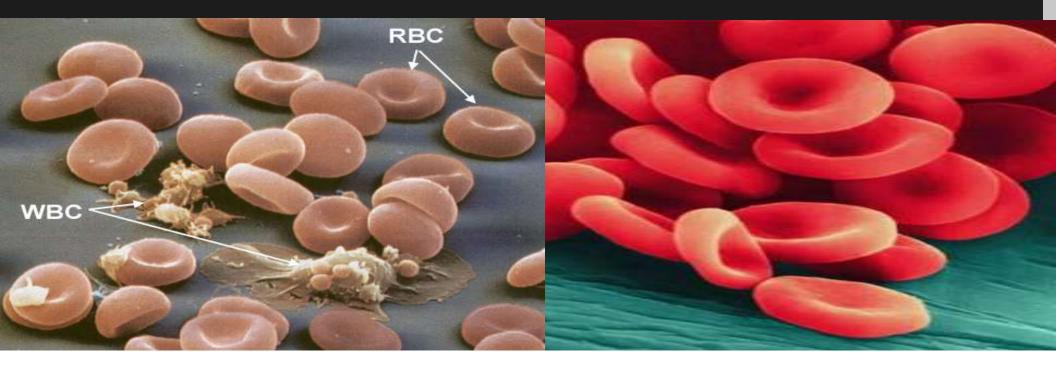
MICROSCOPY ANALYSIS OF BLOOD

Danil Hammoudi. MD



Steps in Blood Microscopy Analysis

1.Sample Collection

- 1. Blood is typically collected in an EDTA tube (to prevent clotting).
- 2. A drop of blood is placed on a glass slide.

2.Smear Preparation

- 1. A second slide is used to spread the drop across the first slide, creating a "feathered edge."
- 2. The smear is air-dried.

3.Staining

- 1. Common stains include **Wright**, **Giemsa**, or **Leishman** stain.
- 2. These help differentiate cellular components by color.

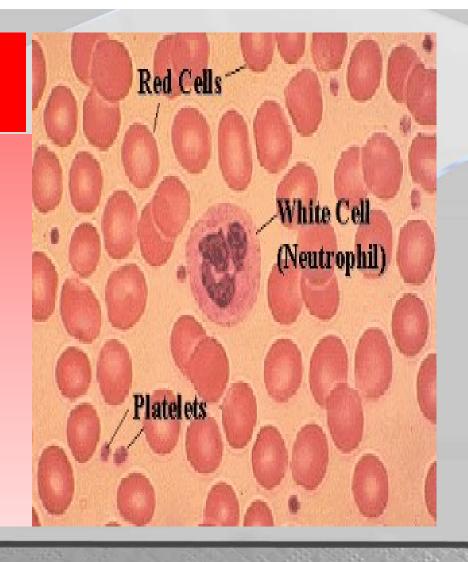
4.Microscopic Examination

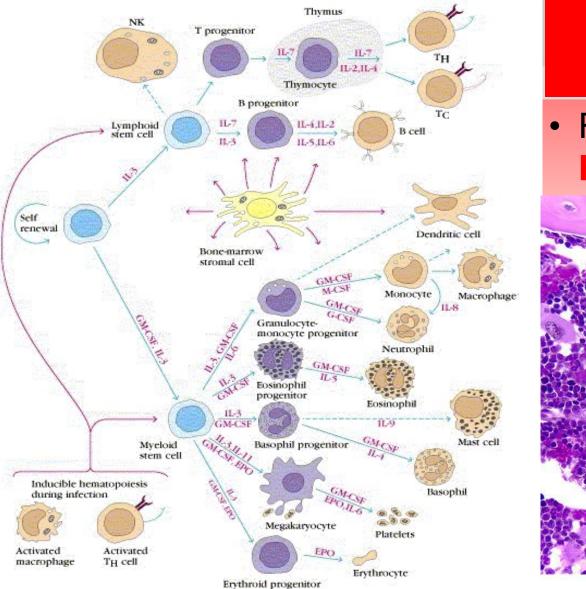
- 1. Using **low power (10x)**: to locate the optimal area for examination.
- 2. High power (40x, 100x oil immersion): to examine individual cells in detail.



Components of Blood

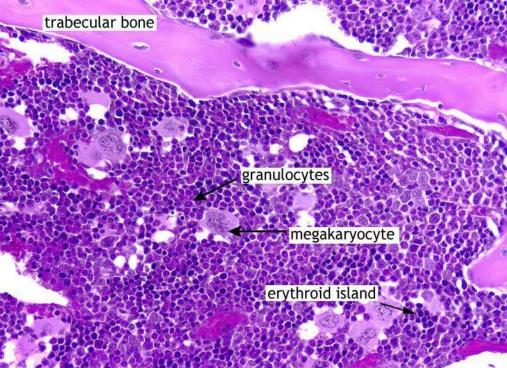
- average adult has about 5 liters (about 5 qts):
- 1 <u>Formed elements</u>:
 - Red blood cells (or erythrocytes)
 White blood cells (or leucocytes)
 Platelets (or thrombocytes)
- 2 <u>Plasma</u> = water + dissolved solutes



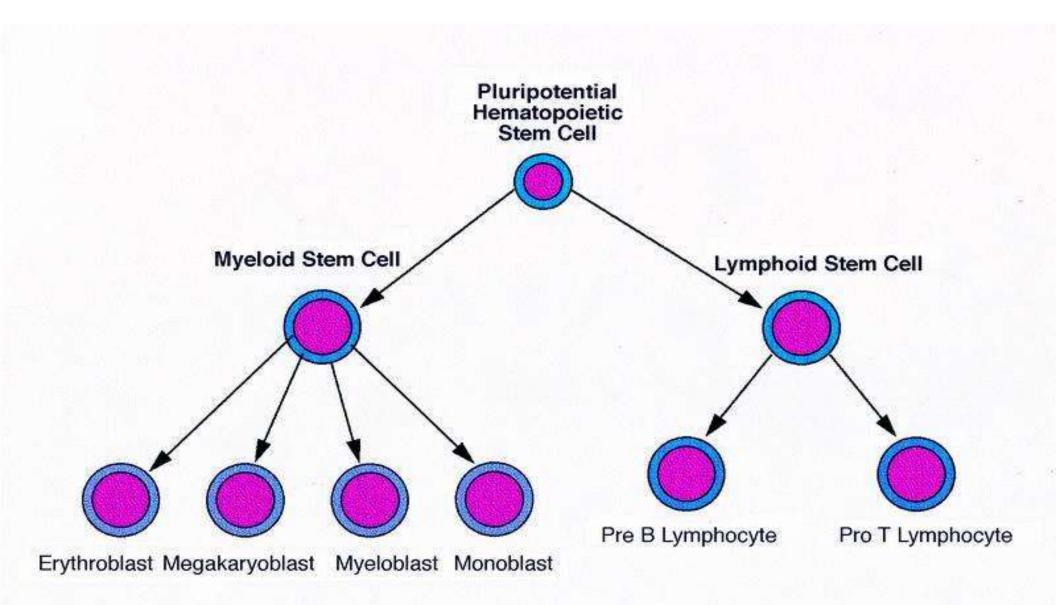


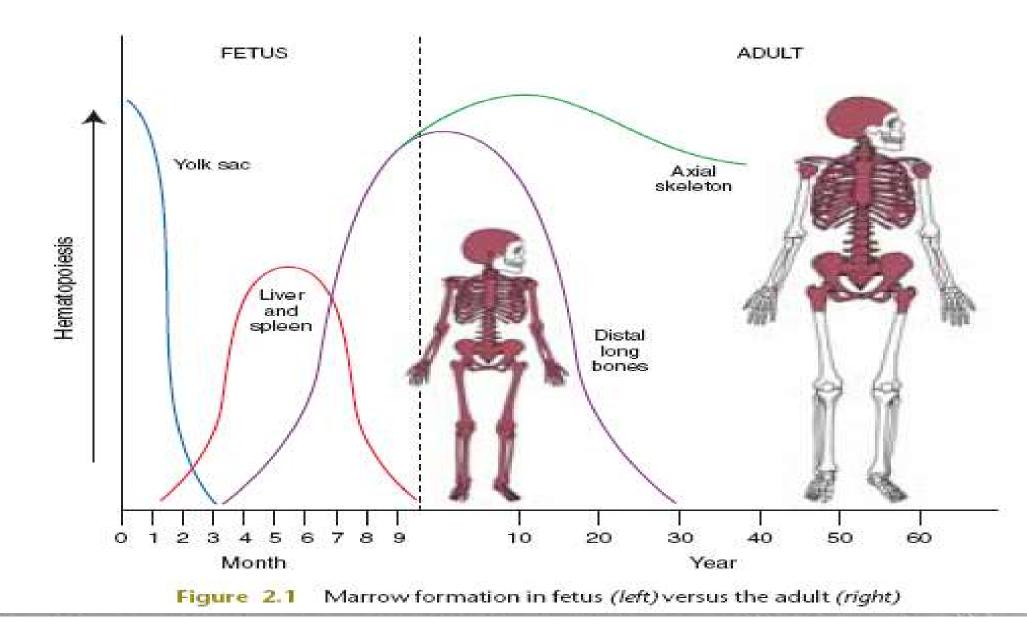
Formed elements: ORIGIN

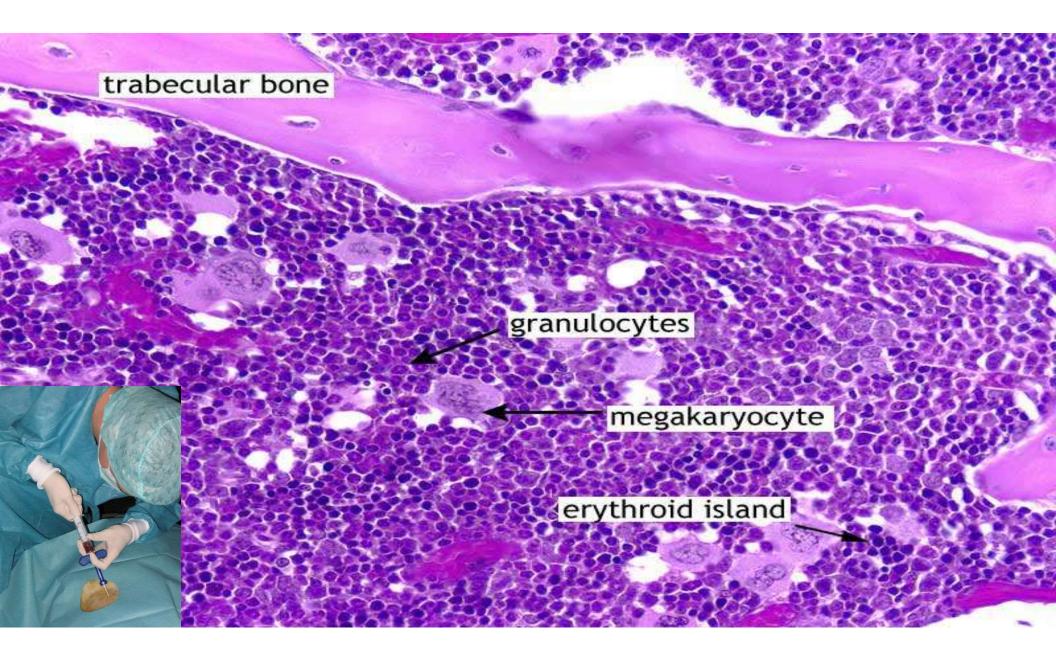
RED Bone MARROW : HEMATOPOIESIS

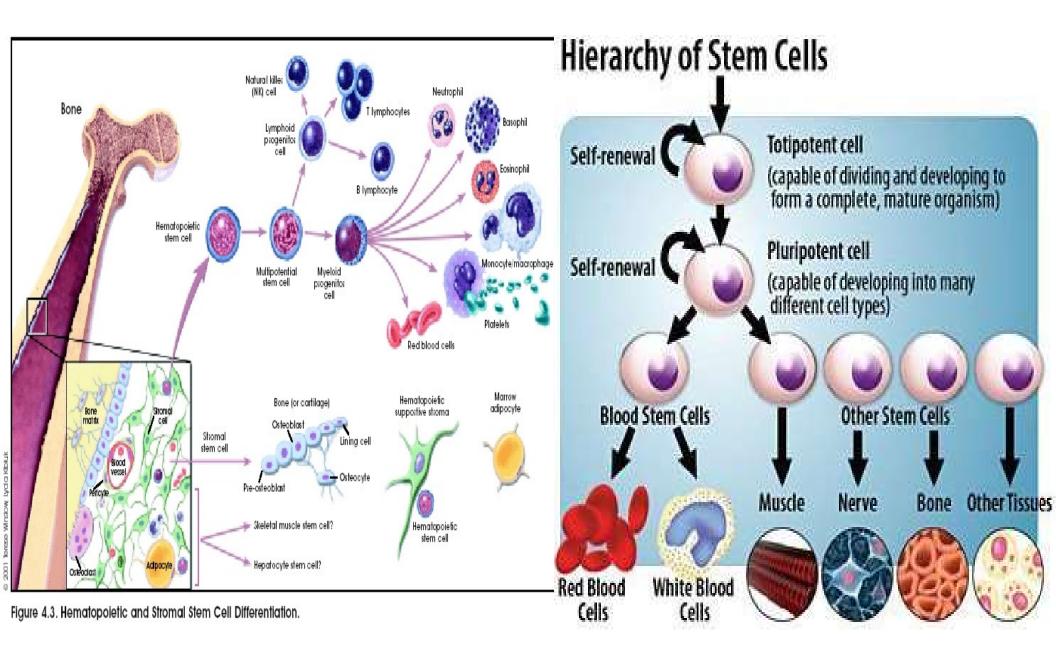


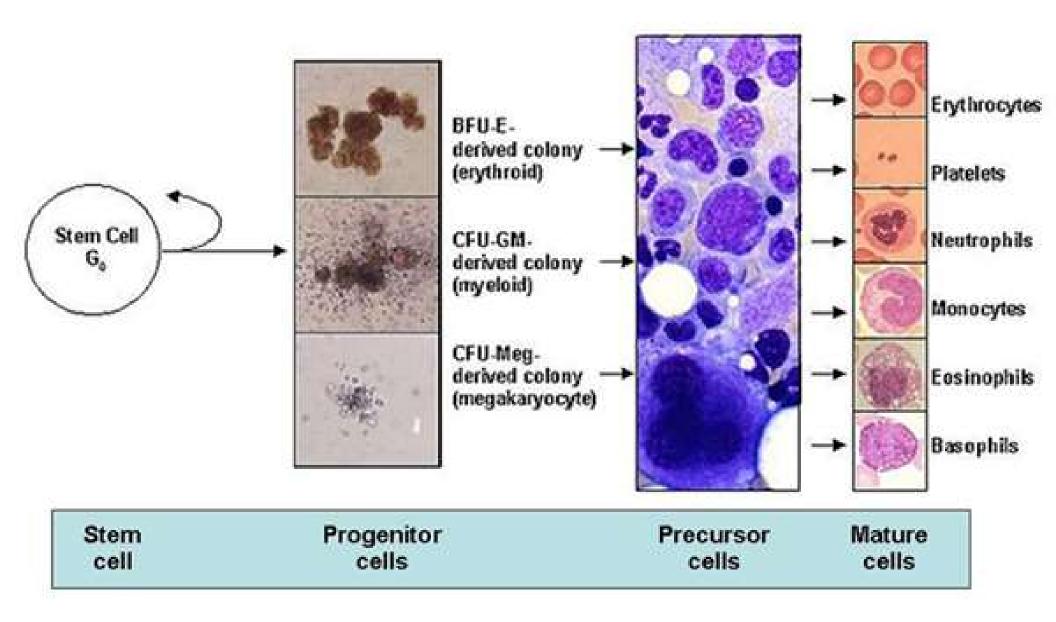
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Functions of Blood:

• 1 - Transportation:

•Oxygen from lungs to tissues
•Carbon dioxide from tissues to lungs
•Nutrients from the digestive tract to cells
•Hormones from endocrine glands to target organs
•Waste products (e.g., urea, creatinine) to kidneys/liver for excretion
• 2 - Regulation –

Body temperature: Distributes heat throughout the body
pH balance: Buffers like bicarbonate help maintain stable pH
Fluid balance: Plasma proteins (like albumin) maintain osmotic pressure and prevent fluid loss

• 3 - Protection -

•Immune defense: White blood cells (WBCs) identify and destroy pathogens

•Antibody transport: Plasma carries antibodies and immune proteins •Clotting: Platelets and clotting factors stop bleeding and seal injuries 4. Communication
•Hormones and cytokines travel in the bloodstream to coordinate body responses (e.g., stress, growth, inflammation)

Functions of Blood

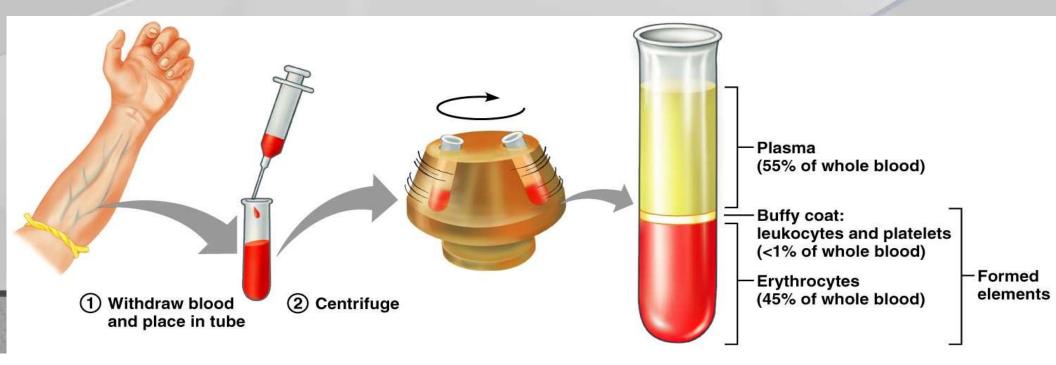


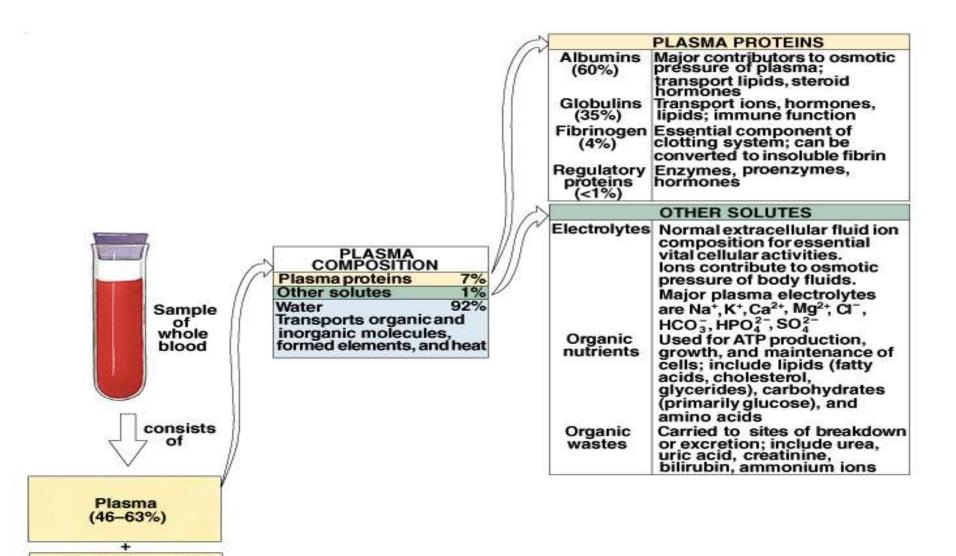
- In human adults about 5 liter of blood contribute 7-8 % to the body weight of the individual.
- The contribution of red blood cells (erythrocytes) to the total volume of the blood (hematocrit) is about 43%.
- Erythrocytes are the dominant (99%) but not the only type of cells in the blood.
- Erythrocytes, leukocytes and blood platelets are also being referred to as the formed elements of the blood.
- Erythrocytes and blood platelets perform their functions exclusively in the blood stream.
- In contrast, <u>leukocytes reside only temporarily in the blood.</u>
- Leukocytes can leave the blood stream through the walls of capillaries and venules and enter either connective or lymphoid tissues.

Components of Whole Blood

Whole blood is a living tissue that circulates through the heart, arteries, veins, and capillaries carrying nourishment, electrolytes, hormones, vitamins, antibodies, heat, and oxygen to the body's tissues.

Whole blood contains red blood cells, white blood cells, and platelets suspended in a fluid called plasma.





Formed elements (37–54%)

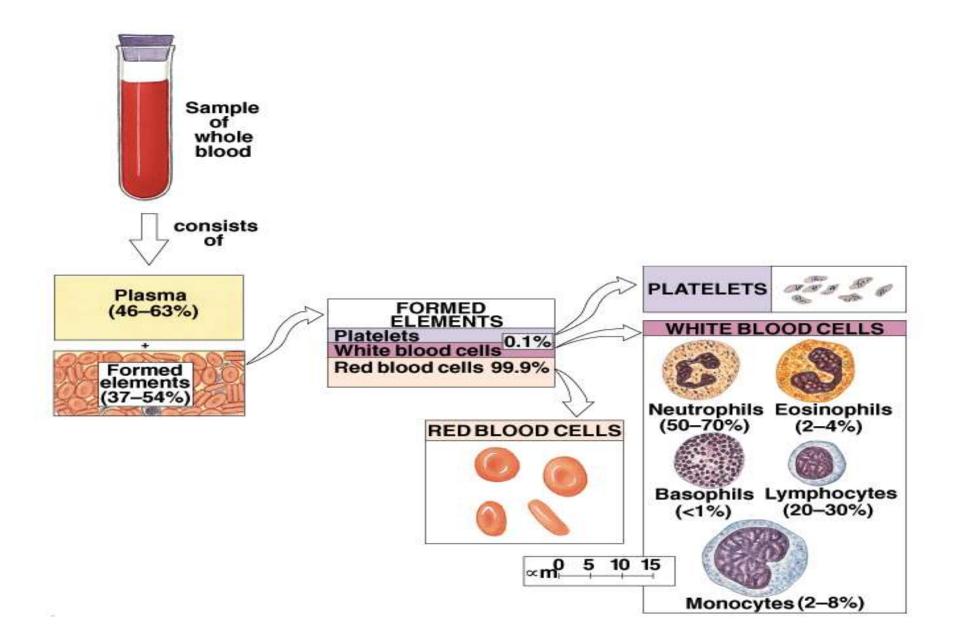
Component	% of Blood	Function
Plasma	~55%	Transport nutrients, hormones, waste
RBCs	~44%	Oxygen/CO ₂ transport
WBCs	<1%	Immune defense
Platelets	<1%	Clotting

Plasma (≈ 55% of total blood volume)

•Definition: The liquid portion of blood.

•Composition:

- Water (≈ 90–92%): Acts as a solvent and medium for transport.
- Plasma proteins ($\approx 7\%$):
 - Albumin maintains oncotic pressure.
 - **Globulins** immune function (e.g., antibodies).
 - Fibrinogen key role in blood clotting.
- Solutes (≈ 1%): Electrolytes (Na⁺, K⁺, Ca²⁺), nutrients (glucose, amino acids), gases (O₂, CO₂), hormones, metabolic waste (urea, creatinine).



2. Formed Elements (≈ 45% of total blood volume)

These are the cellular components of blood:

A. Erythrocytes (Red Blood Cells / RBCs)

Function: Transport oxygen (via hemoglobin) and a small amount of CO₂.
Features: No nucleus, biconcave shape, lifespan ~120 days.
Count: ~5 million per microliter.

B. Leukocytes (White Blood Cells / WBCs)

•Function: Defense against infections and immune responses. •Types:

- Granulocytes:
 - Neutrophils phagocytosis of bacteria (most abundant).
 - **Eosinophils** combat parasites and modulate allergies.
 - **Basophils** release histamine during allergic reactions.
- Agranulocytes:
 - Lymphocytes B cells (antibodies), T cells (cell-mediated immunity), NK cells (kill virus-infected cells).
 - Monocytes differentiate into macrophages; phagocytic.

C. Thrombocytes (Platelets)

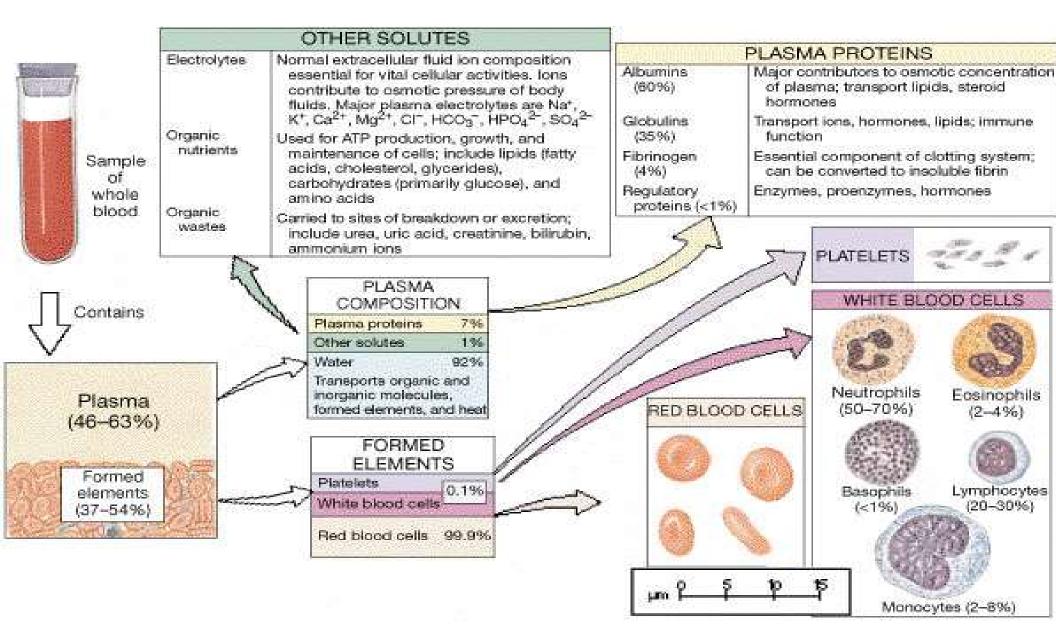
Function: Essential for blood clotting (hemostasis).
Origin: Fragments of megakaryocytes from bone marrow.
Lifespan: ~7–10 days.

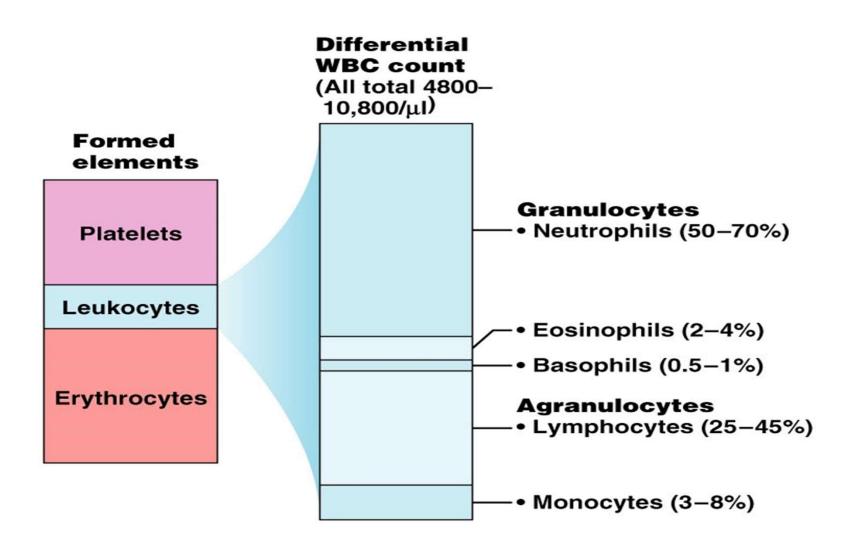
Blood Plasma

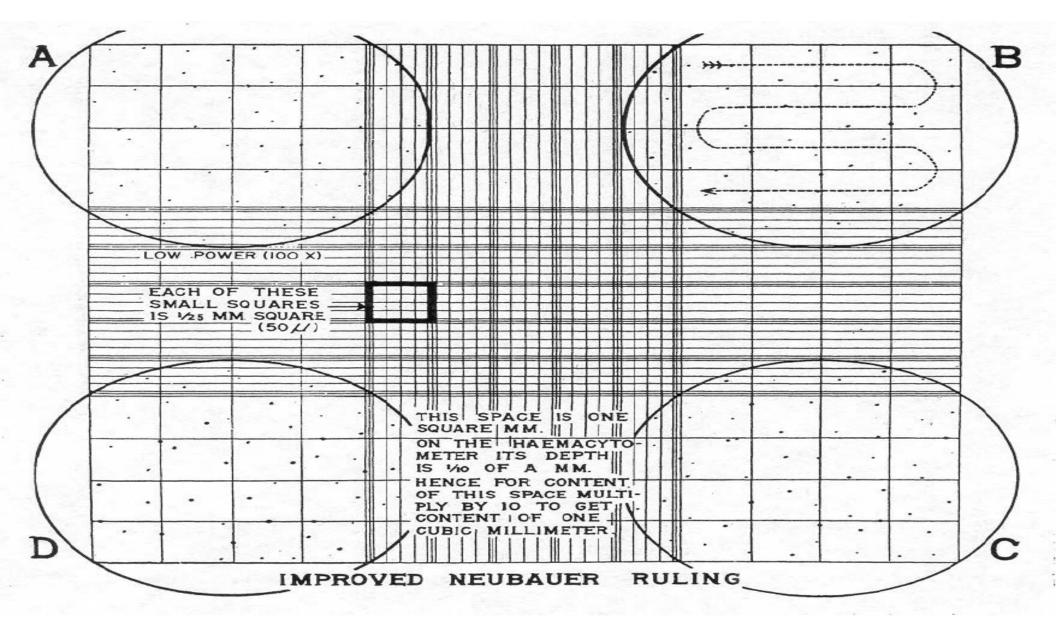
- Blood plasma contains over 100 solutes, including:
 - Proteins albumin, globulins, clotting proteins, and others
 - <u>Lactic acid, urea, creatinine</u>
 - Organic nutrients glucose, carbohydrates, amino acids
 - Electrolytes sodium, potassium, calcium, chloride, bicarbonate
 - Respiratory gases oxygen and carbon dioxide

Plasma

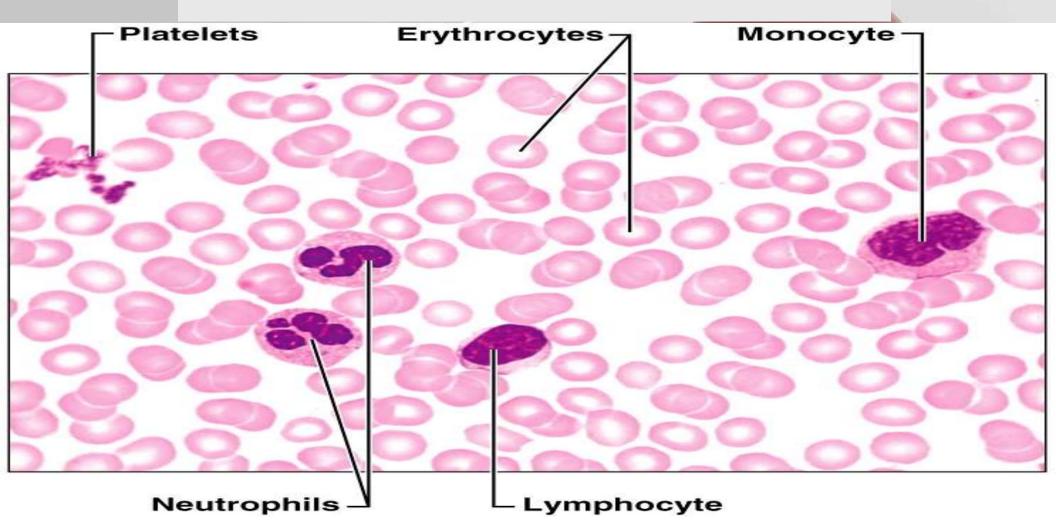
- it's a straw-colored, clear liquid that is 90 percent water, and it is an essential ingredient for human survival.
- It might seem like plasma is less important than the blood cells it carries. But that would be like saying that the stream is less important than the fish that swims in it. You can't have one without the other.
- Besides water, plasma also contains dissolved salts and minerals like calcium, sodium, magnesium, and potassium. Microbe-fighting antibodies travel to the battlefields of disease by hitching a ride in the plasma.
- Without plasma, the life-giving blood cells would be left floundering without transportation. Never underestimate the importance of plasma.



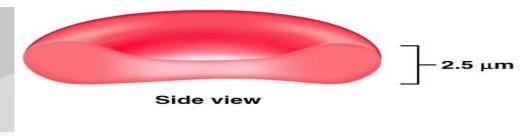




Components of Whole Blood

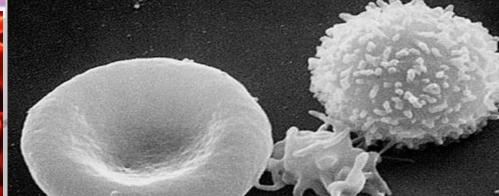


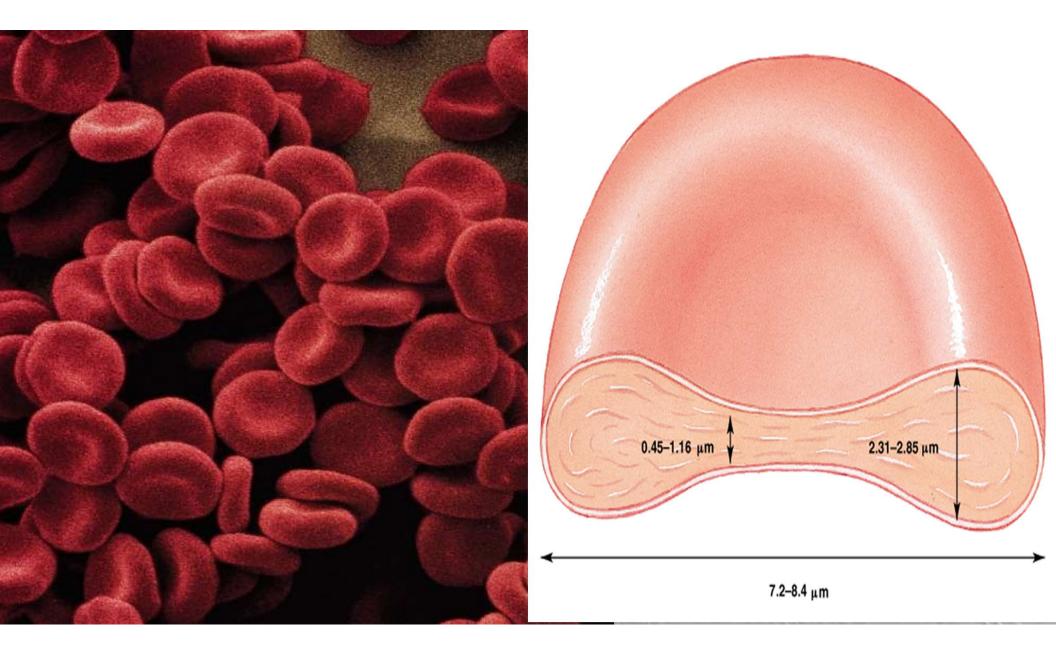
Erythrocytes (RBCs)

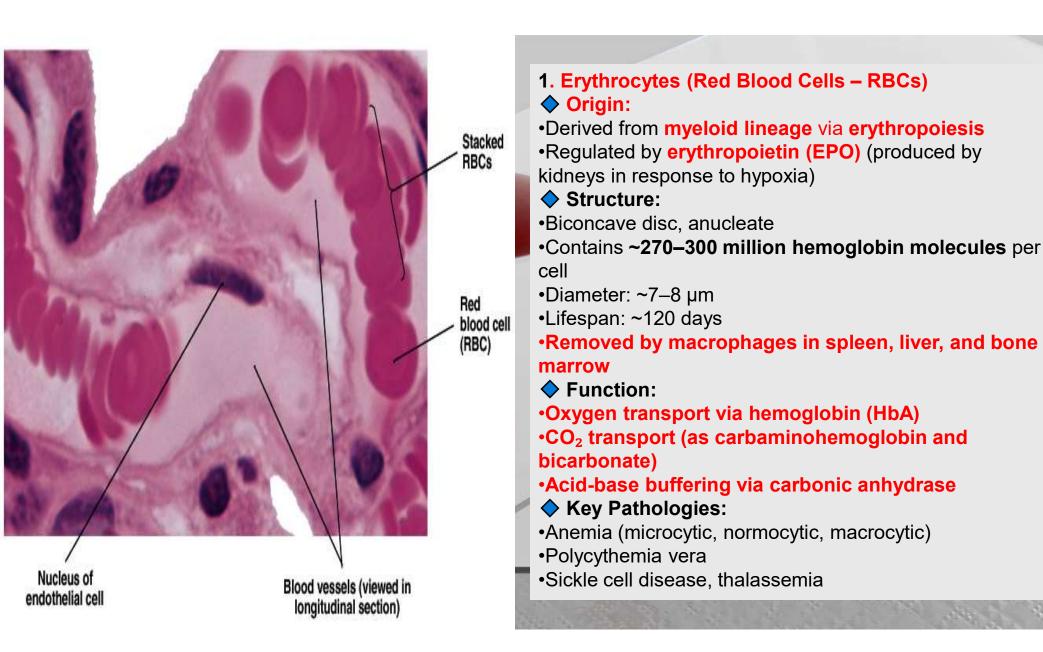


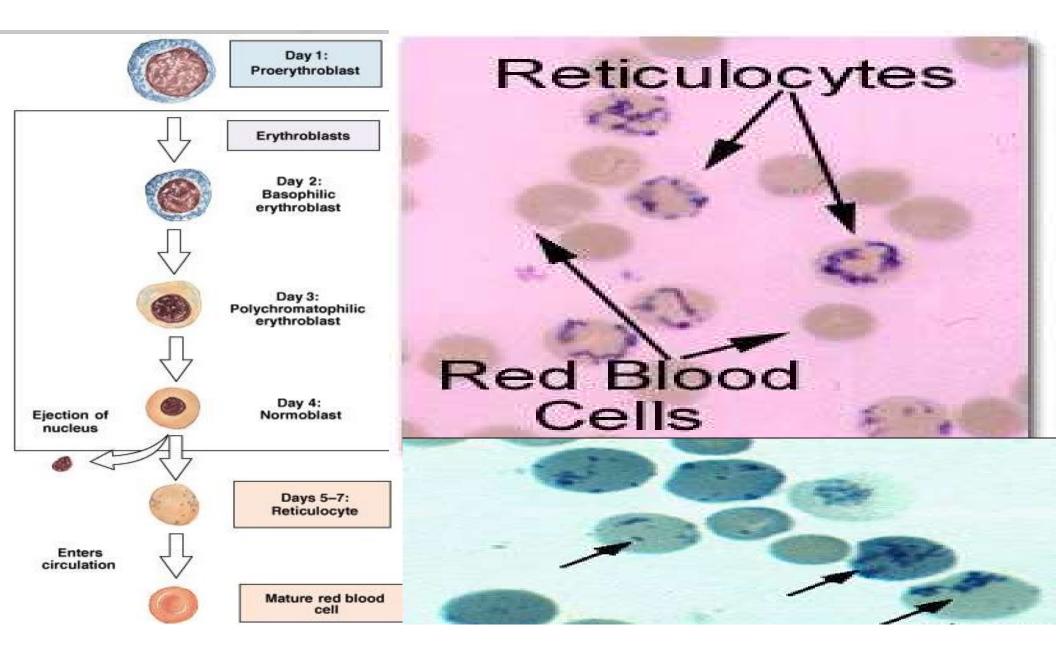












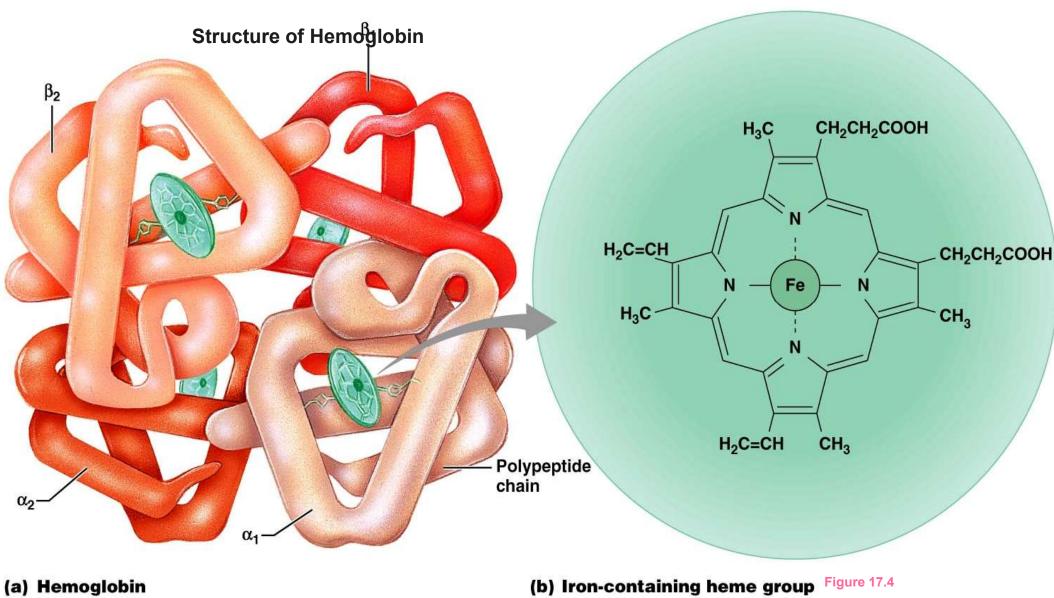
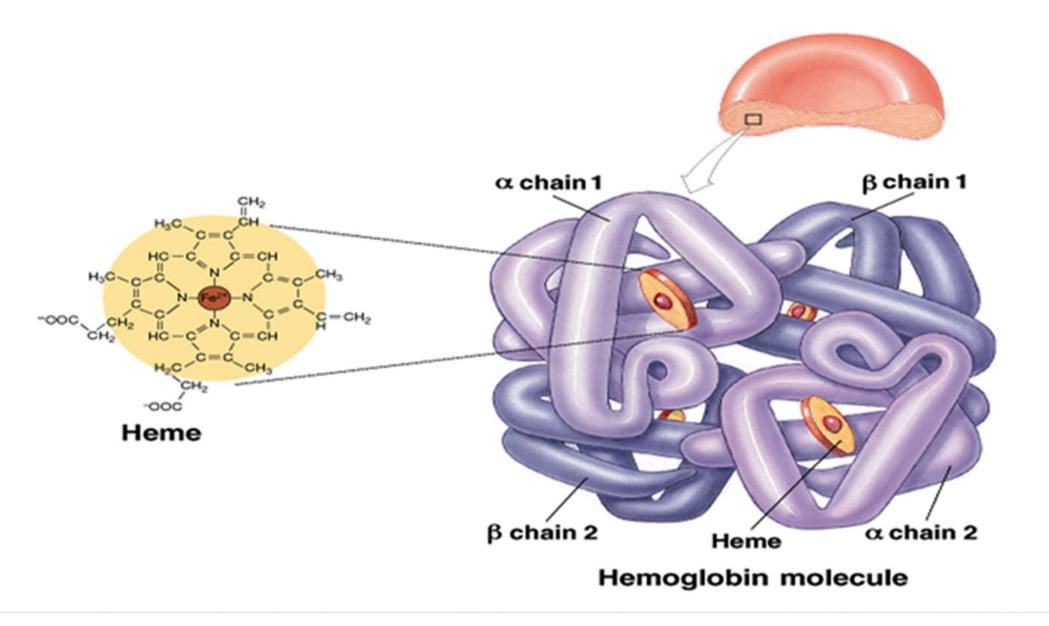
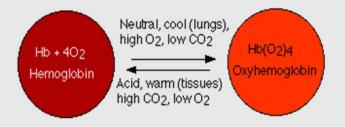


Figure 17.4 (b) Iron-containing heme group



Hemoglobin (Hb)

- Oxyhemoglobin Hb bound to oxygen
 Oxygen loading takes place in the lungs
- <u>Deoxyhemoglobin</u> Hb after oxygen diffuses into tissues (reduced Hb)
- Carbaminohemoglobin Hb bound to carbon dioxide
 - Carbon dioxide loading takes place in the tissues



Men: 13.5 to 16.5 g/dl

- Women: 12.1 to 15.1 g/dl
 Children: 11 to 16 g/dl
 Pregnant women: 11 to 12 g/dl

Name of Hemoglobin	Subunit Structure	Time of Expression
Hemoglobin Portland	$\zeta_2 \gamma_2$	Embryonic
Hemoglobin Gower I	$\zeta_2 \epsilon_2$	Embryonic
Hemoglobin Gower II	$\alpha_2 \epsilon_2$	Embryonic
Hemoglobin F	$\alpha_2 \gamma_2$	Fetal
Hemoglobin Barts	Y4	Fetal (pathologic Hb secondary to absence of all 4 α globulin genes; fatal in utero)
Hemoglobin A ₂	$\alpha_2 \delta_2$	Minor adult hemoglobin
Hemoglobin A	$\alpha_2\beta_2$	Major adult hemoglobin

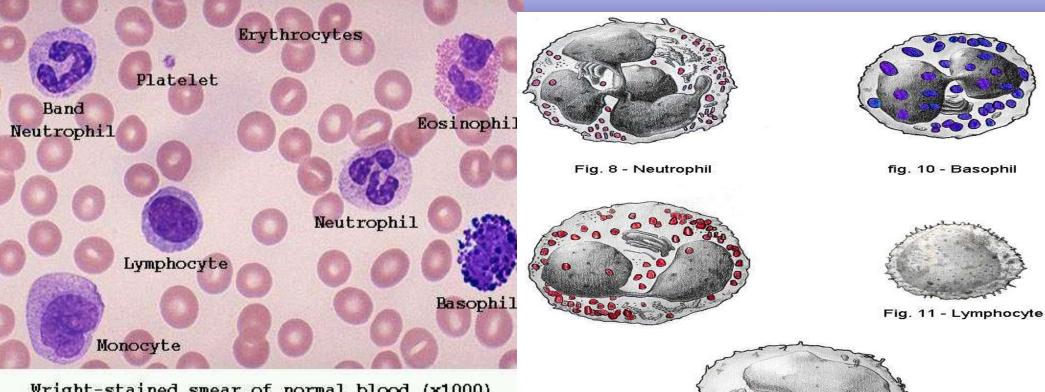
Granulocytes



Granulocytes –

- neutrophils,
- eosinophils,
- and basophils
- » Contain cytoplasmic granules that stain specifically (acidic, basic, or both) with Wright's stain
- » Are larger and usually shorter-lived than RBCs
- » Have lobed nuclei
- » Are all phagocytic cells

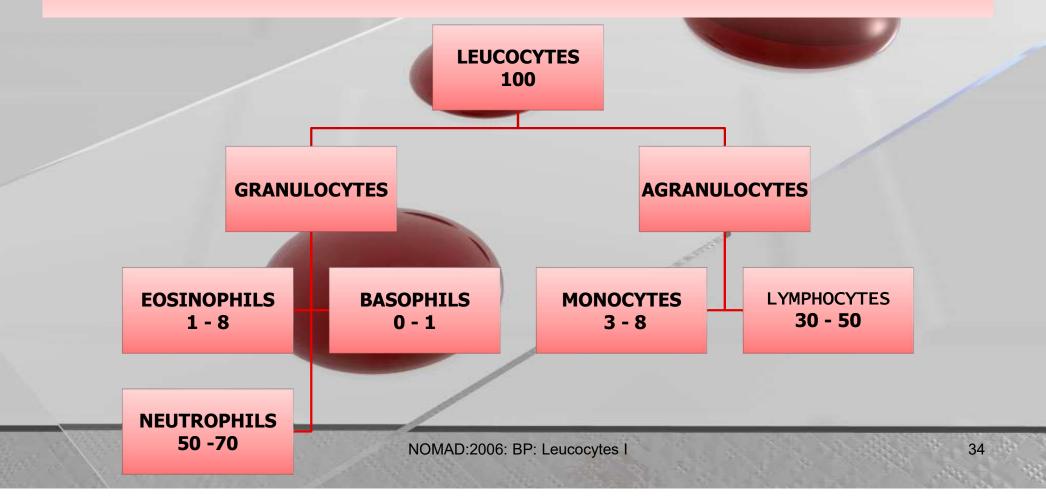
White cells, leukocytes



Wright-stained smear of normal blood (x1000) The RBC's are biconcave discs stained buffpink, and the WBC's nucleus and cytoplasmic granules and platelet stain varying degrees of blue and pink.

Fig. 12 - Monocyte

CLASSIFICATION OF LEUCOCYTES



```
WBC count: 4,500-11,000/µL
```

```
Polymorphonuclear neutrophils: 1800-7800/µL; (50-70%)
```

```
Band neutrophils : 0-700/µL; (0-10%)
```

```
Lymphocytes : 1000-4800/µL; (15-45%)
```

```
Monocytes : 0-800/µL; (0-10%)
```

```
Eosinophils : 0-450/µL; (0-6%)
```

```
Basophils : 0-200/µL; (0-2%)
```

WBC Туре	Normal %	Absolute Count (/µL)	Function
Neutrophils	55–70%	2,500–7,000	Acute bacterial infection, inflammation
Lymphocytes	20–40%	1,000–4,000	Viral infection, adaptive immunity
Monocytes	2–8%	200–800	Chronic inflammation, antigen presentation
Eosinophils	1–4%	50–500	Parasites, allergies, asthma
Basophils	<1%	15–100	Allergic reactions, histamine release

To find the **absolute number** of each WBC type: **Absolute count** = (% cell type) × (Total WBC count) ÷ 100

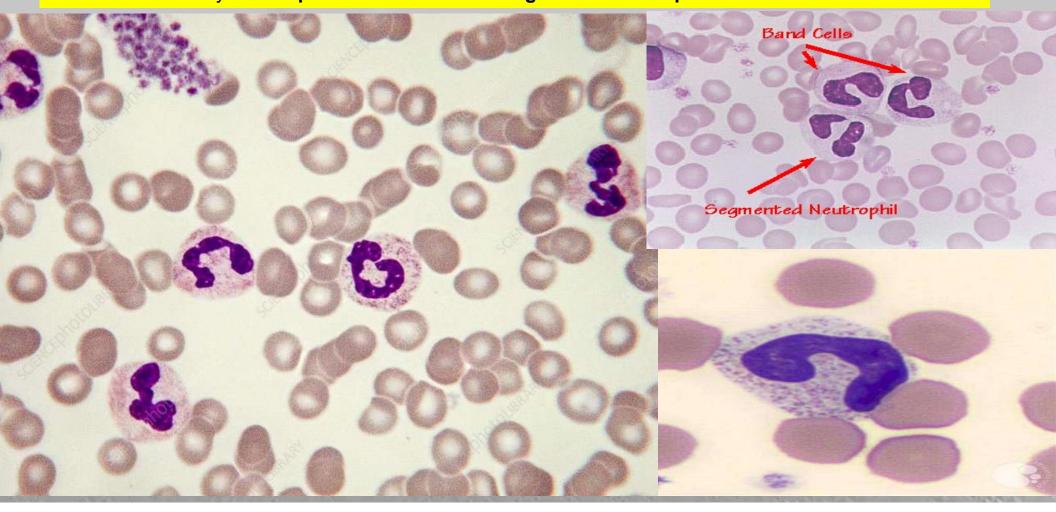
Example:

If WBC = $12,000/\mu$ L and neutrophils = 65%

 \rightarrow Absolute neutrophil count (ANC) = 0.65 × 12,000 = 7,800/µL

Blood Smear - Leishman	^{B1} Neutrophils			
O A	Neutrophils (50–70%) •Polymorphonuclear (segmented nucleus)			
neutrophil	 •Primary function: Phagocytosis of bacteria and debris, acute inflammation, are on site within 4h 			
	 Contain azurophilic (primary) and specific (secondary) granules Rapid responders to acute bacterial infection (↑ in bacterial infections) Lifespan: Hours to days 			
	B2			
	Neutrophils have two types of granules that:			
	 Take up both acidic and basic dyes 			
blood platelet	 Give the cytoplasm a lilac color 			
neutrophi	 – Contain peroxidases, hydrolytic enzymes, and defensins (antibiotic- like proteins) 			
	Neutrophils are our body's bacteria slayers			
	The most abundant wbcs			
	Differential neutrophil count = <u>#neutro</u> x 100			
	total # wbc			

What Are Band Cells? Band cells are immature neutrophils released from the bone marrow during acute infection or inflammation. They are the precursors to mature segmented neutrophils.

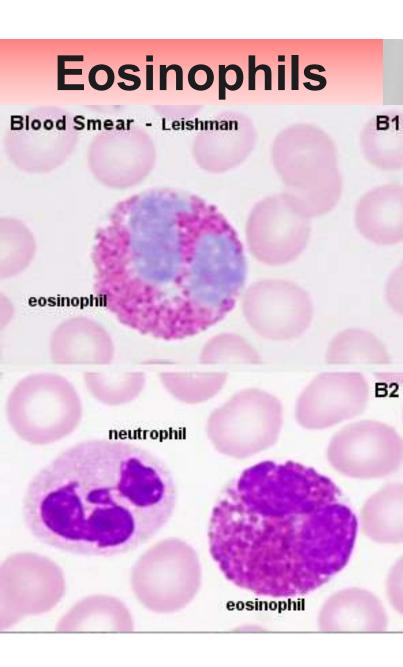


Neutrophilia

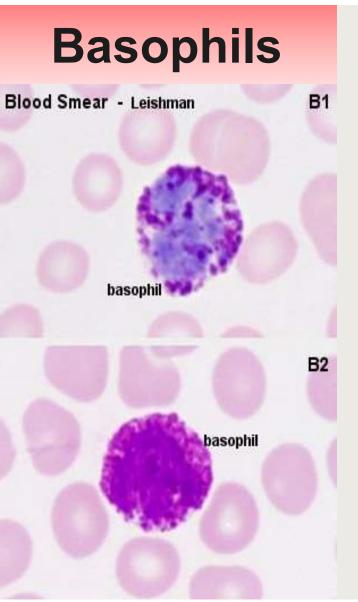
Bacterial infections
Inflammation, trauma, stress
Myeloproliferative disorders
Steroid use (demargination)

Neutropenia

•Viral infections
•Bone marrow suppression
•Chemotherapy
•Aplastic anemia



- Eosinophils account for 1–4% of WBCs
 - Have red-staining, bilobed nuclei connected via a broad band of nuclear material
 - Have red to crimson (acidophilic) large, coarse, lysosomelike granules
 - Lead the body's counterattack against parasitic worms
 - Lessen the severity of allergies by phagocytizing immune complexes
 - Defense against parasites and involved in allergic reactions
 ↑ in parasitic infections, asthma, eosinophilic esophagitis



- Have U- or S-shaped nuclei with two or three conspicuous constrictions
- Are functionally similar to mast cells
- Have large, purplish-black (basophilic) granules that contain histamine
 - <u>Histamine</u> inflammatory chemical that acts as a vasodilator and attracts other WBCs (antihist amines counter this effect)
 - Heparin
 - Leukotrienes (ipid-based inflammatory mediators derived from arachidonic acid via the lipoxygenase pathway. They play key roles in inflammation, bronchoconstriction, chemotaxis, and vascular permeability.

Basophils (<1%)

- •Bilobed nucleus obscured by large basophilic granules
- •Contain histamine, heparin, leukotrienes
- Involved in hypersensitivity reactions (Type I)
- Functionally similar to mast cells

Agranulocytes

• Agranulocytes :

<u>lymphocytes and monocytes</u>:

- Lack visible cytoplasmic granules
- Are similar structurally, but are functionally distinct and unrelated cell types
- Have spherical (lymphocytes) or kidney-shaped (monocytes) nuclei

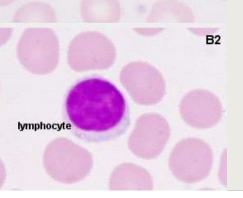
Lymphocytes

Blood Smear - Leishman

an

B1

lymphocyte



Account for 25% -30% or more of WBCs and:

- Have large, dark-purple, circular nuclei with a thin rim of blue cytoplasm
- Are found mostly enmeshed in lymphoid tissue (some circulate in the blood)

•B cells: Humoral immunity (differentiate into plasma cells → secrete antibodies)
•T cells:

•CD4⁺ T-helper: Orchestrate immune response

•CD8⁺ Cytotoxic T cells: Kill infected/tumor cells

•Natural Killer (NK) cells: Innate immune cytotoxicity

 in viral infections and chronic inflammation

Long-lived, memory-capable

Lymphocytosis
 Viral infections (EBV, CMV)
 CLL (chronic lymphocytic leukemia)
 TB, pertussis

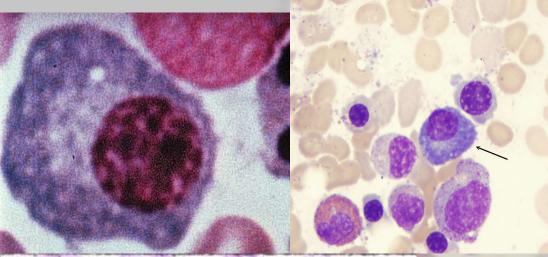
Lymphopenia
 HIV/AIDS
 Steroids
 Immunodeficiency
 SLE

LYMPHOCYTES: IMMUNOCYTES

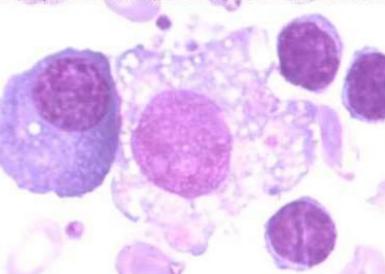
Physiological Classification: T and B <u>T' LYMPHOCYTES :</u>

- Thymus trained or schooled cells
- Responsible for Cell mediated immunity.
- Provide protection against intracellular pathogens

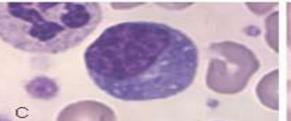
B lymphocytes, plasma cells

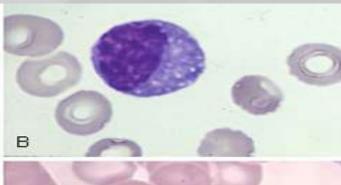


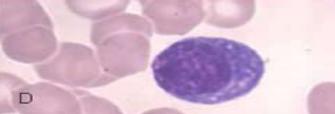
	Origin & Maturation		
の一般になっていてい	Stage	Location	
	Origin	Bone marrow (hematopoietic stem cell)	
	Maturati on	Bone marrow (unlike T cells which mature in thymus)	
	Activatio n	Peripheral lymphoid tissues (lymph nodes, spleen, MALT)	

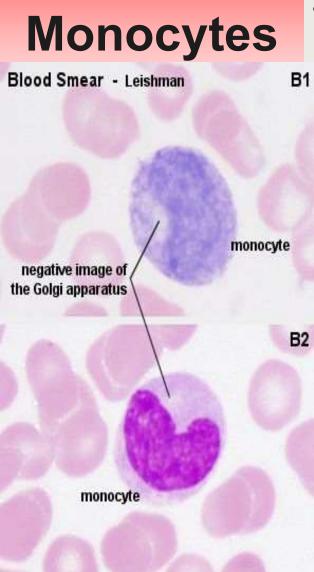












- Monocytes account for 4–8% of leukocytes
 - They are the largest leukocytes
 - They have abundant pale-blue cytoplasms
 - They have purple-staining, U- or kidney-shaped nuclei
 - They leave the circulation, enter tissue, and differentiate into macrophages and dendritic cells

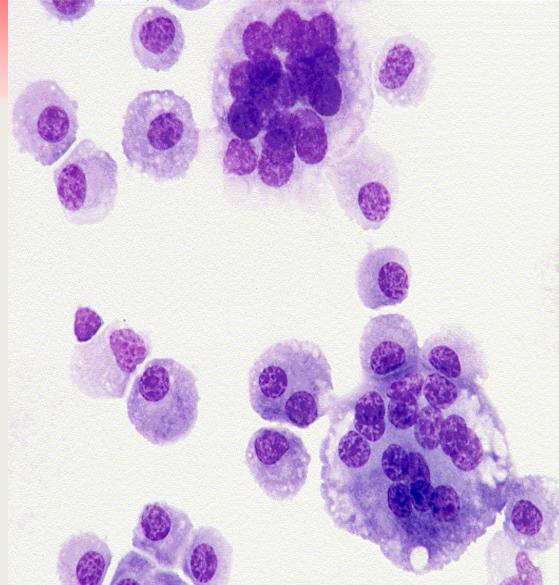
•Circulate in blood, then differentiate into **macrophages** or **dendritic cells** in tissues

•Phagocytose pathogens, present antigens (APCs)

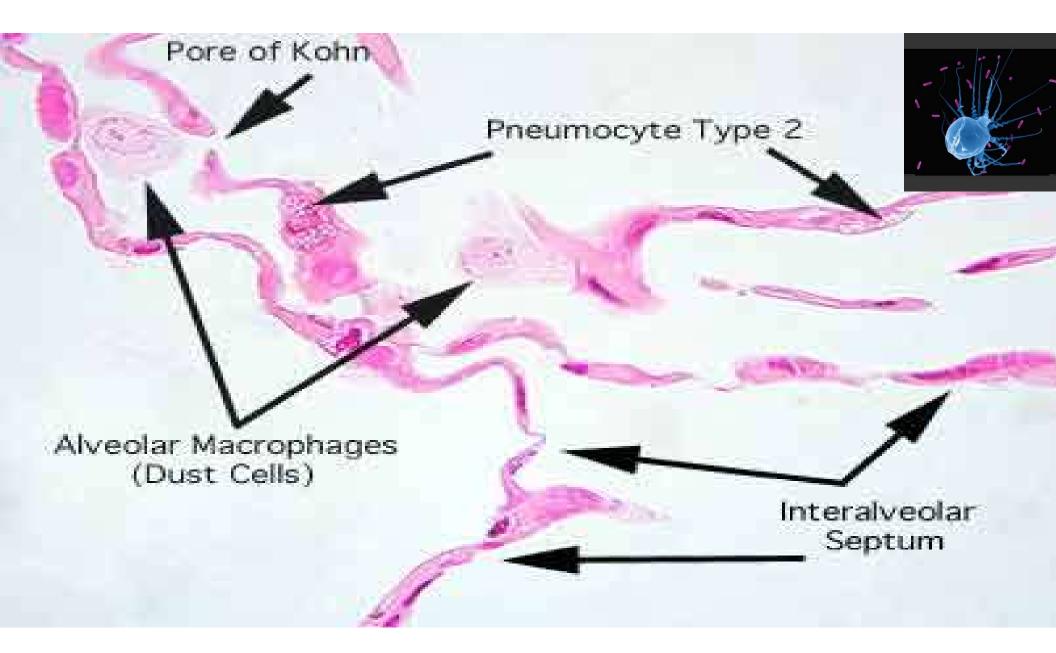
↑ in chronic infections (e.g., TB), certain leukemias
•Macrophage can become giant cells in chronic inflammation

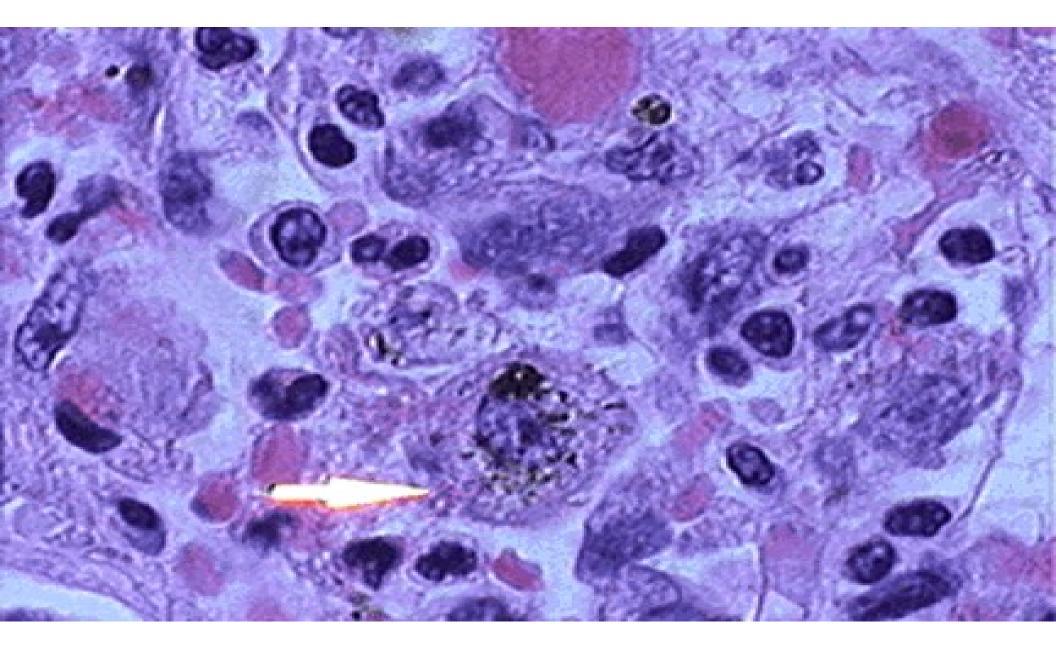
Macrophages

- Macrophages:mononuclear phagocyte system or reticuloendothelial system
 - Are highly mobile and actively phagocytic
 - Activate lymphocytes to mount an immune response
 - Will have different names depending on the location



Tissue / Organ	Macrophage Type	Key Functions		
Liver	Kupffer cells	Phagocytose bacteria, debris, and old RBCs from portal blood		
Lung (alveoli)	Alveolar macrophages	Clear inhaled particles and pathogens, regulate inflammation		
Brain & CNS	Microglia	Immune surveillance, synaptic pruning, neuroinflammation		
Skin	Langerhans cells	Antigen presentation to T cells (also considered dendritic cells)		
Spleen	en Red pulp macrophages Remove old RBCs and blood-borne pathogens			
Bone	Osteoclasts	Resorb bone matrix, involved in calcium homeostasis		
Lymph nodes	Subcapsular sinus macrophages	Trap and present antigens from afferent lymph		
Peritoneum	Peritoneal macrophages	Immune defense in peritoneal cavity, produce cytokines		
Placenta Hofbauer cells		Fetal macrophages, involved in development and immune tolerance		
Intestines (lamina propria)		Tolerate gut flora but respond to pathogens		
Thymus	Thymic macrophages	Clear apoptotic thymocytes during T cell development		
Heart	Cardiac macrophages	Tissue repair and regulation of electrical conduction		





1 Monocytosis

Chronic infections (TB, endocarditis)
Recovery phase of neutropenia
Leukemias (AML-M5)

1 Eosinophilia

•Parasitic infections (especially helminths)

- •Allergies, asthma
- •Eosinophilic leukemia
- •Autoimmune diseases (e.g., Churg-Strauss)

1 Basophilia

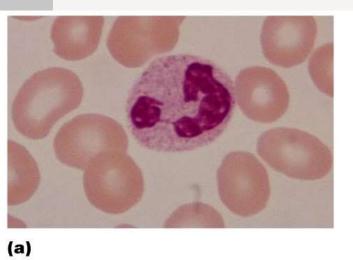
•Rare; seen in:

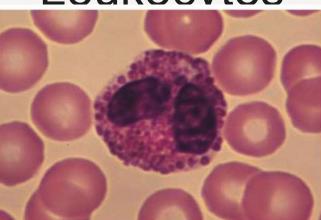
- Myeloproliferative disorders (esp. CML)
- Allergic responses
- Hypothyroidism (occasionally)

Neutrophils & Macrophages

- **Diapedesis:** They squeeze through the pores of the blood vessels.
- Amoeboid movement: They move at rates several times their own length!
- Chemotaxis: Directed movement cells move to wards infected areas.

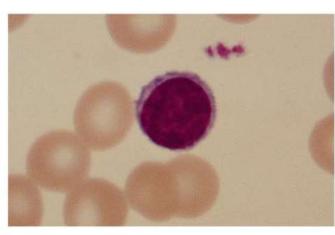
Leukocvtes

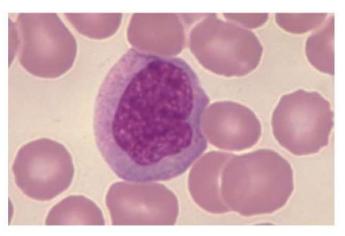




(b)







(d)

(e)

Clinical Red Flags

•Left shift: ↑ bands (immature neutrophils) → acute infection or leukemia

•Right shift: ↑ hypersegmented neutrophils → megaloblastic anemia

•Leukemoid reaction: Marked neutrophilia with left shift, toxic granules, but no blasts

•Blast cells: Always suspicious for leukemia

Left Shift

↑ Band cells in blood = "left shift" (a term from hematopoiesis charts: immature cells to the left)
•Seen in:

- Acute bacterial infections
- Sepsis
- Hemorrhage
- Tissue necrosis (e.g., MI, burns)
- Inflammation
- Myeloproliferative diseases (if persistent)

Toxic Granulation (often coexists with bandemia)

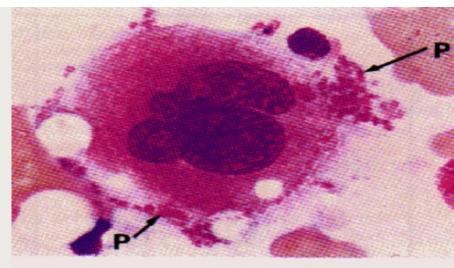
- •Seen in severe infection or sepsis
- •Granules are darker and coarser

Bandemia = >10% band cells or absolute band count >1,000/µL

 \rightarrow Suggests the bone marrow is under stress and releasing immature neutrophils early.

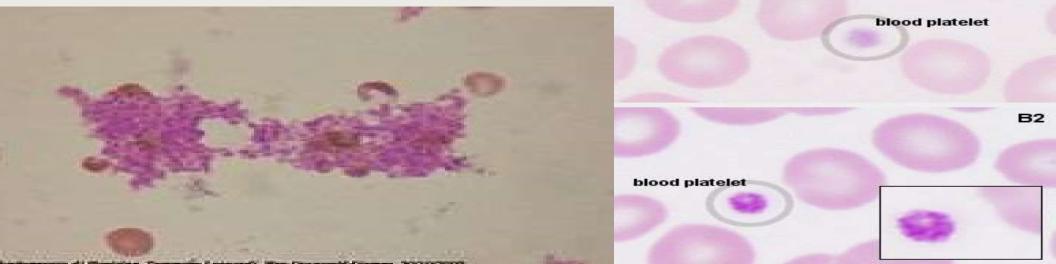
Platelets = Thrombocytes

- Platelets are fragments of **megakaryocytes** with a blue-staining outer region and a purple granular center
- Their granules contain
 - serotonin, Ca²⁺,
 - enzymes, ADP,
 - and platelet-derived growth factor (PDGF)
- Platelets function in the clotting mechanism by forming a temporary plug that helps seal breaks in blood vessels
- Platelets not involved in clotting are kept inactive by NO and prostacyclin



Megakaryocytes and platelet formation Blood Smear - Leishman

B1



Thrombocytes (Platelets)

Origin:

•Derived from **megakaryocytes** via fragmentation in bone marrow •Stimulated by **thrombopoietin (TPO)**

Structure:

Small, anucleate cell fragments (~2–3 μm)
Contain α-granules (fibrinogen, PDGF) and dense granules (ADP, Ca²⁺, serotonin)

♦ Function:

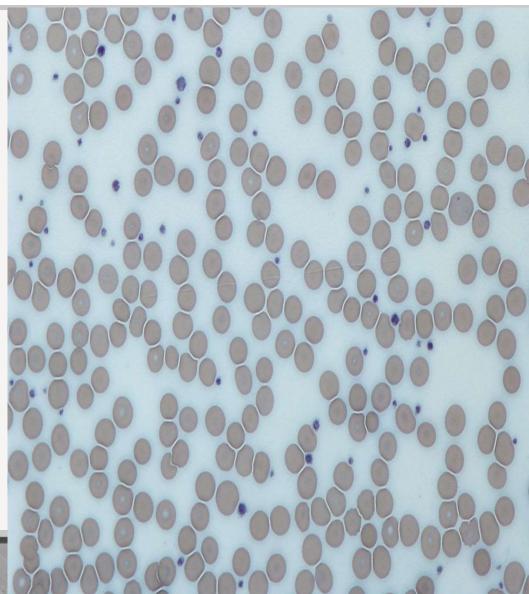
Hemostasis: adhere to damaged endothelium (via vWF), activate, and aggregate to form platelet plug
Provide surface for coagulation cascade activation
Release factors that stabilize clot and promote repair

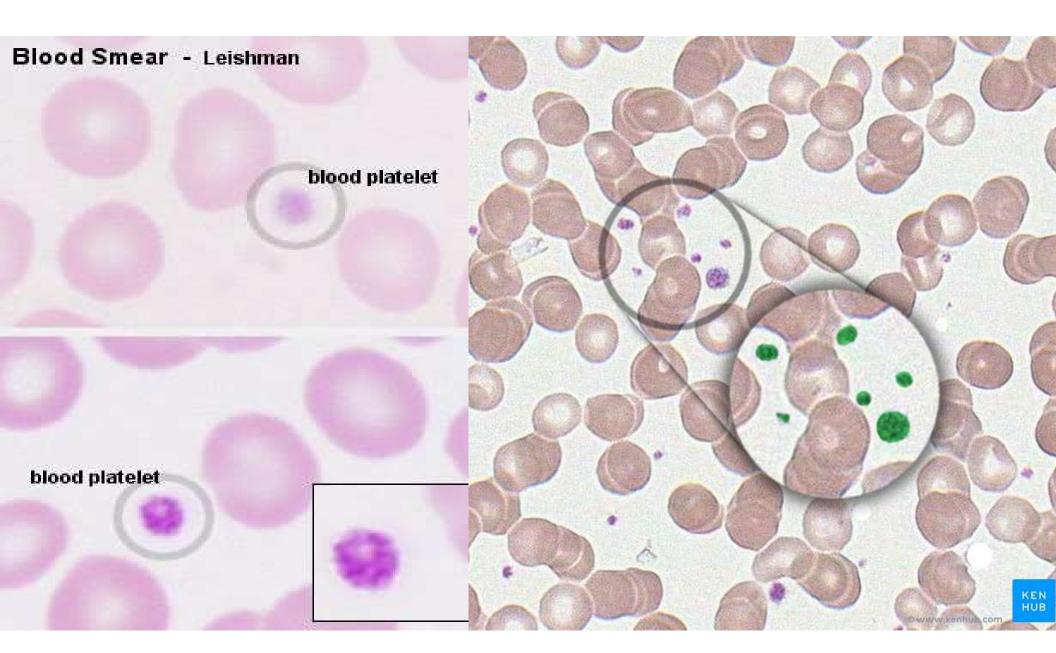
Lifespan:~7–10 days

♦ Key Disorders:

•Thrombocytopenia (low count)

Thrombocytosis (essential thrombocythemia)Platelet function disorders (e.g., Glanzmann thrombasthenia)





Functions of the Spleen

largest secondary lymphoid organ, located in the LUQ (left upper quadrant), protected by ribs 9–11.

•Hematopoietic function Can produce white cell, red cells, and platelets if necessary

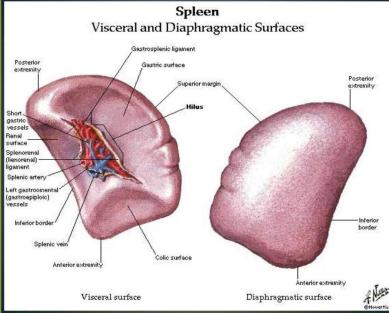
•Reservoir function One third of platelets and granulocytes are stored in the spleen

•Filtration function Aging red cells are destroyed,

spleen removes inclusion from red cells, if red cell membrane is less deformable or antibody-coated spleen presents a hostile environment leading to production of spherocytes

•Immunologic function Opsonizing antibodies produced, trapping and processing antigens from encapsulated organs





Spleen: Functions Overview

1. Blood Filtration •Red Pulp (80%)

- Removes senescent or damaged RBCs (especially those with membrane defects like in spherocytosis)
- **Splenic macrophages** phagocytose opsonized bacteria and parasites
- Filters particles like inclusion bodies (e.g., Howell-Jolly bodies)

2. Immune Surveillance •White Pulp

- Contains lymphoid follicles (B cells) and periarteriolar lymphoid sheaths (PALS – T cells)
- Functions similarly to a **lymph node for the blood** (rather than lymph)
- Initiates adaptive immune responses to bloodborne antigens

3. Reservoir Function

•Stores:

- Platelets (~30% of total)
- **Monocytes** (rapidly mobilized after injury to form tissue macrophages)

4. Defense Against Encapsulated Organisms

 Particularly vulnerable in asplenic or hyposplenic patients

•Important in clearing encapsulated bacteria:

- S. pneumoniae
- H. influenzae type B
- Neisseria meningitidis
- Salmonella spp.

Clinical Tip: These patients require vaccination and prophylactic antibiotics.

5. Hematopoiesis (Fetal Life)

•During the **1st–2nd trimesters**, the spleen contributes to **fetal erythropoiesis**

In adults: can resume hematopoiesis in diseases like myelofibrosis (extramedullary hematopoiesis → splenomegaly)

	Cell Type	Lineage	Nucleus	Key Markers / Features	Function	Clinical Note
	RBC	Myeloid	None	Hemoglobin, biconcave	O_2/CO_2 transport	Anemia, polycythemia
	Neutrophils	Myeloid	Multilobed	CD15 ⁺ , CD16 ⁺	Phagocytosis	Acute infection
	Eosinophils	Myeloid	Bilobed	Major basic protein	Parasite defense, allergies	Asthma, parasitosis
	Basophils	Myeloid	Obscured	Histamine, heparin	Hypersensitivity	Anaphylaxis
	Lymphocytes	Lymphoid	Round	CD3 ⁺ (T), CD19 ⁺ (B), CD56 ⁺ (NK)	Adaptive immunity	HIV, leukemia
	Monocytes	Myeloid	Kidney-shaped	CD14⁺	APC, phagocytosis	TB, chronic infection
Cited and Cited	Platelets	Myeloid	None	GPIIb/IIIa, α and dense granules	Clot formation	ITP, TTP, thrombosis