Histology - HLS

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Blood Cells

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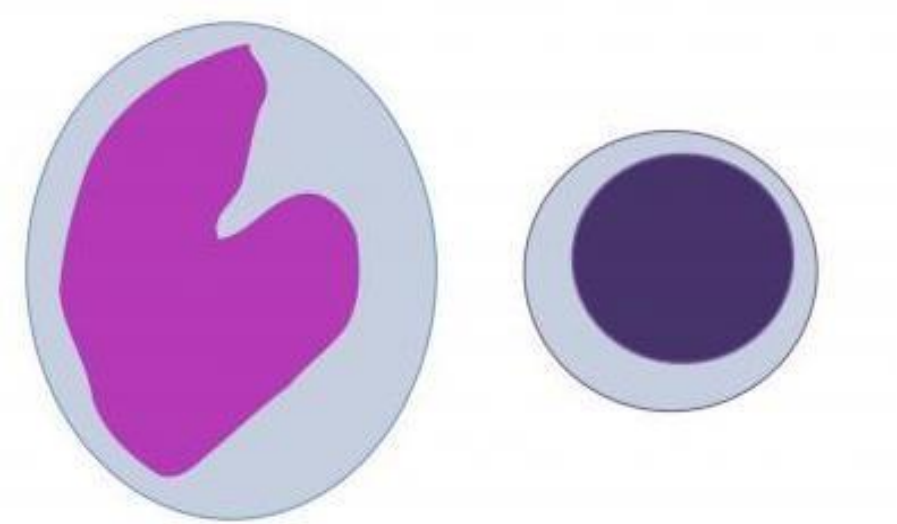
Agranulocytes

They're WBCs that don't have specific granules inside their cytoplasms

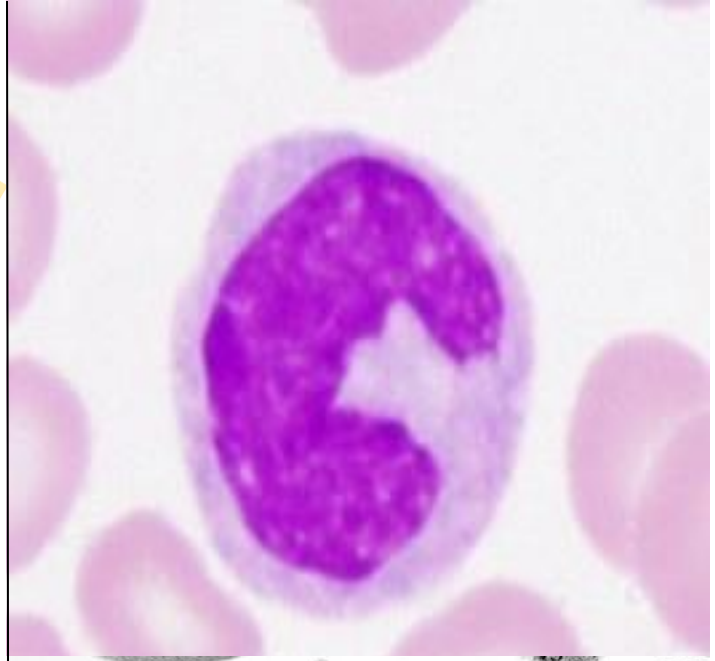
- Single non-lobulated nucleus
- Granules in cytoplasm are too small to see (nonspecific granules, azurophilic granules, primary granules, lysosomes)

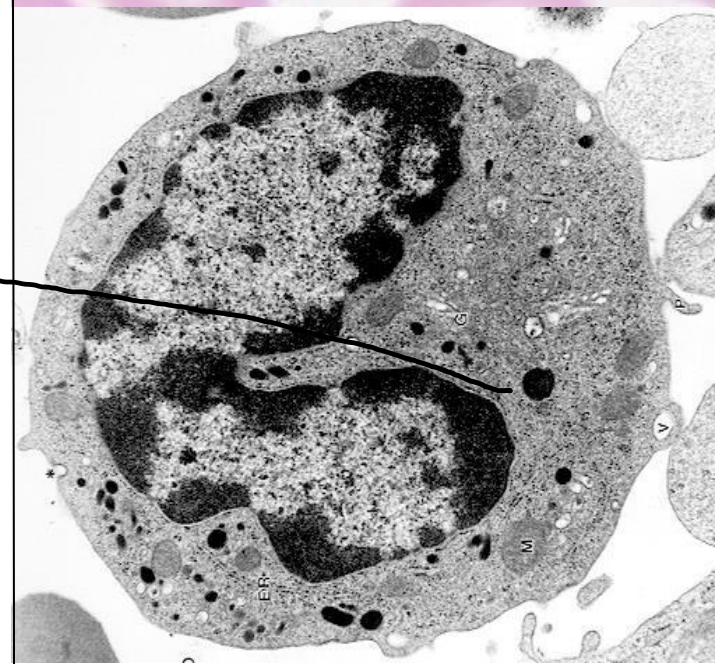
- 2 types based on structure (not cell lineage):
  - **Lymphocytes**
  - **Monocytes**

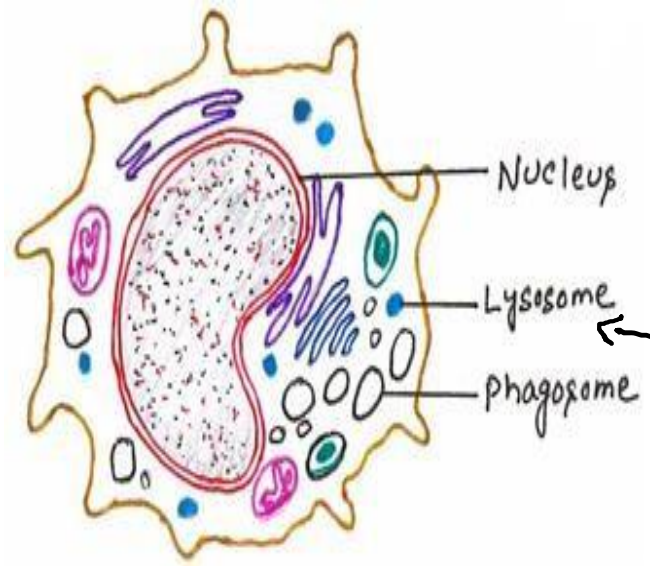


Monocytes

- 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)







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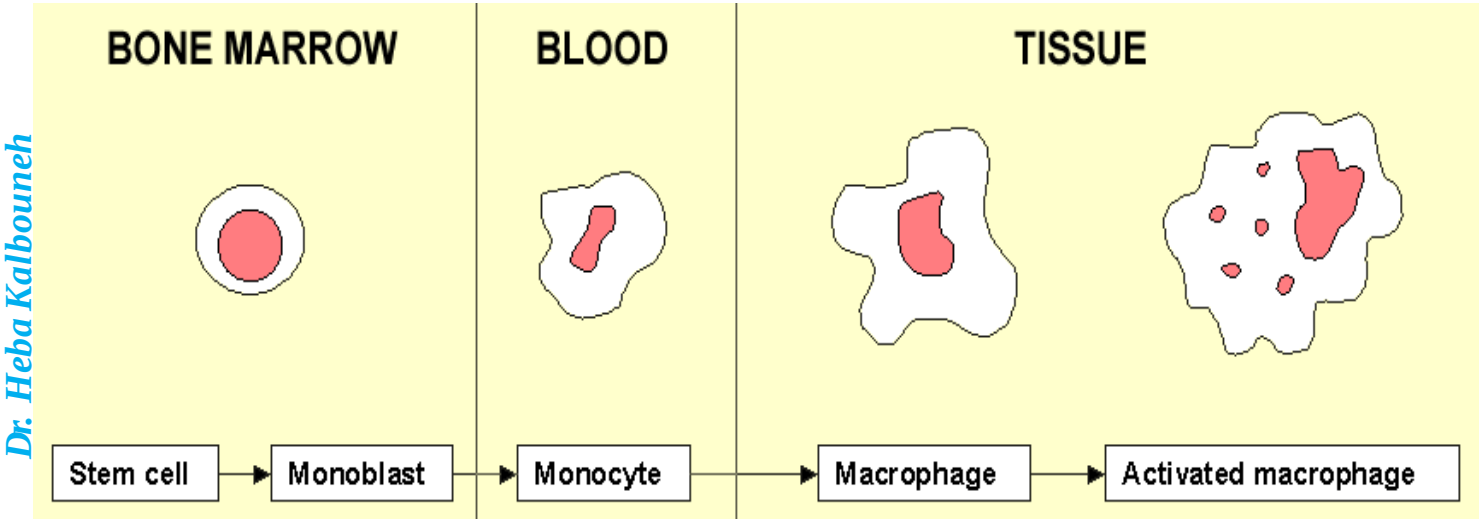
Are precursor cells of macrophages, osteoclasts, microglia, and other cells of the **mononuclear phagocyte system** in connective tissue

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

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



1. Monocytes **originate** from stem cells in **bone marrow**.
2. Once monocytes are released to the blood, they circulate for a few days(1-3) then enter connective tissue, where they become macrophages.
3. They are activated when exposed to certain antigens to perform their function (phagocytosis)

MONONUCLEAR PHAGOCYTIC SYSTEM

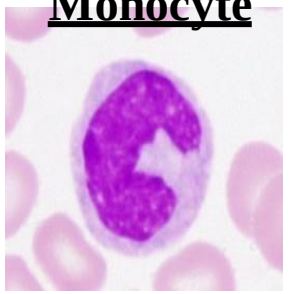
Kupffer Cells
Liver

Macrophages
Bone marrow
Connective tissue

Osteoclasts
Bone resorption

Dust cells
Lung

Monocyte



They are able to phagocytose foreign materials, degrade them with lysosomal enzymes and eliminate them.
(All are characterized by antigen presenting ability too)

(all characterized by phagocytic activity)

Microglia
CNS

Dendritic cells
Lymph node
Spleen

Langerhans cell
Epidermis

Lymphocytes

Ribosomes are the only organelles that produce **basophilia** inside the cytoplasm (using the ordinary stains)

- Smallest leukocytes
- Round nucleus occupies most of cell volume
- Cytoplasm is light clear blue (basophilic because it's rich in free 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

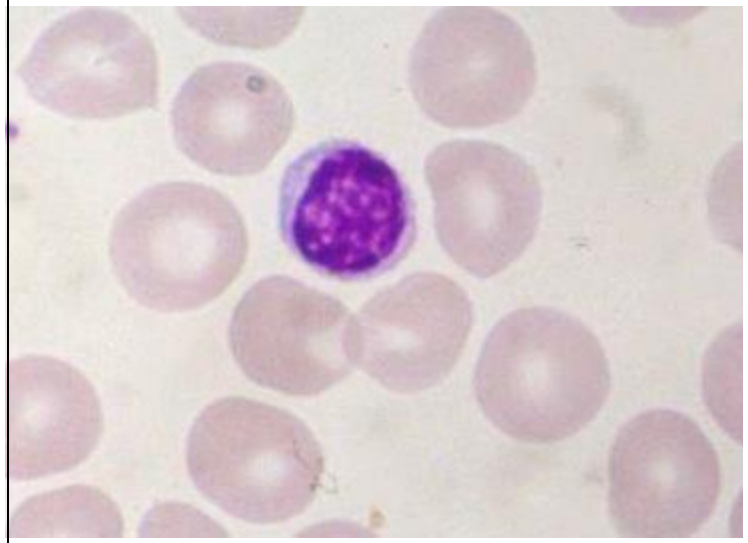
Humoral immunity

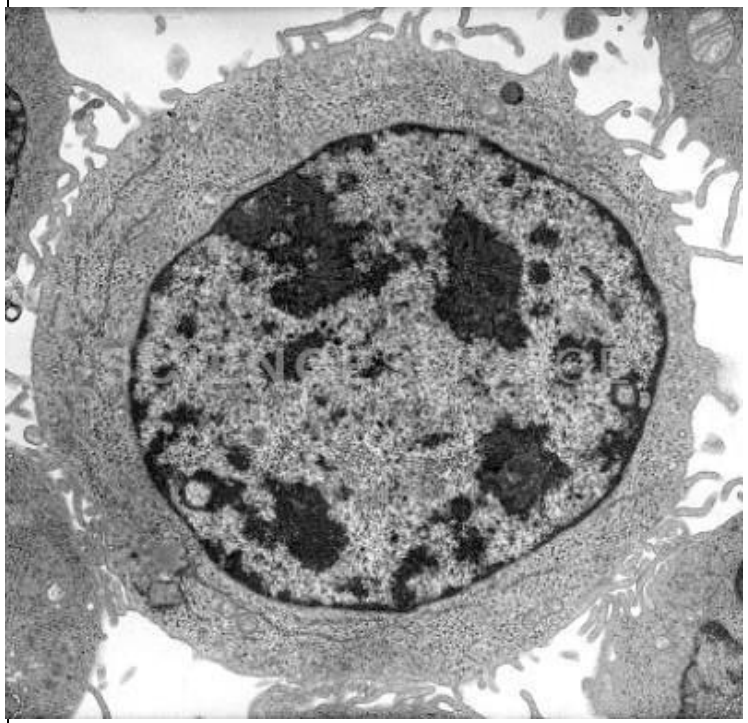
- **B cells**
  - Differentiate into plasma cells
  - Secrete antibodies that bind to specific antigens and mark them for destruction by phagocytic cells

Long term immunity

Humoral means body fluids

Almost same size as RBC





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Lymphopoiesis: the process by which lymphocytes are formed

Precursor cells in bone marrow

Thymus

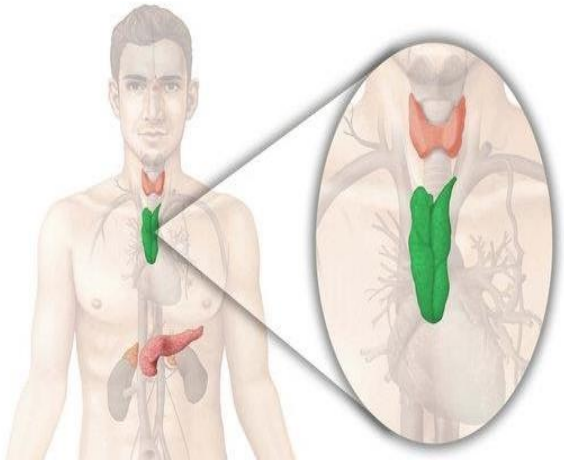
BM

Directly into blood

T Lymphocytes

B Lymphocytes

Natural killer lymphocytes



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Morphologically lymphocytes can be classified into:

Euchromatic nucleus

Heterochromatic nucleus

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

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

We can't differentiate between these lymphocytes under the microscope using ordinary stains but we can by cell surface receptors

T and B lymphocytes can be large, active or small, inactive lymphocytes

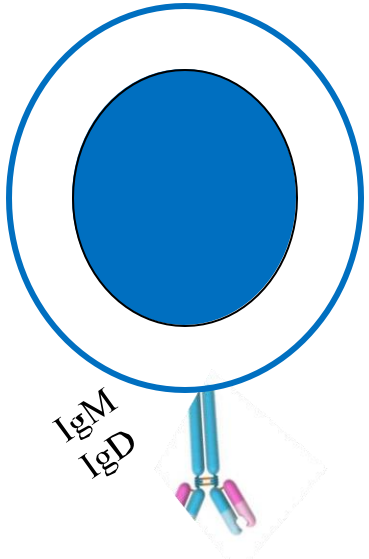
Large
(9-18 µm)
Active lymphocyte

Small
(6-9 µm)
Inactive lymphocyte

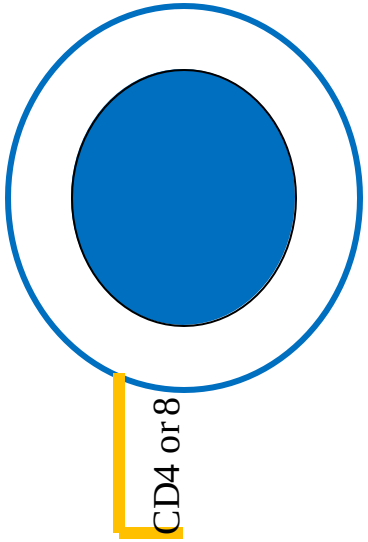
Natural killer cells are always large, active cells

Different types express specific cell surface proteins

On the surface of the B lymphocyte there's BCR (B cell receptor) which is an immunoglobulin

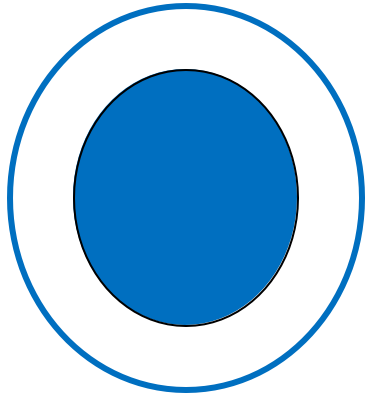


B lymphocyte



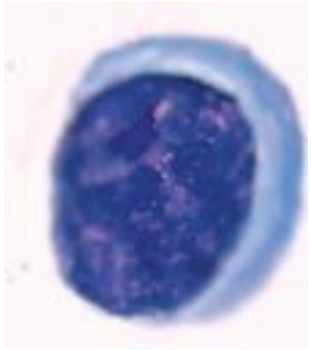
On the surface of T lymphocyte there is CD4 or CD8 (cluster of differentiation)

T lymphocyte



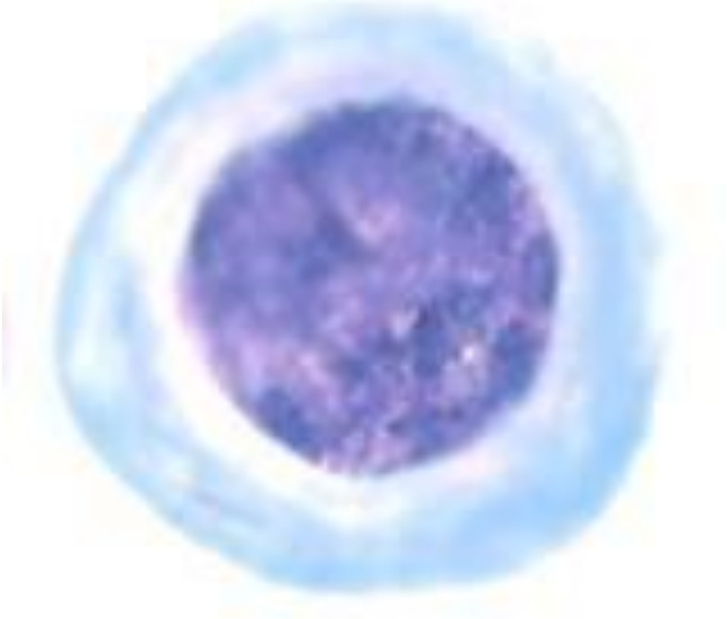
NK cells have specific cell surface receptors, but not T or B cell receptors (not shown in the figure), so they were called non B non T lymphocytes

Natural killer lymphocyte



Small
(6-9 µm)
Inactive lymphocyte

Darkly stained cell



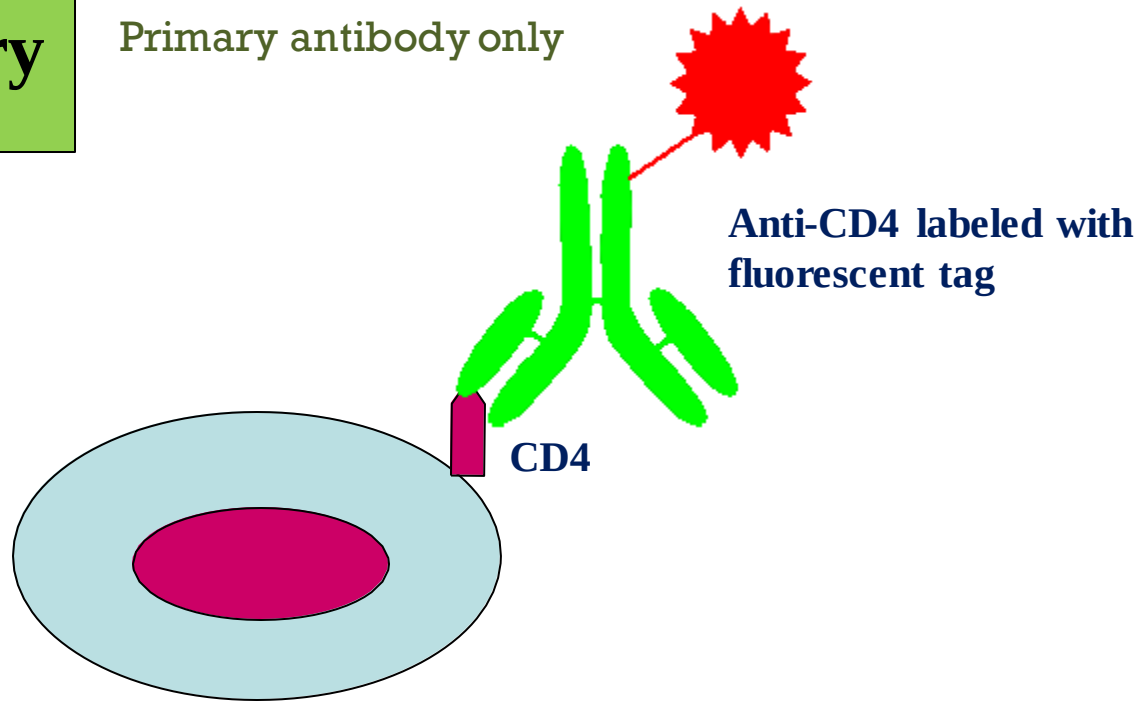
Large
(9-18 µm)
Active lymphocyte

Lightly stained cell

Immunohistochemistry

Direct method

- This technique depends on having specific binding between an antigen and its antibody, using a fluorescence microscope.
- We get the antibody from an animal's blood sample after injecting it with the receptor/antigen of the cell of interest
- Then we label the antibody with a fluorescent tag



Immunohistochemistry

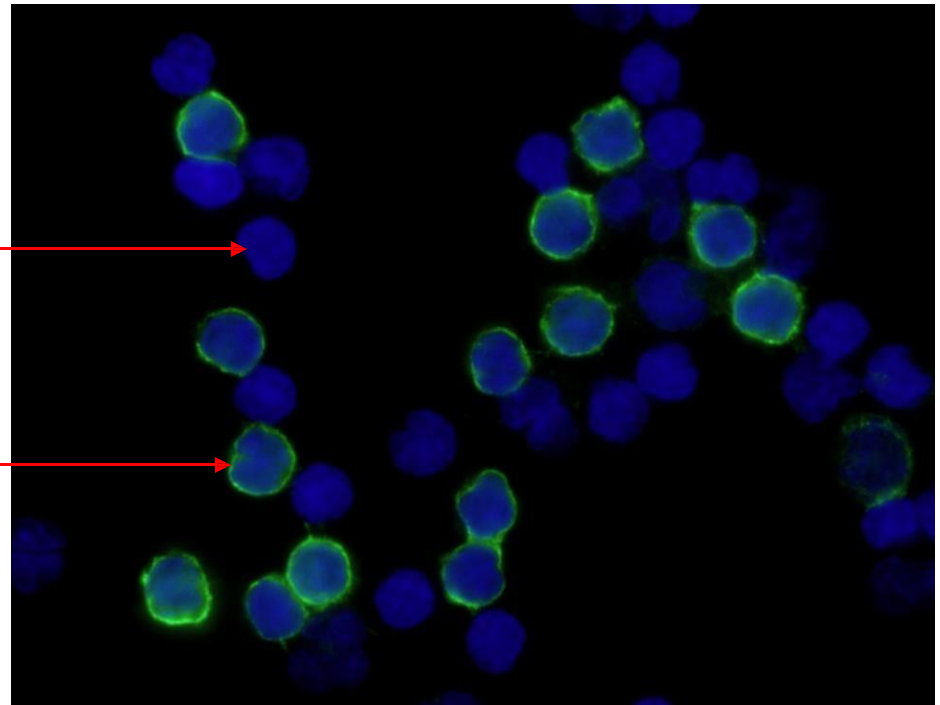
Using CD 4 Antibody

May be CD8+ cells (cytotoxic T lymphocytes) or B lymphocytes

???????

CD 4 +
T Helper lymphocyte

Green tag



We use a special stain to localize the nuclei of the cells (blue in color)

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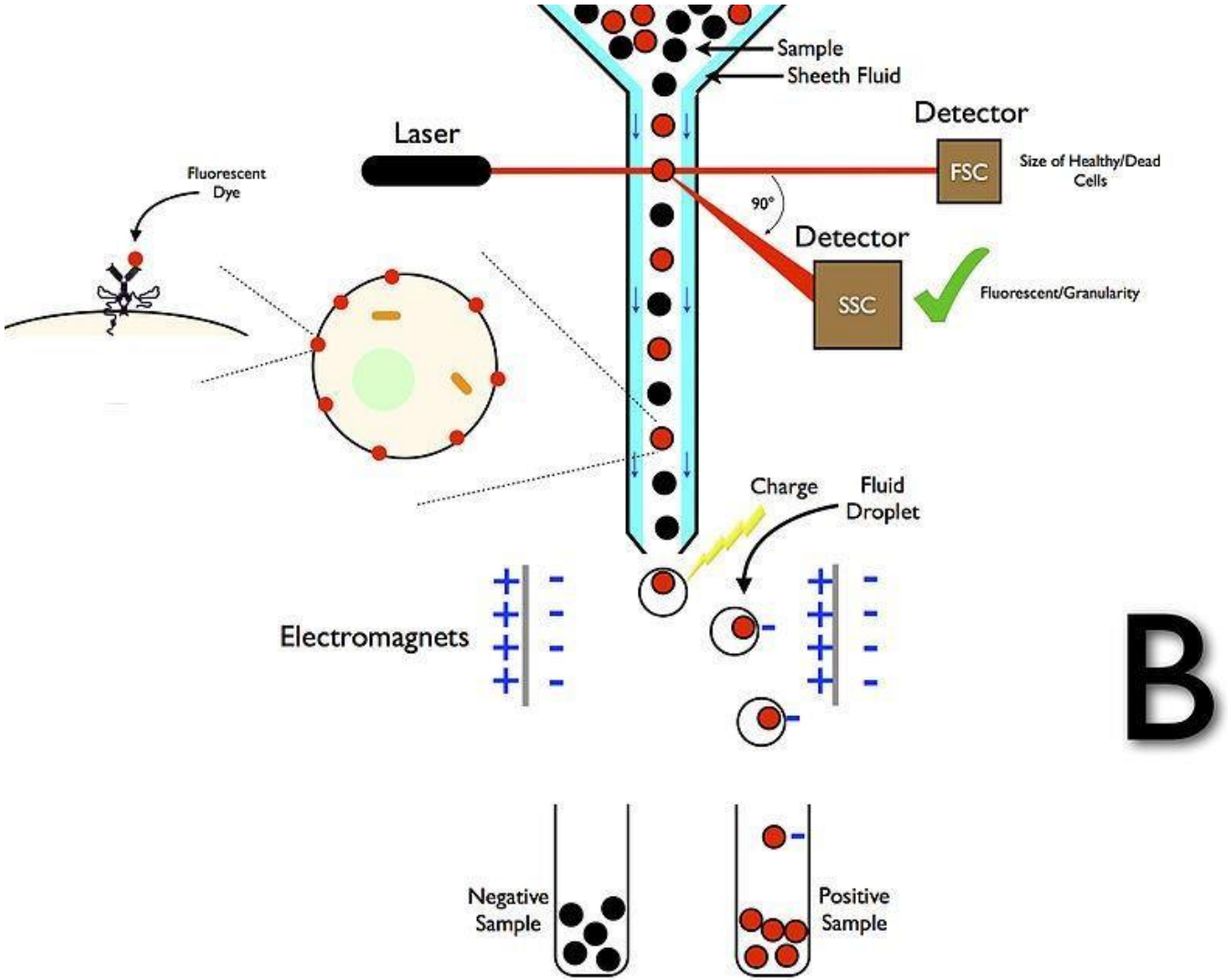
Flow cytometry (FACS)



- We have a fluid sample (could be peripheral blood sample or BM sample)
- We label the cell of interest with specific antibodies and these antibodies are labeled with fluorescent tags
- As these cells are passing through the tube, a laser light measures the intensity of the fluorescent color, separating the cells into positive cells and negative cells (positive for the cells of interest, and negative for the others)
- FACs can be used to diagnose different types of blood cancers, like the different types of leukemias (we can identify the origin of this leukemia)

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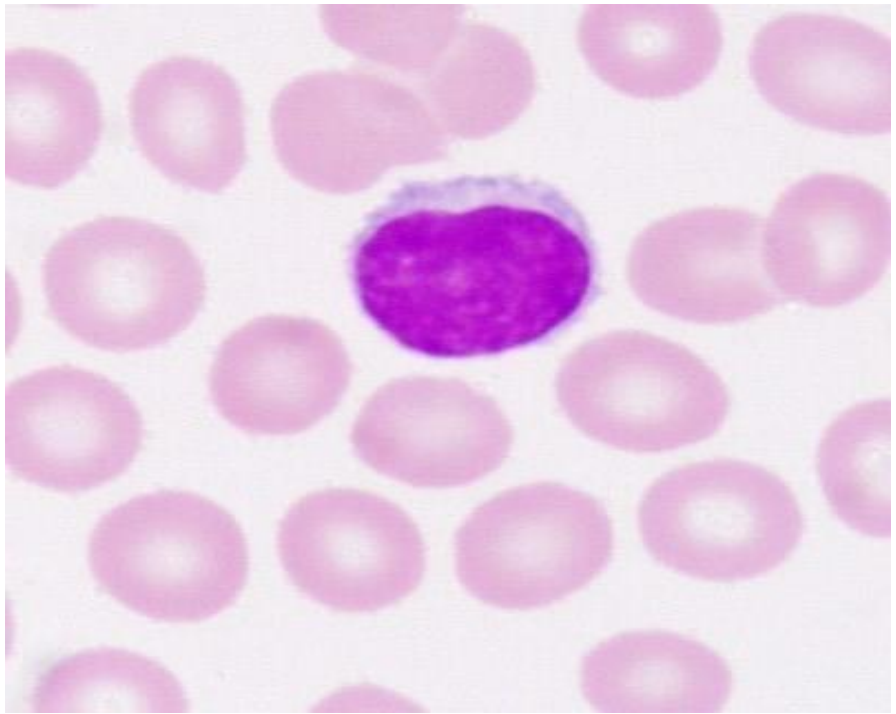
Neutrophils and monocytes are highly phagocytic and engulf microorganisms and cell debris in a **NON-SPECIFIC** manner **(Innate immunity)**

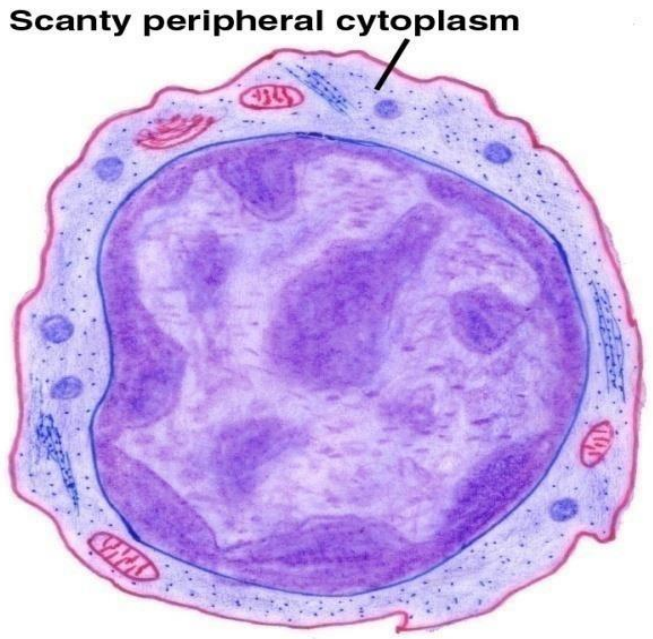
While

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

B Lymphocyte

Memory cell **Plasma cell**

Produces antibodies

into body fluids (blood, lymph and interstitial fluid), that's why the immunity provided by the B cells is called **humoral immunity**

T Lymphocyte

Cytotoxic

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

The immunity provided by them is called **cell mediated immunity** in which they create pores in the cell walls of the cells they're attacking, inducing their apoptosis

Suppressor

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

When we have damage to suppressor cells we will have auto immune diseases

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

- Same mechanism of cytotoxic T cells but they don't need to be activated
- Part of innate immunity (they make sure that all nucleated cells present MHC1 molecules on their surfaces and kill cells that don't present MHC1 (viruses and some types of cancers suppress the transcription of MHC1 genes)

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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 dentritic 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.

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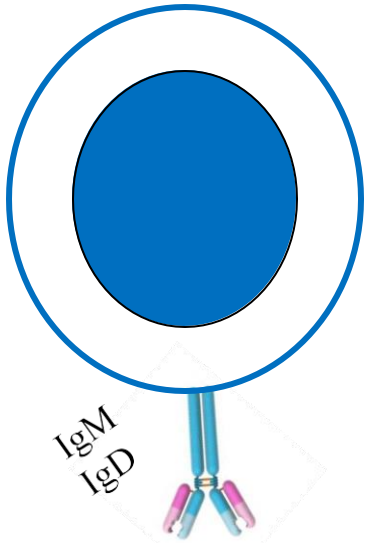
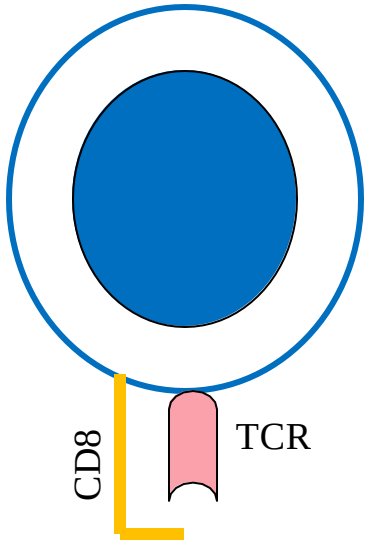
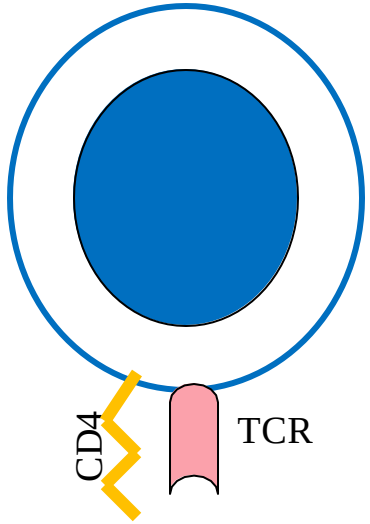
Different types express specific cell surface proteins



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*

- T lymphocytes differentiate inside the thymus and undergo thymic education (learn how to differentiate between self and non-self antigens and acquire specific T lymphocyte receptors that can only recognize certain type of antigens)
- We have thousands of T lymphocytes with different TCRs where each TCR can only recognize a single specific antigen

Major histocompatibility complex
MHC

MHC is basically like molecular fingerprints on our cell surfaces; unique for each individual

(involved in graft rejection}

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)

Also called human leukocyte antigens (HLAs)

Because they previously thought that these molecules are present only on the surface of leukocytes, but later on found that these molecules are present in all nucleated cells

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.

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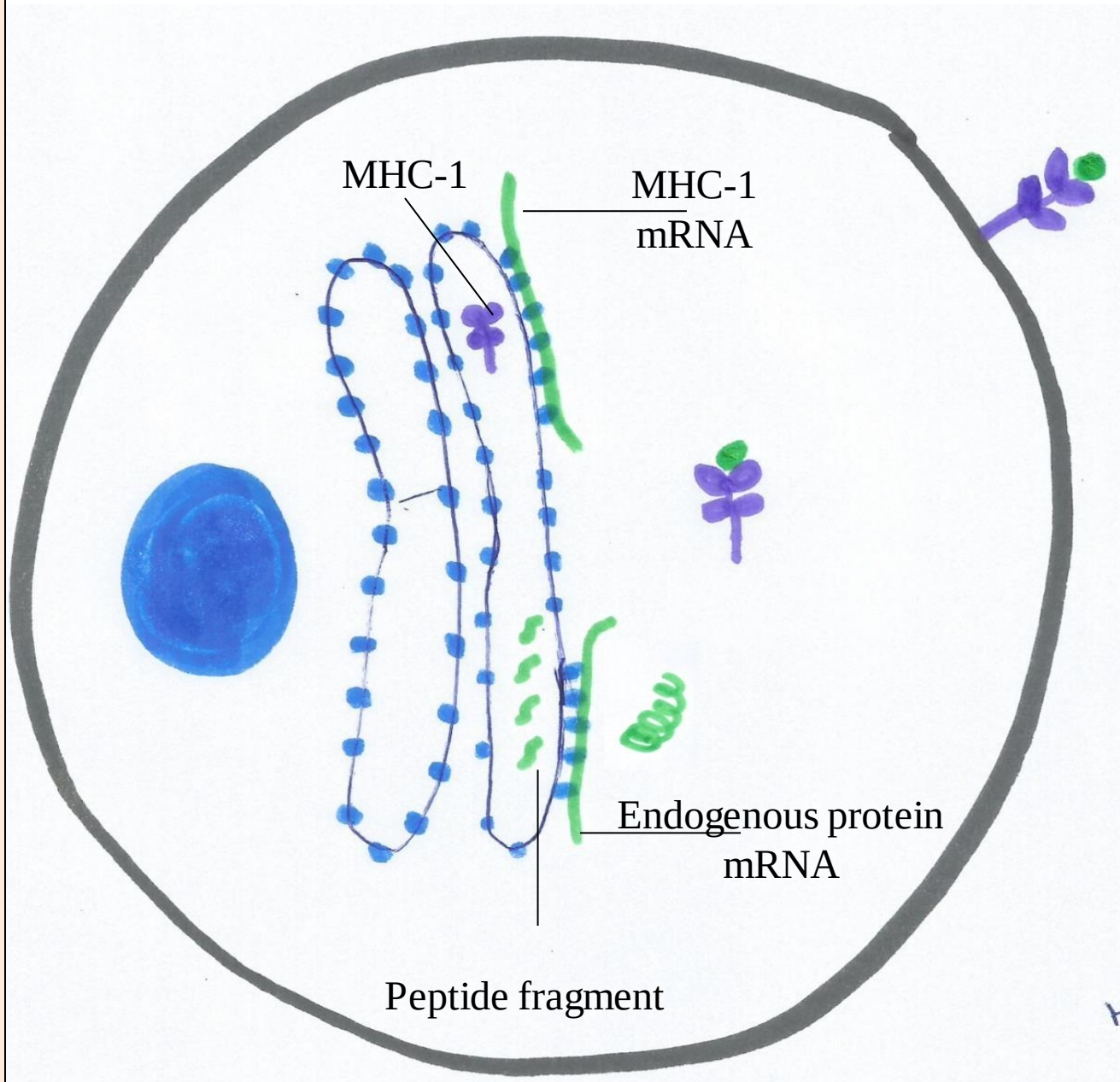
1. In the nucleus of the cell, there are genes of MHC 1 molecule which are transcribed into mRNA
2. mRNA is transported into the cytoplasm, then translated into protein using ribosomes of RER
3. MHC1 protein is transported into the lumen of RER, and at the same time our cells are continuously synthesizing many proteins
4. The mRNA of an endogenous protein is translated using ribosomes of RER.
5. A special enzyme in the RER called proteosome cuts a small sample from the endogenous protein, and the sample is transported into the lumen of the RER
6. MHC1 combines with this sample and the complex is transported into the cytoplasm and then to the cell surface

It's like our cells provide continuous reports to the immune cells about the proteins beings synthesized within the cells, so that cytotoxic T cells don't attack this cell when recognizing it as a self cell (holding self peptide or antigen)



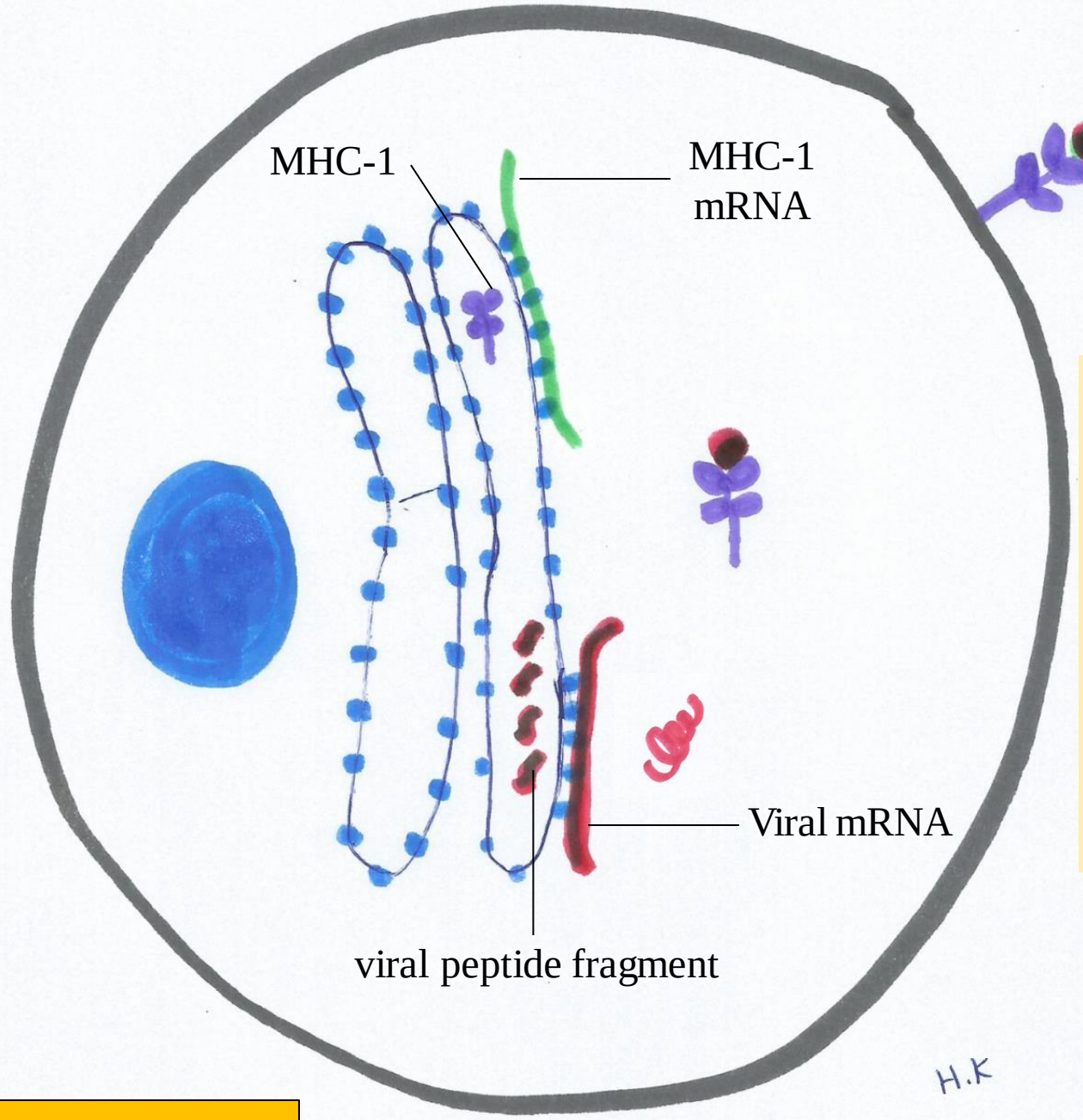
Self- peptide bound to MHC-1

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

Normal cell

We know that viruses use the host cell's organelles to multiply and synthesize their proteins.

(in red) A viral mRNA is translated into a viral protein using RER ribosomes, the proteosome cuts a small sample from the viral protein, then the sample is transported into the lumen of RER and combines with MHC1.
This complex moves into the cytoplasm then into the surface of the cell, marking this cell as a viral infected cell, so that it
gets recognized as a viral infected cell by cytotoxic T cell which then attacks it.



Viral- peptic
bound to
MHC-1

Viral peptic
is derived
from the vir
proteins tha
are
synthesized
by the vira
infected ce
(non-self
Antigen)

Viral infected cell

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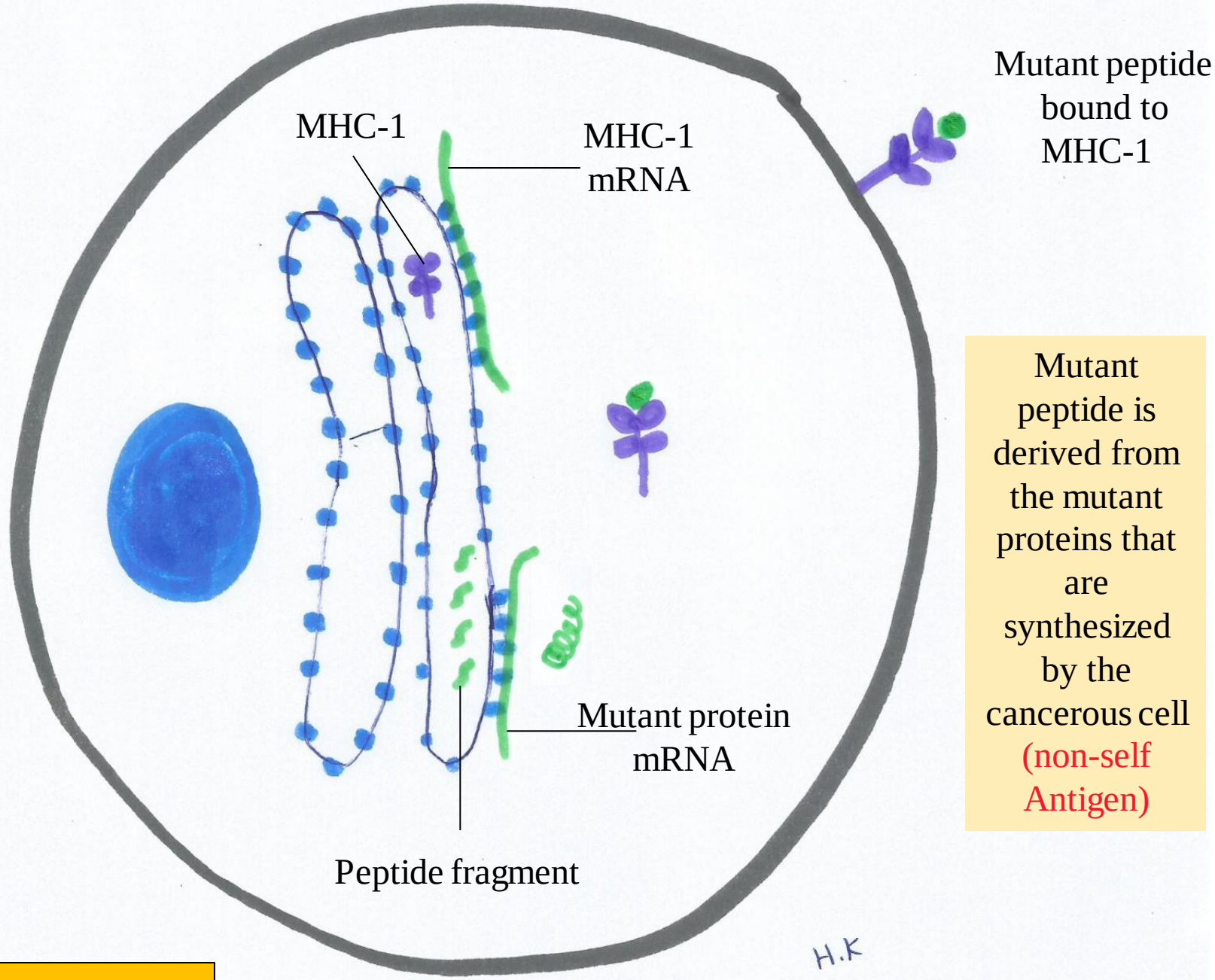
In cancer cells we have multiple mutations inside the DNA, producing abnormal proteins.
Mutant DNA >> mRNA >> mutant protein by RER ribosomes, then proteosome cuts a small peptide fragment from the mutant protein, and the fragment combines with MHC1. Again, as we said earliler, the complex is transported and presented on the cell
surface, marking it as a cancer cell, causing the cytotoxic T cells to attack it and initiate an immune response against this cancer cell



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

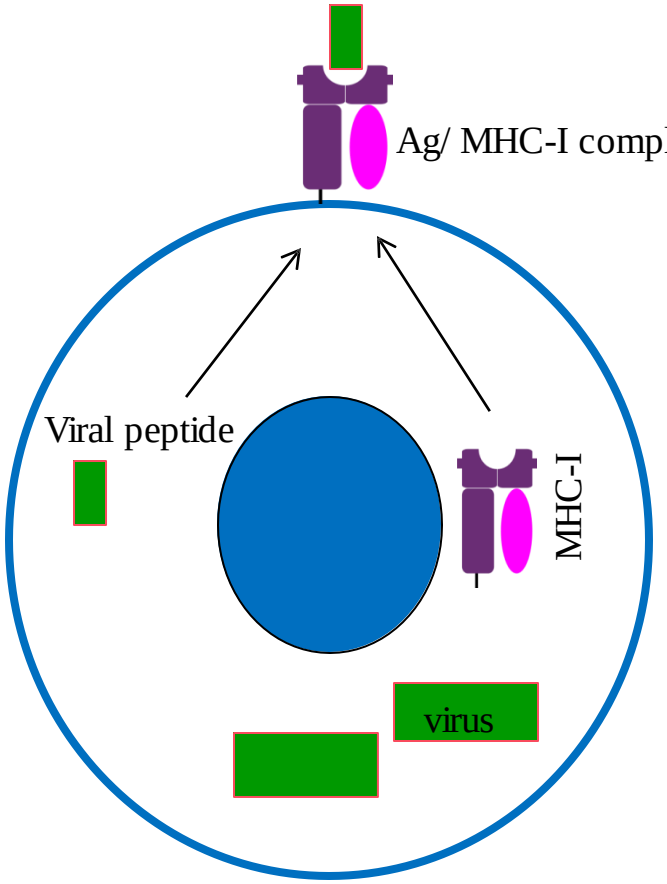
Cancerous cell

Cytotoxic CD8 T cells:

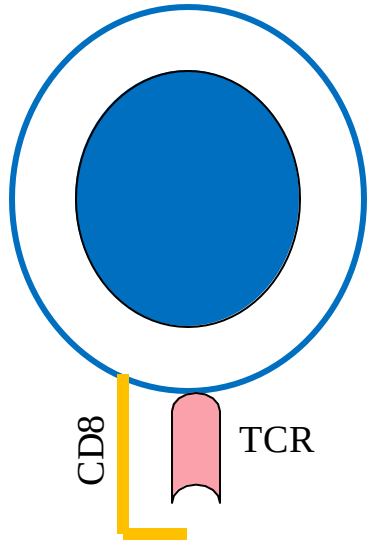
Antigen in virus infected, transplanted or neoplastic cells bind to MHC-I molecules

Cell- mediated immunity

Ag-MHCI complex



Viral infected cell

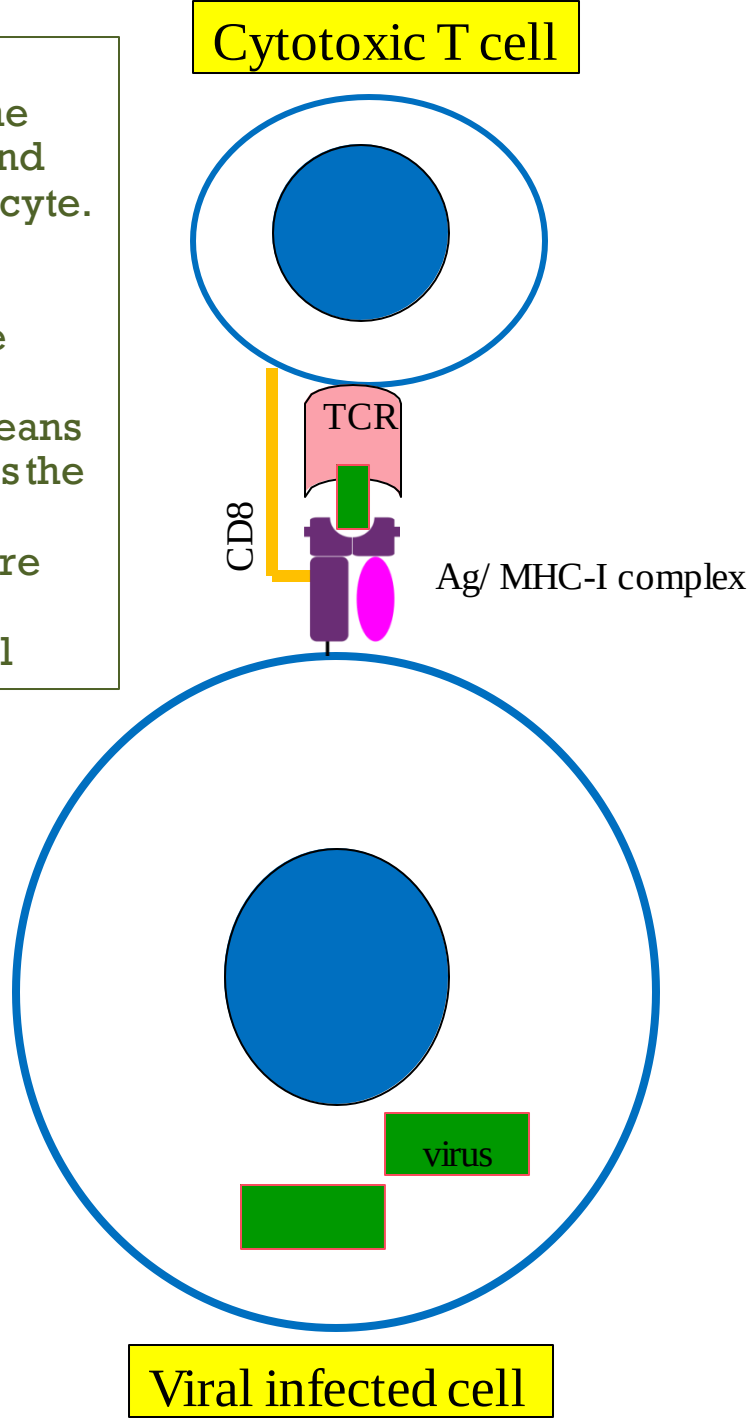


Cytotoxic T cell

This is a viral infected cell presenting on its surface a viral peptide coupled to MHC1; the cell will be recognized by cytotoxic T lymphocyte which has TCR specific to this viral peptide.

CD8+ cells only recognize antigens presented on MHC1 molecules

Here we have the binding between the viral infected cell and cytotoxic T lymphocyte. The Cytotoxic T lymphocyte recognizes both the antigen and MHC1 molecule, which means that TCR recognizes the antigen, while CD8 molecule makes sure that this antigen is presented on MHC1



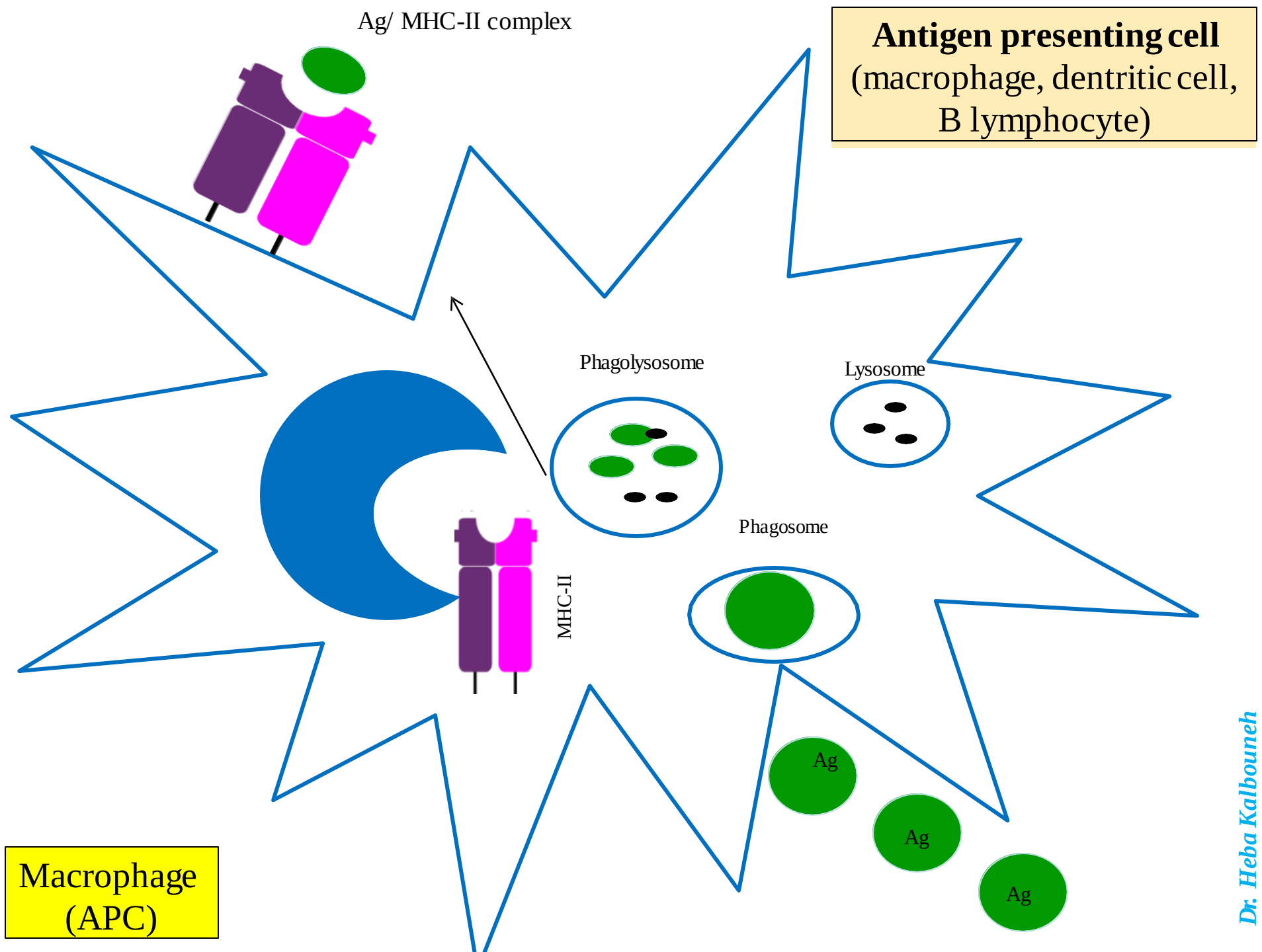
When the Ag- MHCI complex binds to receptors on cytotoxic CD8 T cells They

Proliferate
Activate

Will increase in number to form a clone, and be activated and larger in size

Release
Perforins and granzymes (proteases)

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



About the previous slide

- Inside the nucleus, we have genes for MHC2 molecules, of which mRNA is translated into proteins by ribosomes of RER.
- Let's say that this is a macrophage (rich in lysosomes which contain hydrolytic enzymes, and performs phagocytosis, eventually forming a phagolysosome).
- In a phagolysosome, degradation and digestion of antigens occur, producing fragments.
- A fragment of this antigen combines with MHC2 and is presented on the cell surface as antigen-MHC2 complex.
- Now, MHC2 is recognized by helper T cells.

REMEMBER: APCs present MHC1 molecules on their surfaces like all nucleated cells as we mentioned before.

So what's the difference between antigens presented on MHC1 and MHC2 molecules?

Antigens presented on **MHC1** are samples of proteins **synthesized within the cell,** but antigens presented on **MHC2** molecules are **foreign materials** that have undergone phagocytosis and degradation then presentation.

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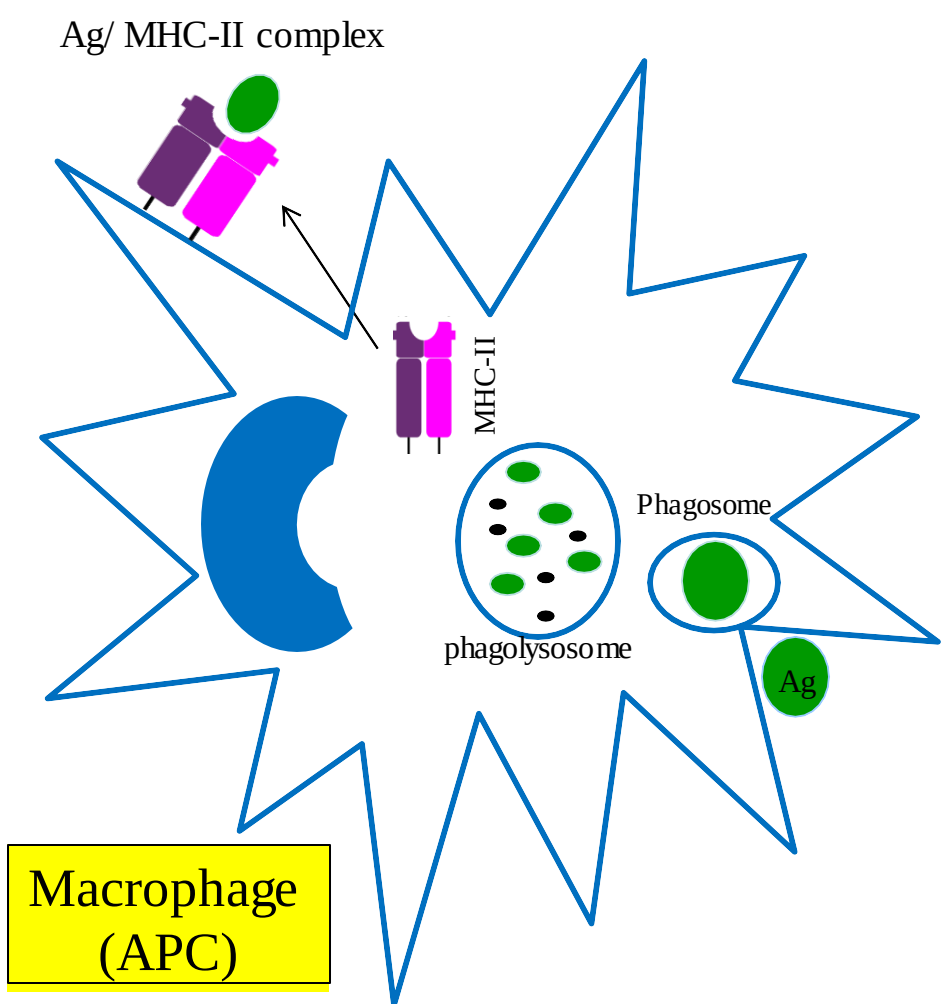
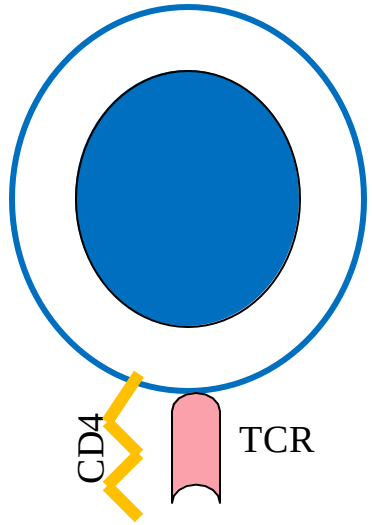
Helper CD4 T cells:

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





These APCs can either present a ntigens within the tissue to T helper cells, or pass through the lymph in order to reach the nearest lymph node to present antigens to T helper cells there.

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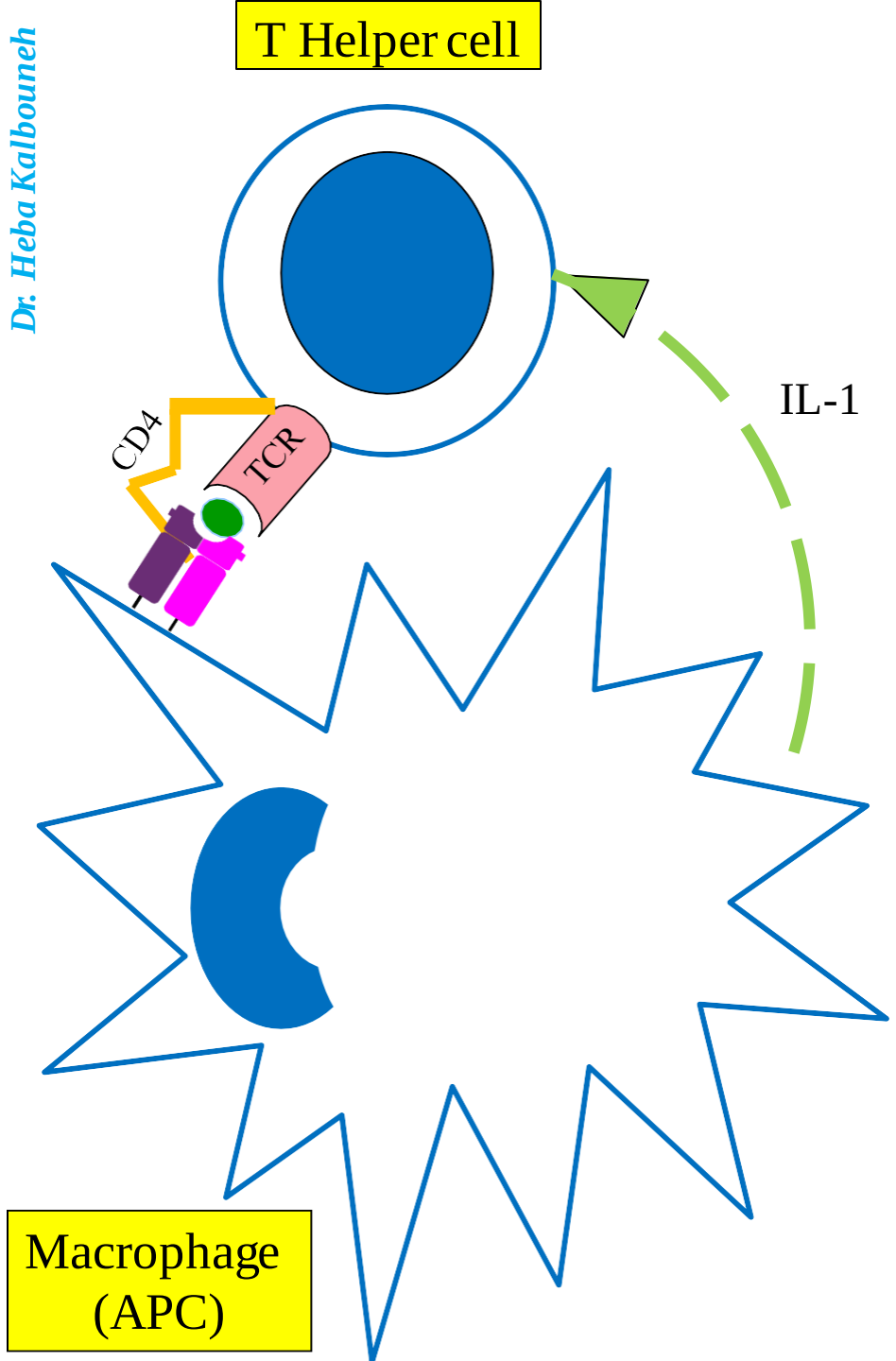
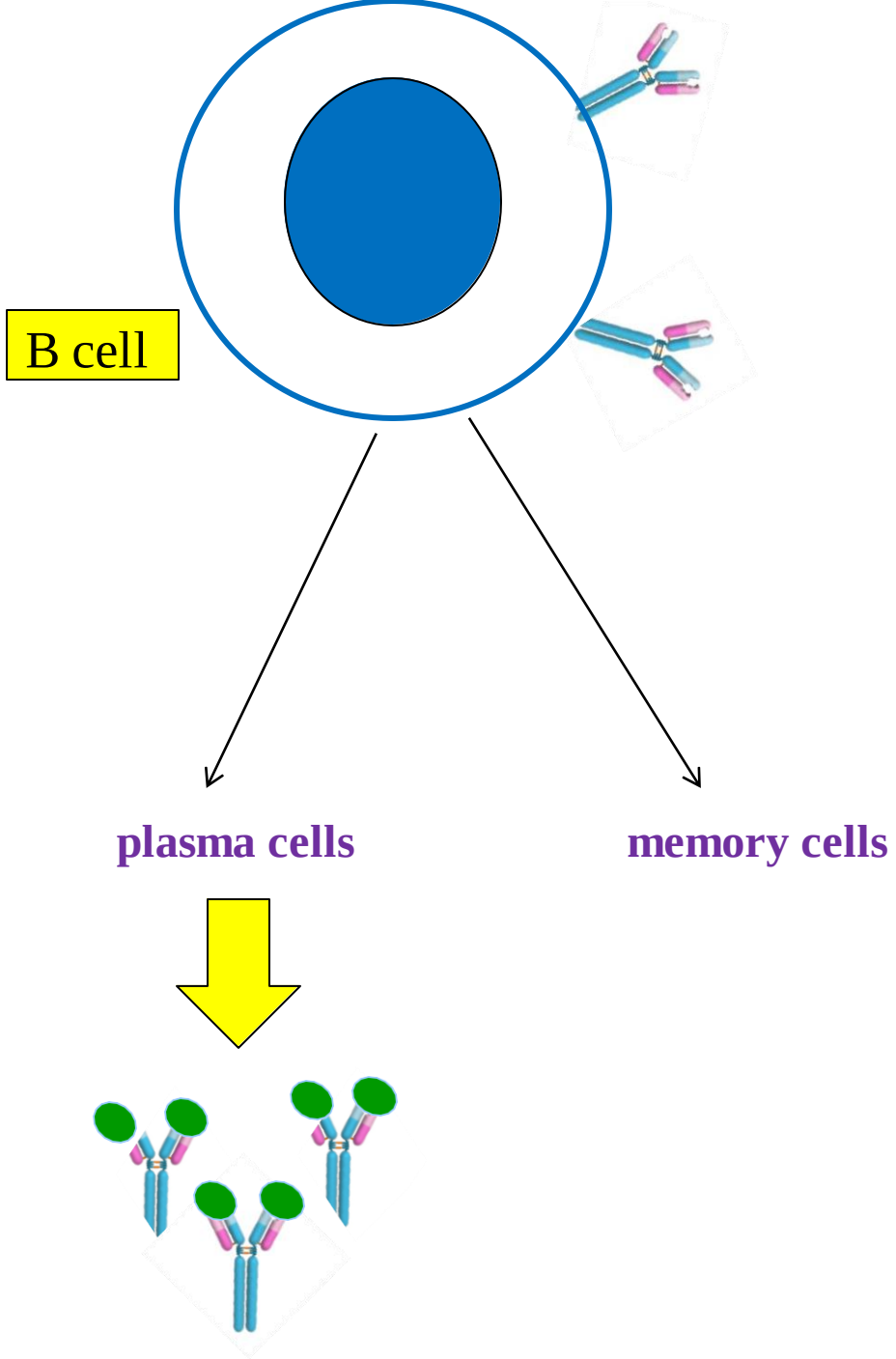


When the Ag- MHCII complex binds to receptors on Helper CD4 T cells
They

Proliferate
Activate

Secrete Lymphokines (cytokines) to Stimulate T and B cells

TCR recognizes the antigen, and CD4 molecule binds MHC2 to make sure that the antigen is presented on MHC2 molecule
(so CD4+ and CD8+ lymphocytes recognize only antigens presented on MHC 2 or MHC 1 molecules, respectively)



When a B lymphocyte is stimulated by T helper cells

↓

Proliferate
Activate

↓

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

↓

Life long immunity (vaccination)

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Antibodies secreted are specific to the presented antigens; they form complexes, deactivate the antigens and facilitate the phagocytosis

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

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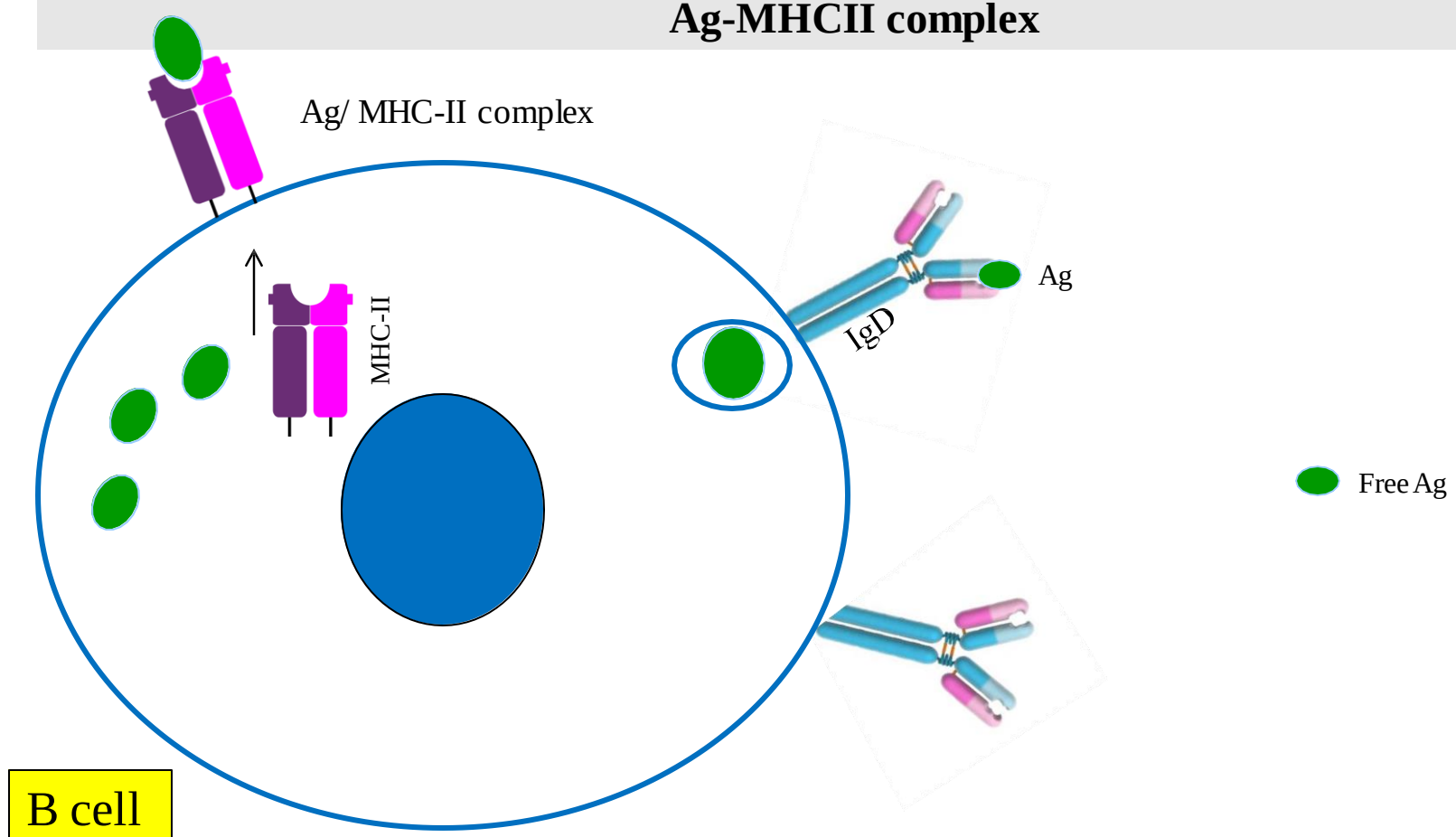
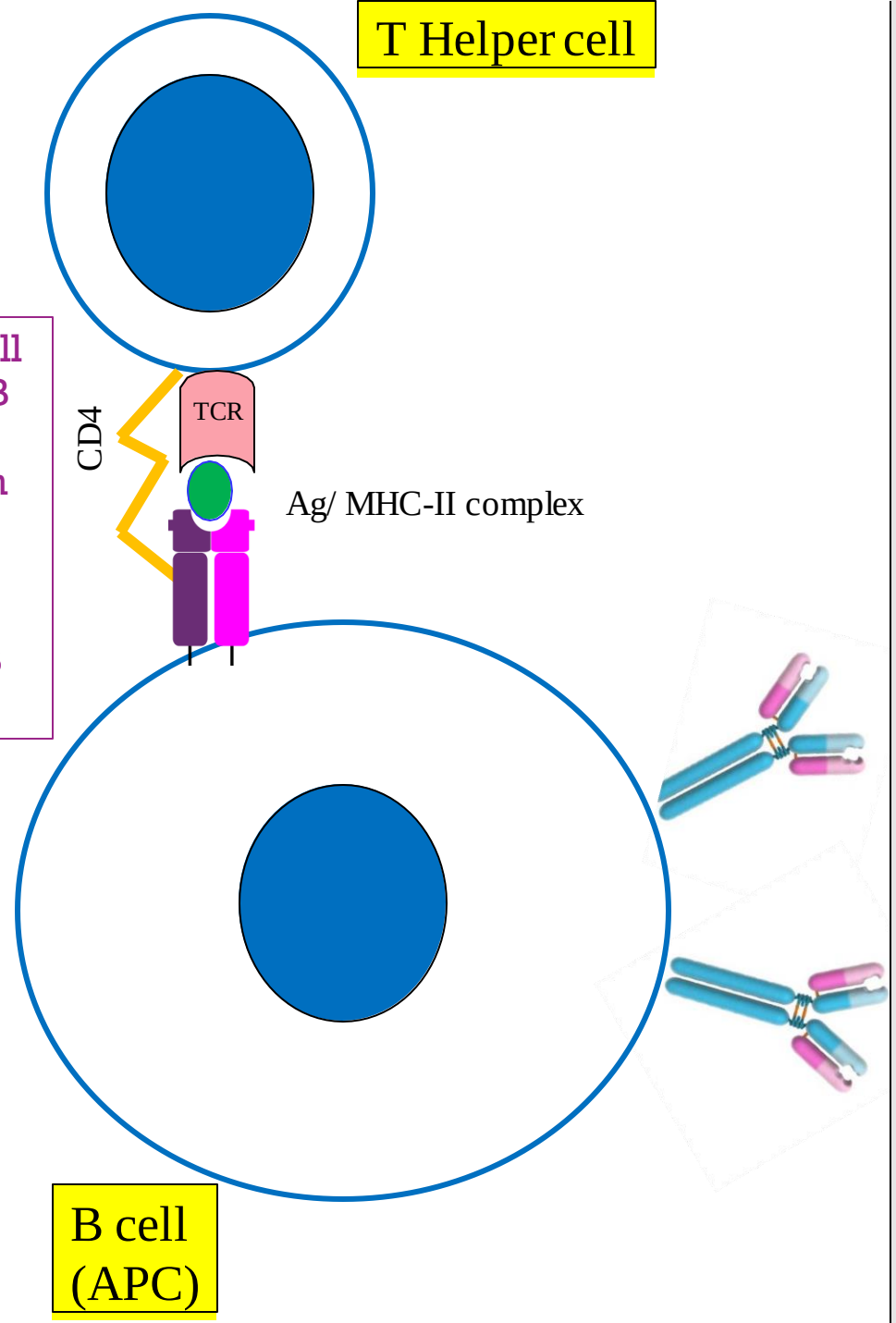


B cells are APCs, so they express MHC2 molecules and have BCRs on their surfaces specific for certain antigens.
This BCR is an immunoglobulin and can only recognize free antigens (Not presented on MHC1 or MHC2)



This binding will stimulate both B and T helper lymphocytes, in which the T lymphocyte directly stimulates the B lymphocyte

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)

Side note: Most of the circulating lymphocytes are small inactive T lymphocytes

Lymphocytes

Monocytes

Eosinophils

Neutrophils

Never **L**et **M**onkeys **E**at **B**ananas

Basophils

Most common to least

WBCs

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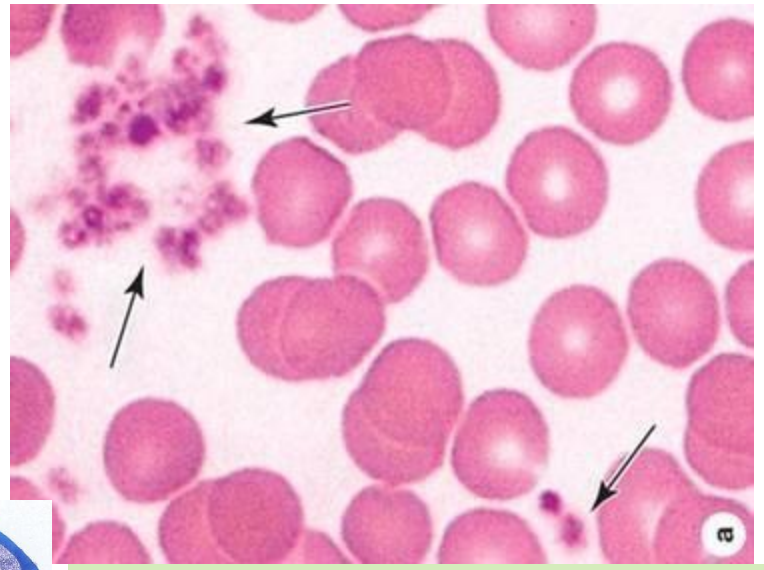
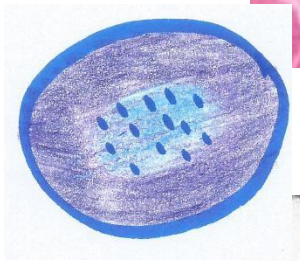
Thrombocytes (Platelets)

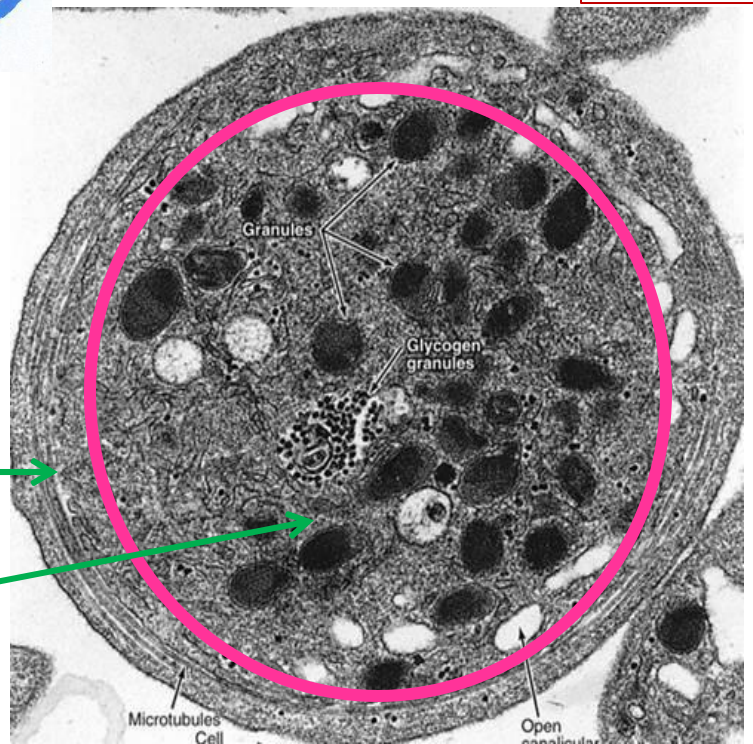
- Small non-nucleated cytoplasmic fragments
- Formed by fragmentation of the cytoplasm of **megakaryocytes** in the bone marrow
- **Number: 200,000-400,000/mm3**
- **Shape: biconvex discs**
- **Cytoplasm: purple, granular**
- **Diameter: 2-4 um**
- **Lifespan about 10 days**
- Control the bleeding by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



Plateletes appear like basophilic dots in staining reaction



In stained blood smears, platelets often appear in clumps Because of thick glycocalyx



Platelete has 2 zones

➢Outer pale basophilic (clear) perpheriral zone: **hyalomere** Glassy

➢ Central dark granular zone: **granulomere** Rich in granules

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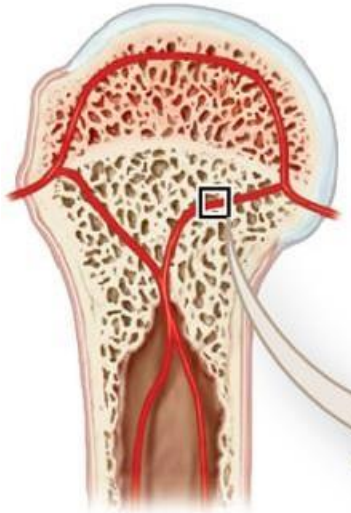
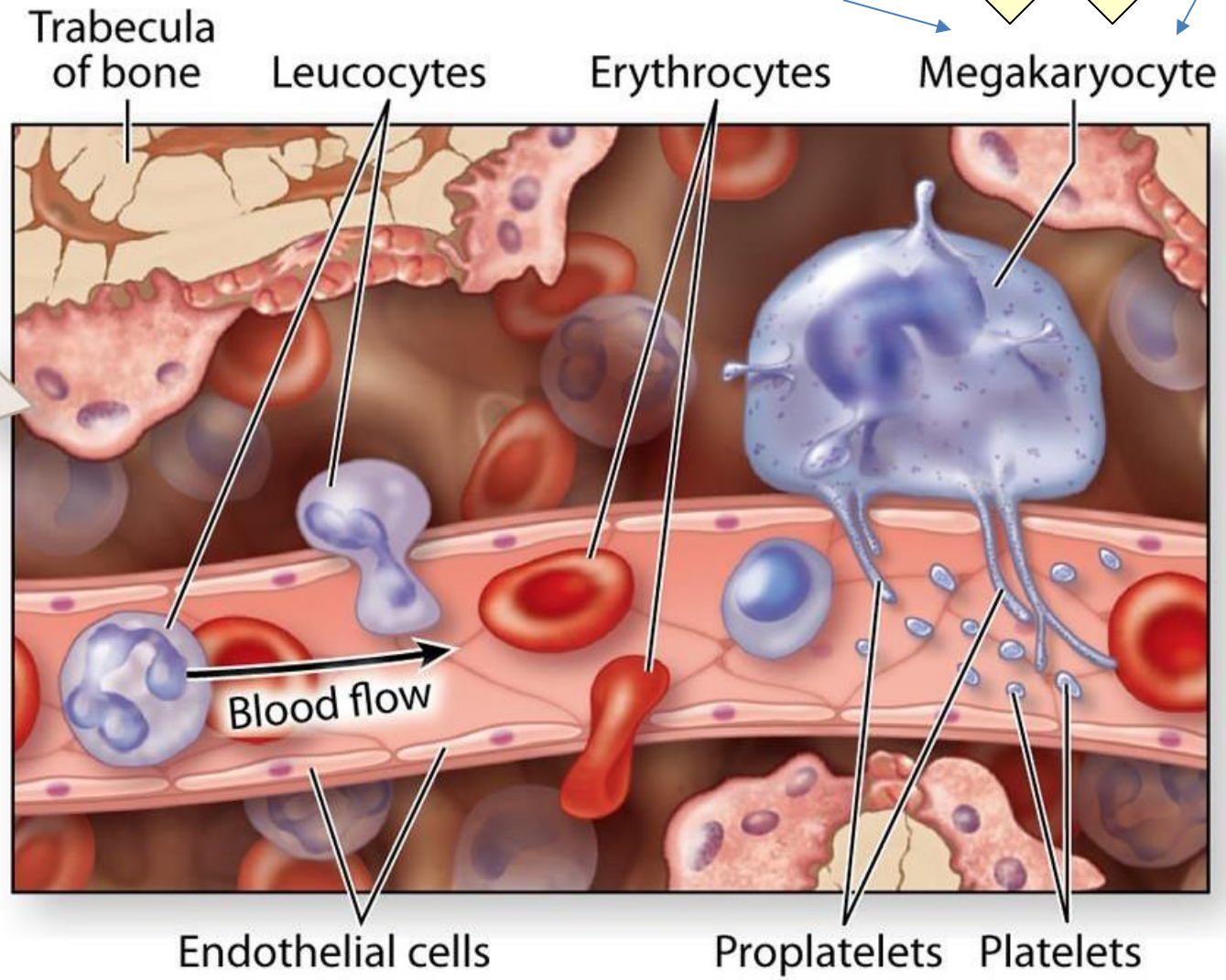
This cell has a large nucleus, inside which are multiple rounds of DNA duplication without nuclear division (polyploid nucleus)

This large cell extends its cytoplasmic processes into the lumen of the capillary, and as the blood flows, fragments of these cytoplasmic processes break down, forming platelets





- Inside the BM, we have this capillary that is lined with endothelial cells.
- In between these endothelial cells, we have wide gaps (blood cells leave the BM and squeeze through them to enter the blood stream)

Invaginations of plasma membrane deep inside, reaching granulomeres (to provide a pathway to quick endocytosis or exocytosis of materials from granulomere to plasma or vice versa

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

Granulomere: contains granules and organelles

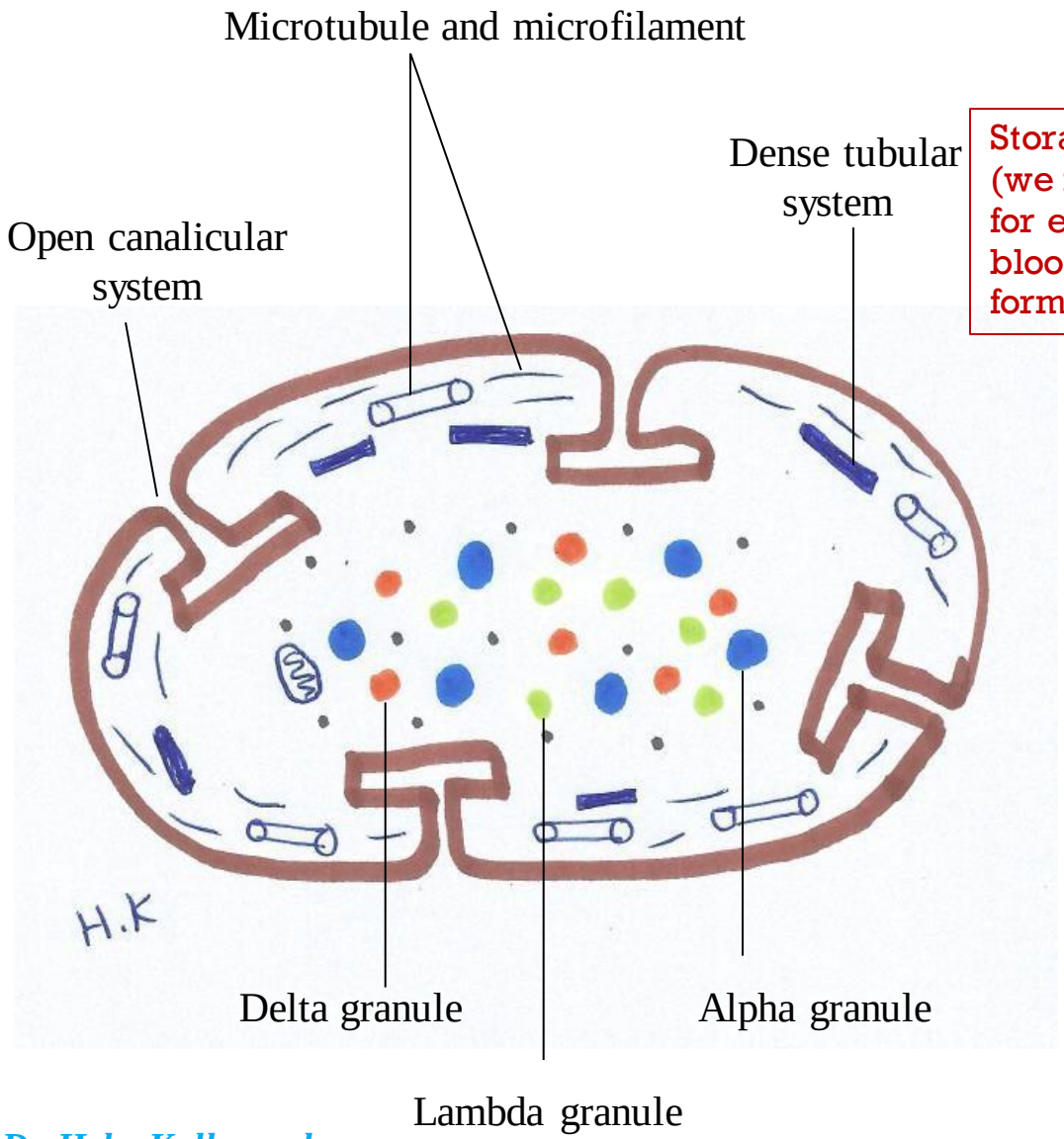
Alpha granules: clotting factors, growth factors

Don't synthesize serotonin; just store it (serotonin acts as vasoconstrictor)

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

Lambda granules: lysosomes (aid in clot resorption)

Have thick glycocalyx

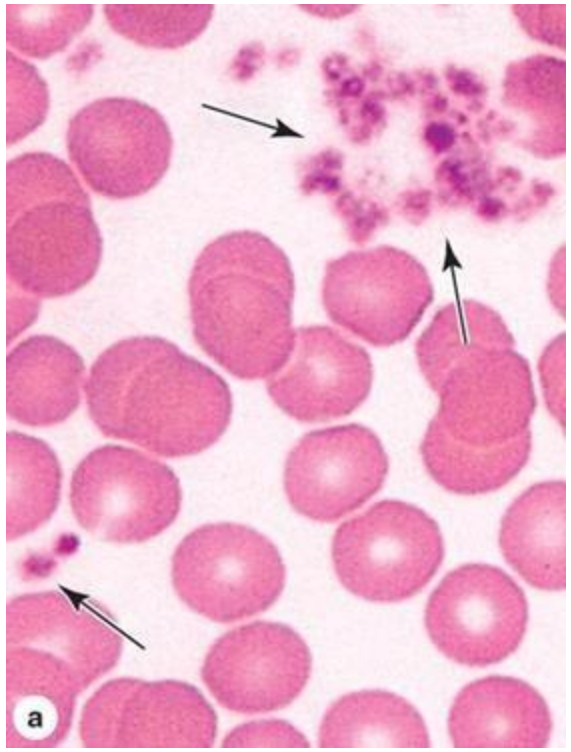


Storage of calcium (we need calcium for exocytosis and blood clot formation)

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

After the bleeding stops, this clot must be removed (clot retraction and removal)



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/Cytoxic_T_cell_activity_against_target_cells.swf

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

Some basic concepts in general histology

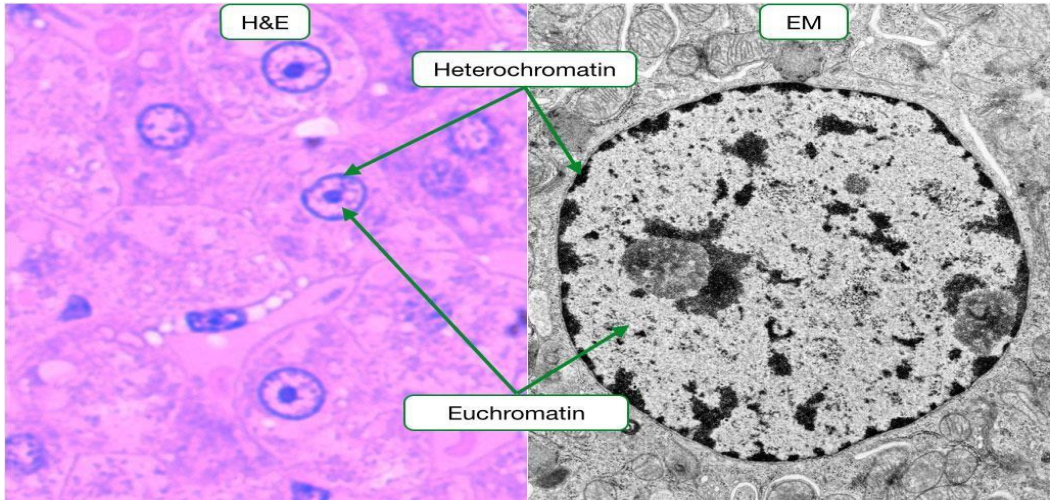
Chromatin

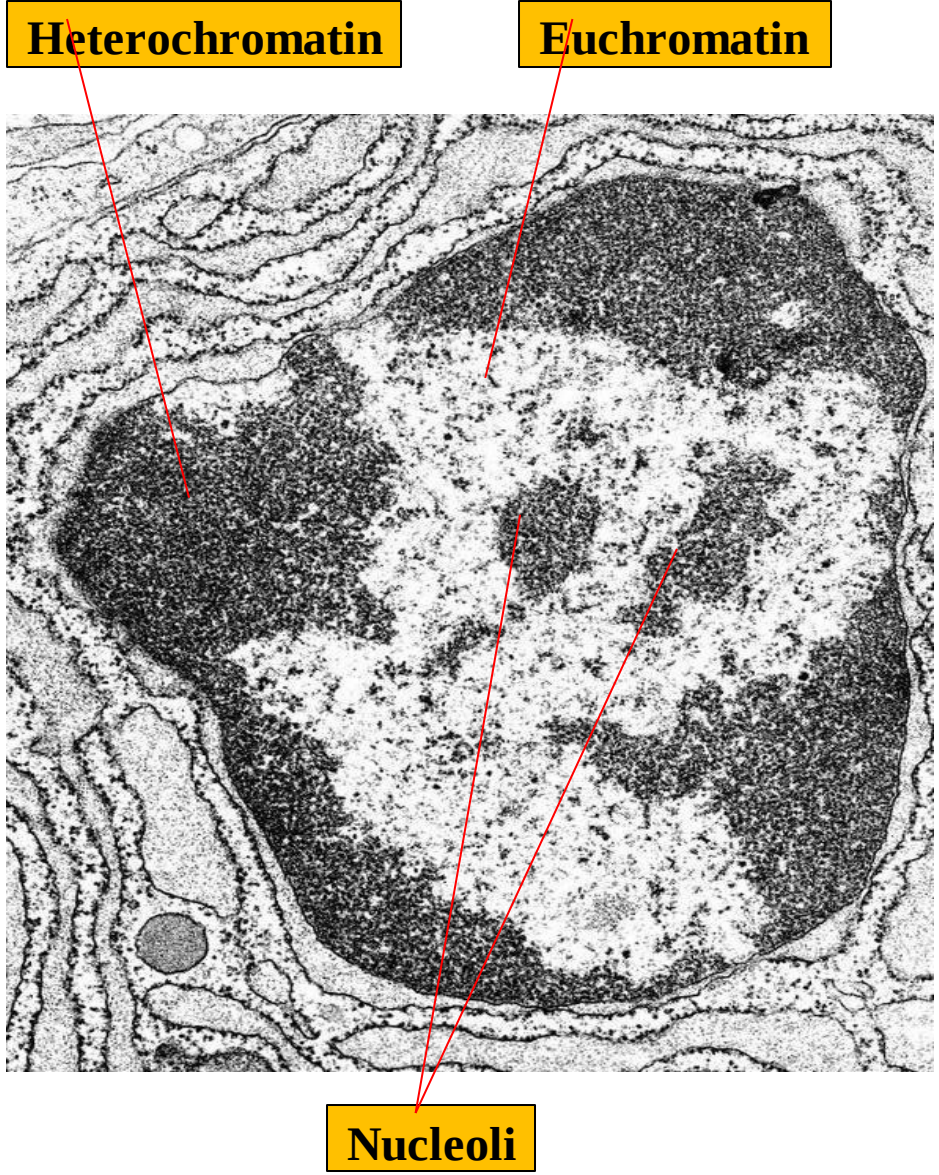
Formed of **DNA**.

- **2 Forms:**
  - **Euchromatin**: extended active chromatin (pale). (Electron lucent)
  - **Heterochromatin:** condensed inactive chromatin (dark) (it's usually clumped in the inner aspect of the nuclear membrane)

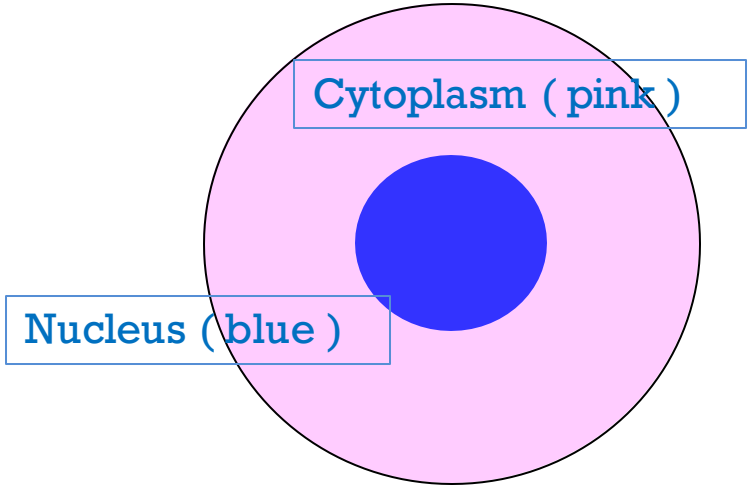
Nucleolus

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





Under LM nucleus is basophilic; if there is more heterochromatin, nucleus appears dark blue, and if euchromatin is more, it appears light blue

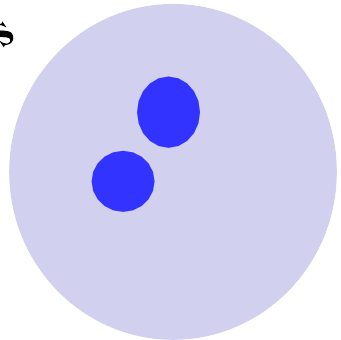


H & E

Large light blue nucleus has two prominent nucleoli

Active nucleus

Nucleolus is a spherical dark basophilic mass



Inactive nucleus

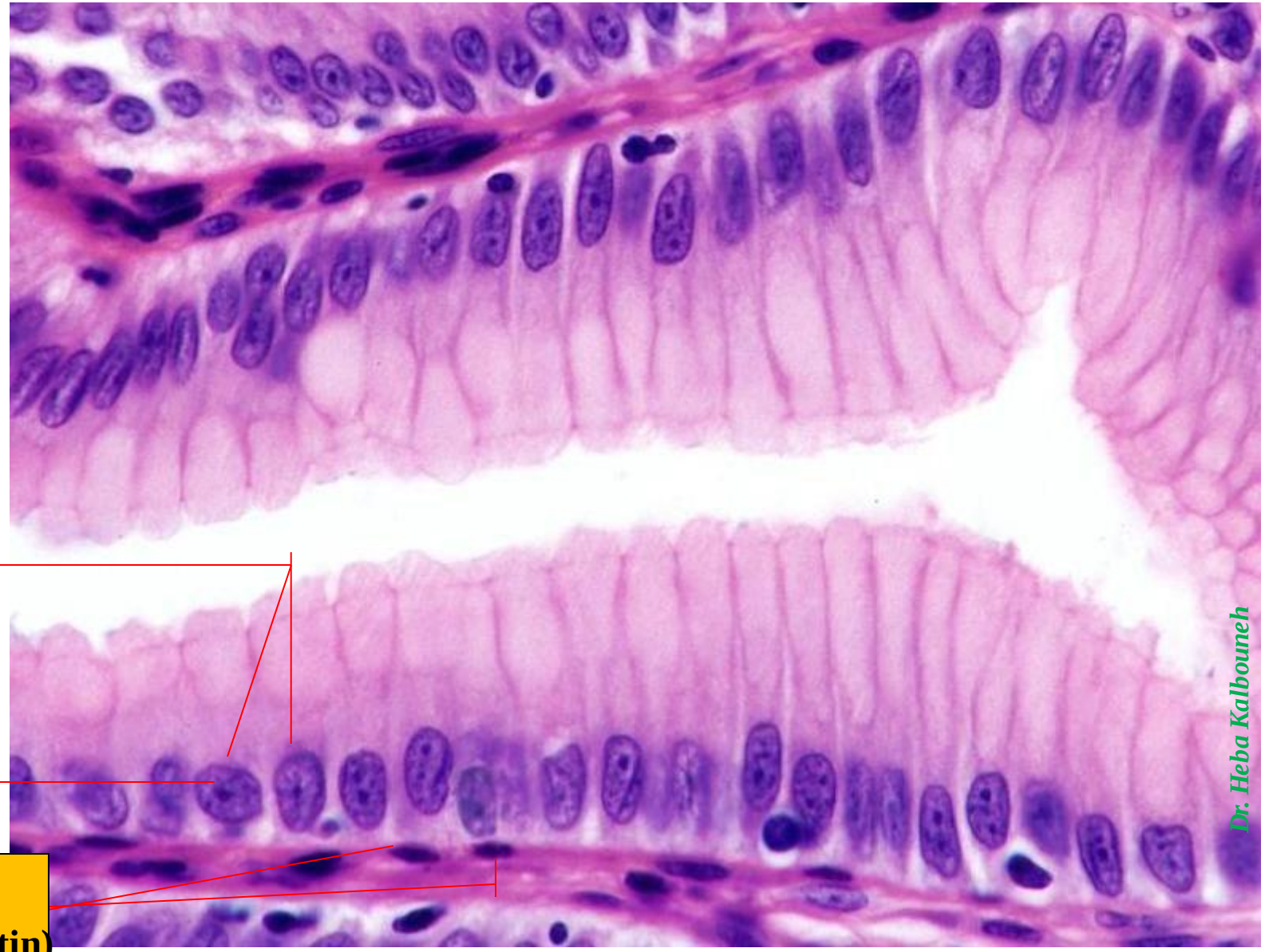
Small dark nucleus

Note:

The nucleus stains blue (basophilic) using H&E

Lightly basophilic: active **Deeply basophilic and small: inactive**

More cytoplasm >>more organelles >> cell is more active



Note:

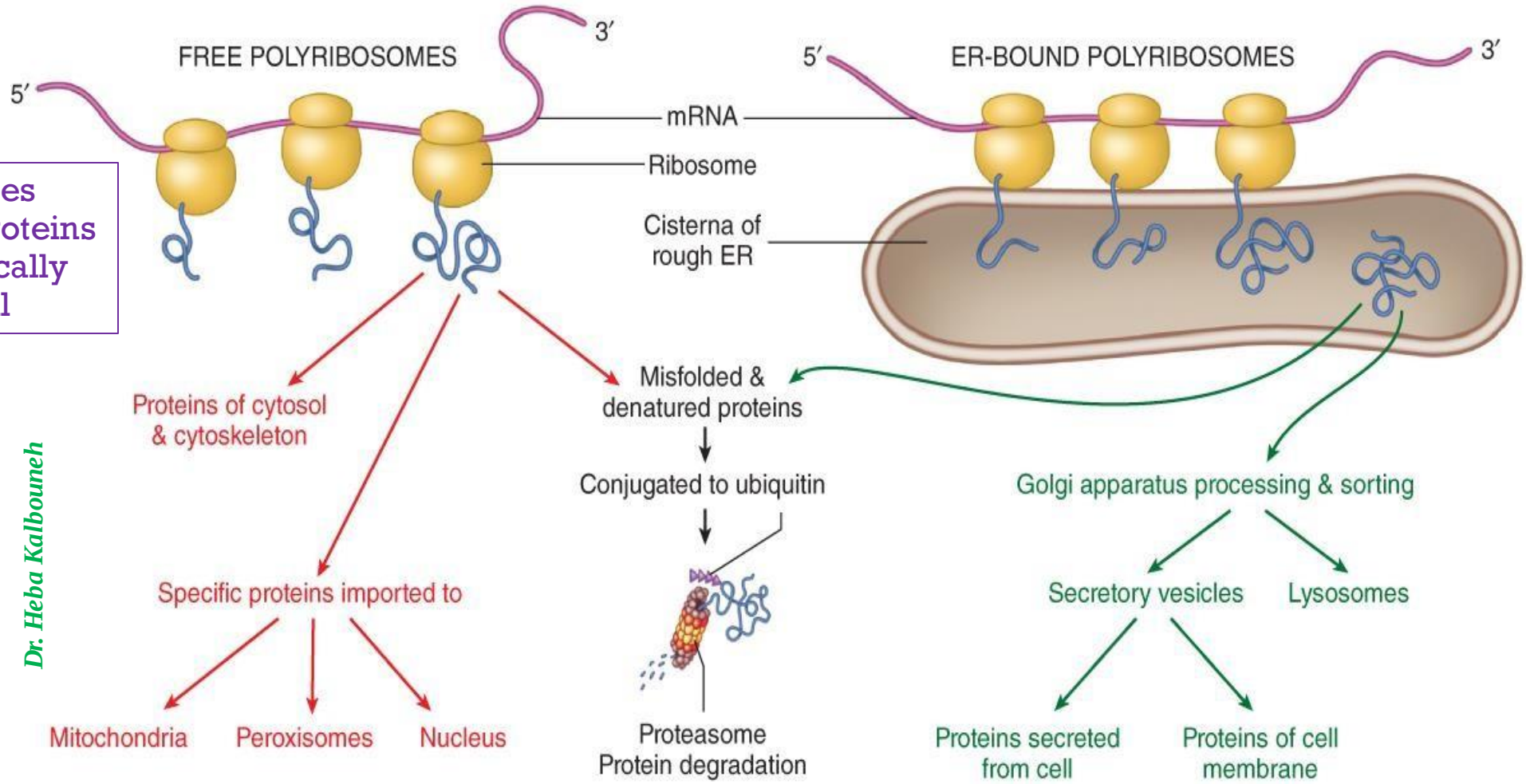
The cytoplasm stains pink/red (acidophilic) using H&E

The organelle (when prominent) that produces basophilia in the cytoplasm is the ribosome

Ribosome is formed mainly by ribosomal RNA >> stained blue

Ribosomes are the site of protein synthesis, we have two types of ribosomes (free and attached)

Free ribosomes synthesize proteins to be used locally within the cell



Attached proteins synthesize proteins for export or to be incorporated in the plasma membrane

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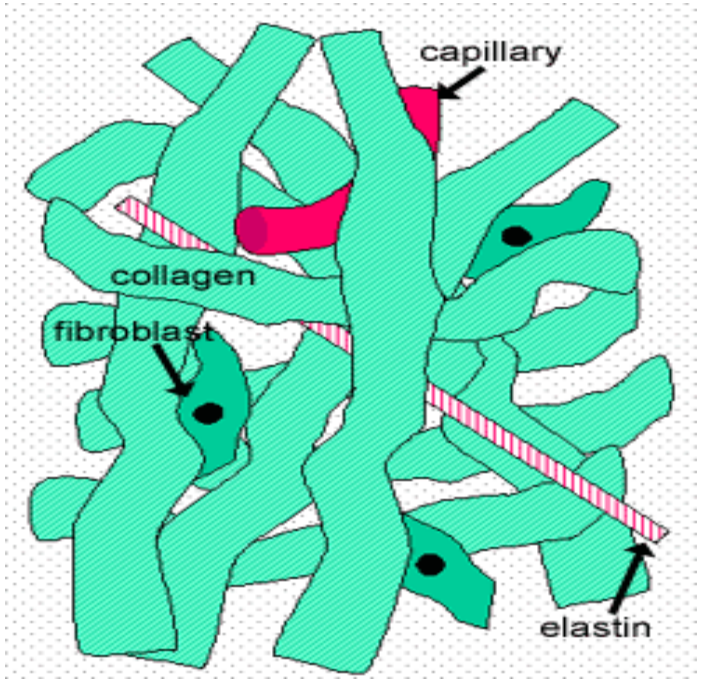
- Connective tissue proper is classified into loose and dense connective tissue
- Loose connective tissue contains cells, fibers, and ground substance in almost the same proportions
- Dense connective tissue is mainly composed of collagen type 1 fibers (more than the cells and ground substance)

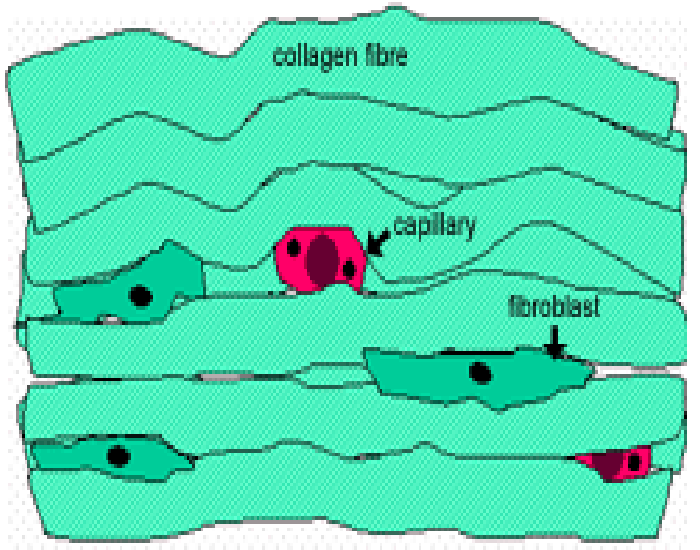
Dense irregular connective tissue

- ✓ 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*.

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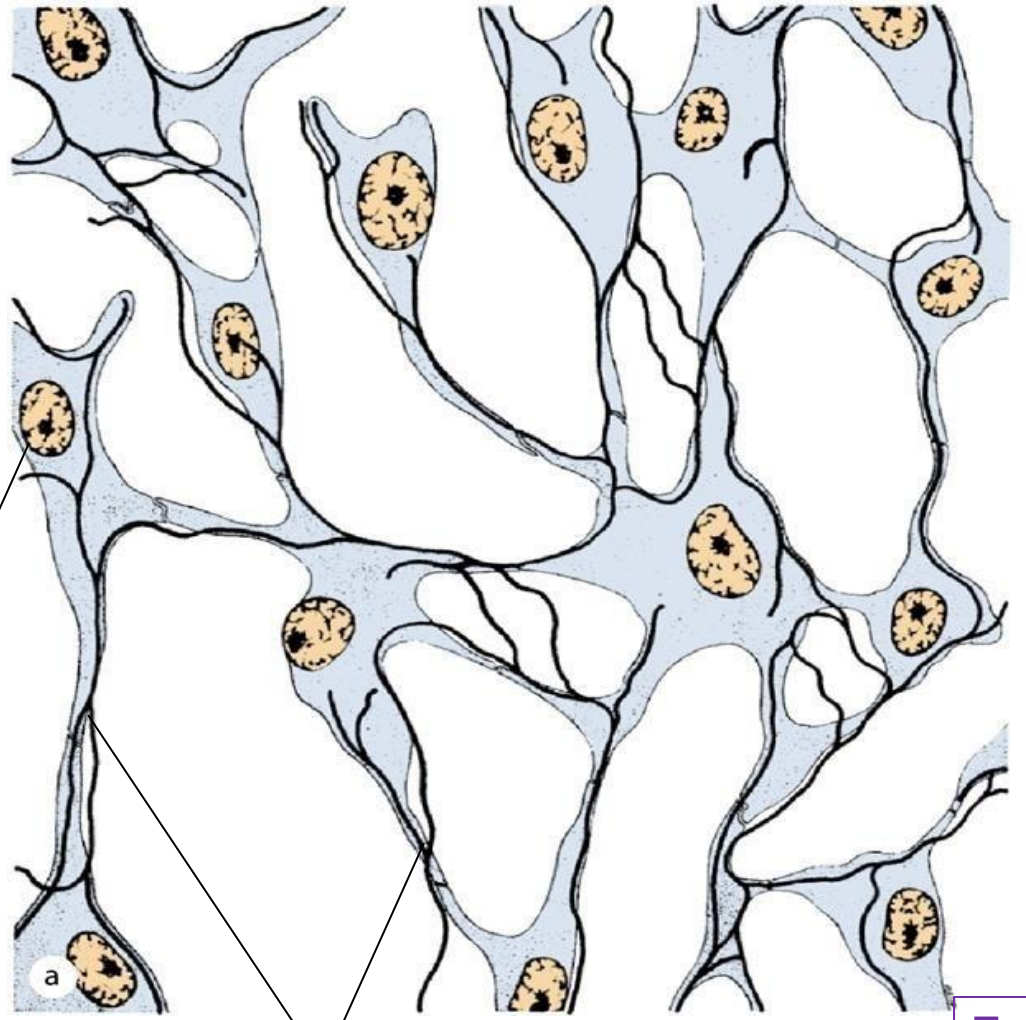
Reticular connective tissue

- 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 fibers are collagen type 3 fibers

Because these organs contain wide spaces filled with blood and lymph and lined with epithelium and supported by reticular tissue



Reticular cell

Reticular fibers are thin and branching forming a network

To permit movement of large molecules and cells

Types of capillaries

Capillary is the smallest blood vessel where fluids exchange; it's simply a single layer of endothelial cells supported by basement membrane surrounded by a thin layer of loose connective tissue

Continuous capillaries

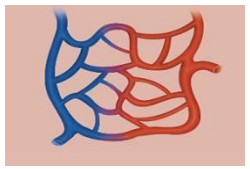
- **Are most common**
- **Endothelium forms solid lining**
- **Adjacent cells are held together with tight junctions**
- **Found in most organs**

Continuous layer of endothelial cells supported by a continuous layer of basement membrane

Fenestrated capillaries

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

Supported by continuous layer of basement membrane
It's more leaky and permeable compared to continuous type of capillaries



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**

Single layer of endothelial cells with wide gaps or clefts in between supported by discontinues layer of basement membrane