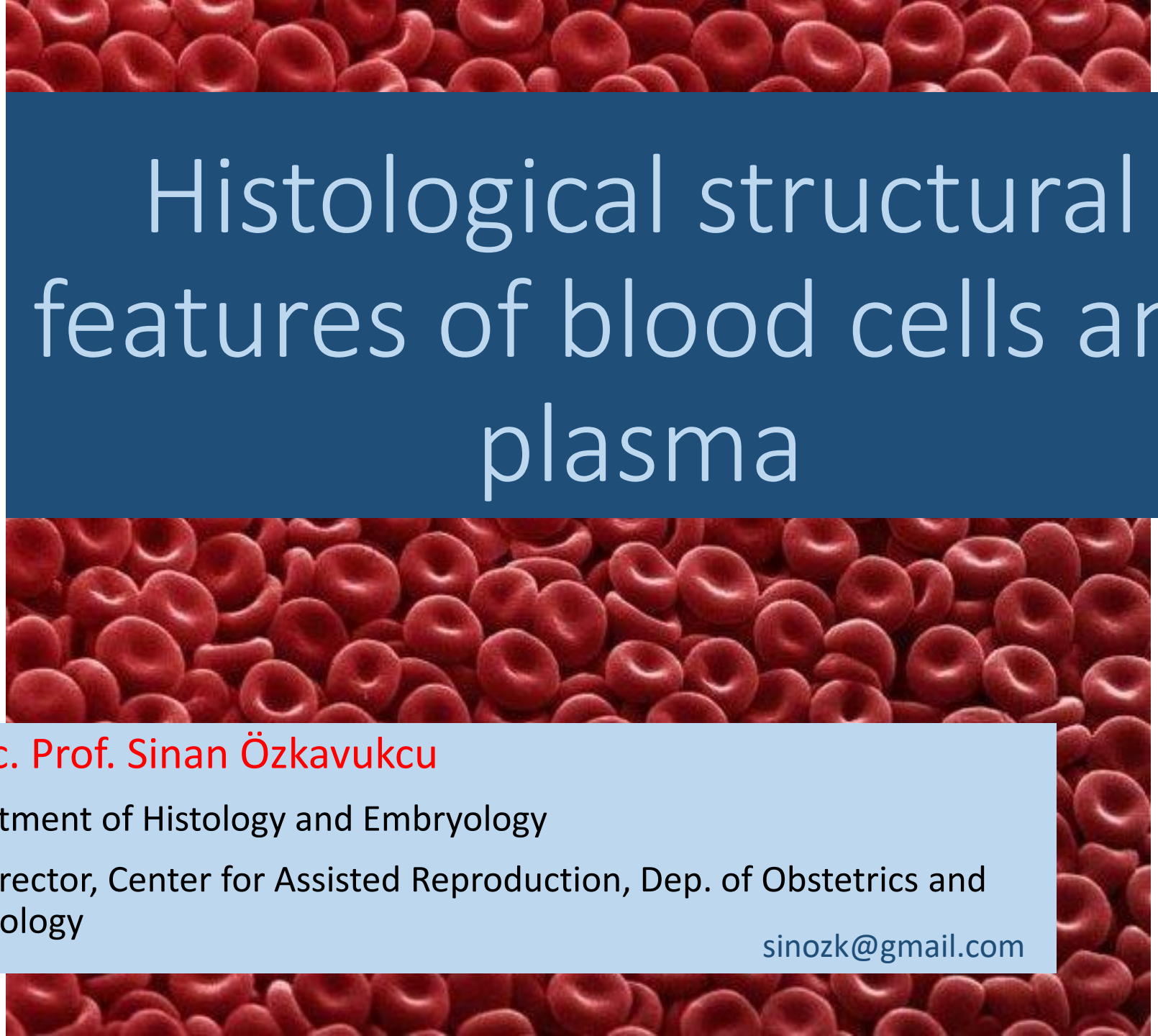

Histological structural features of blood cells and plasma

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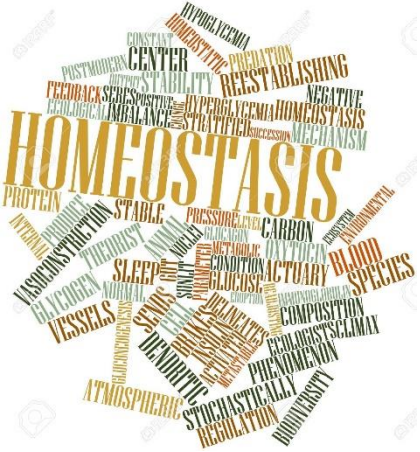
BLOOD

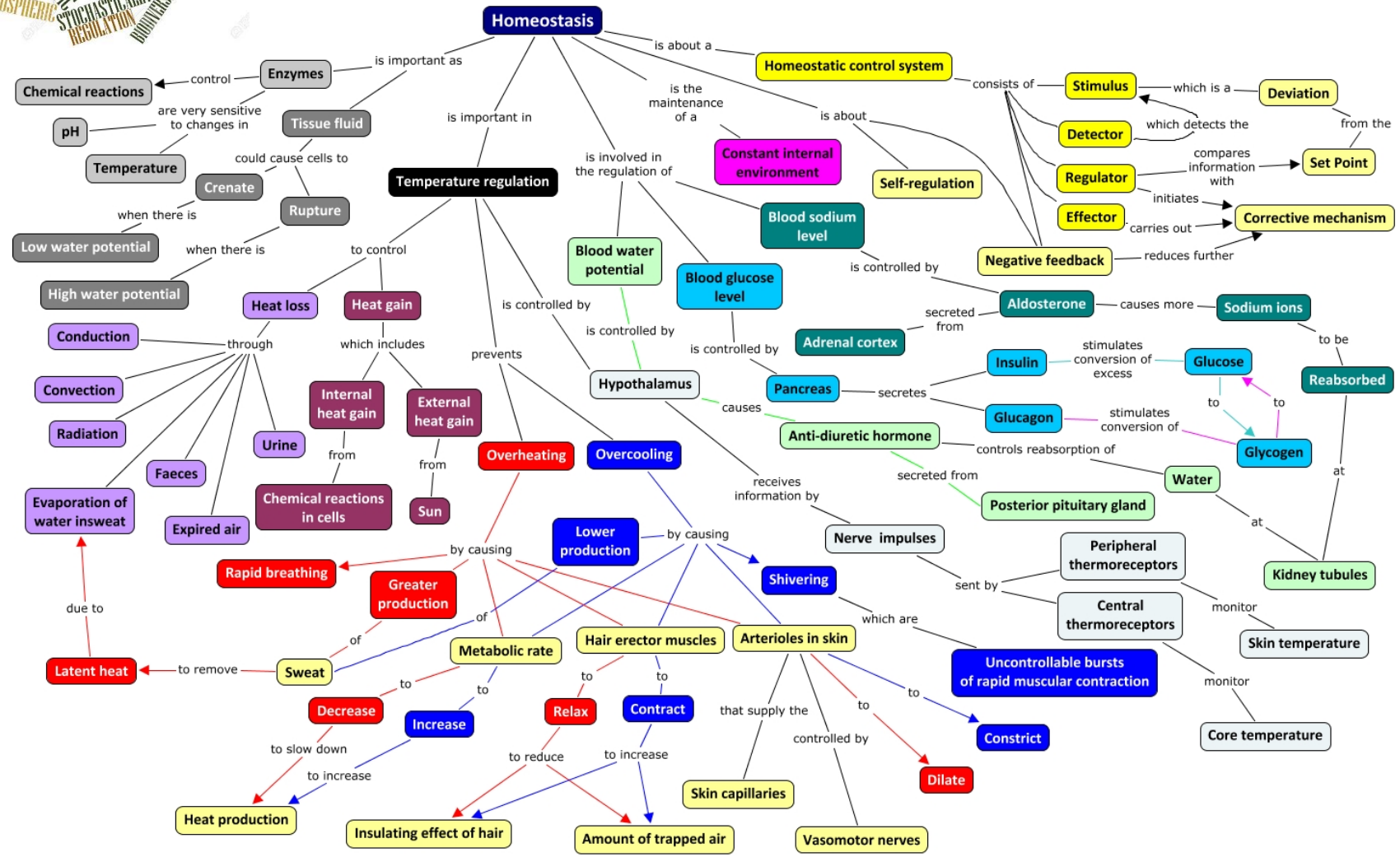
- Blood; is a liquid connective tissue, contains cells and extracellular compounds.
- There are about 5-6 liters of blood in adults and it constitutes 7-8% of body weight.

Duties:

- Delivering oxygen and nutrients directly or indirectly to the cells
- Removal of carbon dioxide and waste materials from cells
- Transmitting hormones and regulatory substances to tissues and cells
- To maintain homeostasis with tasks such as; acid-base balance (protection of pH), coagulation, thermoregulation
- Defensing body against pathogenic agents, proteins and cancer cells by transporting cells and molecules of the immune system



Homeostasis is the state of steady internal physical and chemical conditions maintained by living systems



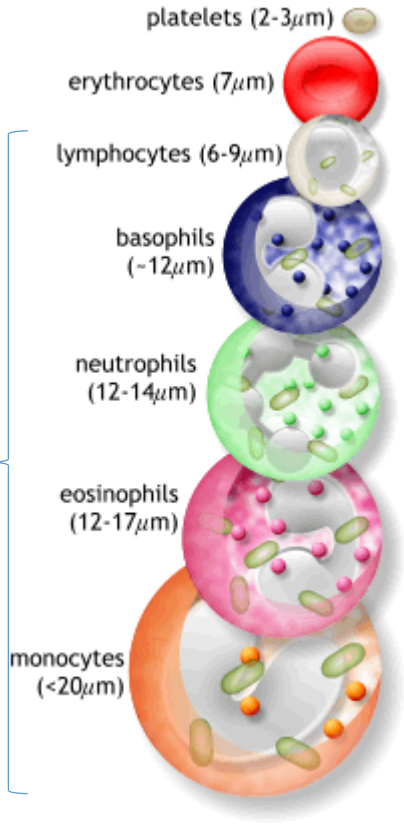
Not to be confused with hemostasis.

BLOOD TISSUE

CELLS

- Erythrocytes → Red Blood Cells (RBC)
- Leukocytes → White Blood Cells (WBC)
- Thrombocytes → Platelets

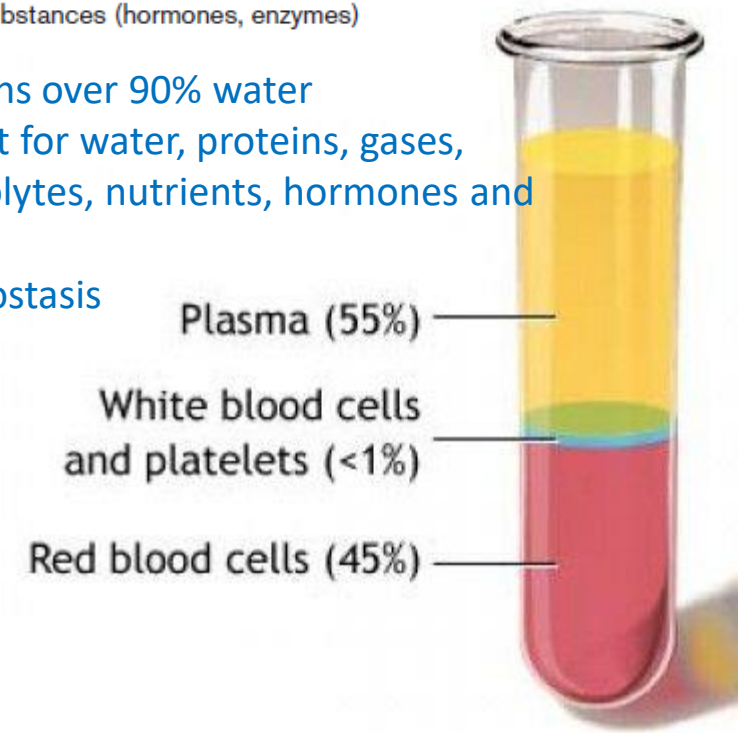
- Leukocytes:
  - Agranulocytes
    - Lymphocytes
    - Monocytes
  - Granulocytes
    - Neutrophils
    - Basophils
    - Eosinophils



PLASMA

Component	%
Water	91–92
Protein (albumin, globulins, fibrinogen)	7–8
Other solutes: • Electrolytes (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, HCO_3^-, PO_4^{3-}, SO_4^{2-}) • Nonprotein nitrogen substances (urea, uric acid, creatine, creatinine, ammonium salts) • Nutrients (glucose, lipids, amino acids) • Blood gases (oxygen, carbon dioxide, nitrogen) • Regulatory substances (hormones, enzymes)	1–2

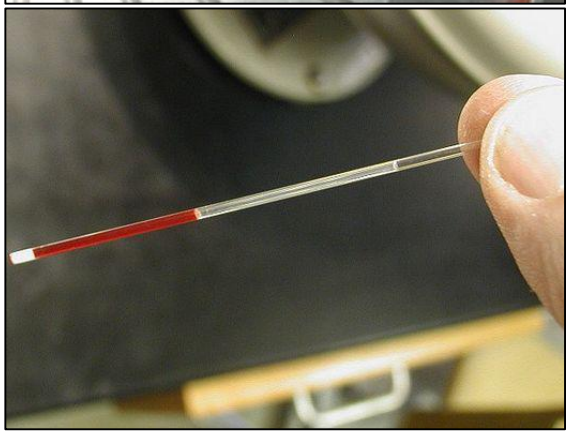
- Contains over 90% water
- Solvent for water, proteins, gases, electrolytes, nutrients, hormones and waste
- Homeostasis

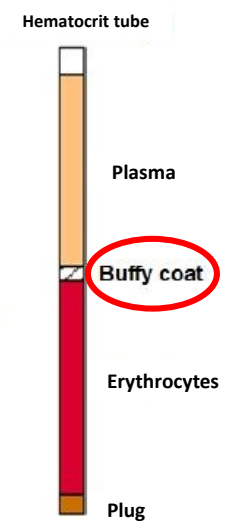


Hematocrit

= a blood test that measures the volume percentage (vol%) of red blood cells (RBC) in blood

Centrifugation of a blood sample, after addition of anticoagulant agents (heparin), to calculate the liquid and cellular parts by means of percentage which gives information about the amount of erythrocytes.





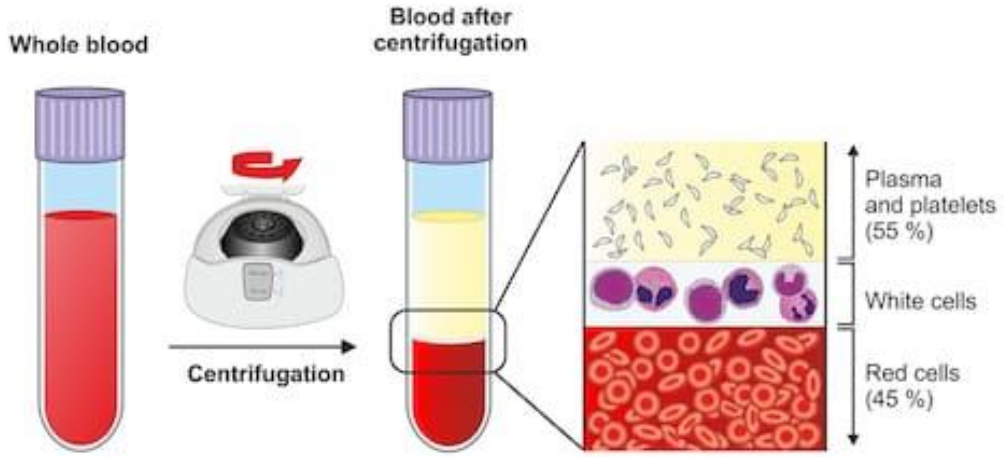
Optimal hematocrit measures:

- Males 39-50%
- Females 35-45%

⬇ Anemia

⬆ Polycythemia

Buffy-coat



- The buffy coat is the fraction of an anticoagulated blood sample that contains most of the white blood cells and platelets following centrifugation of the blood.

- Leukocytes are less dense and small in number (1% of the blood volume).
- They form a thin white or grayish layer on the erythrocytes in the tube, which is called "buffy coat".
- The upper surface of the buffy coat contains platelets as a thin layer.

Plasma
the extracellular matrix of the blood tissue

- Water
- Proteins
- Solutes
  - electrolytes
  - non protein nitrogen
  - nutrients
  - gas
  - hormones, enzymes

Plasma Albumin

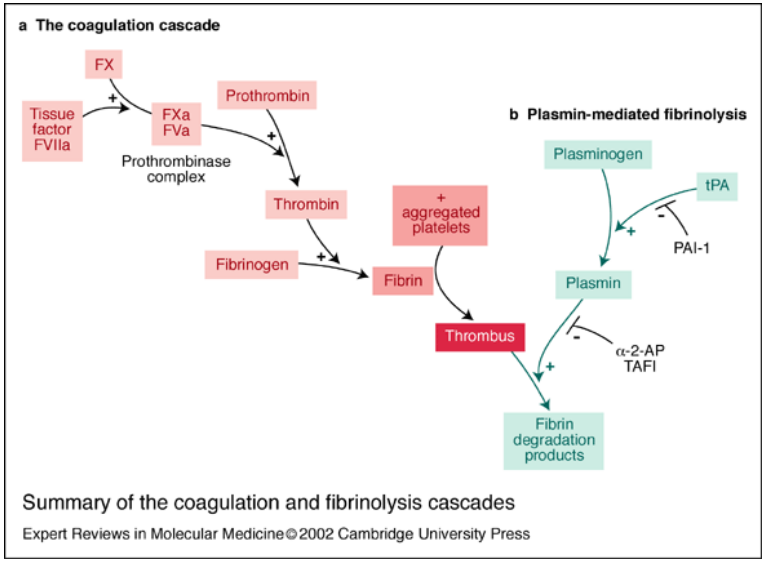
- The main protein component of plasma is ALBUMIN (50%).
- Smallest plasma protein (70kd), produced in the liver
- It is responsible for colloid osmotic pressure (oncotic pressure) and maintains fluid balance between tissue ECM and vessel lumen.
- Albumin also acts as carrier protein in the blood. Many hormones (thyroxine), metabolites (bilirubin) and drugs (barbiturates) are transported by binding to albumin.
- Colloid osmotic pressure decreases in the diseases with albumin loss (impaired production in the liver, excessive excretion from the kidneys), fluid accumulates in the tissues and in the body cavities (edema, pleural effusion, ascites).

Plasma Globulins

- γ (gamma) Globulins = immunoglobulins
  - They are functional immune system molecules called antibodies and produced by plasma cells
- Nonimmune globulins (α and β globulins)
  - Produced by the liver
  - Provide intravenous oncotic pressure
  - Bind and transport some substances
    - Ceruloplasmin (copper)
    - Transferrin (iron)
    - Haptoglobin (hemoglobin)
    - Fibronectin, lipoproteins, coagulation factors

Plasma Fibrinogen

- It is the largest plasma protein (340 kd) and is produced in the liver
- Normally present as dissolved in plasma, it is transformed into insoluble fibrin structure as a result of stepwise reactions of coagulation factors and enables clotting



PROTEIN	PLACE SYNTHESIZED	FUNCTION
Albumin (Mostly found)	Liver	1. Adjusts colloidal osmotic pressure 2. Plays a role in substance transport
α, β Globulins (Nonimmune globulins)	Liver	Carries metals and fat-soluble vitamins
δ Globulins (immune globulins)	Plasma Cells	Immune system
Coagulation proteins (fibrinogen)	Liver	Important in the formation of fibrin during coagulation
Plasma lipoproteins:		
chylomicrons	Intestinal epithelium	Carries triglyceride to the liver
VLDL	Liver	Carries triglyceride between liver and cells
LDL	Liver	Carries cholesterol between liver and cells

Serum

= has the same content as plasma, obtained by removing coagulation factors

- Plasma = Serum + Coagulum
- Serum = Plasma – Coagulation factors

- Clotting occurs as soon as blood comes out of the vein
- Blood is drawn into tubes with citrate or heparin (anticoagulant) to get accurate measurements for laboratory studies
- High volume of serum can be obtained after centrifugation

PHYSICAL PROPERTIES OF BLOOD

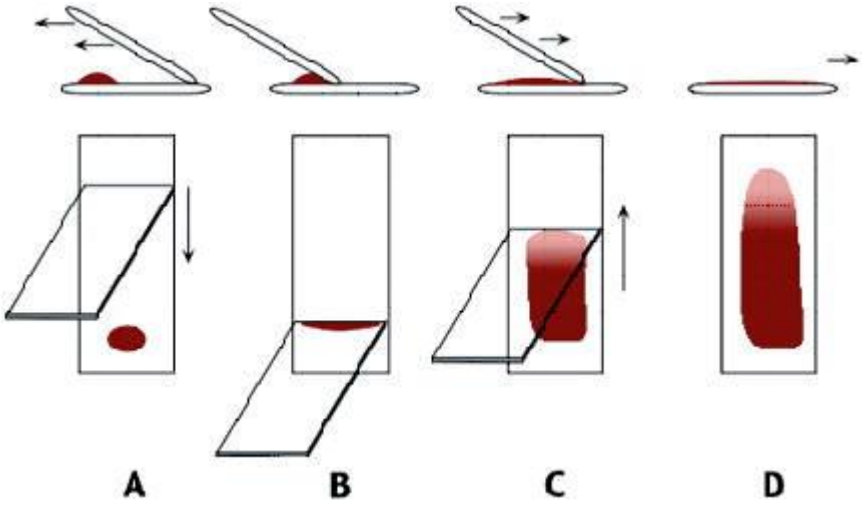
- COLOR: Red color originates from hemoglobin; As hemoglobin is saturated with oxygen, the color gets brighter.
- DENSITY: A mean density value of plasma and specific cells (1050-1060).
- REACTION: pH is around 7.35-7.45; higher in arterial blood and lower in venous blood
- VISCOSITY: Around 3.5-5.4; it is higher in venous blood due to the increased carbon dioxide.
- *** Blood plasma is isotonic at 0.9% NaCl

Sedimentation

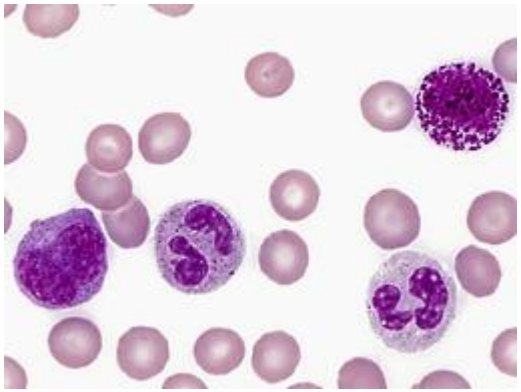
- It is the gravitational pelleting of erythrocytes in the blood kept in a tube after preventing coagulation.
- Normally 0 -15 mm/h
- Increases in cases of infection, rheumatic diseases, pregnancy, menstruation, senescence, etc.

Blood smear

- The easiest method used in the examination of blood cells
- A drop of blood is placed directly on a glass slide and spread over its surface with the edge of another slide.
- After air drying, it is stained with modified Romanovsky type staining mixtures (Wright or Giemsa).
- Methylene blue (basic dye)
- Azure (basic dye)
- Eosin (acidic dye)



The granules of leukocytes can be distinguished as they have different staining properties (nuclei and basophil granules receive basic dyes)

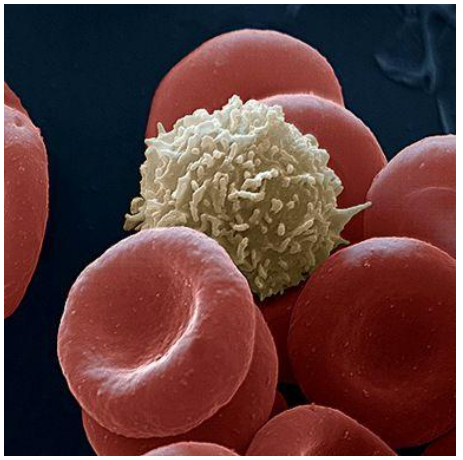


Cellular elements of blood

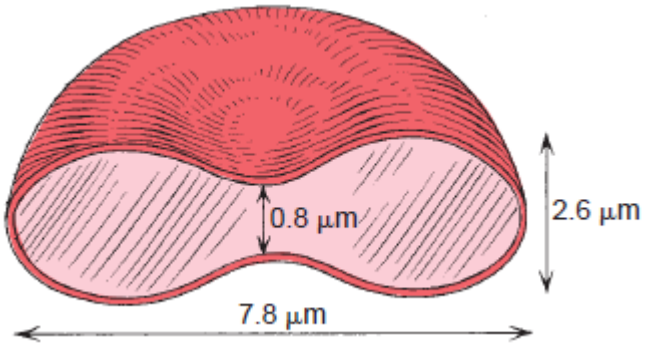
Formed Elements	Cells/L			
	Male	Female	%	
Erythrocytes	$4.3–5.7 \times 10^{12}$	$3.9–5.0 \times 10^{12}$		4,5-5,5 million/mm³
Leukocytes	$3.5–10.5 \times 10^{9}$	$3.5–10.5 \times 10^{9}$	100	5-9 thousand/mm³
Agranulocytes				
Lymphocytes	$0.9–2.9 \times 10^{9}$	$0.9–2.9 \times 10^{9}$	25.7–27.6[a]	
Monocytes	$0.3–0.9 \times 10^{9}$	$0.3–0.9 \times 10^{9}$	8.6[a]	
Granulocytes				
Neutrophils	$1.7–7.0 \times 10^{9}$	$1.7–7.0 \times 10^{9}$	48.6–66.7[a]	
Eosinophils	$0.05–0.5 \times 10^{9}$	$0.05–0.5 \times 10^{9}$	1.4–4.8[a]	
Basophils	$0–0.03 \times 10^{9}$	$0–0.03 \times 10^{9}$	0–0.3[a]	
Thrombocytes (platelets)	$150–450 \times 10^{9}$	$150–450 \times 10^{9}$		250 thousand/mm³

[a]Percentage of leukocytes.

Erythrocytes

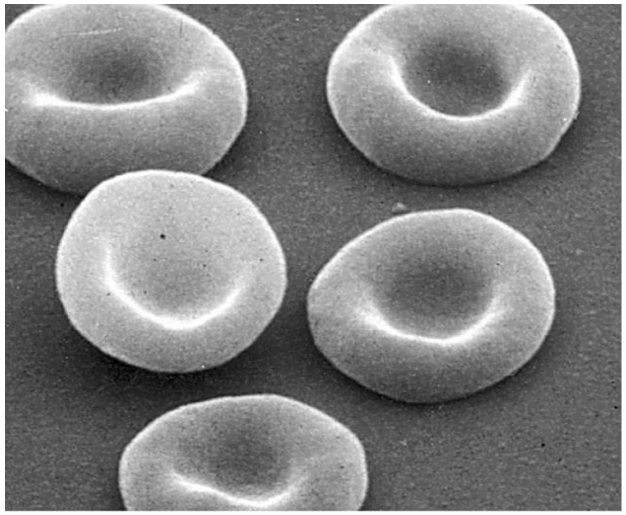


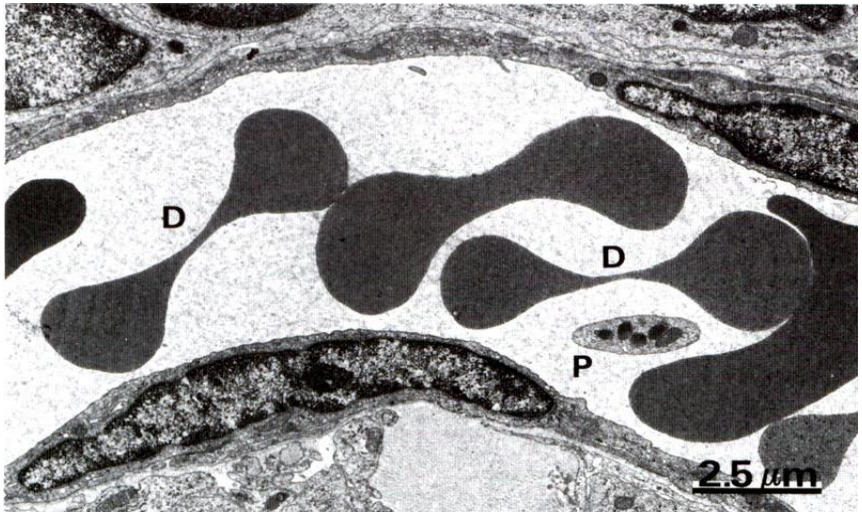
- Red blood cells
- No typical organelles and nuclei (anuclear)
- Transports oxygen to tissues and removes released carbon dioxide from tissues
- It is in the form of a biconcave disc, thus increasing surface area (advantage in gas exchange)
- Total surface area of erythrocytes is 2000 times higher than the whole body surface area



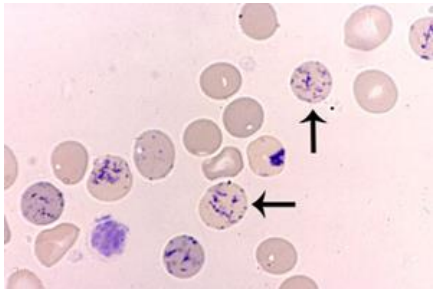
microscopic ruler

- Their lifetime is up to 120 days
- They are eliminated especially in the spleen and also in bone marrow and liver, with phagocytosis.
- A small part of it is eliminated in the circulation and releases hemoglobin
- Extremely flexible and capable folding in the capillaries

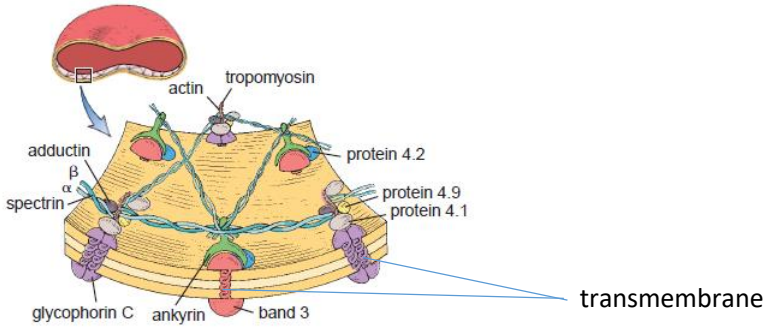




- Mature erythrocytes lack the nucleus and cytoplasmic organelle and lose them during differentiation.
- In cases of increased production (such as erythrocyte loss), reticulocytes, which are young erythrocytes, can join the circulation. These cells may contain a small amount of residual GER and ribosomes (rRNA) and these structures appear to be blue network (reticle) with cresyl blue.
- If more than 1% of circulating erythrocytes are reticulocytes, it is obvious that the need for oxygen carrying capacity is increased. Ex. hemorrhage or living in high altitude.
- Circulating reticulocytes turn into mature erythrocytes after 24-48 hours.
- Since erythrocytes do not contain mitochondria, they have to provide the energy from anaerobic glycolysis to maintain the hemoglobin in a functional state.
- Since they do not have ribosomes, glycolytic enzymes and other important enzymes cannot be replaced. Therefore, mature erythrocytes have a certain life span

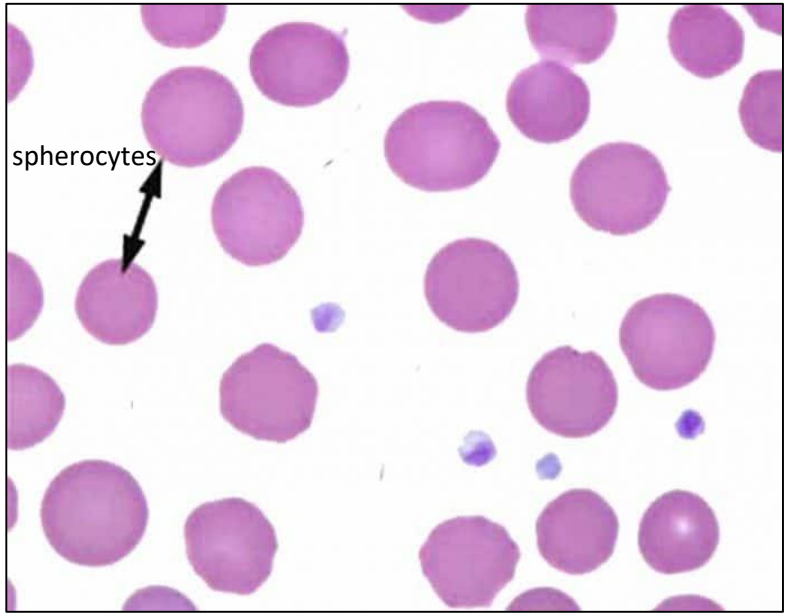


Biconcave disc

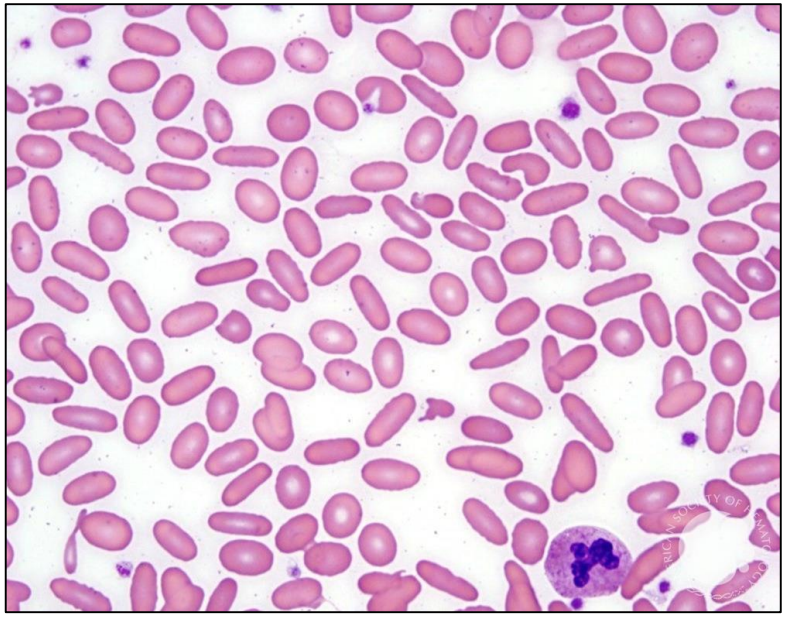


- In order to maintain its shape, an erythrocyte contains two large groups of protein families in their membrane structure
  - Integral membrane proteins– extracellular domains of these integral membrane proteins express specific blood group antigens
    - Glycophorins – Glycophorin C
    - Band 3 proteins – binds hemoglobin
  - Peripheral membrane proteins– reside on the inner surface of the cell membrane.
    - Spectrin tetramers, actin, band 4.1 protein, adducin, band 4.9 protein, and tropomyosin are organized into a two-dimensional hexagonal lattice network that laminates the inner layer of the membrane
    - The lattice is anchored to the lipid bilayer by the globular protein ankyrin, which interacts with band 4.2 protein as well as with band 3
- This unique cytoskeletal arrangement contributes to the shape of the erythrocyte and imparts elastic properties and stability to the membrane.
- As it passes through the smallest vein, it becomes flat with pressure, and when it enters into the larger vessel, it regains biconcave disc structure back.

Any defect in the expression of genes that encode these cytoskeleton proteins can result in abnormally shaped and fragile erythrocytes.



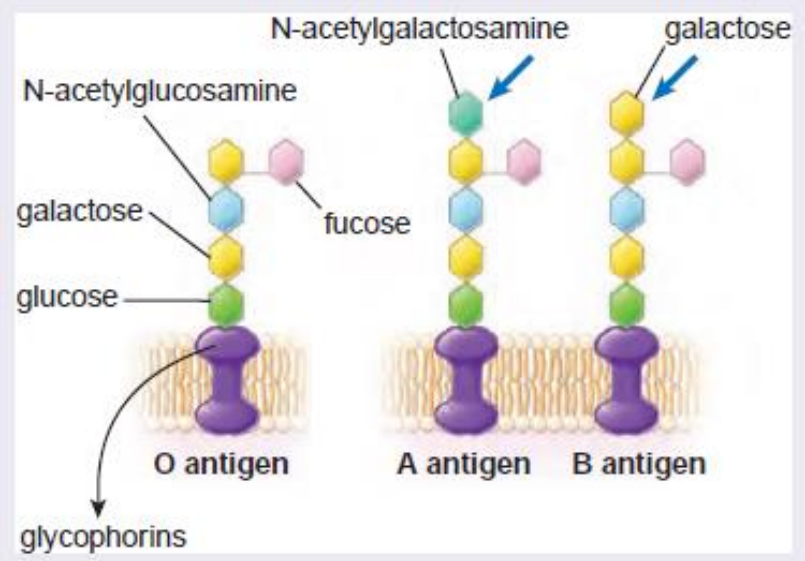
Hereditary spherocytosis
Defect in spectrin gene expression



Hereditary elliptocytosis
Deficiency in band 4.1 proteins

ABO Blood Group Systems

- ABO group compliance is important in blood transfusions..
  - It is determined by the surface antigens on erythrocytes
  - These antibodies are glycoprotein and glycolipid structures that extend outside the cell on the internal membrane protein glycophorins.
  - O antigen can be synthesized in all individuals
  - Individuals with group A add N-acetylgalactosamine on the O group with N-acetylgalactosamine transferase (A-glycosyltransferase)
  - Individuals with group B add galactose on group O with galactose transferase (B-glycosyltransferase)



- People with AB group have both enzymes, whereas those with O group do not have any of these enzymes.
- Therefore, there are antibodies against:
  - B antigen in A group,
  - A antigen in B group, and
  - both antigens in O group.
- AB group individuals can be a general recipient as they produce no antibody.

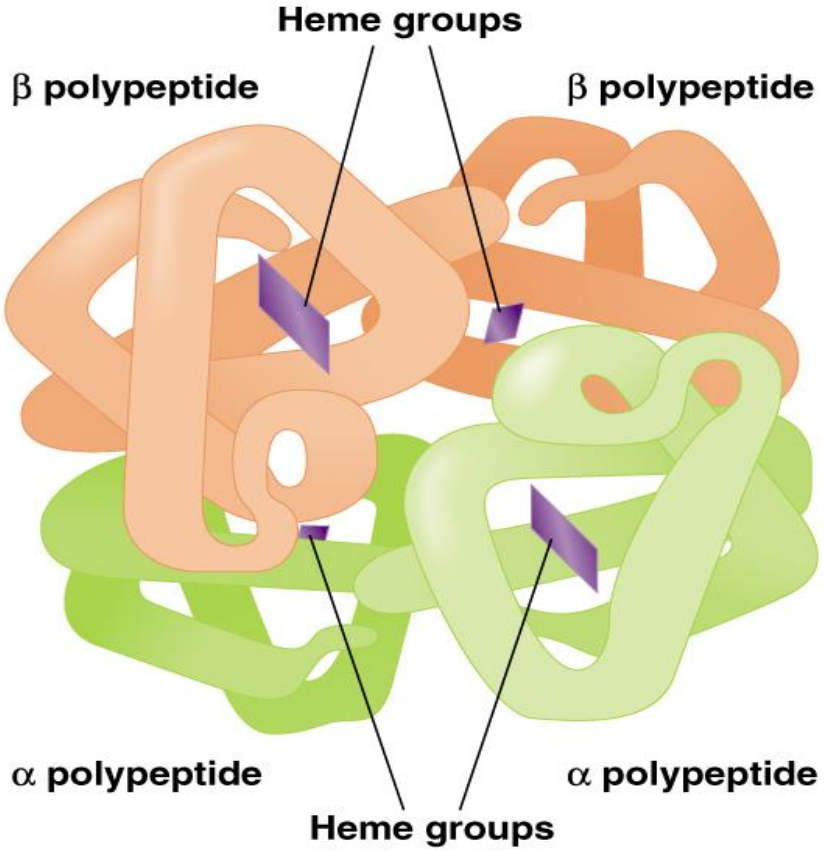
Rh Blood Group Systems

- Rhesus (Rh) antigen is also important for transplantation and in newborn infants
- Expressed with D, C, E antigens identified by Rh30 polypeptide and Rh50 glycoprotein
- Immunoglobulins develop against erythrocytes of Rh (D +) babies carried by Rh (D-) mothers.
- These antibodies cause erythroblastosis fetalis by hemolizing the erythrocytes of the baby in the second pregnancy.

- Administration of anti-D antibodies (RhoGAM) to the mother during pregnancy and after parturition destroys any circulating Rh(D+) fetal erythrocytes that persist in the mother's blood, thus preventing Rh-incompatibility reactions in future pregnancies.

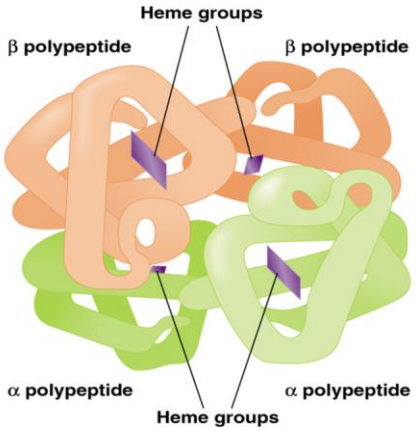
Hemoglobin

- A specialized protein for transport, by binding oxygen and carbon dioxide
- The erythrocytes contain 33% dissolved hemoglobin surrounded by cell membranes.
- It is found more intense close to the cell membrane for gas exchange
- It makes erythrocytes stain acidophilic with eosin.
- Hemoglobin is a natural colored compound protein.
- It is made of globulin, a colorless protein, and 4 *Heme* molecules attached to it.



- Hemoglobin molecule has 4 globulin complexes
- The globulin complexes are formed by the binding of α, β, δ or γ globulin to a group of iron-containing heme groups in the form of a polypeptide subunit pair.
- Hemoglobin can bound reversibly to oxygen and forms oxyhemoglobin, and forms carbominohemoglobin when bound to carbon dioxide.

- Hemoglobin forms an **irreversible** bond with carbon monoxide (carboxyhemoglobin). In this case, the oxygen carrying capacity of the blood is reduced. It is seen in cases of asphyxia during fire, suffocation from the chimney and stove.



In humans, only 3 forms are considered normal in postnatal life.

Types of hemoglobin

HbA is found in 97% of normal adults. It carries two beta chains.

- HbA ⟶ 2α + **2 beta chains**, 2α,2β

HbA_2 is found 2%. Carries two delta chains.

- HbA_2 ⟶ 2α + **2 delta chains**, 2α,2δ

- HbF ⟶ 2α + **2 gama chains**, 2α,2γ

HbF is present in 1% of healthy adults.
HbF is 80% in the newborn. However, this goes down to the normal adult level at about 8 years old.
It carries two gamma chains.

Although 550 types of abnormal hemoglobin molecule types have been identified, the majority of these do not cause any clinical conditions.

Hemoglobin A_1c

- Hb is one of the 4 subtypes of A (HbA1a1, HbA1a2, HbA1b, and HbA1c)
- The most important feature is the irreversible binding of the glucose molecule
- Measurement of HbA1c values, also called glycosylated hemoglobin, allows the determination of one's blood sugar levels 2-3 months ago.
- HbA1c levels should not exceed 7% of total hemoglobin
- Fasting is not needed during measurement

Thalassemia

It is an autosomal recessive disorder characterized by abnormal Hb production.

Symptoms: Anemia, splenomegaly, dark urine, yellow skin, growth retardation

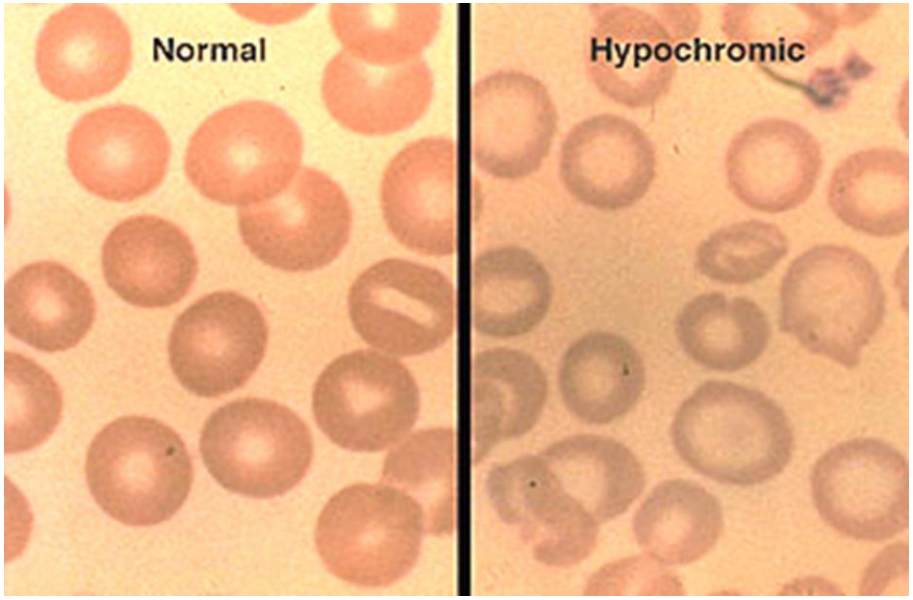
Named according to the affected genes (alpha or beta globülin) and prognosis varies depending on how many genes are affected.

Treatment: Blood transfusions, iron-binding therapy, folic acid, splenectomy, bone marrow transplantation

Sickle cell anemia

- Because each polypeptide chain is controlled by a gene, a single gene disorder causes abnormal type Hb synthesis.
- For example; HbS is an abnormal form of HbA and is found in sickle cell anemia patients.
- 1 amino acid in the beta chain is different (valine in HbS, glutamine in HbA).
- Unlike HbA, HbS becomes insoluble at low oxygen pressure and crystallizes into inflexible rods.
- This deforms the erythrocytes, and the erythrocytes characteristically turn into sickle shape.
- Rigid cells cannot adapt to narrow passages (thin capillaries) because they are not flexible like normal erythrocytes.
- They cause obstruction and tearing of the capillaries.
- This results in a further decrease in the number of oxygen-carrying erythrocytes, leading to anemia.

- The changes in diameter and shape of erythrocytes as well as the staining properties observed in peripheral blood smear are important criteria for the diagnosis of some diseases.
- The reduction of normal pinkish color of erythrocytes and the enlargement of the pale area in the middle is called hypochrome.
- Normal staining (indicating that erythrocytes carry a normal amount of Hb) is referred to as normochrome.
- Hypochromia indicates that erythrocytes contain less than normal Hb amount.

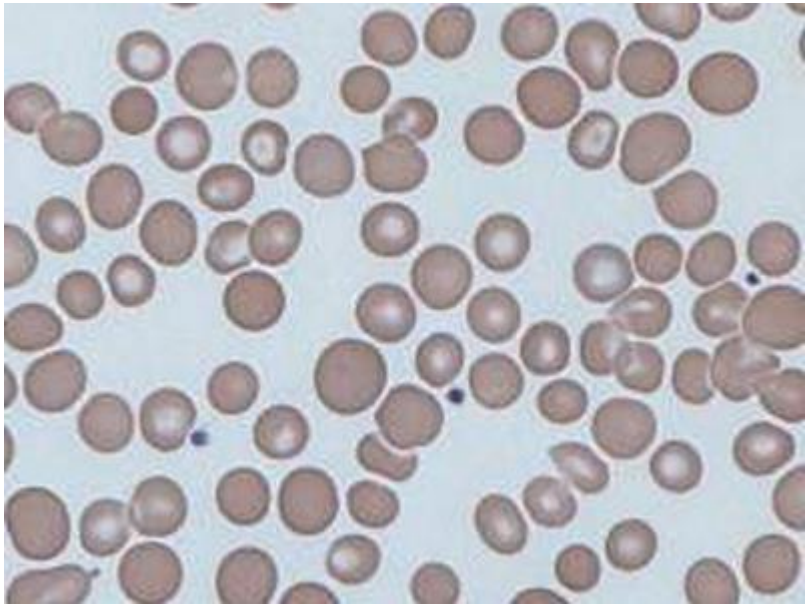


Change of diameter in erythrocytes

9 µm ↑ macrocytosis

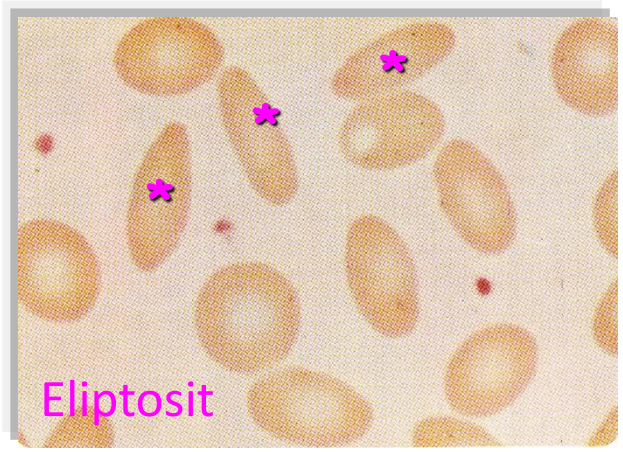
6 µm ↓ microcytosis

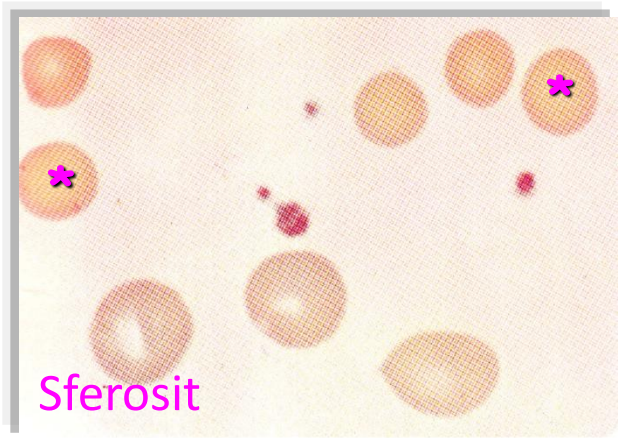
Anisocytosis: The difference between the dimensions of erythrocytes in peripheral smear (unequal size)

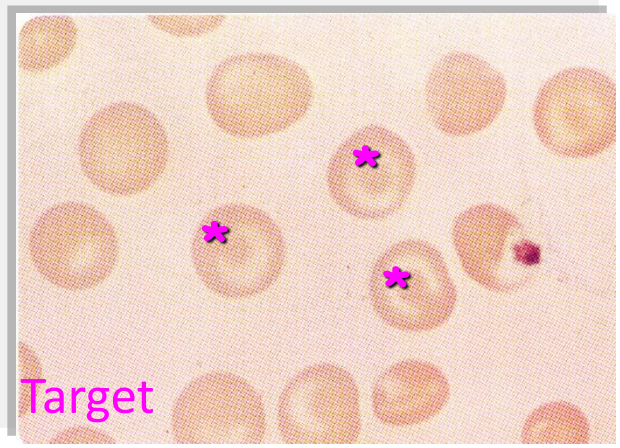


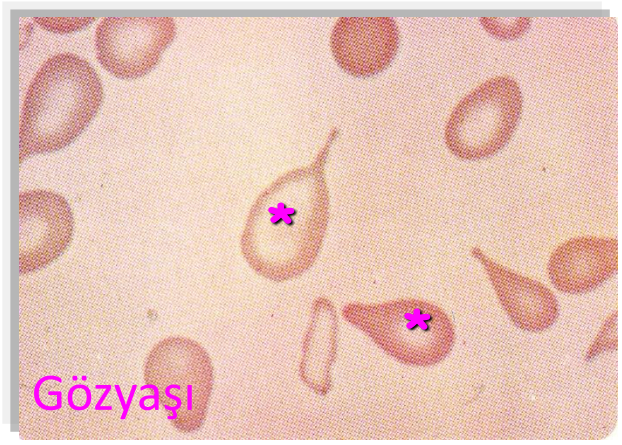
Variation in cell shape of erythrocytes = Poikilocytosis

Poikilocytosis: The presence of erythrocytes in different shapes in peripheral smear (cell skeletal disorder)









Leukocytes

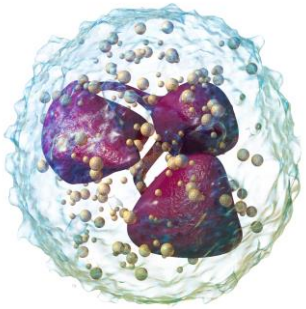
White Blood Cells-WBC

They are subdivided according to the presence or absence of specific granules.

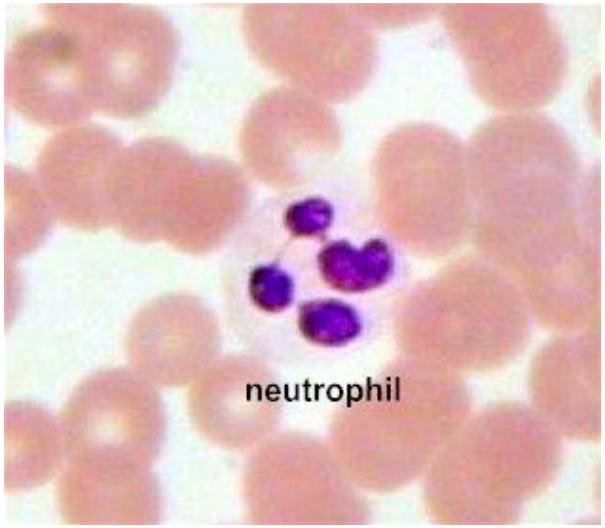
However, all leukocytes have non-specific azurophilic granules of lysosome structure.

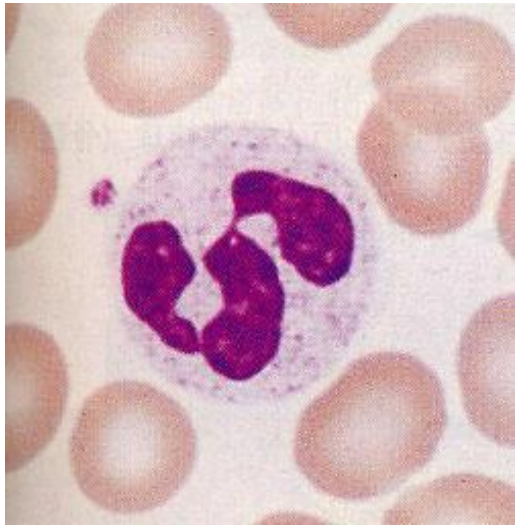
- Granulocytes: Neutrophils, Eosinophils, Basophils
- Agranulocytes: Lymphocytes, Monocytes

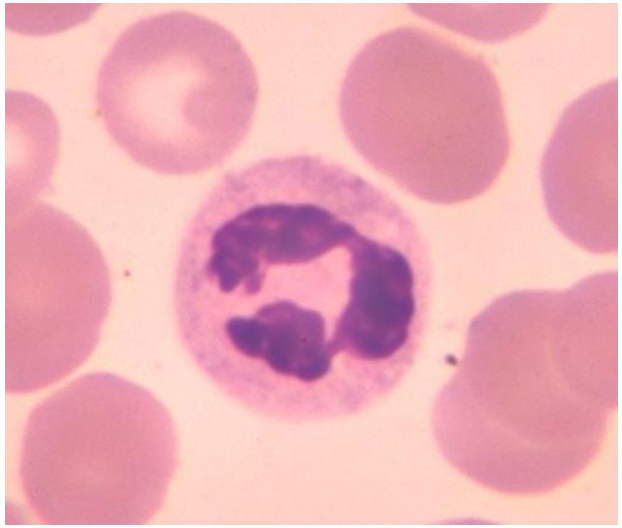
Neutrophils



- They are the largest number of leukocytes, they are 10-12 µm in diameter.
- Since they have no characteristic staining in their cytoplasm, they are named neutral.
- Because their nuclei are multi-lobed, they are also called polymorphonuclear neutrophils (PMN / PNL).
- The Barr body (named after discoverer Murray Barr) in which the inactive X chromosome in females, is concentrated and stored can be detected in the PMN leukocyte nucleus (small **drumstick**-like projection).


neutrophil





Granules of neutrophils

- Specific (*secondary*) granules
  - They are the smallest granules
  - Their number is more than double the azurophilic granules
  - They are difficult to observe under the light microscope, they are monitored in the form of an ellipse with EM
  - They contain enzymes (type IV collagenase, phospholipase) and complement activators.
- Azurophilic (*primary*) granules
  - They are larger but fewer
  - They are bactericidal lysosomes containing myeloperoxidase
  - They also contain acid hydrolase, defensin (helps antibodies), cathelicidin (antimicrobial)
- Tertiary granules
  - There are two types: those containing phosphatases that remove a phosphate group from a molecule and metalloproteinases such as gelatinase, collagenase, which wash away connective tissue and helps migration into the tissue.

Motile

- Neutrophils have migration - movement abilities and are the first cells to reach the area after any tissue damage.
- Circulating neutrophils express adhesion molecules on their surface under the action of cytokines, and these molecules match their counterparts on the endothelium and slow them down, allowing them to enter into the connective tissue.

Neutrophils' surface		Endothelial cells of the postcapillary venule
Sialyl Lewis^x (s-Le^x) carbohydrates	⟷	**E-selectin and P-selectin** (types of cell adhesion molecules)

initial phase – the recognition
partially tethered to the endothelial cell, slow down and roll on the surface of the endothelium

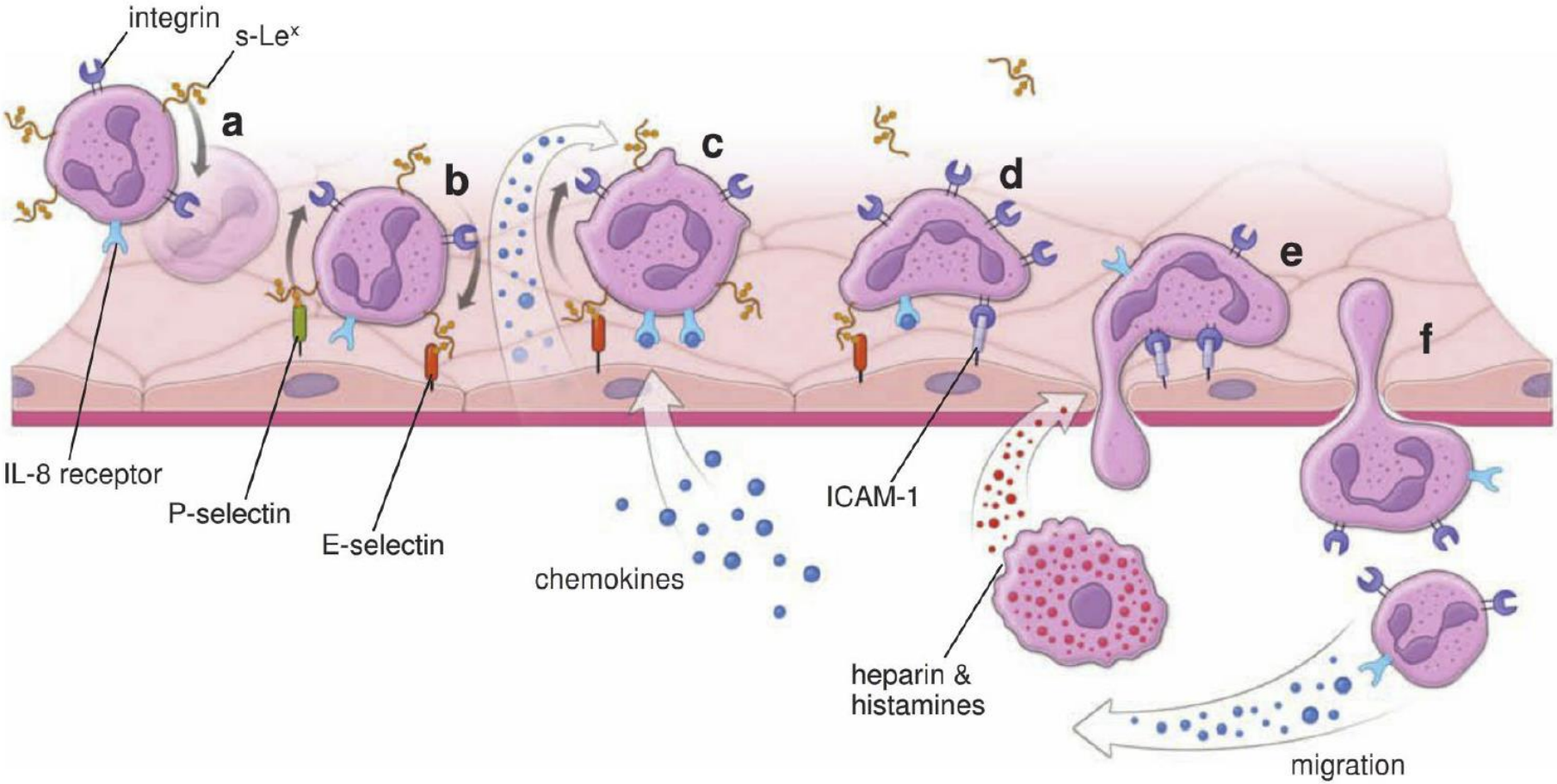
IL-8 receptors	⟷	**chemokines – IL-8**

adhesion
expression of other adhesion molecules on the neutrophil, such as integrins (VLA-5)

Integrins (VLA-5)	⟷	**ICAM-1** (intercellular adhesion molecule-1) (immunoglobulin superfamily of adhesion molecules)

second phase - provide tight bonds – firm adhesion

third phase (Diapedesis) - extends a pseudopod to an intercellular junction previously opened by histamine and heparin released from the mast cells in the connective tissue, allowing the neutrophil to migrate through the vessel wall.


integrin
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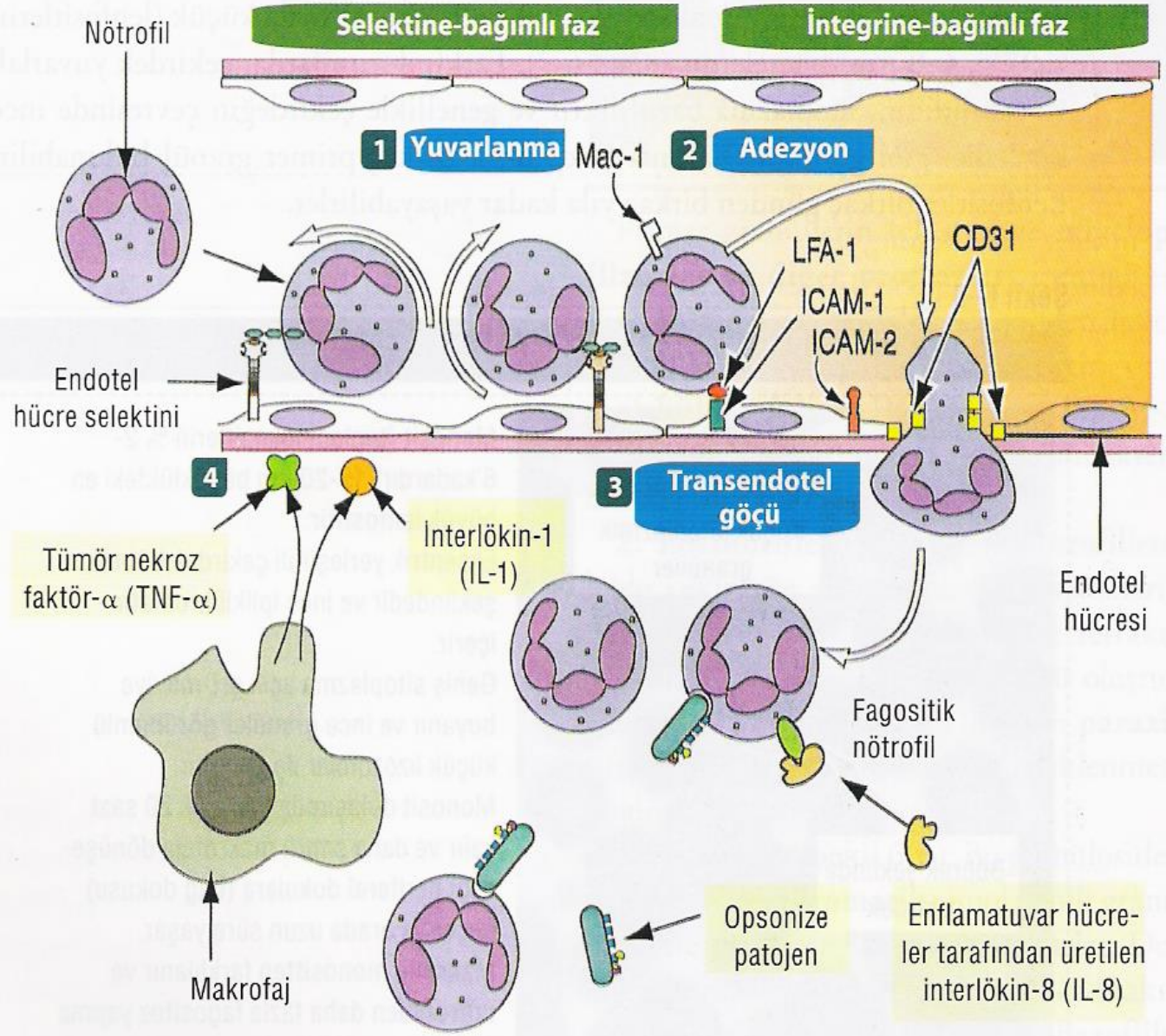
chemokines
ICAM-1
heparin &
histamines
migration

Homing ve inflamasyon



1 Yuvarlanma ve bağlanma
Lökositler (şekilde nötrofil) endotel hücre yüzeyinde bulunan selektinler ve nötrofil yüzeyinde bulunan karbonhidrat ligandları ile geri dönüşümlü bir bağlanma meydana getirirler. Bu bağlanma güçlü değildir ve hücre yuvarlanmasına devam eder.

2 Adezyon (yapışma)
Nötrofil ve endotel (yapışma) hücre arasında güçlü bir etkileşim meydana gelir. Bu etkileşim endotel üzerindeki intersellüler adezyon molekülleri **ICAM-1 ve ICAM-2** ile **Mac-1** ve **LFA-1 integrinleri** (lenfosit fonksiyonuna-bağlı antijen) aracılığı ile olur. ICAM-1 inflamasyon varlığında salınır.

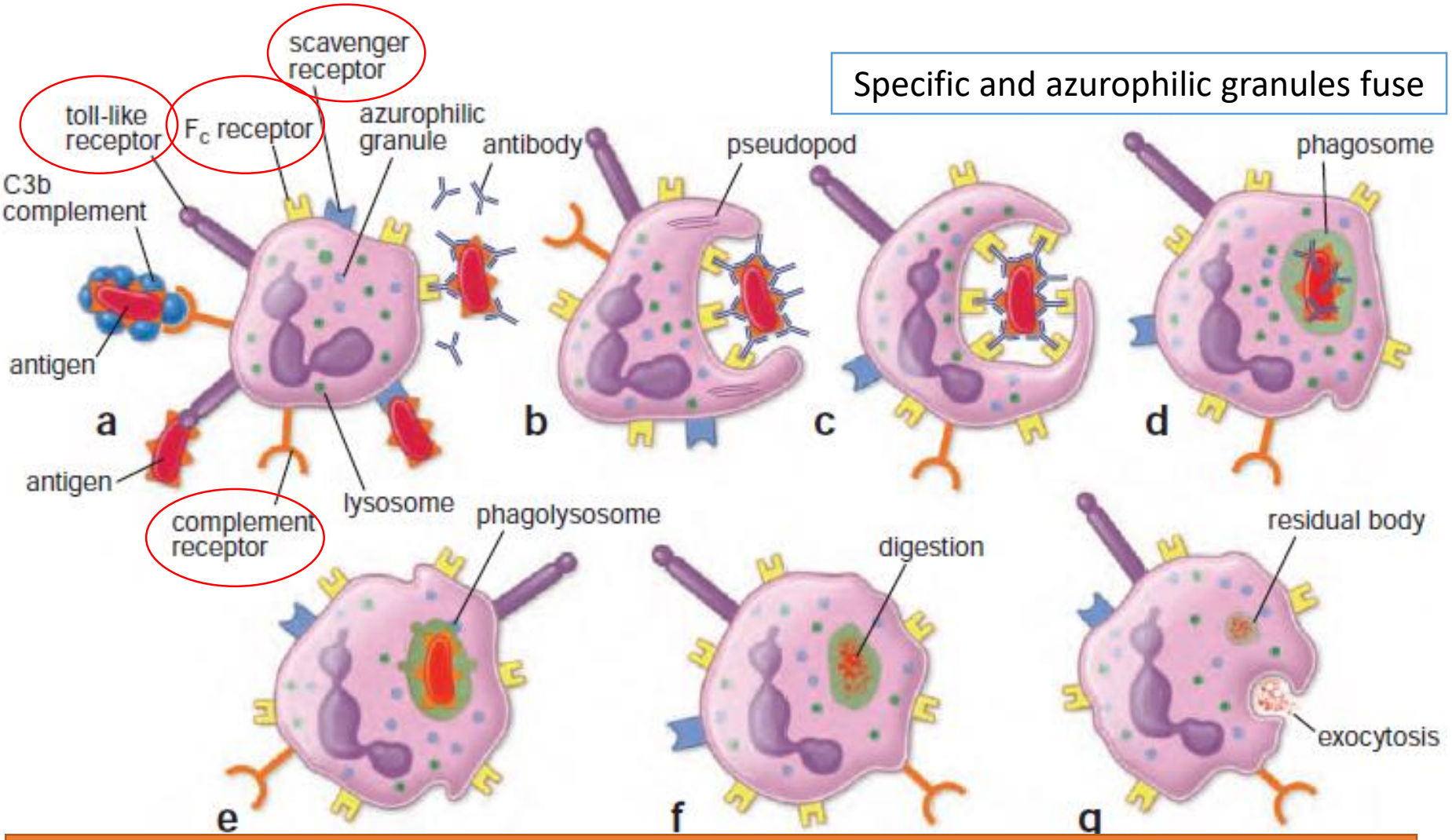
3 Transendotel göçü
T hücresi enflamatuar hücreler tarafından üretilen **IL-8** konsantrasyonunun artışı ile endotelden göç eder. **CD31** diapedezi kolaylaştırır.

4 Aktive olmuş makrofajlar endotel hücrelerden selektinlerin salgılanmalarını uyaran **TNF-α ve IL-1**'i salgılarlar.

Active phagocytosis

- Neutrophils are cells involved in active phagocytosis mediated by their surface receptors.
- Bacteria and pathogens can be attached directly to neutrophils, as well as they may need to be opsonized (coated with antibodies or complement) to make them more attractive to the neutrophil.
- After binding, phagocytosis and secretion of various cytokines take place: Interleukin-1 (IL-1), interleukin-3 (IL-3) and Tumor necrosis factor alpha (TNF-α)
- IL-1 plays a thermoregulatory role in fever by creating prostaglandins and act on hypothalamus

Fc receptor → Fc region of IgG
CR → binds complement C3b
SR → Gr +/- bacteria



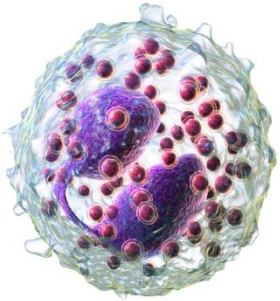
Specific and azurophilic granules fuse

- reactive oxygen species and free radicals → Oxygen-dependent intracellular killing
- defensins and antimicrobial peptides called cathelicidins→ oxygen-independent killing

Pus

the accumulation
of dead bacteria and dead neutrophils
constitutes the thick exudate called **pus.**
The yellow—green color of the
pus comes from the heme pigment of
myeloperoxidase enzyme in azurophilic
granules of neutrophils.

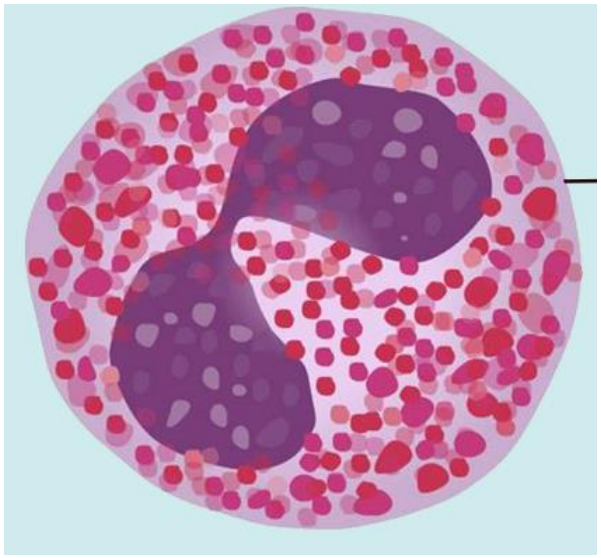
Eosinophils

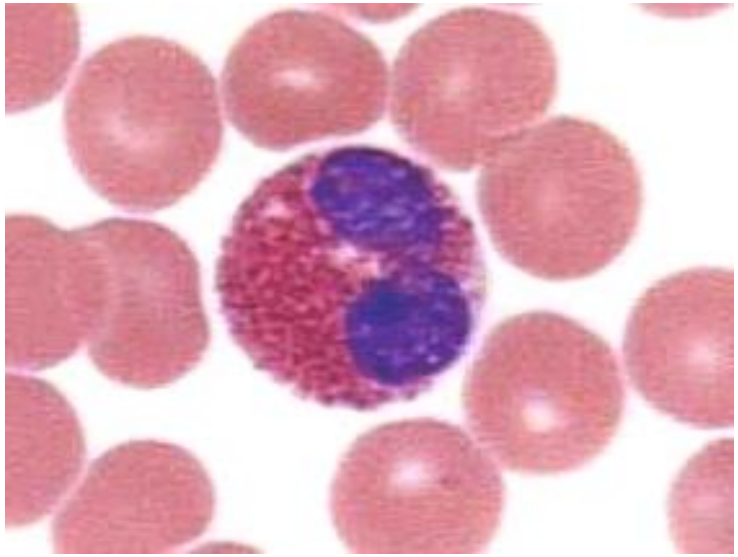


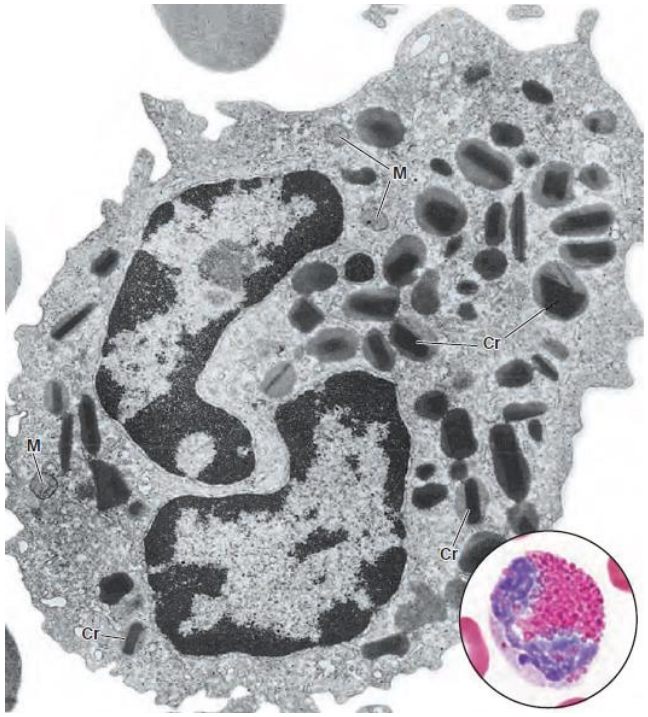
- Its dimensions are neutrophil-sized, and its nuclei have typically two lobes (*bi-lobed*).
- They contain large, eosinophilic, refractile granules in their cytoplasm
- Large, elongated specific granules
  - They contain crystalloid body that can be seen with TEM (refractivity in the light microscope)
    - Major basic protein (inside crystalloid body), eosinophil cationic protein, eosinophil peroxidase, eosinophil-derived neurotoxin
    - Toxic activity against parasites (protozoa and helminths)
    - Neurotoxin against parasites
    - Histaminase, arylsulphatase, collagenase, cathepsin
- Azurophilic granules (lysosomes)
  - Lysosomal acid hydrolases

Functions of eosinophils

- They take part in the allergic reactions and defense of parasitic infections (protozoans and helminthic parasites)
- After production in bone marrow, they move into the connective tissue from blood and get activated with IgG, IgA and secretory IgA.
- The count of eosinophils in blood samples of individuals with allergies and parasitic infections is usually high (eosinophilia).
- Abundant in the lamina propria of lung and intestines and in patients with asthma
- Participates in other immunologic responses and phagocytoses antigen-antibody complexes



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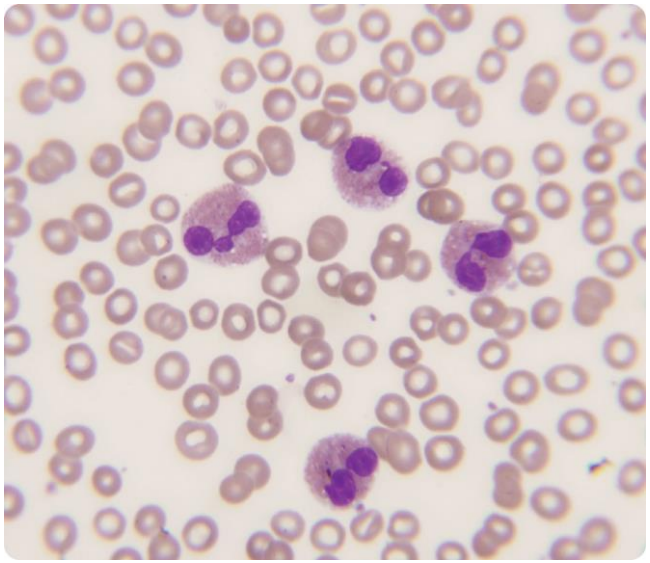
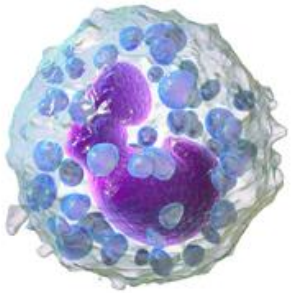


Basophils



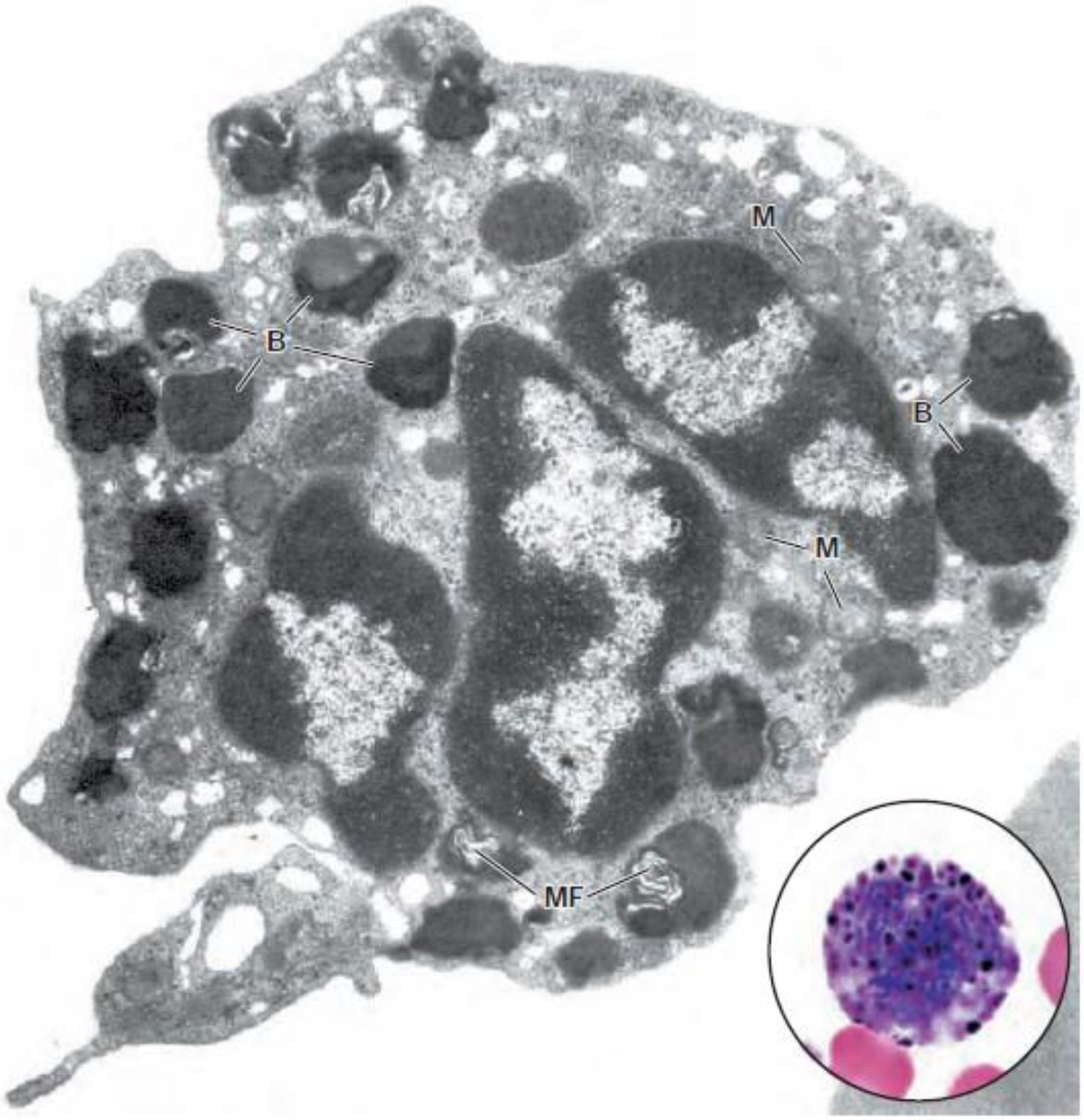
- They are cells of the same size as neutrophils, with a large number of basic stained granules.
- They are the least numerous white blood cells, up to 0.5% of the total leukocytes.
- It has a lobed, hardly selected nucleus due to dense granules in stained blood smears
- The basophil plasma membrane possesses numerous high-affinity **Fc receptors** for IgE antibodies.
- In addition, a specific protein **CD 40L** is expressed on the basophil's surface.
- CD40L interacts with a complementary receptor (CD40) on B lymphocytes, which results in increased synthesis of IgE.

Granules of basophils

- Specific granules
  - Granules are larger than neutrophil's
  - They contain heparin, histamine, heparan sulfate, leukotrienes, IL-4, and IL-13.
  - Anticoagulant, vasodilation, smooth muscle contraction, stimulation of IgE synthesis
  - Dark staining property is caused by dense sulfate groups in heparin and heparan sulfate.
- Azurophilic granules
  - Lysosomal acid hydrolases

Functions of basophils

- functionally related to, but not identical with, mast cells of the connective tissue
- Both mast cells and basophils bind an antibody secreted by plasma cells, **IgE,** through high-affinity Fc receptors expressed on their cell surface. Exposure to a specific antigen (allergen) instantly or later in life triggers the activation of the basophil and release of vasoactive substances from granules
- Responsible for hypersensitivity reactions and anaphylaxis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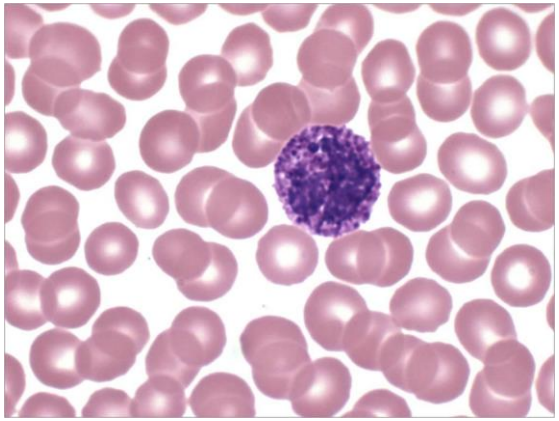
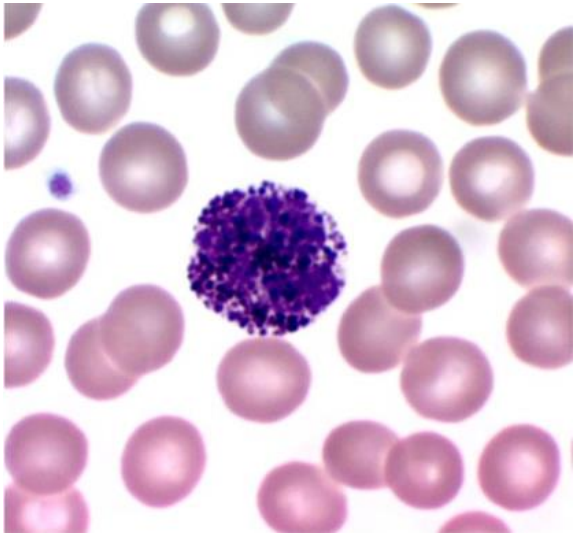
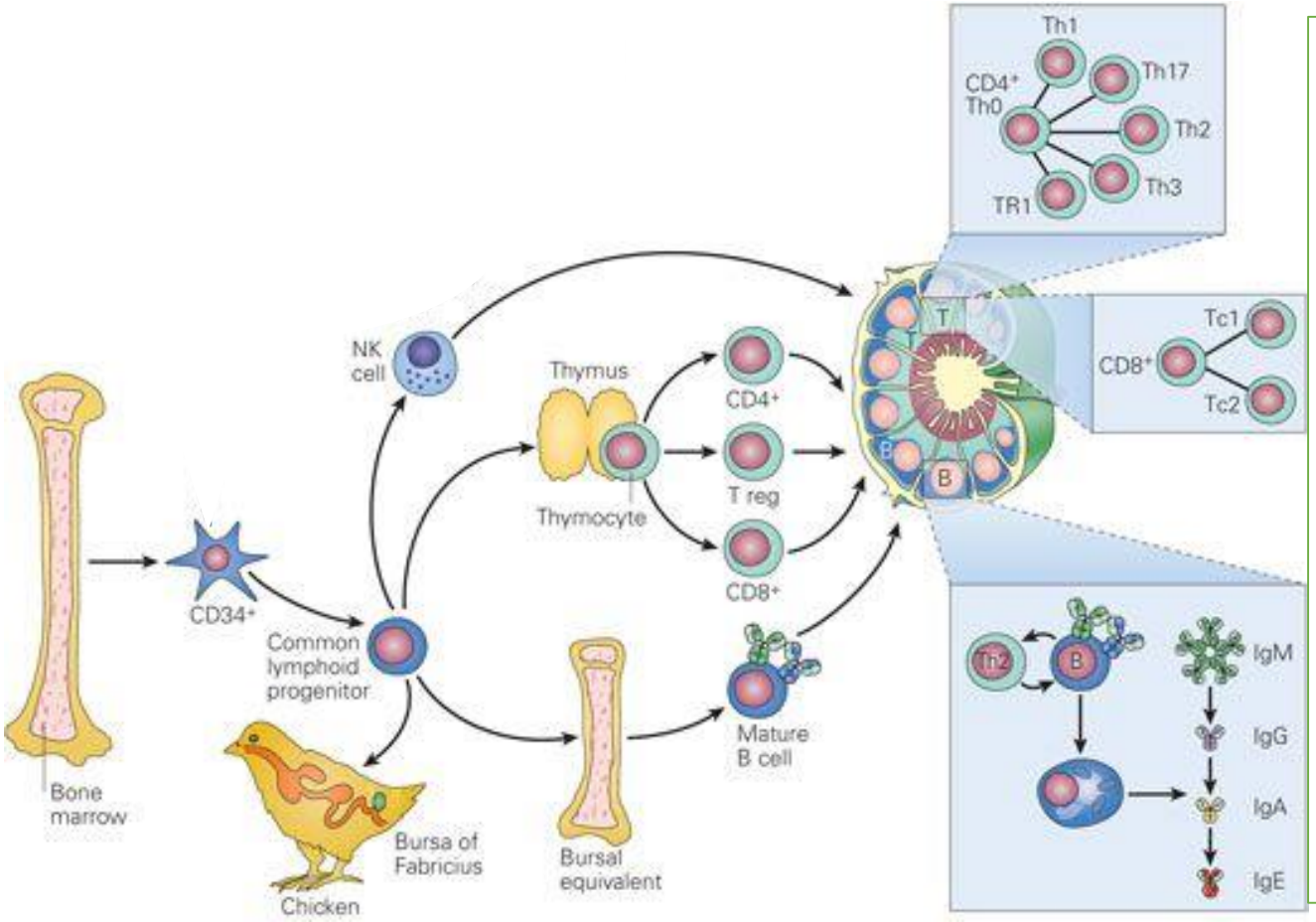




Lymphocytes

- They are the basic cells of the lymphatic and immune system
- Constitutes 30% of all blood leukocytes
- Lymphocytes circulating in the blood and lymph fluid represent immunocompetent cells (capable of recognizing and reacting antigens) in transit from one lymphatic tissue to another.
- Three types of lymphocytes are defined according to their size in the immune system: small, medium and large (activated lymphocytes or natural killer (NK) cells).
- 6-30 µm

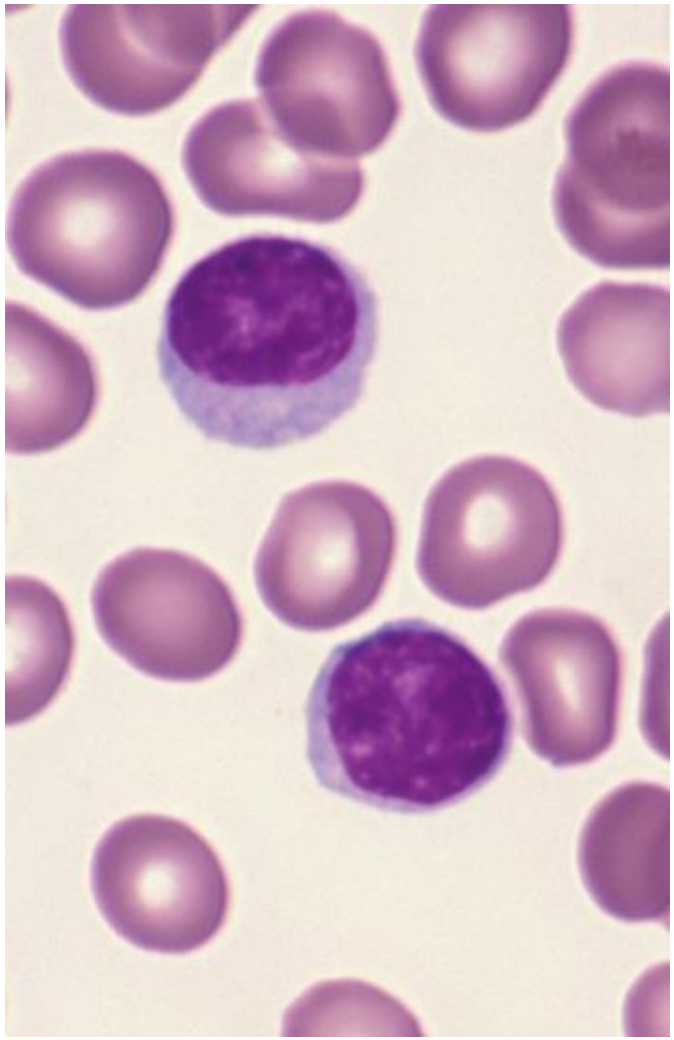
Origins of Lymphocytes

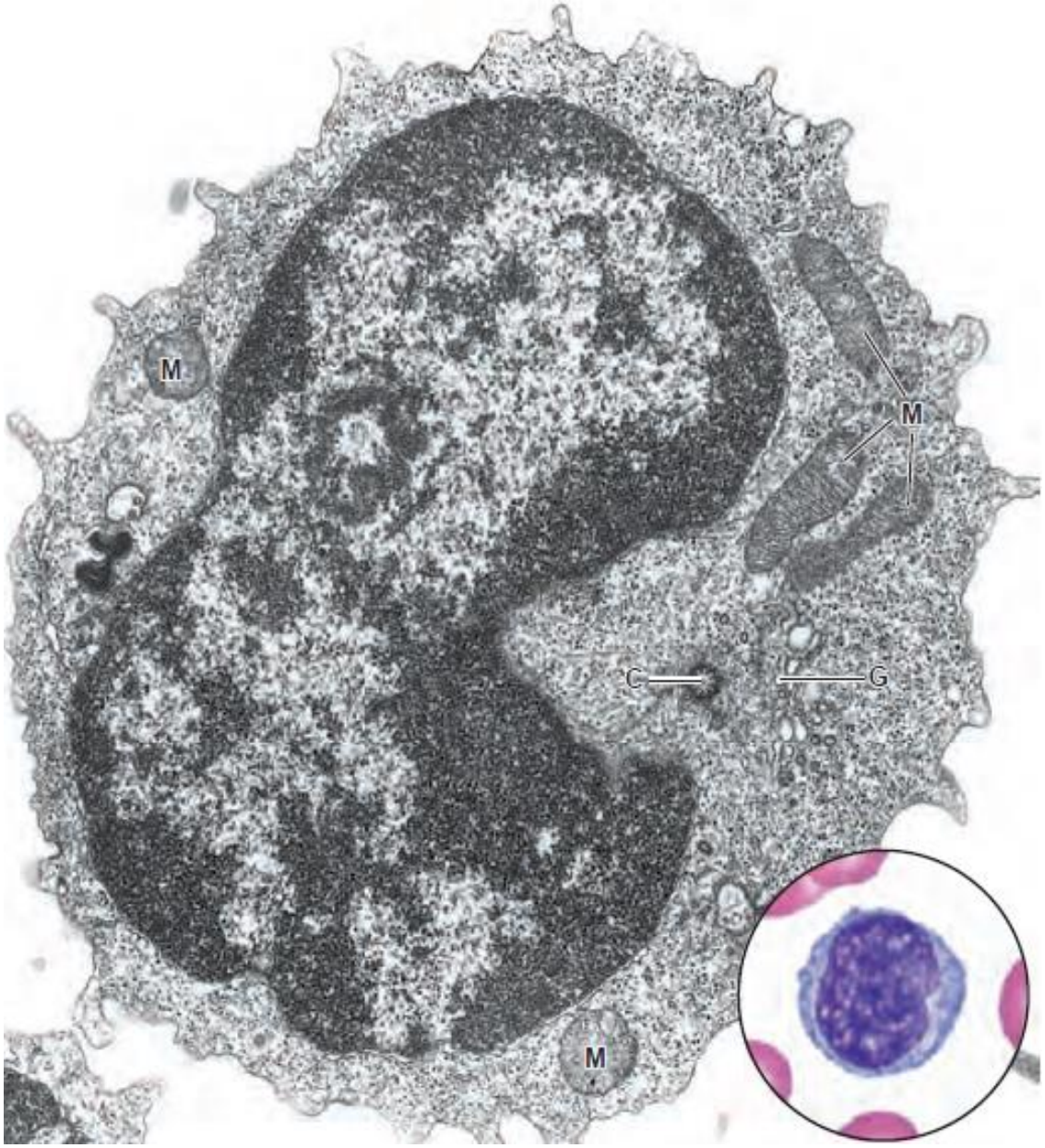


- not terminally differentiated cells
- capable of undergoing divisions and differentiations into other types of effector cells
- originate in the bone marrow, lymphocytes are capable of developing outside the bone marrow in tissues associated with the immune system

Morphology

- Small lymphocytes are the 90% of the circulating lymphocytes and they are around the size of an erythrocyte.
- They have an intensely staining, slightly indented, spherical nucleus almost filling the cytoplasm
- The cytoplasm appears as a very thin, pale blue rim surrounding the nucleus

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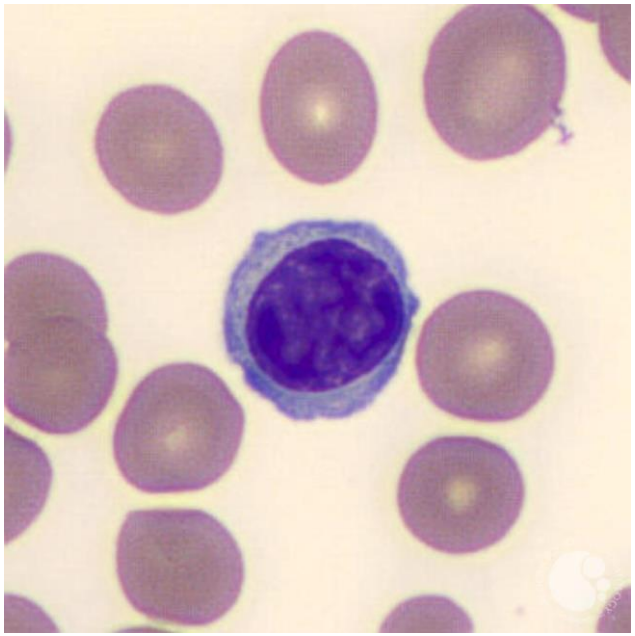


Three functionally distinct types of lymphocytes are present in the body

1. T lymphocytes (matured in Thymus)
2. B lymphocytes (matured in Bursa fabricius in birds and Bone marrow in mammals)
3. NK cells

CD: Cluster of Differentiation
a protocol used for the identification and investigation of cell surface molecules providing targets for immunophenotyping of cells

Type of cell	CD markers
stem cells	CD34+, CD31-, CD117
all leukocyte groups	CD45+
Granulocyte	CD45+, CD11b, CD15+, CD24+, CD114+, CD182+[16]
Monocyte	CD4, CD45+, CD14+, CD114+, CD11a, CD11b, CD91+,[16] CD16+[17]
T lymphocyte	CD45+, CD3+
T helper cell	CD45+, CD3+, CD4+
T regulatory cell	CD4, CD25, FOXP3 (a transcription factor)
Cytotoxic T cell	CD45+, CD3+, CD8+
B lymphocyte	CD45+, CD19+, CD20+, CD24+, CD38, CD22
Thrombocyte	CD45+, CD61+
Natural killer cell	CD16+, CD56+, CD3-, CD31, CD30, CD38

T-Lymphocytes

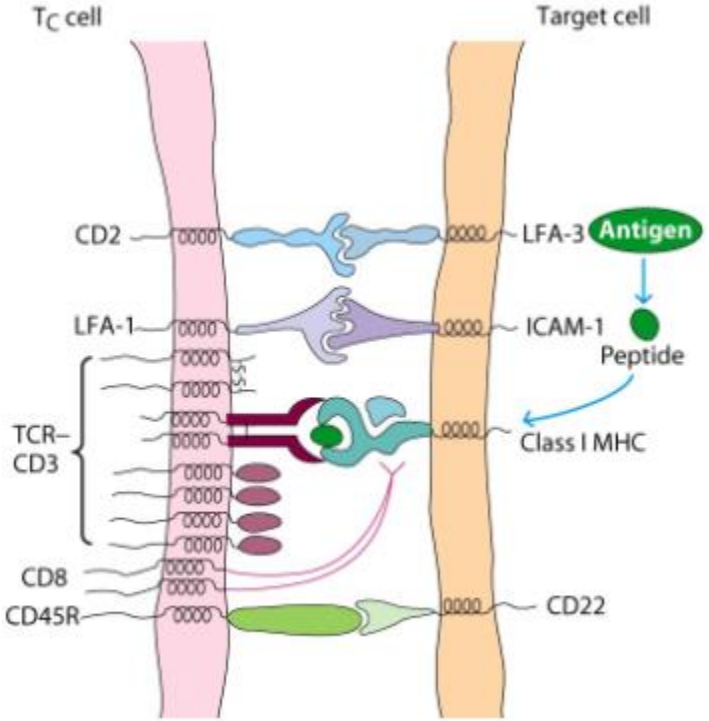
- They are responsible for cell-mediated immunity
- They are recognized by cell surface receptors called TCR (α and β glycoprotein chain)
- Express the CD2, CD3, CD5, CD7 markers on their surfaces.
- There are cytotoxic, helper, suppressor and gamma/delta types.
- However, the main functional distinction depends on CD4 and CD8 expressions.
  - CD4+ T_{helper} lymphocytes: recognize antigens presented with MHC-II
  - CD8+ $T_{cytotoxic}$ lymphocytes : recognize antigens bound to MHC-I

MHC: major histocompatability complex

MHC-I molecules are expressed by all nucleated cells

$CD8^+$ T-lymphocytes

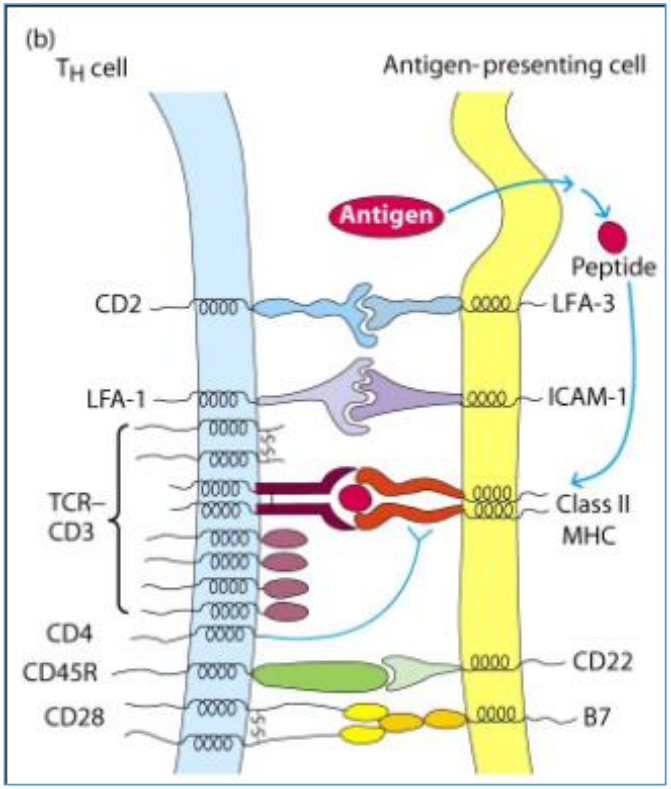
- It is the main effector cell of cell-mediated immunity
- Sensitive to foreign, virus-infected and neoplastic cell surface antigens



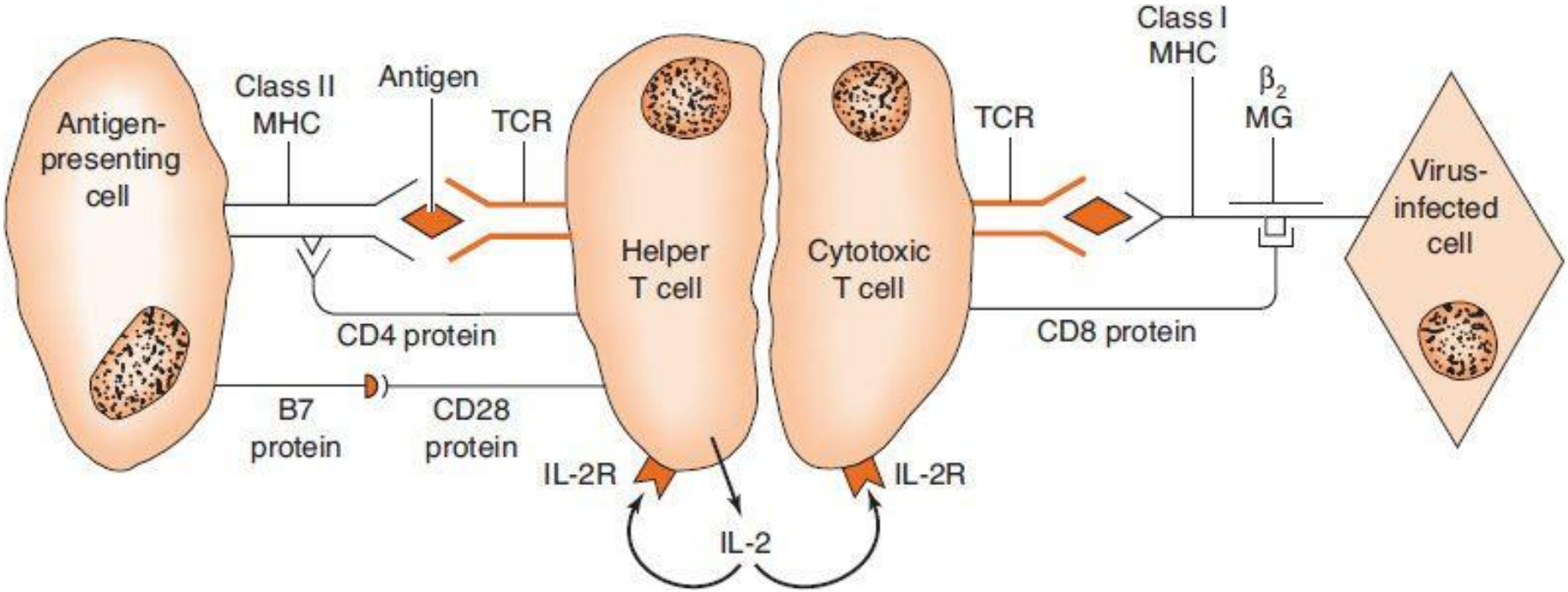
- When TCR binds to the MHC-I-antigen complex, Tc cells secrete substances called lymphokines and perforin, causing lysis in enemy cells, killed by membrane damage.
- Cytotoxic T lymphocytes play a major role in organ and tissue rejection and tumor immunity.

$CD4^+$ T-lymphocytes

- Important in initiating an immune reaction response to a foreign antigen



- Antigen bound to MHC II molecules is presented by «antigen-presenting cells» such as macrophages
- Activated helper CD4 T-lymphocytes produce interleukins (IL-2) to stimulate more T-lymphocytes and to differantiate B-lymphocytes into plasma cells for antibody production


Antigen-presenting cell
Class II MHC
Antigen
TCR
Helper T cell
Cytotoxic T cell
TCR
Class I MHC
β_2 MG
Virus-infected cell
CD4 protein
CD8 protein
B7 protein
CD28 protein
IL-2R
IL-2R
IL-2

Other T cells

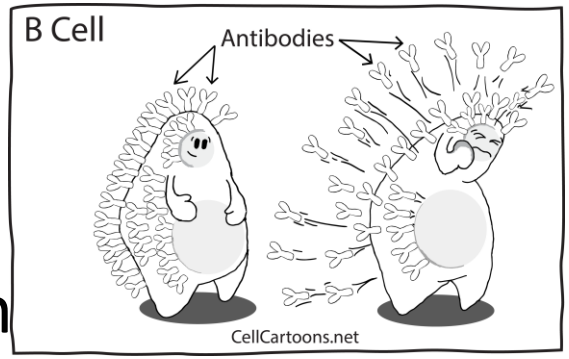
Regulatory / Suppressor T cells

- Their function is to suppress the immune system
- $CD4^+$, $CD25^+$, $FOXP3^+$ regulatory T cells
- $CD8^+$, $CD45RO^+$ suppressor → secretes IL-10

Gamma/delta T cells

- It is a group of T lymphocytes that go to the epithelial tissues (skin, oral mucosa, vagina, intestine) after being matured in the thymus.
- They are thought to form the first line of immune defense

B cells

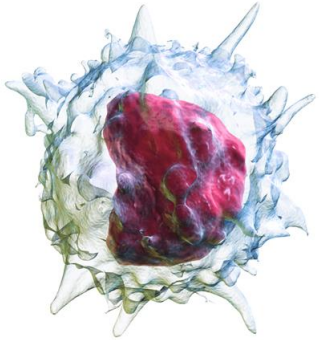


- Responsible for antibody production
- Mature B cells in the blood express IgM, IgD and MHC-II molecules on their surface
- Specific markers are CD9, CD19, CD20, CD24

NK cells

- Programmed to kill virus-infected cells and malign cells
- They secrete an antiviral substance: interferon γ
- Also referred to as large granular lymphocyte (LGL)
- (~ 15 µm)
- Specific markers are CD16, CD56, CD94

Monocyte

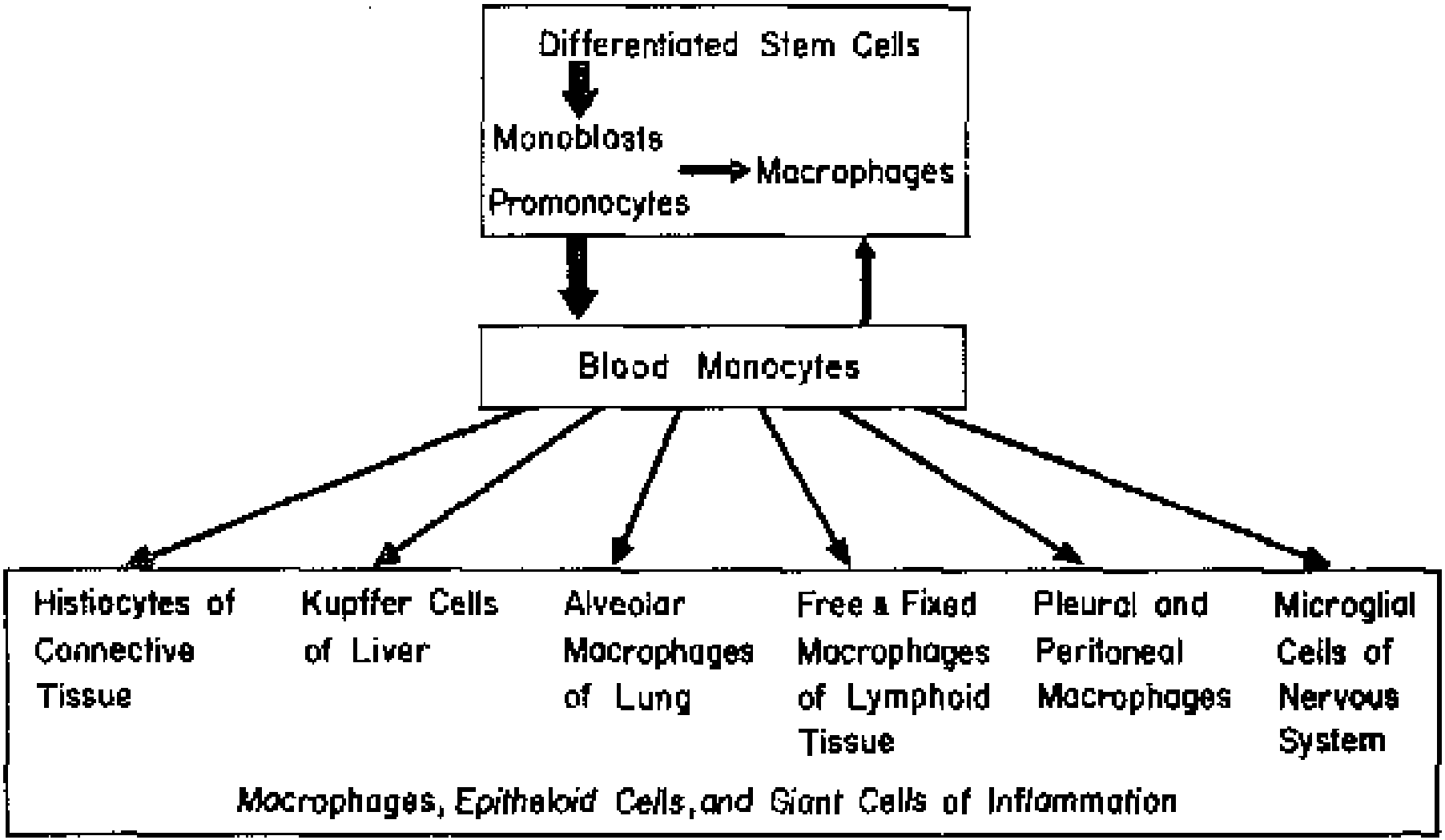


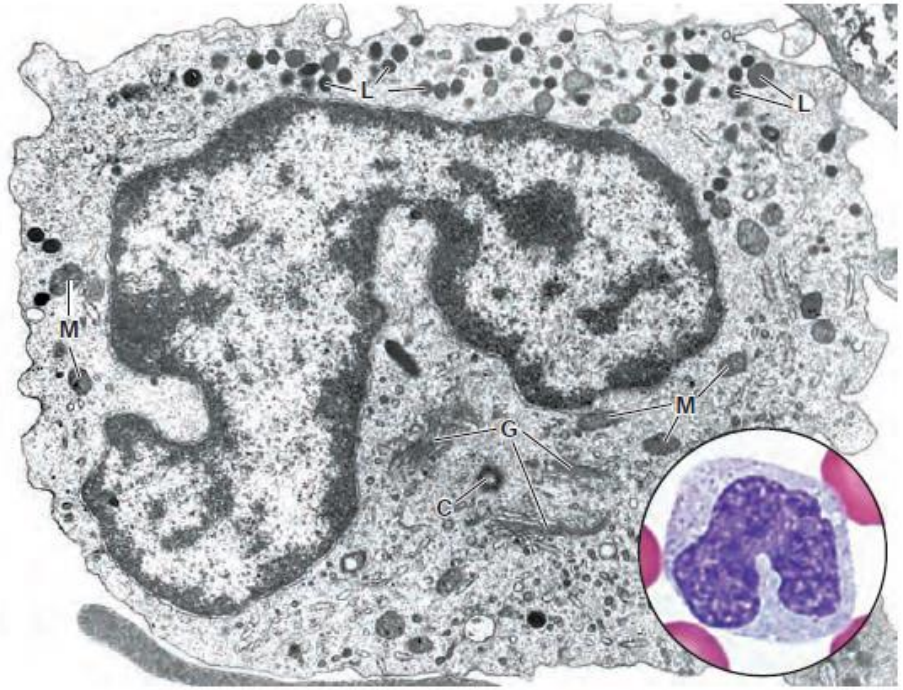
- They are the precursors cells of the **mononuclear phagocytotic system**
- They are the largest sized leukocytes in blood smear
- 18 µm
- After it is produced in the bone marrow, it remains in the circulation for 1-3 days and transits into the tissues, where it turns into cells responsible for phagocytosis: Mononuclear phagocytotic system:
  - Macrophages
  - Osteoclasts
  - Alveolar macrophage
  - Kupffer cells

Macrophages function as antigen-presenting cells in the immune system

professional antigen-presenting cells are **dendritic cells, macrophages and B cells**

mononuclear phagocytotic system

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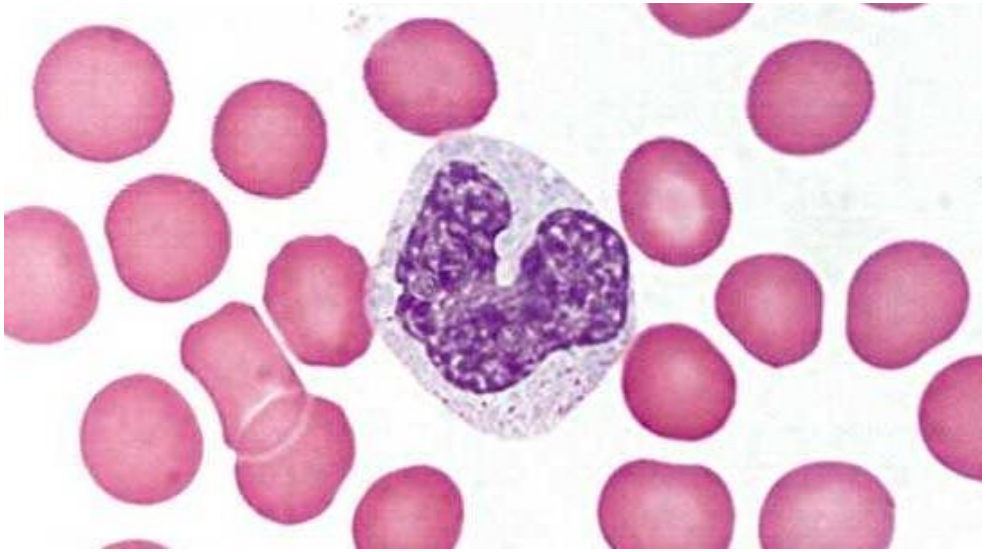
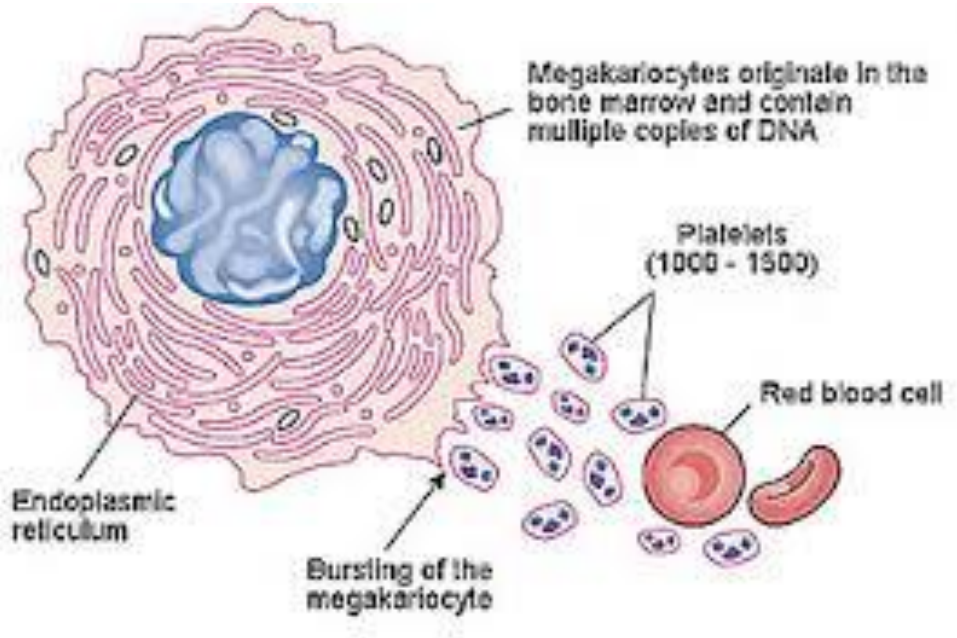
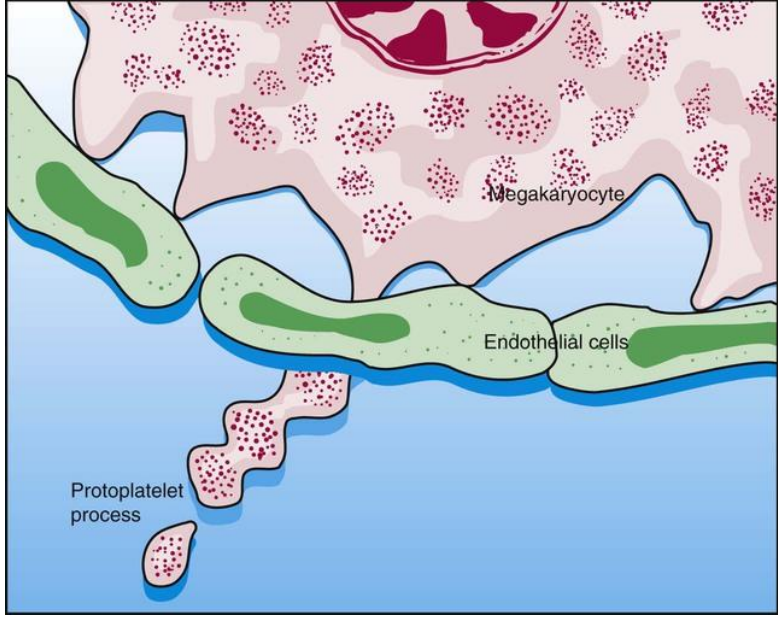


Thrombocytes = Platelets

- **Thrombocytes are small, membrane-bounded, anucleate cytoplasmic fragments derived from megakaryocytes**
- 150.000-400.000/mm^3 (2-4 µm sized)
- They are colorless, oval or biconvex disc shaped structures. Life expectancy in circulation is 8-10 days
- Platelets are formed under the control of thrombopoietin produced in liver and kidneys

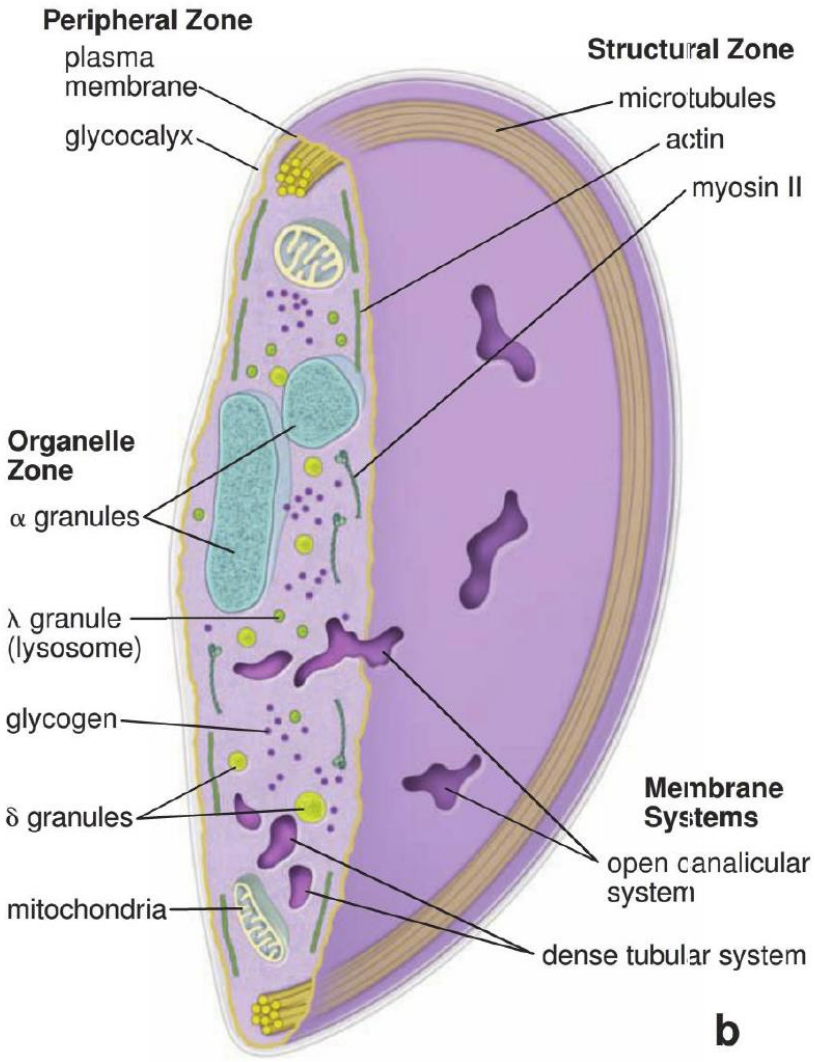


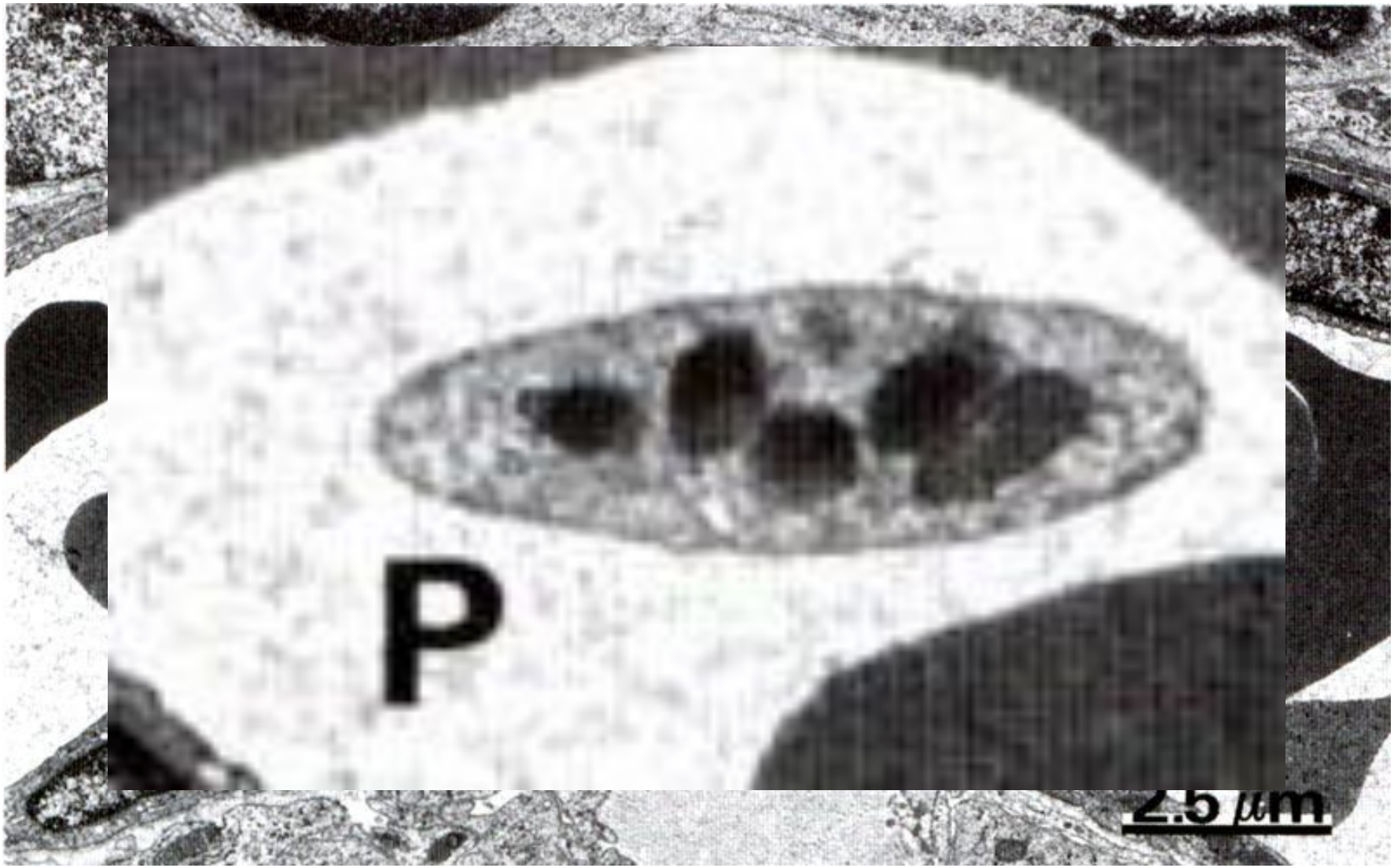


Formation: small bits of cytoplasm are separated from the peripheral regions of the megakaryocyte by extensive **platelet demarcation channels.** The breakdown of the megakaryocyte cytoplasm in the bone marrow and the surrounding of these parts by the membrane, cytoplasmic fragments form individual platelets

Platelet Morphology in 4 Zones

1. **Peripheral zone**
   - cell membrane covered by a thick surface coat of glycocalyx (=glycoproteins, GAGs, coagulation factors)
2. **Structural zone**
   - microtubules, actin filaments, myosin, and actin-binding proteins that form a network supporting the plasma membrane
3. **Organelle zone**
   - mitochondria, peroxisomes, glycogen particles, and at least three types of granules
4. **Membrane zone**
   - two types of membrane channels:
     - open canalicular system (OCS)
     - dense tubular system (DTS)
       - sER –Ca storage

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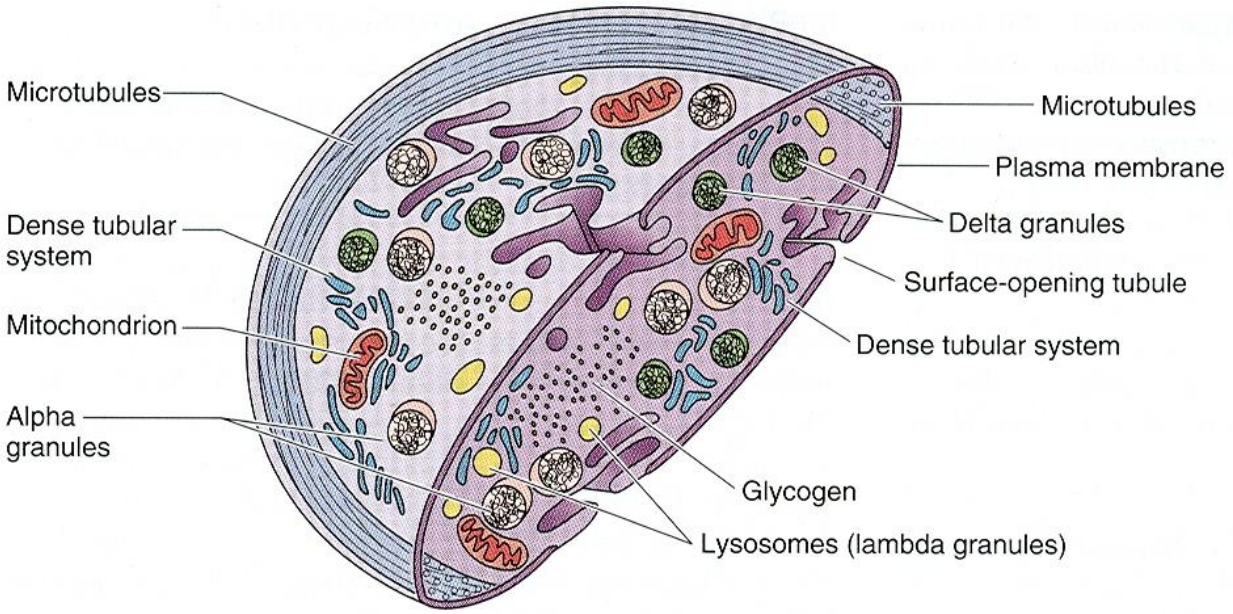
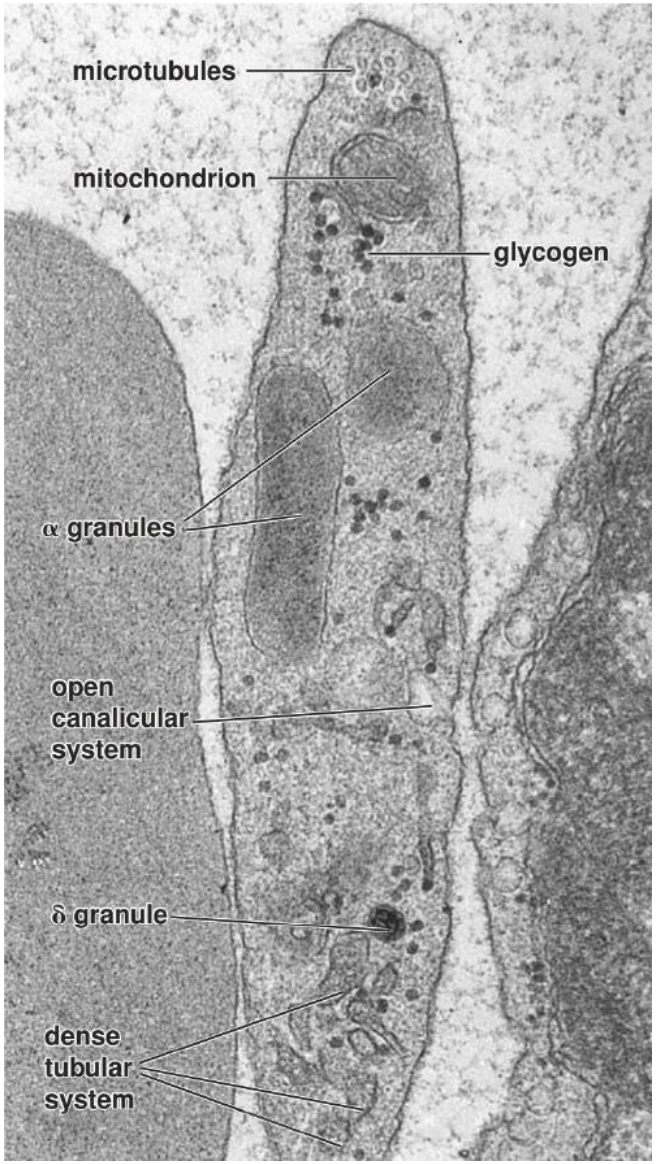
Organelle Zone

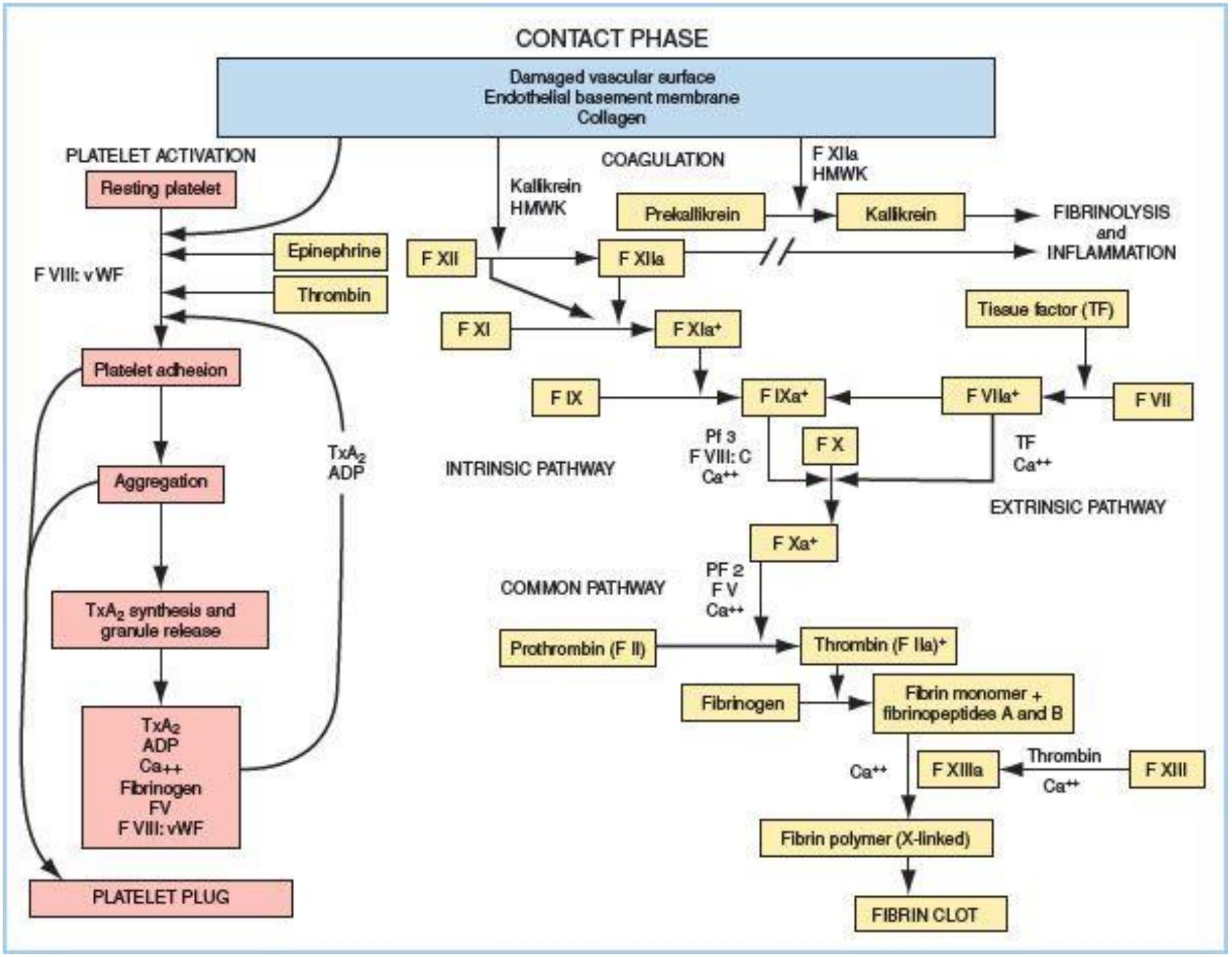
- The purple colored central part where the granules settle. There are few mitochondria, glycogen particles and granules of different properties (alpha, delta and lambda granules).



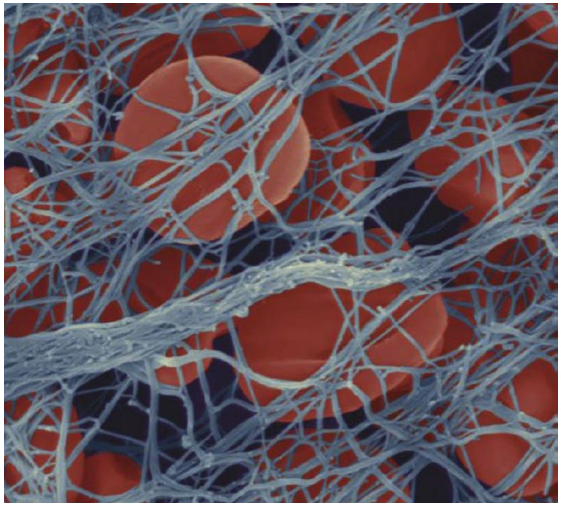
Granules of thrombocyte

- Alpha granules
  - 300–500 nm diameter, most numerous granules
  - contain mainly fibrinogen, coagulation factors, plasminogen, plasminogen activator inhibitor, and platelet-derived growth factor
  - vessel repair, blood coagulation, and platelet aggregation.
- Delta granules
  - 250–300 nm, smaller, denser, and less numerous
  - mainly contain adenosine diphosphate (ADP), adenosine triphosphate (ATP), serotonin, and histamine.
  - facilitate platelet adhesion and vasoconstriction
- Lambda granules (thrombocyte lysosomes);
  - 175–200 nm
  - contain several hydrolytic enzymes
  - function in clot resorption during the later stages of vessel repair




CONTACT PHASE
Damaged vascular surface
Endothelial basement membrane
Collagen
PLATELET ACTIVATION
Resting platelet
F VIII: vWF
Epinephrine
Thrombin
Platelet adhesion
Aggregation
TxA2 synthesis and granule release
TxA2
ADP
Ca++
Fibrinogen
FV
F VIII: vWF
PLATELET PLUG
TxA2
ADP
COAGULATION
Kallikrein
HMWK
F XIIa
HMWK
Prekallikrein
Kallikrein
FIBRINOLYSIS and INFLAMMATION
F XII
F XIIa
F XI
F XIa+
Tissue factor (TF)
F IX
F IXa+
F VIIa+
F VII
INTRINSIC PATHWAY
Pf 3
F VIII: C
Ca++
F X
TF
Ca++
EXTRINSIC PATHWAY
F Xa+
COMMON PATHWAY
PF 2
F V
Ca++
Prothrombin (F II)
Thrombin (F IIa)+
Fibrinogen
Fibrin monomer + fibrinopeptides A and B
Ca++
F XIIIa
Thrombin
Ca++
F XIII
Fibrin polymer (X-linked)
FIBRIN CLOT

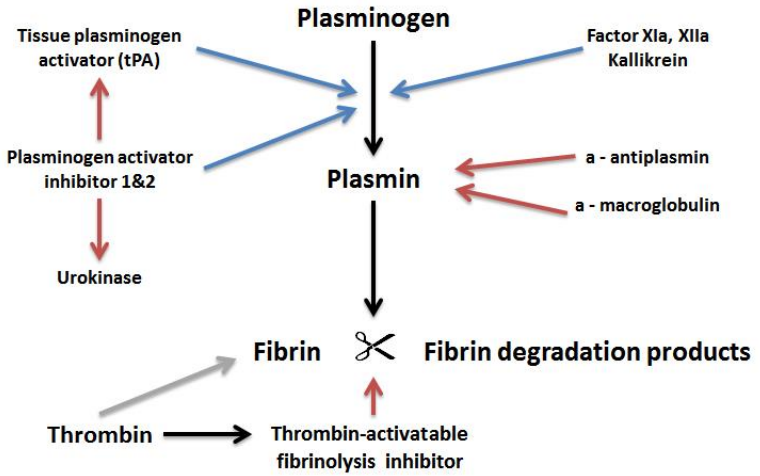
Platelet function



- **continuous surveillance of blood vessels, blood clot formation, and repair of injured tissue**.
- **hemostasis** (control of bleeding)

- Blood vessel wall injury → exposure to collegene (ECM) → platelet adhesion
- Triggering of degranulation → release of serotonin, ADP, **Thromboxane A2**
- **Aggregation, vasoconstriction → primary hemostatic plug**
- **Platelet thromboplastic factor (PTF3) and other coagulation factors**
- **Soluble fibrinogen → fibrin → loose mesh production → secondary hemostatic plug**

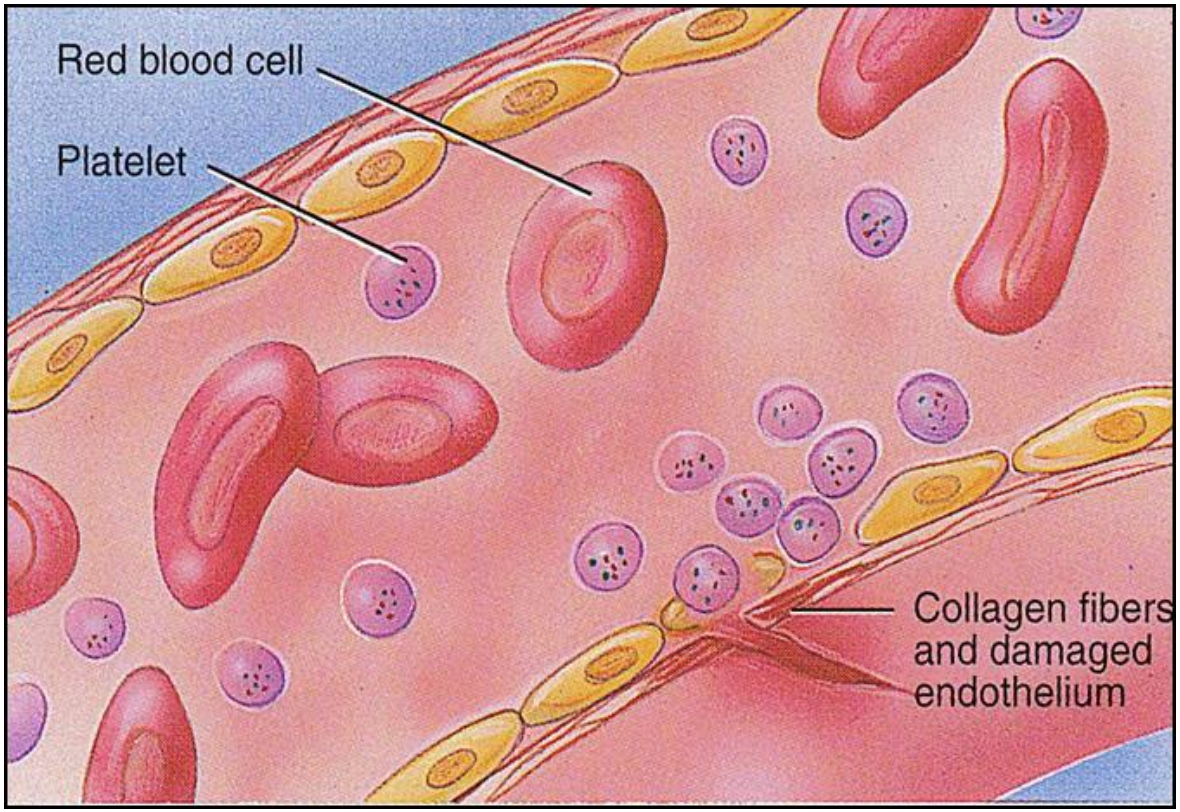
- **Actin and myosin in structural zone contraction → clot retraction**
- **Plasminogen → plasmin**
- **Lambda granules → lysosomal enzymes**
- **Endothelial cells → tissue plasminogen activator (TPA)**
- **Fibrinolysis**
- **Tissue repair – P-DGF**



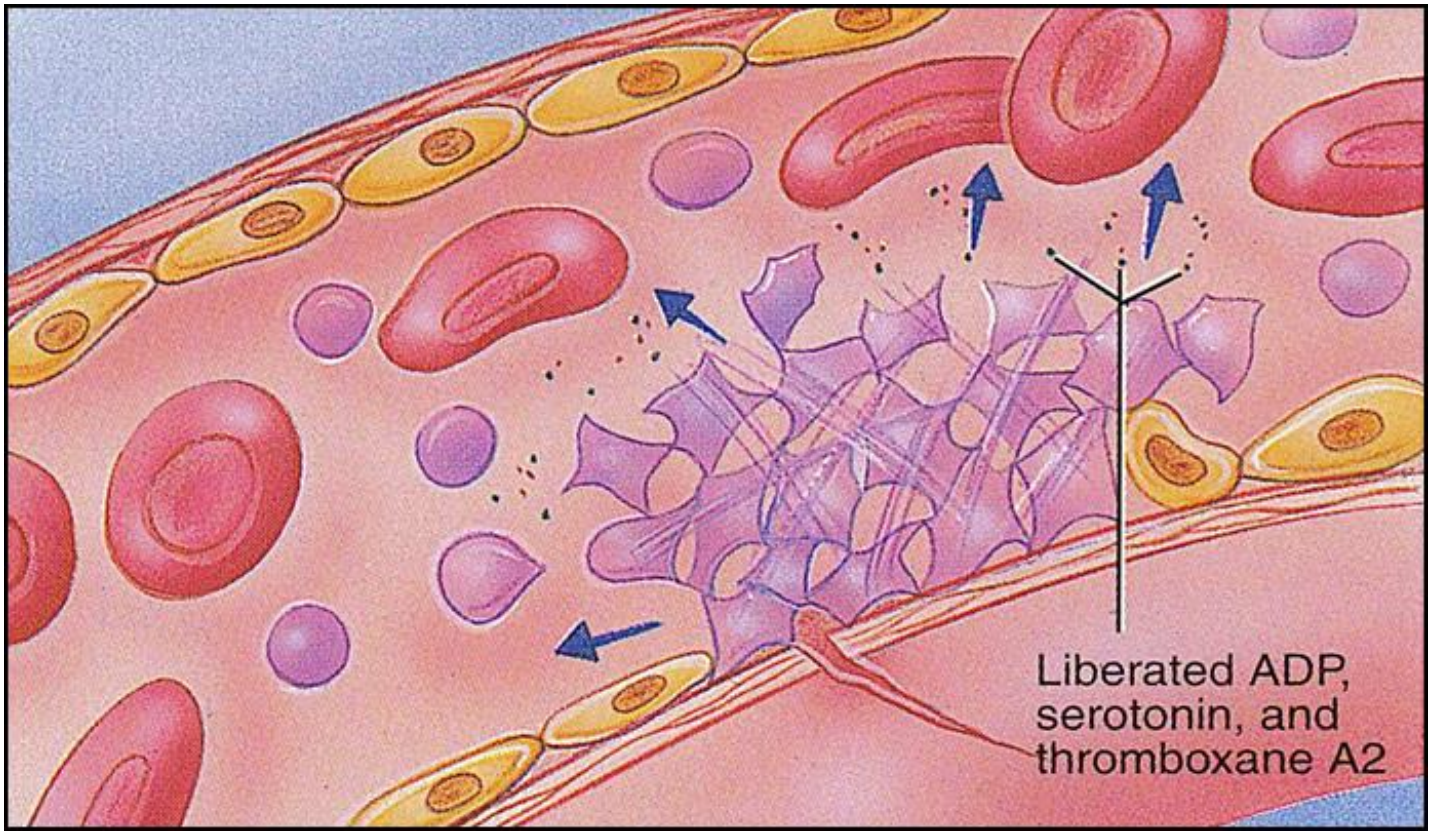
Fibrinolysis (simplified) - Blue arrows denote simulation, red arrows inhibition

1– Primary aggregation:

- Plasma proteins accumulate on the subendothelial collagen fibers, which are exposed in places where endothelial cells damage in blood vessels. Following this, platelets cluster on the damaged area and form thrombotic plugs.

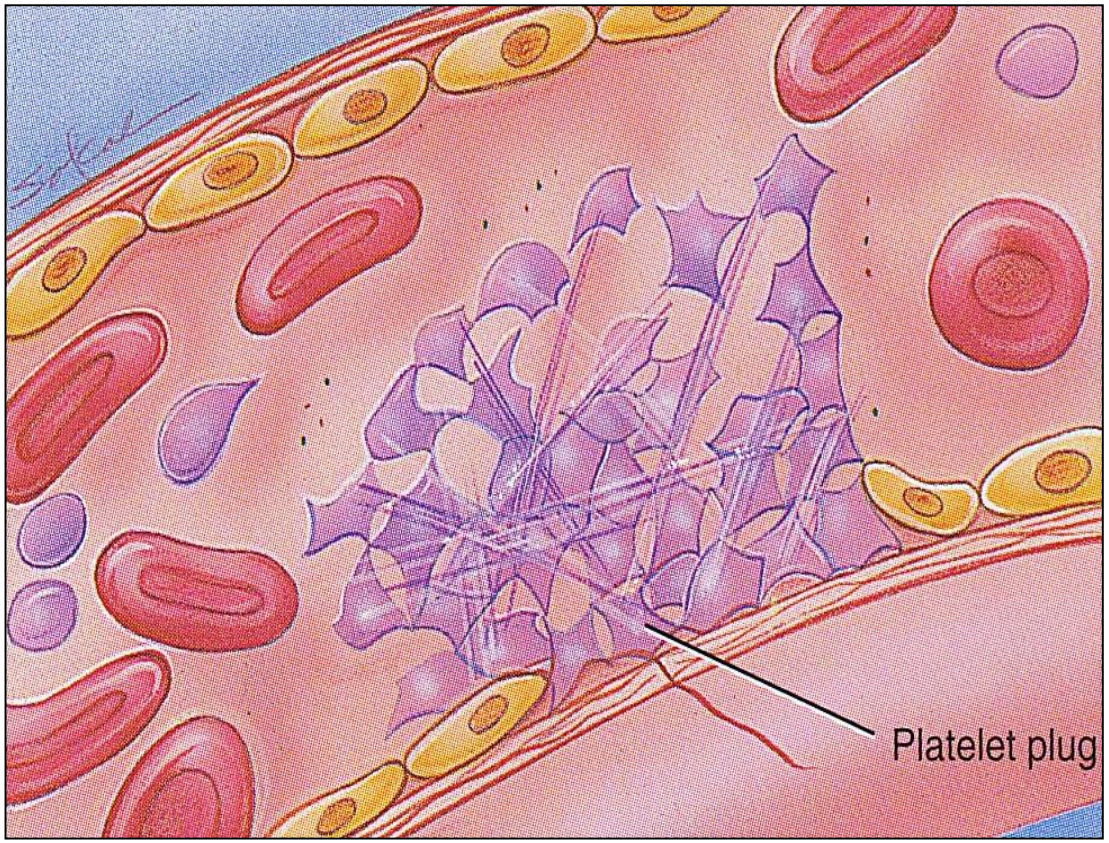


- 2– Secondary aggregation:
- Platelets in the thrombotic plug; empty the content of alpha and delta granules.
- Serotonin is a vasoconstrictor, causing contraction of smooth muscles in the vessel wall, blocking blood flow towards the damaged area

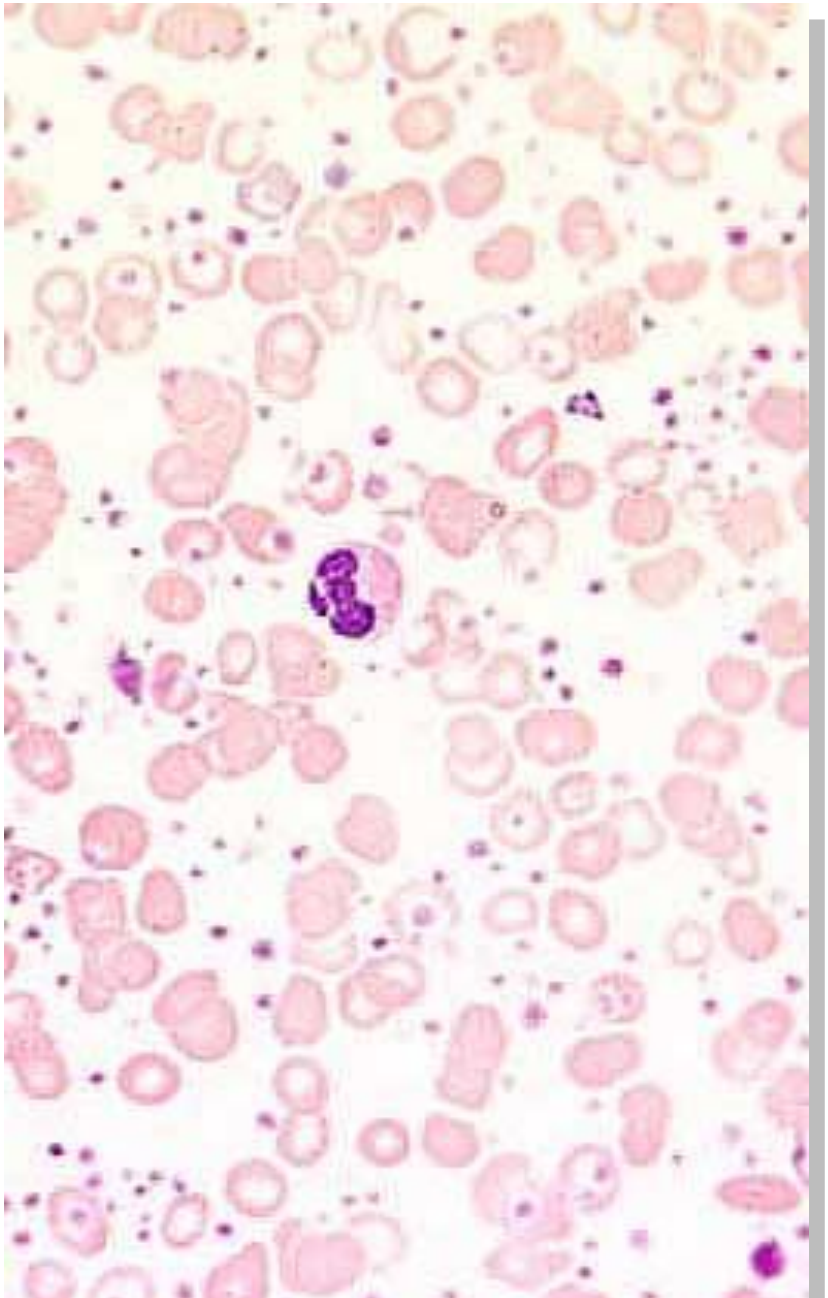


3– Coagulation:

- In addition to the fibrinogen normally found in plasma, platelets secrete additional fibrinogen.
- Thromboplastin converts protrombin to trombin and trombin converts fibrinogen to fibrin
- Fibrin forms a dense fibrous network where more platelets and other blood cells will attach, and the wound in the vascular wall is blocked.



Thrombocytosis



Thrombocytopenia