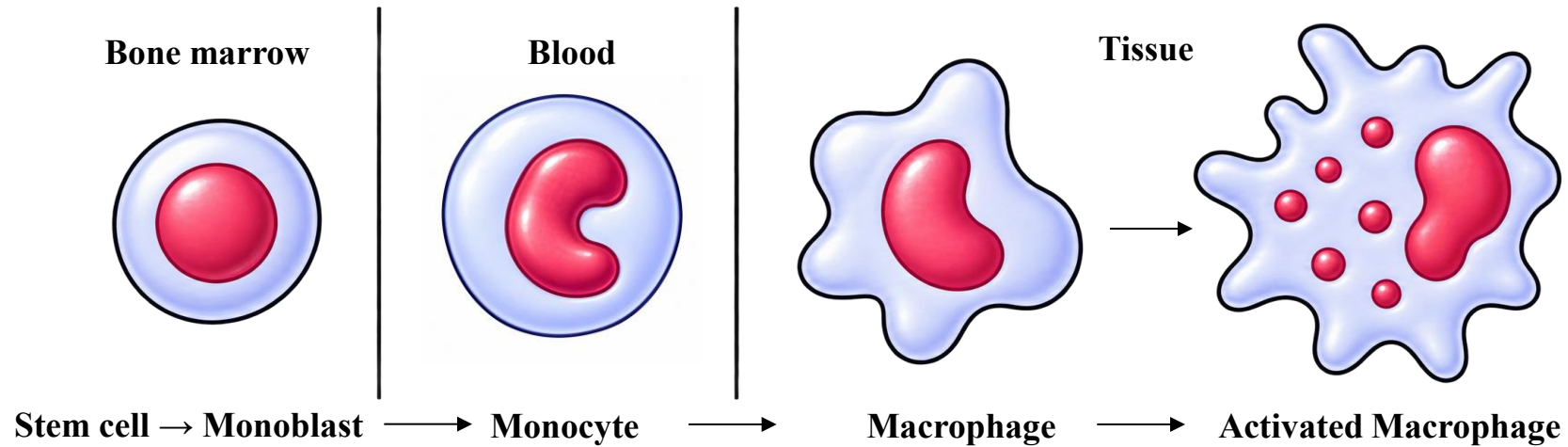


Are precursor cells of macrophages, osteoclasts, microglia, and other cells of the **mononuclear phagocyte system** in connective tissue



Many monocyte-derived cells function as **antigen-presenting cells**.

Monocytes and macrophages are the same cells at different stages of maturation



Kupffer Cells

Liver

Microglia

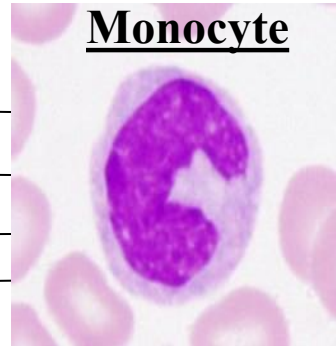
CNS

Macrophages

Bone marrow
Connective tissue

MONONUCLEAR PHAGOCYtic SYSTEM

Monocyte



Dendritic cells/ macrophages

Lymph node
Spleen

Osteoclasts

Bone
resorption

Alveolar (dust) cells

Lung

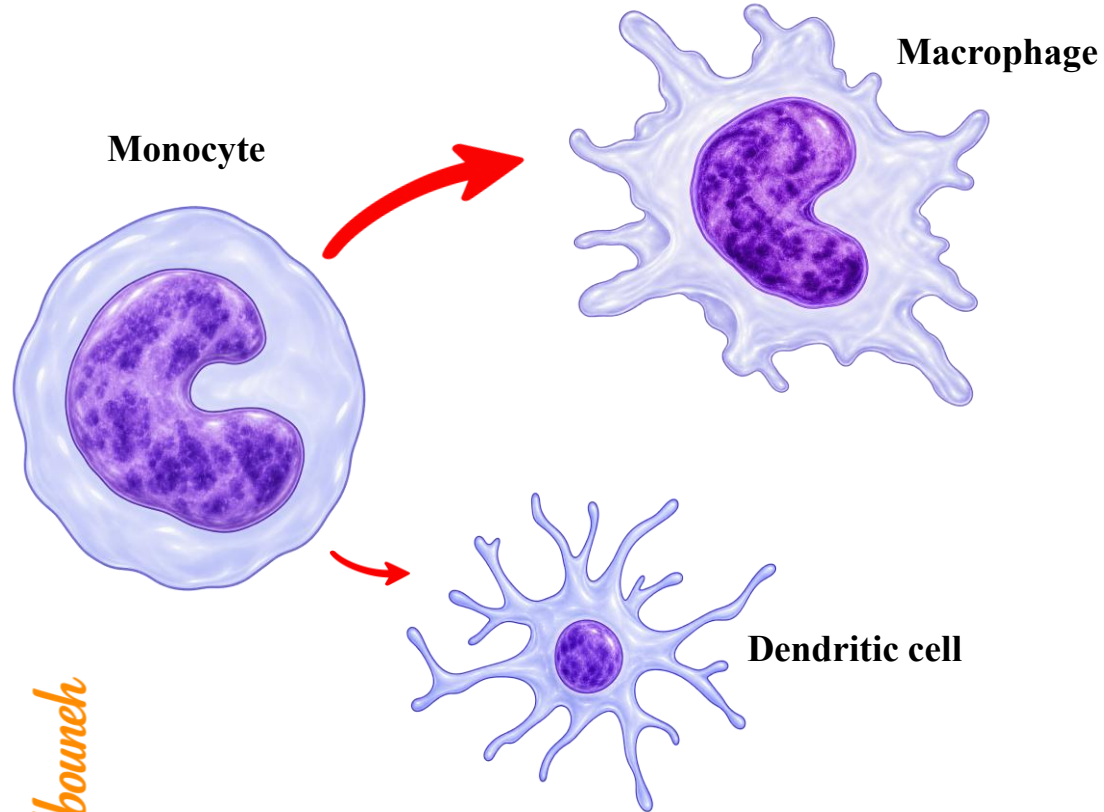
Langerhans cells

Epidermis

Morphologic changes occur upon differentiation of monocytes into macrophages:

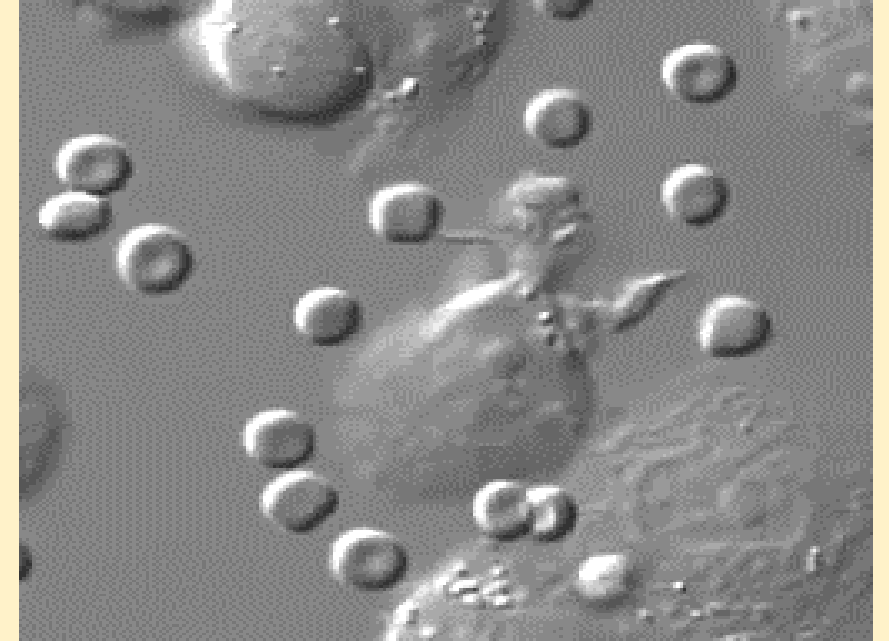
- The cell increases in diameter more than threefold
- The cell acquires more cytoplasmic granules and vacuoles
- Cell surface receptors increase in number
- The phagocytic activity improves

NOTE: This differentiation involves profound changes in cell morphology, gene expression, and metabolic activity,



Monocyte-derived cells generally have **phagocytic activity** and can function in **antigen presentation**.

A macrophage eating bacteria



Monocytes circulate in the blood and, after migrating into tissues, generally differentiate into **macrophages** or **dendritic cells** (particularly under inflammatory conditions). These cells contribute to immune defense through phagocytosis, antigen processing and presentation, and cytokine secretion, thereby linking innate and adaptive immune responses.

Lymphocytes

- Smallest leukocytes (Lymphocytes vary considerably in size !!)
- Round nucleus occupies most of cell volume
- Cytoplasm is light clear blue
- Increased numbers are commonly seen in **viral infections**
- Lymphocytes vary in life span according to their specific function, some live for a few days and some live for many years

T cells

Cell mediated immunity

- Has different types, some directly kill foreign or infected cells; others activate phagocytes to destroy microbes

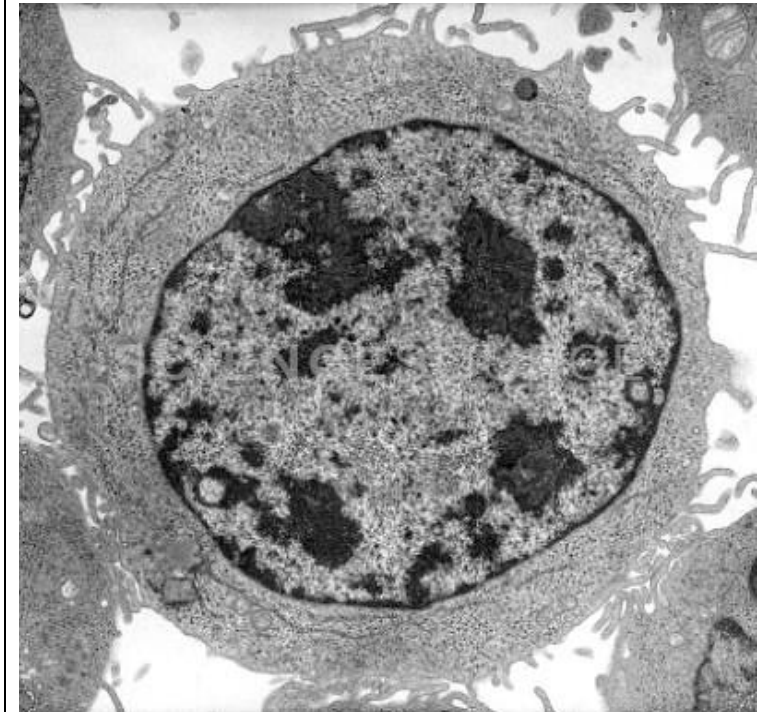
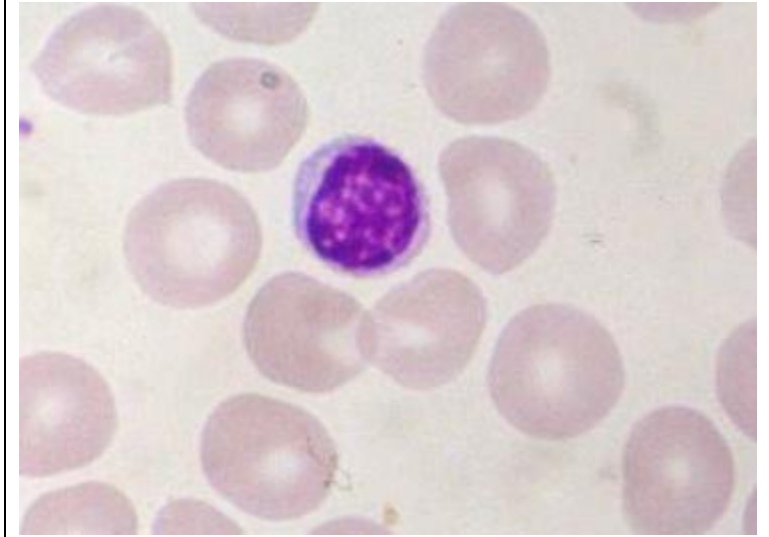
B cells

Humoral immunity

Humoral means
body fluids

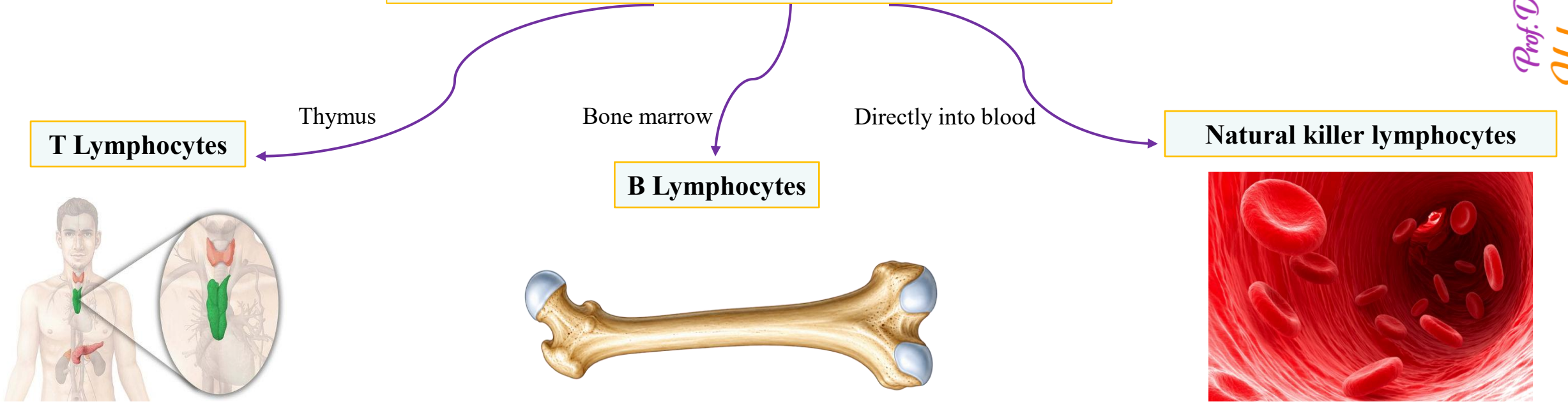
- Differentiate into plasma cells
- Secrete antibodies that bind to specific antigens and mark them for destruction by phagocytic cells

Memory B and T cells provide long-term immunologic memory



Lymphopoiesis: the process by which lymphocytes are formed

Precursor cells in bone marrow



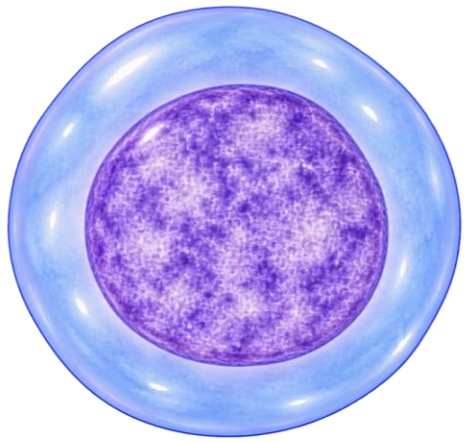
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B and T lymphocytes recognize **SPECIFIC** antigens (**Adaptive immunity**)

NK cells act **NON-SPECIFICALLY** and are part of **Innate immunity**.

Note:

Neutrophils and monocytes/ macrophages are highly phagocytic and engulf microorganisms and cell debris in a **NON-SPECIFIC** manner (**Innate immunity**)

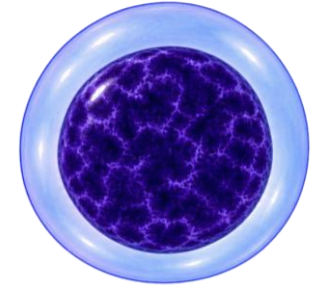


Large
(9-18 μm)
Active lymphocyte

Morphologically lymphocytes can be classified into:

The amount of cytoplasm depends upon **state of activity** of the lymphocyte

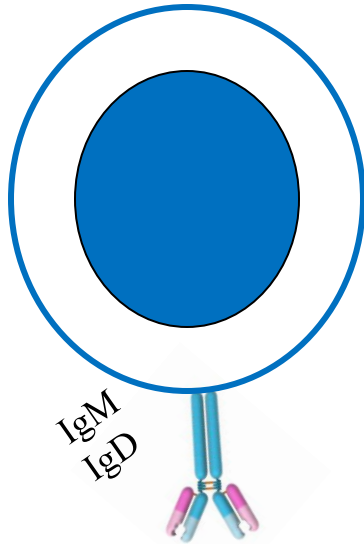
In circulation blood there is **predominance of small inactive lymphocytes**



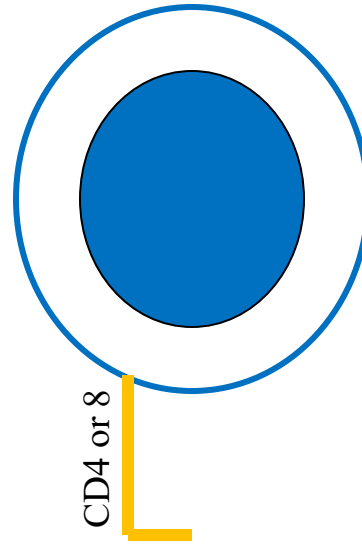
Small
(6-9 μm)
Inactive lymphocyte

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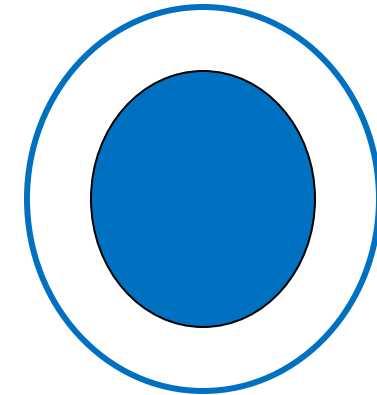
Different types express specific cell surface proteins



B lymphocyte



T lymphocyte

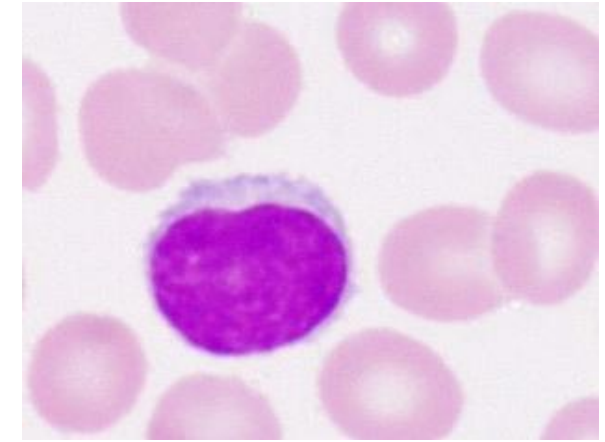
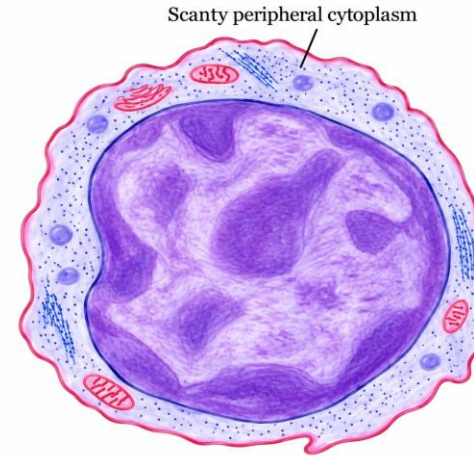


Natural killer lymphocyte

Small lymphocytes have scant cytoplasm containing few organelles but numerous free ribosomes (polyribosomes).



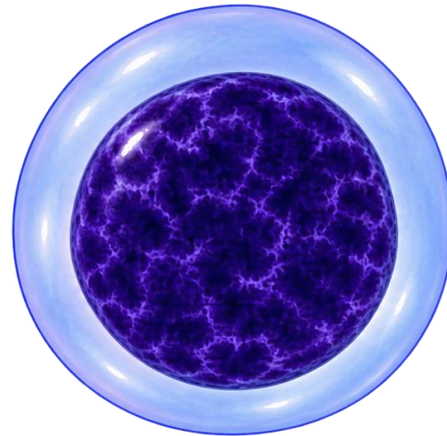
The abundant ribosomes account for the **basophilic cytoplasm**.



**Small
(6-9 μm)**

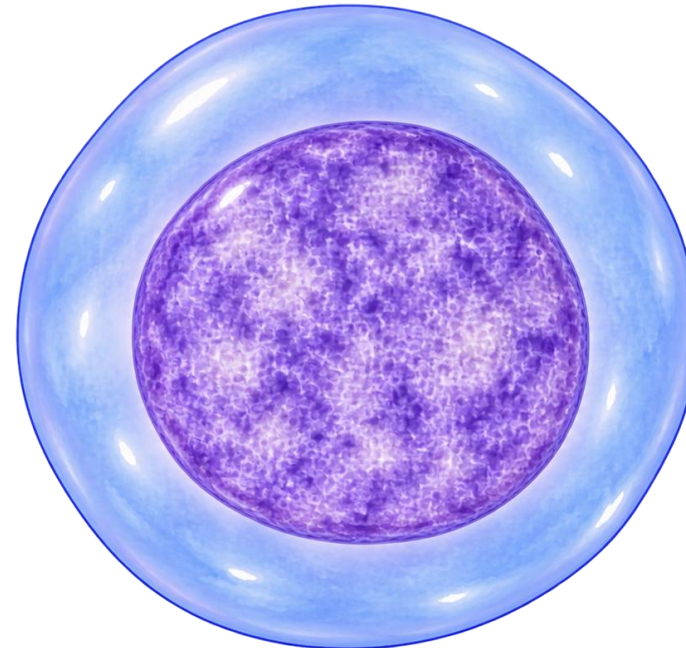
Inactive lymphocyte

Darkly stained cell



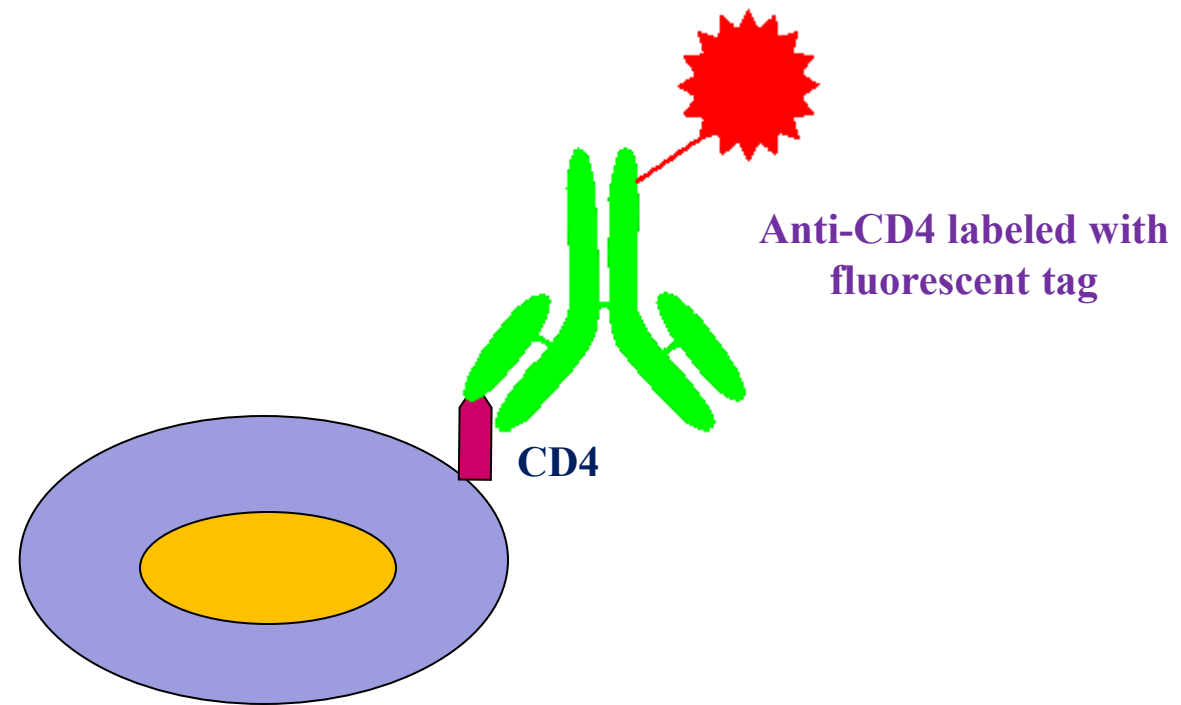
**Large
(9-18 μm)
Active lymphocyte**

Lightly stained cell



Immunocytochemistry

Direct method



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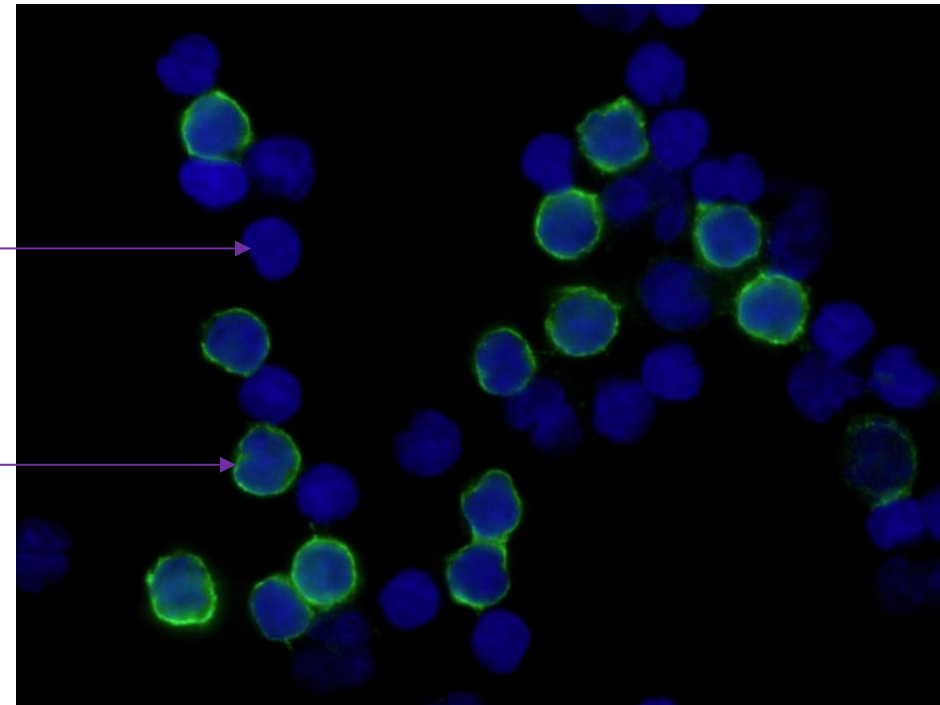
Immunocytochemistry

Using CD 4 Antibody
DAPI staining

???????

CD 4 +
T Helper lymphocyte

Immunofluorescence staining
of CD4⁺ peripheral blood
lymphocytes



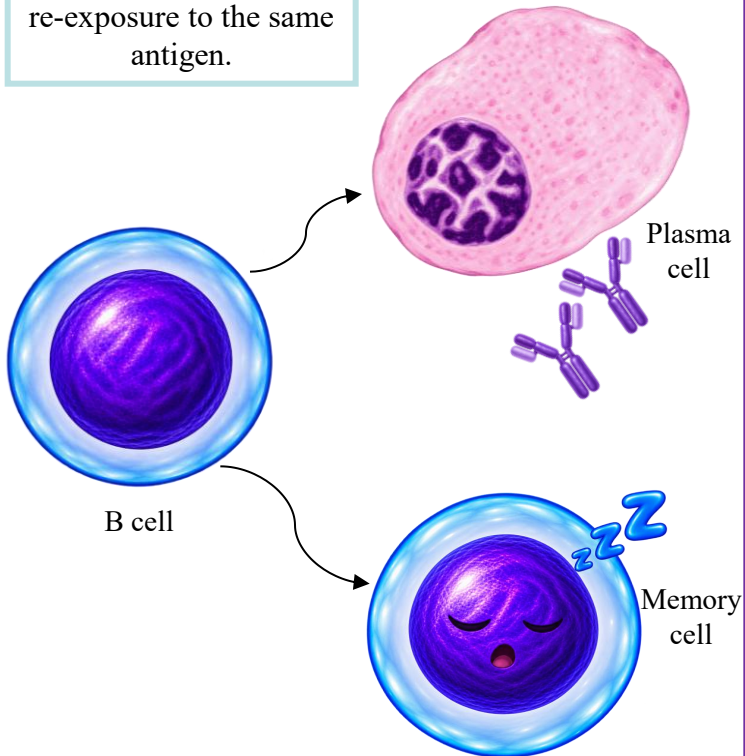
B Lymphocytes

Memory cells

Produce a faster antibody response upon re-exposure to the same antigen.

Plasma cells

Produce antibodies



Note: Memory Cells: Include memory B cells and memory T cells (helper, cytotoxic, and regulatory memory T cells)

T Lymphocytes

Cytotoxic

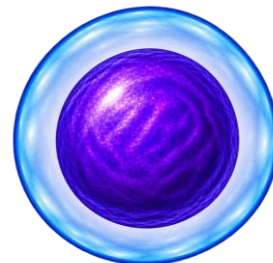
Kill virus-infected, transplanted and neoplastic cells

Helper

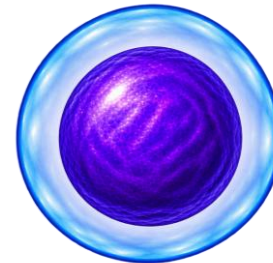
Coordinate immune responses by activating B cells, cytotoxic T cells, macrophages, and other immune cells

Regulatory

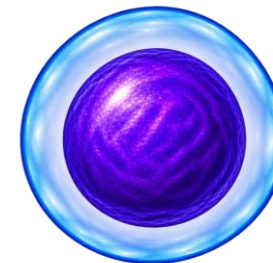
- Suppress immune response to self Ag
- Suppress excessive immune responses



Cytotoxic T cell
CD8+



Helper T cell
CD4+



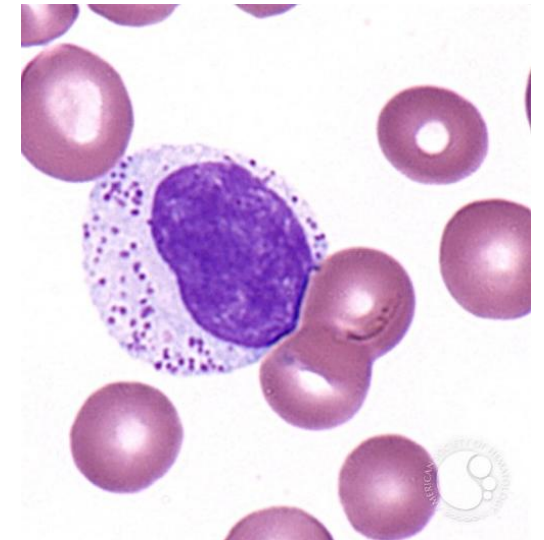
Regulatory T cell
CD8+/- CD4+

Natural killer cells

Kill virus-infected, transplanted and neoplastic cells without prior antigen-specific exposure (innate immunity)

Large granular lymphocytes

Activated lymphocytes



Innate immunity: We are born with innate immunity. It is non-specific, which means that the innate cells are not able to distinguish one type of pathogen from another.

Cells of innate immunity: Neutrophils, Basophils, Eosinophils, Mast cells, Monocytes (macrophages and dendritic cells), Natural killer cells

Adaptive (acquired) immunity is the body's ability to recognize and respond to specific foreign substances (antigens: microbes, parts of microbes, or non-microbial substances, such as pollen)

Cells of adaptive immunity: B and T lymphocytes

Regulatory T cells switch off the immune response when the stimulus is removed



Damage to regulatory (suppressor) cells can result in **autoimmune disease**

Memory cells allow a more rapid response if the antigen appears again later



which allows a very rapid response upon subsequent exposure to the same antigen.

Basis of immunity/vaccination

Natural killer cells and T cells play a major role in **graft rejection**

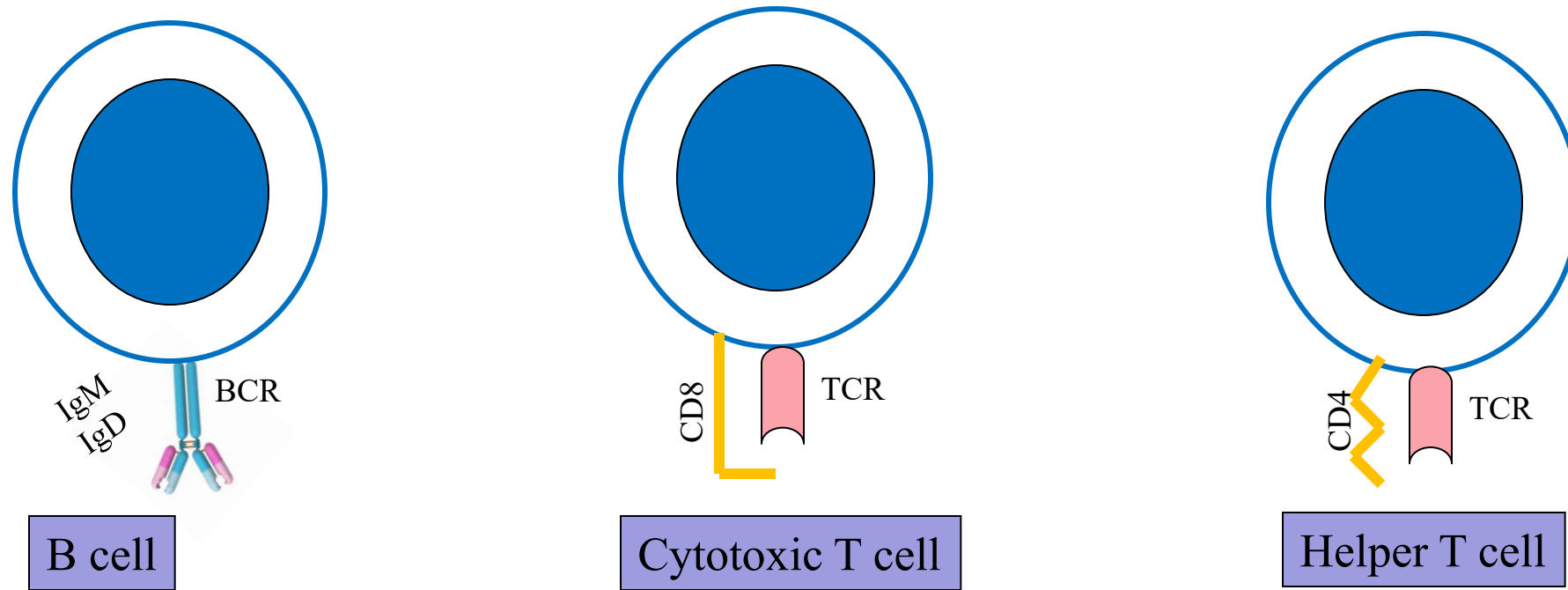
HIV affects Helper T cells

The retrovirus that produces acquired immunodeficiency syndrome (AIDS) infects and rapidly kills helper T cells.



Reduction of this key lymphocyte group cripples the patient's immune system rendering them susceptible to opportunistic bacterial, fungal, protozoan, and other infections that usually dealt with easily in immunocompetent individuals.

Different types express specific cell surface proteins



Note: Receptors of B cells are immunoglobulins that bind antigens directly; those on T cells react only with antigen on MHC molecules and this requires the additional cell surface proteins CD4 or CD8.

T lymphocytes are said to be **MHC restricted**

"CD" stands for "cluster of differentiation": are surface molecules that help differentiate one cell type from another

Major histocompatibility complex

MHC

Glycoproteins on the cell membrane

Two classes:

MHC-I

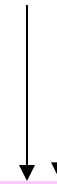
- ✓ Present on the surface of all nucleated cells
- ✓ Presents peptides derived from proteins produced within the cell

MHC –II

- ✓ Present mainly on the surface of antigen-presenting cells (APCs)
- ✓ Presents peptides derived from material taken up and digested by the cell

Helper T cells recognize antigens presented by MHC II, while cytotoxic T cells recognize antigens presented by MHC I.

T lymphocytes recognize antigens presented by MHC molecules.

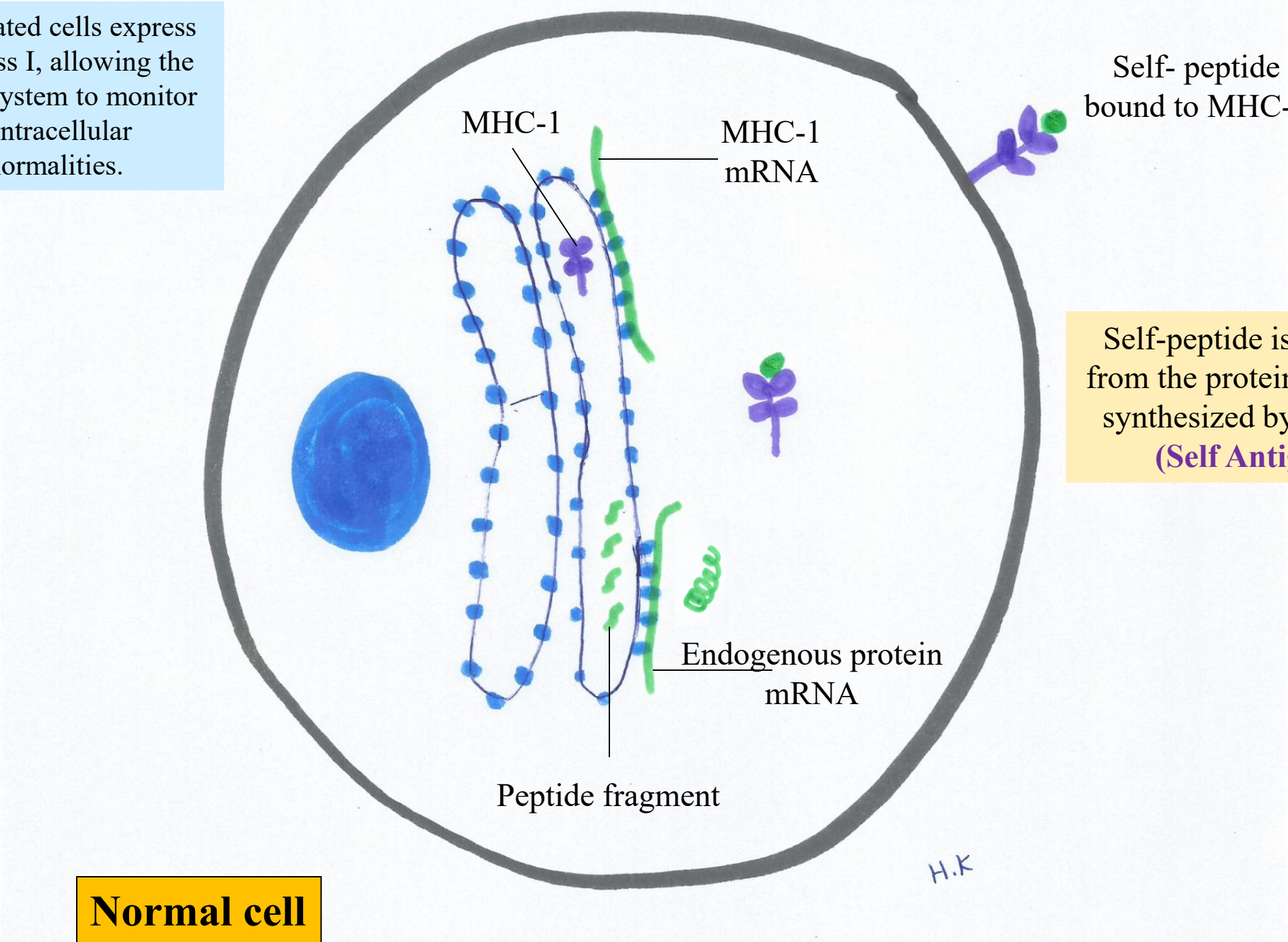


MHC molecules are important in tissue transplantation.

If the MHC molecules on transplanted cells are sufficiently different from those of the recipient, the recipient's T lymphocytes may recognize the transplanted cells as foreign and attack them, leading to graft rejection.

In humans, MHC molecules are called Human Leukocyte Antigens (HLA).

All nucleated cells express MHC class I, allowing the immune system to monitor for intracellular abnormalities.

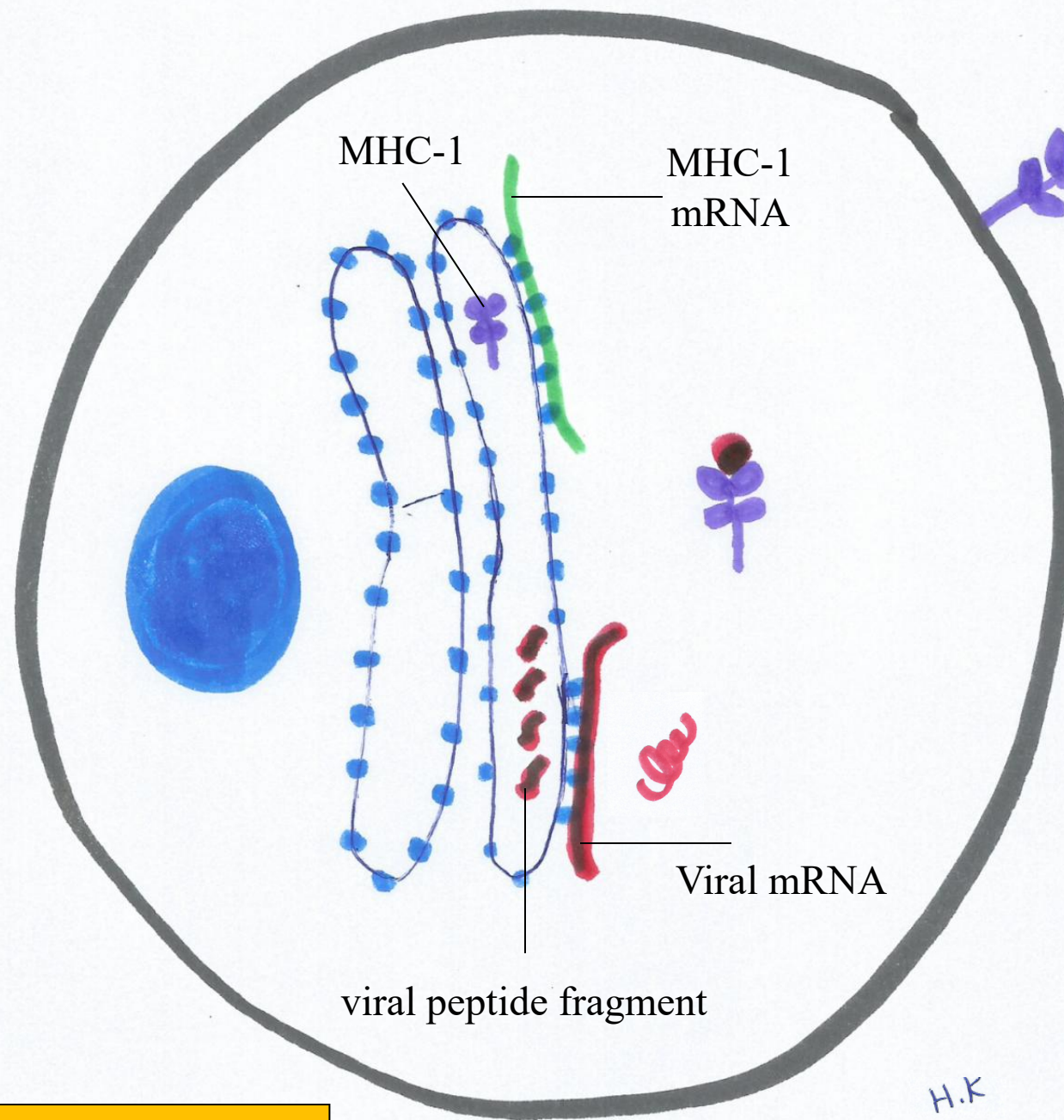


Self- peptide
bound to MHC-1

Self-peptide is derived from the proteins that are synthesized by the cell
(Self Antigen)

Normal cell

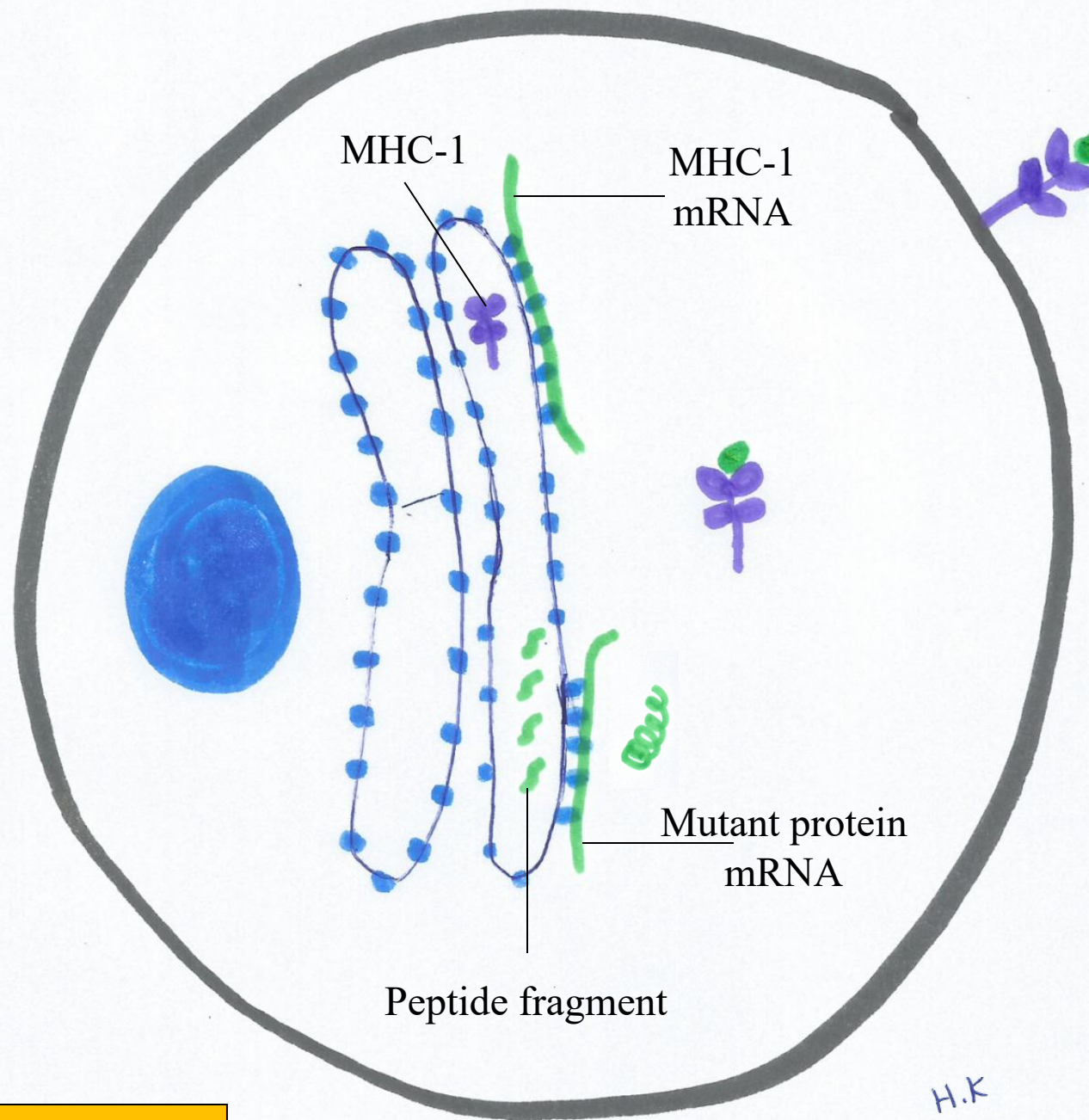
H.K



Viral- peptide
bound to MHC-1

Viral peptide is derived
from the viral proteins that
are synthesized by the
virus- infected cell
(non-self Antigen)

Virus- infected cell



Mutant peptide
bound to MHC-1

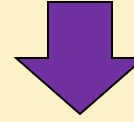
Mutant peptide is derived
from the mutant proteins
that are synthesized by
the neoplastic cell
(non-self Antigen)

Neoplastic cell

H.K

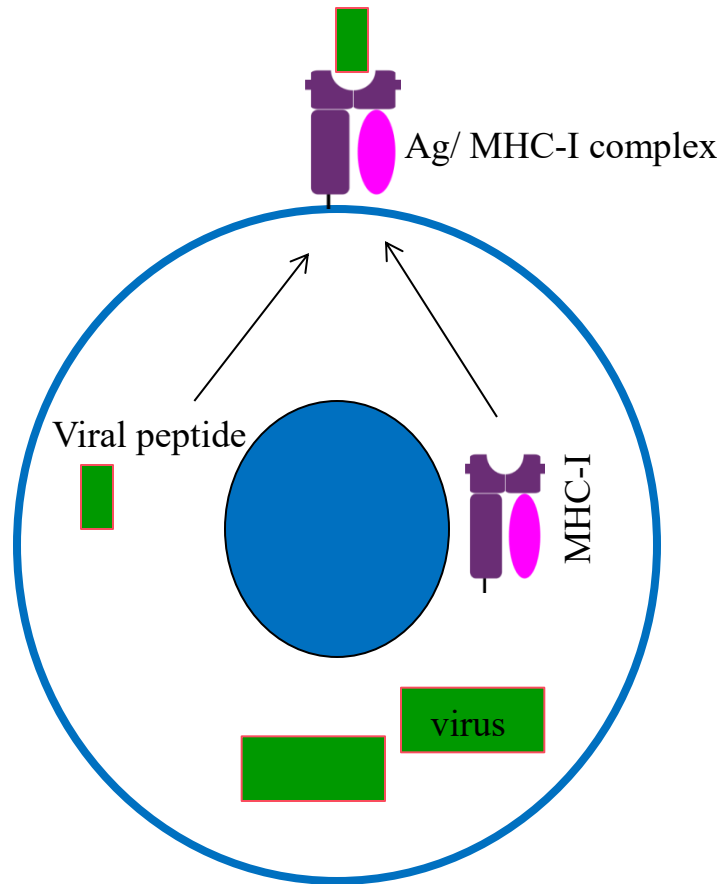
Cytotoxic CD8 T cells:

Antigen in virus infected, transplanted or neoplastic cells bind to MHC-I molecules

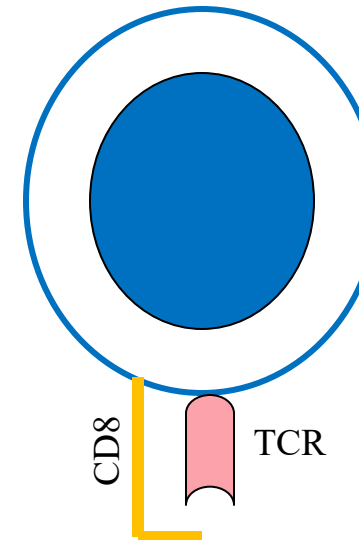


Ag-MHCI complex

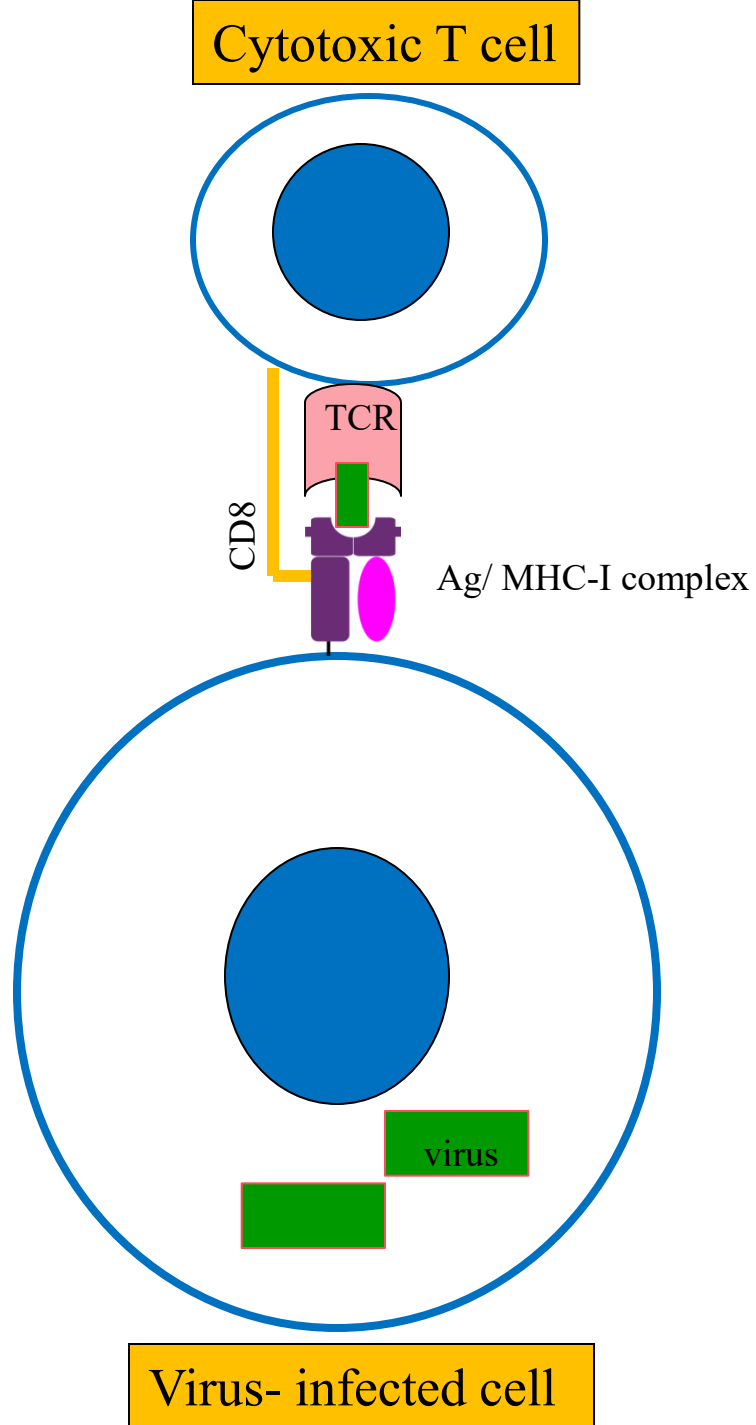
Cell- mediated
immunity



Virus- infected cell

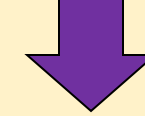


Cytotoxic T cell

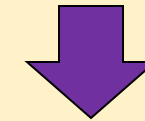


When the Ag- MHC-I complex binds to receptors on cytotoxic CD8 T cells

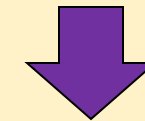
They



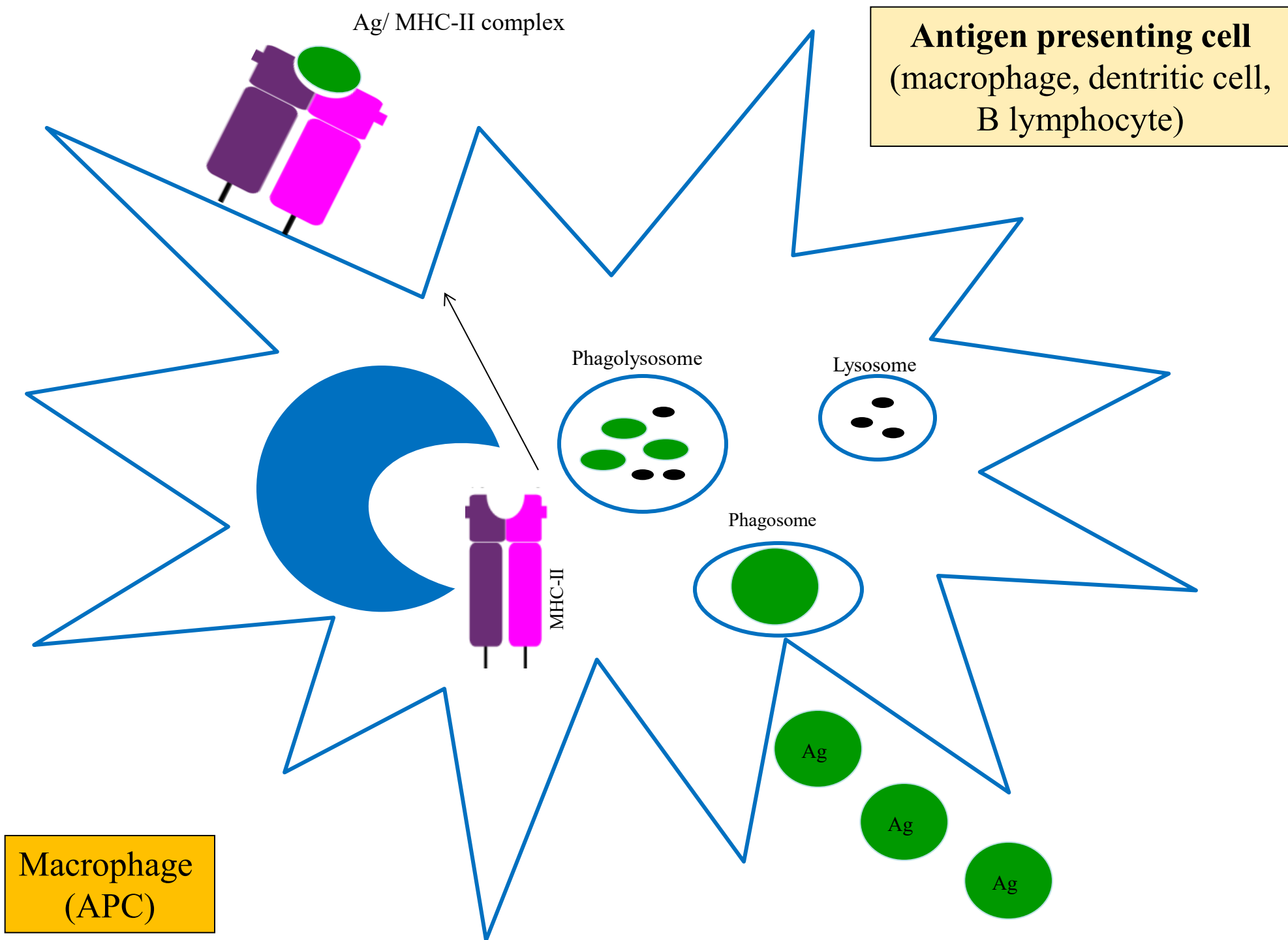
Proliferate
Activate



Release
Perforins and granzymes
(proteases)

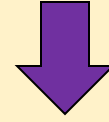


Perforins form pores in the cell membrane through which granzymes can enter, inducing apoptosis

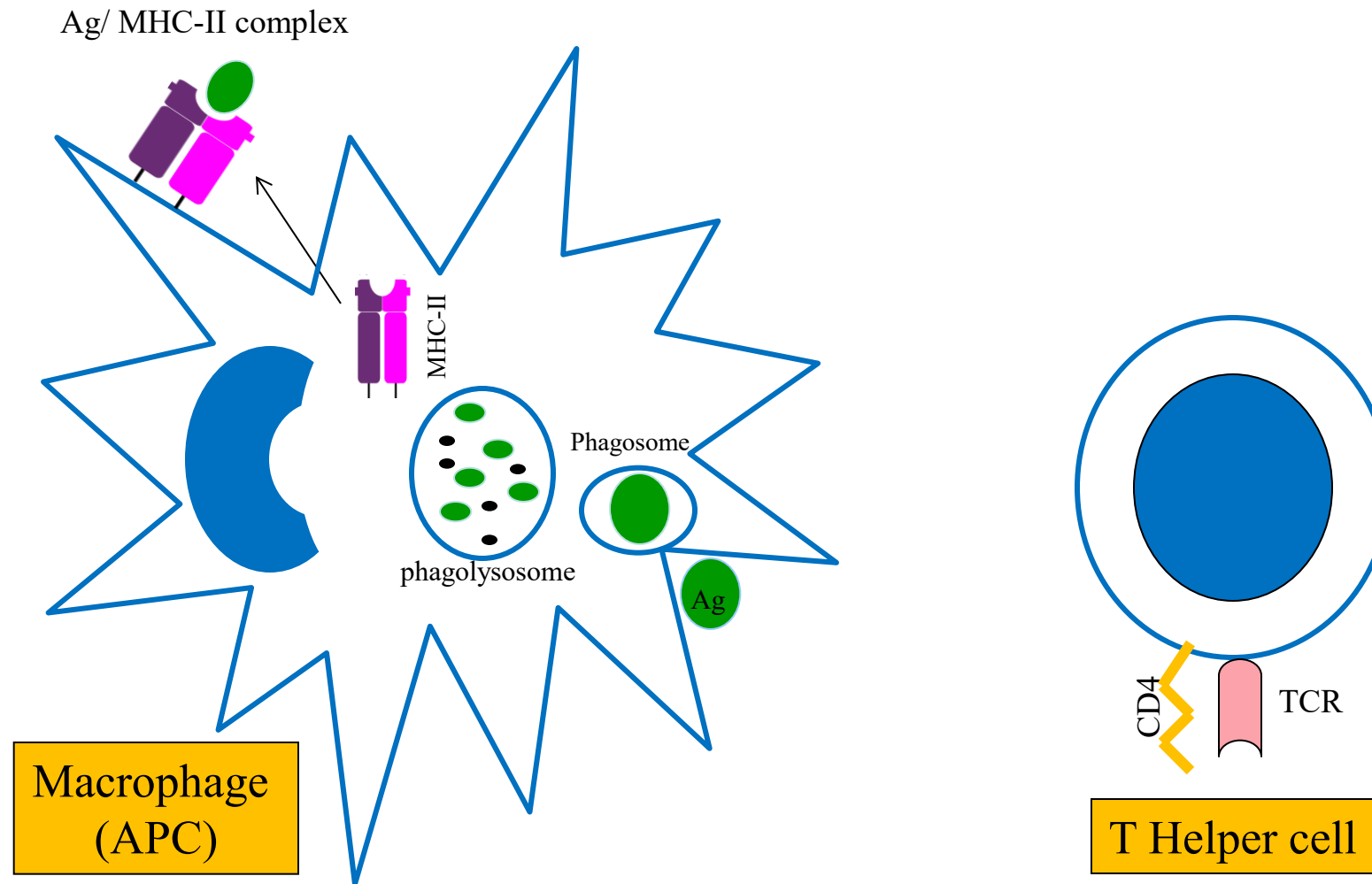


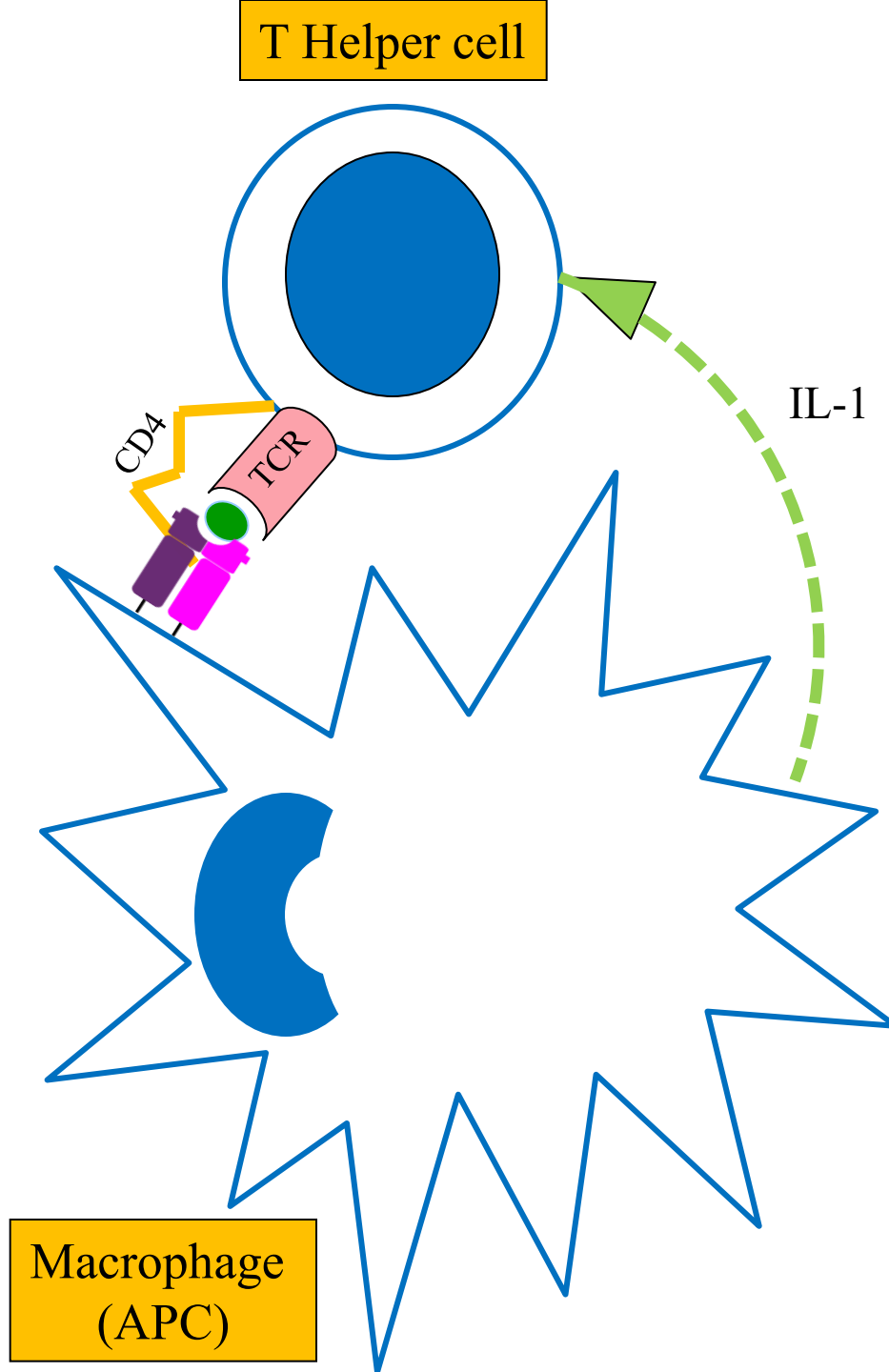
Helper CD4 T cells:

When Ag is phagocytosed by antigen presenting cells (APCs)
e.g macrophages, dendritic cell and B lymphocytes
It binds to MHC-II molecules



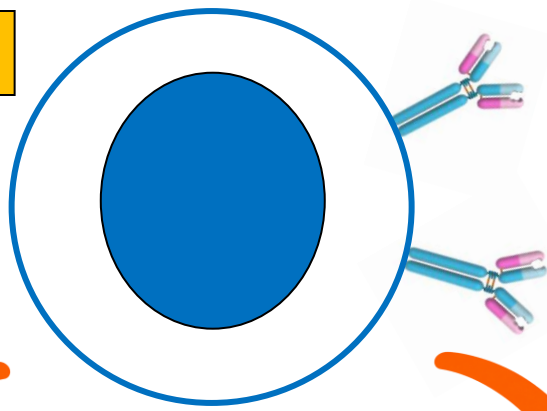
Ag-MHCII complex





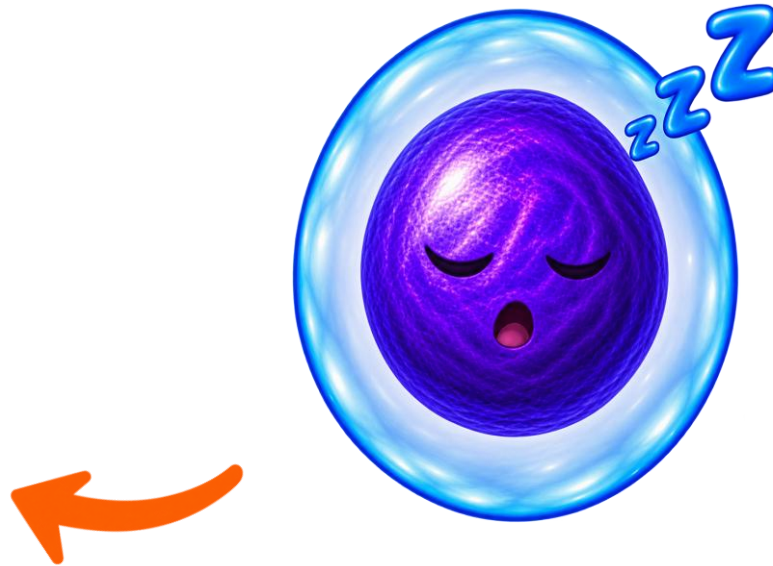
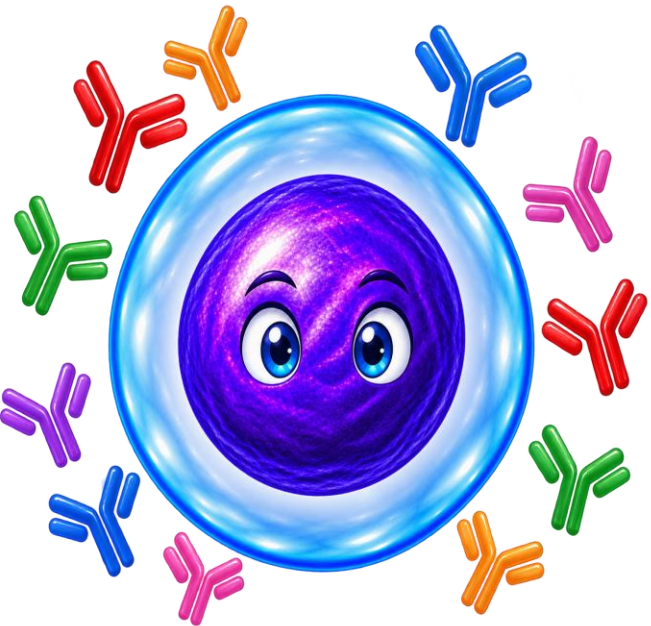
When the Ag- MHCII complex binds to receptors on Helper CD4 T cells
They
Proliferate
Activate
↓
Secrete Lymphokines (cytokines) to Stimulate
T and B cells

B cell

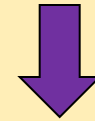


Plasma cells

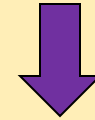
Memory cells



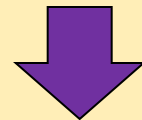
When a B lymphocyte is stimulated by T helper cells



Proliferate
Activate



Activated B lymphocytes:
1- differentiate into **plasma cells** (secrete antibodies)
2- differentiate into **memory cells**
(Rapid response on the 2nd exposure to the same Ag)



Life long immunity (vaccination)

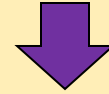
Prof. Dr. Heba Kalbouneh

B cells:

When the specific Ag binds to receptors on B cells

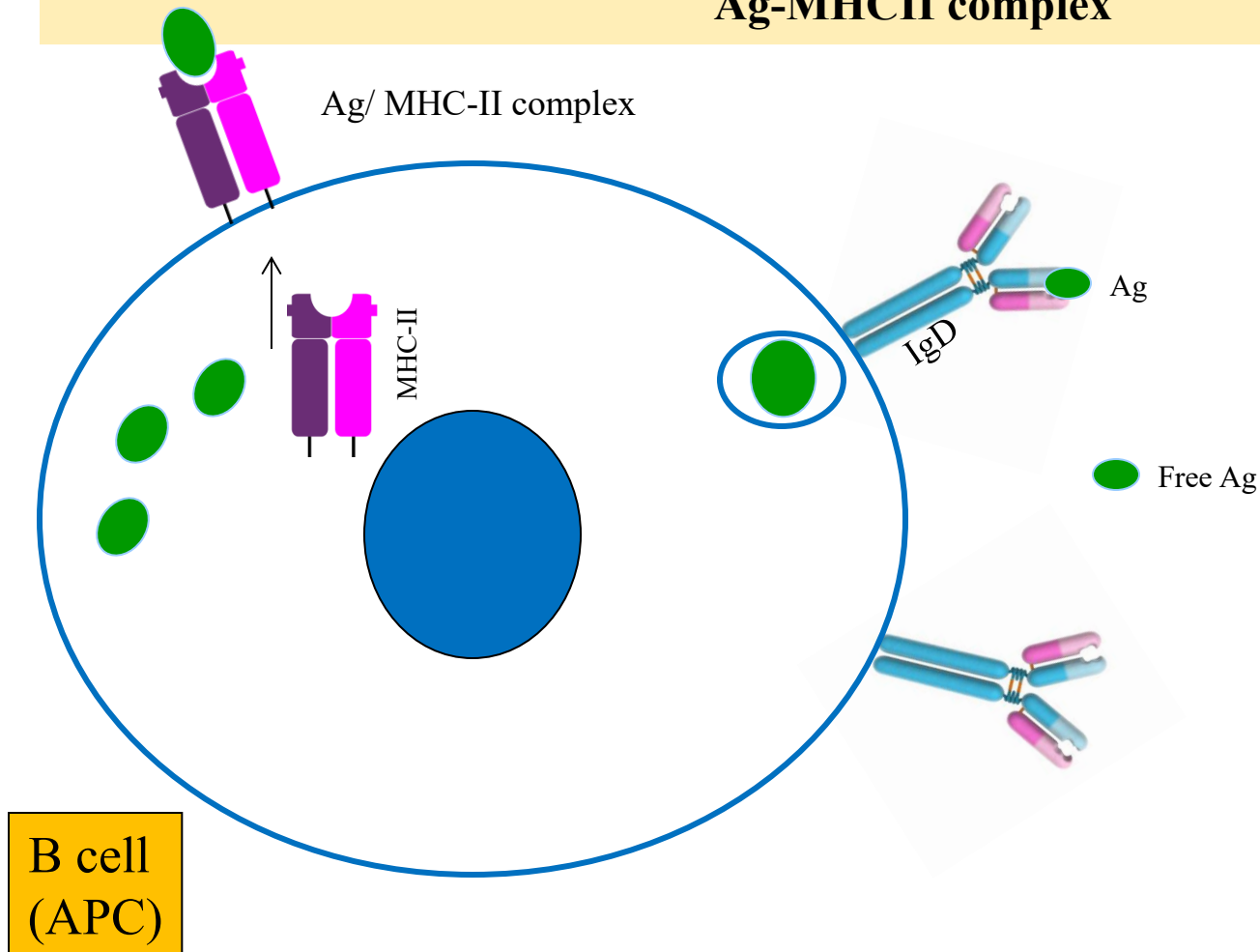


receptor-mediated endocytosis and fragments of the Ag bind to
MHC-II molecules



Ag-MHCII complex

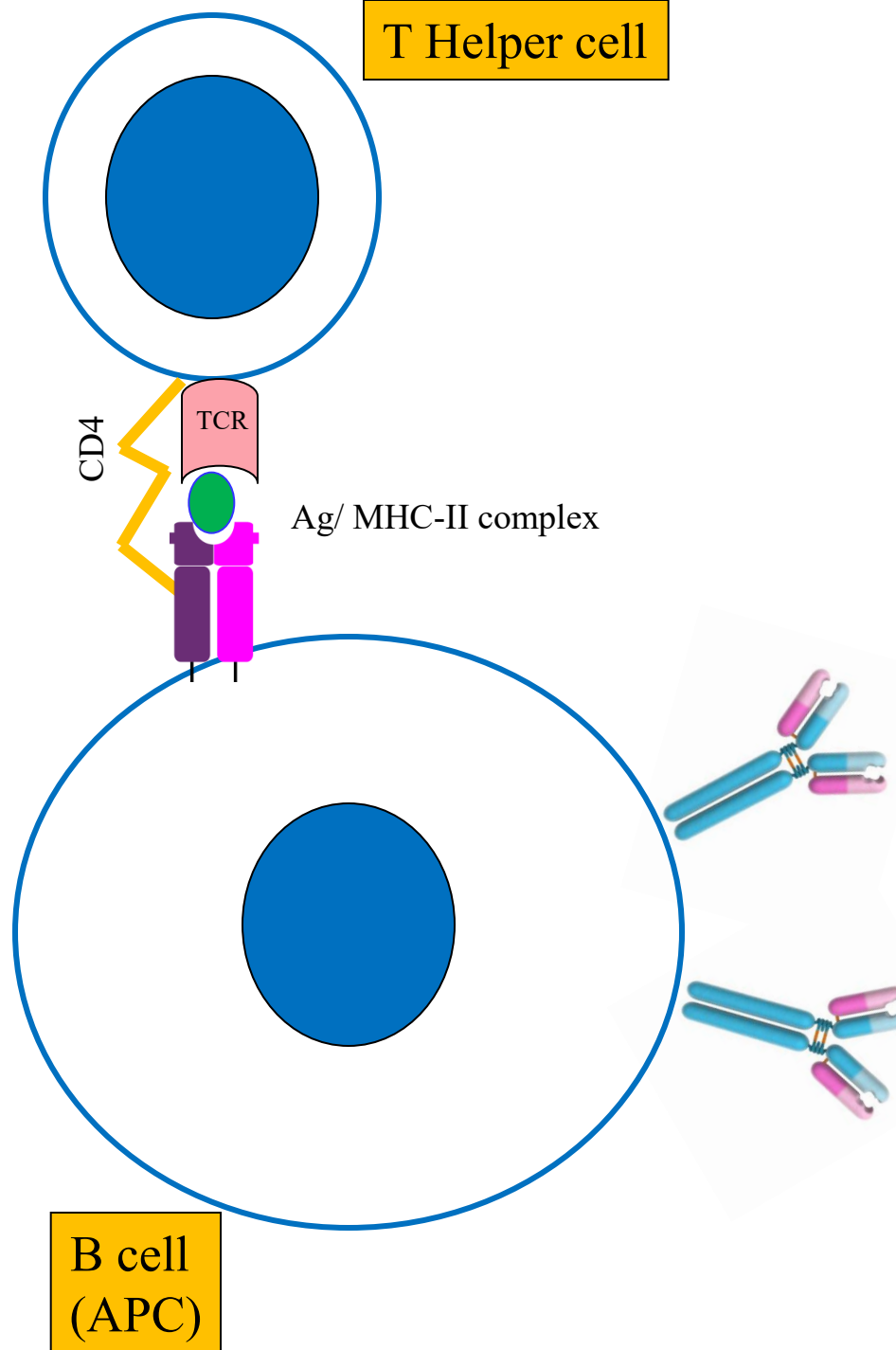
Humoral immunity



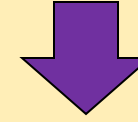
B cells recognize native (intact) antigens directly — meaning antigenic structures that are not processed or presented by MHC molecules.

They can bind to: Proteins, Polysaccharides, Lipids, Nucleic acids, Small molecules (haptens) when attached to a carrier protein

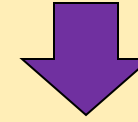
B cell
(APC)



T Helper cells bind to Ag-MHCII complex on the B cells



Stimulates proliferation and differentiation (activation) of B cells



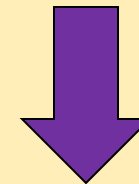
B cells

Proliferate
Activate

Activated B lymphocytes:

1- differentiate into **plasma cells**
(secrete antibodies)

2- differentiate into **memory cells**
(Rapid response on the 2nd exposure to the same Ag)



Life long immunity (vaccination)



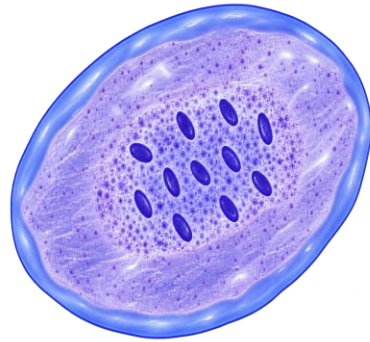
Never Let Monkeys Eat Bananas

Most common to least

Which type of lymphocyte is most abundant in peripheral blood?

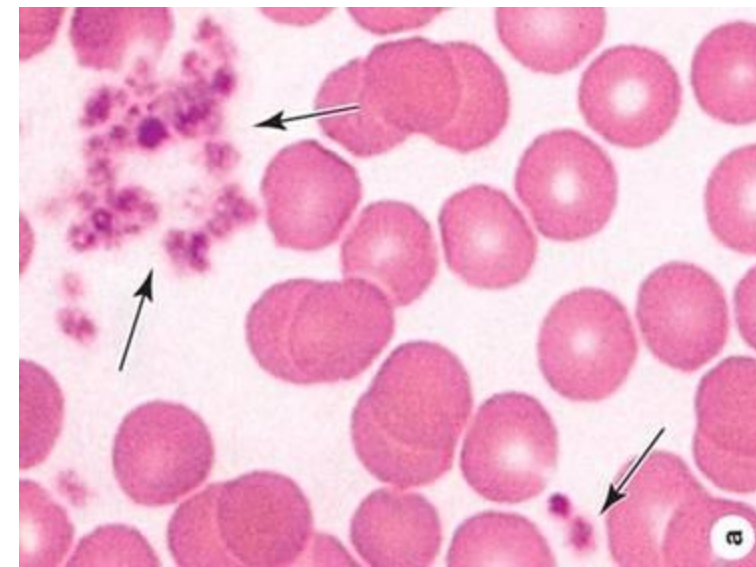
Thrombocytes (Platelets)

- Small anucleate cytoplasmic fragments
- Formed by fragmentation of the cytoplasm of **megakaryocytes** in the bone marrow
- Number: 200,000–400,000/mm³
- Shape: biconvex discs
- Diameter: 2-4 μ m
- Lifespan about 10 days
- Prevent blood loss by forming a platelet plug and promoting blood coagulation.
- Cytoplasm has a pale peripheral zone and a central purple granular zone.

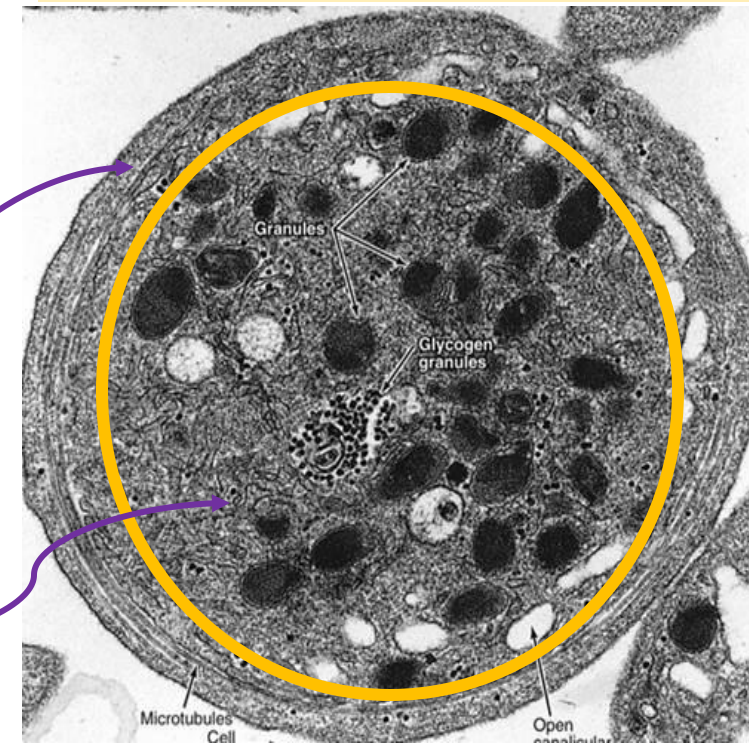


Platelet has 2 zones

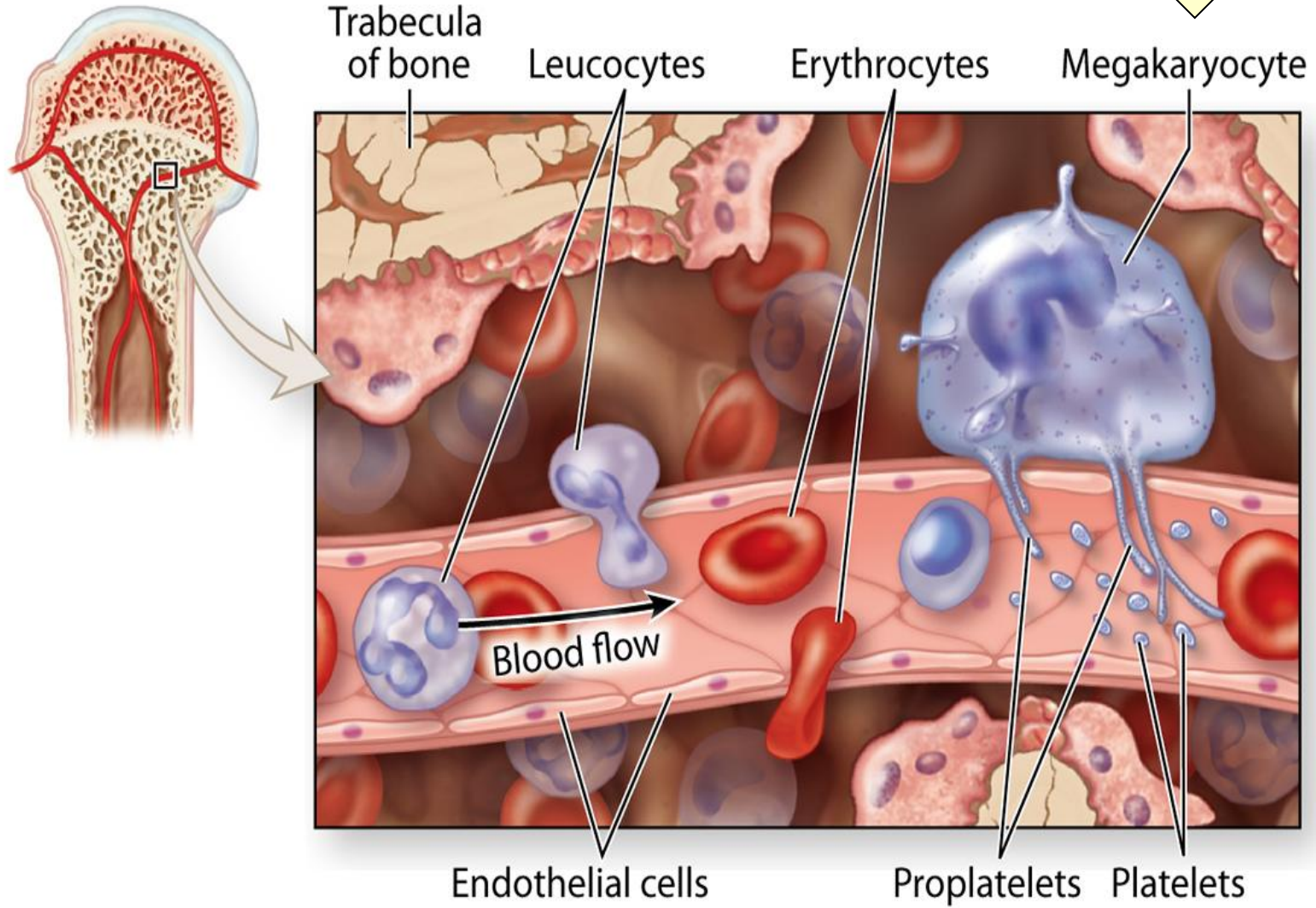
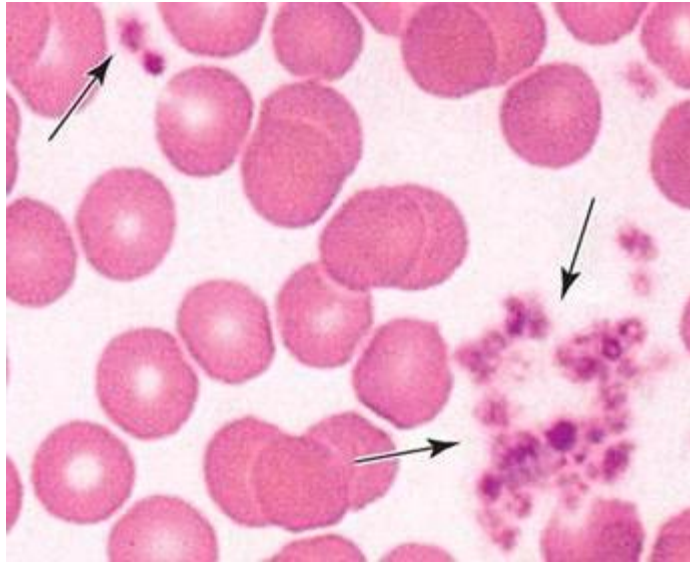
- Pale peripheral zone with few granules: **hyalomere**
- Dark-staining central zone containing granules: **granulomere**



Platelets may appear singly or in small clumps in blood smears.



Platelets continuously monitor the vascular endothelium for injury. When the endothelial lining is damaged, they adhere to the injured site, become activated, aggregate, and promote blood clot formation to stop bleeding.



Hyalomere: contains cytoskeletal elements and membranous systems

Cytoskeletal elements

Microtubules — help maintain the discoid shape

Actin and myosin filaments — involved in platelet contraction, shape change, and clot retraction

Membranous systems

Open canalicular system — communicates with the cell surface and facilitates release of granule contents

Dense tubular system — stores Ca^{2+} and is involved in platelet activation

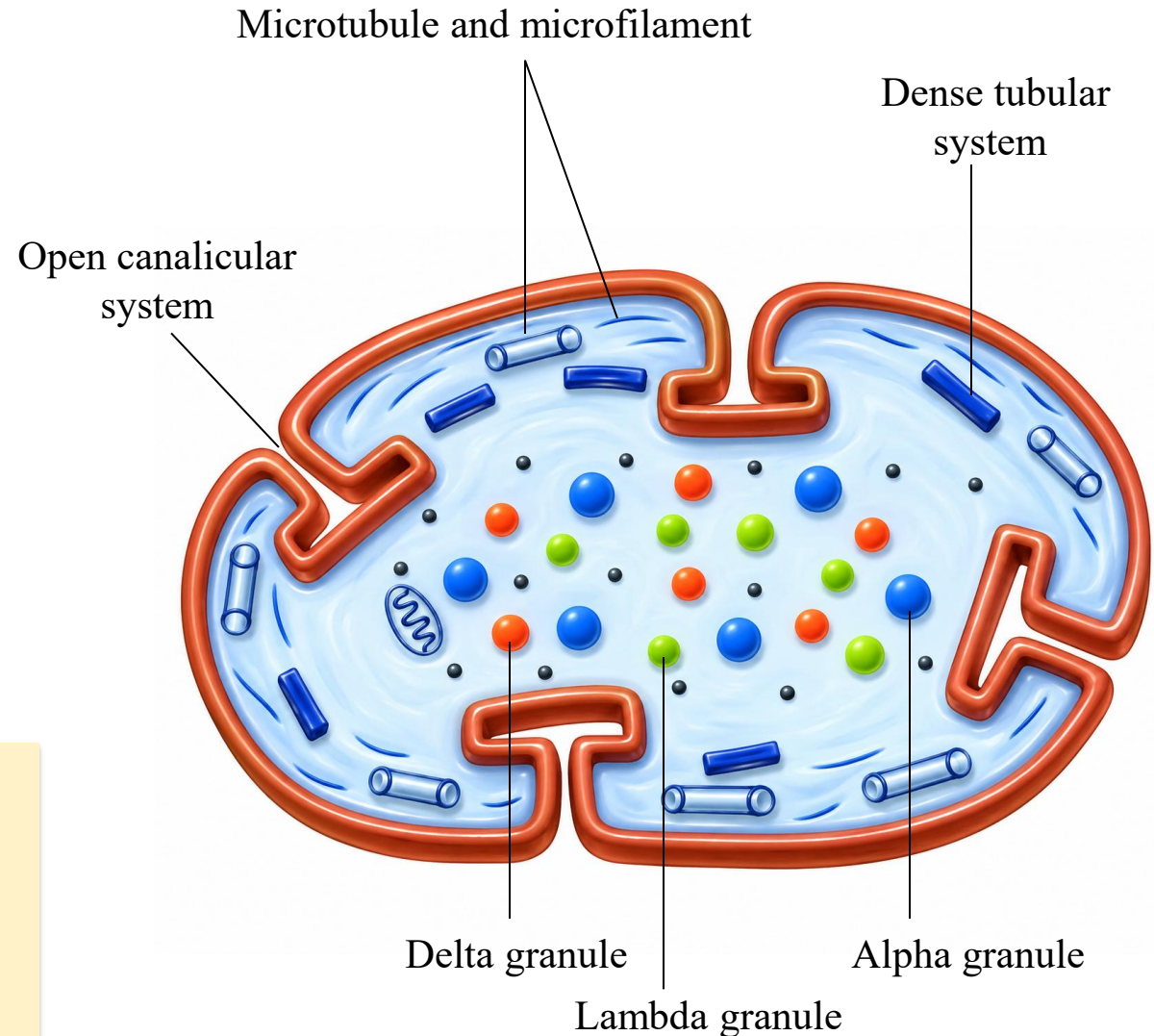
Granulomere: contains granules and organelles

Alpha granules: clotting proteins & growth factors

Delta (Dense) granules: ADP, ATP, Ca^{2+} & serotonin

Lambda granules: lysosomal enzymes

Have thick glycocalyx



Useful links (optional)

http://highered.mheducation.com/sites/dl/free/0072507470/291136/t_cell_dependent_antigens.swf

[http://highered.mheducation.com/sites/dl/free/0072507470/291136/Cytotoxic T cell activity against target cells.swf](http://highered.mheducation.com/sites/dl/free/0072507470/291136/Cytotoxic_T_cell_activity_against_target_cells.swf)

<http://highered.mheducation.com/sites/dl/free/0072507470/291136/immResponse.swf>

For SWF (Adobe Flash) files, [Ruffle Player](#)