



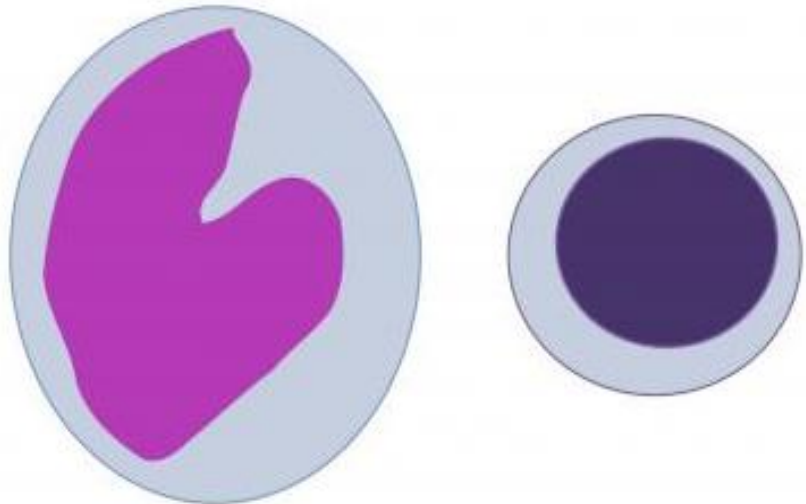
Blood cells

Edited by Sara Anas

Dr. Heba Kalbounch
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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**



Monocytes

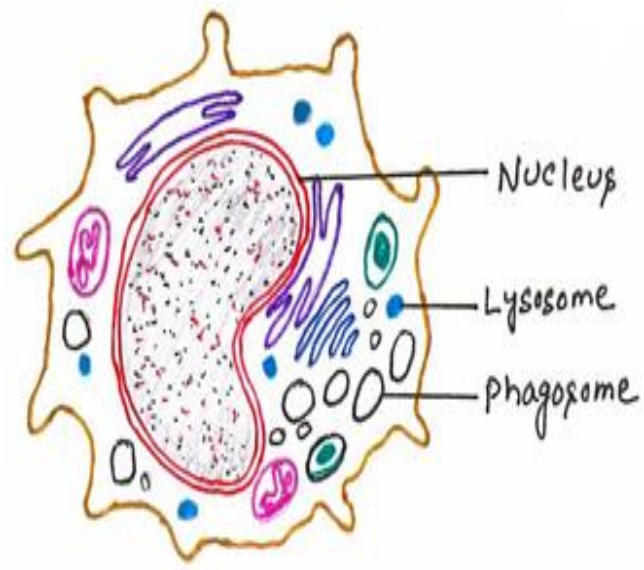
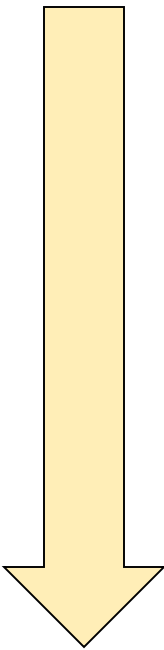
- Largest leukocytes
- Bluish cytoplasm (*frosted glass appearance*) & a large C-shaped nucleus
- Highly motile and phagocytic
- Travel through bloodstream to reach connective tissues, where they transform into **macrophages** (large phagocytic cells)- Chronic infections

Azurophilic granules (lysosomes)



It is a "c" shape nucleus and the cytoplasm is not granular but is still unclear

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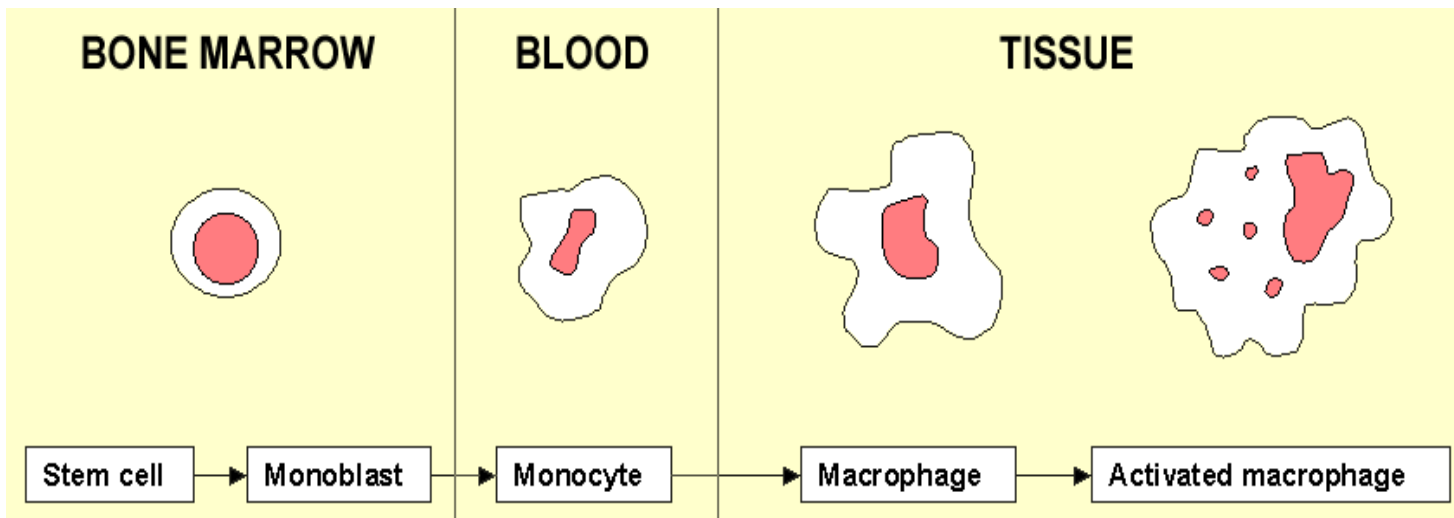
These dense structure are lysosomes

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

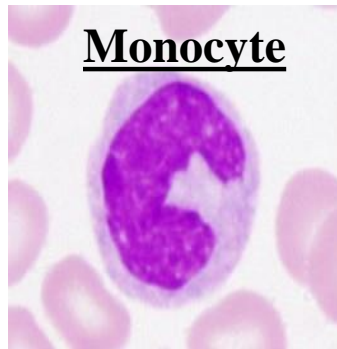


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↓
Kupffer Cells
 Liver
 ↓
Macrophages
 Bone marrow
 Connective tissue
 ↓
Osteoclasts
 Bone resorption

MONONUCLEAR PHAGOCYtic SYSTEM

↓
Dust cells or alveolar cells
 Lung



(all characterized by phagocytic activity)
 And all are antigen presenting cells

↓
Microglia
 CNS
 ↓
Dendritic cells
 Lymph node
 Spleen
 ↓
Langerhans cell
 Epidermis

Lymphocytes

- Smallest leukocytes
- Round nucleus occupies most of cell volume
- Cytoplasm is light clear blue *Basophilic cytoplasm because it is rich of ribosomes*
- 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

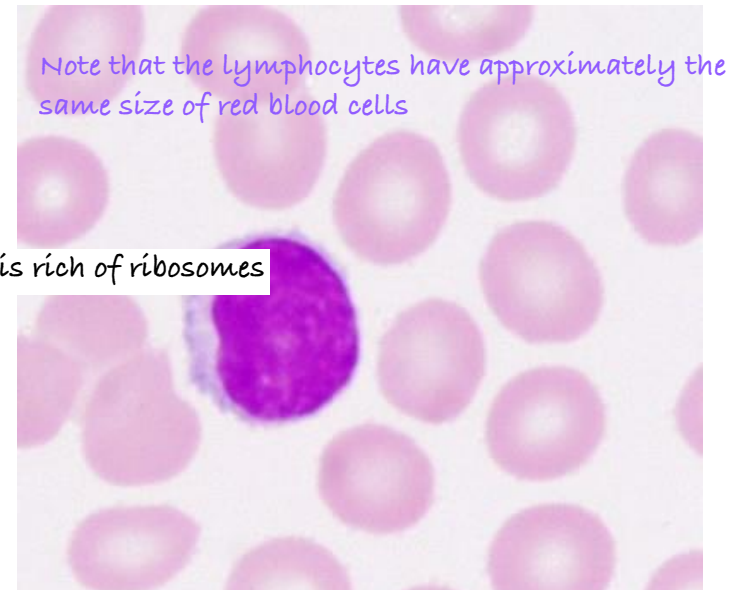
Cell mediated immunity

- **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

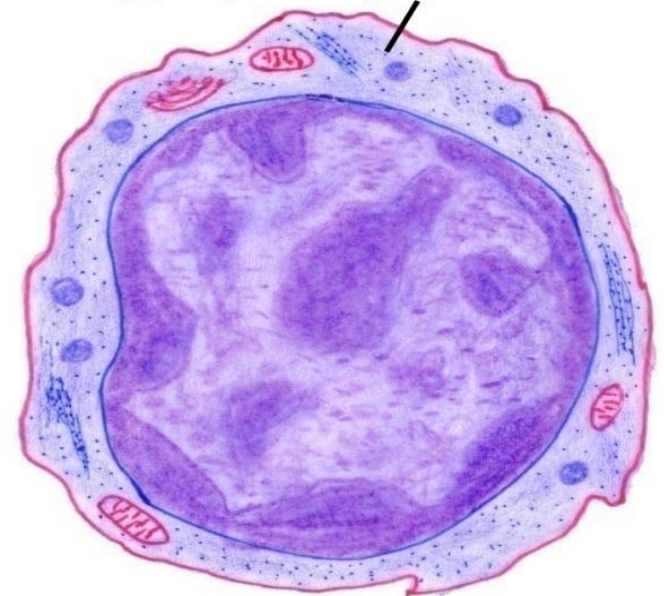
Long term immunity



Note that the lymphocytes have approximately the same size of red blood cells

The ribosome is the only organelle that produces basophilia inside the cytoplasm using the ordinary stain

Scanty peripheral cytoplasm



Humoral means
body fluids

Lymphopoiesis: the process by which lymphocytes are formed

**Precursor cells in
bone marrow**

Thymus

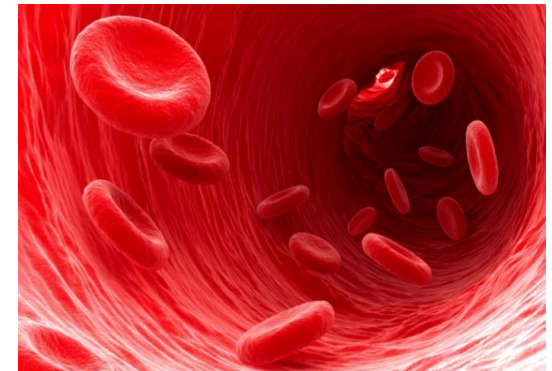
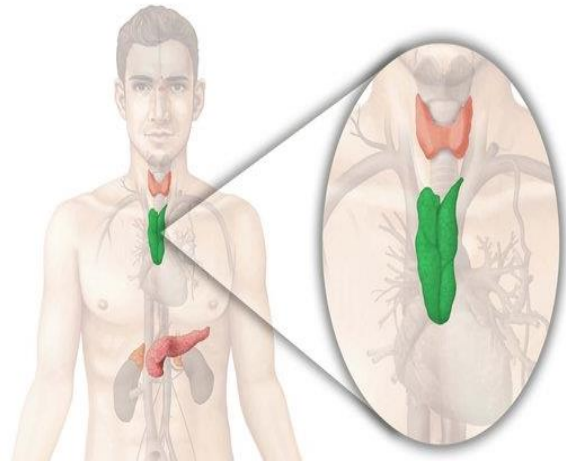
Directly into blood

T Lymphocytes

BM

B Lymphocytes

**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**

The natural killer cell is always large and active cell



Euchromatic nucleus

Large
(9-18 μm)
Active lymphocyte

It could be T or B or natural killer lymphocyte



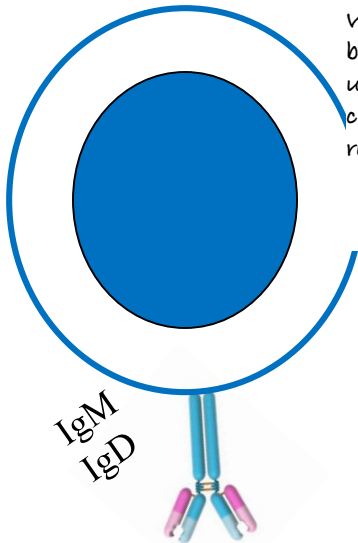
Heterochromatic nucleus

Small
(6-9 μm)
Inactive lymphocyte

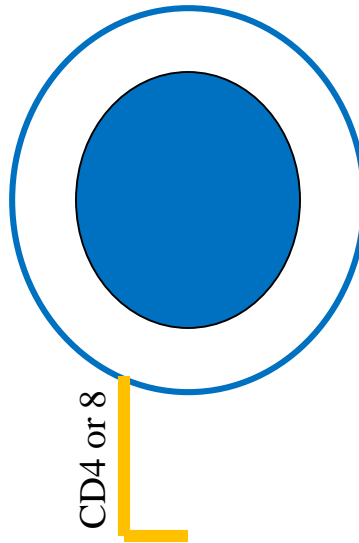
It could be T or B lymphocyte

Different types express specific cell surface proteins

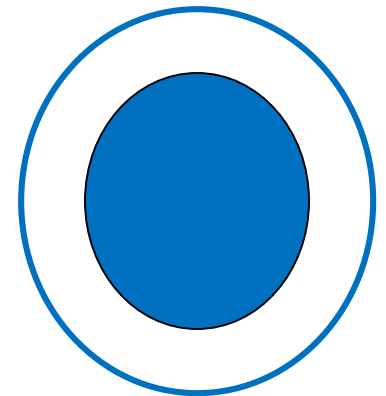
We can not differentiate between these lymphocytes under the microscope, but we can by the cell surface receptors



B lymphocyte

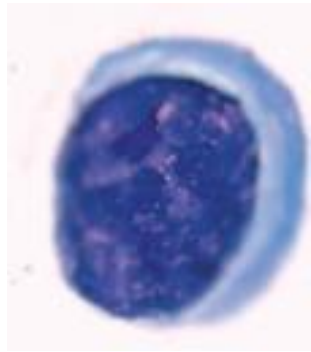


T lymphocyte



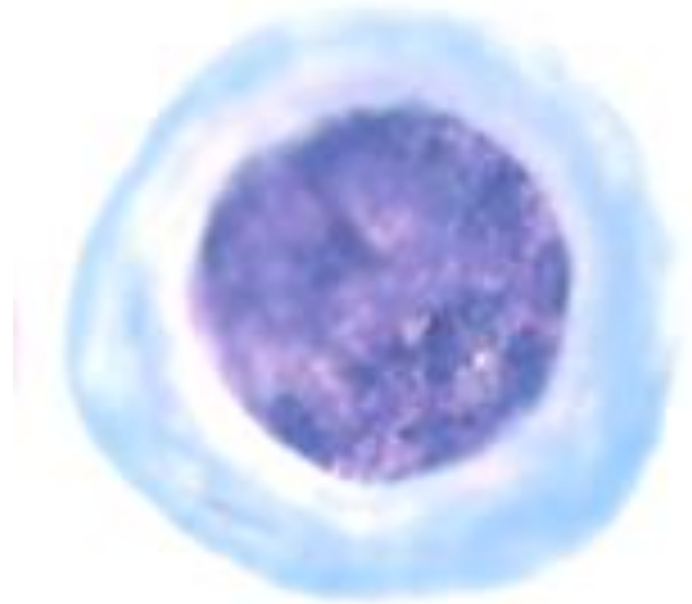
It has receptors but not those in T or B lymphocytes.

Natural killer lymphocyte



**Small
(6-9 μm)
Inactive lymphocyte**

Darkly stained cell



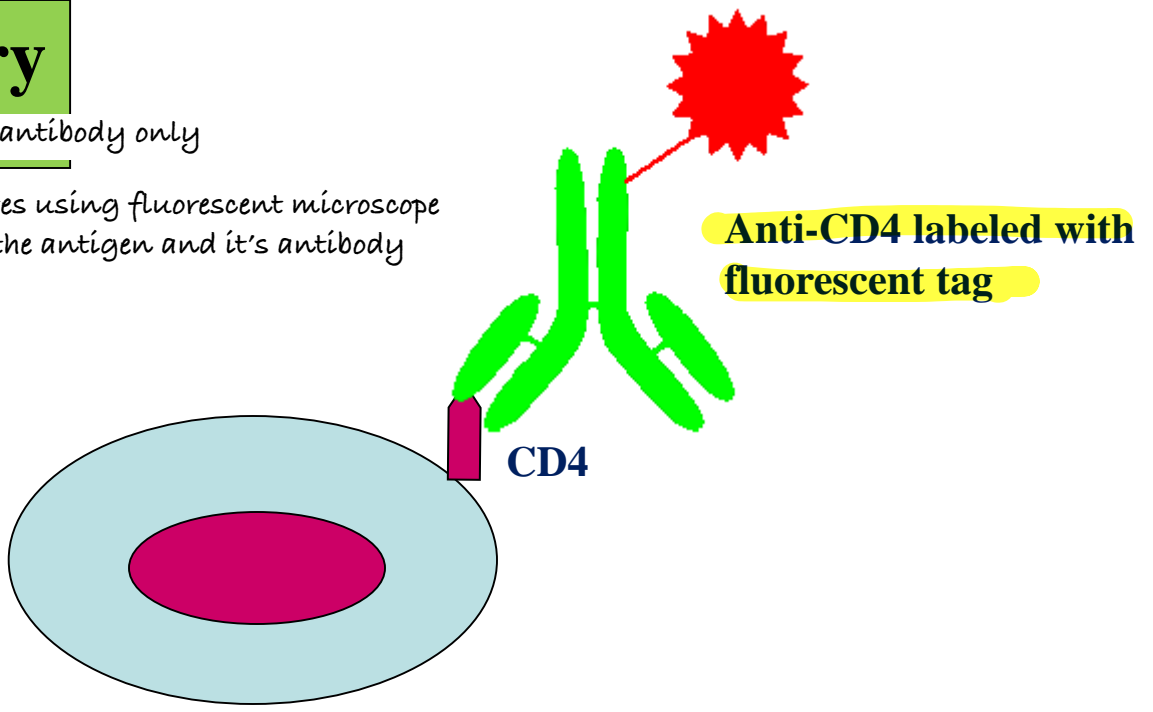
**Large
(9-18 μm)
Active lymphocyte**

Lightly stained cell

Immunohistochemistry

Direct method Primary antibody only

In order to differentiate between type of lymphocytes using fluorescent microscope
This method depends on specific binding between the antigen and it's antibody

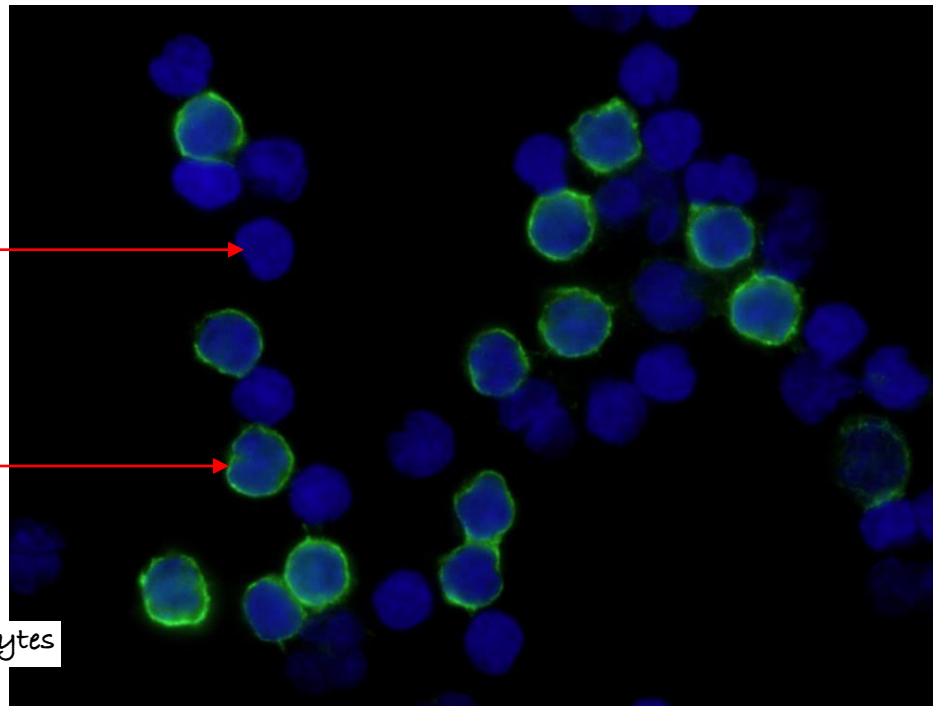


Immunohistochemistry

Using CD 4 Antibody

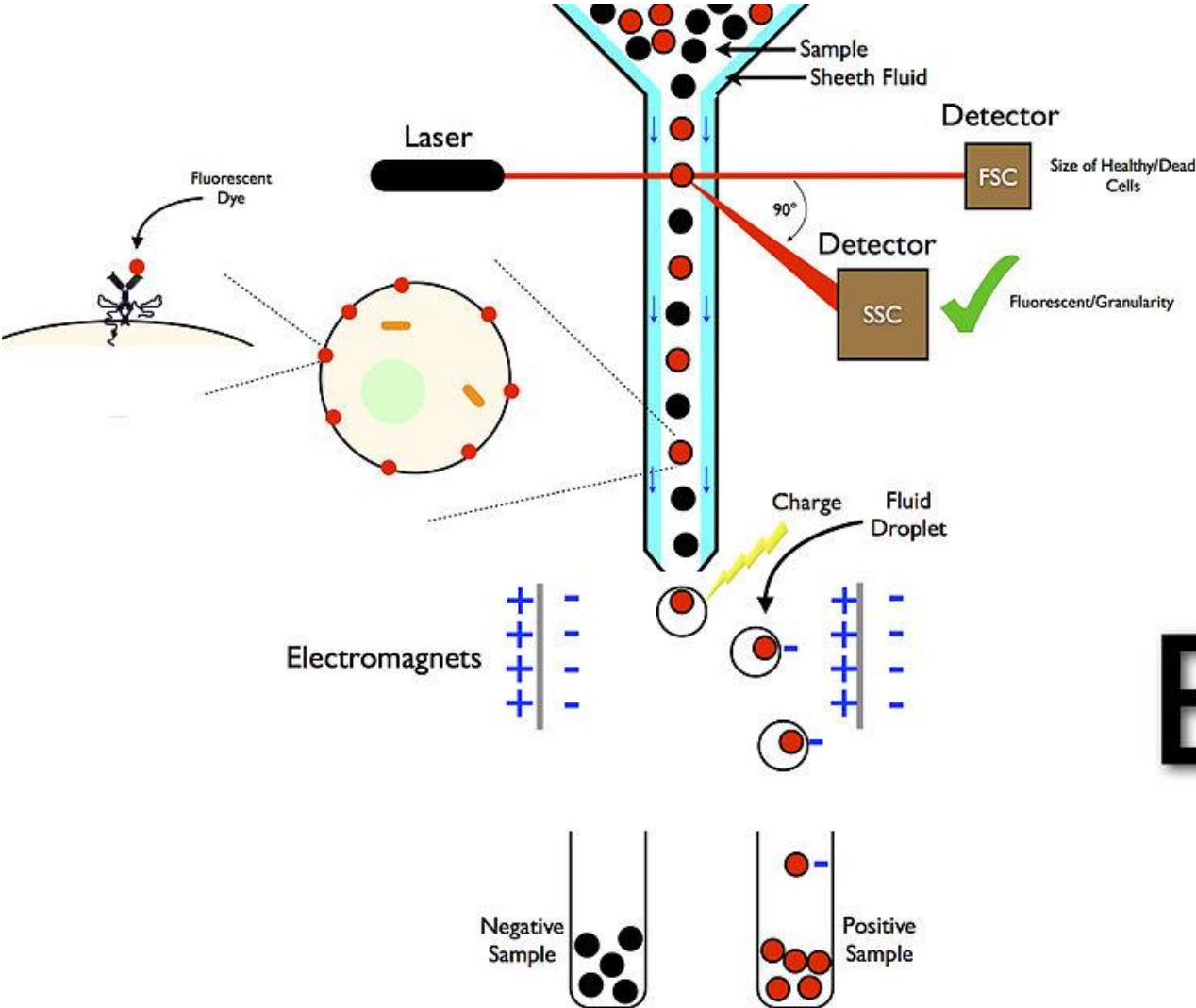
May be cytotoxic T lymphocyte or B cell

**CD 4 +
T Helper lymphocyte**



The blue colour represents the nuclei of different lymphocytes

Flow cytometry (FACS)



B

About a previous slide:

We use FACS for sorting and counting cells.

**fluid sample: taken from BM or peripheral blood.

We label the cell with specific antibodies and these antibodies are labeled with fluorescent material.

As these cells passing through the tube, we have a laser light measures the intensity of the fluorescent colour separating the cells into positive cells and the negative cells.

**in this example: the positive cells are the B cells

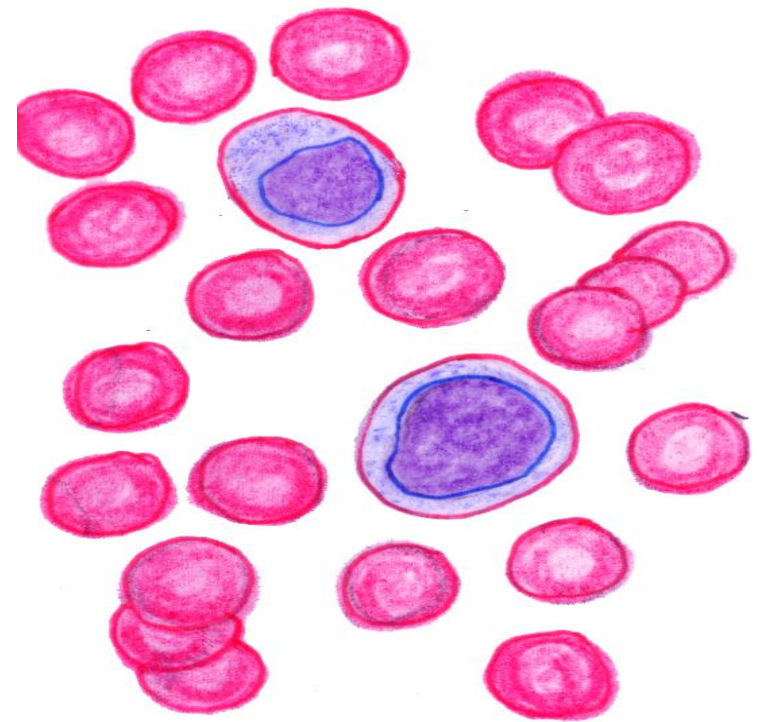
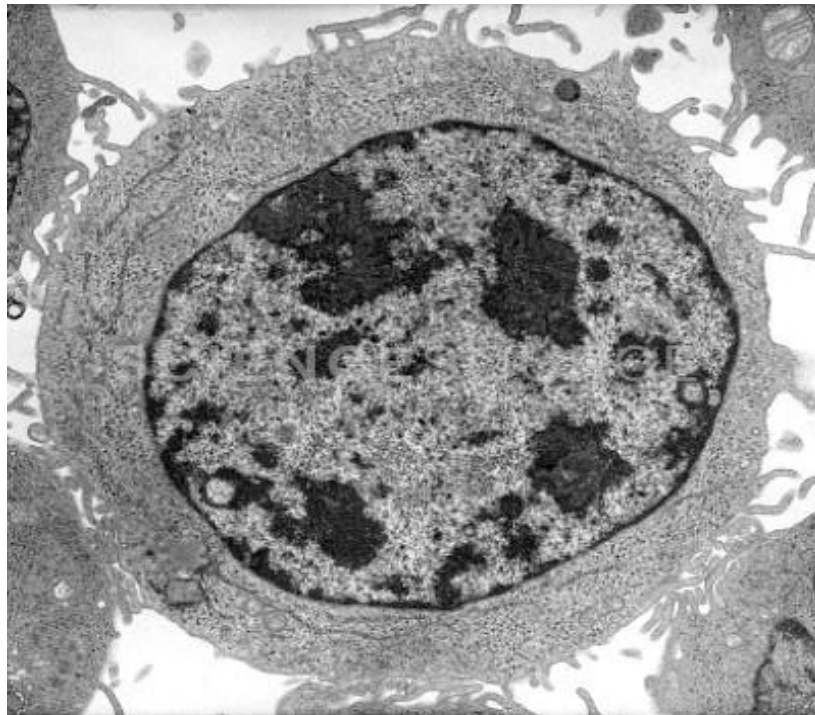
The negative cells are non B cells.

And by the way, we use FACS to diagnosis different type of blood cancers such as different types of leukaemia.

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**)



Size relation to erythrocytes

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



Account for basophilic cytoplasm

Lymphocytes

B Lymphocyte

Memory cell

Plasma cell

Produces antibodies

into body fluid (blood, lymph, interstitial fluid)
That's why it is called humoral immunity

Called cell mediated immunity

T Lymphocyte

Cytotoxic *Harsh cells*

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

Suppressor

Suppresses immune response to self Ag
Suppresses immune response of T and B lymphocytes

Helper

Help cytotoxic T cells and B cells in their immune functions

Natural killer cells

(NULL Lymphocyte)

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

Large granular lymphocytes Activated lymphocytes

These cells can recognise the cells that don't express MHC-1 such as cancer cells and some virus infected cells (hidden MHC-1).
So if the cells in the blood present MHC-1 molecule, the natural killer cells don't attack them but they are able to detect the absence of these molecules

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

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

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

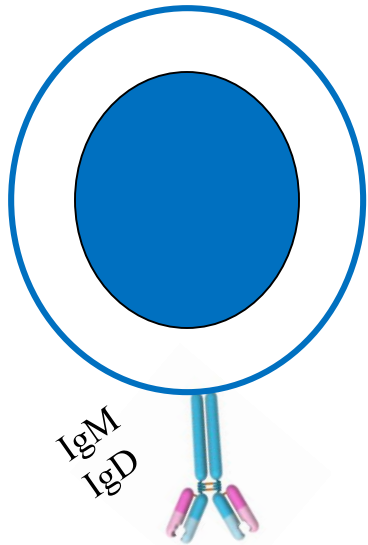
HIV affects **Helper T cells**

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

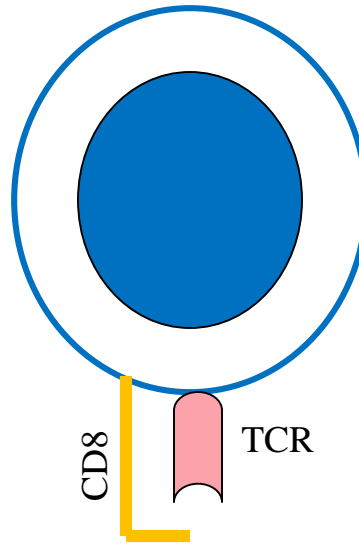


Reduction of this key lymphocyte group cripples the patient's immune system rendering them susceptible to opportunistic bacterial, fungal, protozoan, and other infections that usually dealt with easily in immunocompetent individuals.

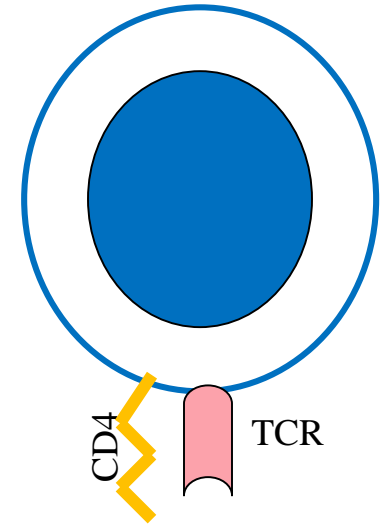
Different types express specific cell surface proteins



B cell



Cytotoxic T cell



Helper T cell

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

About a previous slide: (revision)

The cytotoxic T cells and helper T cells are differentiated inside the thymus.

Inside the thymus they undergo thymic education (learn how to differentiate between self and non self antigen and they get specific T lymphocyte receptors that can only recognise certain type of antigen).

Major histocompatibility complex

MHC Finger print (unique
For each individual.
Hint: (graft rejection)

Glycoprotein on cell membrane

Two classes:

MHC-I

On surface of **all nucleated cells**

Coupled to peptide formed within the cell We don't found in RBCs

MHC -II

On surface of **APCs**

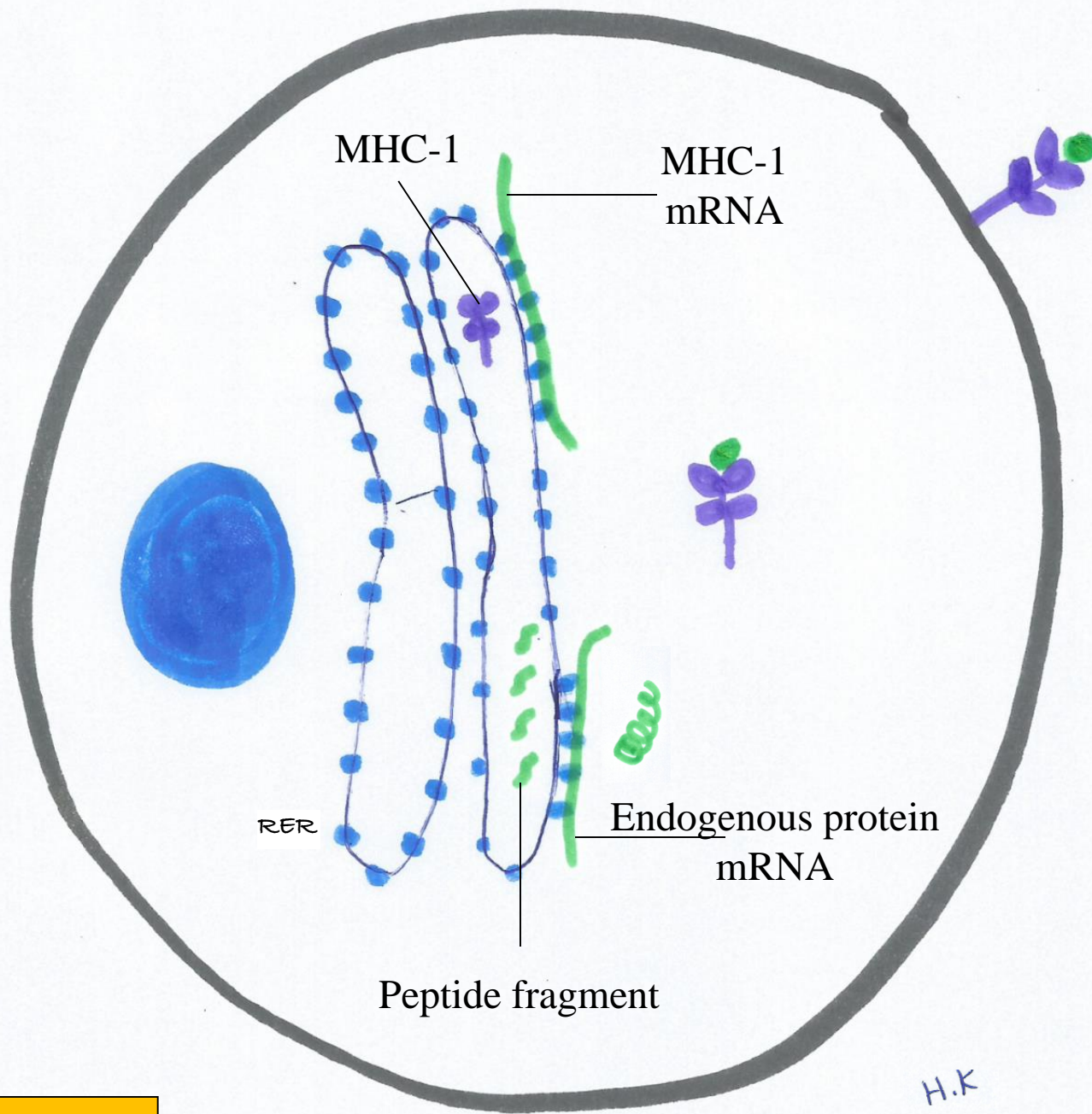
Coupled to peptide product of proteins the cells had ingested (peptide product of Ag digestion)

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

Also called human leukocyte antigens (HLAs)

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



MHC-1

MHC-1 mRNA

RER

Endogenous protein mRNA

Peptide fragment

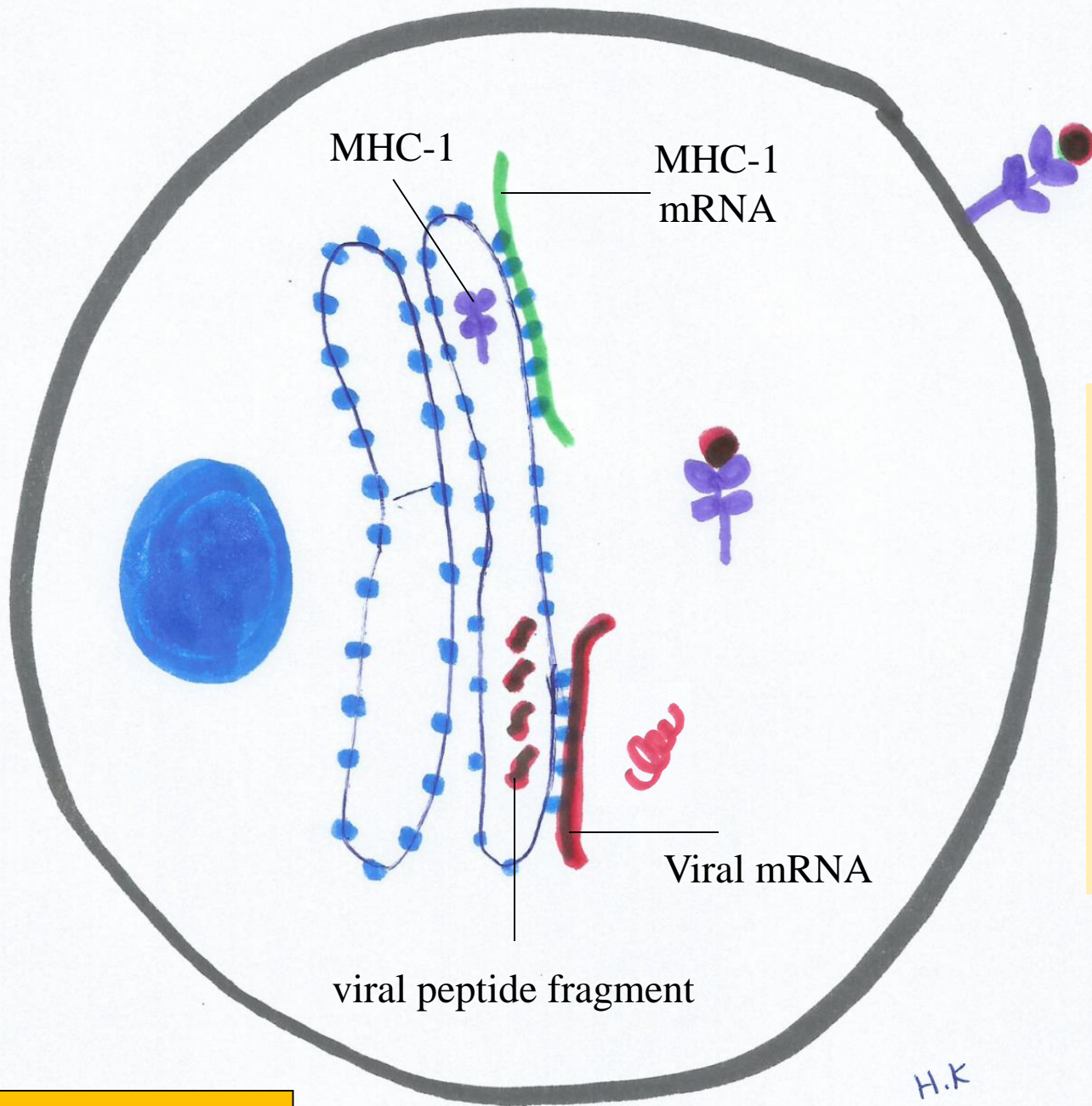
Self-peptide bound to MHC-1

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

Normal cell

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H.K



Viral- peptide
bound to
MHC-1

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

MHC-1
mRNA

MHC-1

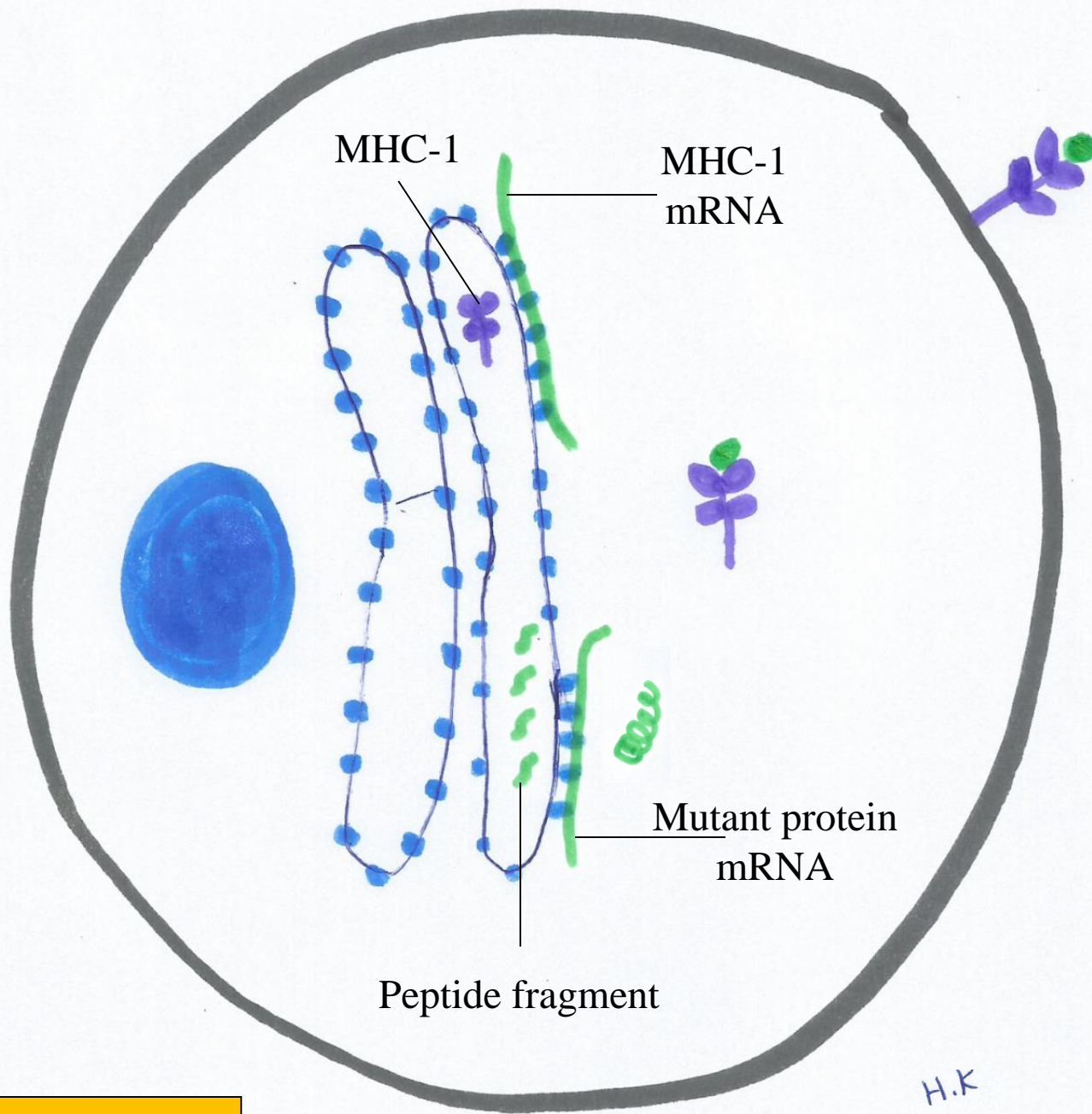
Viral mRNA

viral peptide fragment

H.K

Viral infected cell

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Mutant peptide bound to MHC-1

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

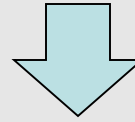
Cancerous cell

H.K

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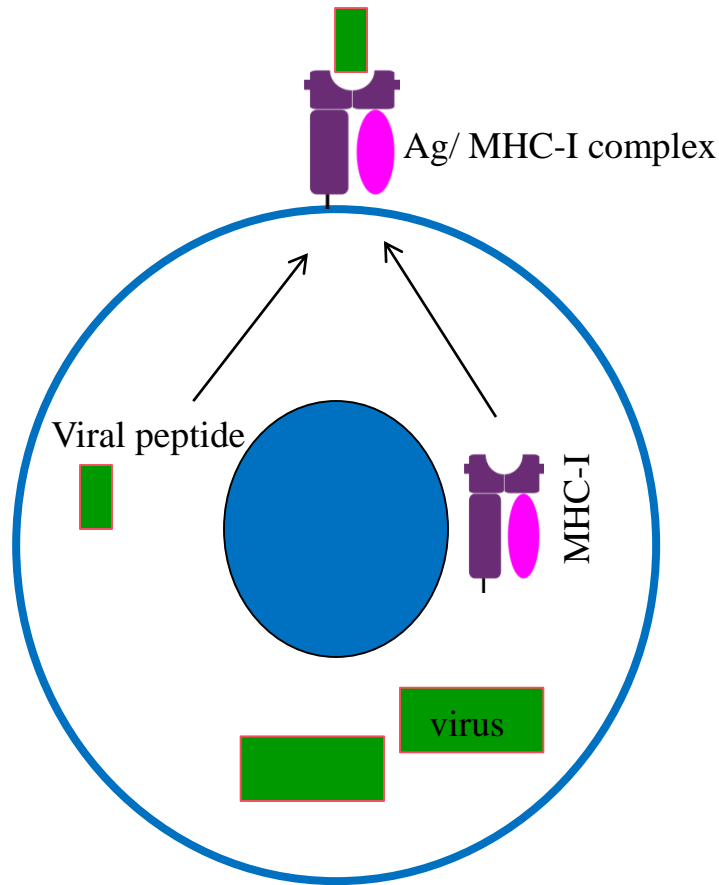
Cytotoxic CD8 T cells:

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

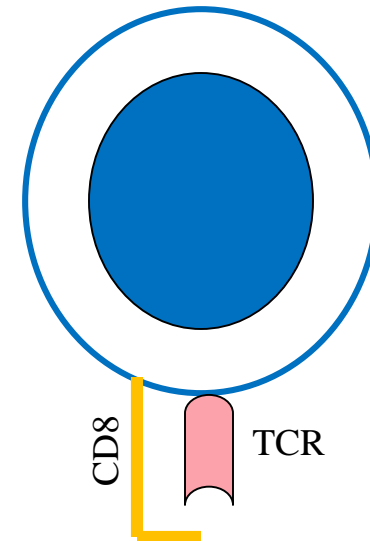


Ag-MHCI complex

Cell-mediated immunity



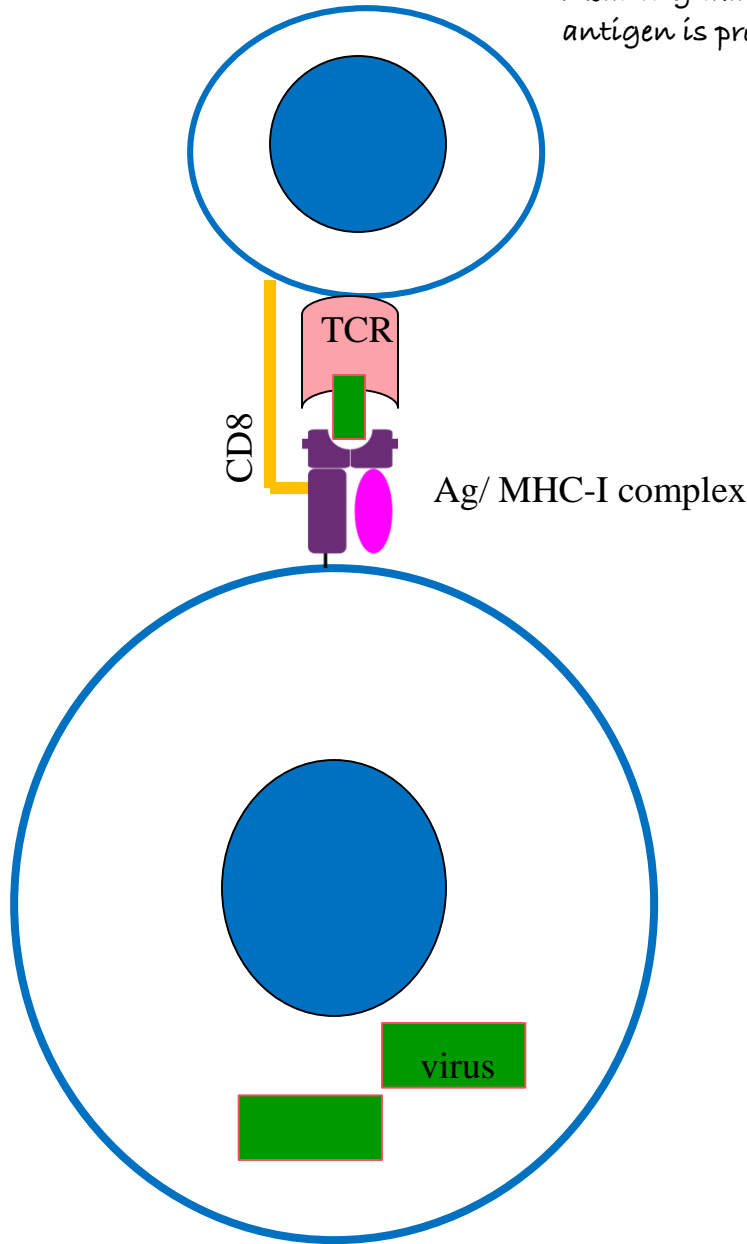
Viral infected cell



Cytotoxic T cell

Cytotoxic T cell

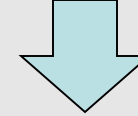
It recognises both (antigen + MHC-I molecule)
Meaning that TCR recognises the antigen while the CD8 makes sure that this antigen is presented on MHC-I



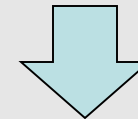
Viral infected cell

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

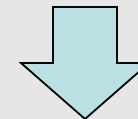
They



Proliferate
Activate



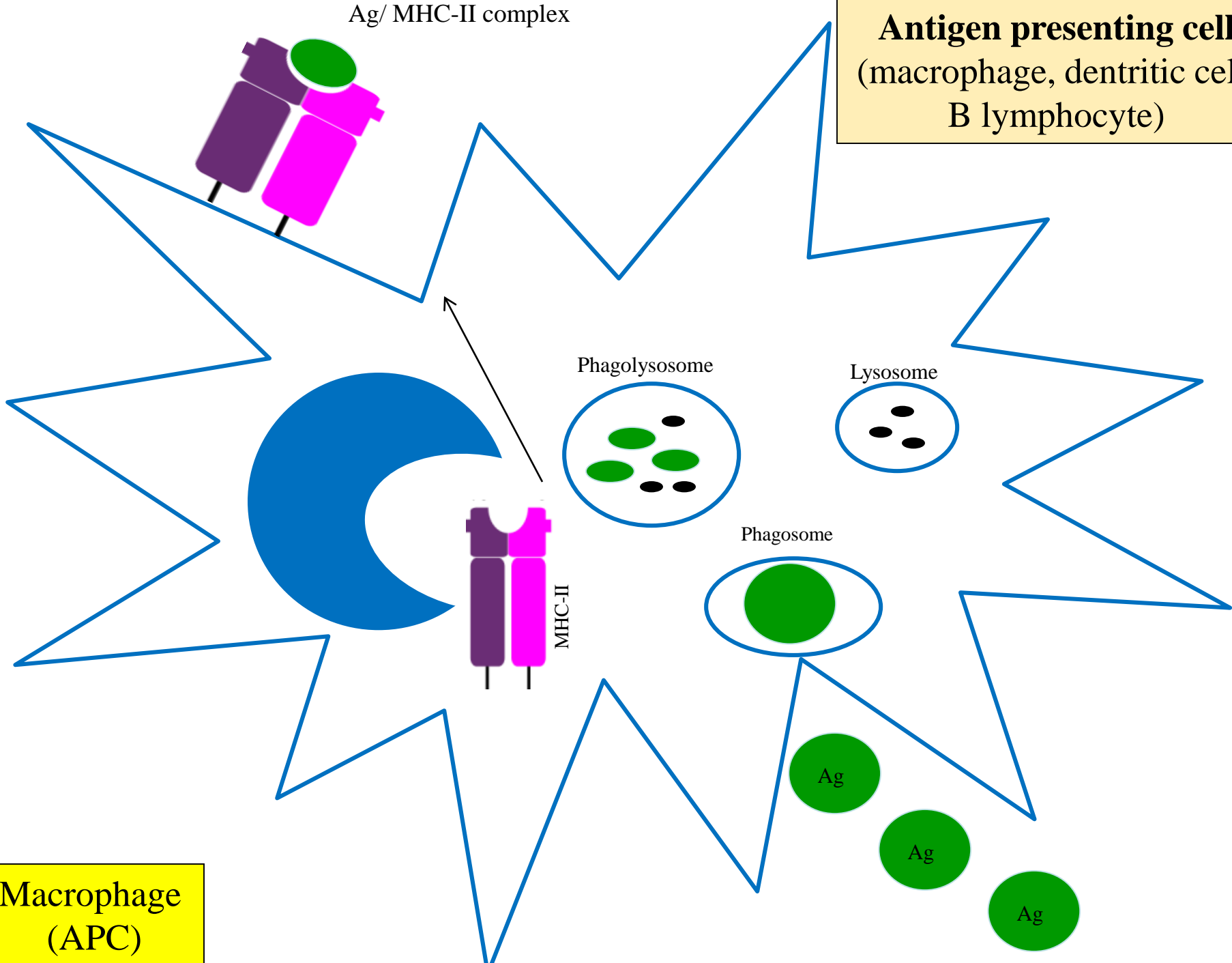
Release
Perforins and granzymes
(proteases)



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

Ag/ MHC-II complex

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

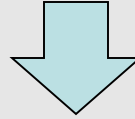


Macrophage
(APC)

Helper CD4 T cells:

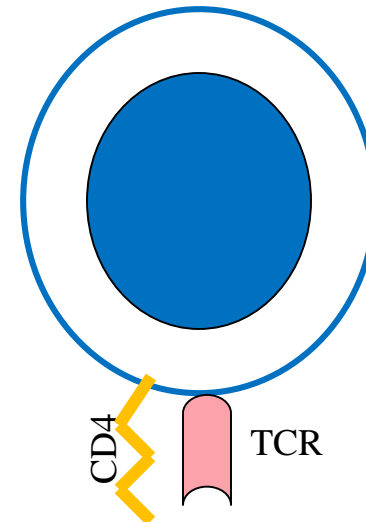
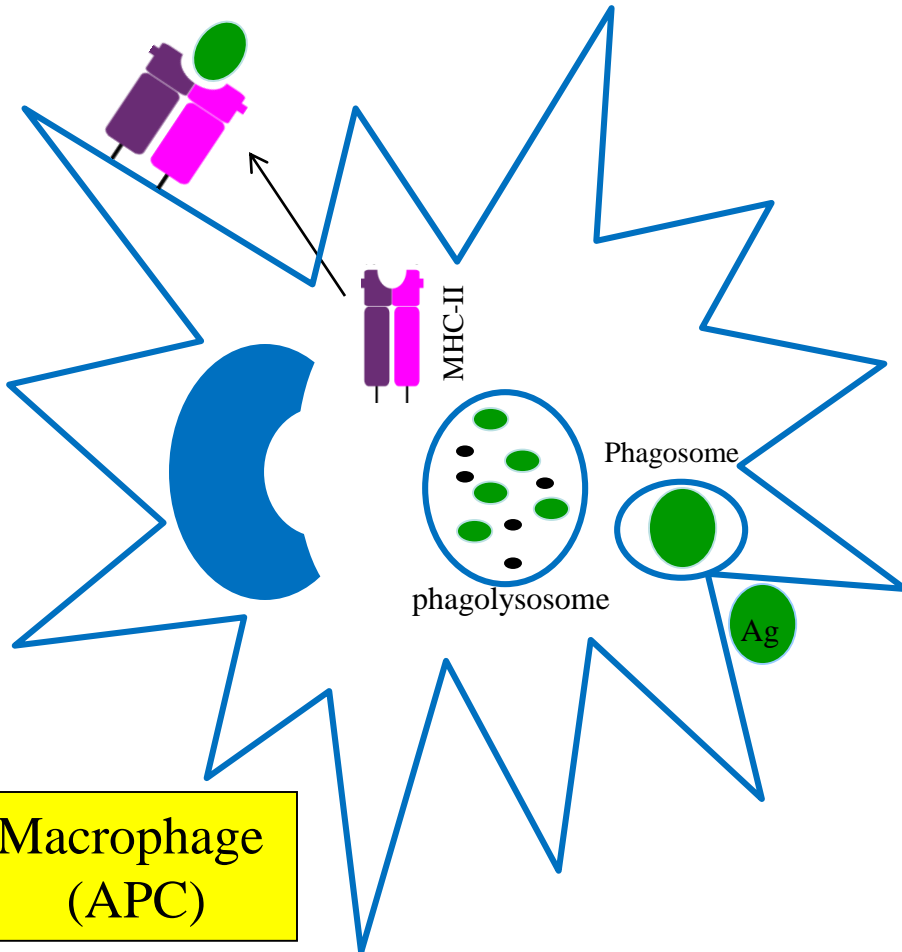
When Ag is phagocytosed by antigen presenting cells (APCs)
e.g macrophages, dendritic cell and B lymphocytes

It binds to MHC-II molecules



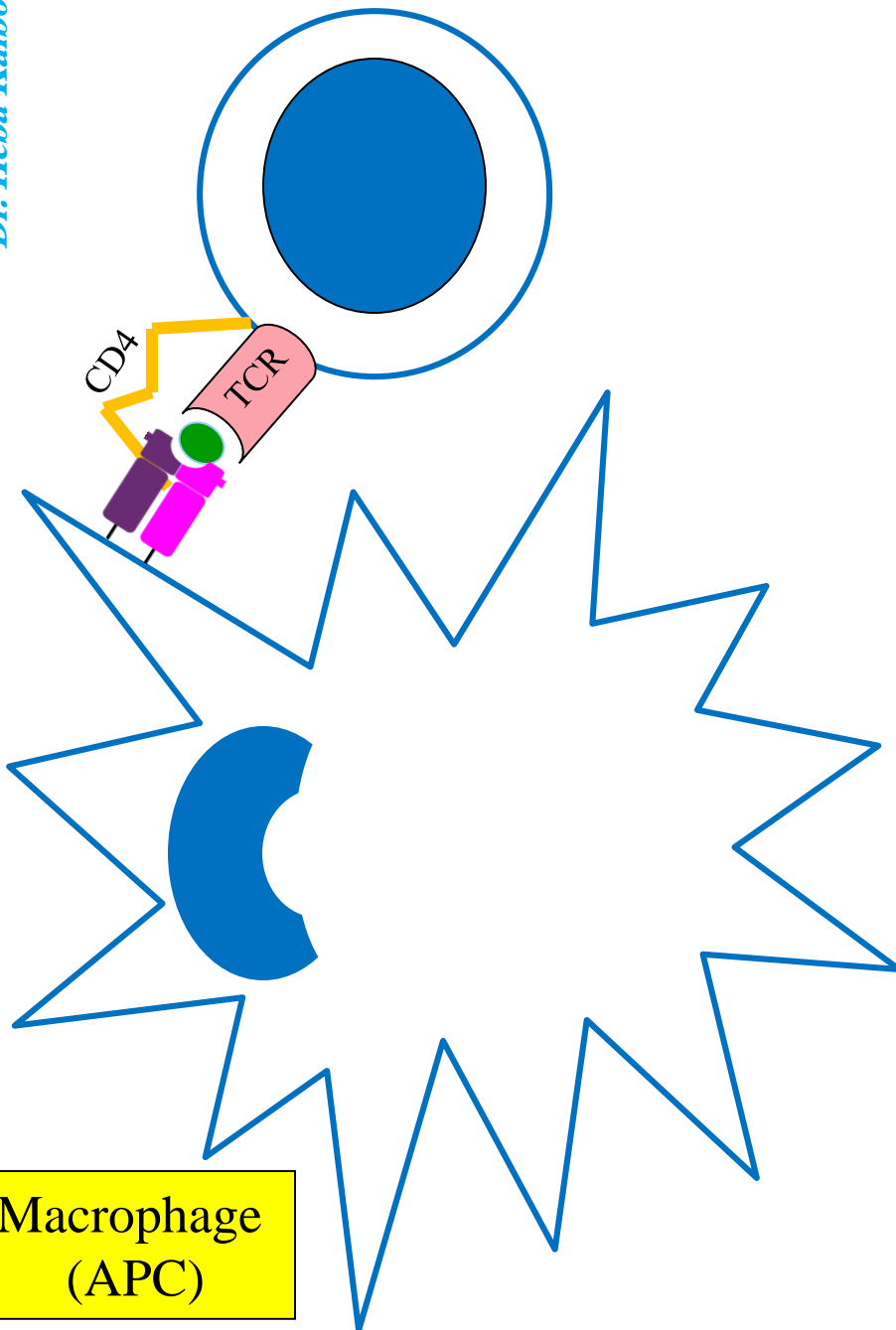
Ag-MHCII complex

Ag/ MHC-II complex



T Helper cell

T Helper cell



Macrophage (APC)

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

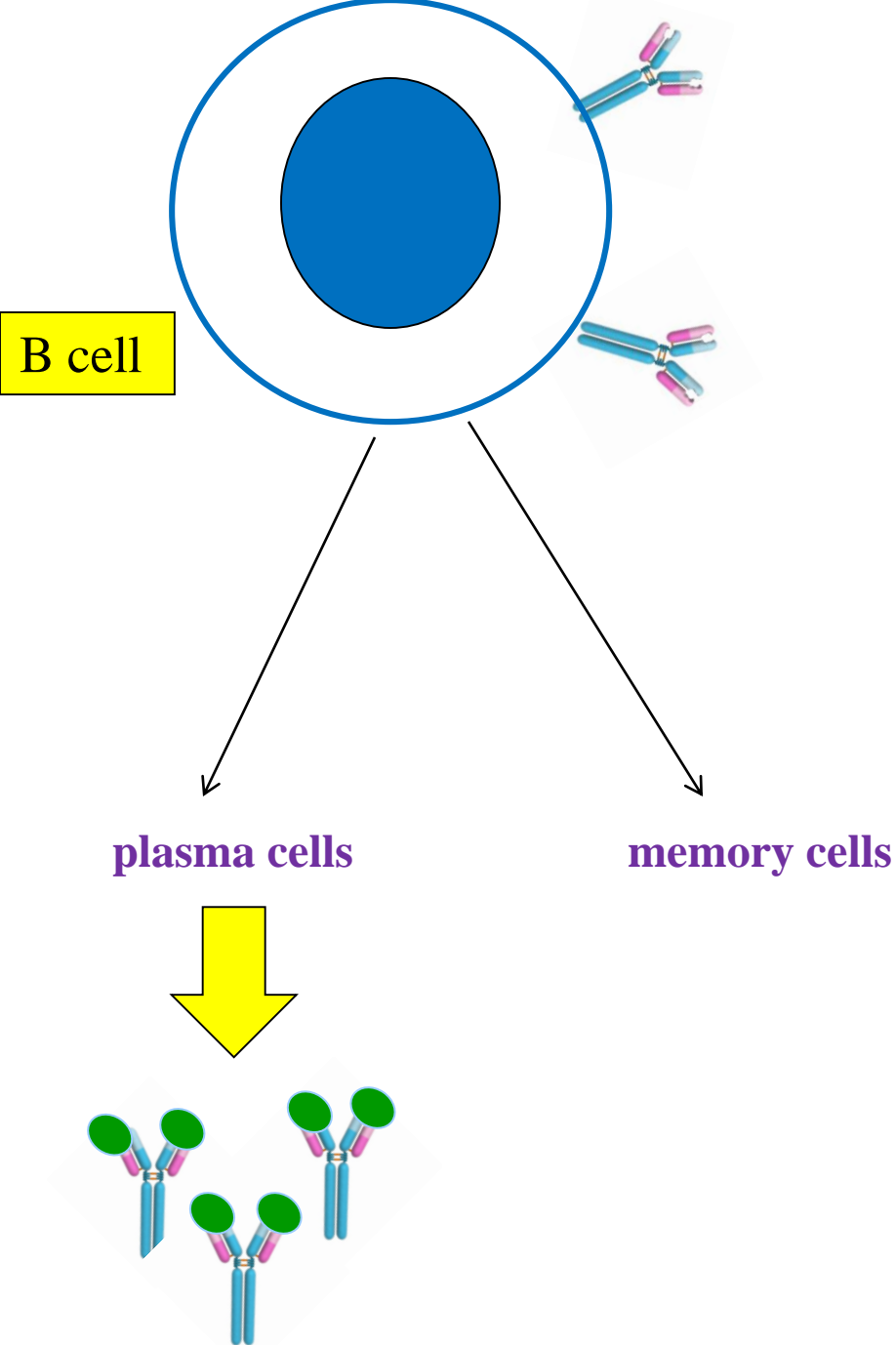
They



Proliferate
Activate



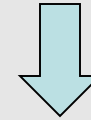
Secrete Lymphokines (cytokines) to Stimulate T and B cells



When a B lymphocyte is stimulated by T helper cells



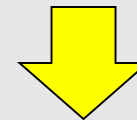
Proliferate
Activate



Activated B lymphocytes:

1- differentiate into **plasma cells** (secrete antibodies Specific for the antigen
recognised by T helper cell)

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



Life long immunity (vaccination)

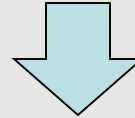
Humoral immunity

B cells:

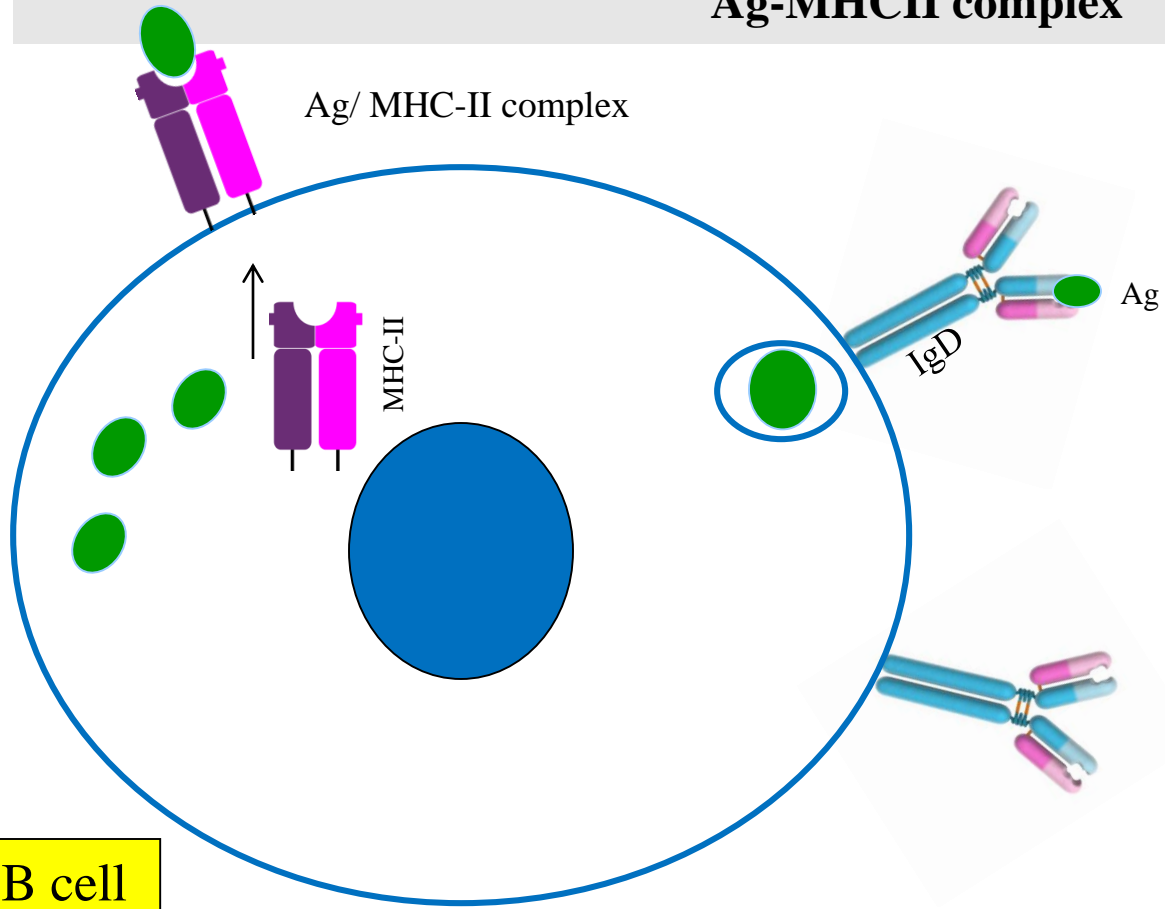
When the specific Ag binds to receptors on B cells



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



Ag-MHCII complex



Ag/ MHC-II complex

MHC-II

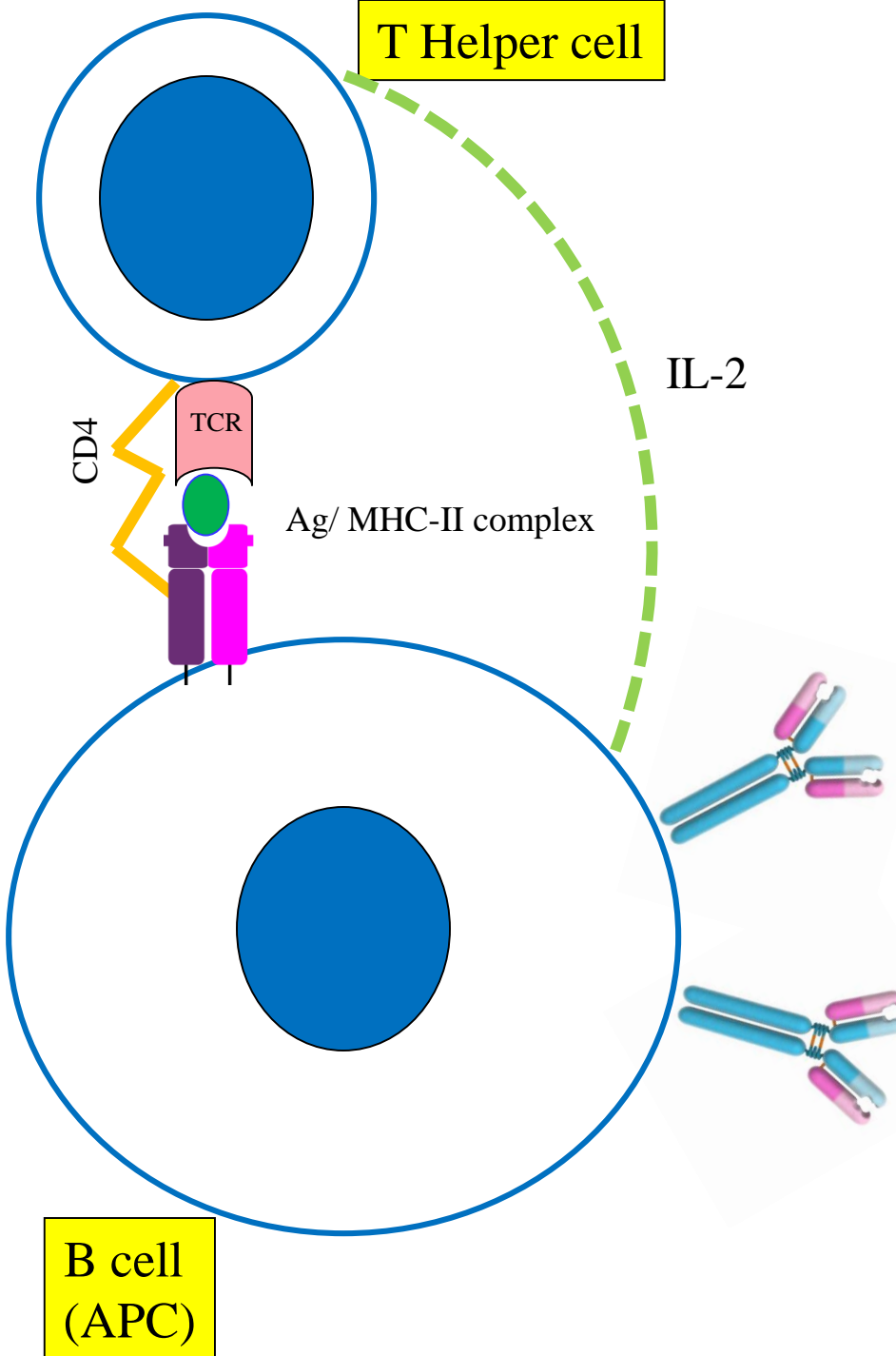
IgD

Ag

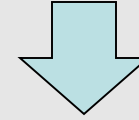
Free Ag

Antibodies can't recognise binding antigen.

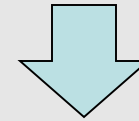
B cell
(APC)



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



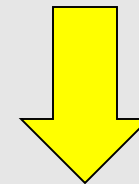
Stimulates proliferation and differentiation (activation) of B cells



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)

Antigen presenting cells have MCH-1 (nucleated cells).

What is the difference between MCH-1 and MCH-2:

1- MCH-1 presents protein synthesised within the cell.

2-MCH-2 completely foreign material not synthesised by the cell, just phagocytosis and degradation and then presentation into the cell surface

Most the circulating lymphocytes are small inactive T lymphocytes.

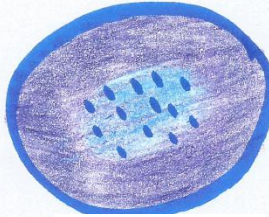
Neutrophile Lymphocyte Monocyte Eiosophile Basophile
Never Let Monkeys Eat Bananas

Most common to least

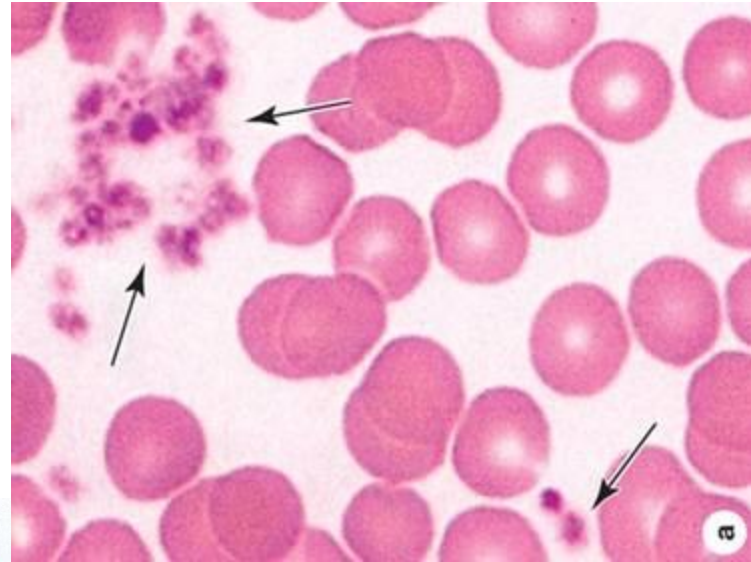


Thrombocytes (Platelets)

- 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



Biconvex in shape

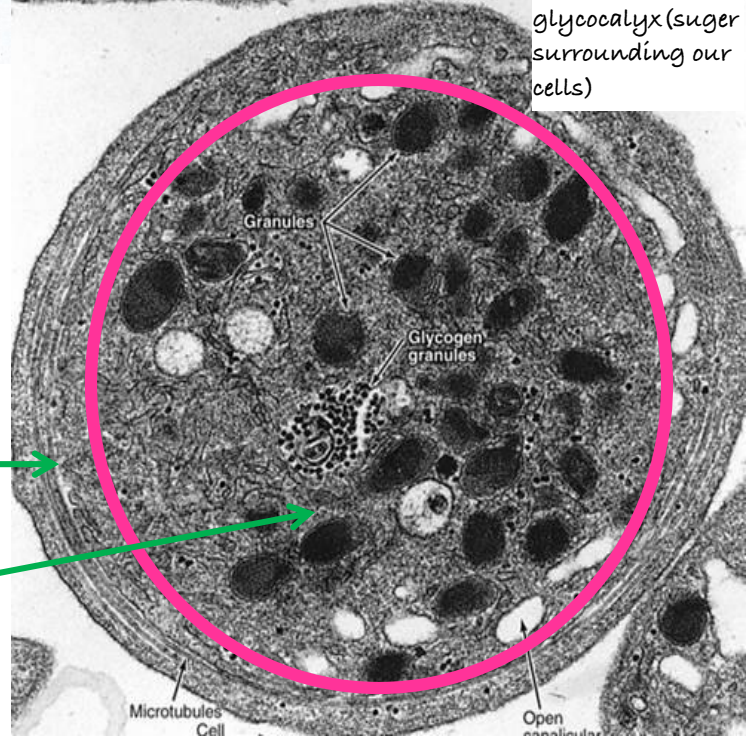


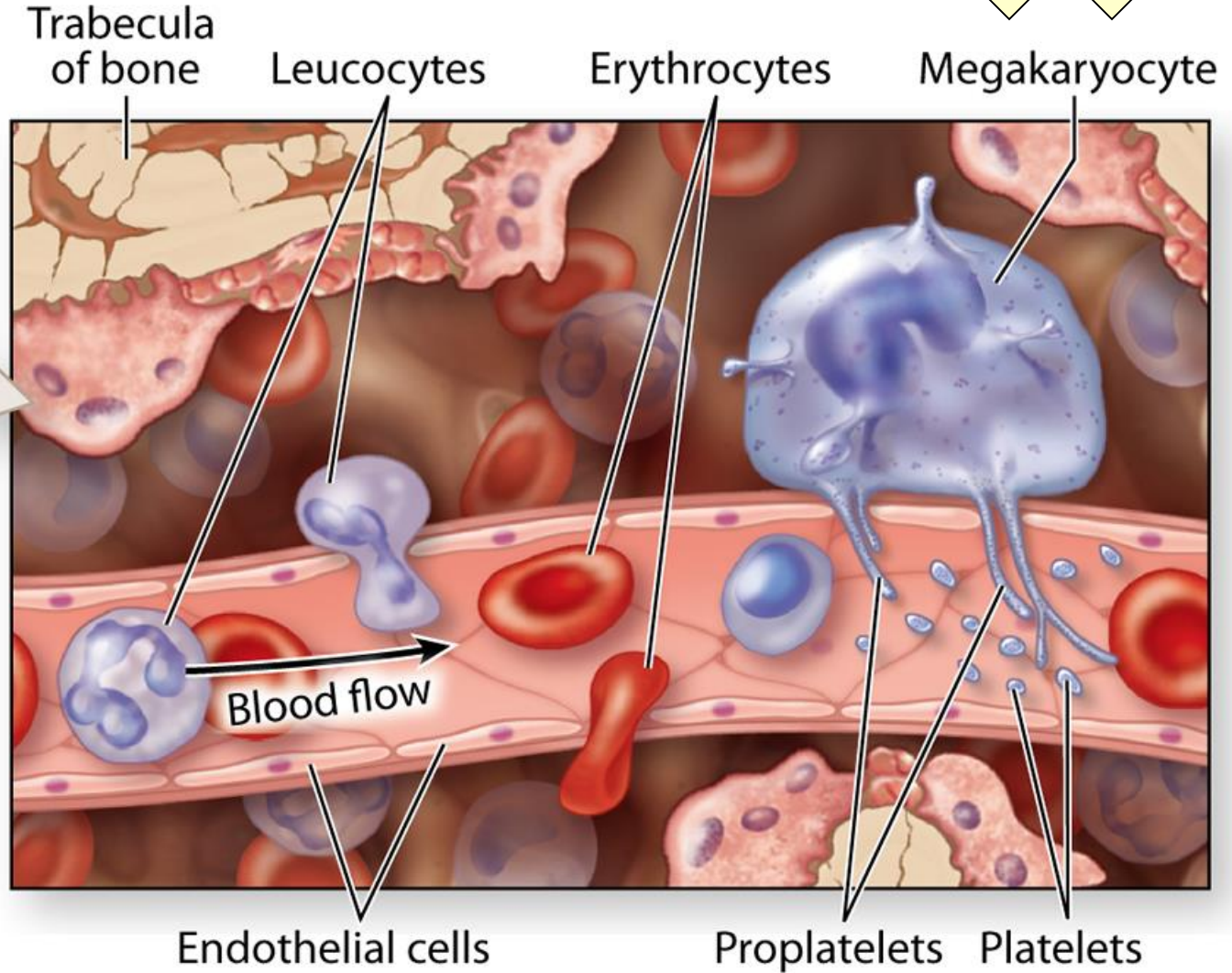
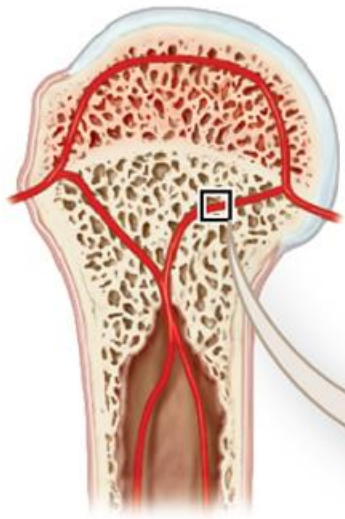
In stained blood smears, platelets often appear in clumps Because it has a thick

glycocalyx (suger surrounding our cells)

- Platelete has 2 zones
- Outer pale basophilic (clear) perpheriral zone: **hyalomere**
- Central dark granular zone: **granulomere**

Hyalo: glassy
Mere: segment





Megakaryocyte has a large nucleus.
Inside the nucleus we have multiple rounds of DNA duplication without nuclear division

Hyalomere: contains cytoskeleton and membranous channels

Cytoskeletal elements

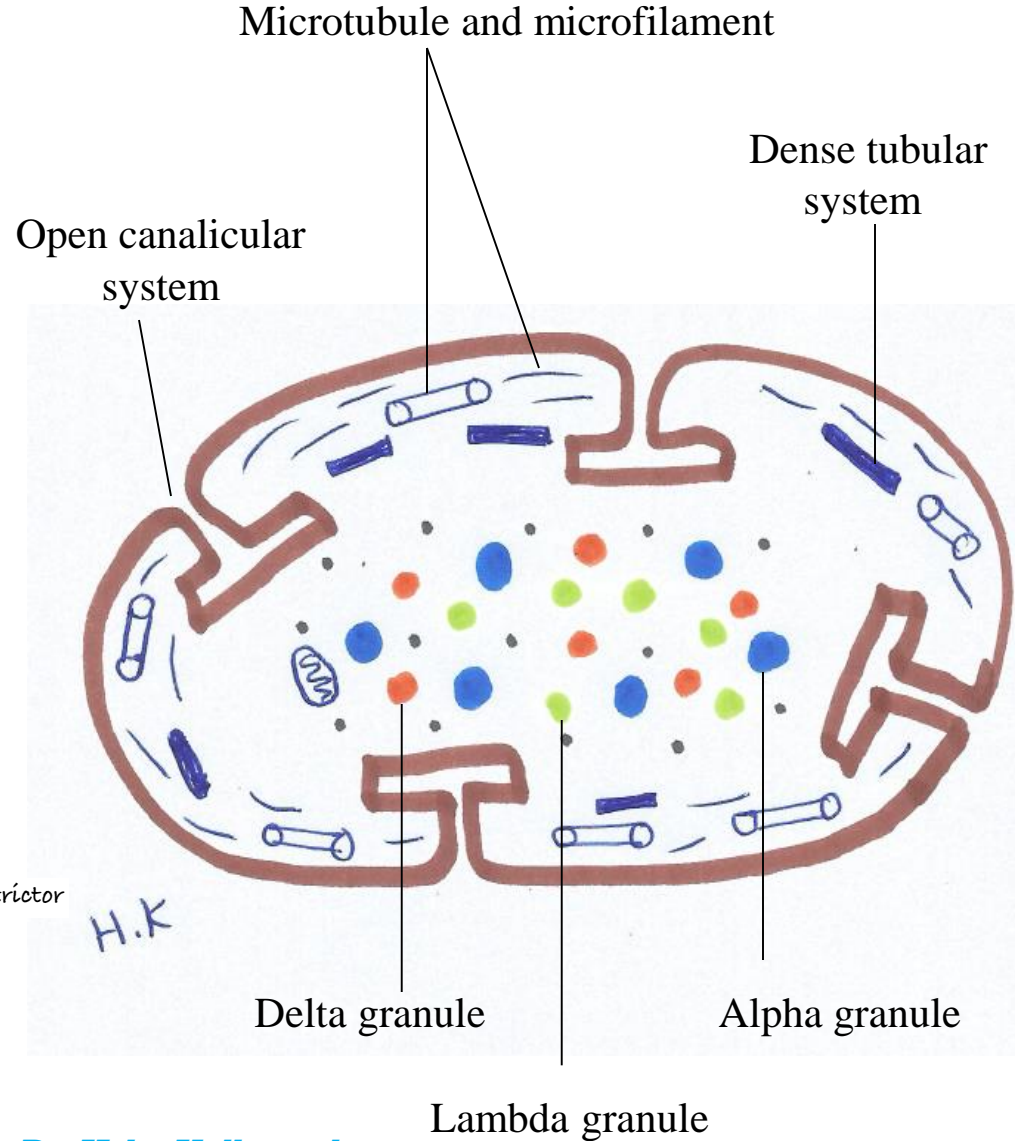
- Microtubule
- Actin filaments contractile proteins

Maintain shape and help contractions of platelets and squeezing

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 vasoconstrictor (absorbed from plasma), ATP, ADP

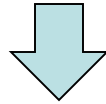
Lambda granules: lysosomes (aid in clot resorbtion)

Open canalicular system: Invagination of the plasma membrane deep inside reaching granulomere.

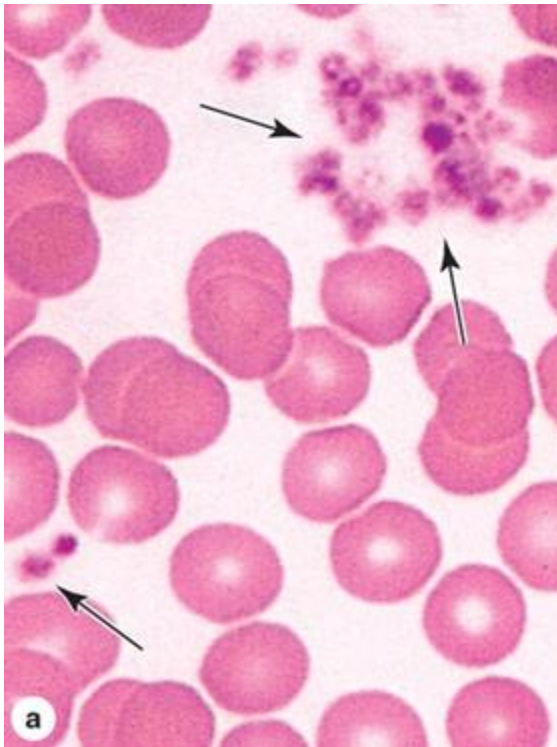
Function: providing a pathway for quick endocytosis or exocytosis of material from granulomere to plasma and vice versa.

Dense tubular system: acts as a storage site for calcium. (We need calcium for exocytosis and in blood clot formation)

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



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>

Some basic concepts in general histology

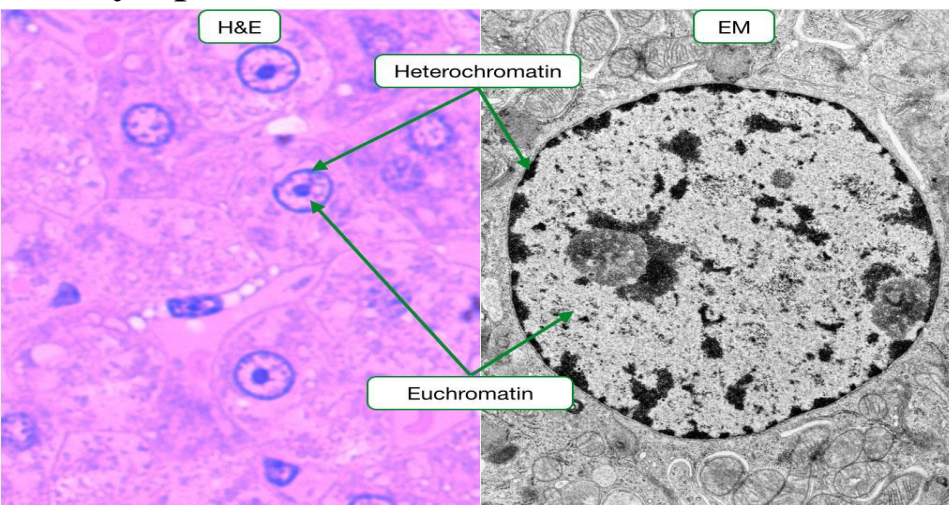
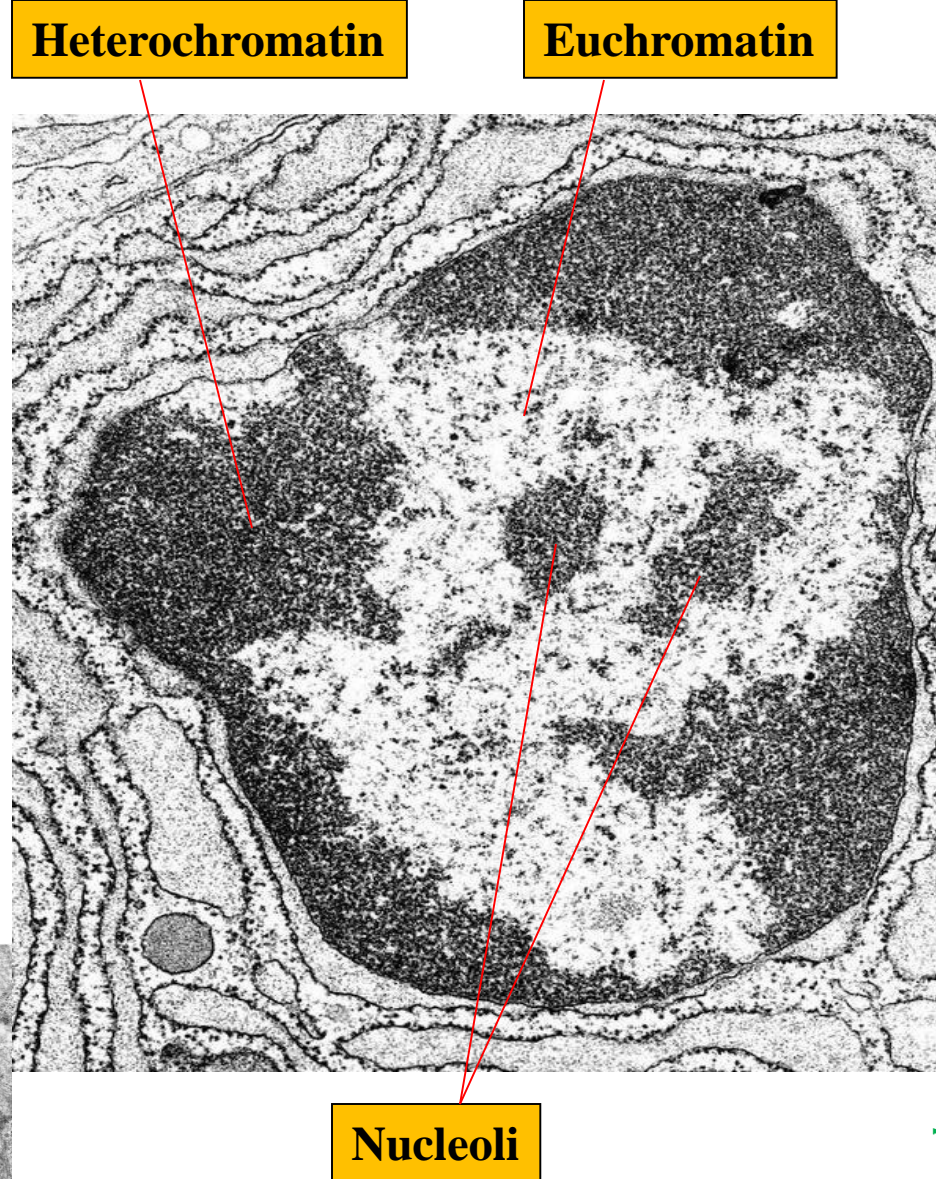
Chromatin

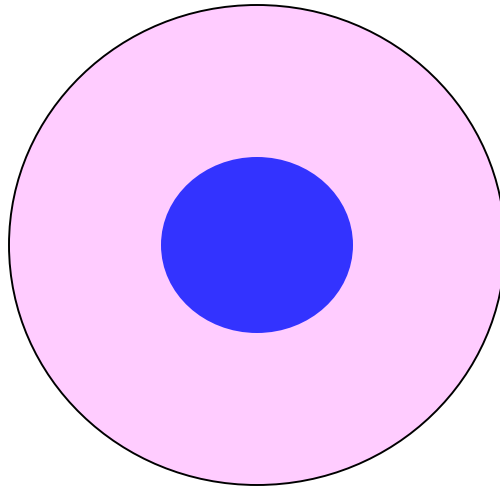
Formed of DNA.

- 2 Forms:
 - Euchromatin: extended active chromatin (pale).
 - Heterochromatin: condensed inactive chromatin (dark)

Nucleolus

- It is a spherical dark mass not surrounded by a membrane.
- Usually one.
- Function: formation and assembly of ribosomal RNA (rRNA), which is responsible for protein synthesis in the cytoplasm



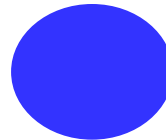
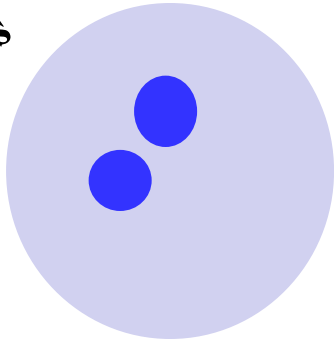


H & E

Active nucleus

Inactive nucleus

Nucleolus is a spherical dark basophilic mass

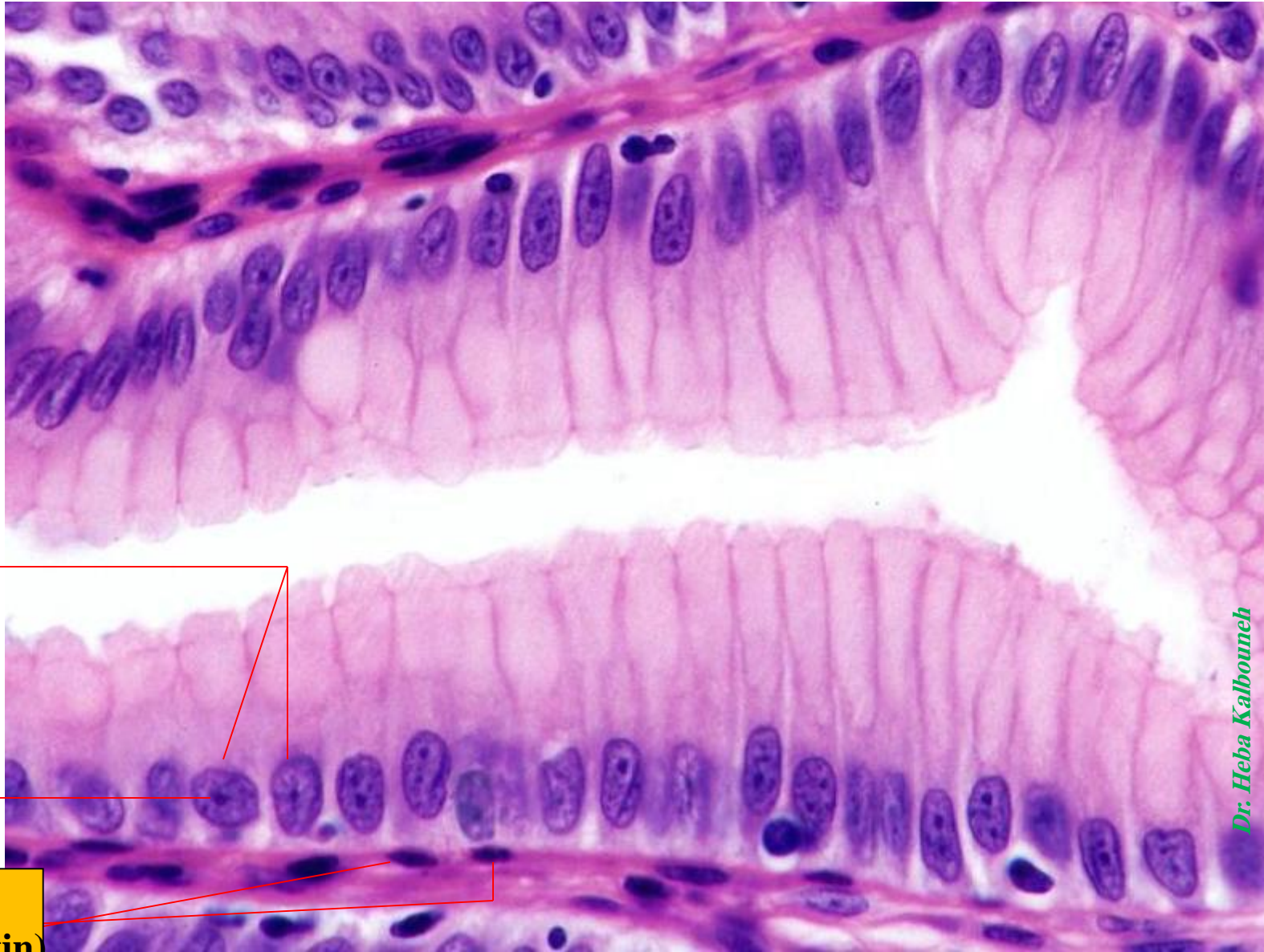


Note:

The nucleus stains **blue (basophilic)** using H&E

Lightly basophilic: active

Deeply basophilic and small: inactive



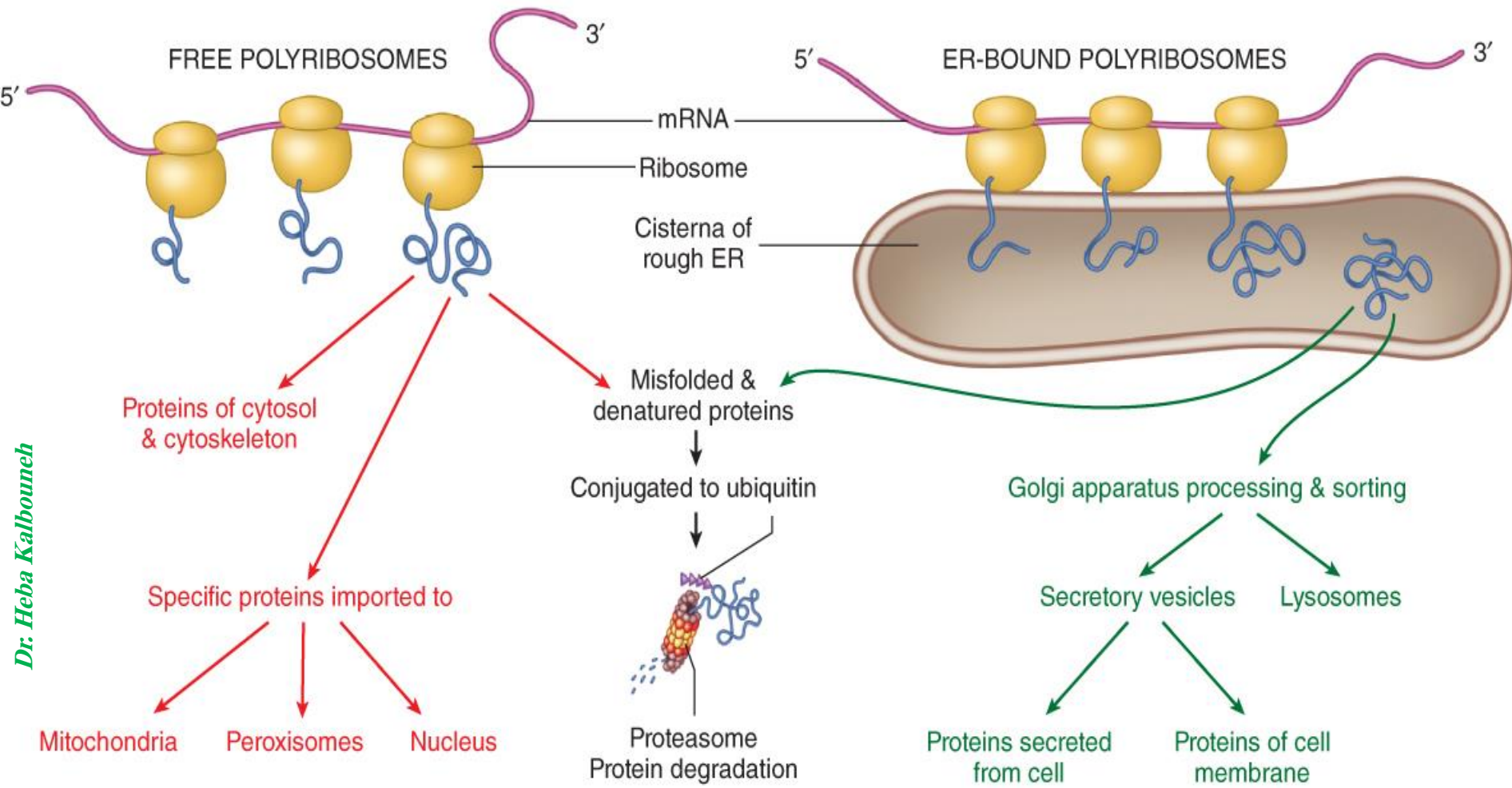
**Active nucleus
(Euchromatin)**

Nucleolus

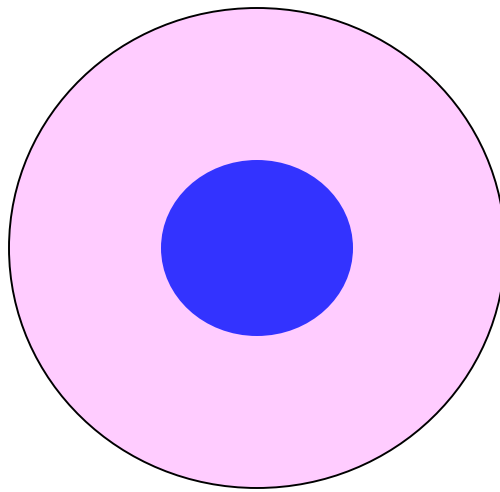
**Inactive nuclei
(Heterochromatin)**

Note:

The cytoplasm stains **pink/red (acidophilic)** using H&E
The organelle (when prominent) that produces **basophilia** in the cytoplasm is the **ribosome**

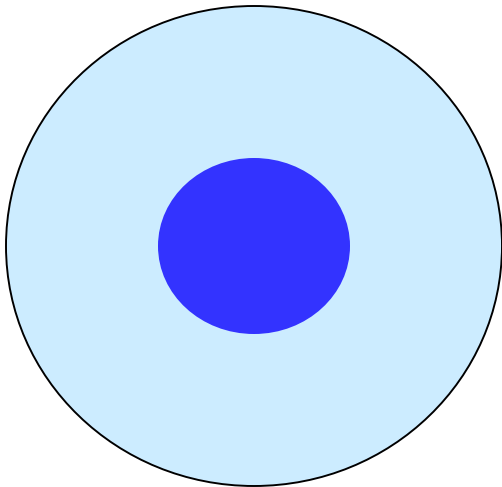


Dr. Heba Kalbouneh

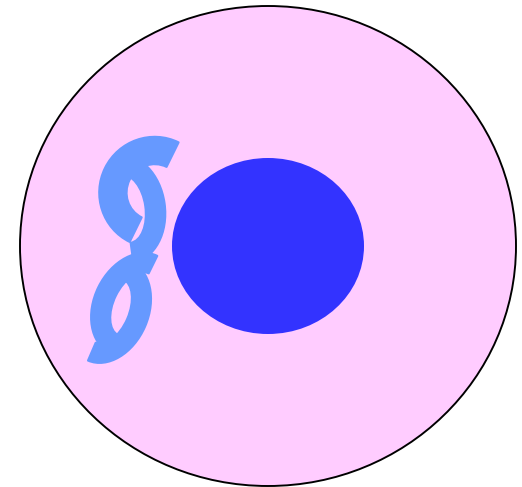


H & E

Free Ribosomes

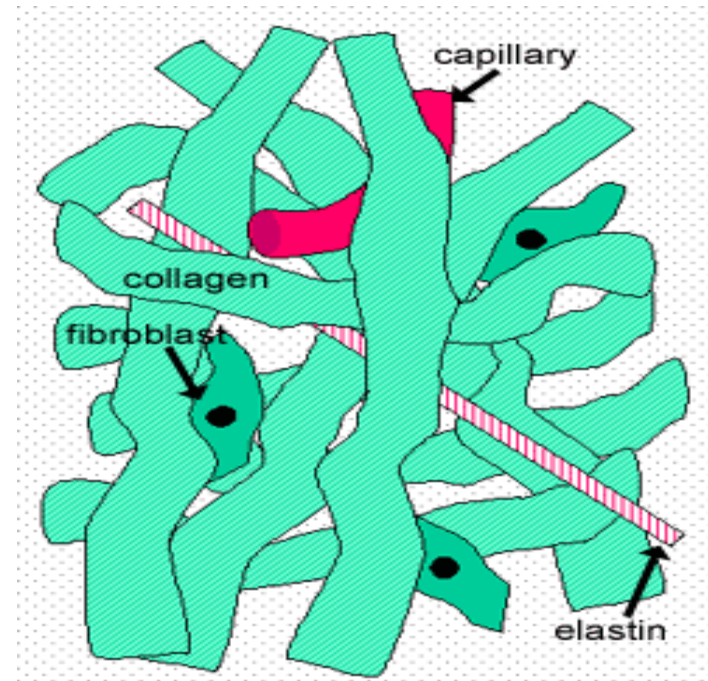


**Attached Ribosomes
(RER)**



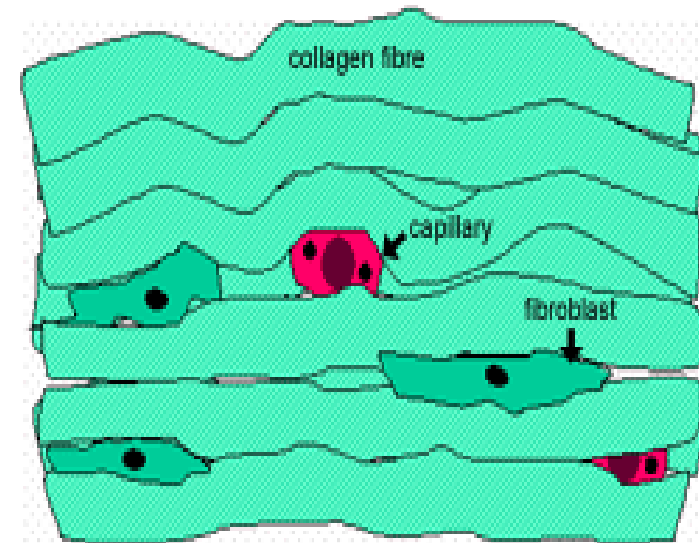
Dense irregular connective tissue

- ✓ Bundles of collagen fibers are randomly interwoven with no definite orientation
- ✓ Provides resistance to stress from all directions
- ✓ Dermis of skin (deeper layer), **organ capsules**, submucosa



Dense regular connective tissue

- ✓ Parallel Bundles of collagen fibers with few fibrocytes aligned with collagen and separated by very little ground substance



Stroma means bed

Parenchyma / Stroma:

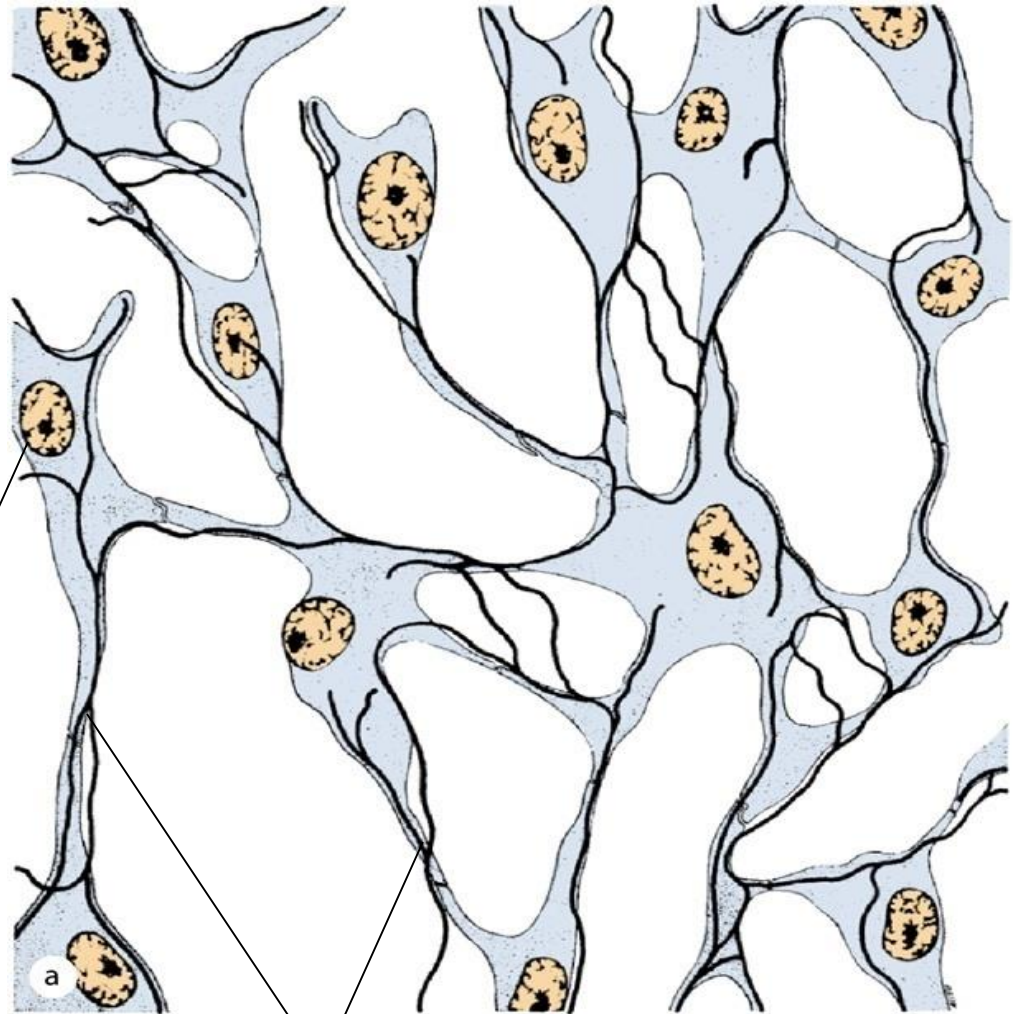
The parenchyma of an organ consists of that tissue which conducts the specific function of the organ and which usually comprises the bulk of the organ. Stroma is everything else -- connective tissue, blood vessels, nerves, ducts. It is made up of all the parts without specific functions of the organ

For Example:

The *parenchyma* of the heart is muscle tissue (cardiac muscle cells). The nerves, intrinsic blood vessels, and connective tissue of the heart comprise the *stroma*.

Reticular connective tissue

- Consists of reticular cells (modified fibroblasts) and the network of reticular fibers formed by them
- Forms the structural framework (stroma) in which the cells of the organ are suspended
- In the liver, bone marrow, lymph nodes and the spleen (**Reticulo-Endothelial organs**)



Reticular cell

Reticular fibers are thin and branching forming a network

Types of capillaries

Continuous capillaries

- Are most common
- Endothelium forms solid lining
- Adjacent cells are held together with tight junctions
- Found in most organs

Fenestrated capillaries

- Endothelium contains pores (fenestrations)
- Found wherever active capillary absorption or filtrate formation occurs
- Found in endocrine glands, small intestine, and kidney

Sinusoidal capillaries

- Exhibit wide diameters with wide gaps between endothelial cells
- Basement membrane incomplete or absent
- Allow large molecules (proteins and blood cells) to pass between the blood and surrounding tissues
- Found in liver, spleen, and bone marrow

