



Blood Cells

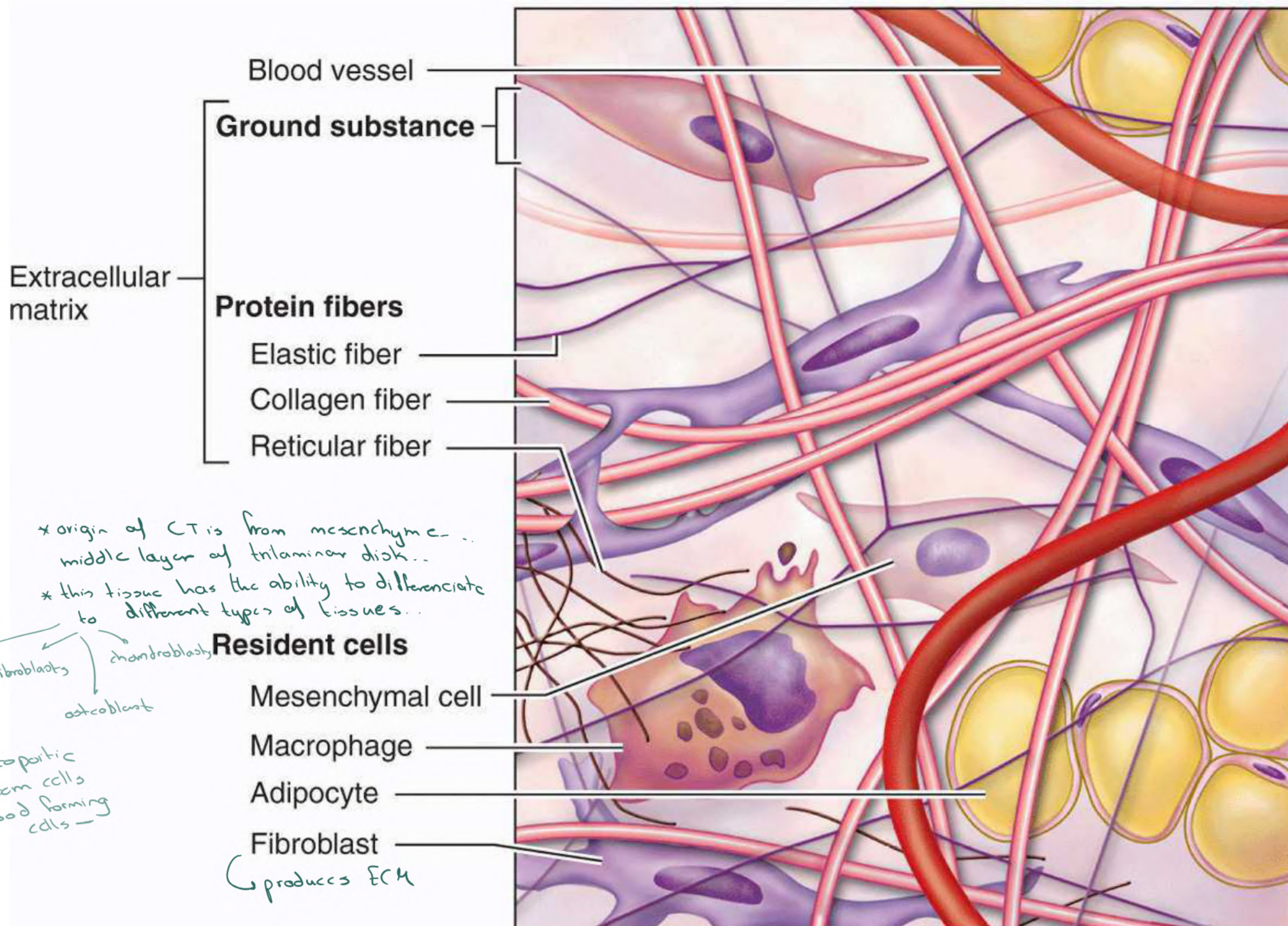
Final
practical
lab...

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Connective tissue proper $\Rightarrow$ cells + ECM

fibers $\begin{cases} \text{collagen} \\ \text{reticular} \\ \text{elastin} \end{cases}$

ground substance $\begin{cases} \text{water + macromolecules that } \uparrow \text{ viscosity of ECM} \\ \text{glycoproteins} \begin{cases} \text{mostly protein} \downarrow \text{adhesive molecules that connect cells with components of ECM} \end{cases} \\ \text{proteoglycans (GAGs)} \begin{cases} \text{mostly sugar} \downarrow \uparrow \text{viscosity of ground matrix} \end{cases} \end{cases}$



* origin of CT is from mesenchyme... middle layer of trilaminar disk...

* this tissue has the ability to differentiate to different types of tissues...

fibroblasts

chondroblasts

osteoblast

hematopoietic stem cells

- blood forming cells -

EC → plasma Clls → WBC
 → RBC
 → platelets

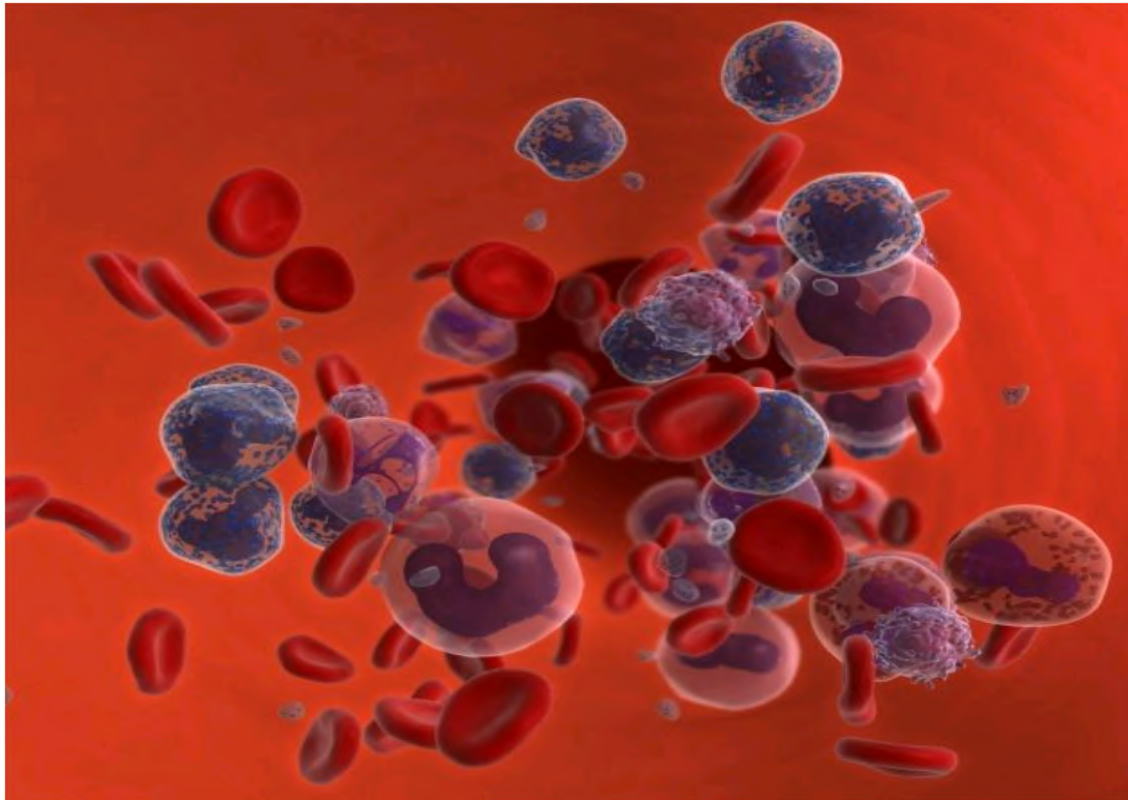
- it has same composition of CT (cells + ECM)

* special CT

* plasma is considered the ECM of blood... but it has a fluid consistency... why?

It lacks collagen fibers,
elastic fibers, reticular
fibers ----

* But also originate from mesenchyme -



Functions of Blood

- Transports nutrients and respiratory gases
ex. glucose
- Transports waste products to organs and tissues where they can be recycled or released
- Transports hormones → *endocrine glands: collection of epithelial cells specialized for secretion & releases hormones directly in blood stream*
- Transports immune cells throughout the body
WBCs
- Helps regulate body temperature
cutaneous → *vasodilation → ↑ heat loss*
→ *vasoconstriction → ↓ heat loss*
- Maintains of acid-base and osmotic balance

7.35 - 7.45

✓ pH of 7.4

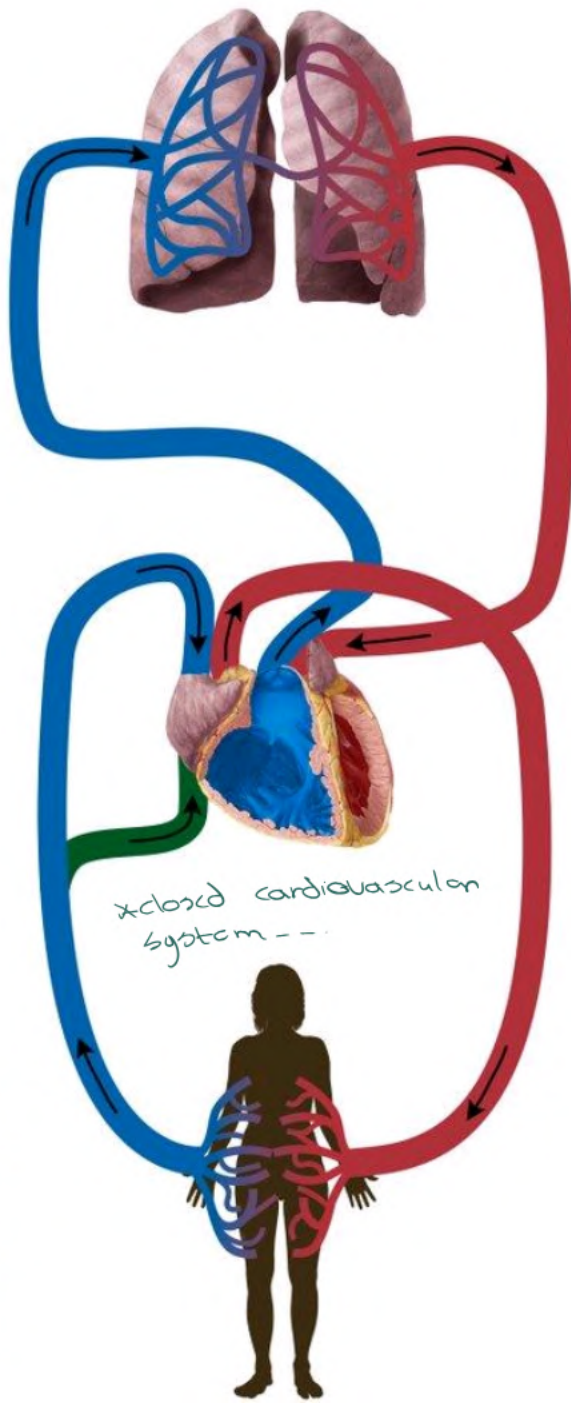
✓ Color is dependent on amount of Oxygen

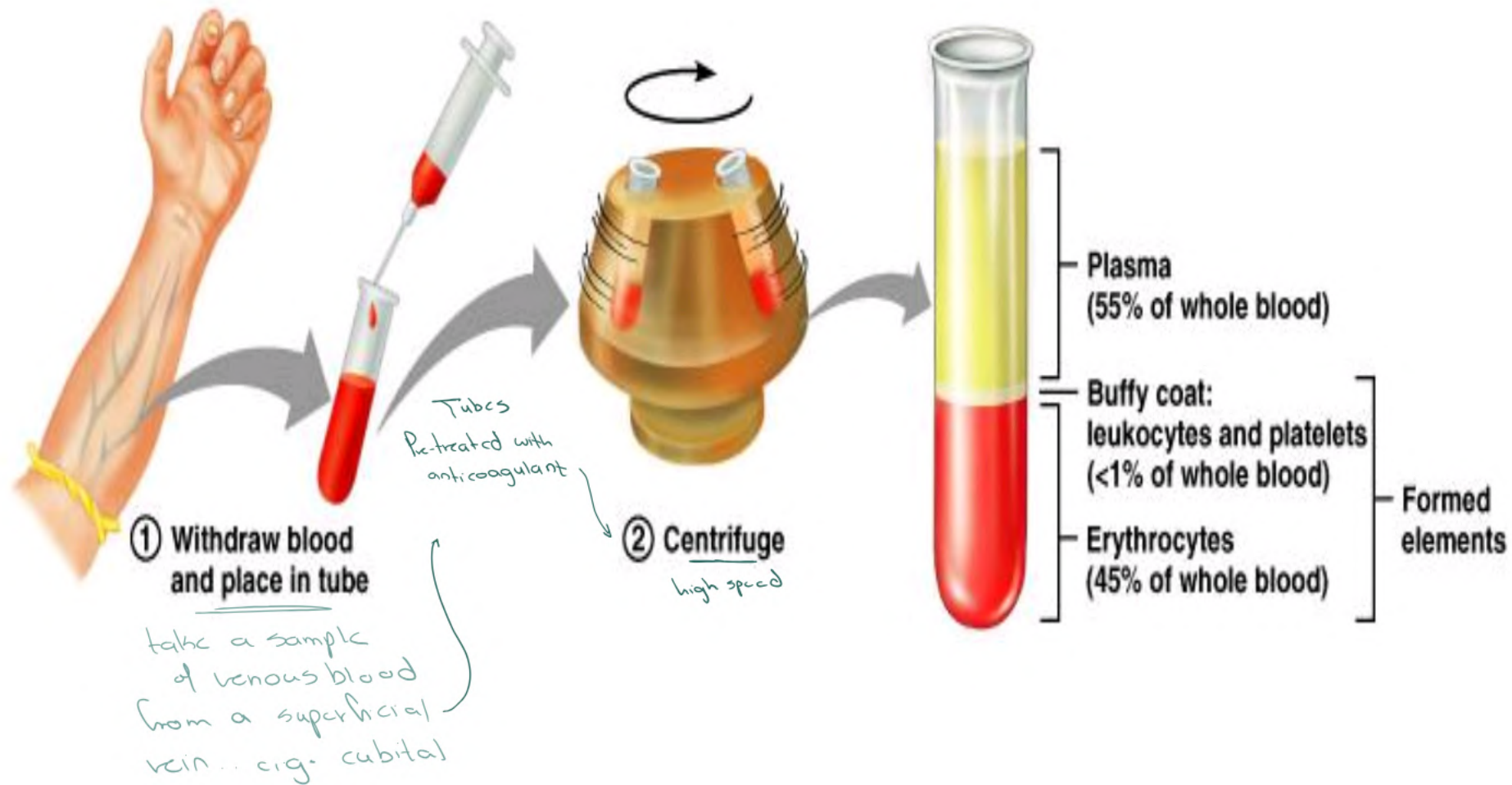
* More oxygen = brighter the red → *most arterials*

* Less oxygen = duller the red → *veins mostly less oxygenated not deoxygenated*

Blood is propelled mainly by rhythmic contractions of the heart

* About **5-6 Liters** of blood in an average adult moves unidirectionally within the closed circulatory system





Collected blood in which clotting is prevented by the addition of anticoagulants (eg, heparin , citrate or EDTA) can be separated by centrifugation into layers that reflect its heterogeneity

Physical Characteristics

Fluid

– Living 45%

Cells (formed elements)

- RBC **Erythrocytes** (carry oxygen)

BUFFY COAT

- WBC **Leukocytes** (immune)
- Platelets **Thrombocytes** (clotting)

– Non living (Matrix) 55%

Plasma (pale yellow fluid)

- 90% water $\approx 91.5\%$
- 10 % (electrolytes, nutrients, proteins (albumin), waste (CO_2 , ammonia, urea), gases, hormones)

HEMATOCRIT: Ratio of the volume of RBCs to the volume of whole blood

Example: a hematocrit value of 40% means that there are 40 ml of RBCs in 100 ml of whole blood

$1 \text{ ml} = 1 \text{ cm}^3$

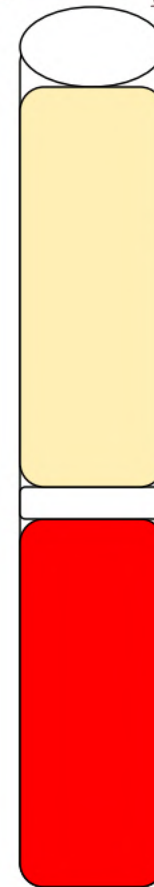
Normal hematocrit:

Males=40-53%

Females= 36-48%

blood formation of hematopoiesis $\Rightarrow$ after maturation they are released in blood stream to perform functions.

due to menstruation & estrogen suppress hematopoietic stem cells in bone marrow.



Top layer= plasma

like collagen fibers giving white color in bulk even though they are colorless...

appears whitish (WBCs) due to condensation of these cells... but they are not white -

Middle layer= buffy coat

$\approx 1 \text{ ml}$

$\approx 1\%$ of Blood volume

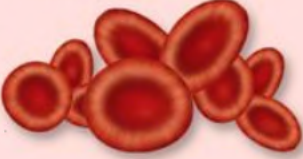
Bottom layer=RBCs

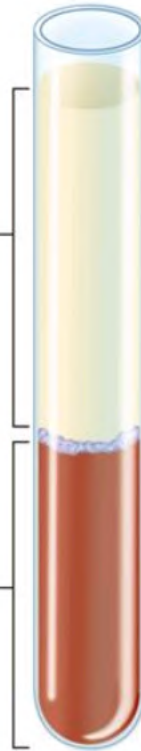
(Packed Cell Volume)

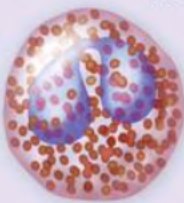
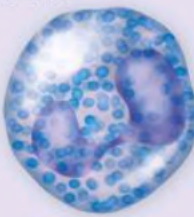
$\mu\text{m} = 1/50$ of a drop

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

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
In each μm of blood... 



Buffy Coat	
Platelets 150,000-400,000/mm ³	Leukocytes 4500-11,000/mm ³
In each μm of blood... * WBCs in thousands while RBCs in millions	 Lymphocytes 25%-33%
	 Neutrophils 54%-62%
	 Monocytes 3%-7%
	 Eosinophils 1%-3%
	 Basophils 0%-0.75%

Serum = everything in plasma, minus the clotting factors

Albumin:

- ✓ The most abundant plasma protein
- ✓ Is made in the liver
- ✓ Helps maintain the osmotic pressure in capillaries
- ✓ Transports steroid hormones and fatty acids

*plasma proteins stay inside BVs to form oncotic osmotic pressure to reabsorb fluid carrying CO₂ & waste in BVs...
*hydrostatic pressure at level of capillary bed forces fluid of plasma to leave blood & enter spaces (interstitial fluid) of tissue carrying O₂ & nutrients...

Hydrostatic pressure that results from contraction force of heart & smooth muscles inside arteries...

Fibrinogen:

- ✓ The largest plasma protein
- ✓ Is made in the liver
- ✓ Important for clot formation

this oncotic pressure is necessary to keep blood inside blood vessels... blood in cardiovascular system has hydrostatic pressure & osmotic...

*plasma proteins are large proteins located inside plasma & usually don't live blood to form an osmotic pressure (oncotic pressure)

Fresh plasma



The importance of proteins inside the plasma is to prevent fluid loss and to create osmotic pressure (to keep the blood inside the blood vessels)

Globulins (α , β and or γ globulins):

α and β globulins

- ✓ Are made mainly by liver
- ✓ Transport fat soluble vitamins, lipids and iron

*plasma cell - (anti-body secreting cell)

γ -globulins (Immunoglobulins (antibodies):

secreted by plasma cells

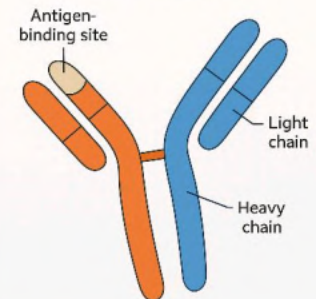
*5 types (GAMED)

IgM, IgA, IgD, IgG, IgE

Plasma cell



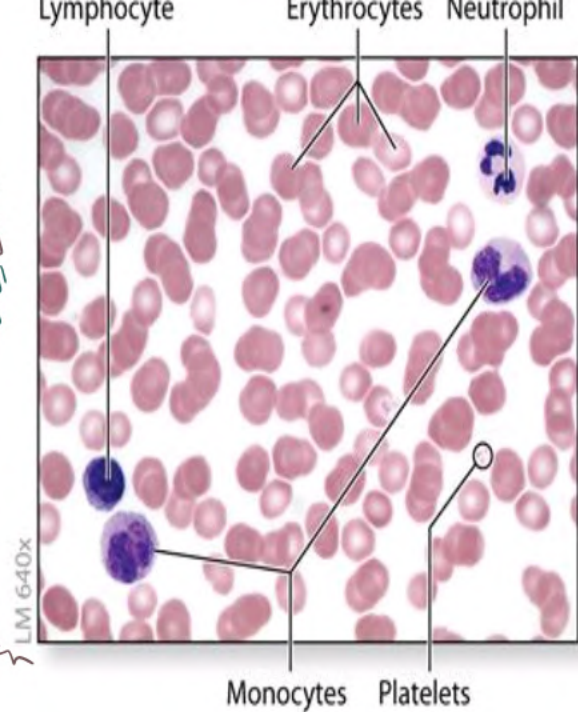
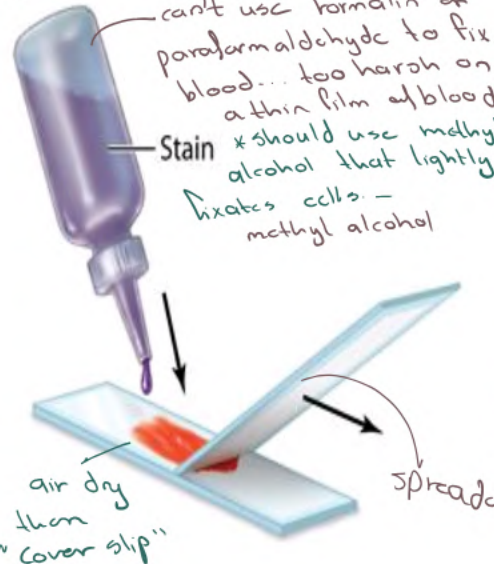
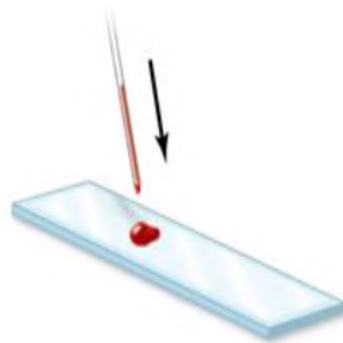
ANTIBODY



Preparing a blood smear



*how to study blood
under light
microscope?



① Prick finger and collect a small amount of blood using a micropipette.

② Place a drop of blood on a slide.

*Preparing tissue :-
(1) fixation
(2) staining

③a Using a second slide, pull the drop of blood across the first slide's surface, leaving a thin layer of blood on the slide.

③b After the blood dries, apply a stain briefly and rinse. Place a coverslip on top.

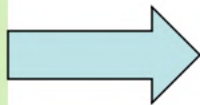
④ When viewed under the microscope, blood smear reveals the components of the formed elements.

light microscope

*can't use H&E → doesn't show RBC components

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 on a glass slide and left dry in air

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

cosin → methylene blue → azure dye
- dissolved in methyl alcohol -

*we use neutral staining
in histology → basic
→ acidic

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

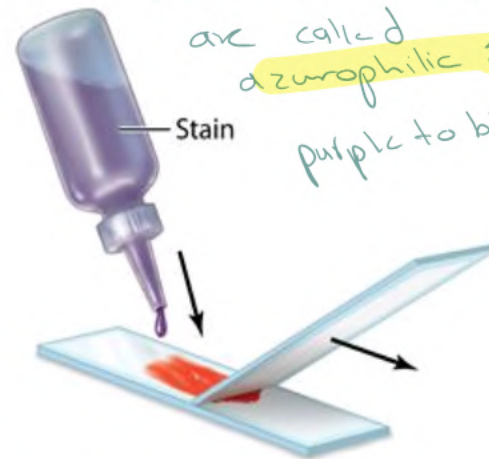
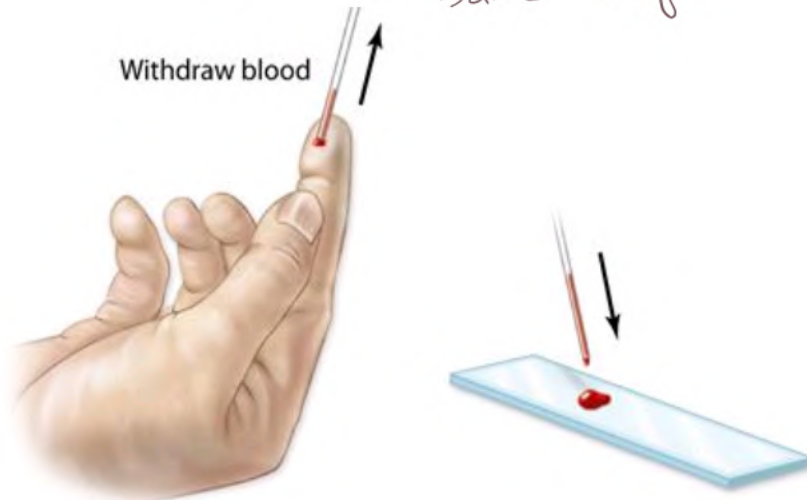
most commonly
→ H & E → acidic dye
red/pink → based on concentration of stain
↑ conc. ⇒ reddish-
& stains basic parts
of tissue (cytoplasm usually)
basic dye
blue
& stains acidic components
of tissue (nucleus)

Dissolved in methyl alcohol (fixative)

Fixation + stain is
same step -

Azure dye!!

structures stained
with azure dye
are called
azurophilic structures
purple to blue



Blood film/smeat

Erythrocytes (RBCs)

كريات الدم الحمراء

not true cells $\Rightarrow$ bags of Hb with no organelle but plasma membrane

no nuclei or organelle

- Small, biconcave discs

* erythropoiesis inside bone marrow
 $\rightarrow$ stem cells (precursors) where nucleated & had organelles that get replaced with Hg

- Transport oxygen and CO_2 , cytoplasm is full of hemoglobin molecules

* In precursors of erythrocytes in bone marrow... to produce a huge amount of Hb need alot of ribosomes $\Rightarrow$ specifically

- Have no nuclei or organelles

* proteins produced by ER are exported - if used locally produced in free ribosome

due to this... the cytoplasm will stain more basophilic due to alot of amino acids & nucleic acids (ribosome)

- Pick up O_2 at lung capillaries and release it at body tissue capillaries

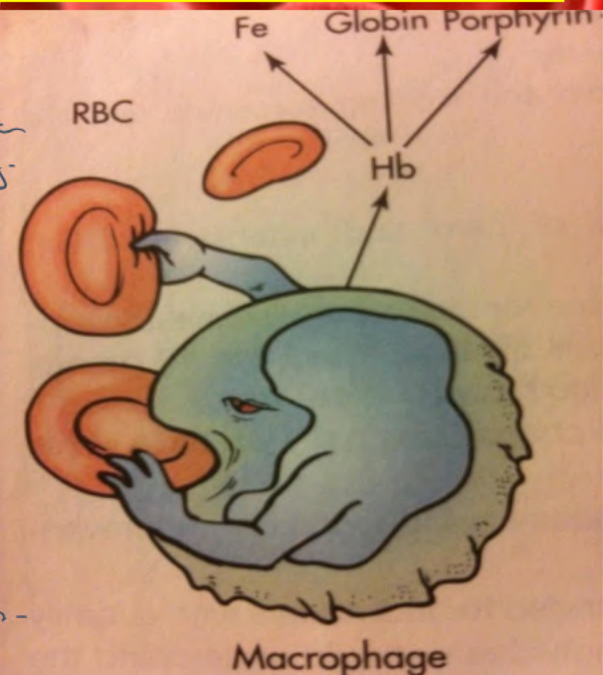
During their maturation process, the erythrocytes extrude their nuclei, and the mature RBCs enter the bloodstream, without their nuclei

- **Fate:** Survive for $\sim 100-120$ days in the circulation. Worn out RBCs are removed by macrophages of the **spleen, bone marrow and liver.**

* old erythrocytes are eliminated inside spleen & bone marrow as lymphatic organs -

Male: 4.5-5.5 million/mm³

Female: 4-5 million/mm³



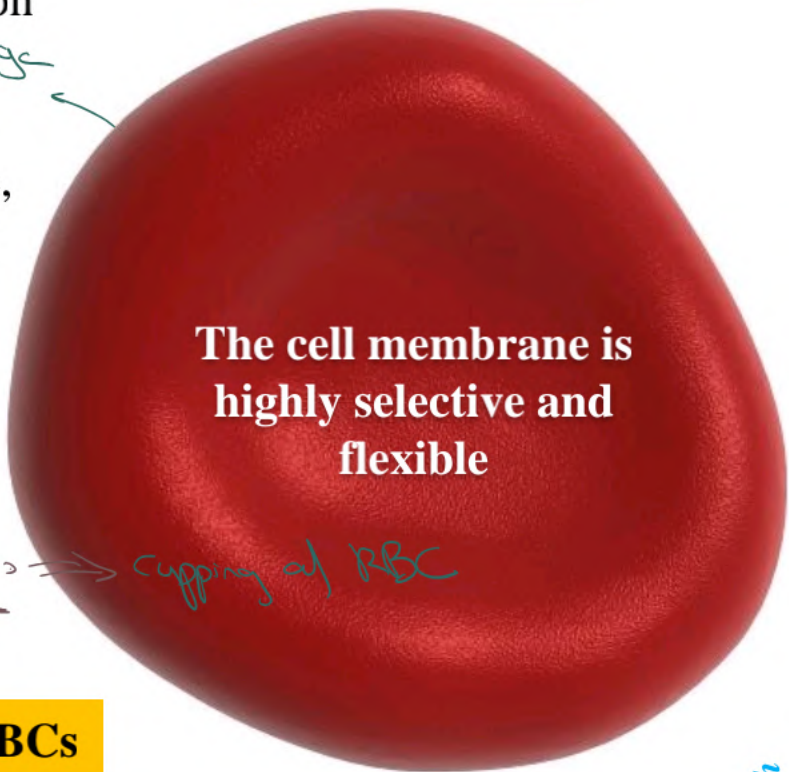
Immediately beneath the plasma membrane is a **meshwork of proteins** (Spectrin and Ankyrin) forming a cytoskeleton

if not present... would cause fragmentation of protein when passing through small bus...
not to stick to walls of bus.
no pointed edge

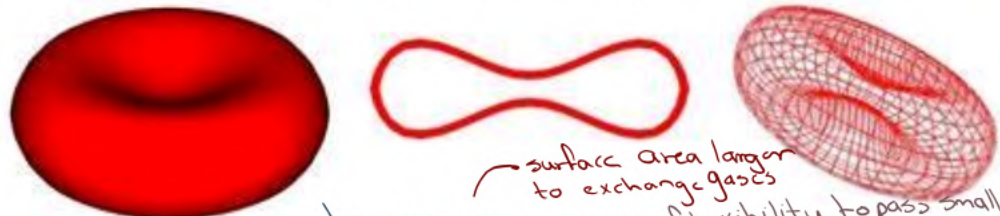
This **submembranous meshwork** stabilizes the membrane, maintains the cell shape, and provides the cell elasticity required for passage through capillaries

proteins
- protect biconcavity, anchors -

Top view

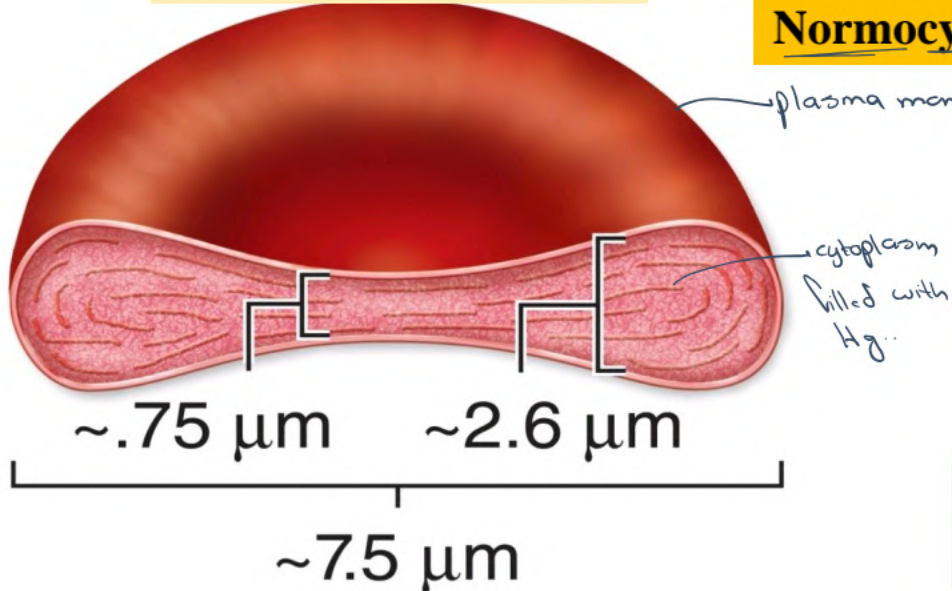


The cell membrane is highly selective and flexible



surface area larger to exchange gases
biconcave → *give flexibility to pass small bus*
- composition gives it flexibility too -
Cupping of RBC

Sectional view



Normocytic RBCs

Size *capillary diameter (4 μm - 10 μm)
 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

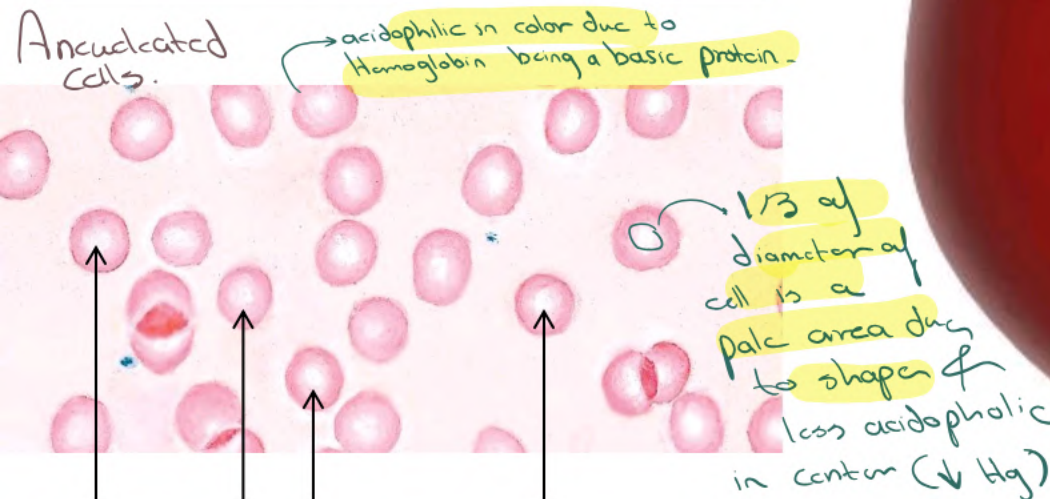
The biconcave shape along with the fluidity of the plasma membrane (50% proteins) permits erythrocytes to bend and adapt to the small diameters and irregular turns of capillaries

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

e.g.
Carbonic
anhydrase

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

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

Normochromic RBCs

normal staining reaction of RBC

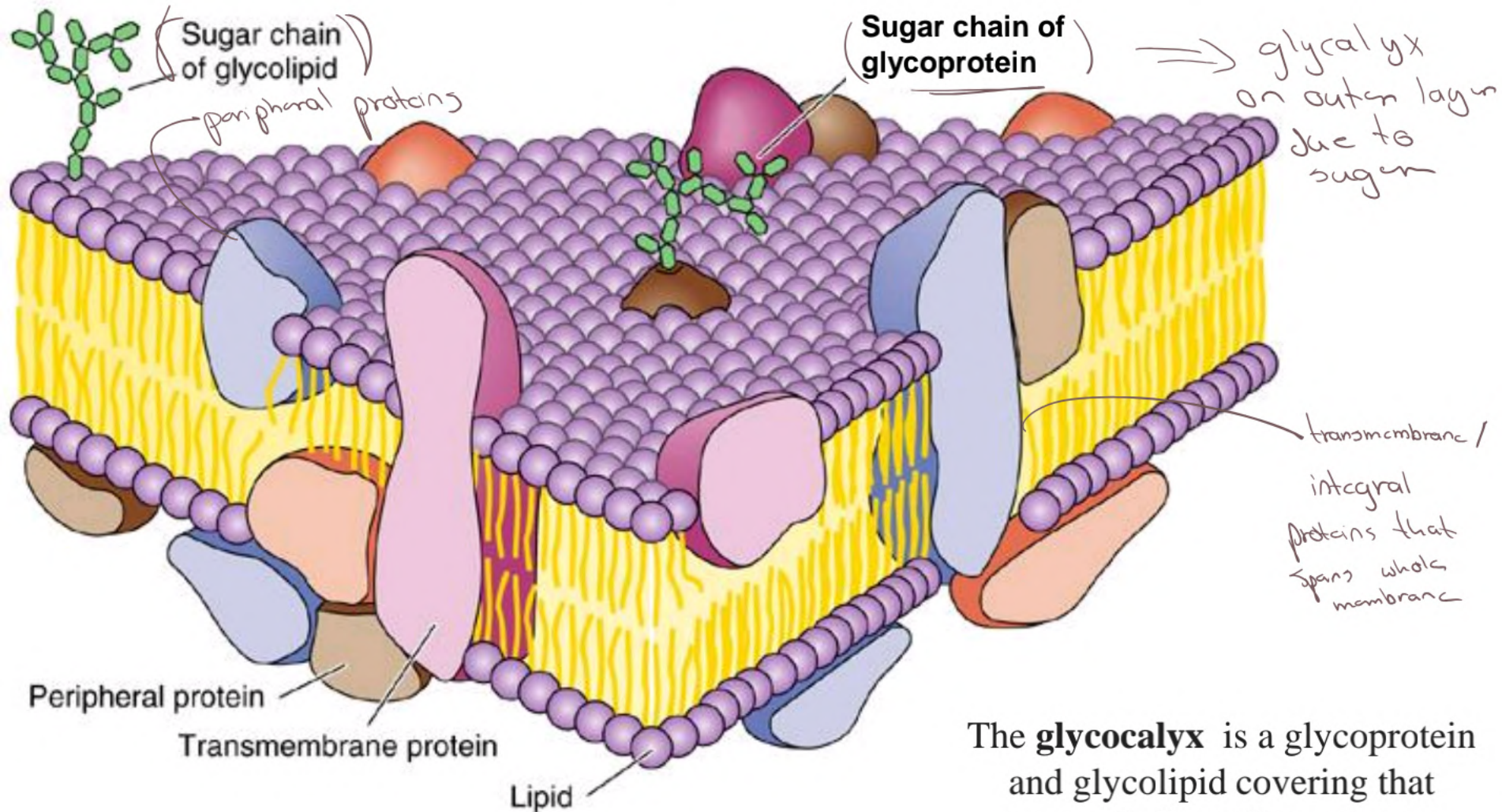
molecular composition of
plasma membrane helps
in RBC flexibility

→ 50% proteins, 40% lipids, 10% sugar.

Cell Membrane

A Carbohydrate chains bound to lipids and proteins

bilayer of phospholipids with
proteins



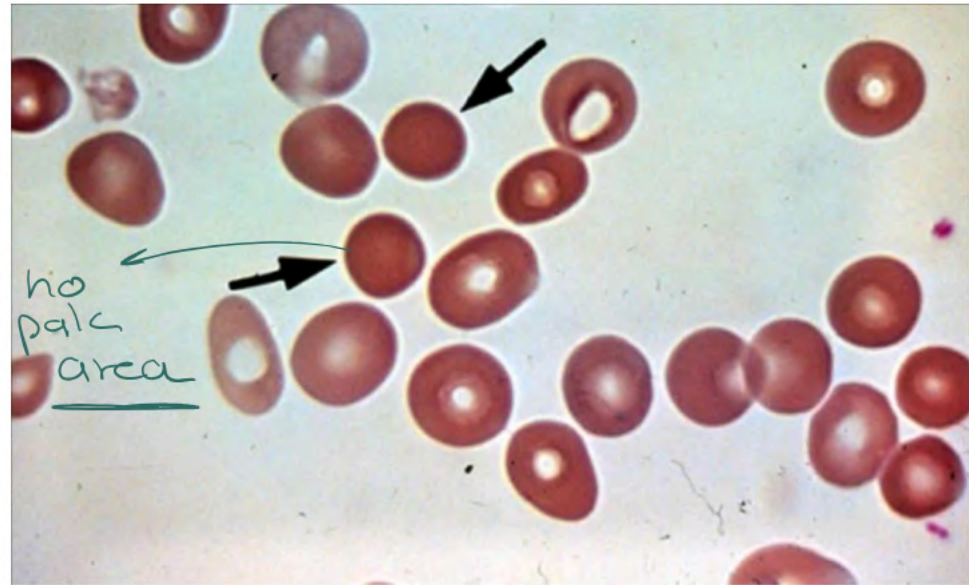
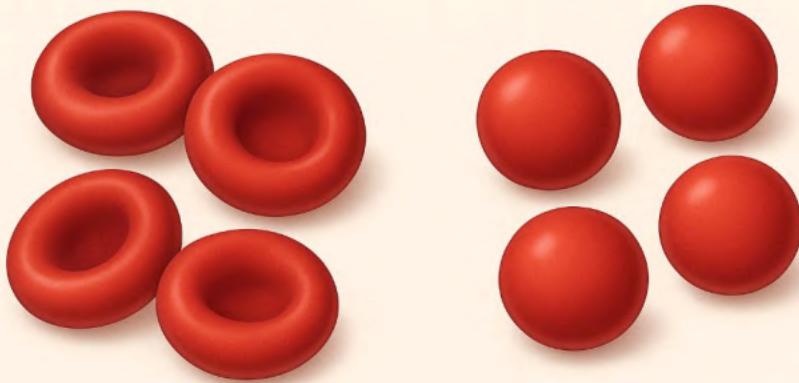
The **glycocalyx** is a glycoprotein and glycolipid covering that surrounds the cell membranes

* Hereditary Spherocytosis



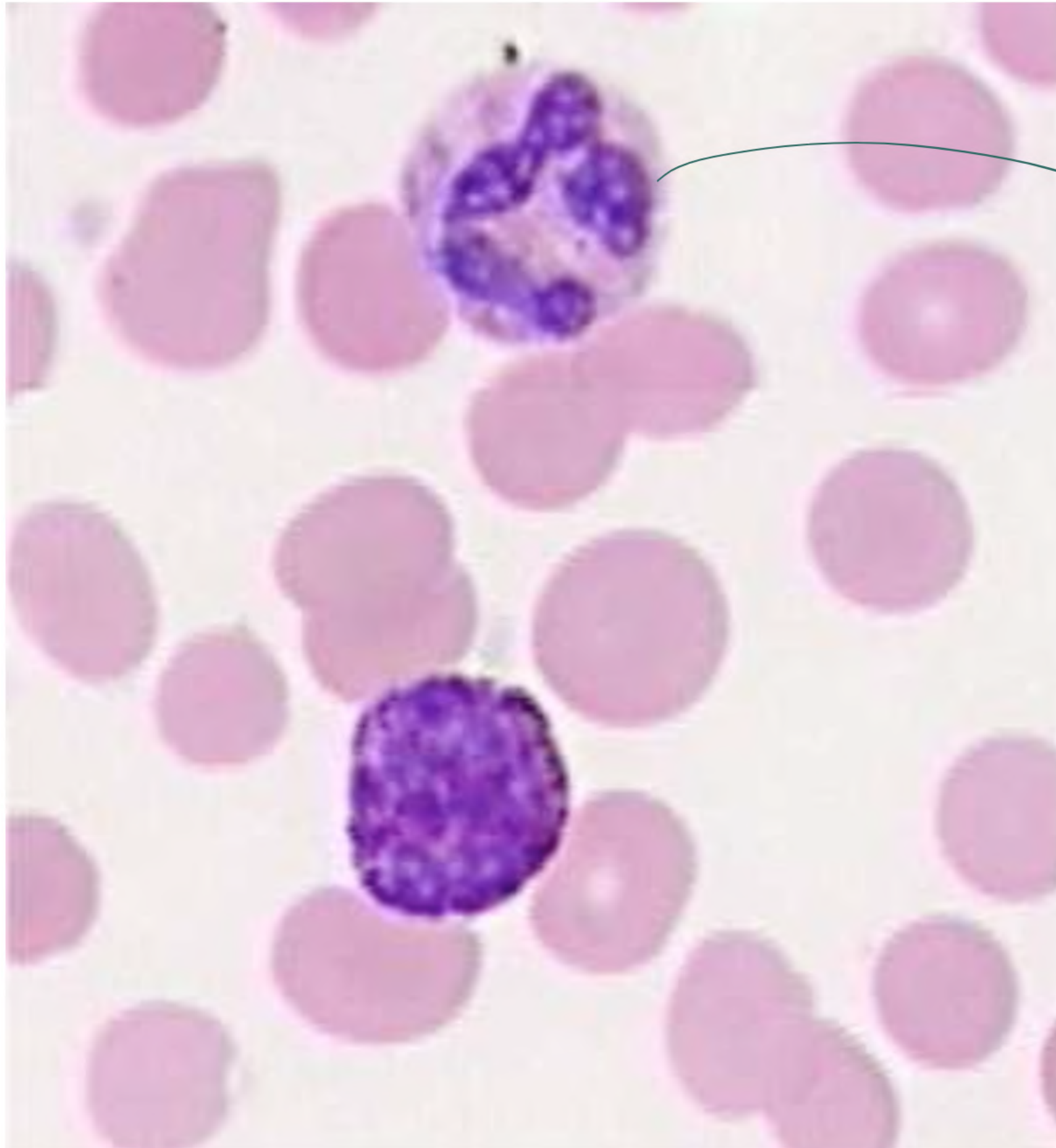
cells are formed normal
in bone marrow & become
spheres when passing BUs.

BICONCAVE RBCs **SPHEROCYTES**



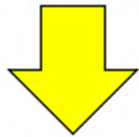
— mutation is submembranous proteins —

Caused by **mutations** in genes relating to membrane proteins (mostly Spectrin and Ankyrin) that allow for the erythrocytes to maintain their biconcave shape



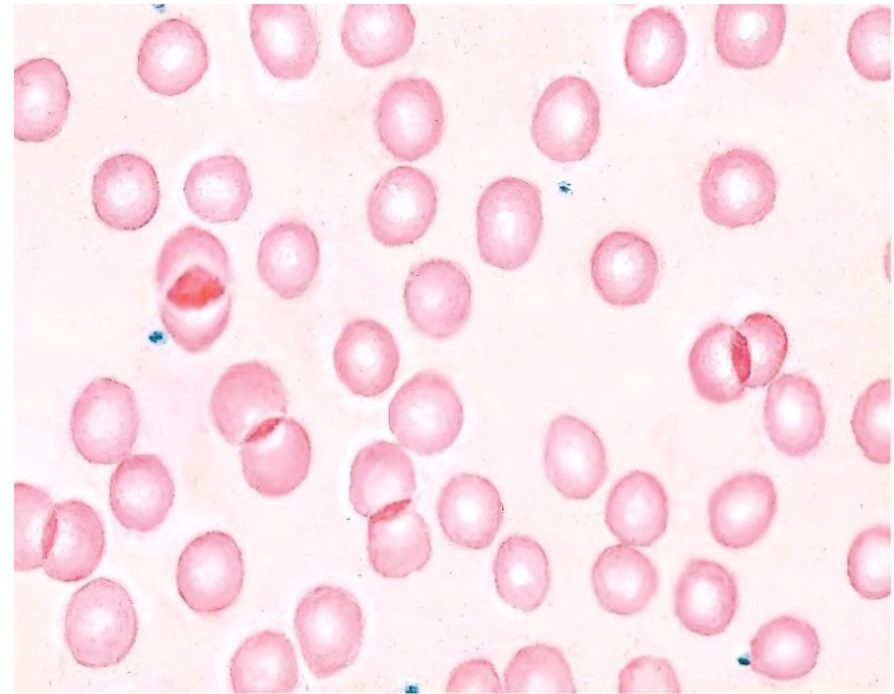
can estimate
size based
on RBCs
 ≈ 2 RBCs
= diameter
 $\approx 14 - 15 \mu m$

LM:
Blood film stained with Leishman:
Rounded
Non nucleated
Acidophilic (with pale central area)



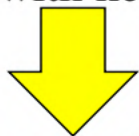
A zone of **central pallor** is about $\frac{1}{3}$
the size of the RBC

LM



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EM:
✓ Have no nucleus or organelles
✓ Filled with hemoglobin

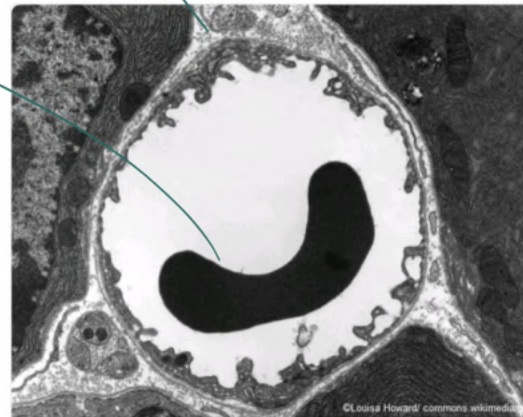


Electron dense and homogenous

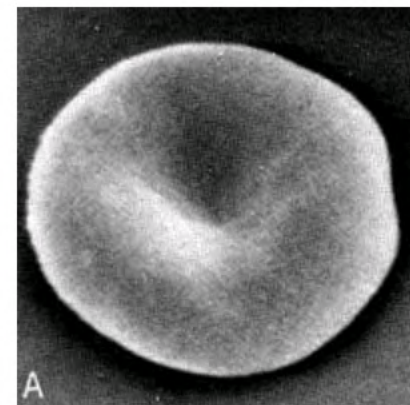
cross-section through capillary

cupping of RBC

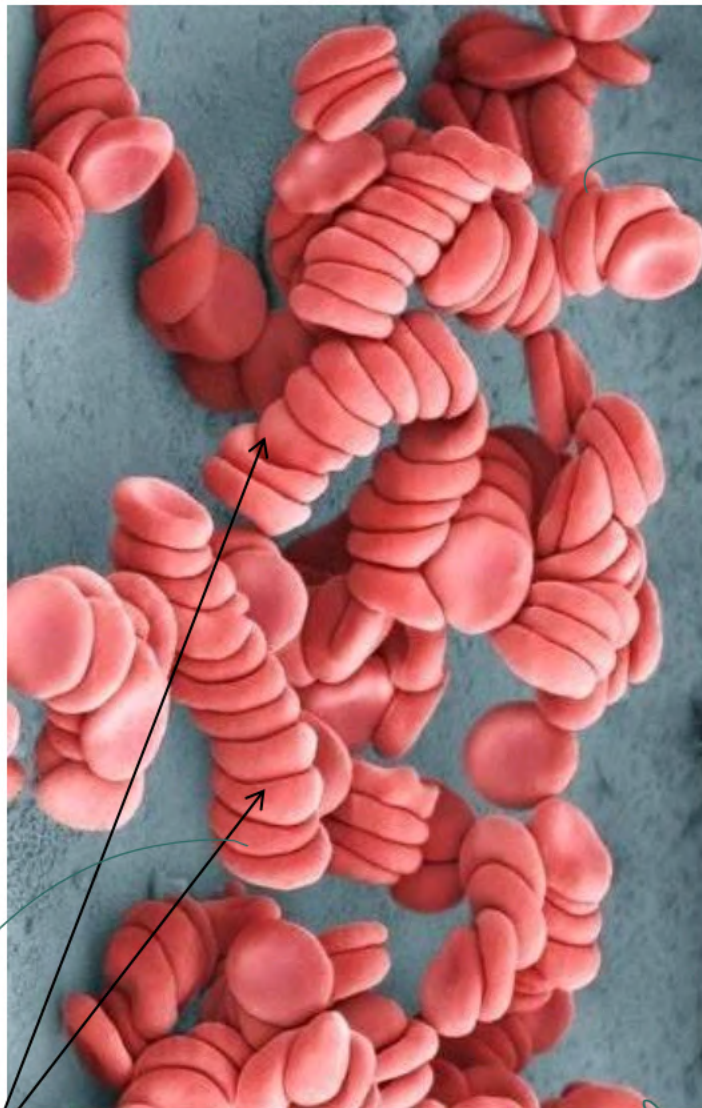
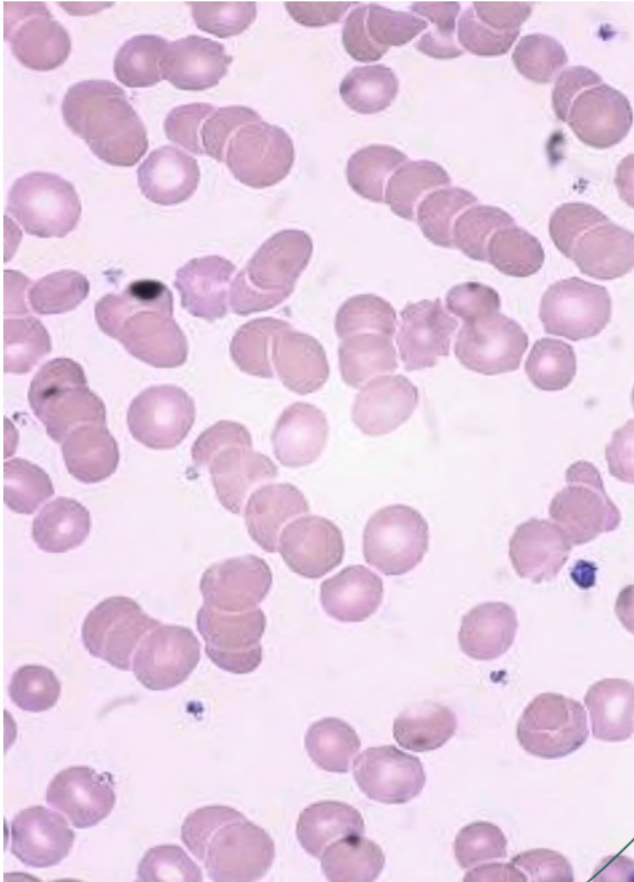
TEM



SEM



Rouleaux appearance occurs to some extent in all films



unless all is rouleaux... could indicate disease
*appears in histological sections in normal sample - artifact -



*can even happen in infections where of circulation ↓ due to ↓ fluid & happen problem of conc. of plasma proteins.

* temporary: not indication of anything specific -

in French roll of coins.

Rouleaux formation:

✓ RBCs may adhere to one another loosely in stacks called Rouleaux (pile of coins)

✓ In slow (not in normal) circulation $\Rightarrow$ or $\uparrow$ viscosity of blood $\Rightarrow$ $\uparrow$ conc. of plasma proteins -

Due to surface tension caused by their biconcave surface (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

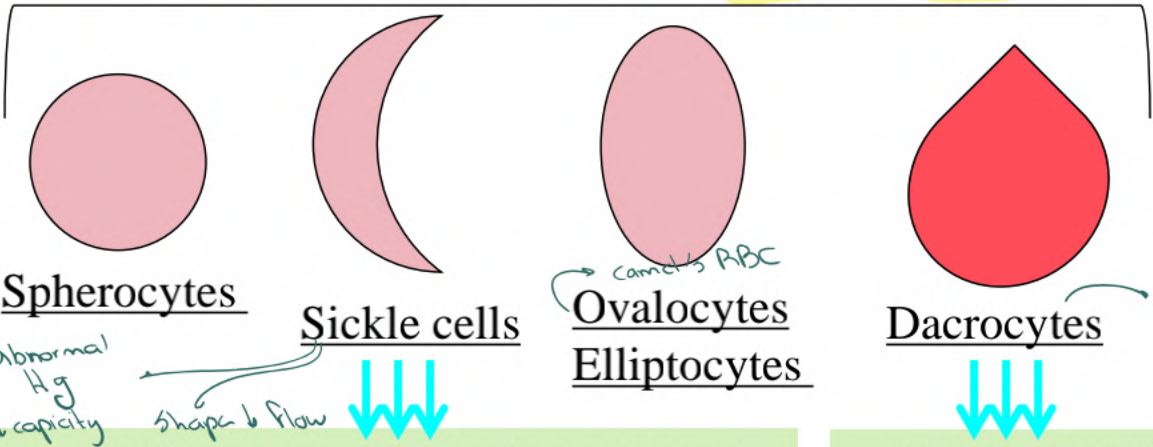
Abnormal sizes:

Microcytes (<6um) → microcytosis if all are
Macrocytes (>9um) → macrocytosis if all RBCs are
Anisocytosis (different sizes) → normocytes / microcytes & macrocytes
Not the same.

Abnormal shapes:

Due to changes either in the cell membrane or Hb content

* Poikilocytosis is variation in the shapes of red blood cells
any abnormal shape of RBC -
Poikilocytes (Poikilocytosis)



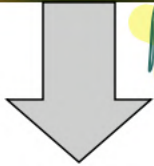
One of the most severe changes in shape occurs during **SICKLING** of RBCs in sickle cell anemia where erythrocytes take on the form of crescents

Abnormal staining:

Hypochromia: Denotes a decrease in the intensity of staining
Indicates a decreased amount of hemoglobin
Frequently accompanies microcytosis



Hypochromic microcytic anemia



Point mutation

Sickle cell anaemia results from abnormal hemoglobin

Based on Hemoglobin Content (color /chromasia):

Normochromic: RBCs have a normal hemoglobin concentration → normal color, normal central pallor (about one-third of the cell).

Hypochromic: RBCs have less hemoglobin than normal → appear paler with an abnormally large central pallor (Common in iron deficiency anemia).

Hyperchromic: RBCs have more hemoglobin concentration than normal → cells look denser and darker with absent central pallor (seen in spherocytosis).



Hypochromia

Normochromia

Hyperchromia

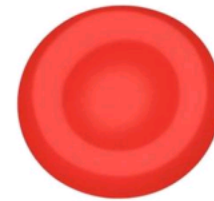
↓ acidophilic

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).

MICROCYTIC

NORMOCYTIC

MACROCYTIC



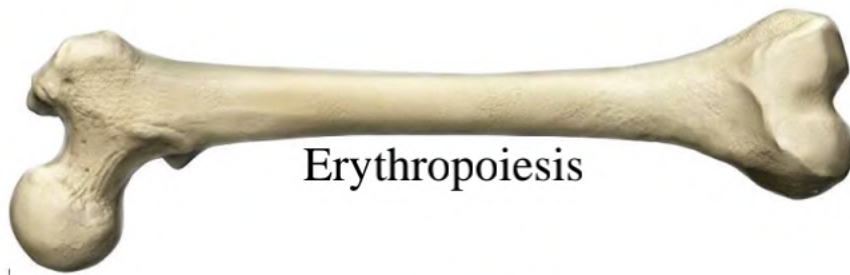
Based on Cell Size (cytometry):

Normocytic: RBCs are of normal size, about 7–8 μm in diameter.

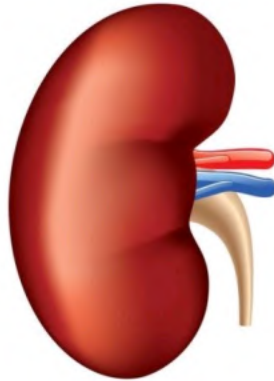
Microcytic: RBCs are smaller than normal (seen in iron deficiency anemia).

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

iron deficiency anemia → hypochromic & microcytic



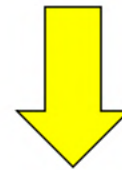
Erythropoiesis



Production of erythrocytes in the bone marrow, is stimulated by erythropoietin

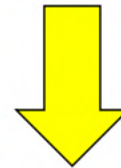


Erythropoietin is produced by the kidneys



supplied by renal artery
specific cells detect O_2 inside blood

When RBC count drops, such as during blood loss, the resulting oxygen-deficiency state, **hypoxemia**, is detected by the kidneys.



erythropoietin hormone in bone marrow

The kidneys respond by increasing their erythropoietin secretion, which leads to increased red blood cell production

↑ production of RBC

Anemia: a decrease in the total number of RBCs (and/or hemoglobin)

Polycythemia: an increase in the total number of RBCs (and/or hemoglobin)

pathology: increase in total RBC mass.

Consequently

→ not always pathological →

People living at high altitudes usually have higher RBC count as a response to lower oxygen levels.

↓ PO₂ in high altitudes → detected by kidneys → ↑ erythropoietin → ↑ erythropoiesis

takes (1-2) weeks to ↑ number.



physiological polycythemia



Athletes whose demand for oxygen is more elevated, also have higher RBC counts.

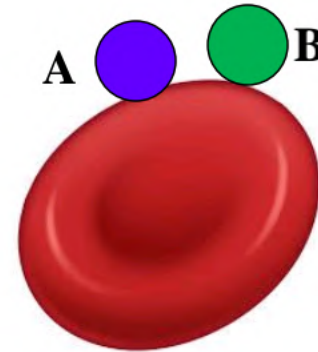
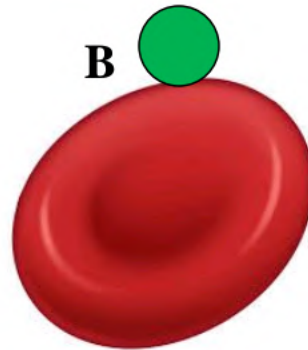
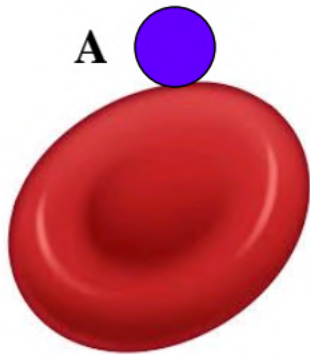
to supply their red muscle



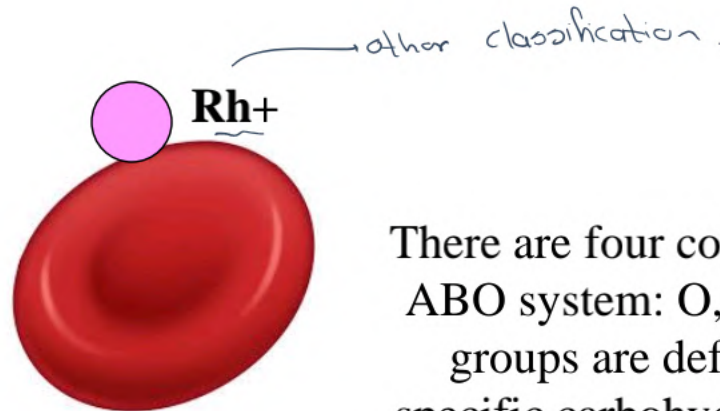
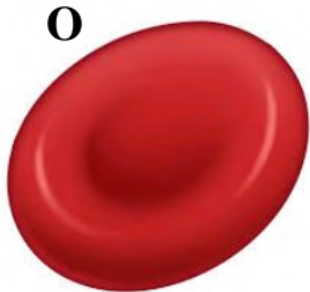
RBC plasma membranes have glycoprotein antigens on their external surfaces

* glycalyx → glycolipid
↳ glycoprotein

* H antigen on glycoprotein → can be modified & different between people



* H antigen with modifications



There are four common blood groups in the ABO system: O, A, B, and AB. The blood groups are defined by the presence of specific carbohydrate sugars on the surface of red blood cells

	RBCs (Erythrocytes)	WBCs (Leukocytes)
Types	1 type	5 types basophils eosinophil neutrophil monocyte lymphocyte
	Not true cells -bags of Hb-	True cells → nucleated with few organelles-
Number	Male: 4.5-5.5 million/mm ³ female: 4-5 million/mm ³ 1 g L = 1 mm ³	<u>4500-11000</u> /mm ³
Diameter	6-9um (7.5um)	wide range ← <u>6-20um</u>
Life span	120 days ≈ 4 months ≈ relatively short	wide range ← Few days-years
Origin and maturation	Bone marrow	Bone marrow and lymphoid tissue extra maturation & differentiation site in thymus for T cells
Shape	Biconcave discs	Spherical in blood spherical once they leave blood to infection site in tissue they become irregular
Function	Gas exchange	Defense
Motility	Non motile flow with blood flow	Motile
	Function exclusively within <u>vascular system</u> outside of blood vessels is BLEEDING or hemorrhage	Function mainly OUTSIDE blood vessels in the tissues

Leukocytes

- Originate in the bone marrow and released continuously into the blood
- Travel in bloodstream but function mainly **outside** blood vessels (in loose CT)
- Leukocytes form a mobile army that helps protect the body from damage by bacteria, viruses, parasites, toxins and tumor cells

- 5 types organized into 2 groups

- **Granulocytes**

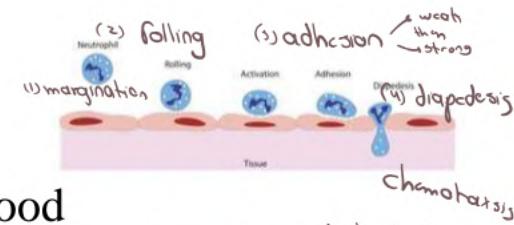
- Neutrophils
- Eosinophils
- Basophils

- **Agranulocytes**

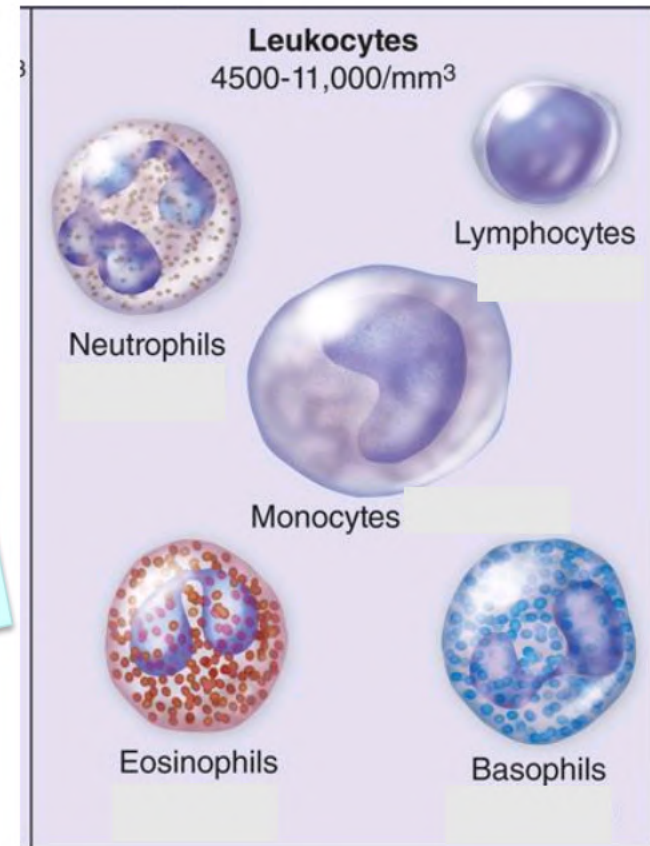
- Lymphocytes
- Monocytes

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

called diapedesis
migration of WBC from
BV to infection
site



toxins of bacteria
activate endothelium
to produce certain
receptors to attract
WBCs to infection
site



Leukocytes (White Blood Cells)

specific granules → which means there are non-specific granules in all leukocytes & most of our cells in the body (primary granules; or lysosomes)

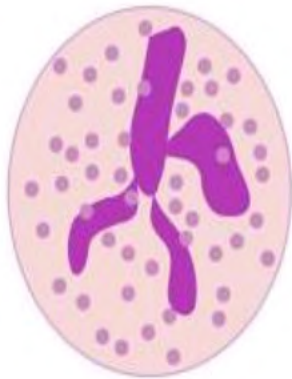
azure dyes stains lysosomes!!

also azurophilic granules

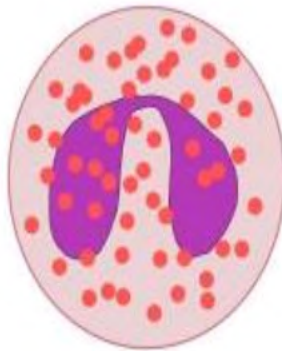
called

Granulocytes

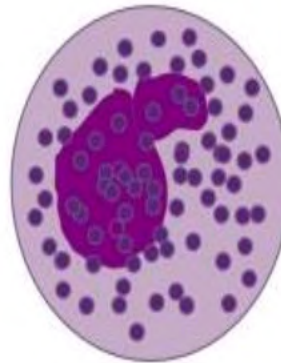
Agranulocytes



Neutrophil



Eosinophil



Basophil



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
- **Single** multi-lobed nucleus (segmented)
none are multi-nucleated
- All are phagocytic; they engulf and consume foreign cells and material
- 3 main types:

Dr. Heba Kalbouneh

Large granules, Red

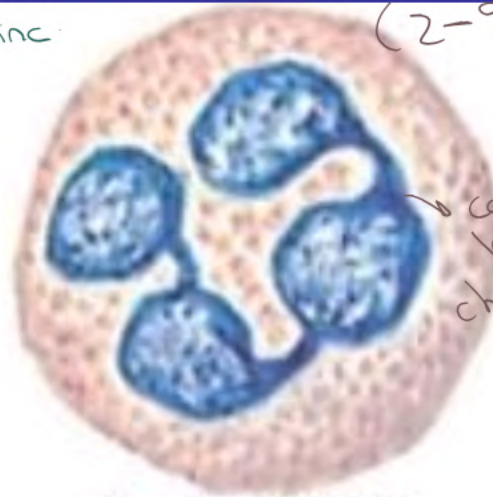


Eosinophil

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

*small,
pale*

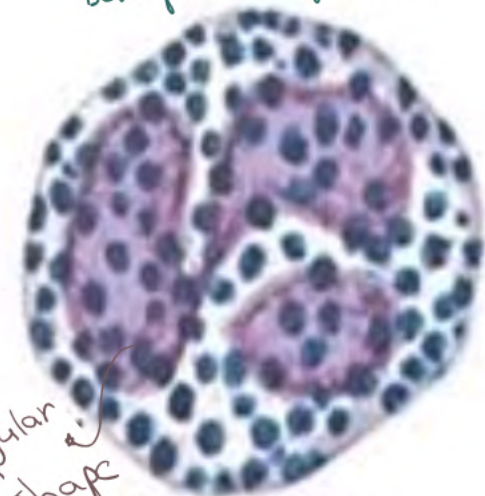
same affinity for acidic & basic dye - (neutrophilic granules)



Neutrophil

Large granules, blue

basophilic granules

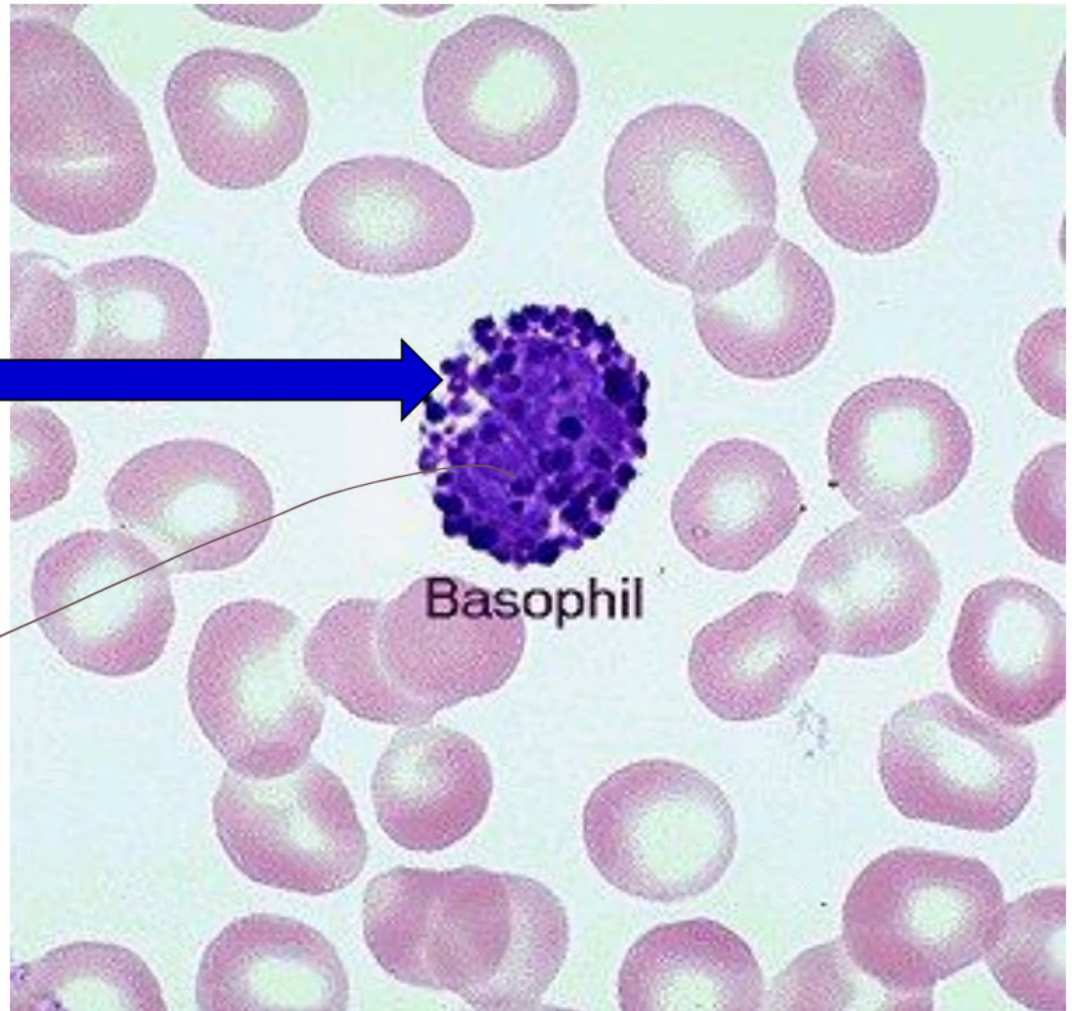


Basophil

Basophil

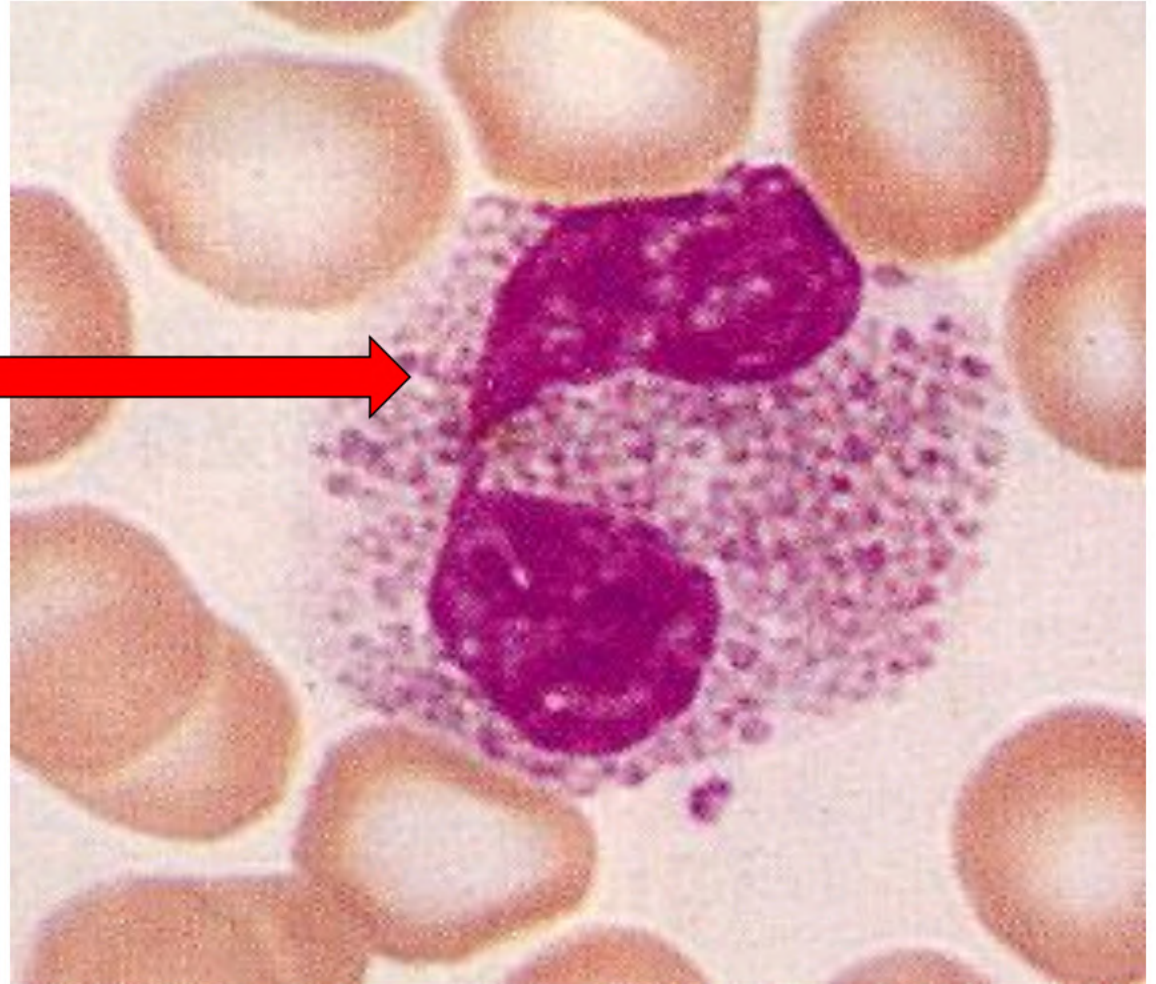
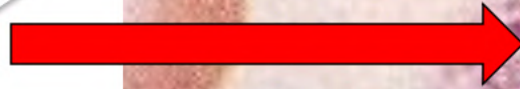
These specific granules
stain with basic stains
blue

*Irregular "5"
nucleus*



EOSINOPHIL

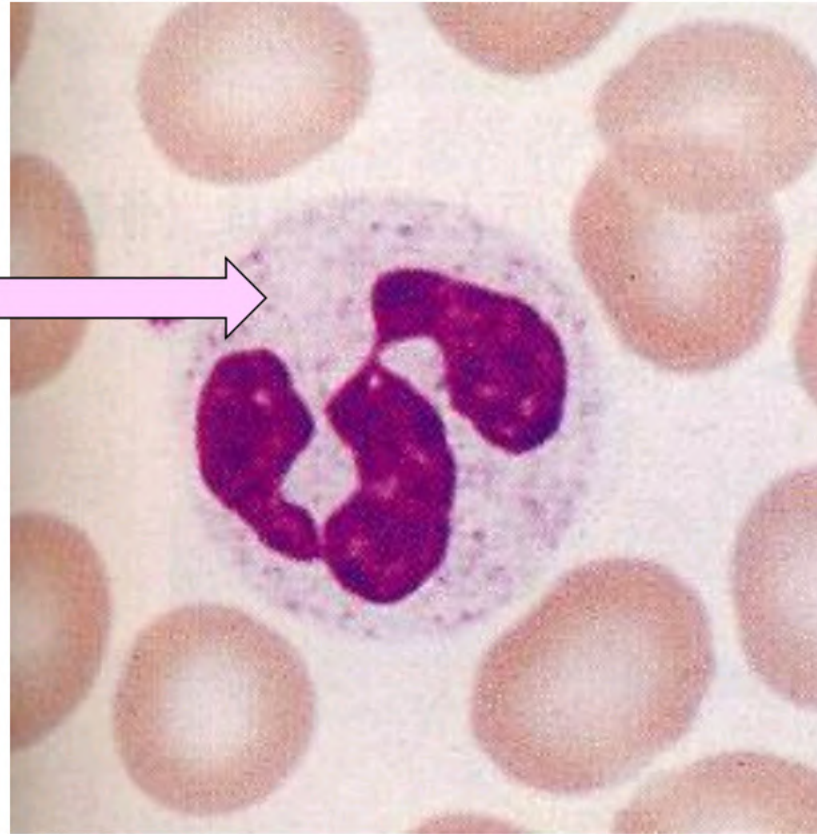
**These specific granules
stain red with acidic
stains such as eosin**



bi-lobulated

Neutrophil

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



Neutrophils

*produced in bone marrow

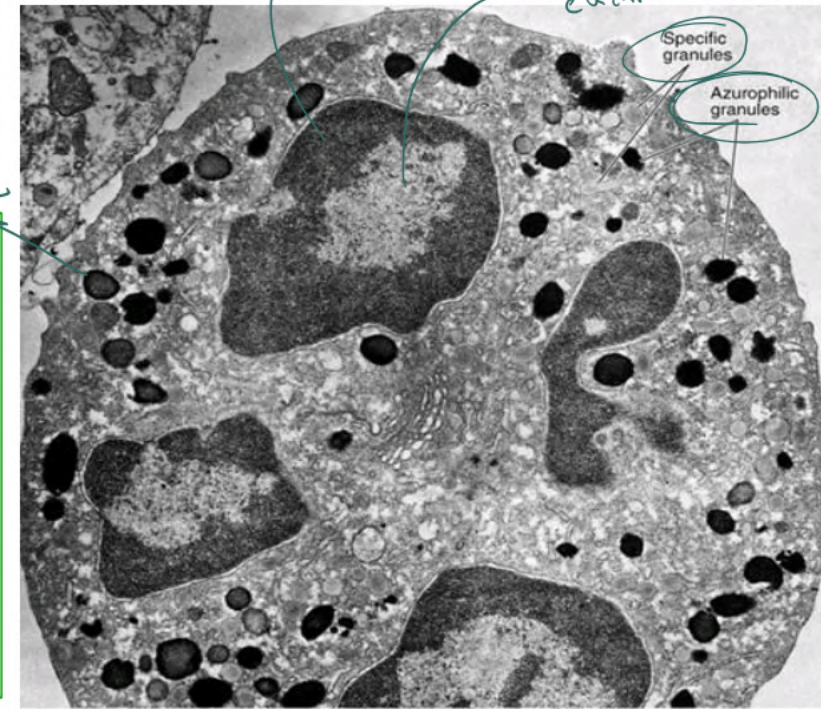
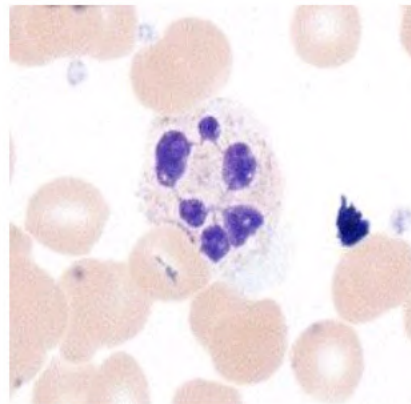
once mature enough... it is released from bone marrow into blood stream... (has two lobes) → circulates few hours in blood (becomes 3 lobes) → about to leave blood to enter CT (4 lobes) → inside CT & about to die (5 lobes)

Poly morphs

No. of lobes indicate maturity state of cells.

- The most common leukocyte → comparing number relative to WBC
- 2-5 lobes in nucleus connected by “threads” of nuclear material (**polymorphs**)
- Light pink cytoplasm → fine granules
- Called neutrophils because cytoplasm takes up red (acidic) and blue (basic) stains equally
- Specialized for responding to Bacterial invasions- Acute infections- Acute pyrogenic infections pyrogenic
- Neutrophils are short-lived cells with a half-life of 6-8 hours in blood and a life span of 1-4 days in connective tissues before dying by apoptosis.

Note: Neutrophils will show increasing segmentation (many segments of the nucleus) as they mature. A normal neutrophil should have 3-5 segments. Hypersegmentation is not normal but occurs in some disorders, most notably vitamin B₁₂ deficiency. ✕



electron micrograph

Specific granules (secondary)

Smaller, more numerous, formed later in development.

- **Lysozyme** → breaks cell wall & kills bacteria

- **Lactoferrin**
(bacteriostatic)

- **Collagenase**

- **NADPH oxidase**

Azurophilic granules (primary)

Larger, formed early in neutrophil development.

- **Myeloperoxidase**

- **Acid hydrolase**

- **Defensins**

- **Protease (eg. Elastase)**

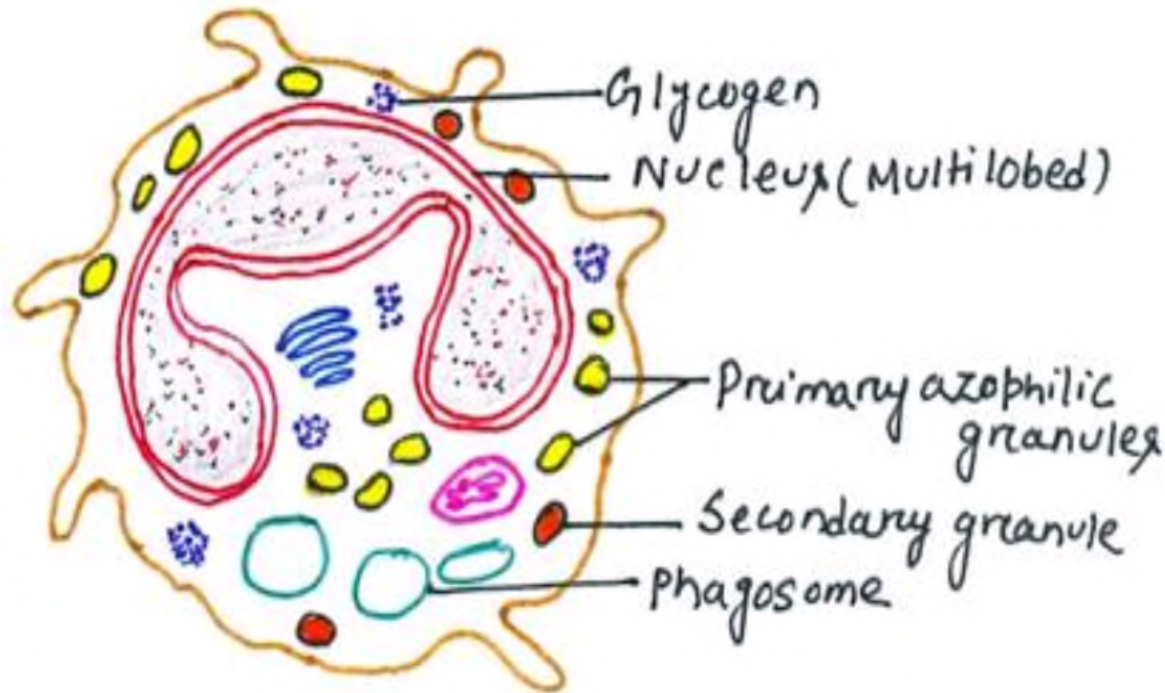
Form H_2O_2 ,
HOCL: powerful
cytotoxin

→ similar to Ig
- protein toxic to antigens

Different names for neutrophils:

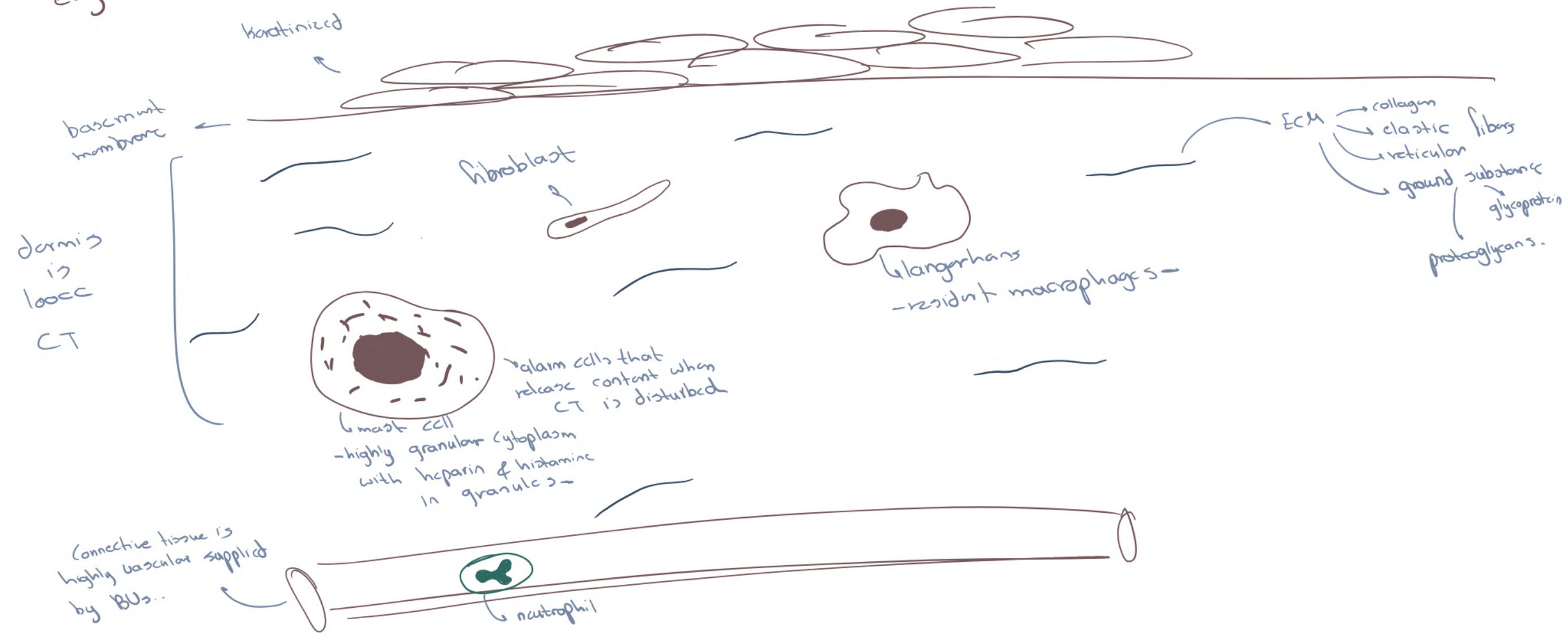
Polymorphs
Pus cells
Myelocytes
Microphages
Cells of acute inflammation

Pus is pyrogenic



*first barrier of our body is "Epithelium"

*skin as
an
e.g.



*if skin breaks

*skin as an e.g.



*bacteria start increasing in numbers & proliferate. they also secrete toxins.

toxins act as chemotactic factors to activate endothelium & BUs locally

also activate macrophage & mast cell to release histamine to ↑ blood flow to area of infection & ↑ permeability ⇒ more fluid leaves blood to CT

first cell that leaves blood in high numbers & enter infection site is

Neutrophils
cell of acute infection -

1st chemotactic factor secrets by infection site is neutrophilic

most common
most motile

- microphage -
able to phagocytose small particles; not in high no.



they also can release granular content of their granules by exocytosis to fight large no. of microbes inside spaces of CT

- neutrophils → polymorph
cells of acute infection, microphage

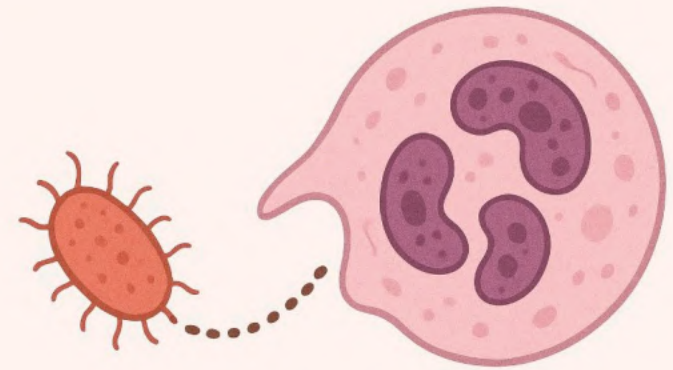
*last WBC recruited to site of infection to clear the area is **monocyte**
monocyte is largest WBC & differentiates into macrophages once it leaves blood..

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.



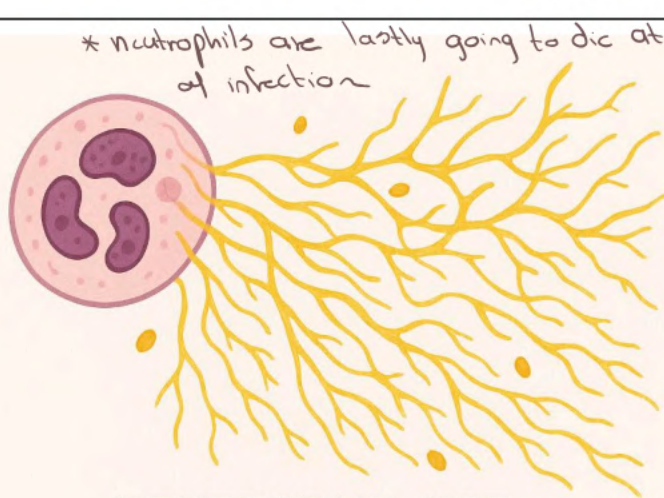
This is extremely toxic to microbes.



PHAGOCYTOSIS

Oxygen-independent killing:

Granules inside the neutrophil release enzymes like defensins, lysozyme, and proteases. These proteins break down microbial walls and deprive bacteria of nutrients.



NEUTROPHIL NETs

** neutrophils are likely going to die at site of infection → can extend chromatin extracellularly ⇒ threads of chromatin attached to specific & non-specific granules.*

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.

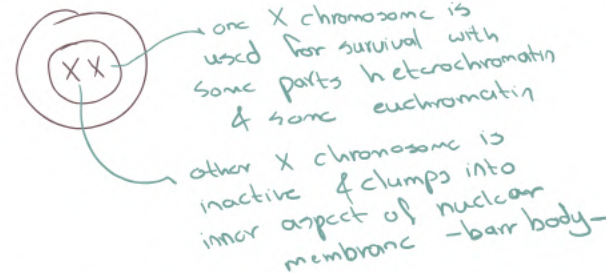


This sticky web traps and kills microbes outside the cell, preventing them from spreading.”

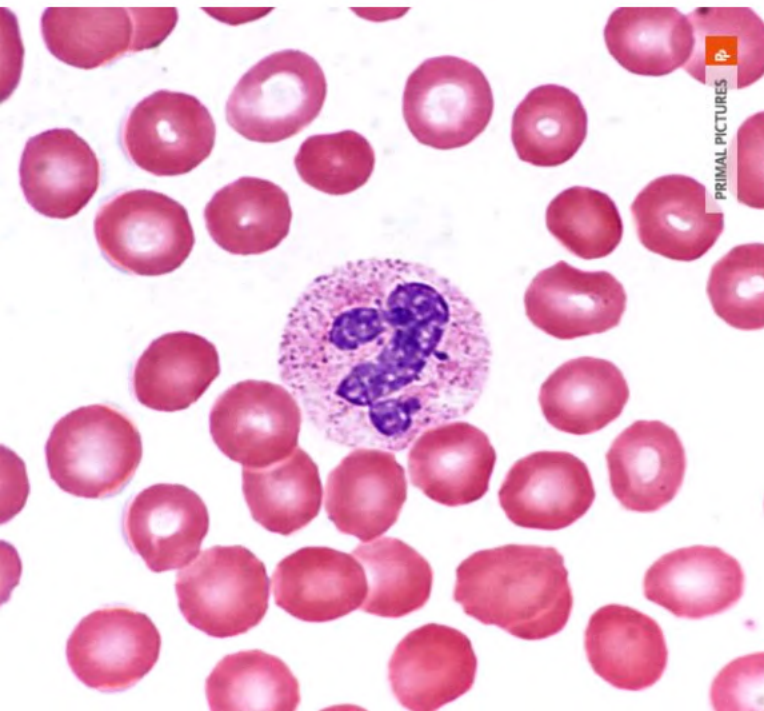


extra X chromosome in female nucleus.

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)



no consequences.



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

Why???

Cells of acute infection

- 1- The most abundant
- 2- The most motile
- 3- Neutrophil chemotactic factors are the first released

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

Few organelles

produce ATP by glycolysis without O_2
energy production even under anaerobic conditions

Glycogen
Nucleus (Multilobed)

Primary azophilic granules

Secondary granule

Phagosome

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.

Neutrophilia
Neutropenia

↑ number of neutrophils in peripheral blood
- acute infection / pyogenic / etc. ...

↓ count of neutrophils
- chemotherapy / bone marrow suppression -



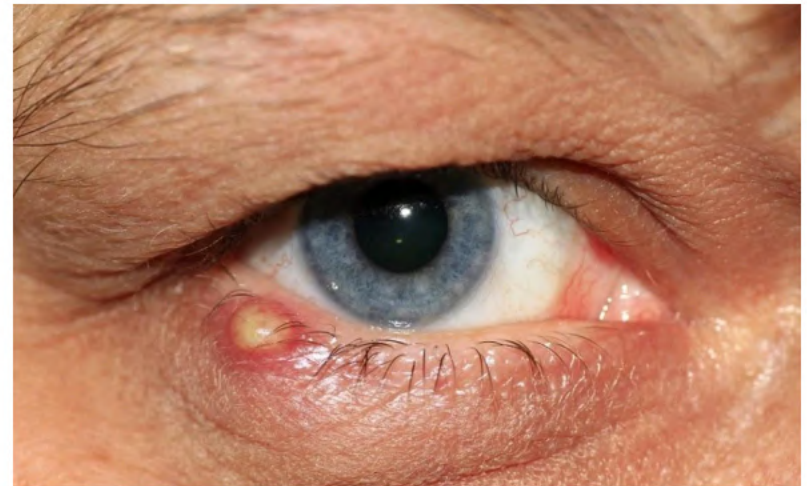
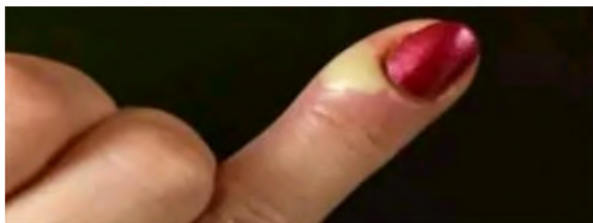
yellowish-greenish due to myeloperoxidase in nonspecific granules produce greenish color.

Pus is a viscous, usually yellow collection of fluid.

pus presence stimulates fever by stimulating hypothalamus
Pyogenic is referring to bacterial infections that make pus while **pyrogenic** is producing heat induces fever



neutrophils → acute infections
 → bacterial infections
 → pyrogenic infections
 → pyogenic infections



Basophils

→ histamine: ↑ blood flow to area & ↑ permeability of BUs so more fluid leaking

→ normal % inside peripheral blood ranges from (0-1)

- Rarest leukocyte – might not see these under the microscopes

→ if we have a blood film & don't see any basophils → normal ✓

*can't outline shape of nucleus

- Usually have bi-lobed, S-shaped nuclei obscured by the large basophilic granules

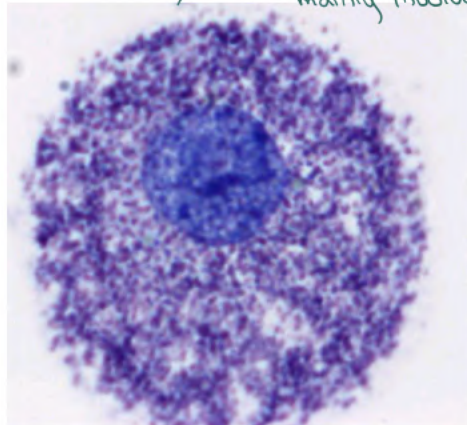
→ mask the shape of the nucleus – both are basophilic –

- Has large granules that stain dark purple/ blue in basic dyes (basophil = basic loving)

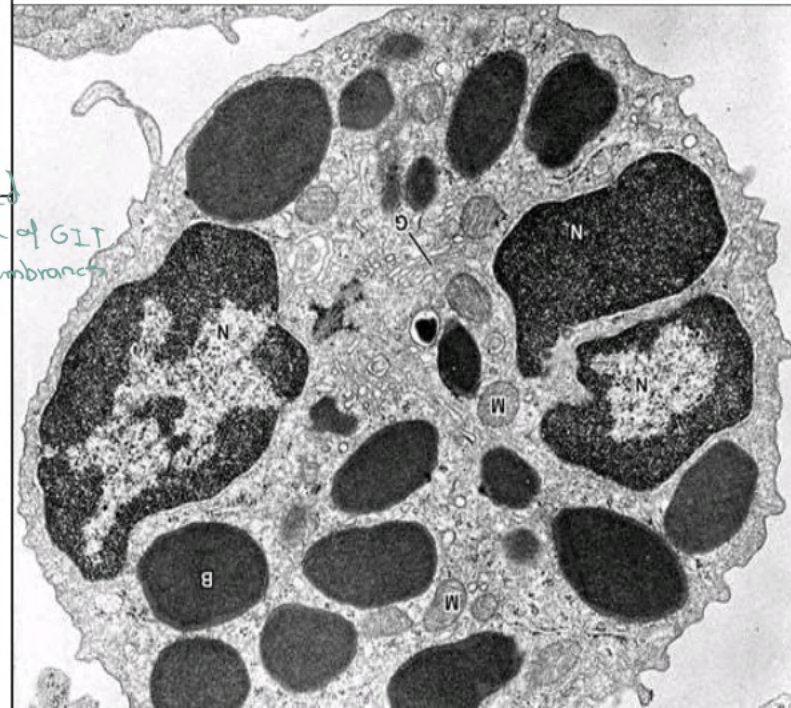
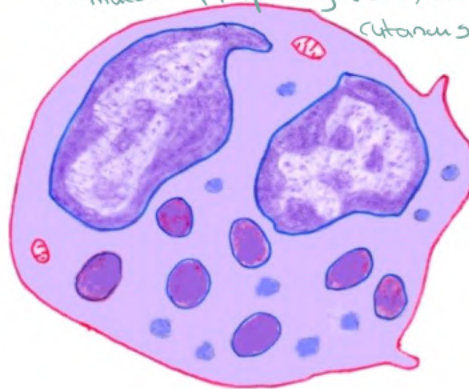
→ similar to mast cells in content of granules.

- Granules contain histamine, heparin and eosinophilic chemotactic factor that mediate inflammation in allergic reactions and parasitic infections

→ mast cells are scattered in the CT & are mainly involved in allergic reactions → concentrated in mucosa of respiratory tract, mucosa of GIT cutaneous membranes



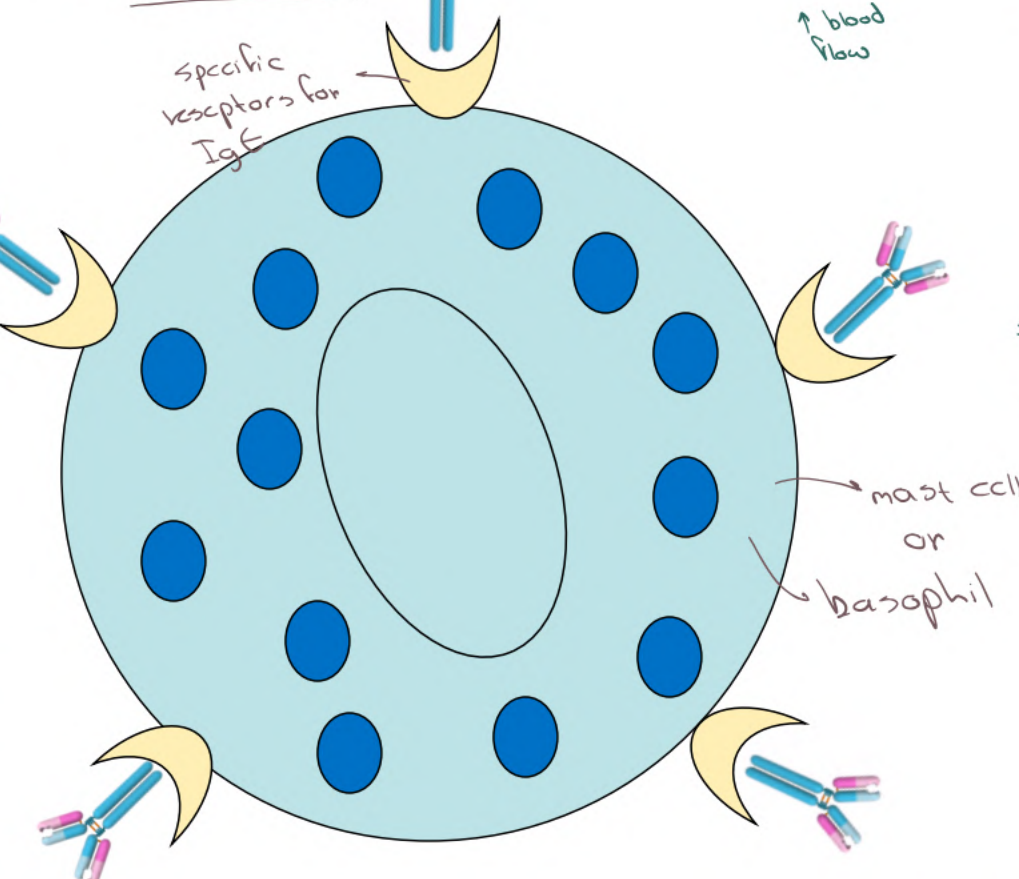
Mast cell



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

Second exposure causes degranulation of mast/basophils & start allergy on 2nd exposure

bind to specific receptors on mast/basophils
IgE antibody



* area of allergy

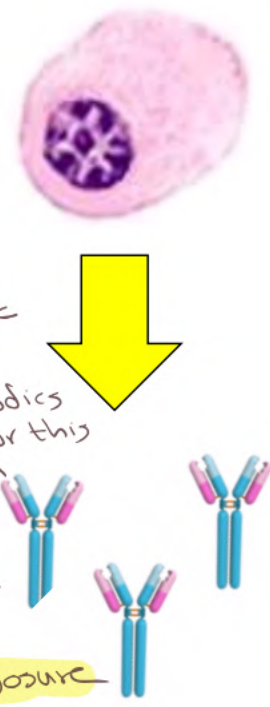
red
↑ blood flow
swollen
↑ permeability of BUs
itchy
inflammatory mediators irritate nerve endings at sight of allergy producing itchy sensation

Allergen - causes allergy -
activate immune system to produce
Plasma cell

* degranulation of mast/basophils is what causes allergy symptoms.

secrete IgE antibodies specific for this allergen during 1st exposure

No reaction in 1st exposure



*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 exocytosis of the cytoplasmic granules.

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

based on area

*itching,
redness/
swelling*

*runny
nose*



Immediate or type 1 hypersensitivity

there are other types.

may be severe

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. *→ can result in death*



Basophils account for up to
15% of infiltrating cells in
allergic dermatitis and skin
allograft rejection

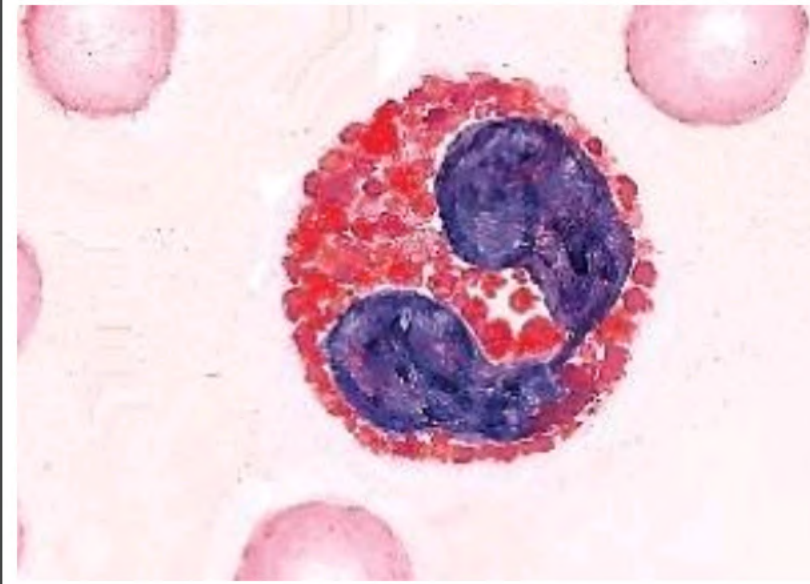


Allergic dermatitis
(eczema) is an itchy skin
rash that develops when you
come into contact with an
allergen



Eosinophils

- Usually have ^{cyceglass in appearance} 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)
 - with central dark area & peripheral pale area
 - ovaloid/ crystalloid in shape
 - specific eosinophilic granules
- Help in ending allergic reactions and in fighting parasitic infections



*All granulocytes are phagocytotic cells
basophil < eosinophils < neutrophils

Specific granules (Crystalloid granules):

- Oval in shape, with flattened crystalloid cores

-Two parts:

Externum (pale): contains histaminase and sulfatase

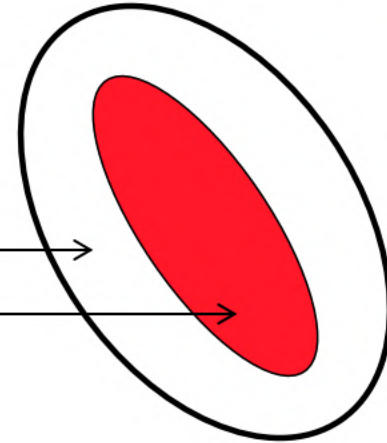
Internum (dark): contains basic protein to kill parasites

stops action of
histamine

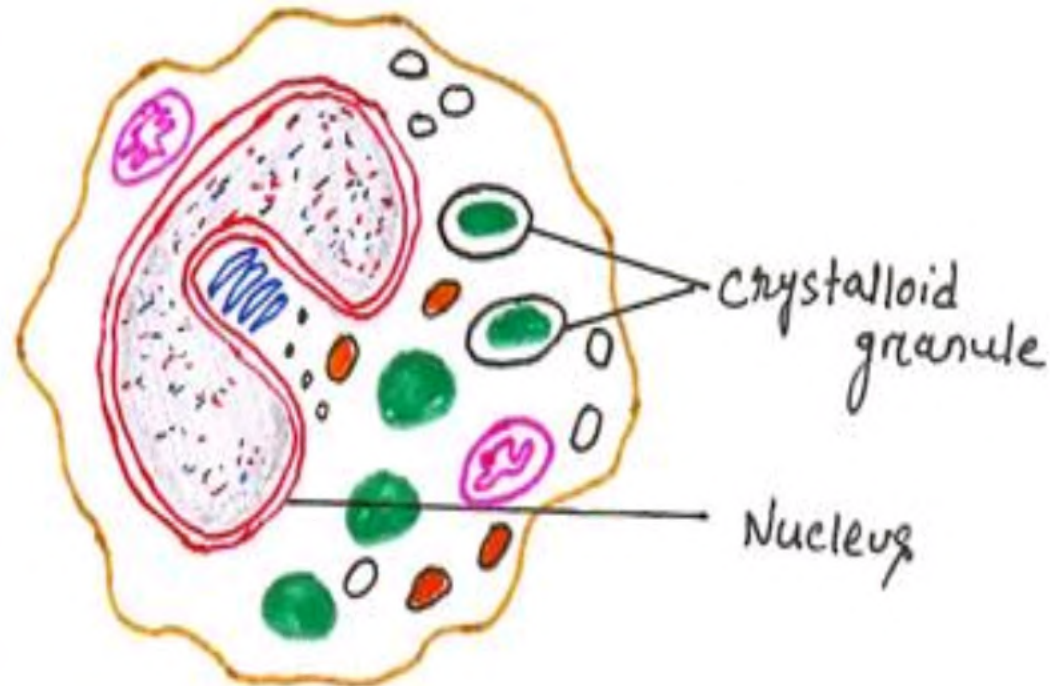
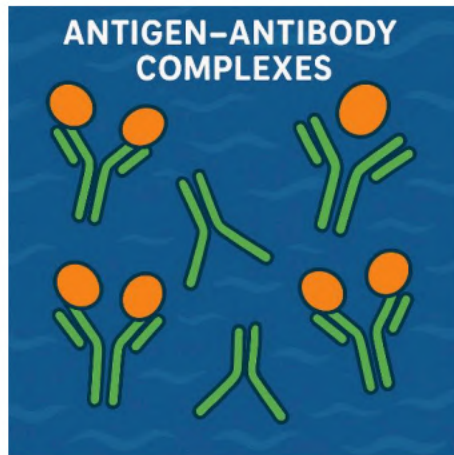
stops action of
heparin & other sulfated
proteoglycans in granules of mast
basophils.

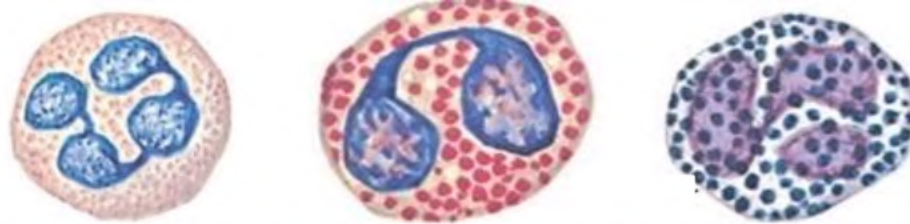
will stain with
acidic dye (red)

Crystalloid granule



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





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

1st ↓
to reach
infection area

General features of granulocytes

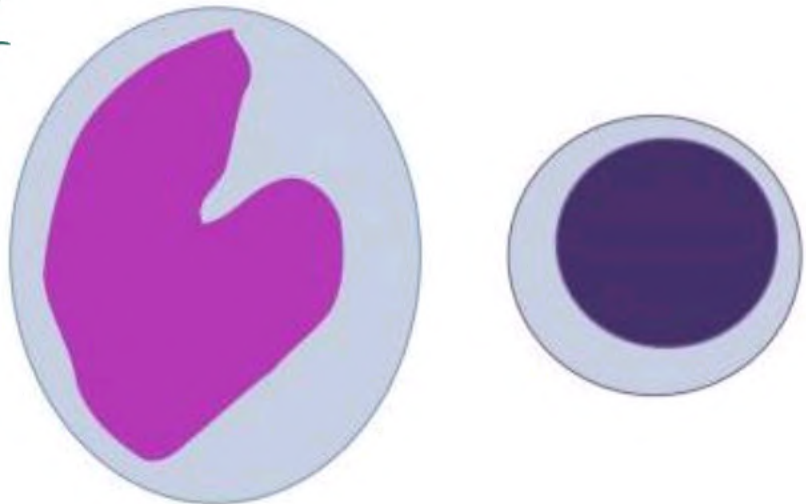
- Spherical in blood stream, irregular in connective tissue
- Highly motile with different shapes due to their amoeboid movement
- Leave blood stream by migrating between the endothelial cells by a process called **diapedesis**

Diapedesis

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

Agranulocytes

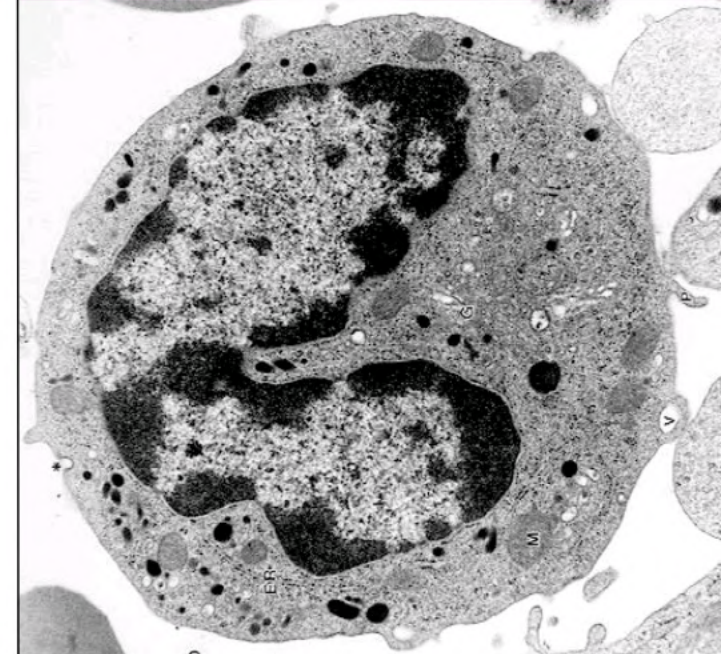
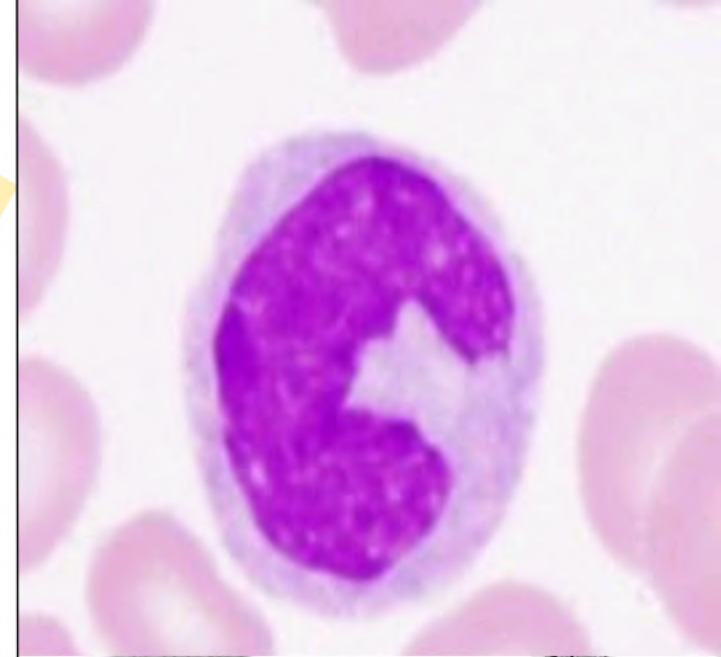
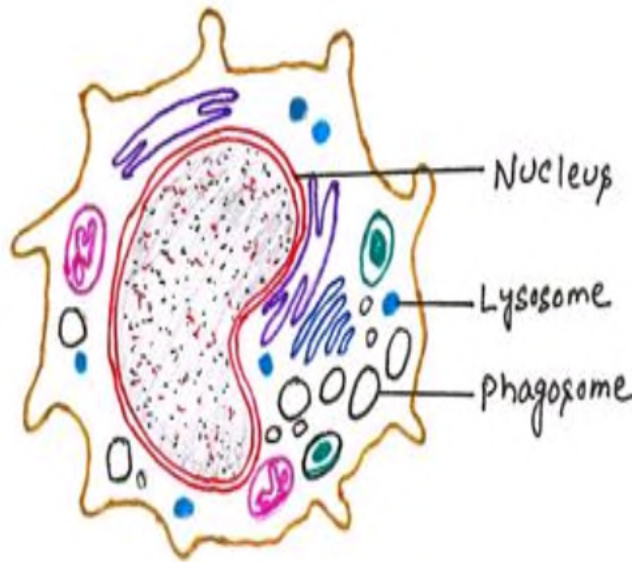
- Single non-lobulated nucleus
- Granules in cytoplasm are too small to see (nonspecific granules, azurophilic granules, primary granules, lysosomes)
- 2 types based on structure (not cell lineage):
 - **Lymphocytes**
 - **Monocytes** → largest WBC
↳ leaves blood to enter CT to become macrophage



Monocytes

- Largest leukocytes
- Bluish cytoplasm (*frosted glass appearance*) & a large C-shaped nucleus *not very clear*
- Highly motile and phagocytic
- Travel through bloodstream to reach connective tissues, where they transform into **macrophages** (large phagocytic cells)

Azurophilic granules
(lysosomes)

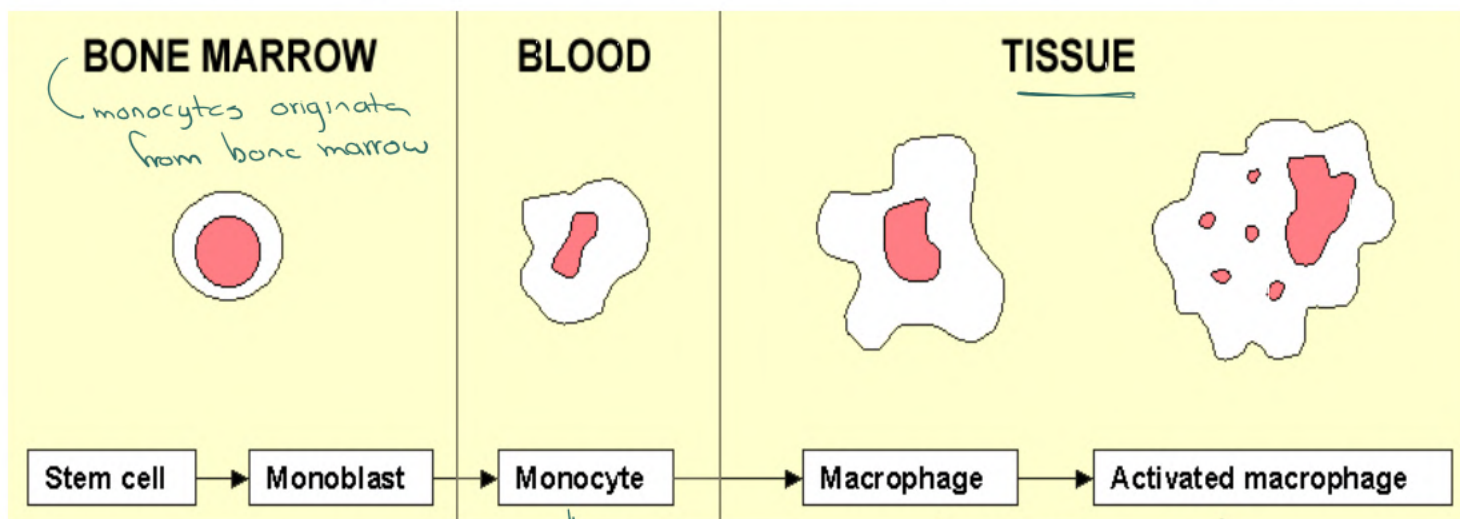


Are precursor cells of macrophages, osteoclasts, microglia, and other cells of the **mononuclear phagocyte system** in connective tissue

All monocyte-derived cells are **antigen-presenting cells**

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

Dr. Heba Kalbouneh



Kupffer Cells

Liver



Macrophages

Bone marrow

Connective tissue



Osteoclasts

Bone resorption

*system of monocyte-derived cells of macrophage like cells in different organs

MONONUCLEAR PHAGOCYTOTIC SYSTEM

*many monocytes have left blood stream to populate in different organs during formation of organs ⇒ resident macrophages.

phagocytosis

antigen-presentation

Dust cells

/alveolar

Lung

Monocyte

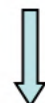


(all characterized by phagocytic activity & antigen-presentation)

all cells derived from monocytes

Microglia

CNS



Dendritic cells

Lymph node

Spleen

very important.



Langerhans cells

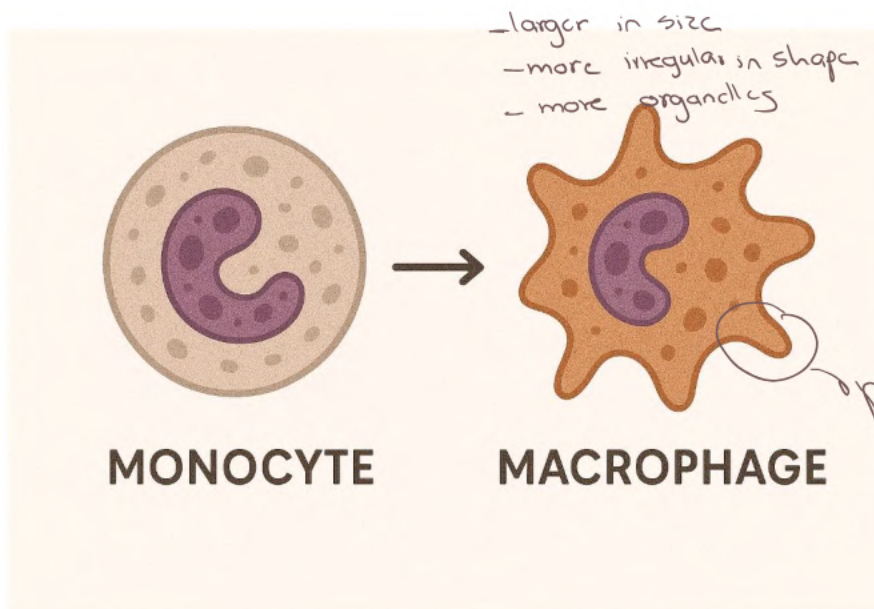
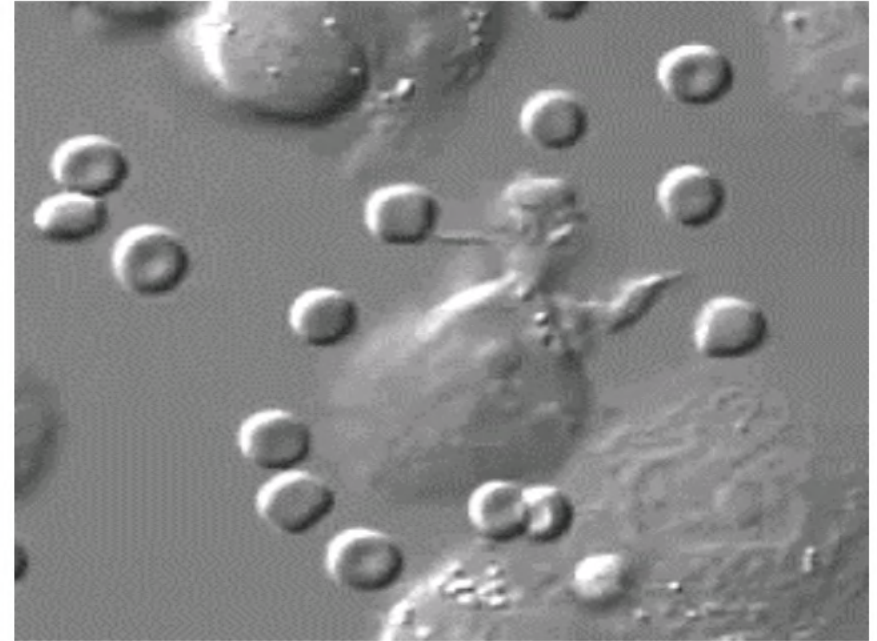
Epidermis

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

- nucleus C or kidney shaped
- cytoplasm not clear due to primary granules
- origin of macrophages.

A macrophage eating bacteria



- larger in size
- more irregular in shape
- more organelles

pseudopodia for
phagocytosis

Lymphocytes

- **Smallest leukocytes** → scant cytoplasm amount
- 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

* diameter of nucleus of lymphocyte is almost same as RBC size.

Cell mediated immunity

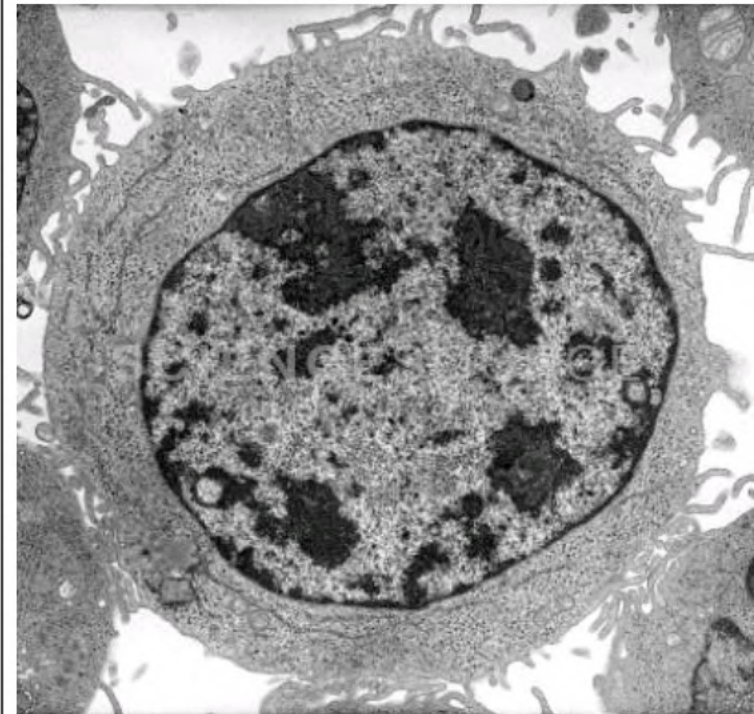
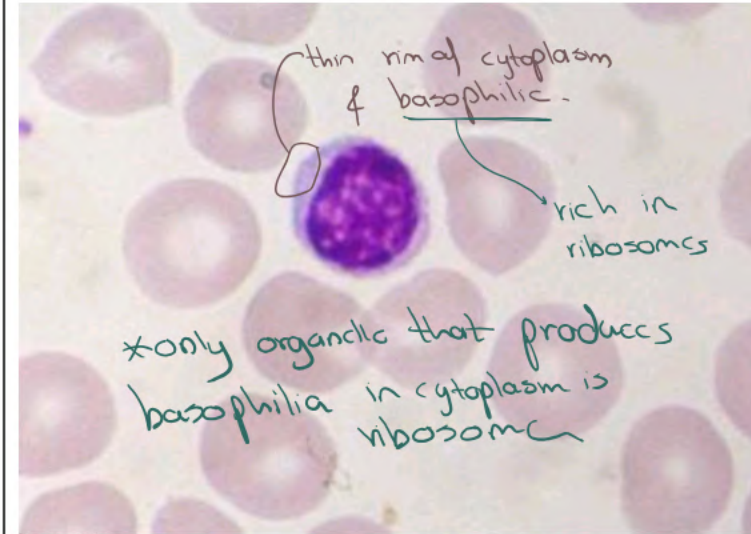
- **T cells**
 - Has different types, some directly kill foreign or infected cells; others activate phagocytes to destroy microbes

Humoral immunity

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

Long term immunity

Humoral means
body fluids



Lymphopoiesis: the process by which lymphocytes are formed

↳ in bone marrow like others

3 options

Precursor cells in bone marrow

*all appear the same using ordinary stain under light microscope.
- large nucleus filling most cytoplasm - → but each express specific cell-surface receptor

Thymus

complete maturation & differentiation inside Thymus

T Lymphocytes

BM

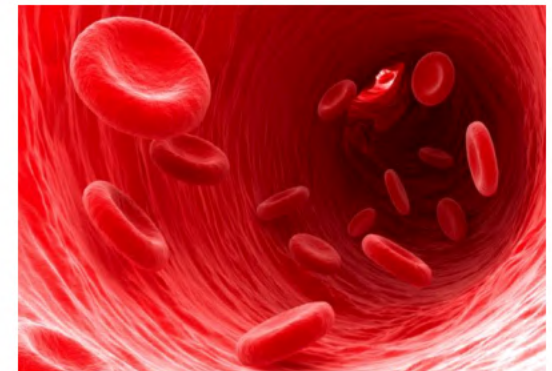
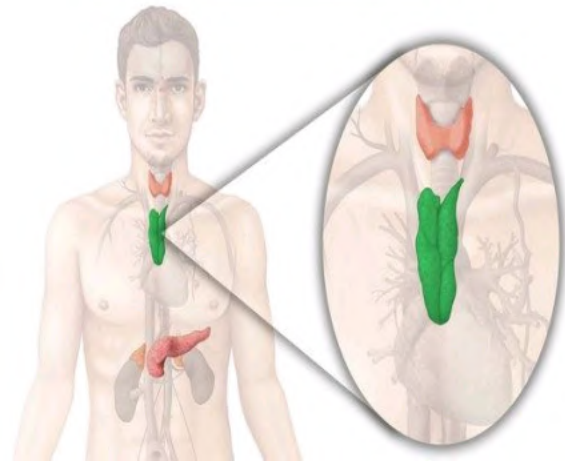
if they complete differentiation in bone marrow

B Lymphocytes

Directly into blood

complete differentiation inside blood...

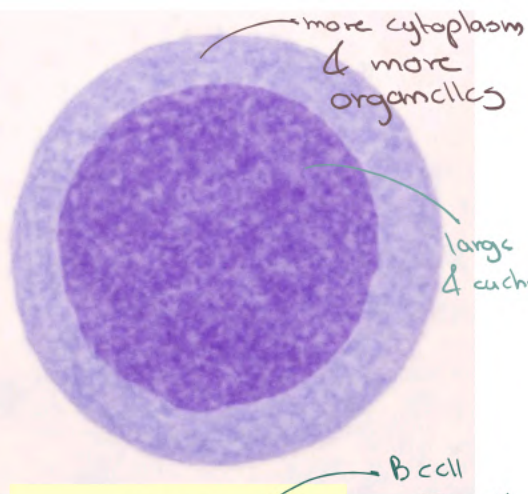
Natural killer lymphocytes



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**

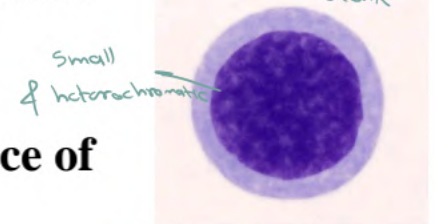


Large
(9-18 μm)
Active lymphocyte

B cell
T cell
natural killer

nucleus
activity
cytoplasm

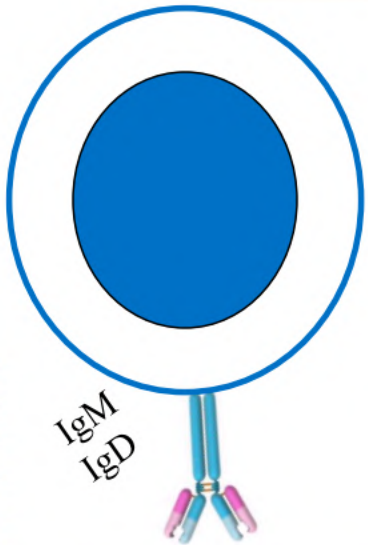
heterochromatin (condensed/inactive)
euchromatin (pale/active) → larger in size nucleus & more lightly stain



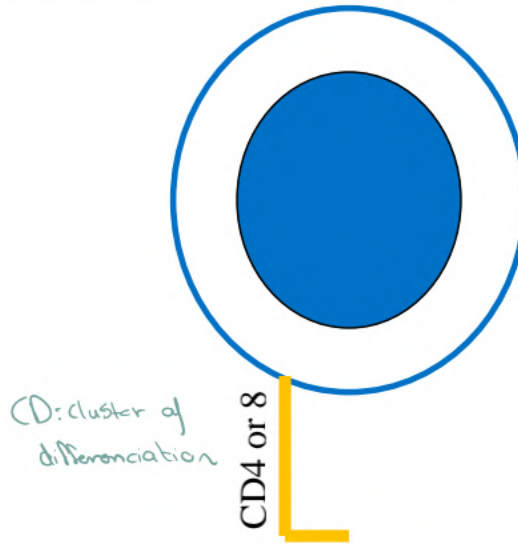
T cell
B cell

Small
(6-9 μm)
Inactive lymphocyte

Different types express specific cell surface proteins

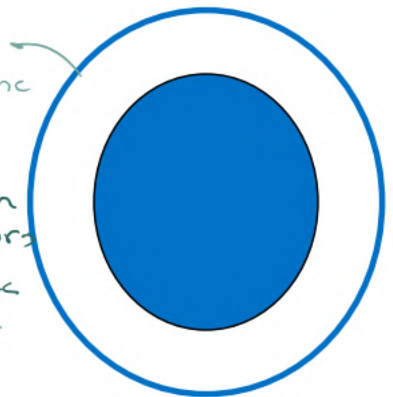


B lymphocyte



T lymphocyte

* former name nonc B nonc T cells... have their own specific receptors other than the ones on B & T cells-



only large & active -innate immunity-

Natural killer lymphocyte

CD4⁺: T-helper
CD8⁺: cytotoxic T cells

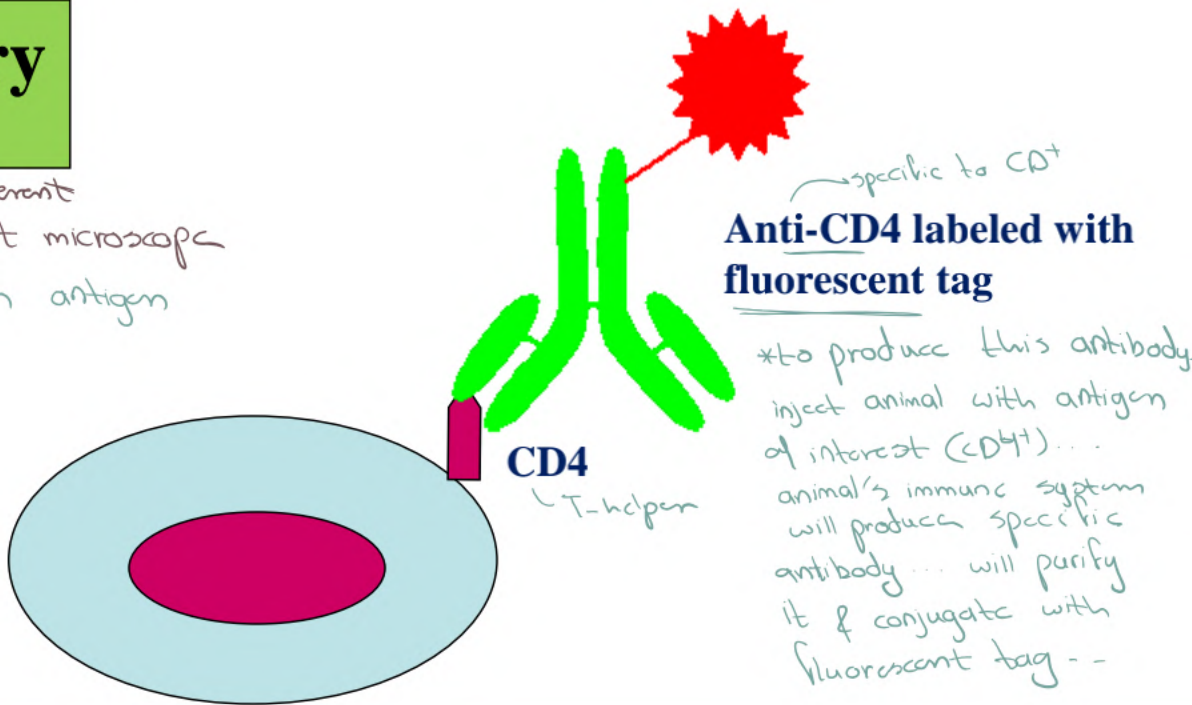
Immunohistochemistry

Direct method

to differentiate between different types of lymphocytes under light microscope

↳ specific binding between antigen & its antibody

- Needs Fluorescence microscope that use UV or infra red light to transilluminate the sample -



Immunohistochemistry

Using CD 4 Antibody

???????

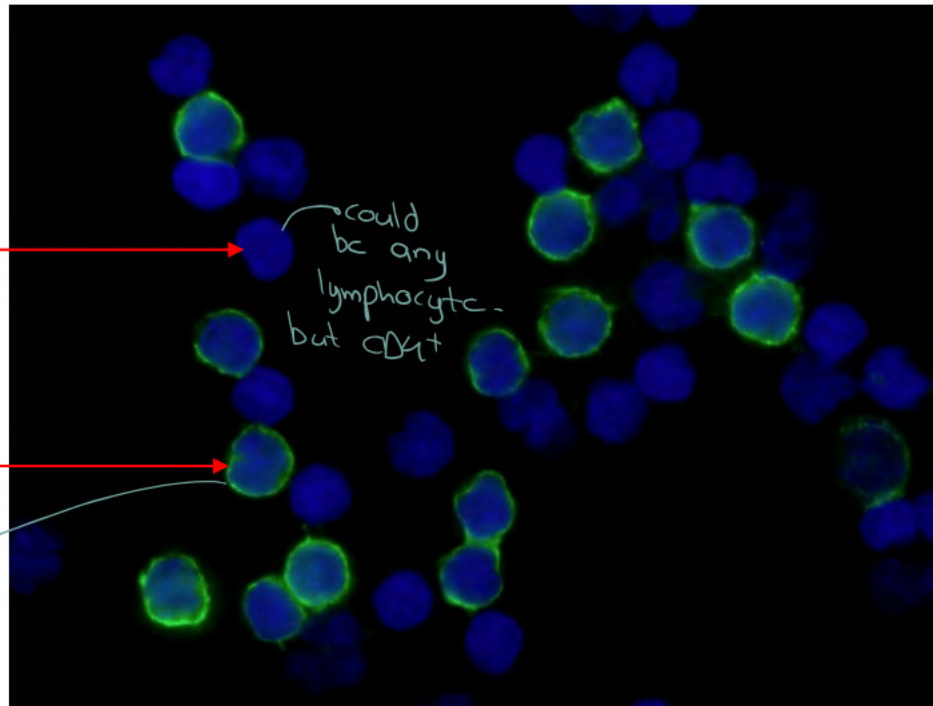
Blue specific to nuclear proteins

CD 4 +

T Helper lymphocyte

green to localize CD4+

CD4+ T-helper



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

While

The activity of lymphocytes is always directed against **SPECIFIC** foreign agents (**Adaptive immunity**)

The small lymphocyte has scanty cytoplasm (contain few organelles but large number of ribosomes)



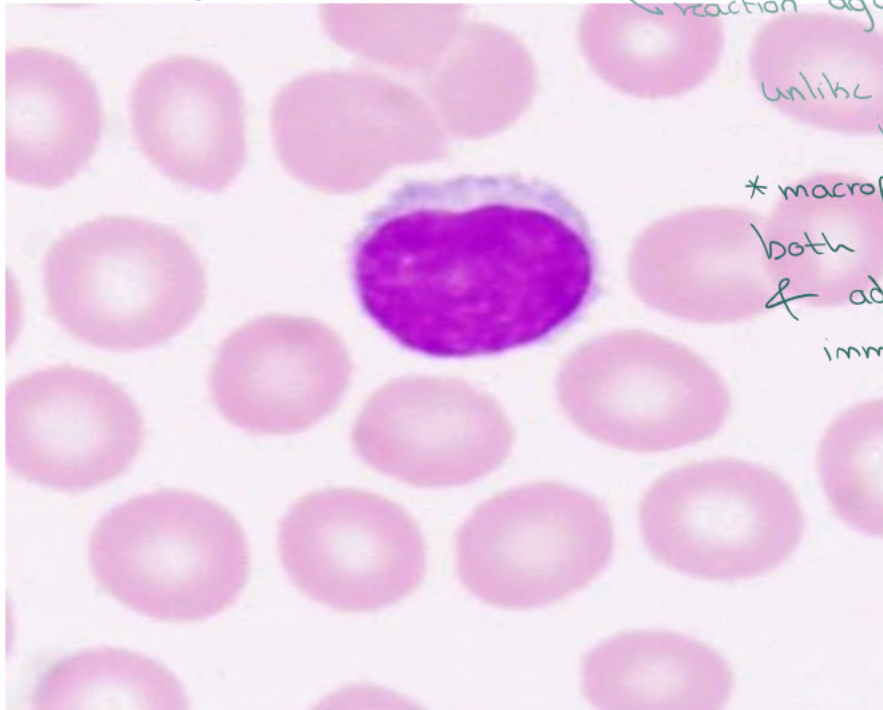
Account for basophilic cytoplasm



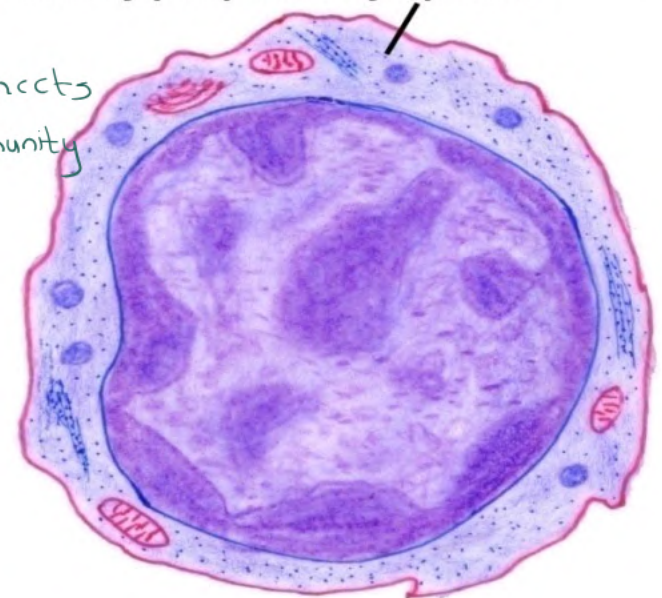
*lymphocytes are highly specialized & advanced.
reaction against specific antigen ..

unlike innate immunity

*macrophage connects both innate immunity & adaptive immunity ..



Scanty peripheral cytoplasm

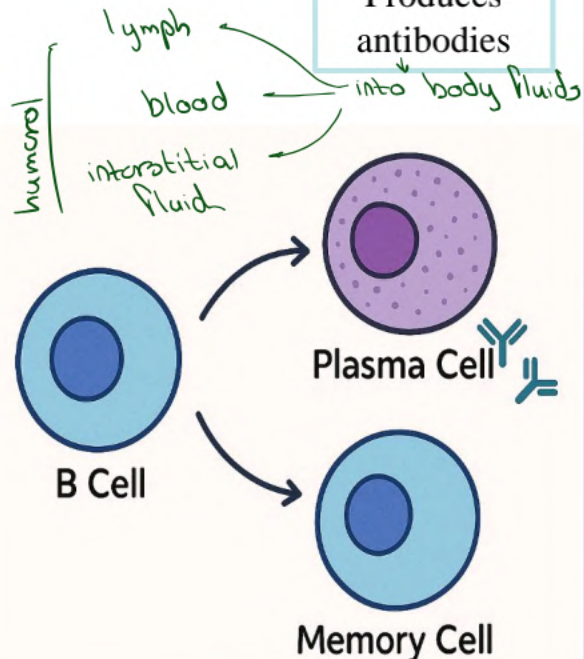


B Lymphocyte

Memory cell

Plasma cell

Produces antibodies



exposure of same antigen in future -- directly differentiates to plasma cell & produce quick immune reaction -- basis for vaccination --

T Lymphocyte

perforins... pores in cell wall thru granzymes that enter cell through pores & induce apoptosis.
Cytotoxic CD8

Kill virus-infected, transplanted and neoplastic cells (cancer) (adaptive immunity)

by dealing cell to cell → cell-mediated immunity

Helper CD4

produce a storm of cytokines

Help cytotoxic T cells and B cells in their immune functions

* helps B cells differentiate to plasma memory cells.

Suppressor CD8 or 4

Suppresses immune response to self Ag

Suppresses immune response of T and B lymphocytes

* immune system over activated often elimination of microbes → suppressed to prevent autoimmune disease against self cells.

T helper (CD4+)

T cytotoxic (CD8+)

Suppressor T cell (CD8+ or CD4+)



Natural killer cells

(NULL Lymphocyte)

~ similar to cytotoxic cells

Kill virus-infected, transplanted and neoplastic cells (innate immunity)

but not specific killing-

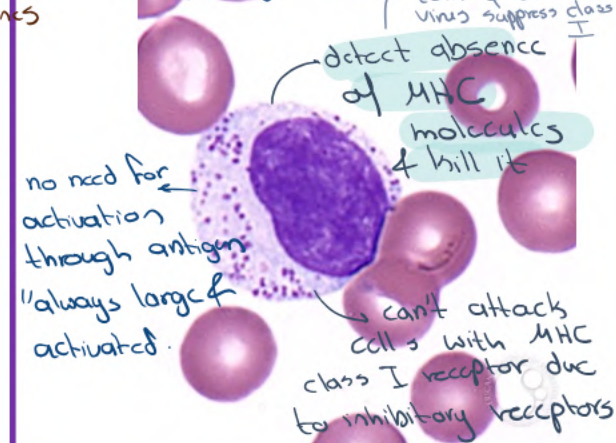
Large granular lymphocytes

Activated lymphocytes

(1) perforins

(2) granzymes

like neoplastic cells & some virus suppress class I



also suppress immune response against self antigen by inducing apoptosis of cytotoxic T cells that are attacking normal cells.

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

**our body continuously produce cancer cells... our immunity is the one responsible of eliminating them*

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

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



Damage to 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

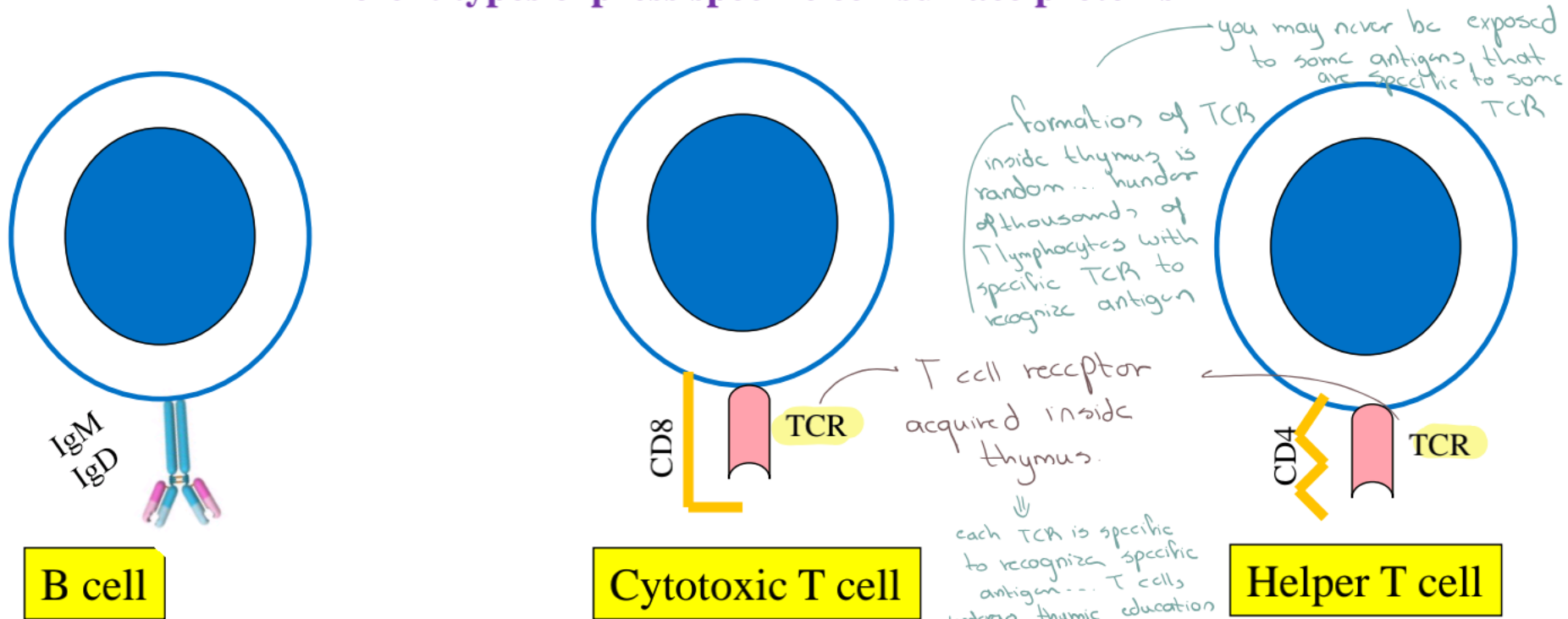
HIV affects Helper T cells

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**

a type of autoimmune disease happens when T cells start attacking self cells (dysfunctional)

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

Major histocompatibility complex

MHC

molecular fingerprints
specific to each person
- why immune systems reject
transplanted organs/tissue
grafts -

Glycoprotein on cell membrane

Two classes:

MHC-I

On surface of **all nucleated cells**

Coupled to peptide formed within the cell

MHC-II

On surface of **APCs**

Coupled to peptide product of proteins
the cells had ingested (peptide product of
Ag digestion)

antigen

antigen presenting cells

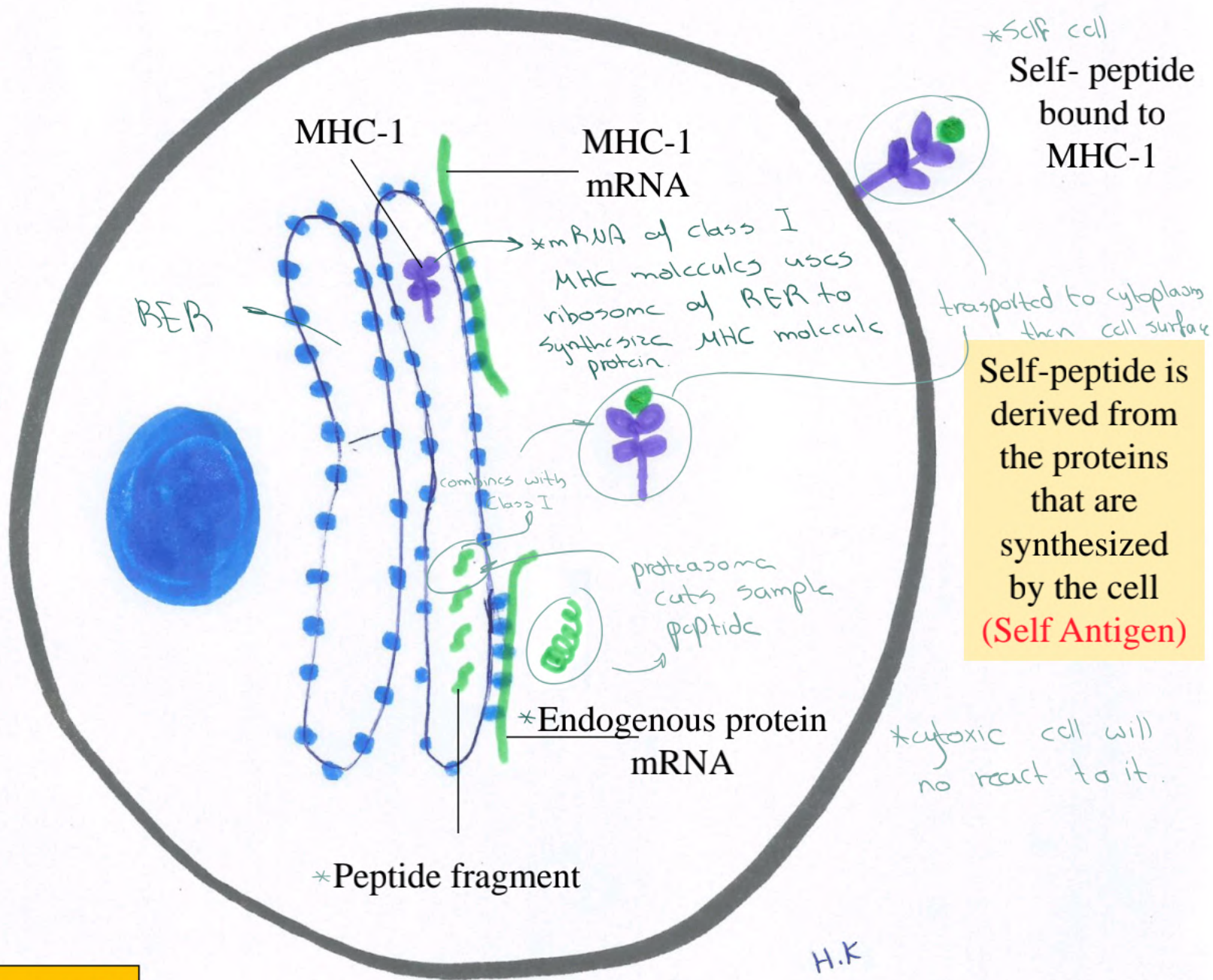
T lymphocytes are specialized to
recognize both classes of MHC
proteins and the antigens they
present

* Also called human
leukocyte antigens
(HLAs)

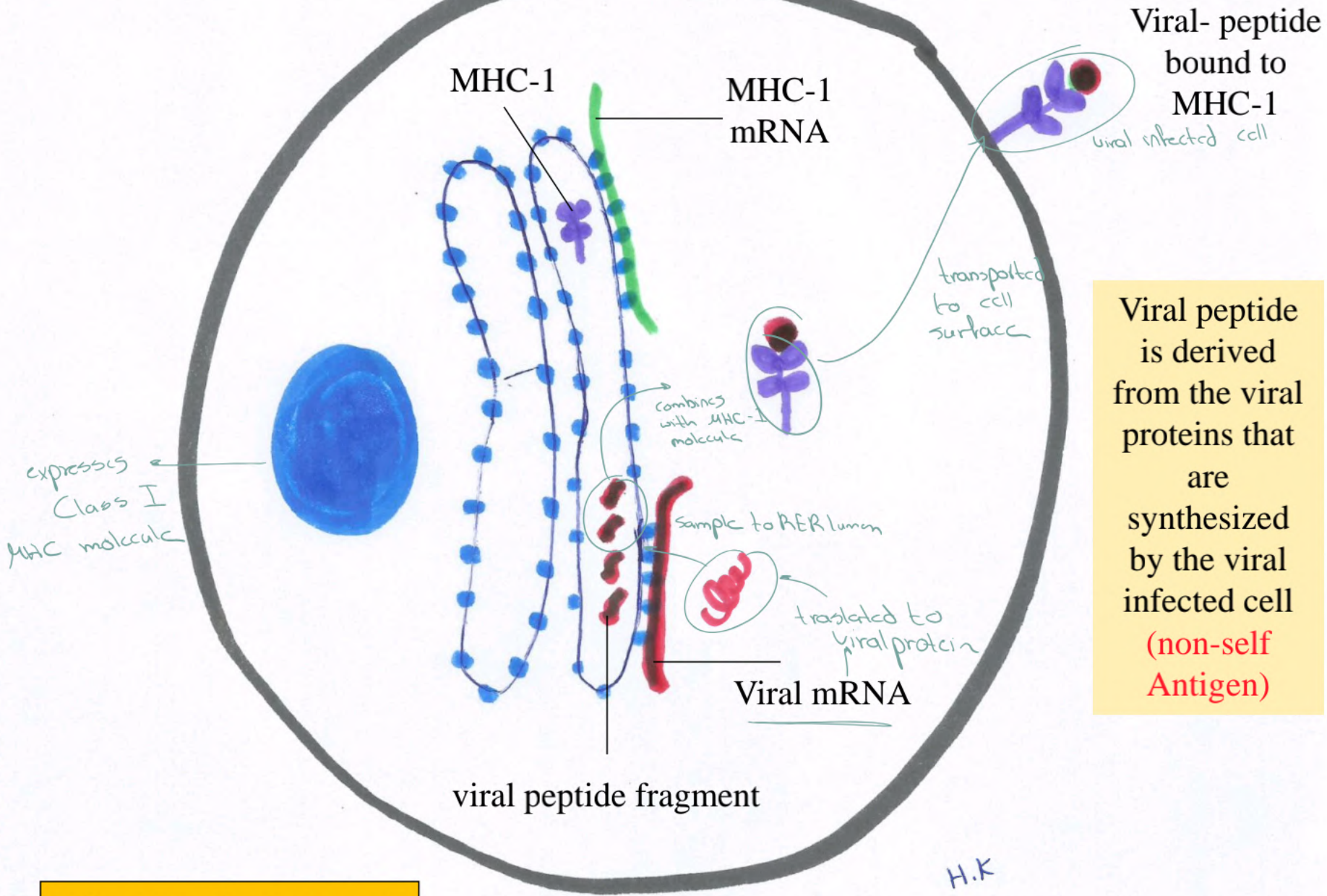
old name
thought to be
only on leukocytes
↓
present on all nucleated
cells on our
body!!
MHC I

If the MHCs on cells
of a tissue graft are not similar to those that T
lymphocytes encountered during their
development, the grafted cells will induce a
strong immune reaction by T cells of the
recipient.

To these lymphocytes, the unfamiliar MHC
epitopes on the graft's cells are recognized as
markers of "non-self" cells that they must
eliminate.



Normal cell



Viral infected cell

Mutant peptide
bound to
MHC-1

marked
as cancer $\Rightarrow$ non-
self cell

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

nucleated $\rightarrow$
so produces
Class I MHC
molecule

mutations
inside nucleus
produce mutant
proteins

coupled with
MHC molecule

sample

MHC-1

MHC-1
mRNA

Mutant protein
mRNA

mutant
Peptide fragment

Cancerous 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

lymph node: masses of T lymphocytes

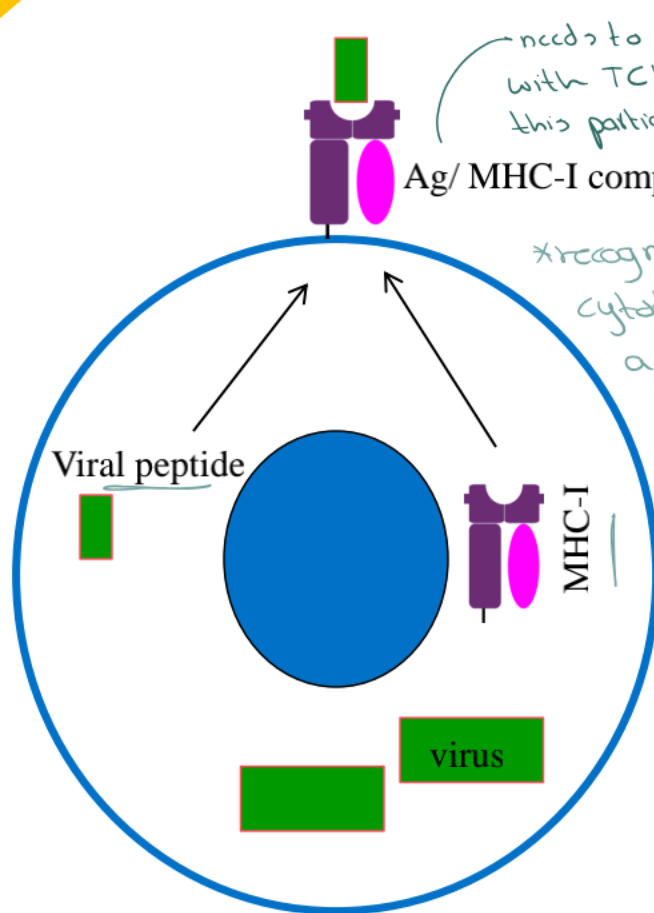
move to nearest lymph node

needs to find a cytotoxic T cell with TCR receptor specific for this particular peptide → antigen presentation may take place within tissue or inside lymph node

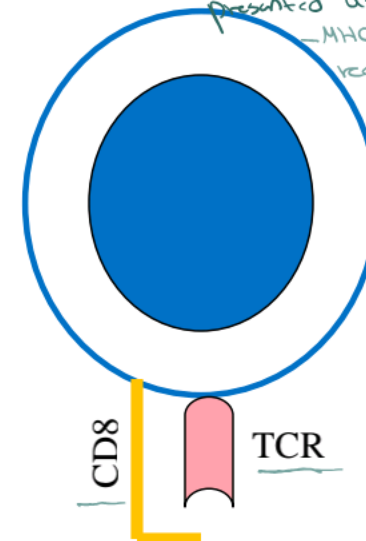
*recognized by cytotoxic T cell as viral infected cell.

TCR receptor specific to recognize the protein on viral infected cell... CD8⁺ binds to MHC I molecule to make sure this antigen is properly presented using class I MHC molecule

MHC restricted cells → only recognize antigens presented on class I (or II for T Helper) MHC molecules

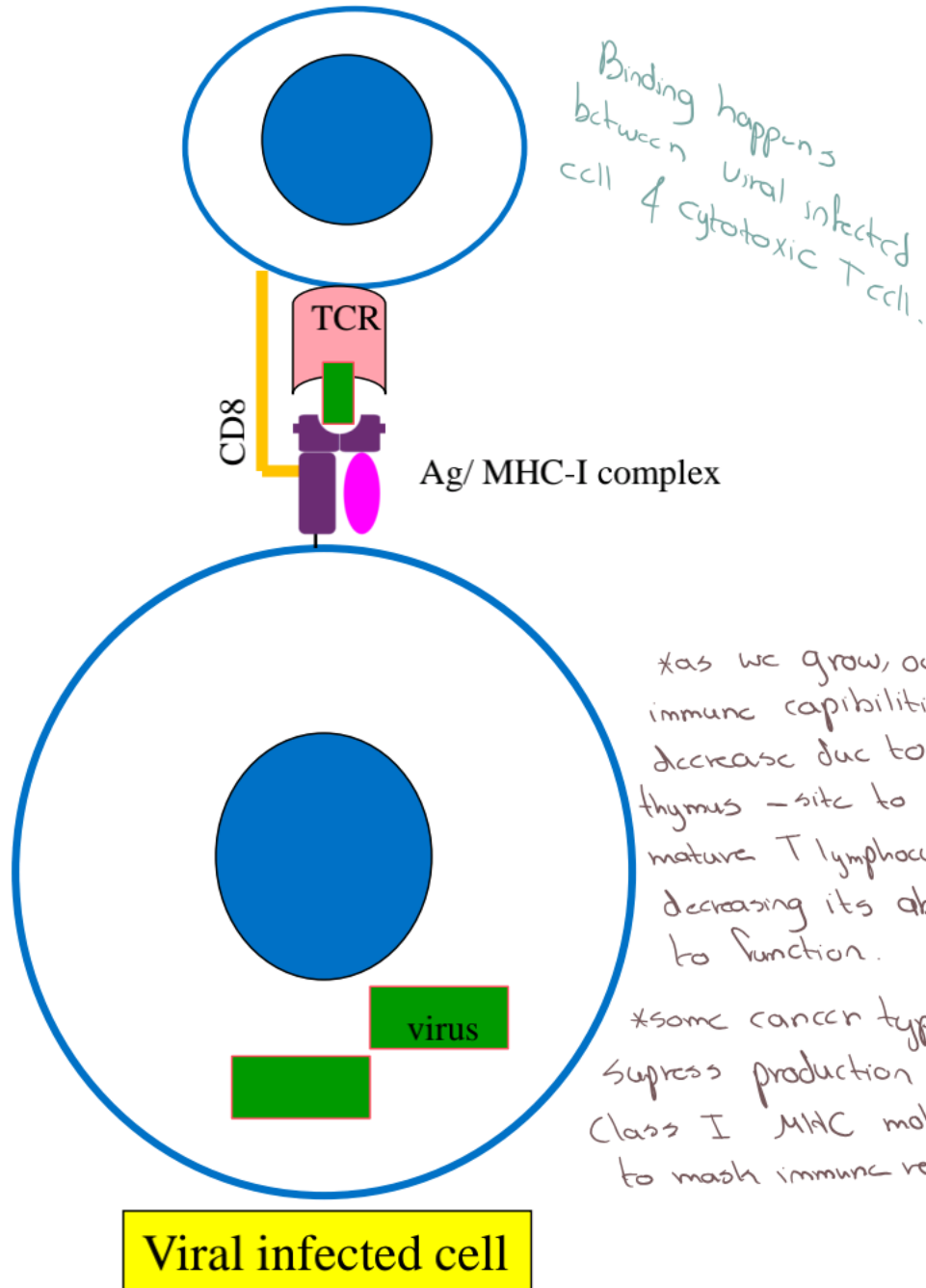


Viral infected cell



Cytotoxic T cell

Cytotoxic T cell



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

They

(1) **Proliferate**

clonal selection & expansion
→ mitosis

(2) **Activate**

large activated cell
↑ perforin & granzyme

Release
Perforins and granzymes
(proteases)

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

Macrophage (APC)

→ derived from monocytes

Ag/ MHC-II complex

only recognized by T helper cells (CD4⁺ cells)

Antigen presenting cell (macrophage, dendritic cell, B lymphocyte)

monocytes in spleen lymph nodes

* express class II & class I

remember nucleated cells!

Phagolysosome

small particles

Lysosome

Phagosome

MHC-II → APC

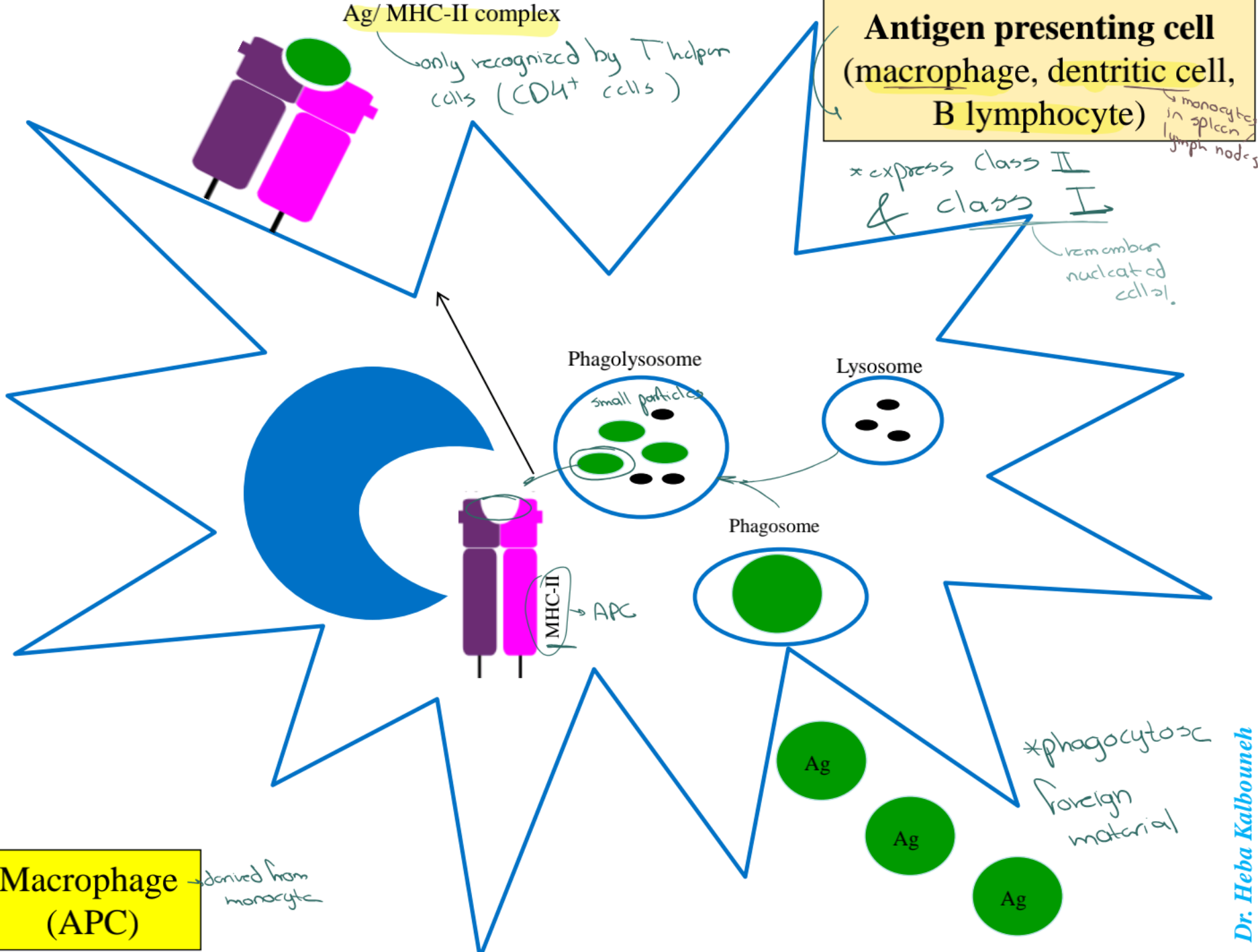
Ag

Ag

Ag

*phagocytose foreign material

Dr. Heba Kalbouneh



Helper CD4 T cells:

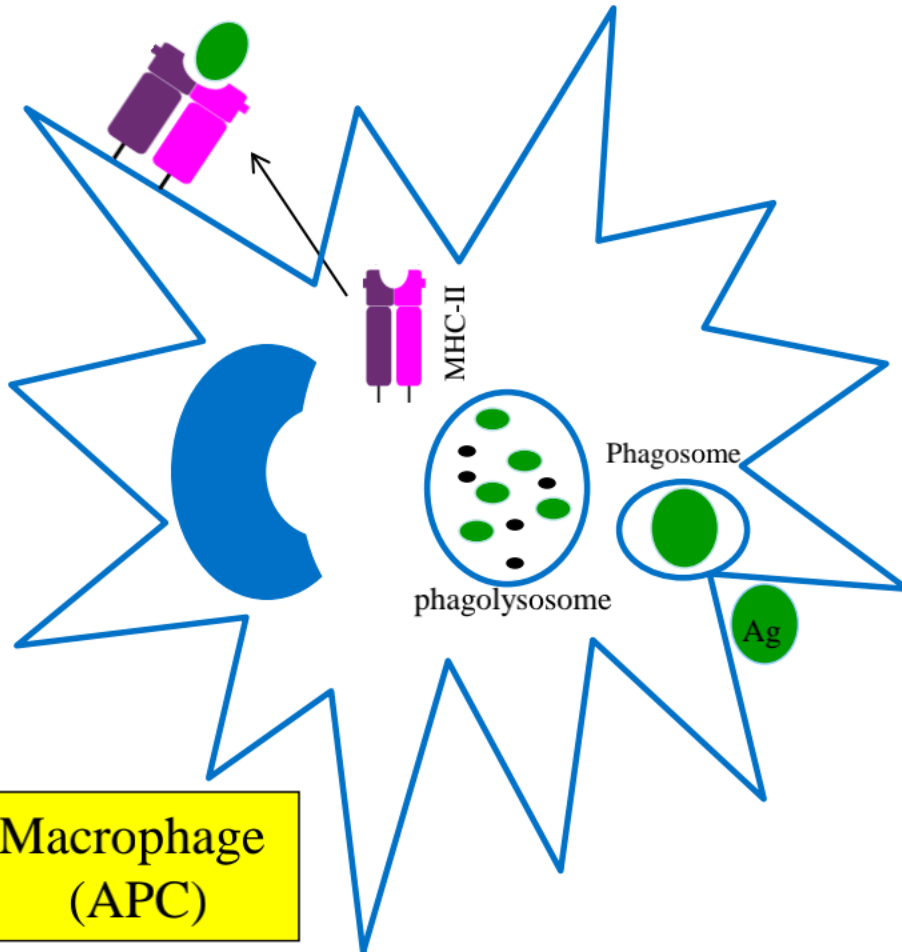
When Ag is phagocytosed by antigen presenting cells (APCs)
e.g macrophages, dentritic cell and B lymphocytes

It binds to MHC-II molecules

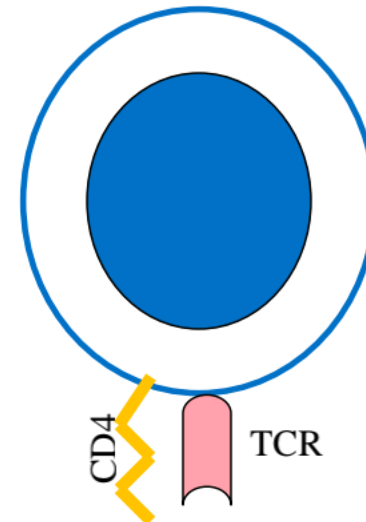


Ag-MHCII complex

Ag/ MHC-II complex

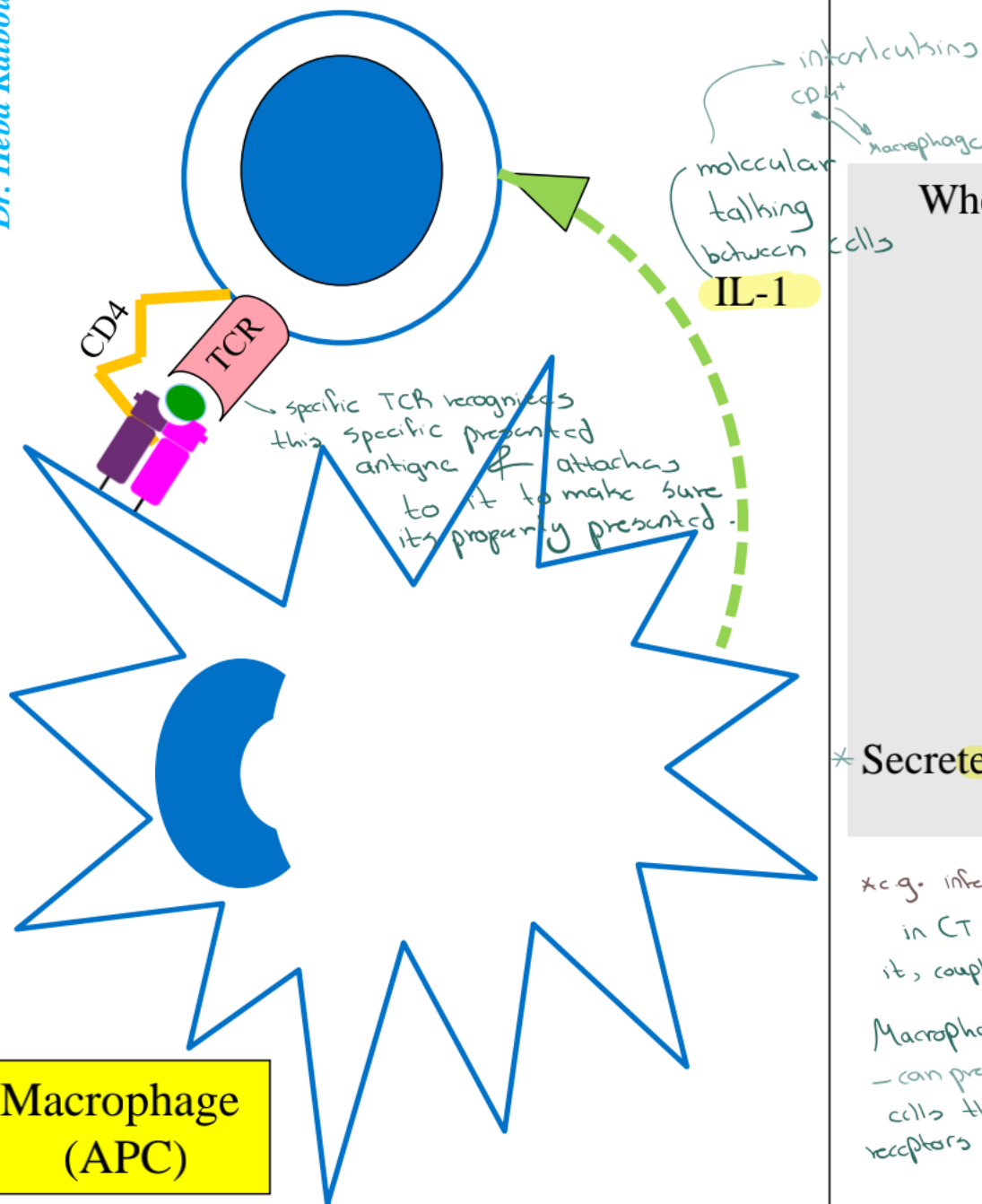


* $CD8^+$ & $CD4^+$
T cells both are MHC
restricted cells → only
recognize antigens on
Class I (cytotoxic) or
Class II (helper)



T Helper cell

T Helper cell



When the Ag- MHCII complex binds to receptors on Helper CD4 T cells

They



- (1) **Proliferate** → ↑ number
- (2) **Activate** → large T helper cells



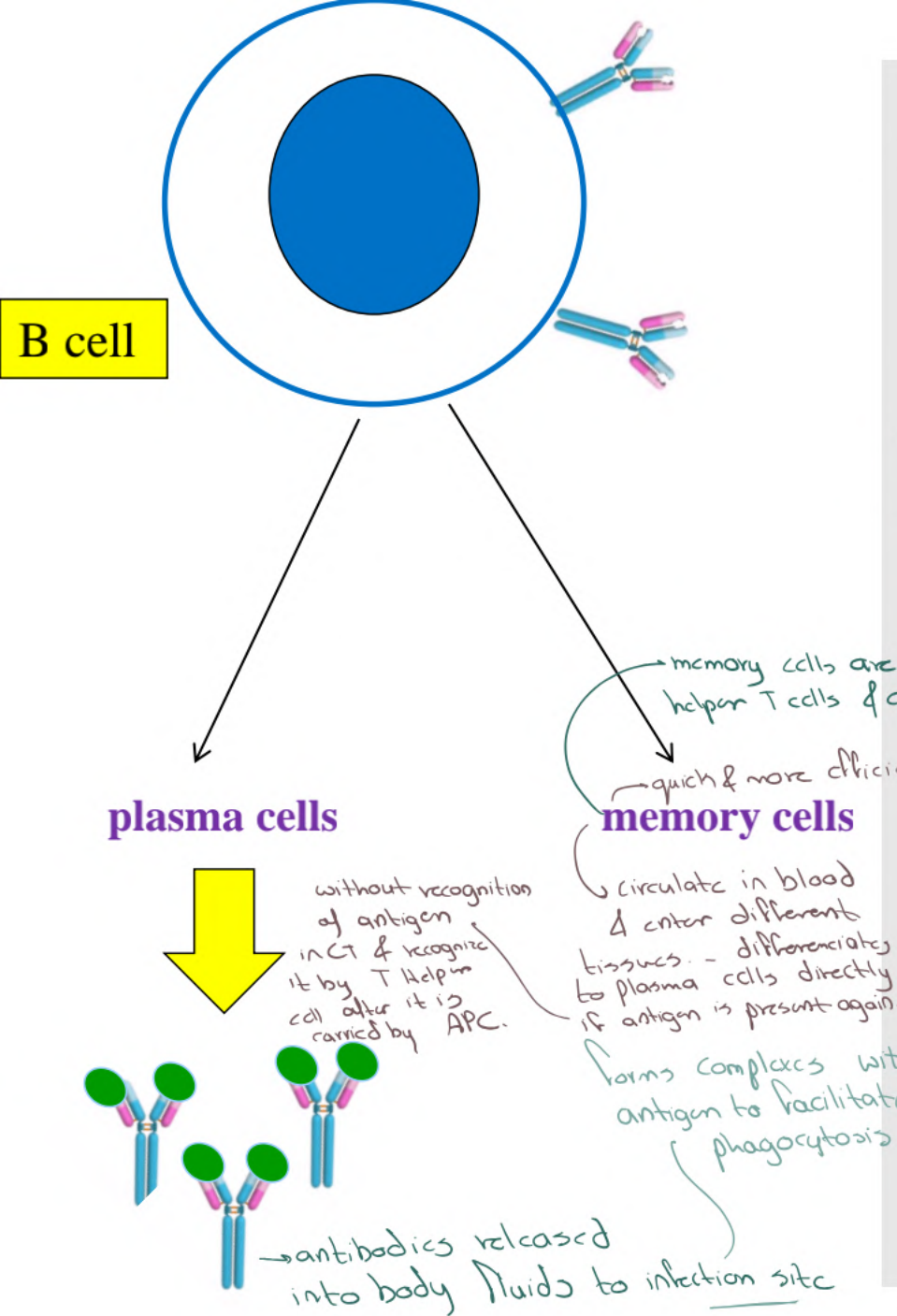
activate all immune cells in our body..

* **Secrete Lymphokines (cytokines) to Stimulate T and B cells**

x.e.g. infection in skin.. macrophage - as resident cell in CT of skin - phagocytose antigen & degrades it, couples with class II MHC molecule - it is APC -

Macrophage then usually travels to lymph node - can present in tissue though - to reach $CD4^+$ helper cells that can recognize specific antigen through TCR receptors on class MHC molecules.

Macrophage
(APC)



When a B lymphocyte is stimulated by T helper cells

usually in the case of bacterial infection → dealt with by antibodies.



- pathway depends on antigen type but everything gets activated —

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)

B cells:

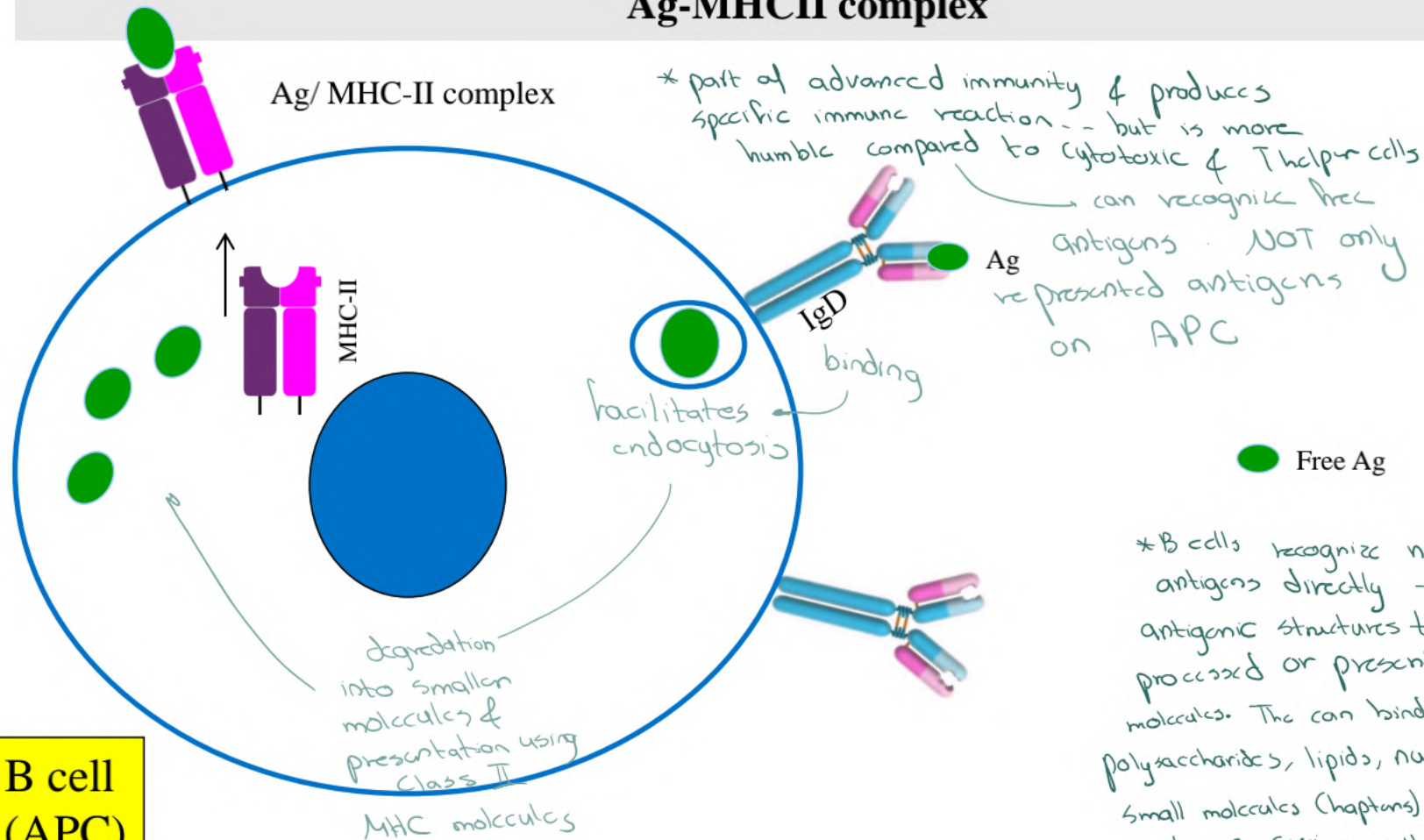
When the specific Ag binds to receptors on B cells



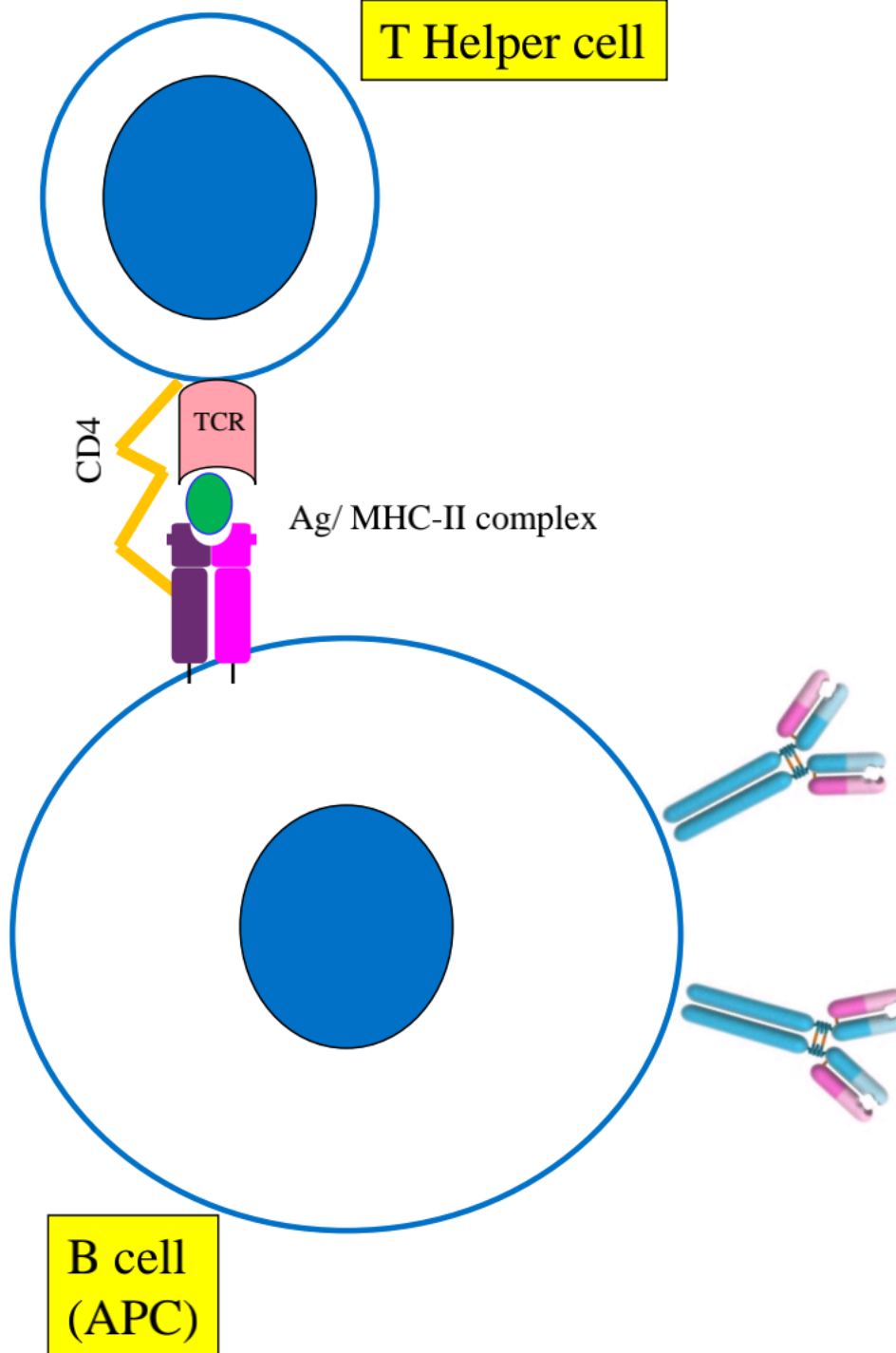
receptor-mediated endocytosis and fragments of the Ag bind to MHC-II molecules



Ag-MHCII complex



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

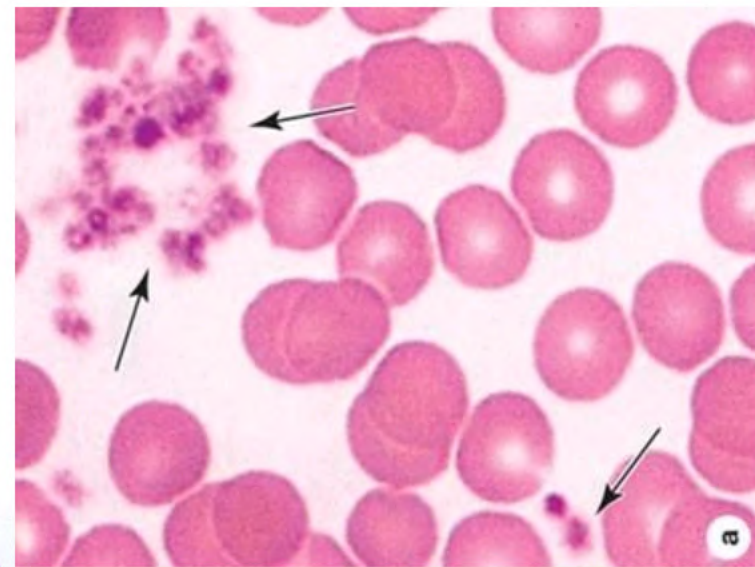
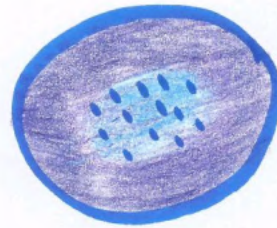


Most common to least

*Which type of lymphocyte is
most abundant in peripheral
blood?*

Thrombocytes (Platelets)

- Small non-nucleated cytoplasmic fragments
- Formed by fragmentation of the cytoplasm of **megakaryocytes** in the bone marrow
- **Number: 200,000-400,000/mm³**
- **Shape: biconvex discs**
- **Cytoplasm: purple, granular**
- **Diameter: 2-4 um**
- **Lifespan about 10 days**
- Control the bleeding by plugging the defects in blood vessels and activating blood clotting cascades

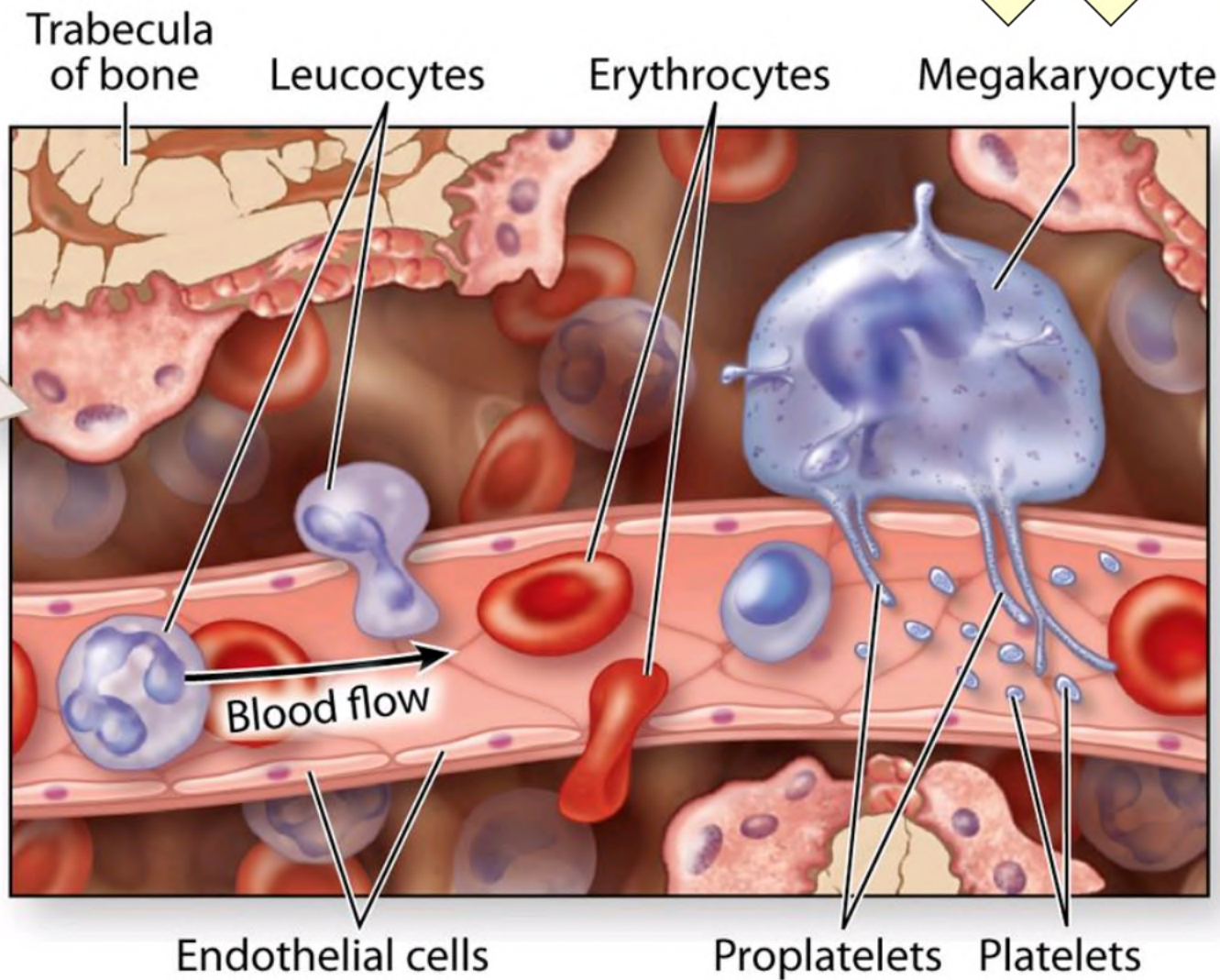
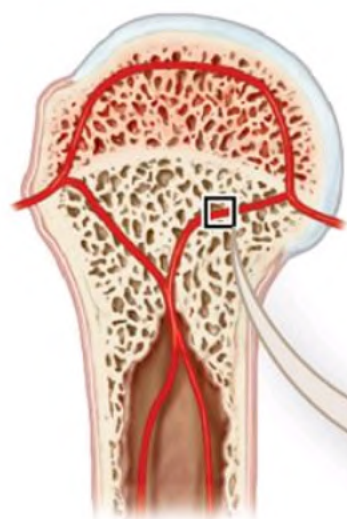


In stained blood smears, platelets often appear in clumps



Platelet has 2 zones

- Outer pale basophilic (clear) peripheral zone: **hyalomere**
- Central dark granular zone: **granulomere**



Hyalomere: contains cytoskeleton and membranous channels

Cytoskeletal elements

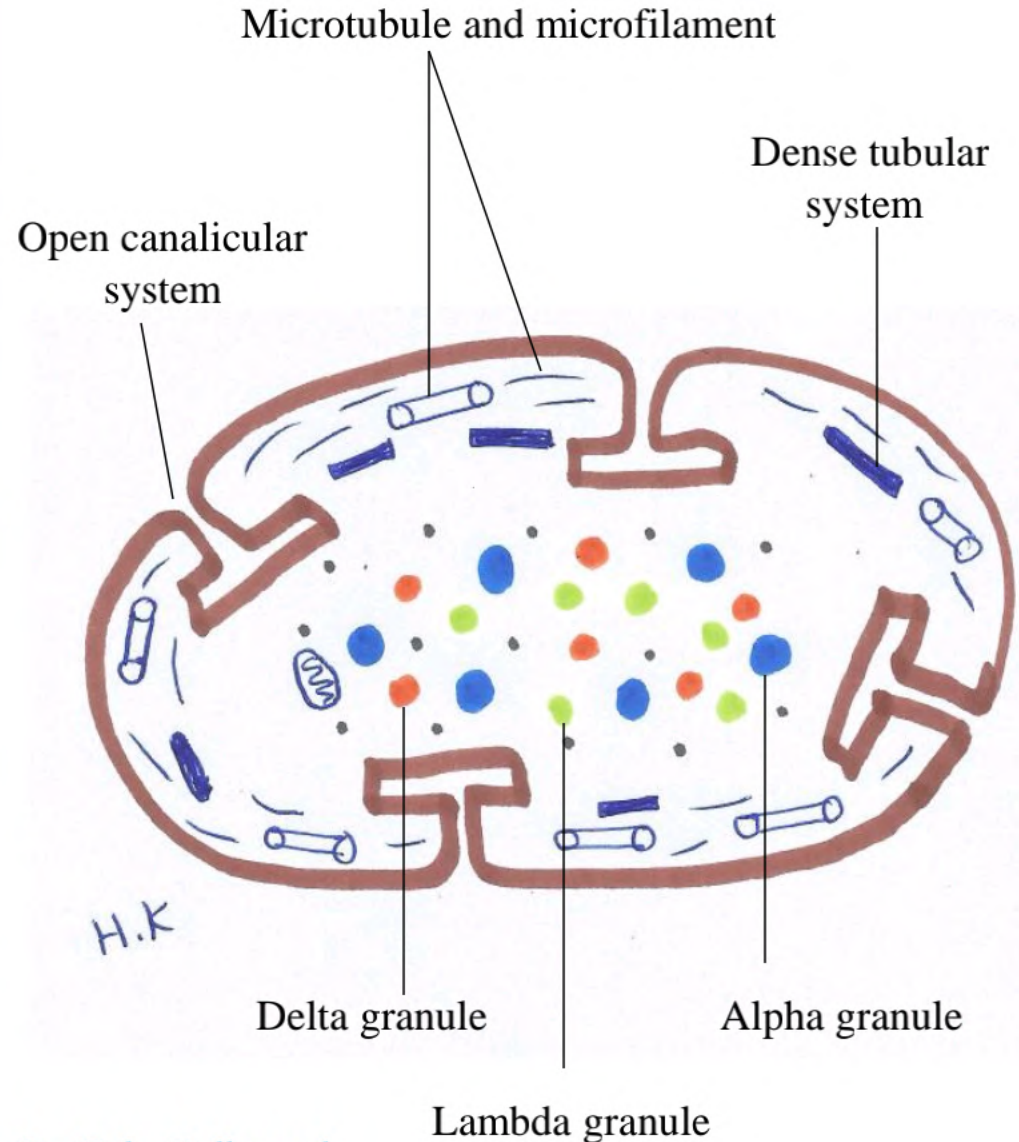
- Microtubule
- Actin filaments

Maintain shape and help contractions of platelets and squeezing, clot retraction

Membrane channels

- Open canalicular system
- Dense tubular system

Have thick glycocalyx



Granulomere: contains granules and organelles

Alpha granules: clotting factors, growth factors

Dense (delta) granules: serotonin (absorbed from plasma), ATP, ADP

Lambda granules: lysosomes (aid in clot resorption)

Their main function is to continually monitor the vascular system and detect any damage to the endothelial lining of the vessels. If the endothelial lining breaks, the platelets adhere to the damaged site and initiate a highly complex chemical process that produces a **blood clot**



Thus preventing blood loss

