LEUKOCYTES

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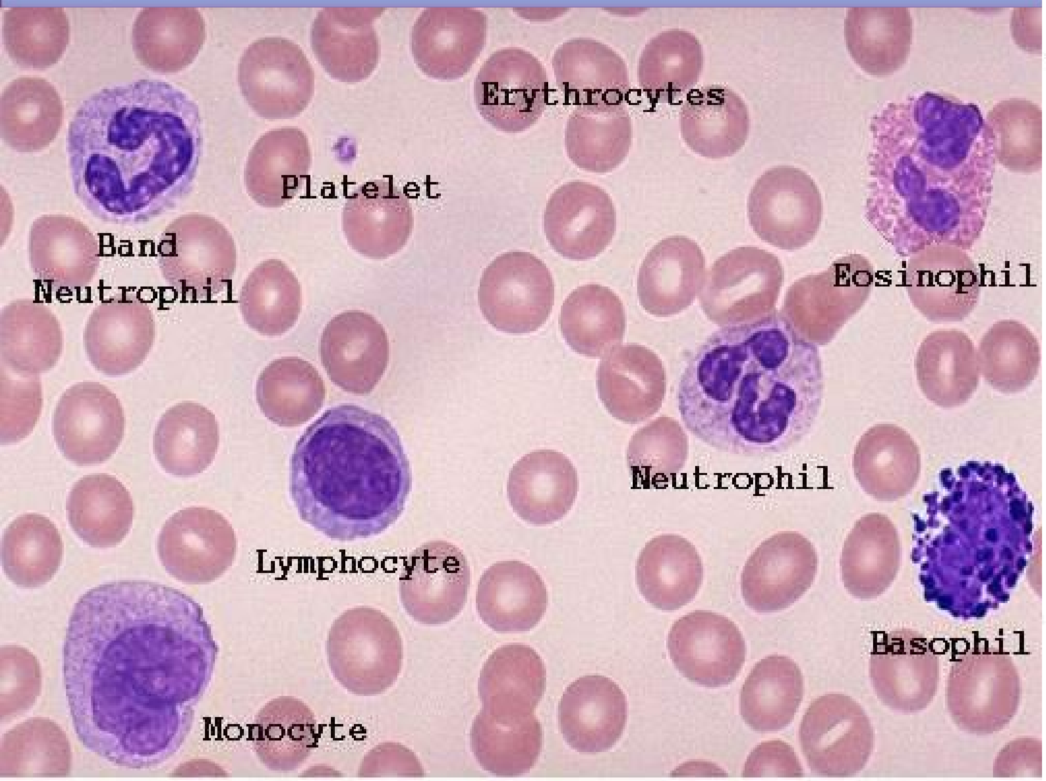
Classification of Leucocytes

- LEUCOCYTES 100
  - GRANULOCYTES
    - EOSINOPHILS 1 - 8
    - BASOPHILS 0 - 1
    - NEUTROPHILS 50 -70
  - AGRANULOCYTES
    - MONOCYTES 3 - 8
    - LYMPHOCYTES 30 - 50

WHITE CELLS, LEUKOCYTES



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

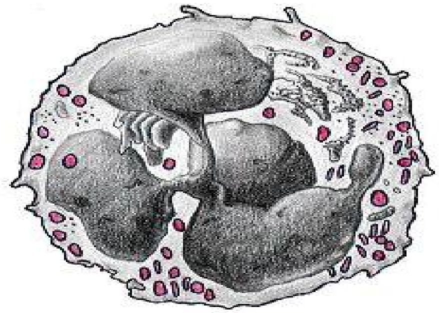


Fig. 8 - Neutrophil

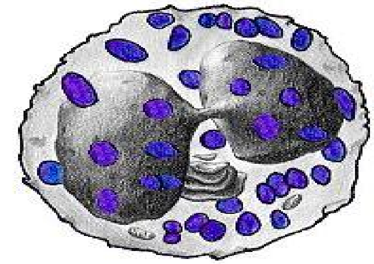
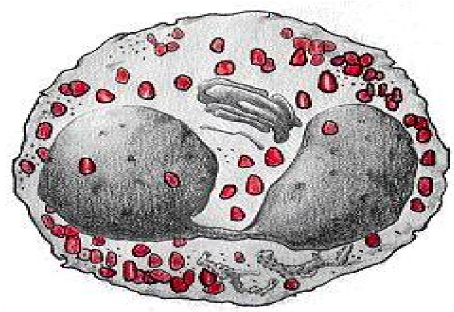


fig. 10 - Basophil



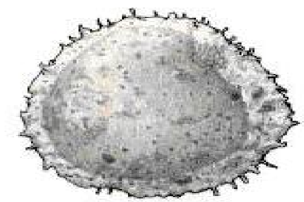


Fig. 11 - Lymphocyte

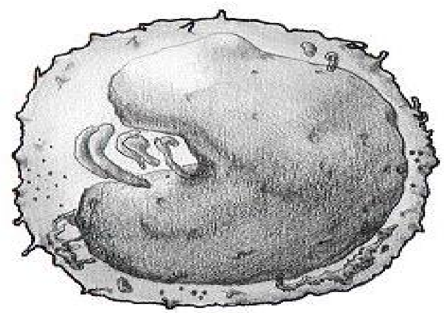
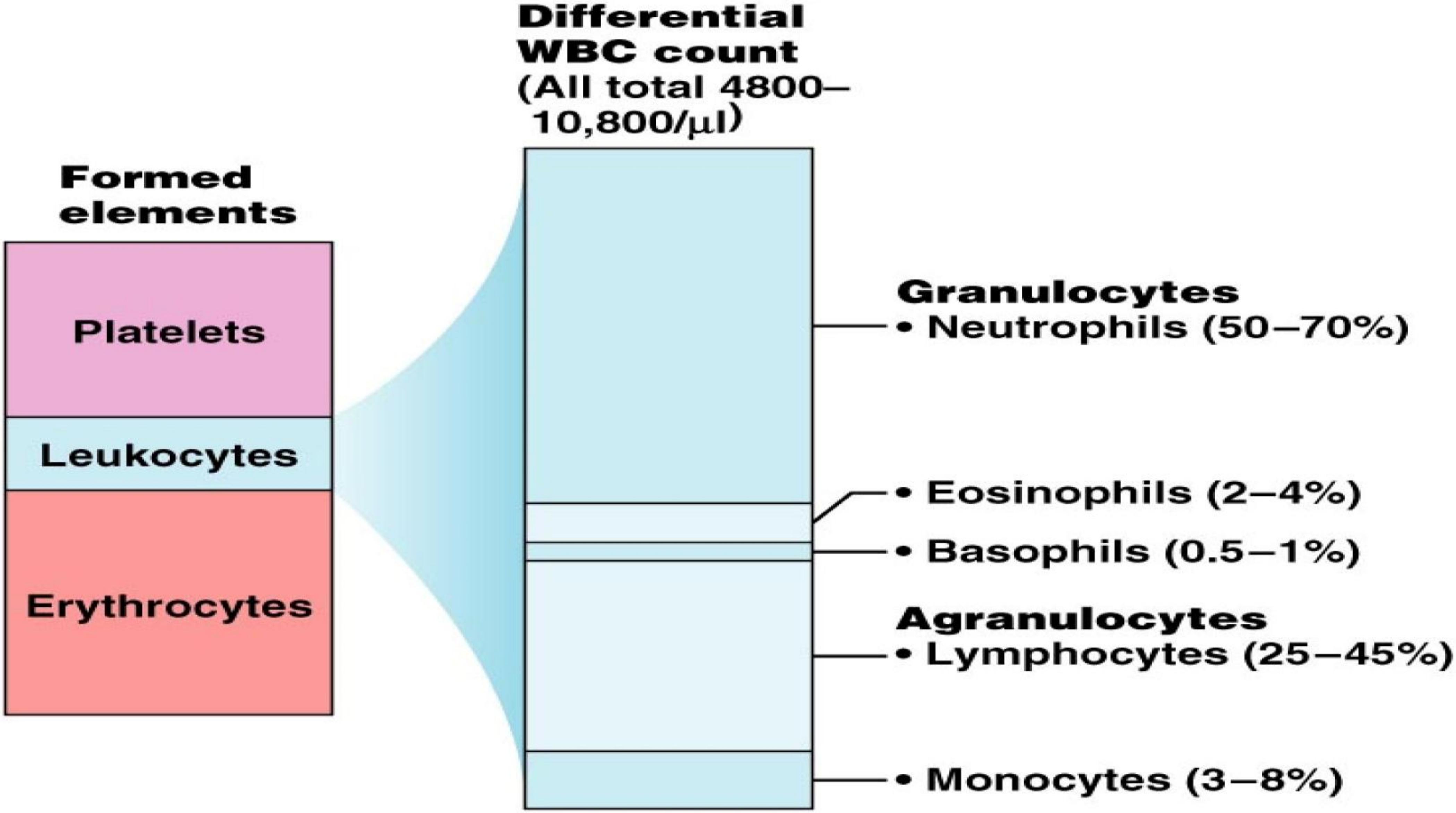


Fig. 12 - Monocyte

LEUKOCYTES (WBCS)

- **Leukocytes, the only blood components that are complete cells:**
  - Are less numerous than RBCs
  - Make up 1% of the total blood volume
  - Can leave capillaries via diapedesis
  - Move through tissue spaces
- **Leukocytosis** – WBC count over 11,000 / mm^3
  - Normal response to bacterial or viral invasion


Differential WBC count (All total 4800–10,800/μl)
Formed elements
Platelets
Leukocytes
Erythrocytes
Granulocytes
• Neutrophils (50–70%)
• Eosinophils (2–4%)
• Basophils (0.5–1%)
Agranulocytes
• Lymphocytes (25–45%)
• Monocytes (3–8%)

GRANULOCYTES

- Granulocytes –
  - **Neutrophils**
  - **Eosinophils**
  - **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**

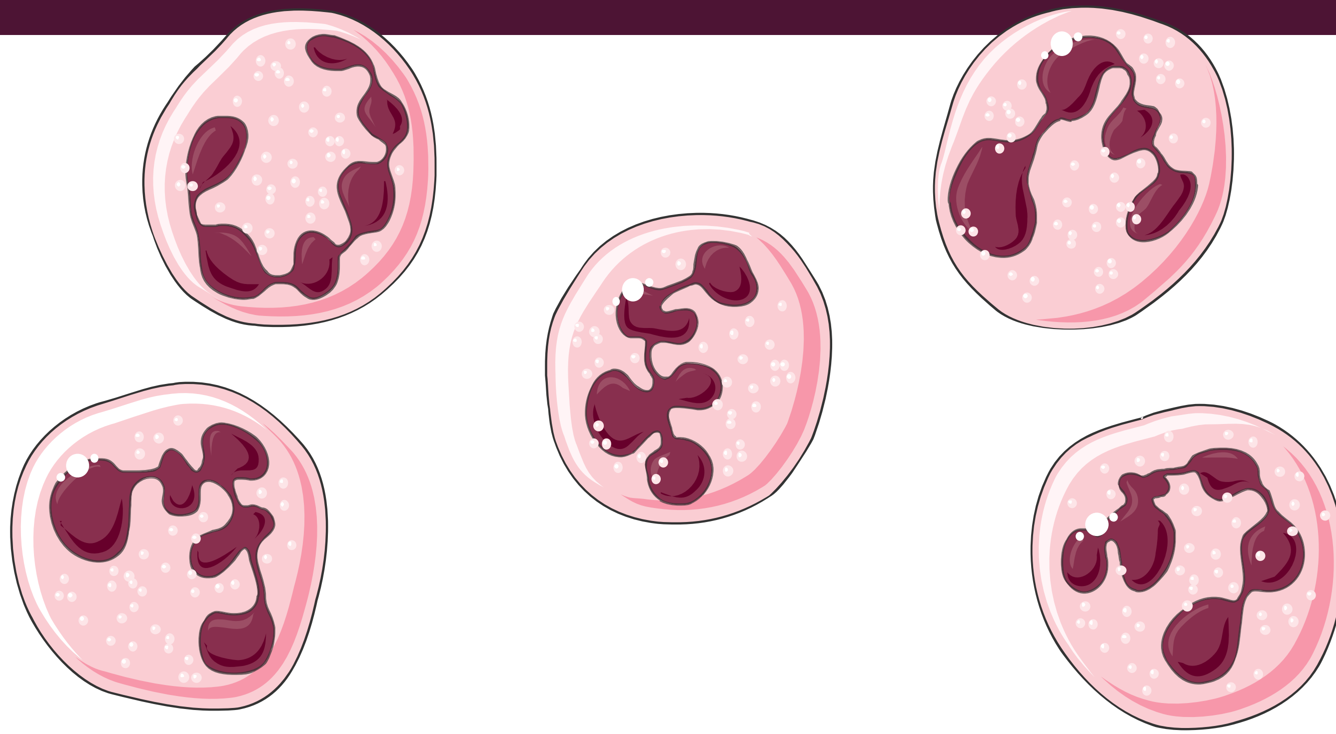
LEUCOCYTES: REVIEW

- Transient life spans in the blood.
- Neutrophils:
  - Phagocytic cells
  - Act as frontline cells for defence along with Macrophages.
  - Show:
    - Diapedesis
    - Amoeboid movement
    - Chemotaxis
    - Phagocytosis.

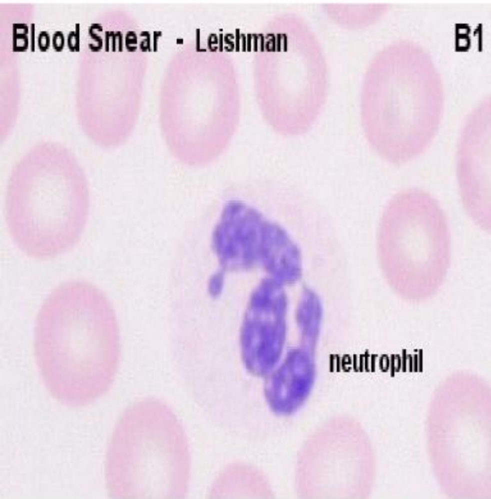
LEUCOCYTES: REVIEW

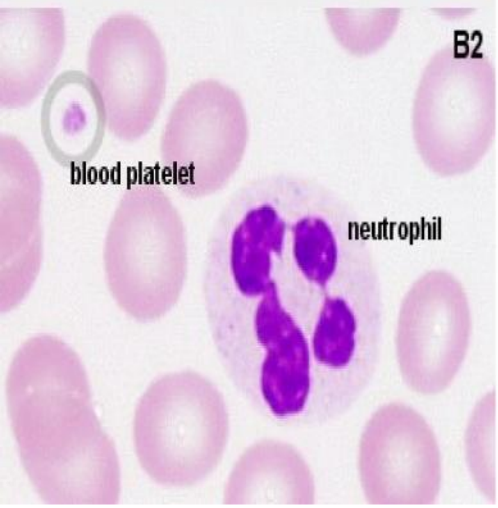
- **Chemotaxis:** Directed movement
- **Chemotaxins:** Cytotaxins & Cytotaxigens

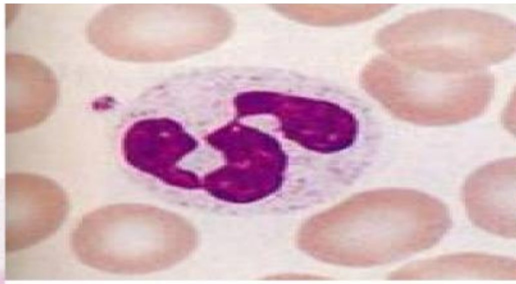
NEUTROPHIL GRANULOCYTES

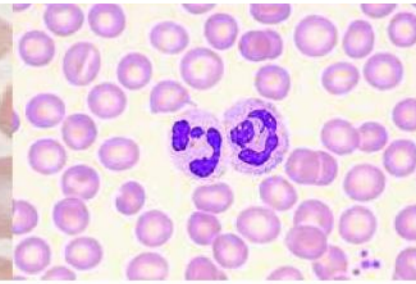


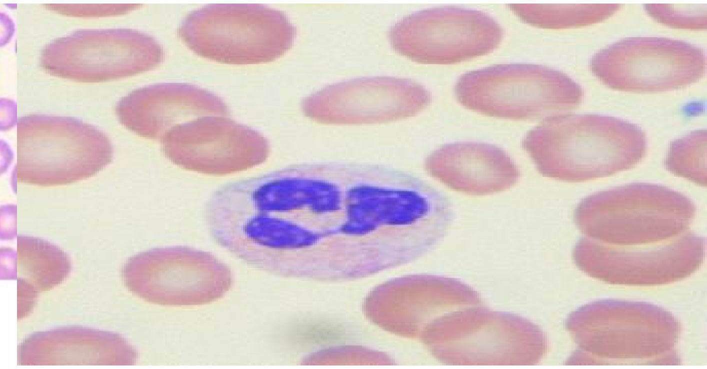
NEUTROPHILS







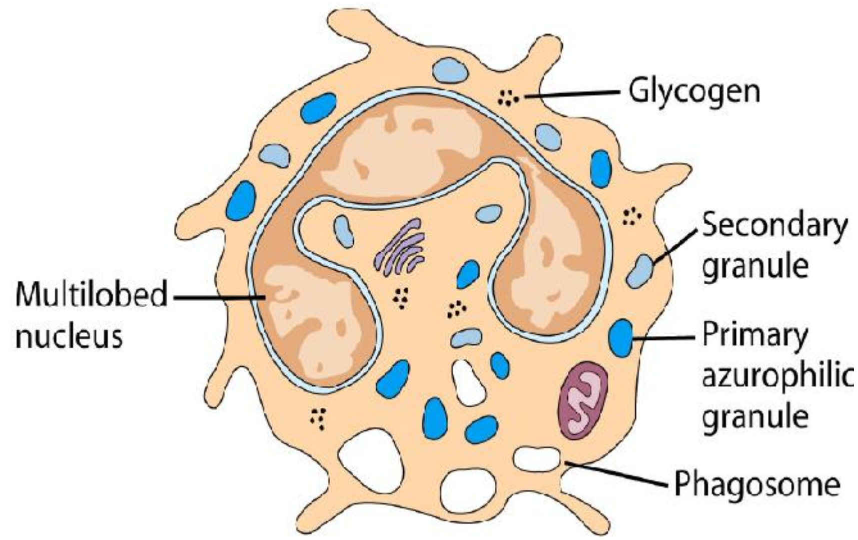




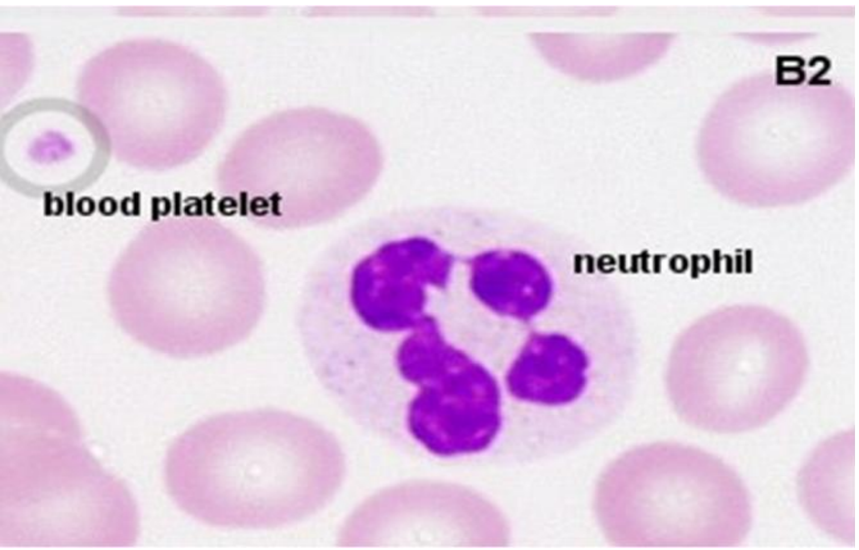
- Neutrophils have two types of granules that:
  - Take up both acidic and basic dyes
  - Give the cytoplasm a lilac color
  - Contain
    - **peroxidases,**
    - **hydrolytic enzymes,**
    - **defensins (antibiotic-like proteins)**
- Neutrophils are our body's bacteria slayers

Granule type Protein

- -**specific granules** (or "secondary granules") Lactoferrin and Cathelicidin
- **azurophilic granules** (or "primary granules")
- myeloperoxidase, bactericidal/permeability increasing protein (BPI)
- Defensins and the serine proteases
- neutrophil elastase and cathepsin G
- **tertiary granules** cathepsin and gelatinase



(a) Neutrophil



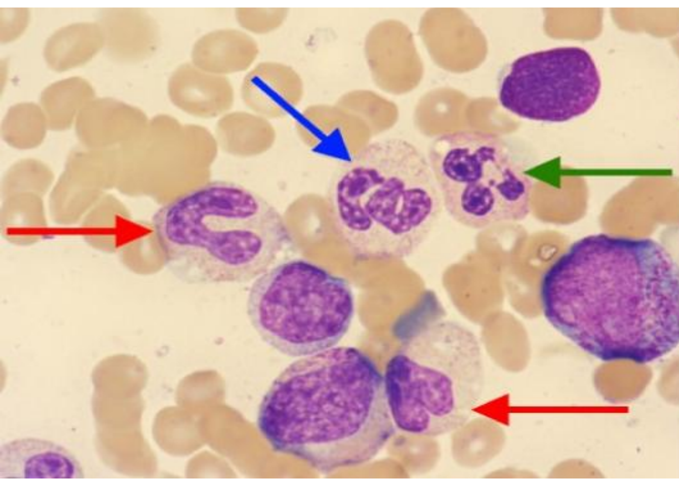
- Circulate in peripheral blood 7-10 hr before migrating into tissue; live only a few days
- "front line of innate defense"
- increased # (leukocytosis) used as an indicator of infection
- extravasate in inflam rxn
- **attracted by chemotactic factors =➔They migrate through the blood vessels, then through interstitial tissue, following chemical signals such as**
  - **-Interleukin-8 (IL-8) and C5a**
  - **-interferon gamma (IFN-gamma),**
  - **-C5a**.
- active phagocytes; digestive enzyme held in 1° and 2° granules
- Use both O2-dep and O2-indep digestive mech's

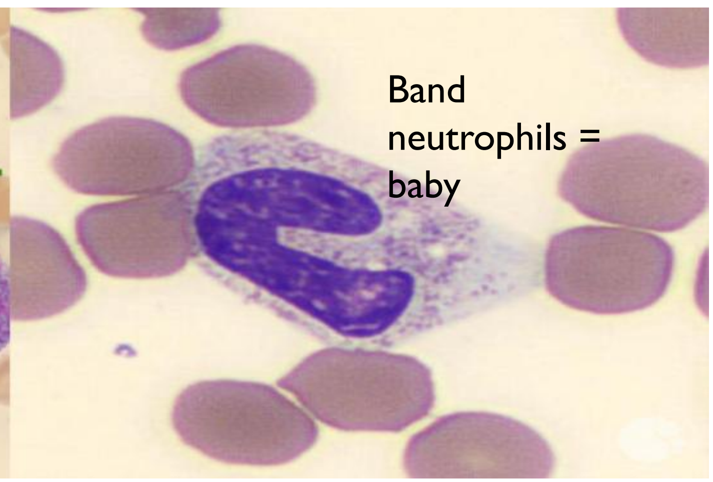
NEUTROPHILIA

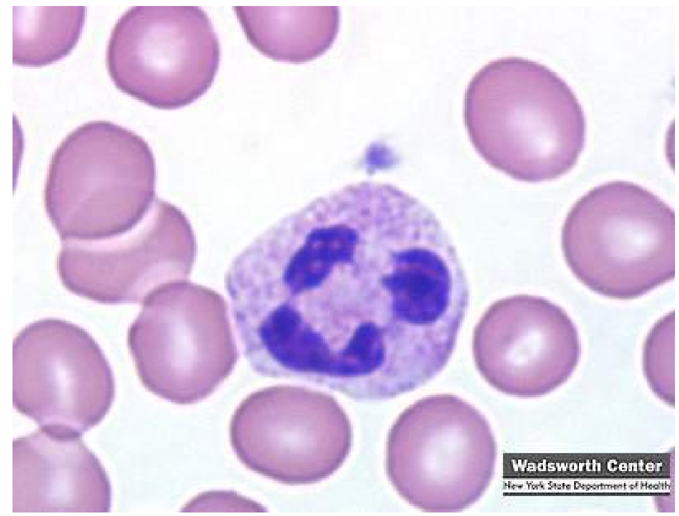
- **NEUTROPHILIA:** **Increased neutrophil count, can be due to:**
  - **Release of stored cells from the bone marrow reserves.**
  - **Bacterial Infections causing increased Neutropoiesis.**
  - **Exercise can cause release of stored neutrophils.**

SEGMENTED NEUTROPHIL (MATURE NEUTROPHILS)

- Neutrophils are produced in bone marrow,
- Released into blood after completing their maturation in marrow, circulate for less than a day, and migrate out of the vessels into tissues or into alveoli and gut lumen.

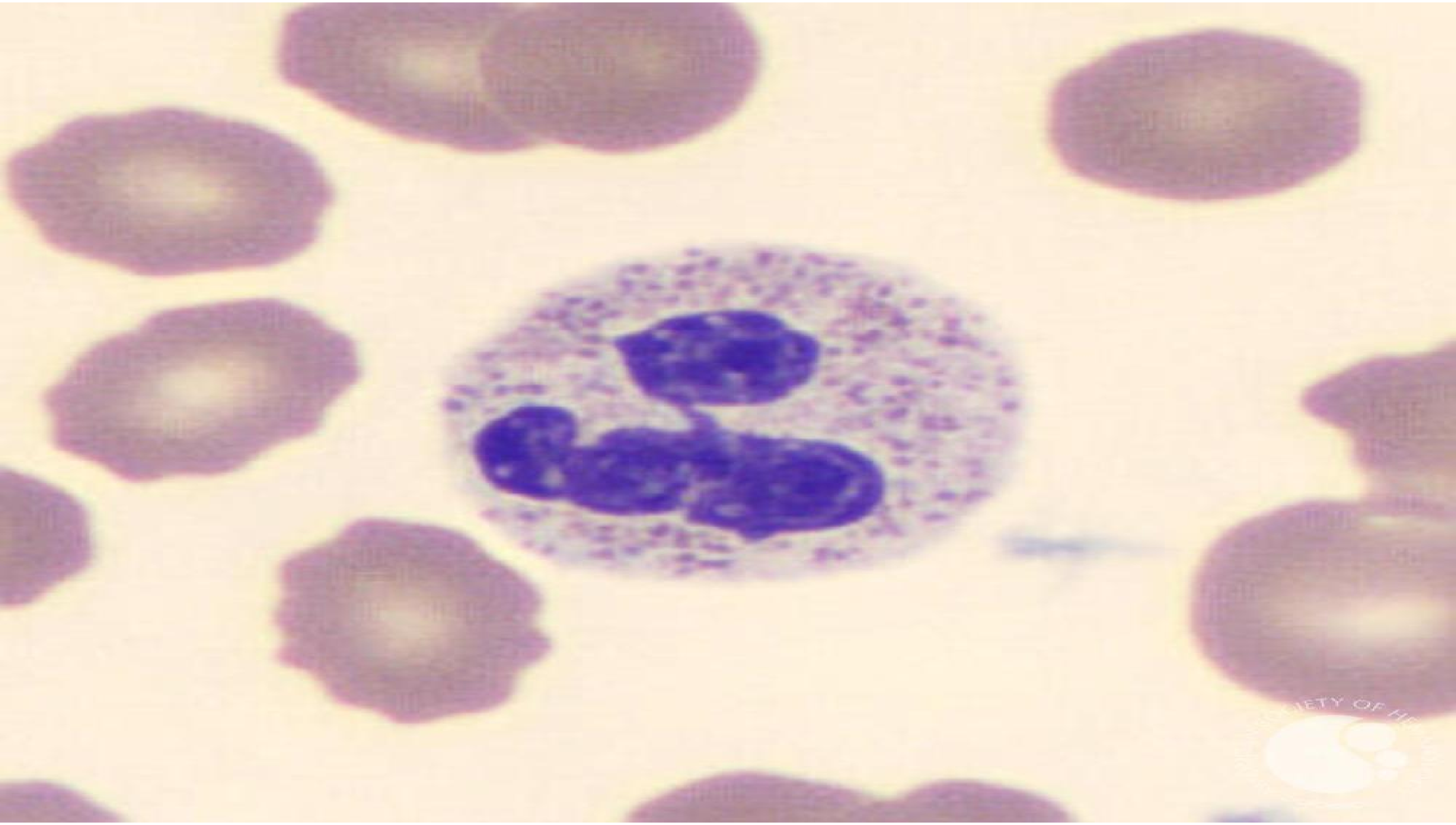






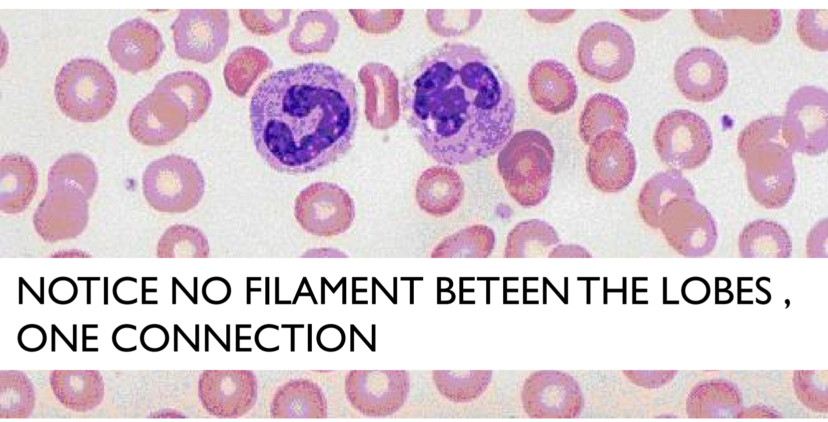
NOTICE THE FILAMENTS BETWEEN THE LOBES

Segmented neutrophils are the most mature **neutrophilic** granulocytes present in circulating blood.

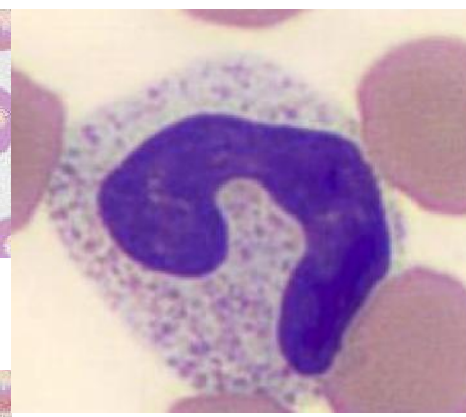


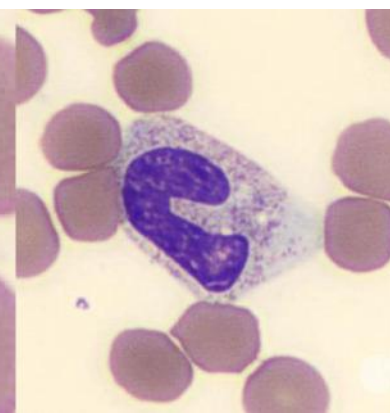
BAND NEUTROPHIL = BABY NEUTROPHILS

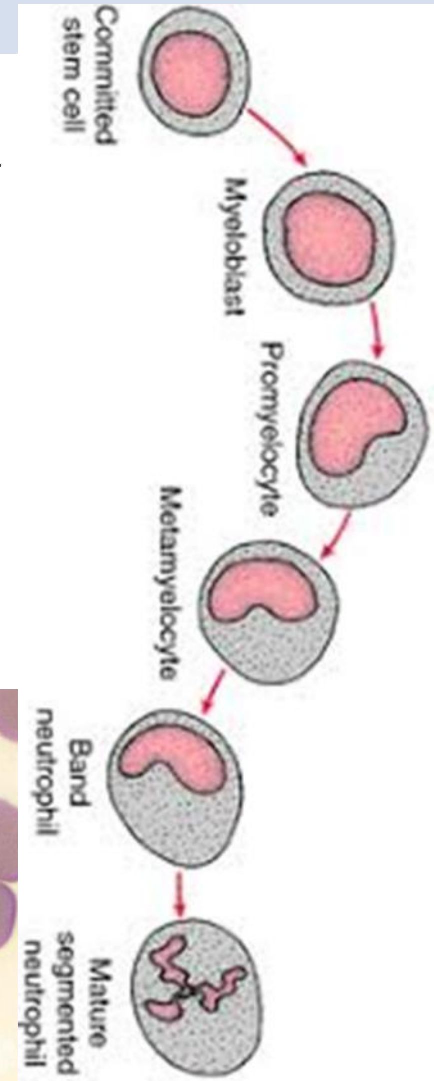
- A **band cell** (**stab cell**) is a cell undergoing granulopoiesis, derived from a metamyelocyte, and leading to a mature granulocyte.
- It is characterized by having a nucleus which is curved, but not lobar.
- Often the term "band cell" implies a neutrophilic lineage.
- However, the term is not used only with neutrophils.
- **A count of band neutrophils is used to measure inflammation.**

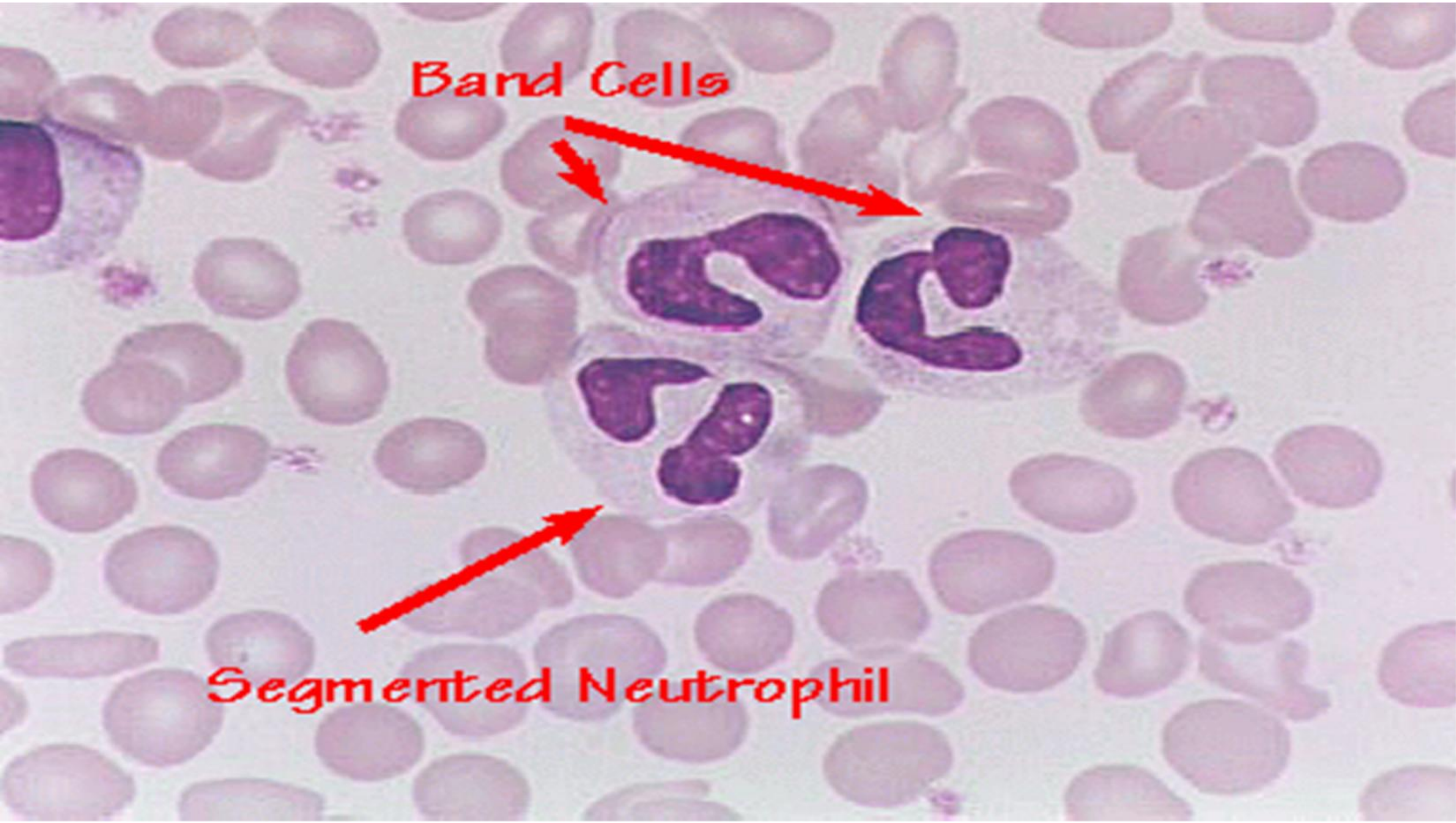


NOTICE NO FILAMENT BETEEN THE LOBES , ONE CONNECTION








Band Cells
Segmented Neutrophil

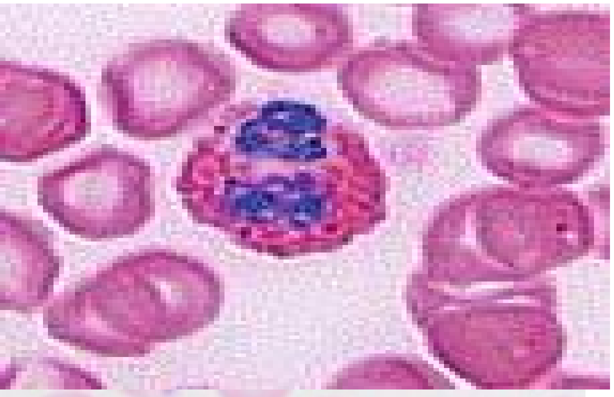
CYTOPLASMIC GRANULES

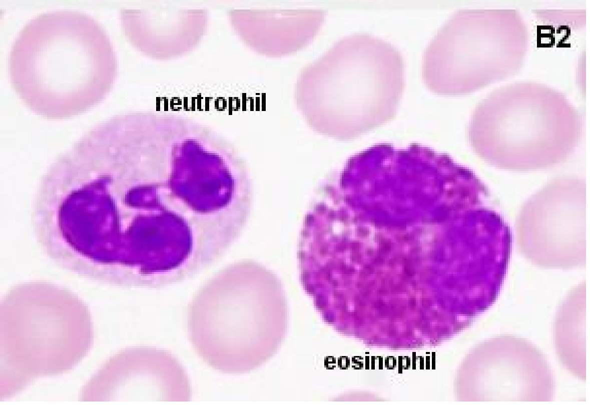
- Fine, azurophilic (Stain with both Eosin & Methylene blue) in nature.
- Contain enzymes such as:
  - Cathepsins.
  - Phosphatases.
  - Nucleases.
- Granules serve as lysosomes.

Neutrophils & Monocytes: Functions

- They seek, attack and destroy invading **bacteria, viruses and other injurious agents**
- Neutropils attack and destroy bacteria and viruses, even in the blood.
- Monocytes are immature until they enter the tissues. There, they swell up to 80 Microns, develop lysosomes, and become Macrophages, capable of defence.

- **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.





EOSINOPHILS

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, lysosome-like granules
- Lead the body's counterattack against parasitic worms
- Lessen the severity of allergies by phagocytizing immune complexes

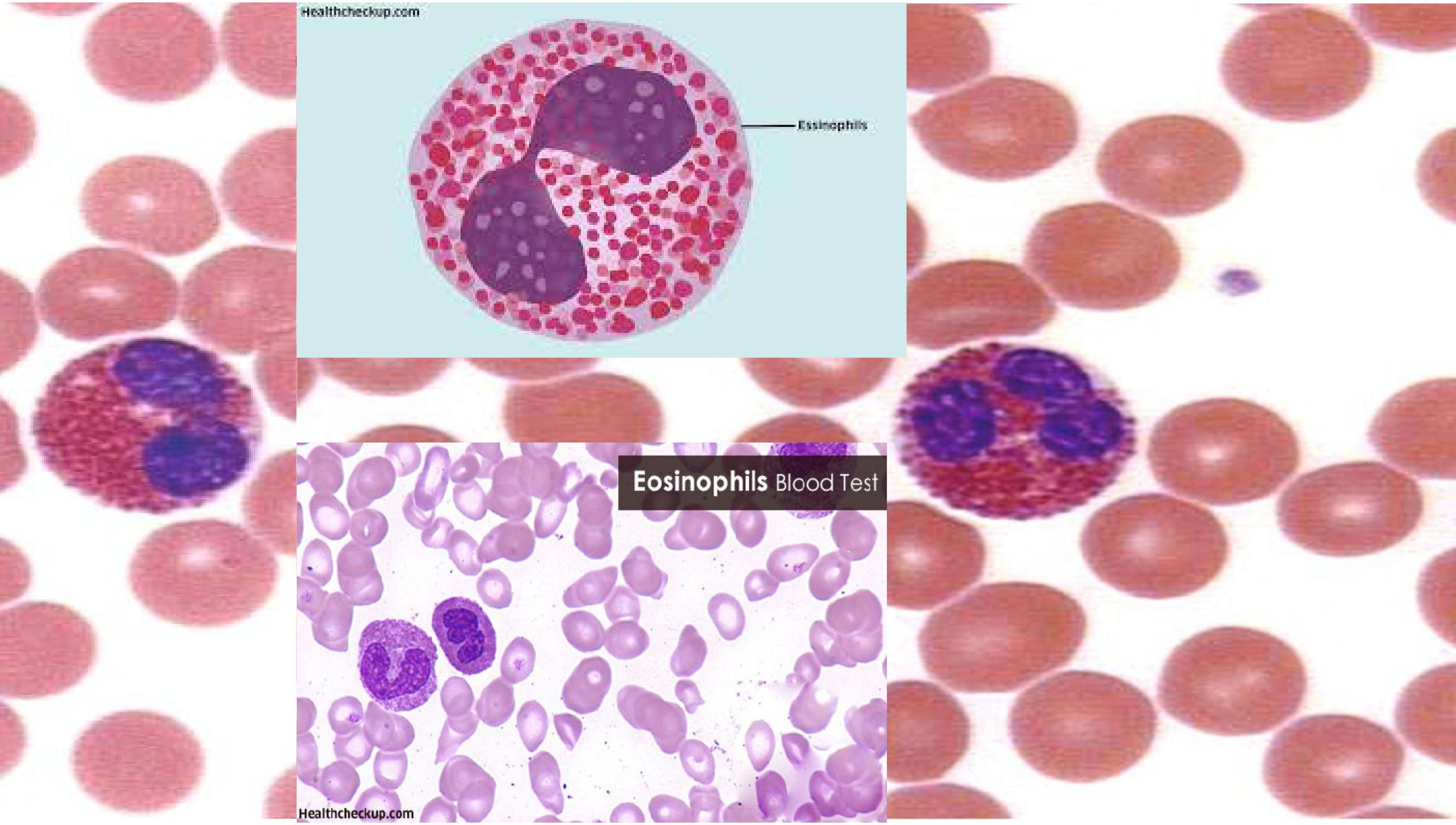
EOSINOPHILS

- Small granules contain many mediators:
  - histaminase and
  - eosinophil peroxidase,
  - ribonuclease (RNase),
  - deoxyribonucleases,
  - lipase, plasminogen,.
  - These mediators are released by a process called degranulation following activation of the eosinophil, and are toxic to both parasite and host tissues

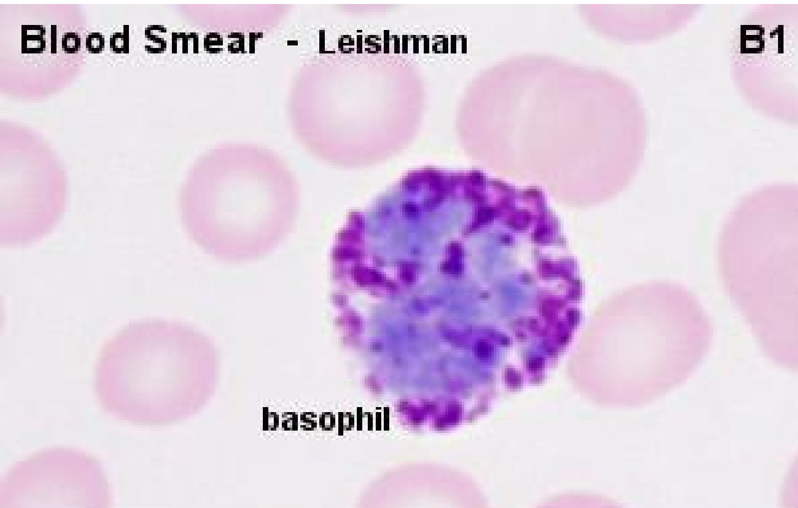
- **They are found in the**
  - **medulla and the junction between the cortex and medulla of the thymus,**
  - **in the lower gastrointestinal tract,**
  - **ovary,**
  - **uterus,**
  - **spleen,**
  - **lymph nodes,**
  - **but not in the lung, skin, esophagus, or some other internal organs under normal conditions.**

The presence of eosinophils in these latter organs is associated with disease.

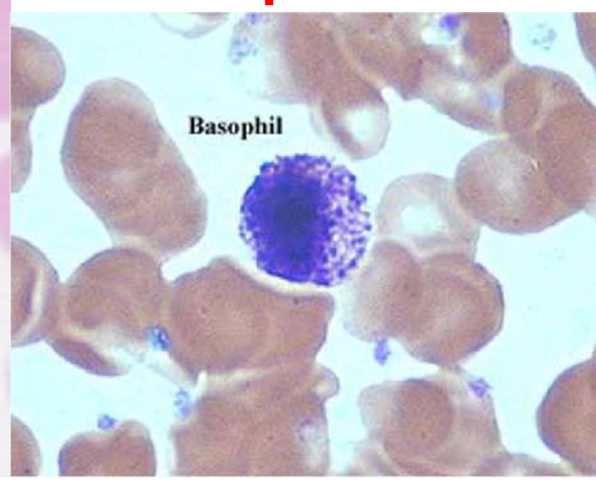
Eosinophils persist in the circulation for 8–12 hours, and can survive in tissue for an additional 8–12 days in the absence of stimulation

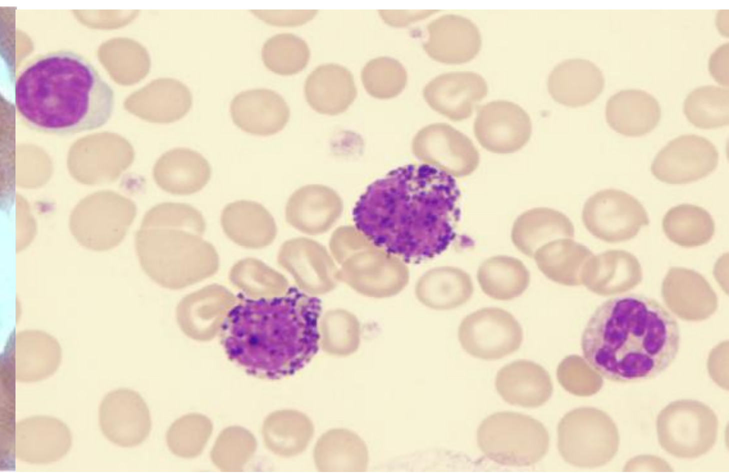

Healthcheckup.com
Essinophils
Eosinophils Blood Test
Healthcheckup.com

BASOPHILS



- Account for 0.5% of WBCs and:
  - 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
    - **Histamine** – inflammatory chemical that acts as a vasodilator and attracts other WBCs (antihist amines counter this effect)
    - **Heparine**



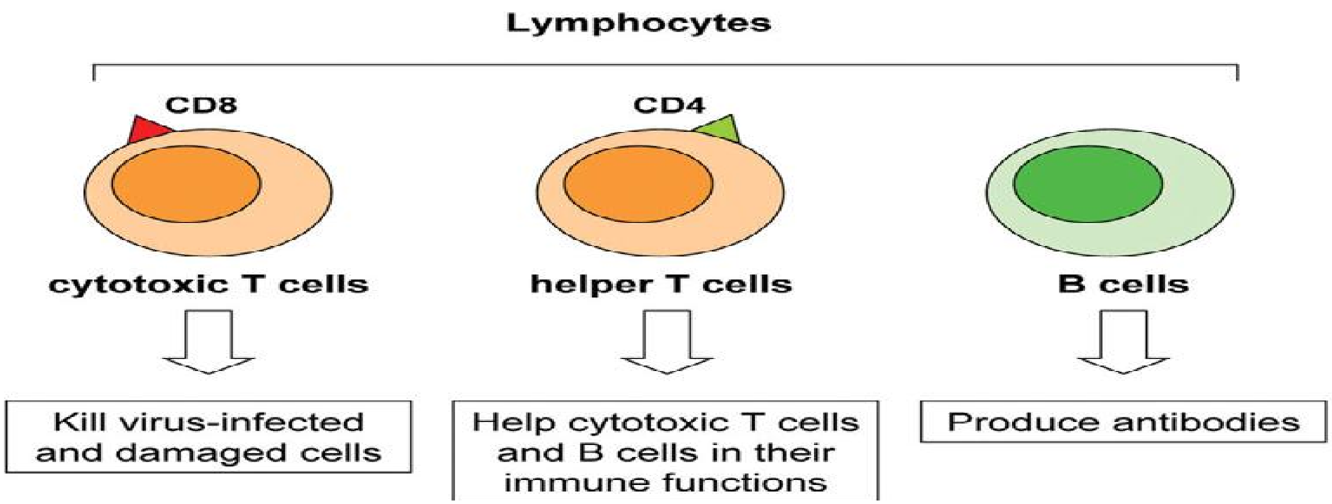


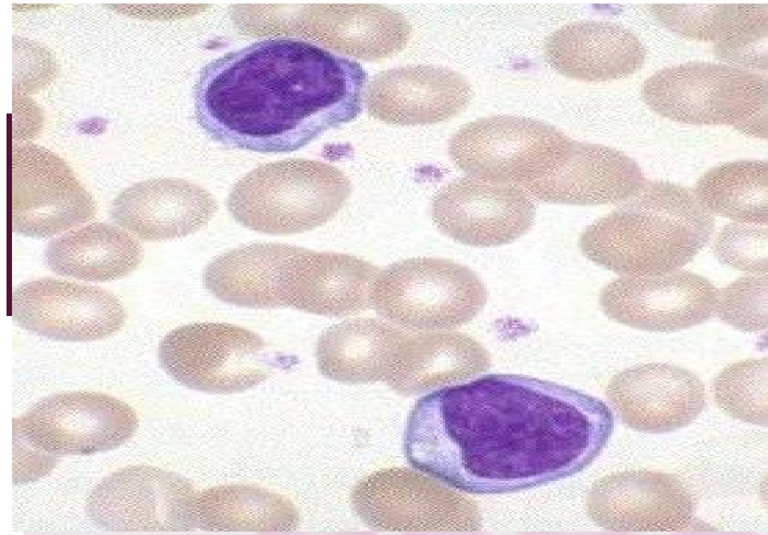
AGRANULOCYTES

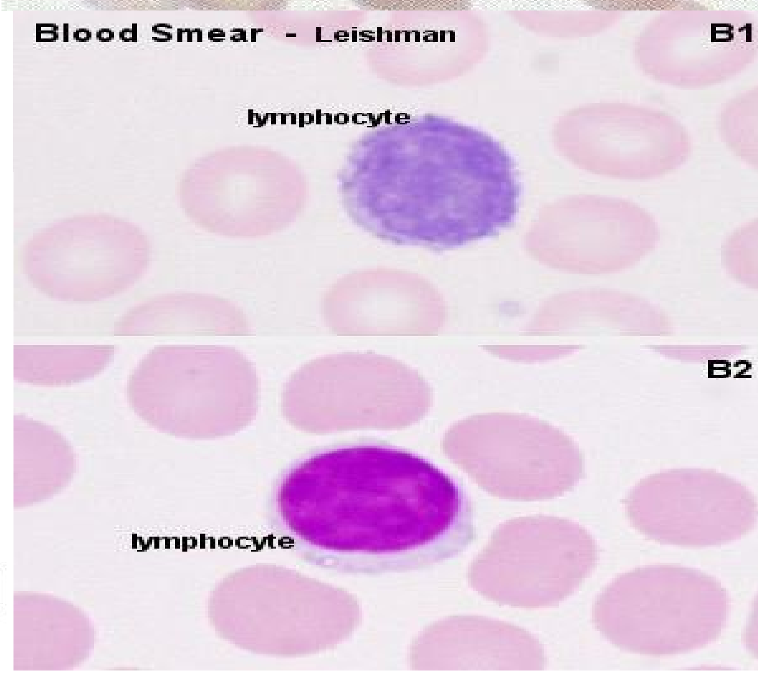
- Agranulocytes :
- **lymphocytes and monocytes**:
  - Lack visible cytoplasmic granules
  - Are similar structurally, but are functionally distinct and unrelated cell types
  - Have spherical (lymphocytes) or kidney-shaped (monocytes) nuclei

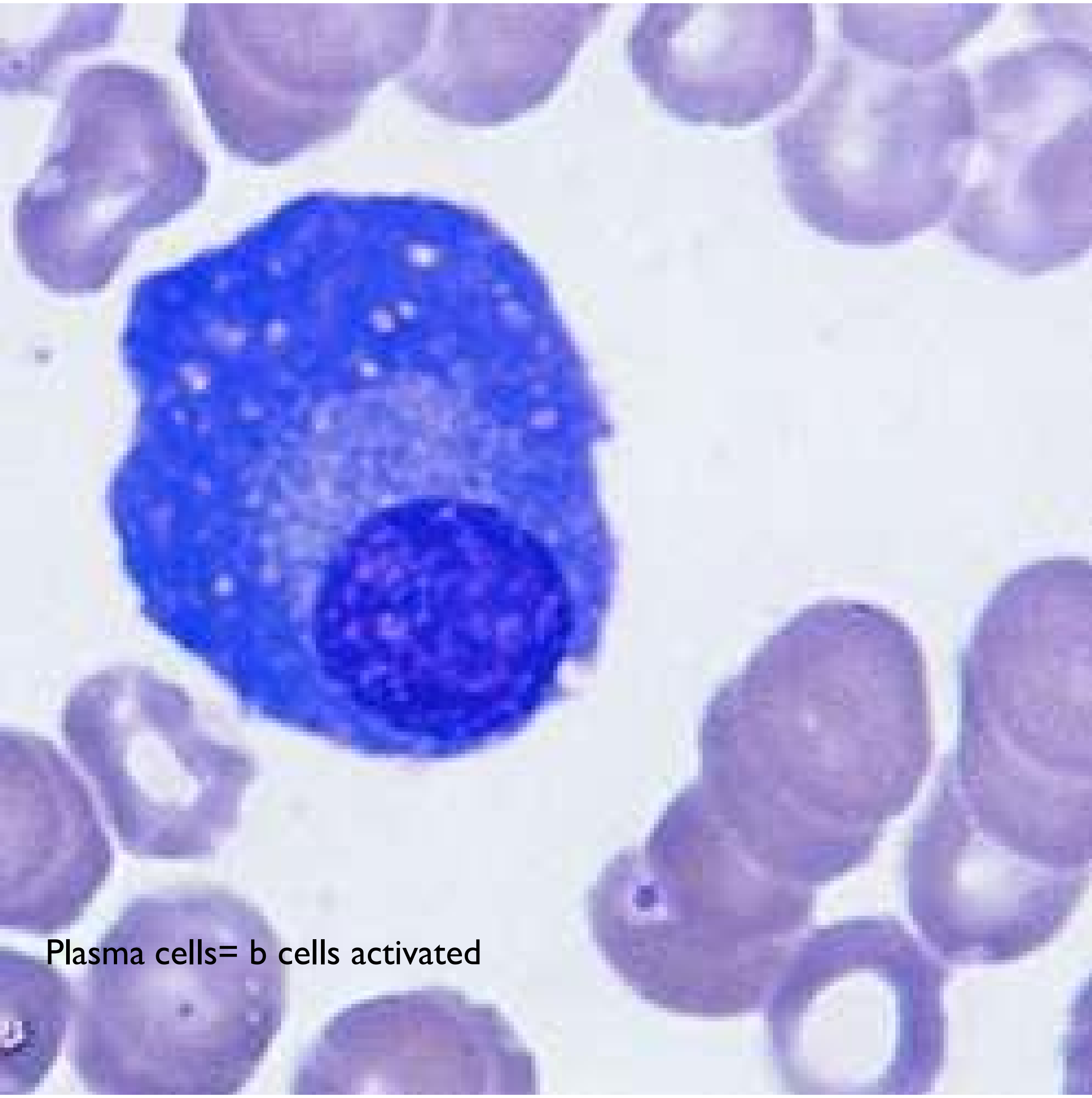
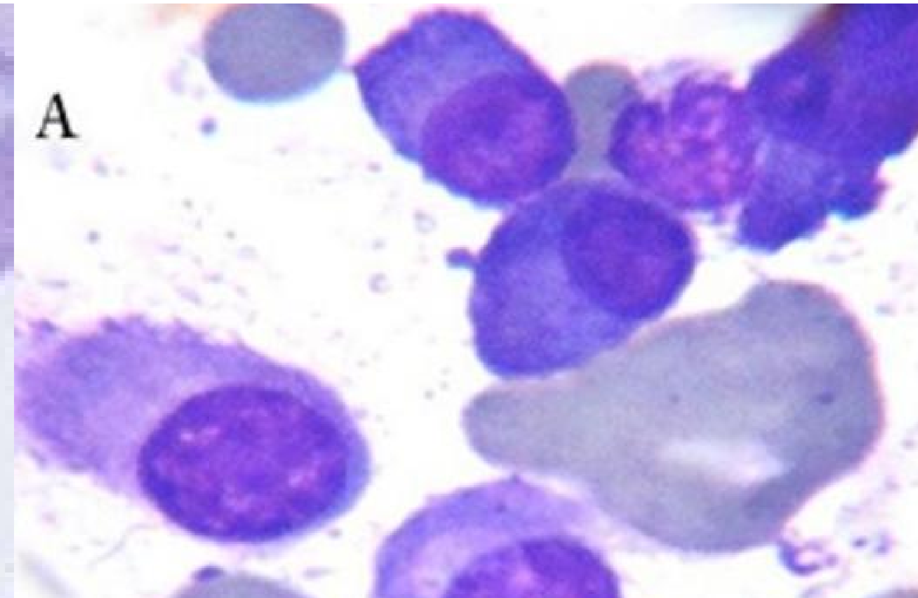
LYMPHOCYTES

- Account for 25% 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)
- There are two types of lymphocytes: T cells and B cells
  - ***T cells function in the immune response***
  - ***B cells give rise to plasma cells, which produce antibodies***








Plasma cells= b cells activated

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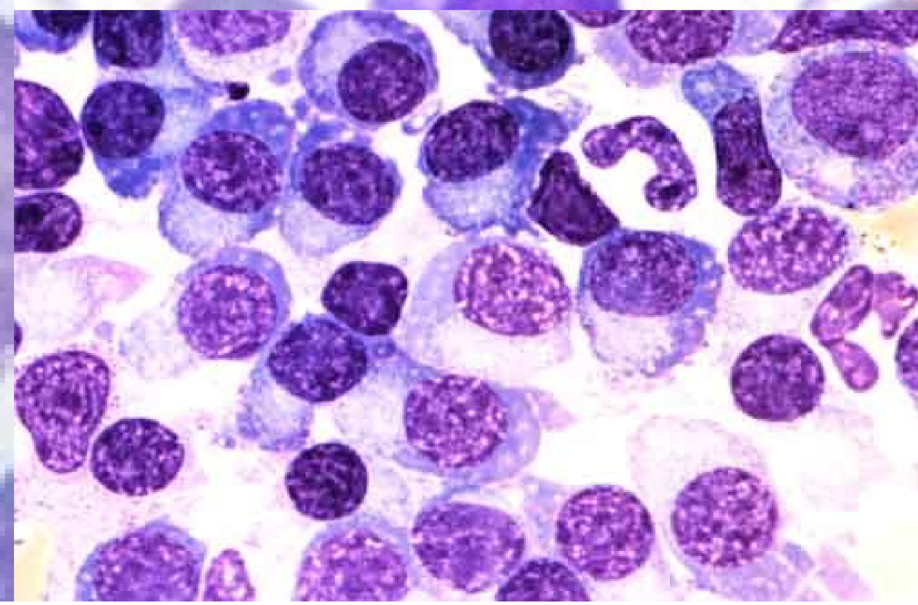
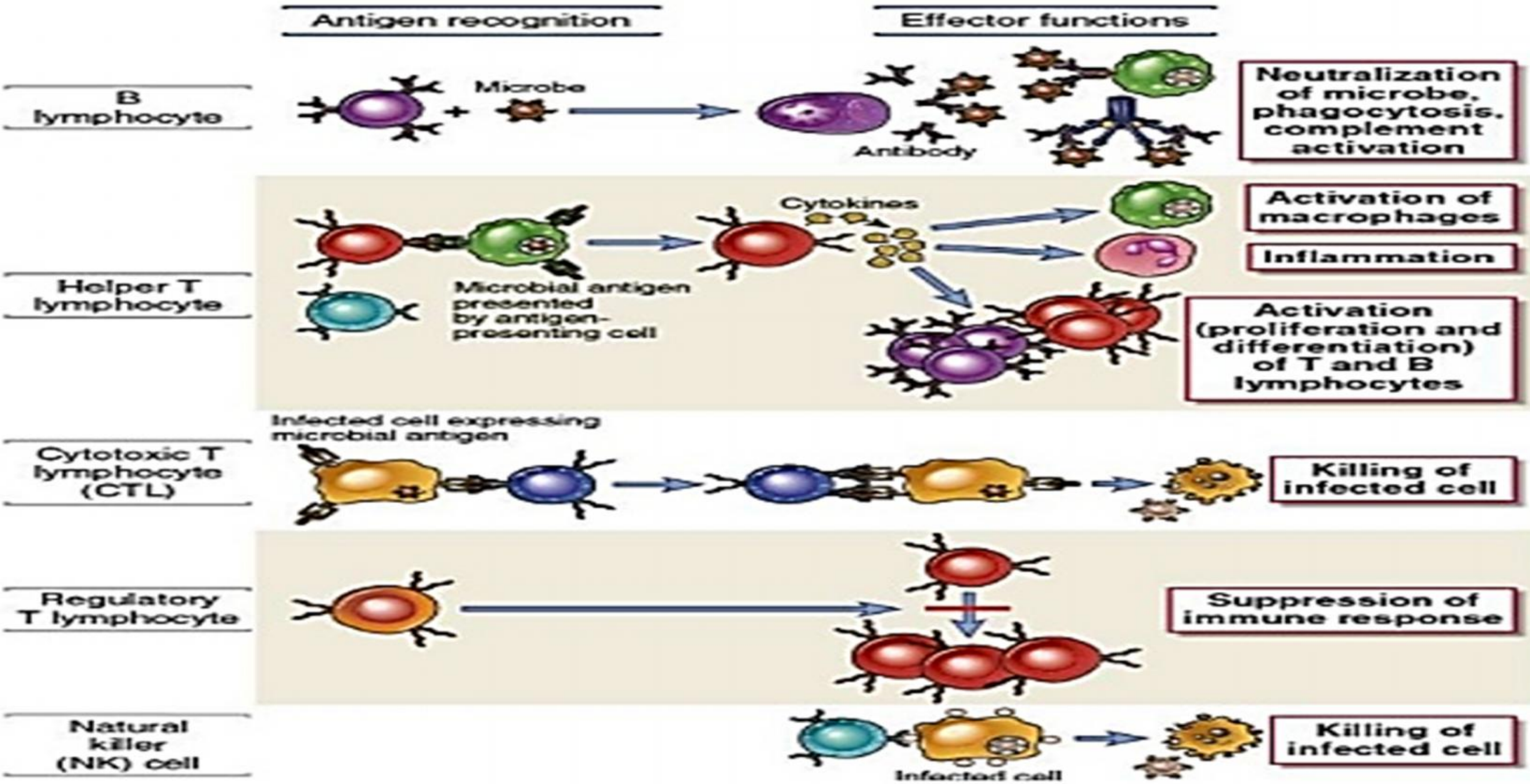
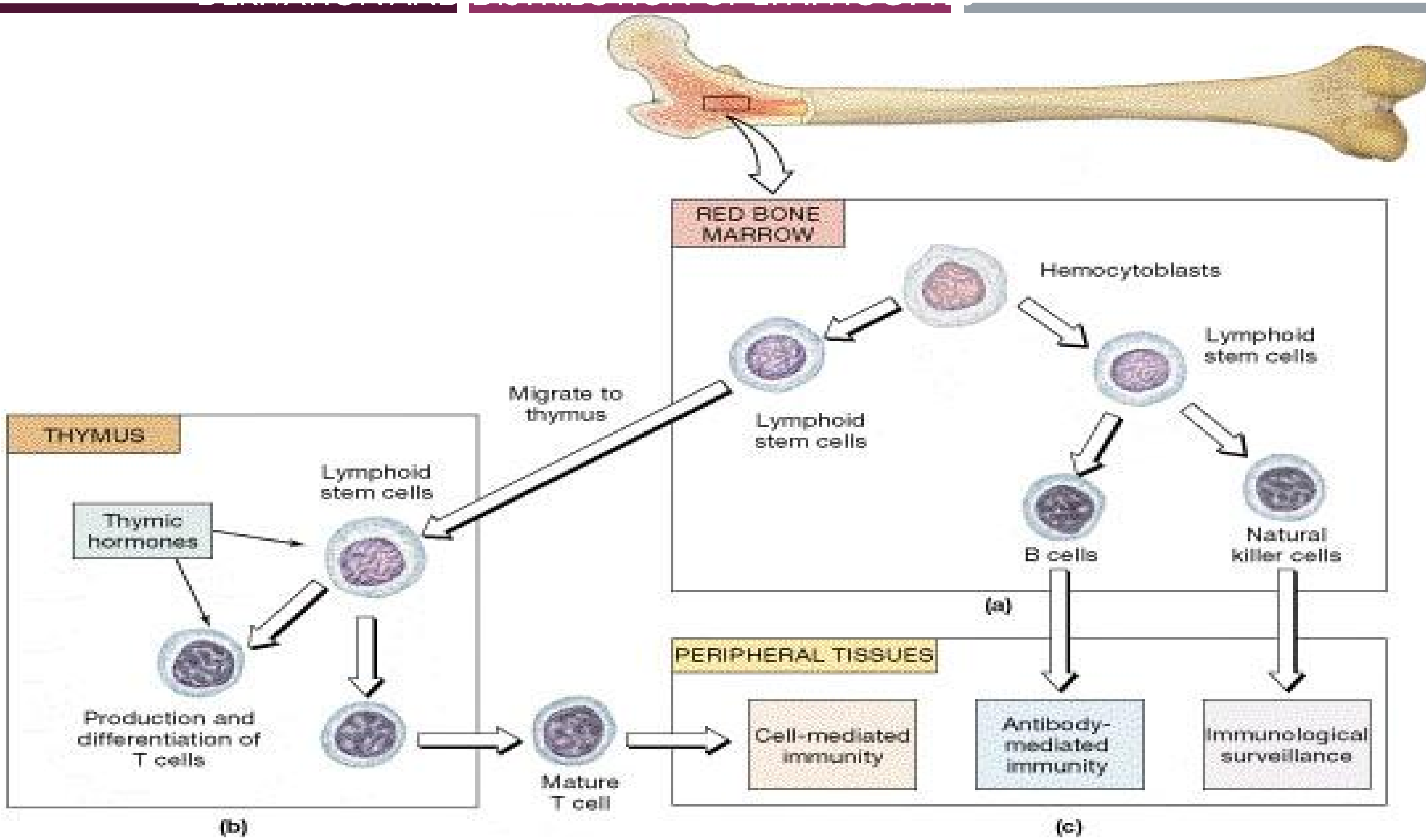


Types of Lymphocytes		
Cell Type	**Function**	**Type of Antigen Response**
T-LYMPHOCYTE		
Helper T-lymphocyte	Initiates and oversees the immune response	Responds to a single antigen
Cytotoxic T-lymphocyte	Directly kills foreign cells; must be activated by a helper T-lymphocyte first	Responds to a single antigen
Memory T-lymphocyte	A type of cytotoxic T-lymphocyte that has already killed; patrols the body looking for the same antigen again	Responds to a single antigen
Suppressor T-lymphocyte	Helps "turn off" the immune response once it has been activated	Responds to a single antigen
B-LYMPHOCYTE		
Plasma cell	Produces and secretes antibodies	Responds to a single antigen
Memory B-lymphocyte	Remembers an initial antigen attack and mounts a faster, more efficient response should the same antigen type attack again	Responds to a single antigen
NK (NATURAL KILLER) CELL		
NK (natural killer) cell	Kills a wide variety of infected and cancerous cells	Responds to multiple antigens

Classes of lymphocytes



Abbas, Lichtman and Pillai. *Cellular and Molecular Immunology*, 7th edition, 2011 (C) Elsevier


RED BONE MARROW
Hemocytoblasts
Lymphoid stem cells
Lymphoid stem cells
B cells
Natural killer cells
(a)
Migrate to thymus
THYMUS
Lymphoid stem cells
Thymic hormones
Production and differentiation of T cells
(b)
Mature T cell
PERIPHERAL TISSUES
Cell-mediated immunity
Antibody-mediated immunity
Immunological surveillance
(c)

CELLS OF IMMUNE SYSTEM

Stem cells of bon marrow

differentiate into

cytokines (IL-&, IL-3)

colony stimulating factor

Lymphoid series

Myeloid series

B-lymphocytes **T-lymphocytes**

NK

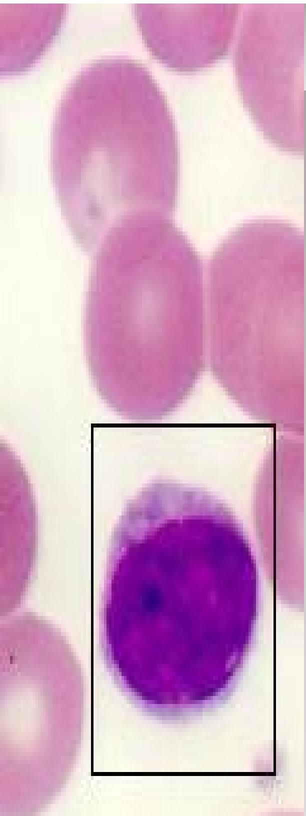
monocytee-macrophages

dendritic cells

eosinophils

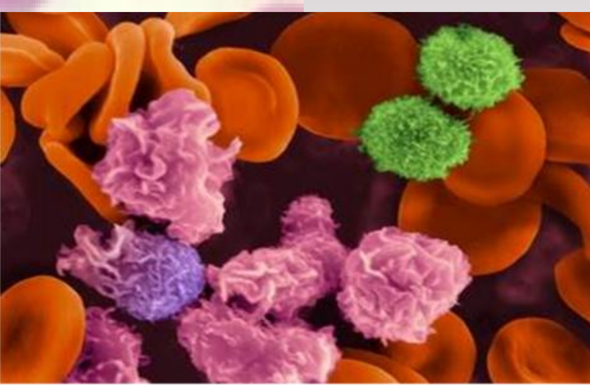
mast cells

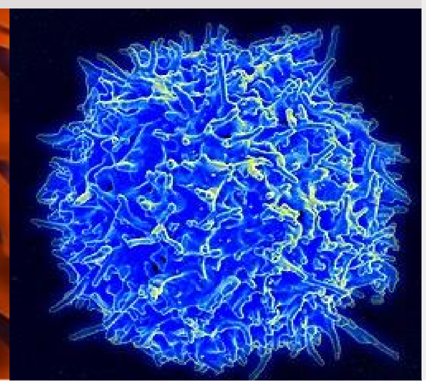
LYMPHOCYTES: IMMUNOCYTES

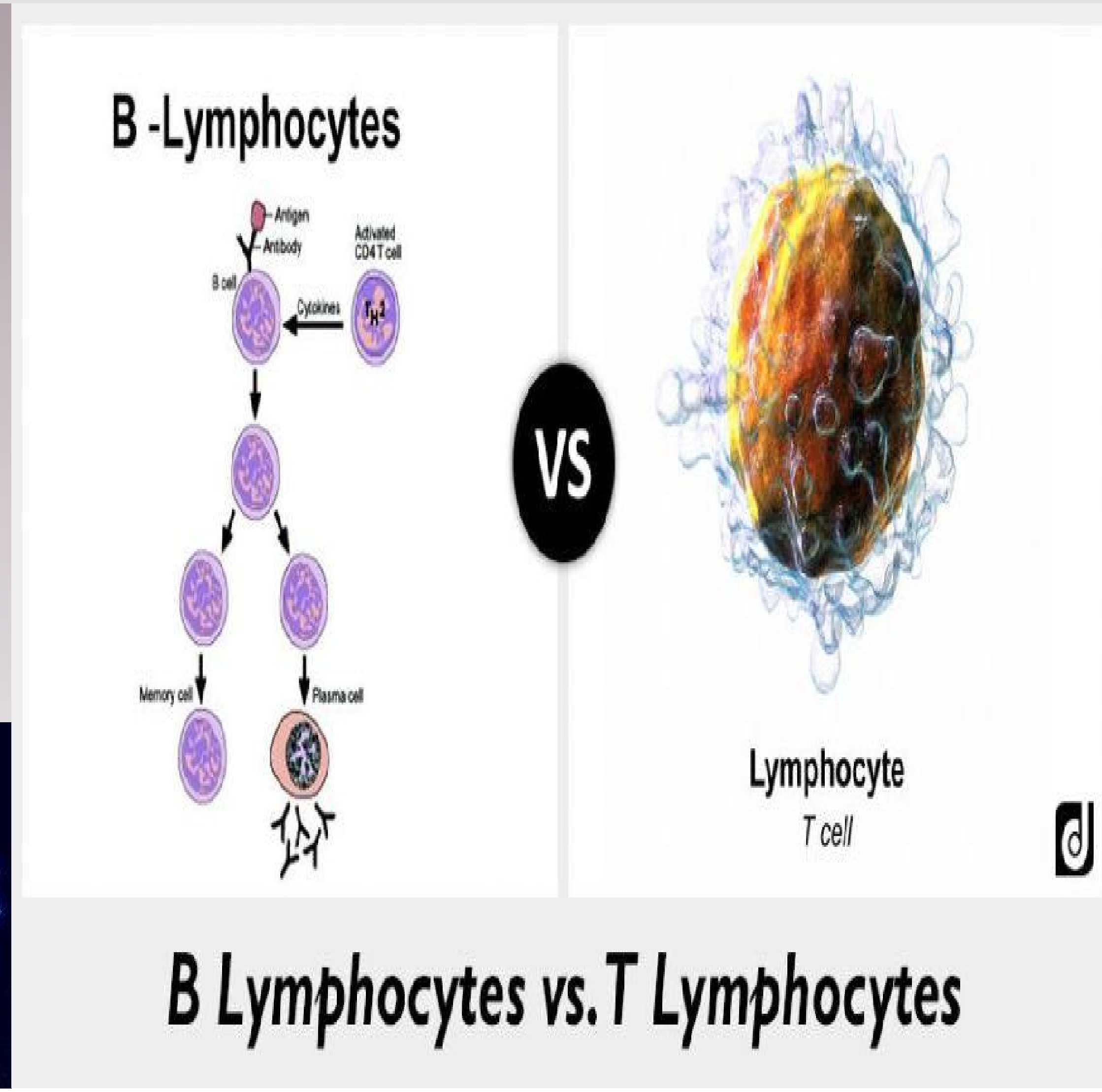


Physiological Classification:T and B

- **‘T’ LYMPHOCYTES :**
  - **Thymus trained or schooled cells**
  - **Responsible for Cell mediated immunity.**
  - **Provide protection against intracellular pathogens**







LYMPHOCYTES

- T cells
  - Manage the immune response
  - Attack and destroy foreign cells
- B cells
  - Produce plasma cells, which secrete antibodies
  - Antibodies immobilize antigens

T-LMPHOCYTES

Immature T-cells express both CD4 and CD8 (DP)

As they mature

* T-cell with TCRs that have affinity to bind to MHC class II will become helper T-cells with CD4 molecule only

* T-cell with TCRs that have affinity to bind with MHC class I will become cytotoxic T-cells with CD8 molecule only

THE LIFE OF THE B CELL

- **B lymphocytes are formed within the bone marrow and undergo their development there**

They have the following functions:

- **To interact with antigenic epitopes, using their immunoglobulin receptors**
- **To subsequently develop into plasma cells, secreting large amounts of specific antibody**, or
- To **circulate as memory cells**
- **To present antigenic peptides to T cells**

B-LYMPOCYTES

* Immature B cells express IgM receptors on the surface

* Mature B cells express IgM, IgD molecules on surfaces

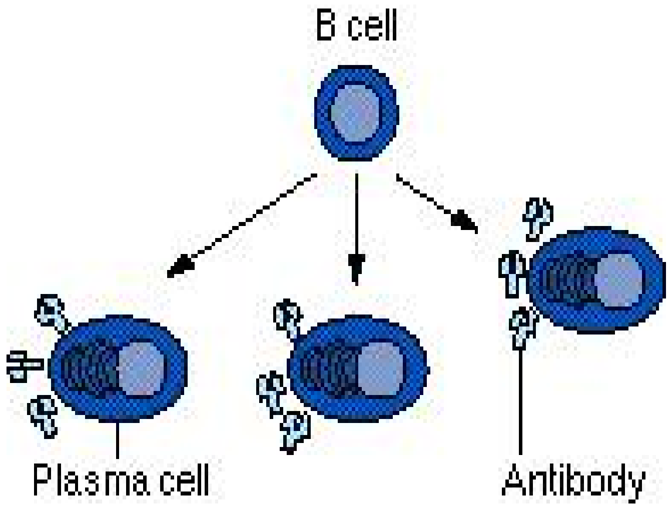
* IgM and IgD molecules serve as receptors for antigens

* Memory B-cells express IgG or IgA or IgE on the surface

* B-cells bear receptors for Fc portion of IgG and a receptor for C3 component of the complement

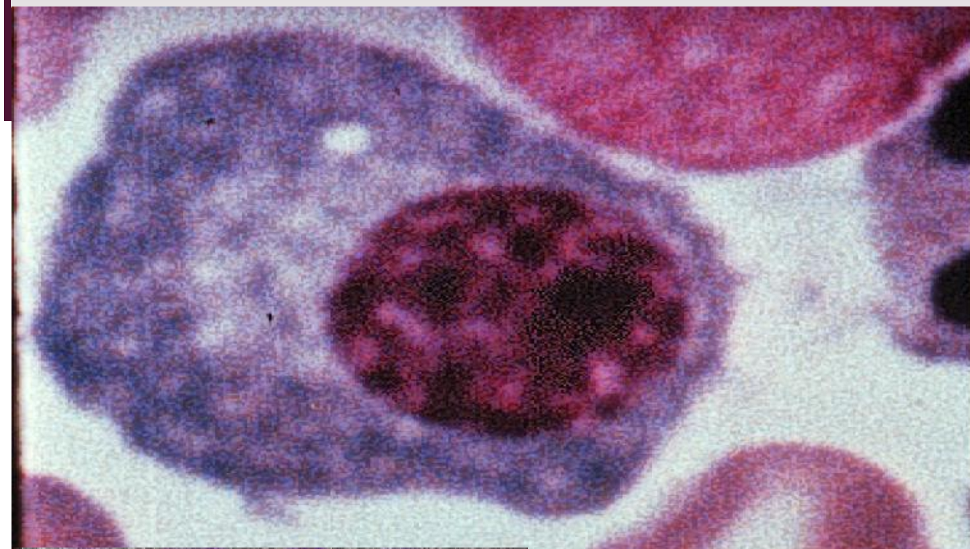
* They express an array of molecules on their surfaces that are important in B-cells interactions with other cells such as MHC II, B7 and CD40

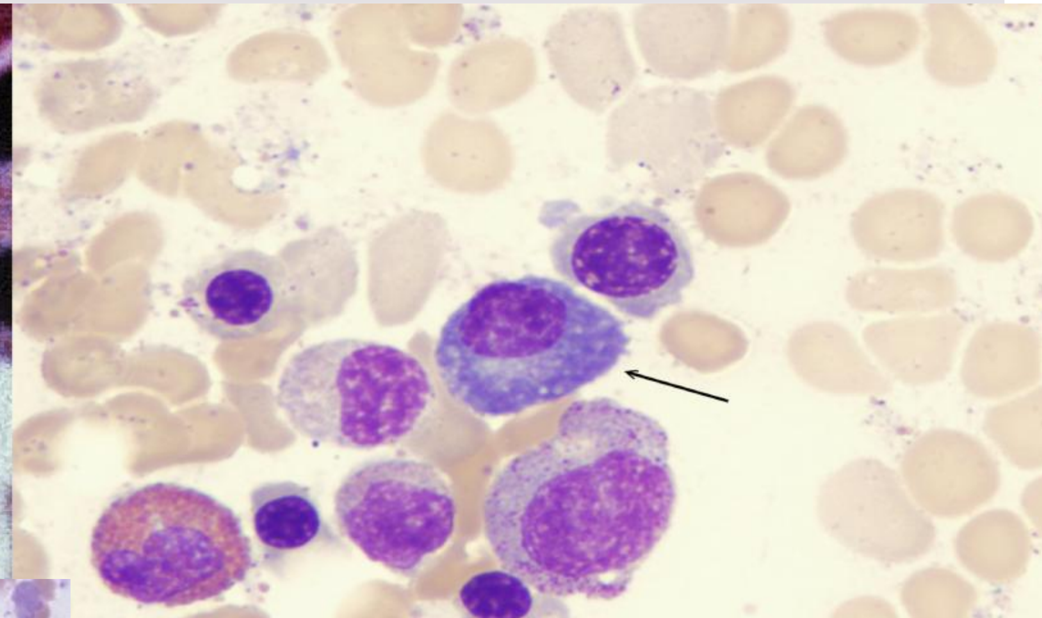
* B CELLS BECOME PLASMA CELLS, WHICH PRODUCE ANTIBODIES WHEN A FOREIGN ANTIGEN TRIGGERS THE IMMUNE RESPONSE

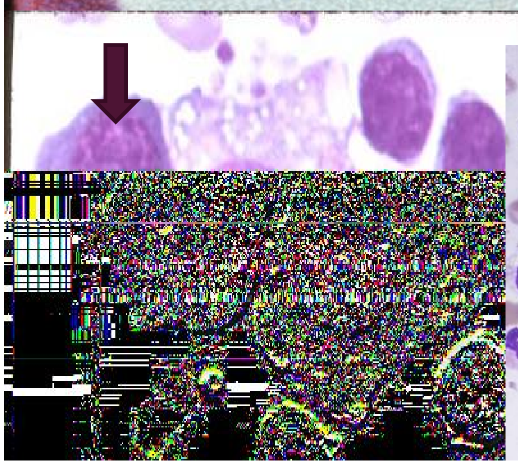


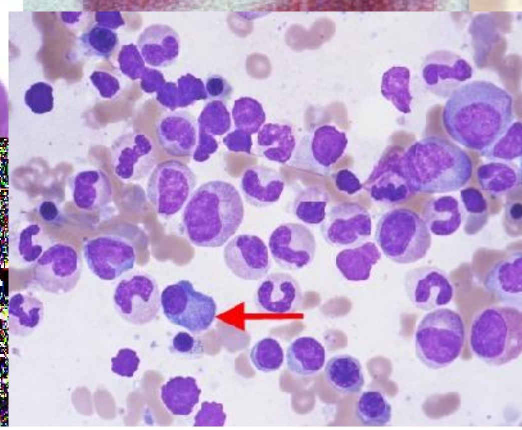
B LYMPHOCYTES , PLASMA CELLS

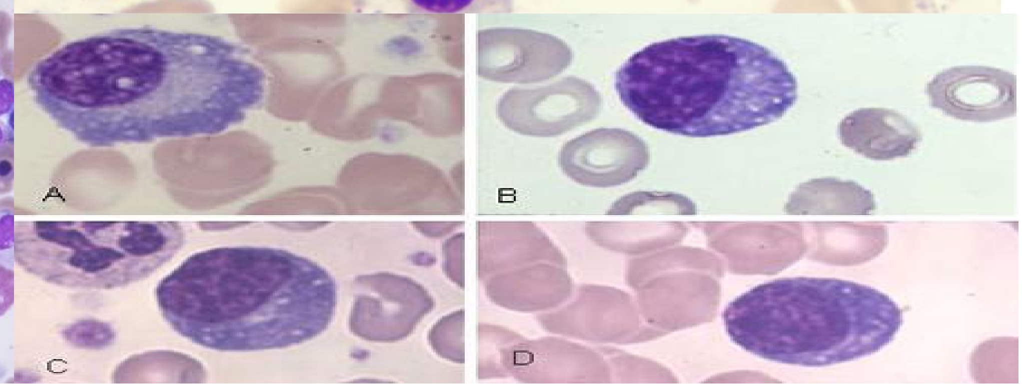
PLASMA B CELLS, PLASMOCYTES, EFFECTOR B CELLS

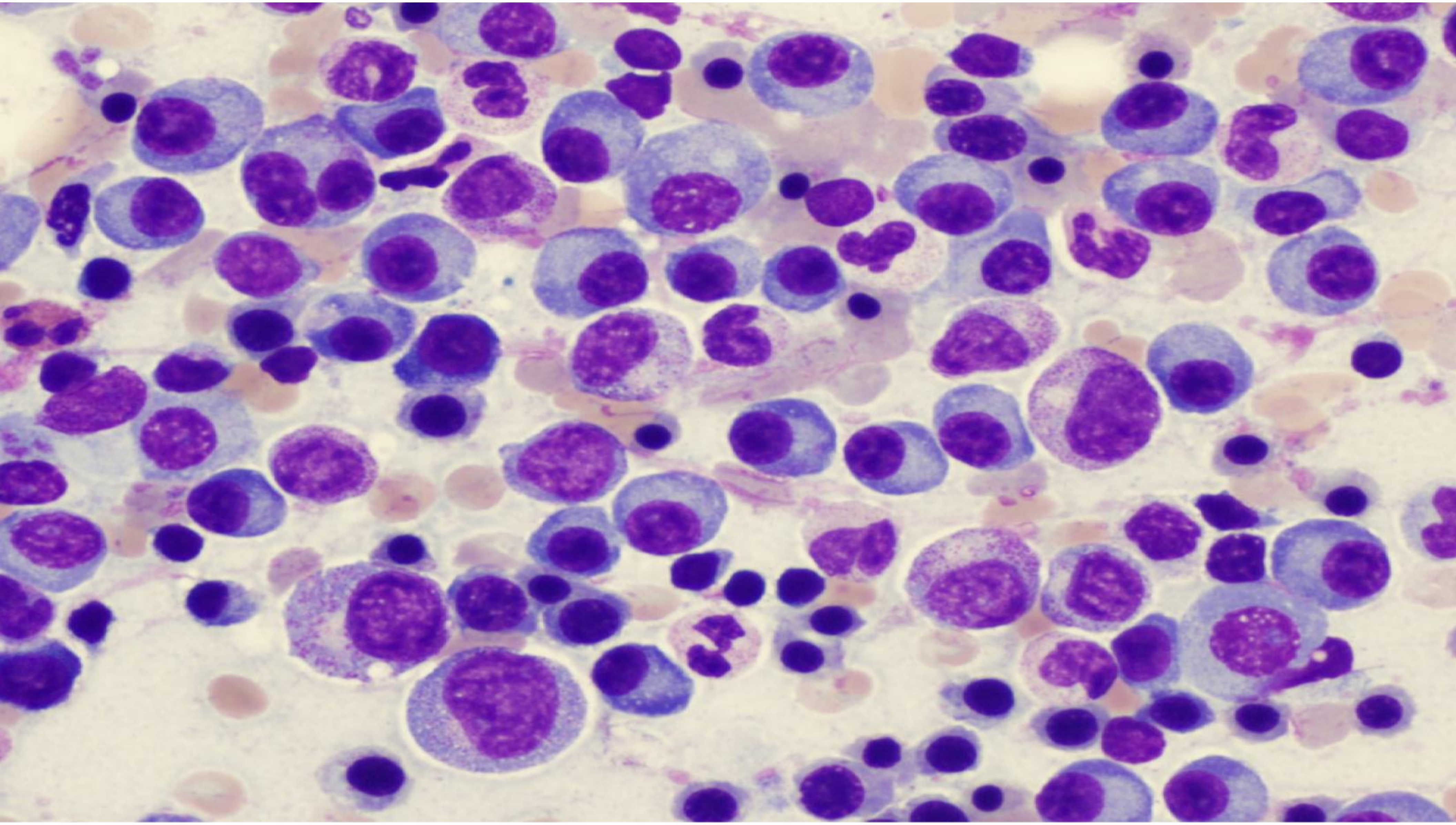


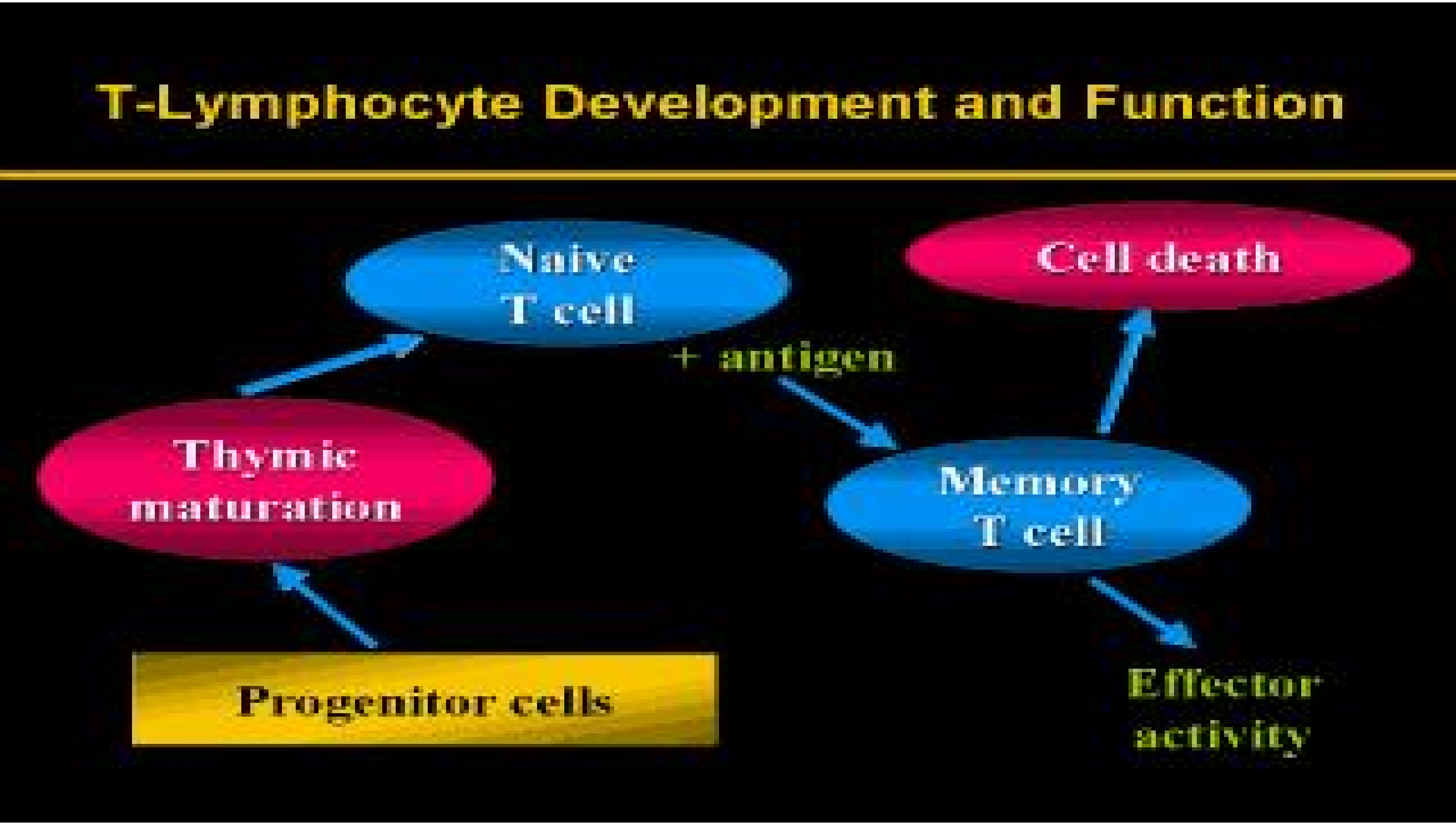


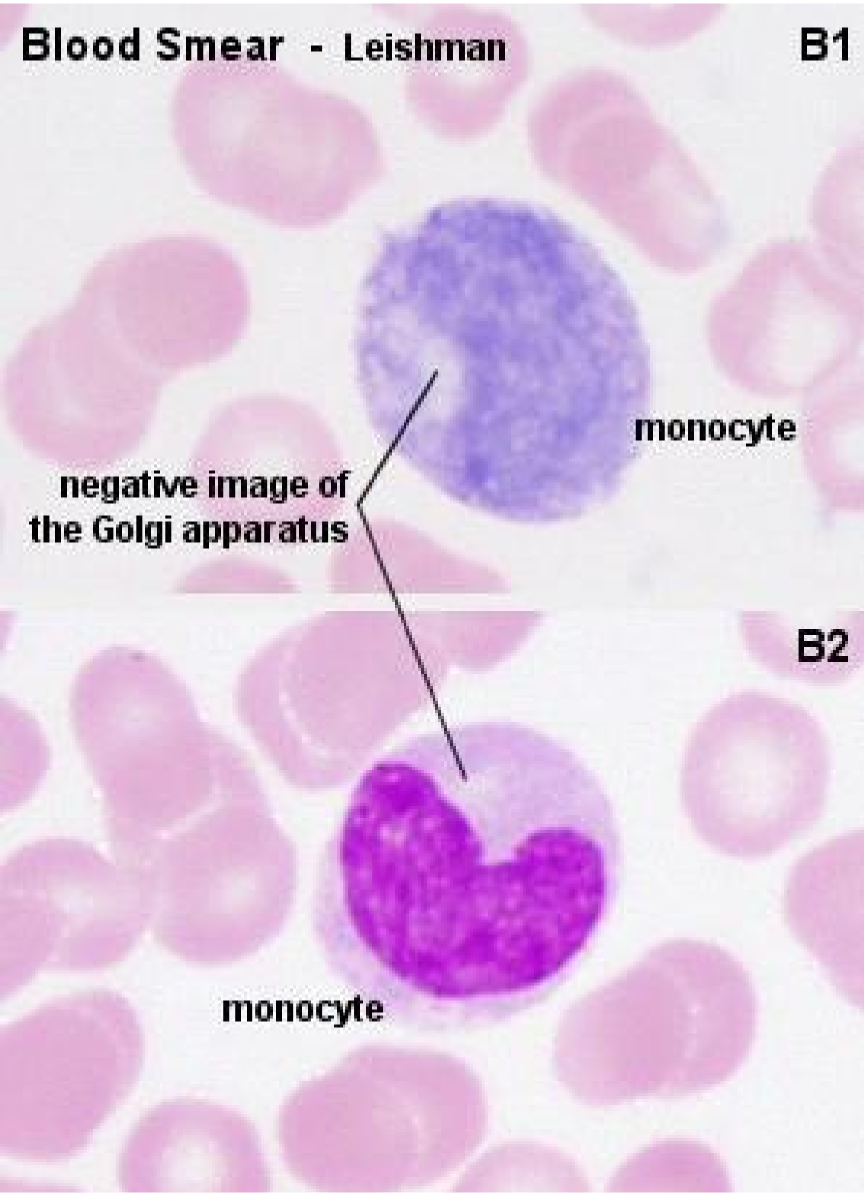








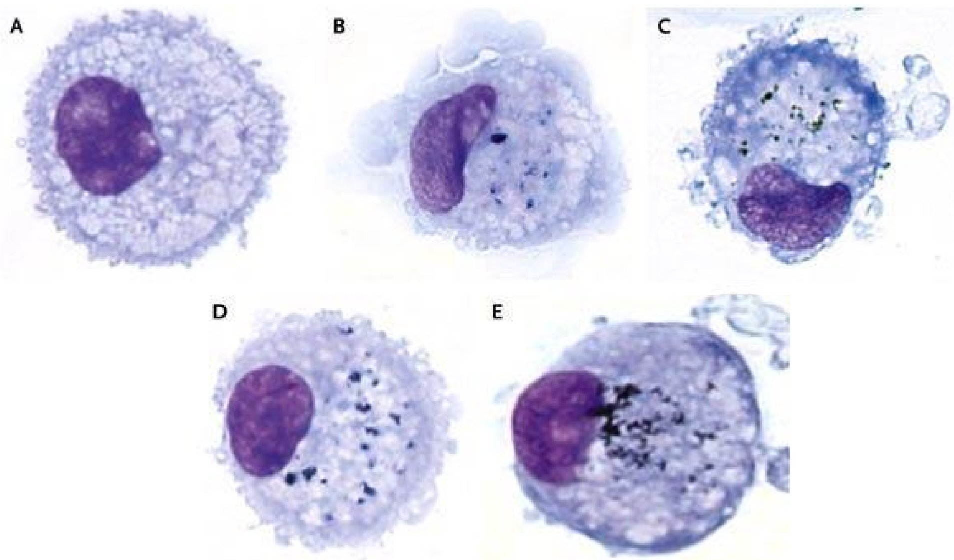

T-Lymphocyte Development and Function
Naive
T cell
Cell death
+ antigen
Thymic
maturation
Memory
T cell
Progenitor cells
Effector
activity

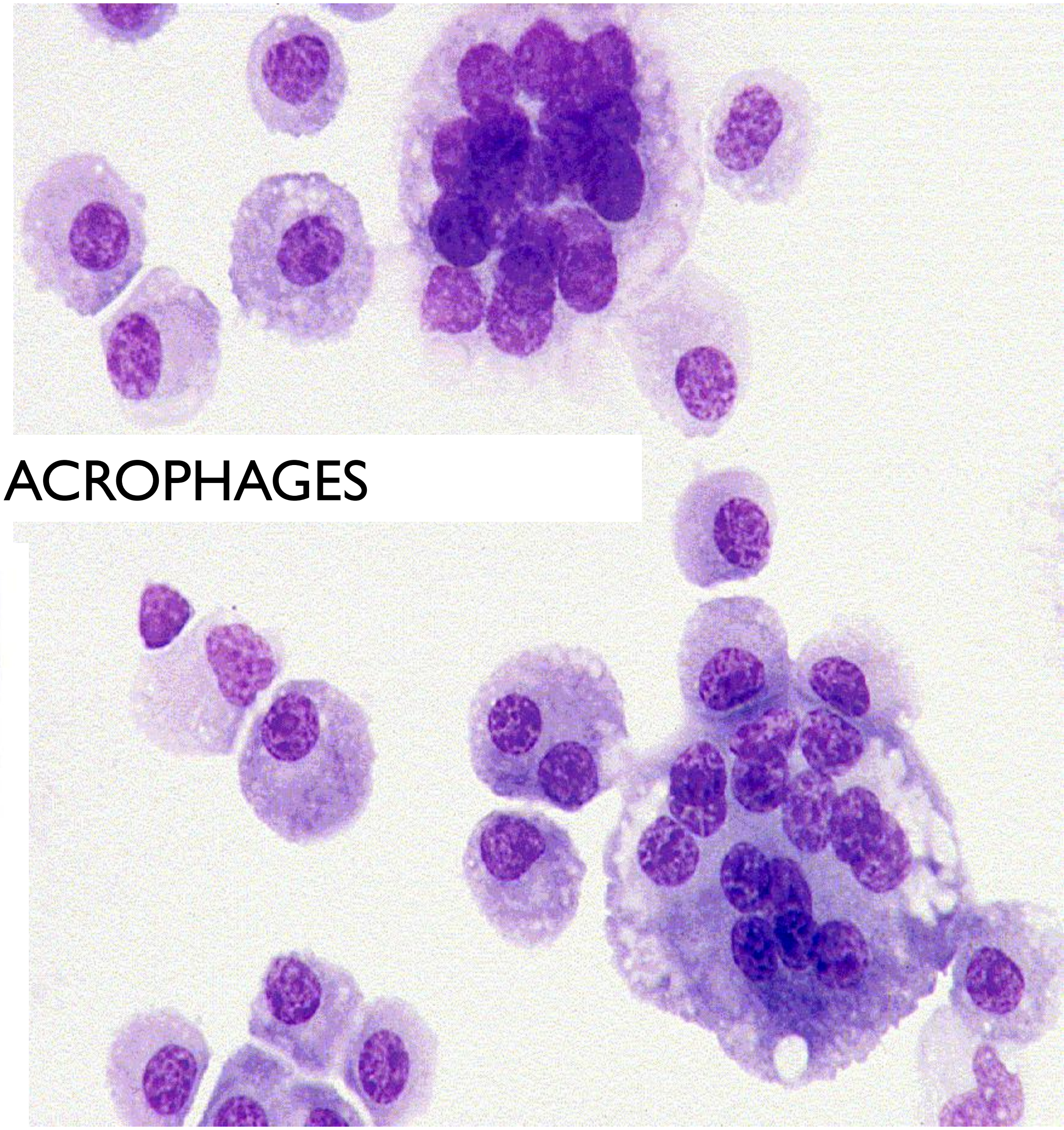


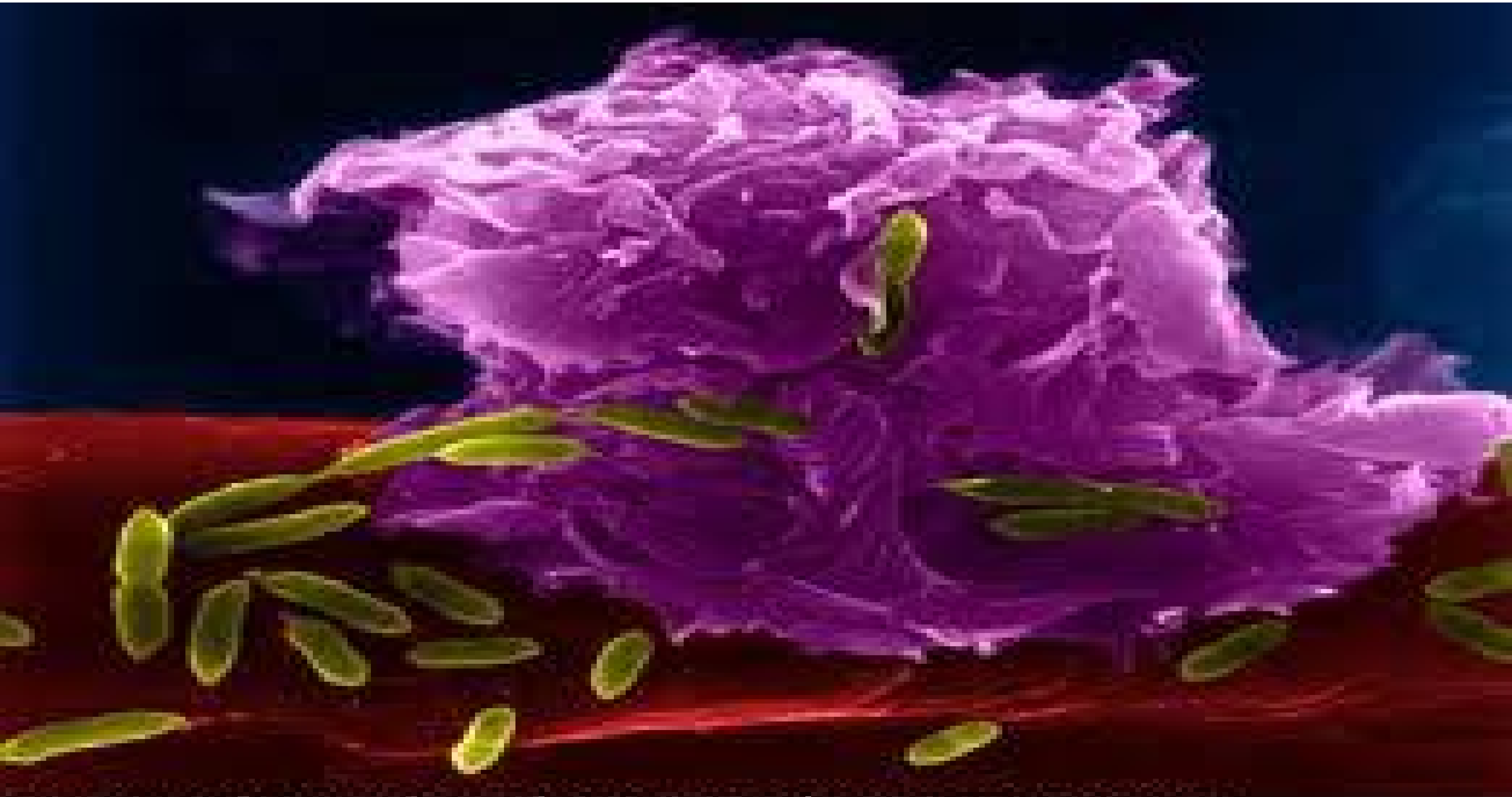
- Monocytes account for 4–8% of leukocytes
  - They are the largest leukocytes
  - They have abundant pale-blue cytoplasms
  - They have purple-staining, **U- or kidney-shaped nuclei**
  - **They leave the circulation, enter tissue, and differentiate into macrophages and dendritic cells**

MACROPHAGES

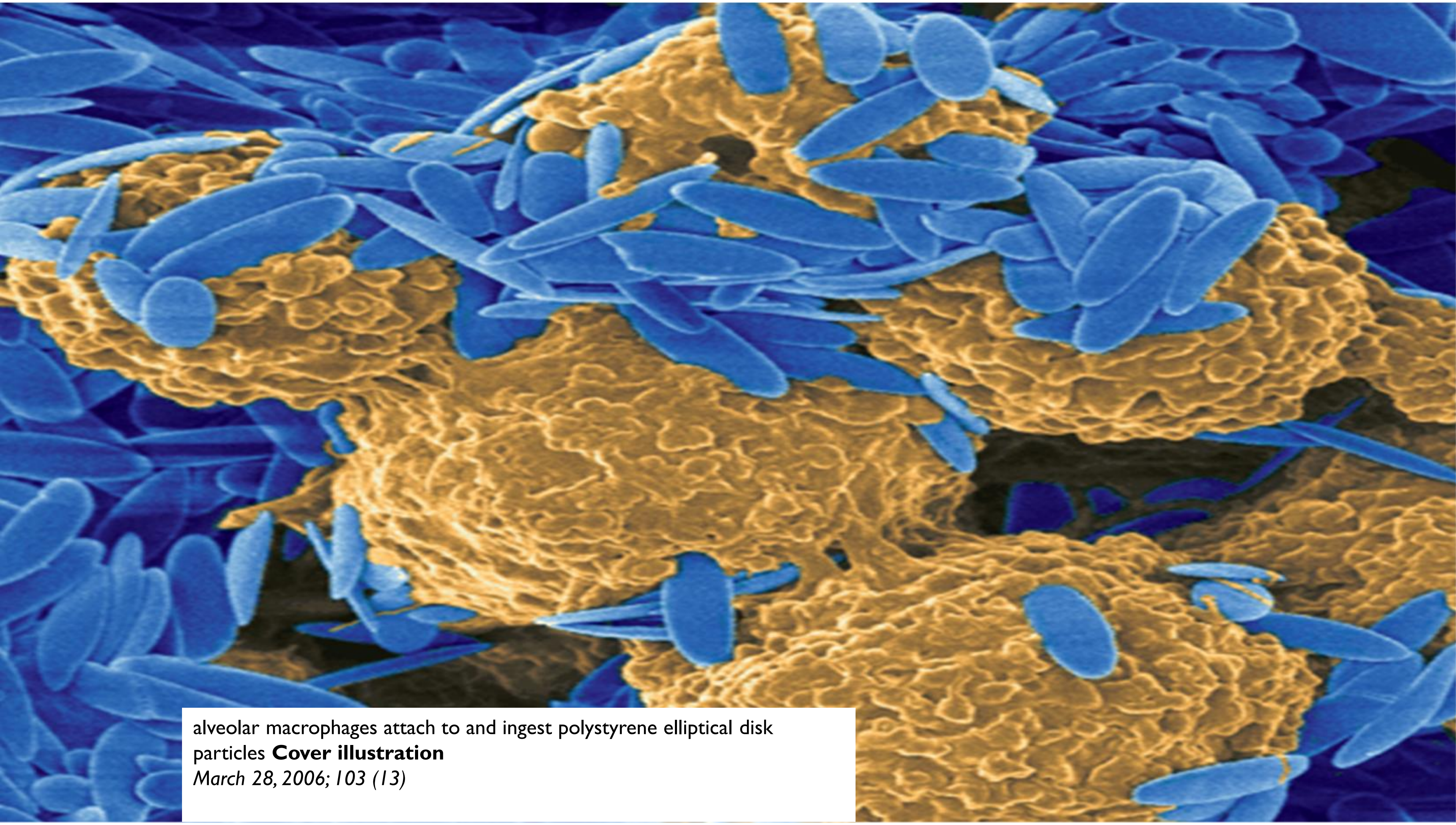
- Macrophages:
  - Are highly mobile and actively phagocytic
  - Activate lymphocytes to mount an immune response
  - Will have different names depending on the location





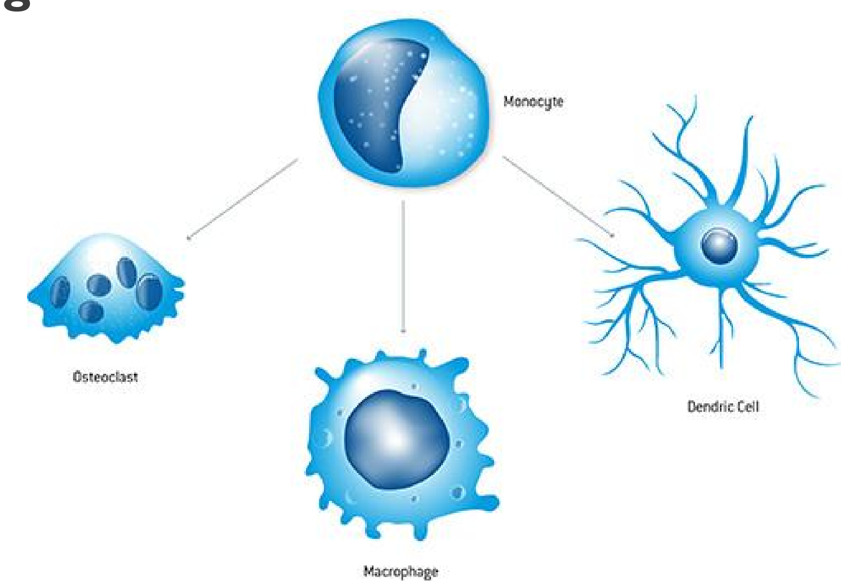


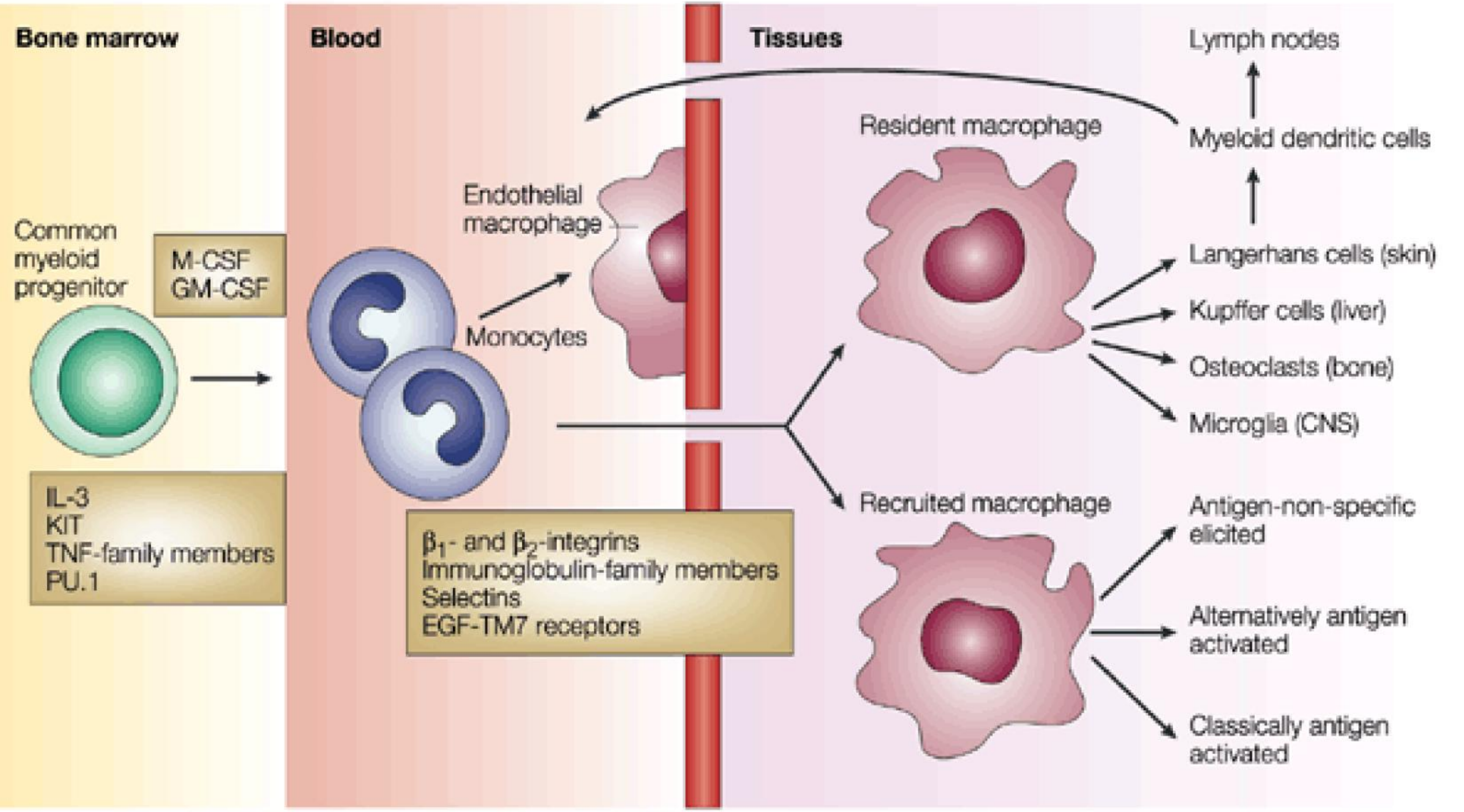
An activated macrophage phagocytosing bacteria upon contact
Photo: courtesy of Dennis Kunkel



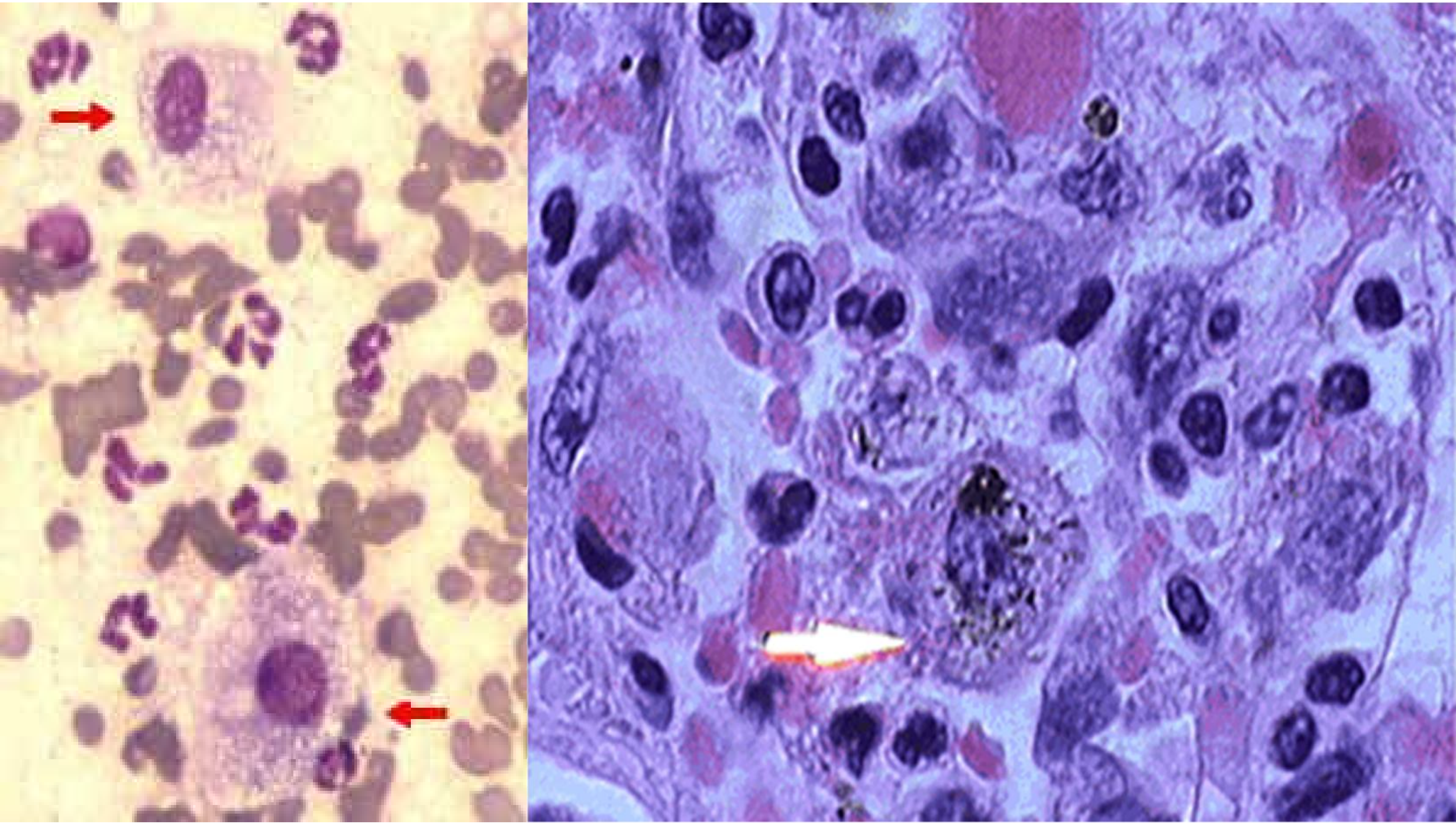
alveolar macrophages attach to and ingest polystyrene elliptical disk particles **Cover illustration**
March 28, 2006; 103 (13)

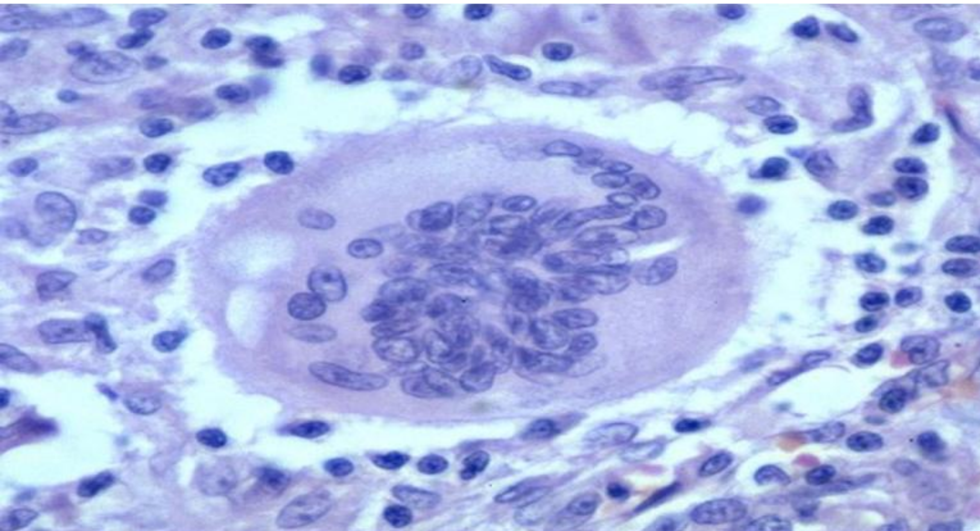
- Normal macrophages **include macrophages located in tissues that include:**
  - **connective tissue –histiocytes**
  - **liver sinusoids – Kupffer's cells**
  - **lung – alveolar macrophages**
  - **lymph nodes – free and fixed macrophages**
  - **spleen – free and fixed macrophages**
  - **bone marrow – fixed macrophages**
  - **serous fluids –pleural and peritoneal macrophages**
  - **skin – histiocytes, Langerhans's cell**
- **Bone : osteoclast**

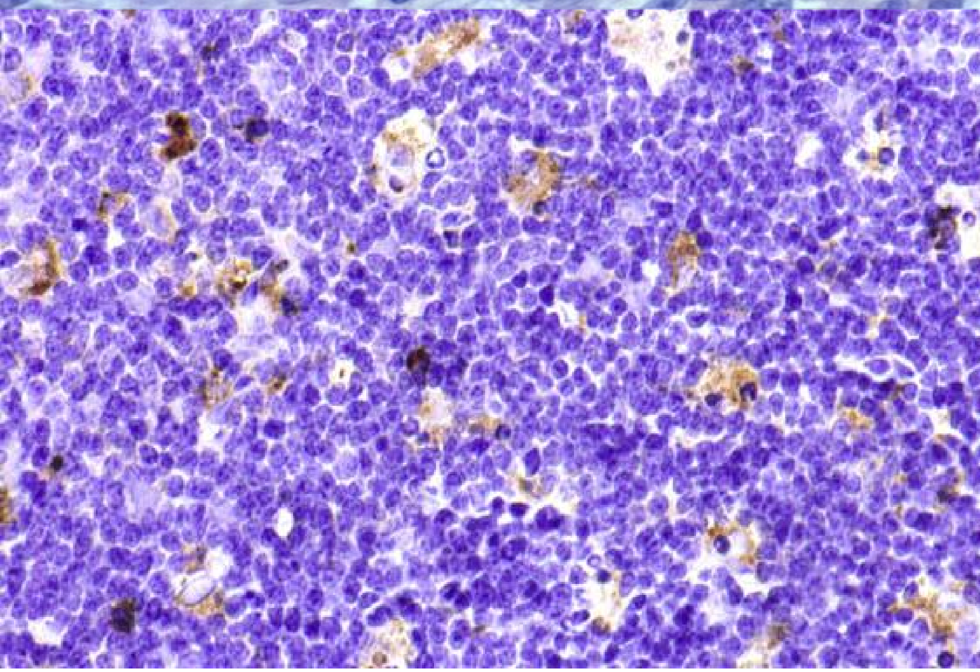


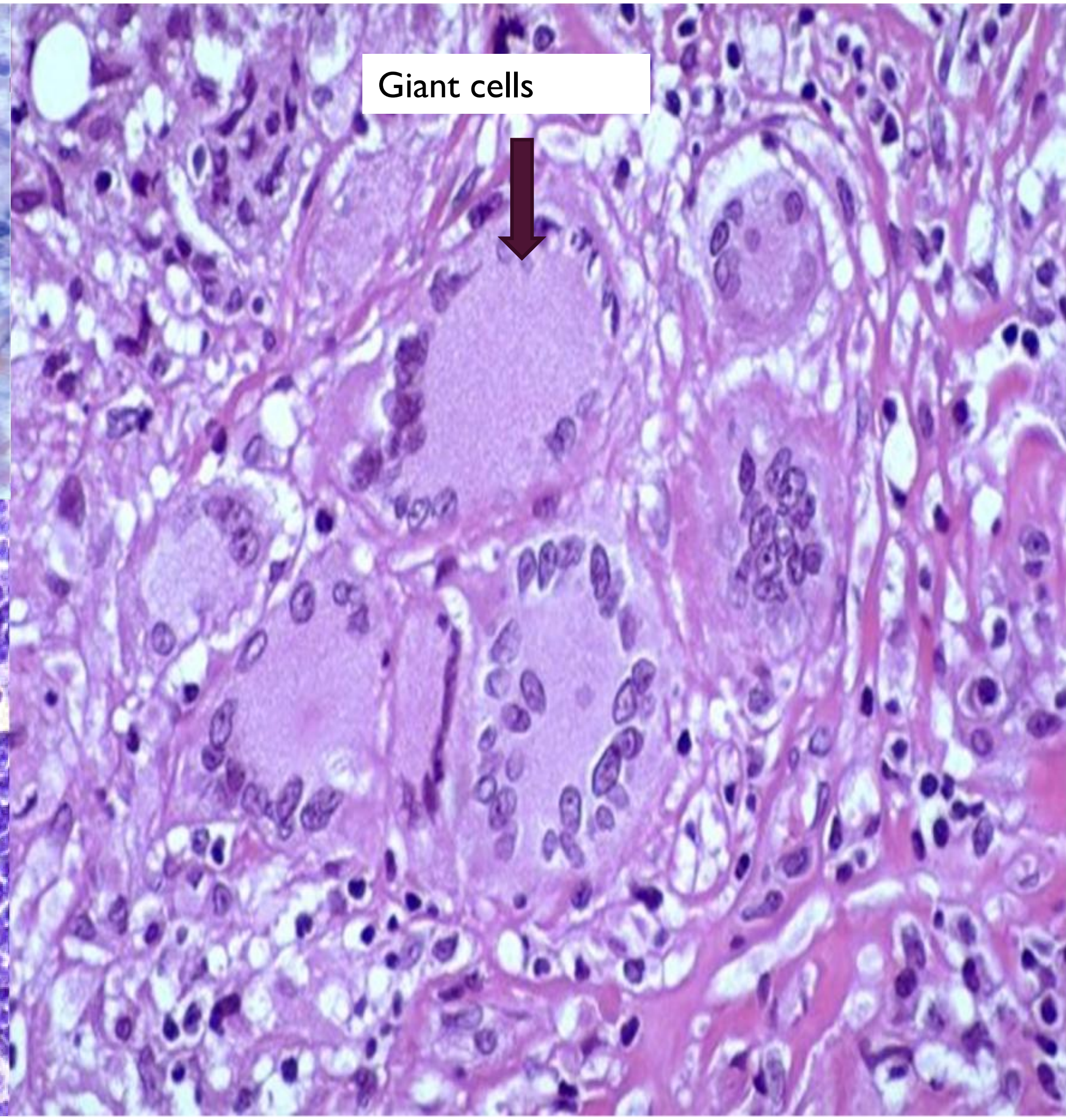

Bone marrow
Blood
Tissues
Lymph nodes
Resident macrophage
Myeloid dendritic cells
Endothelial macrophage
Common myeloid progenitor
M-CSF
GM-CSF
Langerhans cells (skin)
Kupffer cells (liver)
Monocytes
Osteoclasts (bone)
Microglia (CNS)
IL-3
KIT
TNF-family members
PU.1
Recruited macrophage
Antigen-non-specific elicited
β_1- and β_2-integrins
Immunoglobulin-family members
Selectins
EGF-TM7 receptors
Alternatively antigen activated
Classically antigen activated


Alveolar macrophages
("dust cells")
20 um

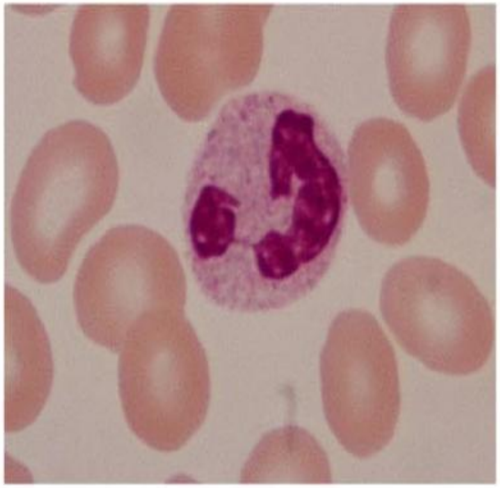


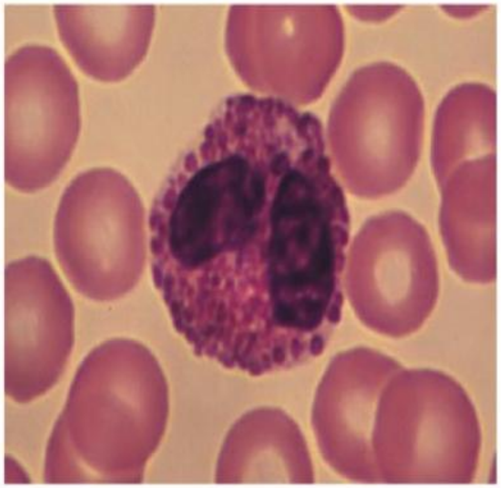


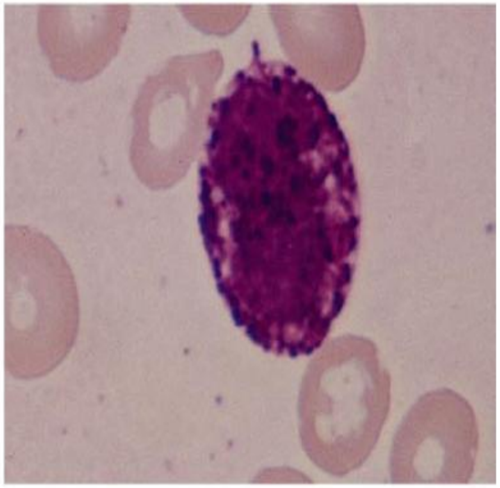


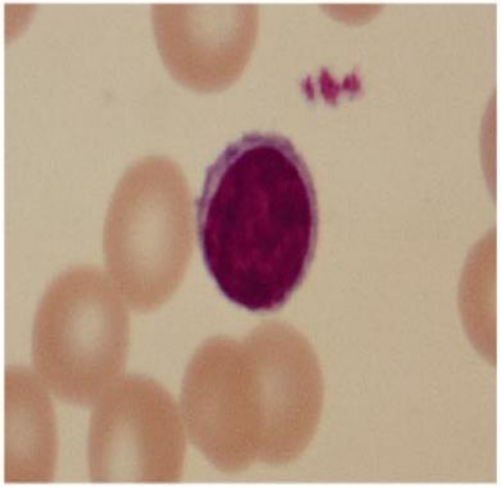

Giant cells

LEUKOCYTES


(a)


(b)


(c)


(d)

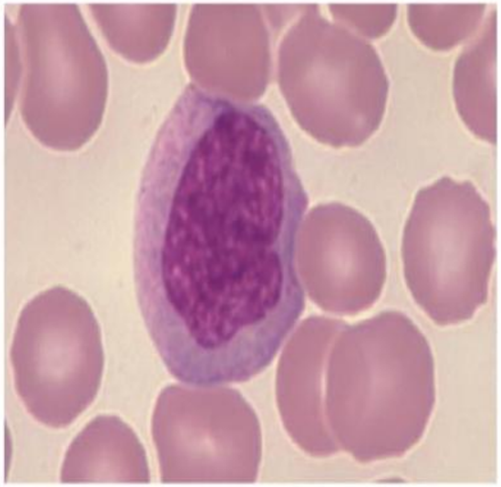
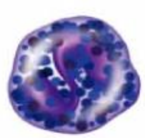
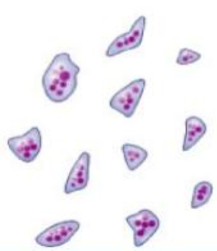

(e)

TABLE 17.2 Summary of Formed Elements of the Blood

CELL TYPE	ILLUSTRATION	DESCRIPTION*	CELLS/μl (mm^3) OF BLOOD	DURATION OF DEVELOPMENT (D) AND LIFE SPAN (LS)	FUNCTION
Erythrocytes (red blood cells, RBCs)		Biconcave, anucleate disc; salmon-colored; diameter 7–8 μm	4–6 million	D: about 15 days LS: 100–120 days	Transport oxygen and carbon dioxide
Leukocytes (white blood cells, WBCs)		Spherical, nucleated cells	4800–10,800		
Granulocytes					
▪ Neutrophil		Nucleus multilobed; inconspicuous cytoplasmic granules; diameter 10–12 μm	3000–7000	D: about 14 days LS: 6 hours to a few days	Phagocytize bacteria
▪ Eosinophil		Nucleus bilobed; red cytoplasmic granules; diameter 10–14 μm	100–400	D: about 14 days LS: about 5 days	Kill parasitic worms; destroy antigen-antibody complexes; inactivate some inflammatory chemicals of allergy
▪ Basophil		Nucleus lobed; large purplish-black cytoplasmic granules; diameter 10–14 μm	20–50	D: 1–7 days LS: a few hours to a few days	Release histamine and other mediators of inflammation; contain heparin, an anticoagulant

*Appearance when stained with Wright's stain.

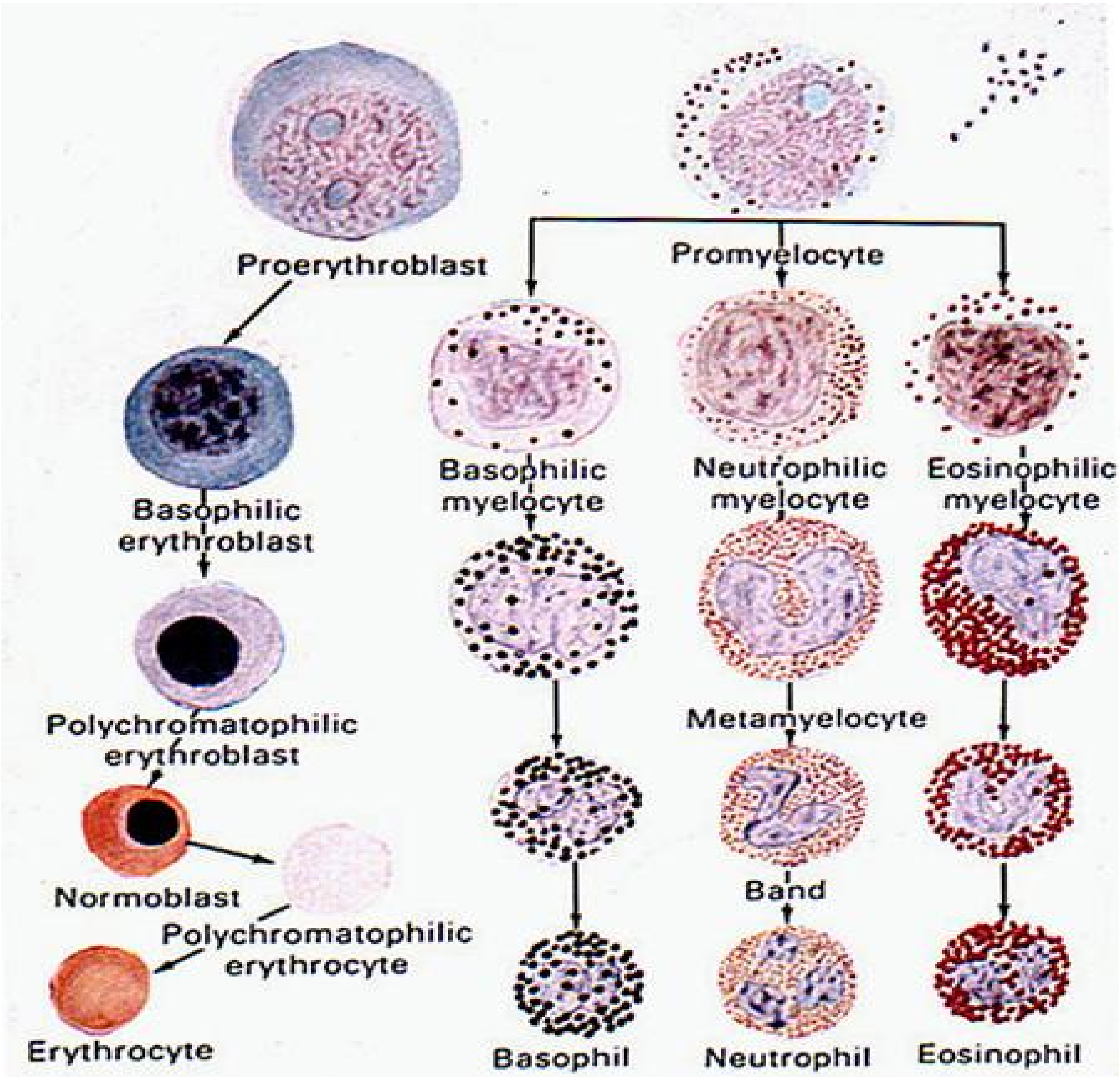
TABLE 17.2 Summary of Formed Elements of the Blood *(continued)*

CELL TYPE	ILLUSTRATION	DESCRIPTION*	CELLS/µl (mm^3) OF BLOOD	DURATION OF DEVELOPMENT (D) AND LIFE SPAN (LS)	FUNCTION
Leukocytes (white blood cells, WBCs)		Spherical, nucleated cells	4800–10,800		
Agranulocytes					
▪ Lymphocyte		Nucleus spherical or indented; pale blue cytoplasm; diameter 5–17 µm	1500–3000	D: days to weeks LS: hours to years	Mount immune response by direct cell attack or via antibodies
▪ Monocyte		Nucleus U or kidney shaped; gray-blue cytoplasm; diameter 14–24 µm	100–700	D: 2–3 days LS: months	Phagocytosis; develop into macrophages in the tissues
Platelets		Discoid cytoplasmic fragments containing granules; stain deep purple; diameter 2–4 µm	150,000–400,000	D: 4–5 days LS: 5–10 days	Seal small tears in blood vessels; instrumental in blood clotting

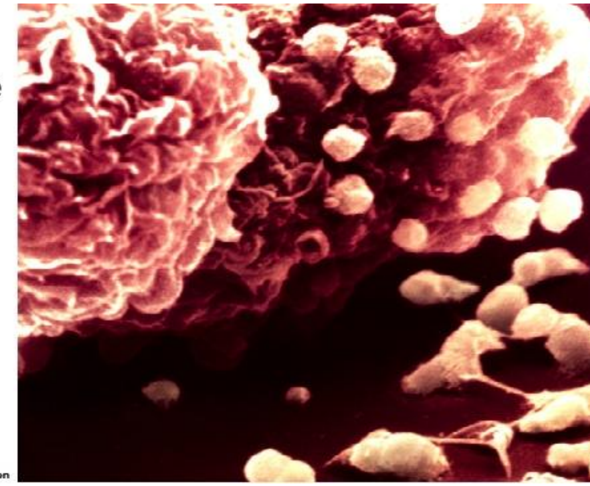
*Appearance when stained with Wright's stain.

PRODUCTION OF LEUKOCYTES

- **Leukopoiesis is stimulated by interleukins and colony-stimulating factors (CSFs)**
  - Interleukins are numbered (e.g., IL-1, IL-2), whereas CSFs are named for the WBCs they stimulate (e.g., granulocyte-CSF stimulates granulocytes)
- **Macrophages and T cells are the most important sources of cytokines**
- Many hematopoietic hormones are used clinically to stimulate bone marrow



Mast cell Releases Histamine Granules



FORMATION OF LEUKOCYTES

- All leukocytes originate from **hemocytoblasts**
- Hemocytoblasts differentiate into myeloid stem cells and lymphoid stem cells
- Myeloid stem cells become myeloblasts or monoblasts
- Lymphoid stem cells become lymphoblasts
- Myeloblasts develop into eosinophils, neutrophils, and basophils
- Monoblasts develop into monocytes
- Lymphoblasts develop into lymphocytes

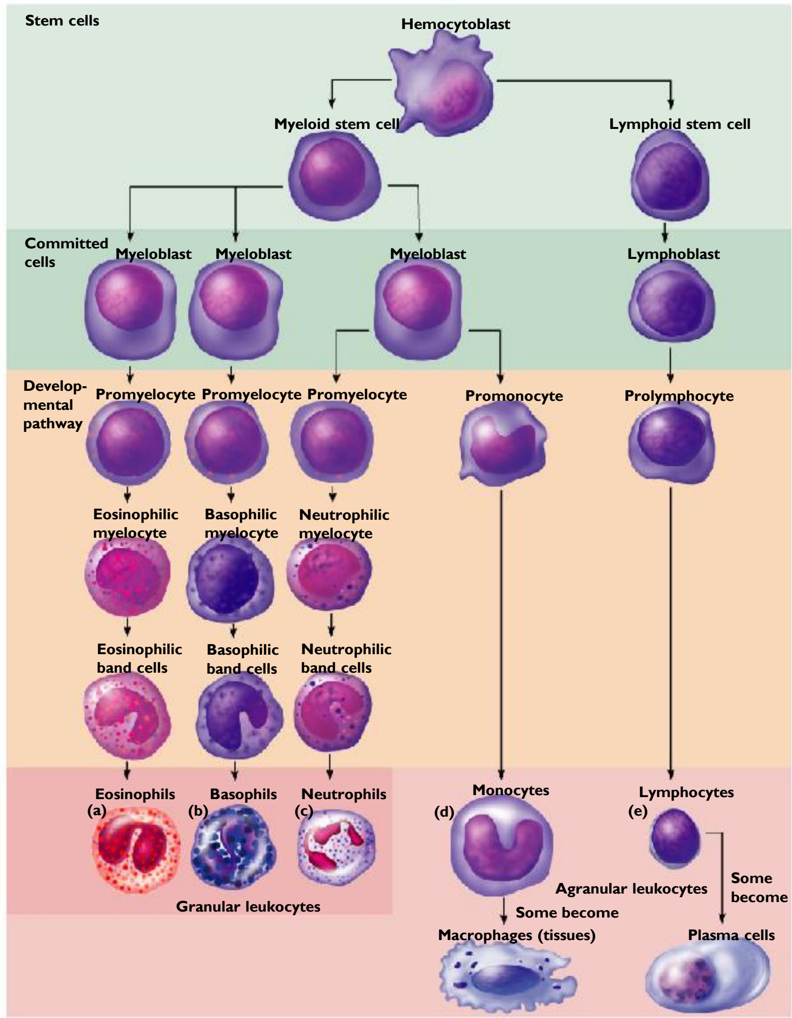
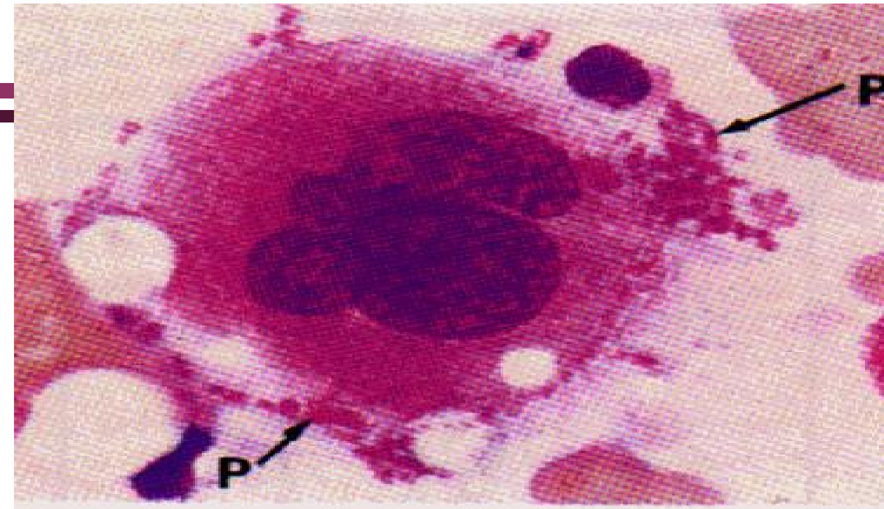

Stem cells
Hemocytoblast
Myeloid stem cell
Lymphoid stem cell
Committed cells
Myeloblast
Myeloblast
Myeloblast
Lymphoblast
Develop-mental pathway
Promyelocyte
Promyelocyte
Promyelocyte
Promonocyte
Prolymphocyte
Eosinophilic myelocyte
Basophilic myelocyte
Neutrophilic myelocyte
Eosinophilic band cells
Basophilic band cells
Neutrophilic band cells
Eosinophils
(a)
Basophils
(b)
Neutrophils
(c)
Monocytes
(d)
Lymphocytes
(e)
Granular leukocytes
Agranular leukocytes
Some become
Some become
Macrophages (tissues)
Plasma cells

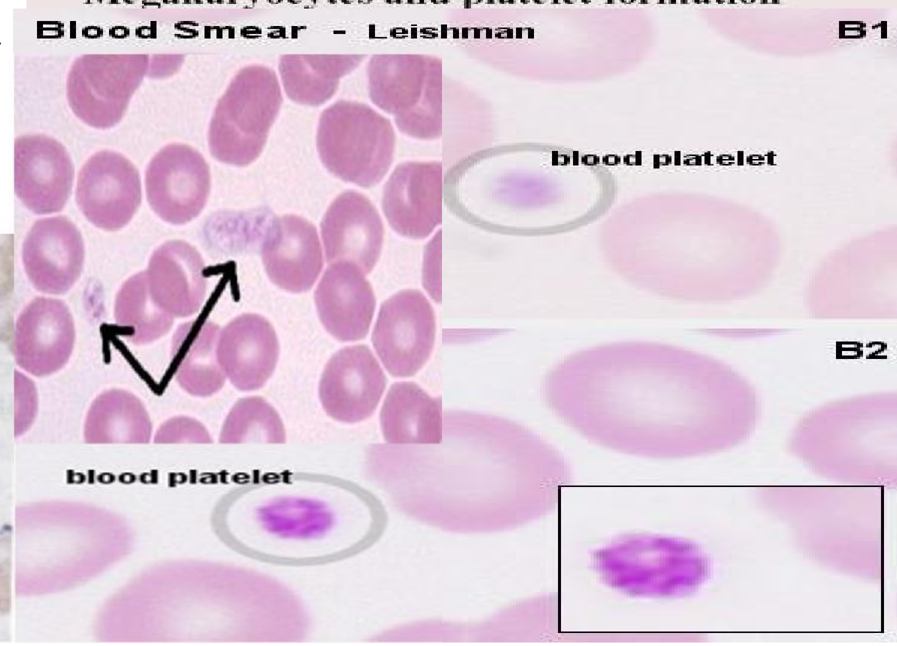
Figure 17.11

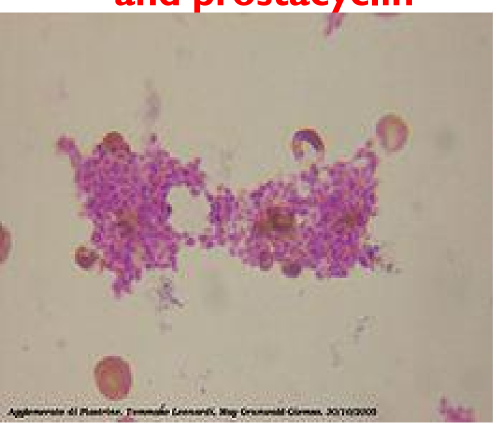
PLATELETS

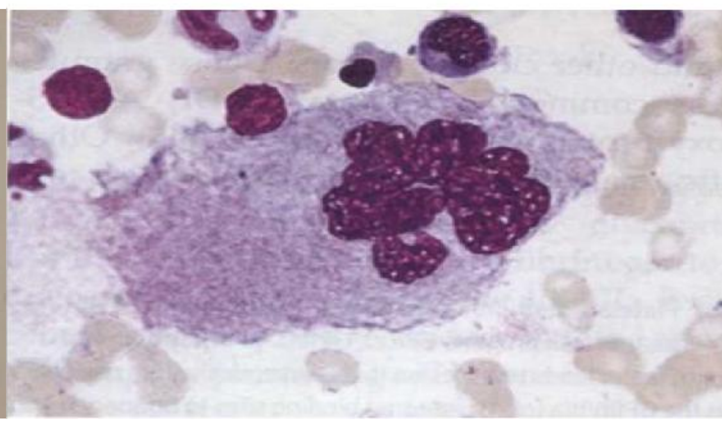
- Platelets are fragments of **megakaryocytes** with a blue-staining outer region and a purple granular center
- Their granules contain
  - **serotonin, Ca^{2+},**
  - **enzymes, ADP,**
  - **and platelet-derived growth factor (PDGF)**
- Platelets function in the clotting mechanism by forming a temporary plug that helps seal breaks in blood vessels
- **Platelets not involved in clotting are kept inactive by NO and prostacyclin**



Megakaryocytes and platelet formation

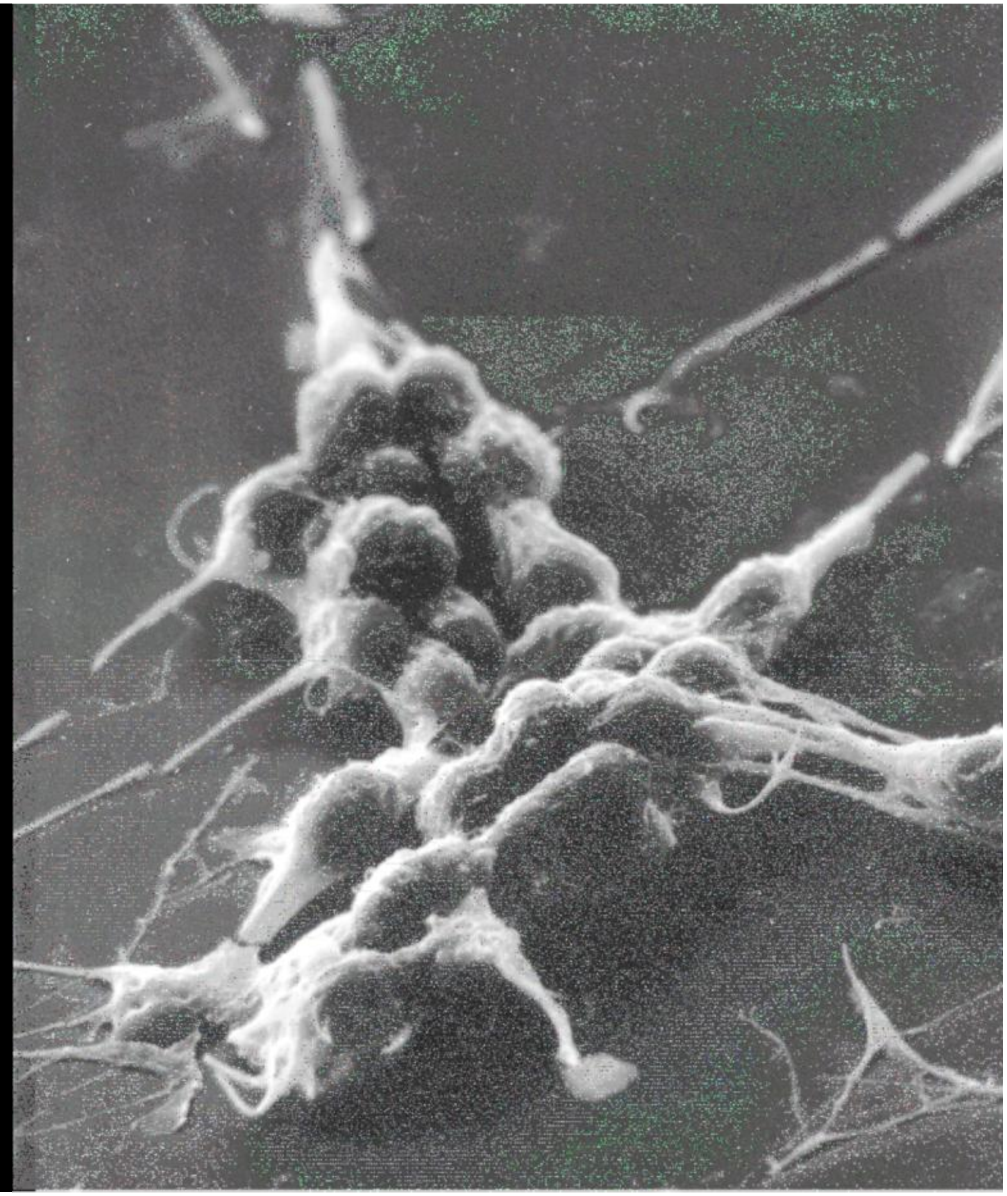






PLATELET FUNCTION STUDIES

- Bleeding Time
- Platelet Count
- Platelet Aggregation Studies



Cytoplasm includes active proteins such as:

- Actin.
- Myosin.
- Thrombesthenin.

- The normal concentration of platelets in the blood is between 150,000 and 300,000 per microliter.
- do not have nuclei and cannot reproduce .
- In their cytoplasm are such active factors as
  - 1) actin and myosin molecules,
    - as well as still another contractile protein, thrombosthenin, that can cause the platelets to contract;
  - (2) residuals of both the endoplasmic reticulum and the Golgi apparatus that synthesize various enzymes and store large quantities of calcium ions;
  - (3) mitochondria and enzyme systems that are capable of forming ATP and ADP;
  - (4) enzyme systems that synthesize prostaglandins, which are local hormones that cause many types of vascular and other local tissue reactions;
  - (5) fibrin-stabilizing factor;
  - (6) a growth factor that can cause vascular endothelial cells, vascular smooth muscle cells, and fibroblasts to multiply and grow, thus causing cellular growth that helps repair damaged vascular walls.

- The cell membrane of the platelets is also important.

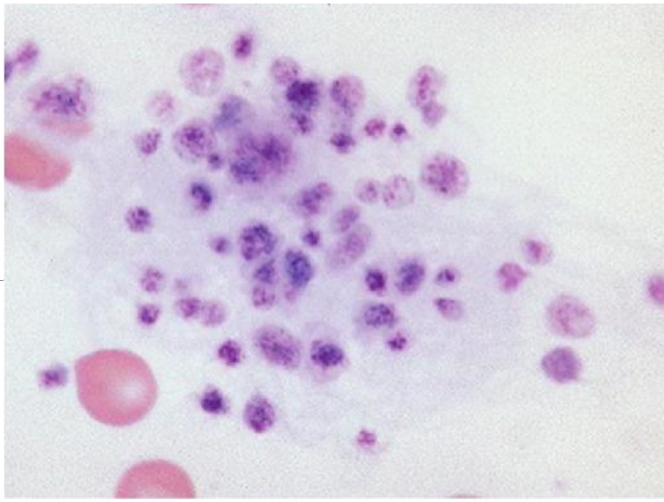
- On its surface is a coat of glycoproteins that causes it to avoid adherence to normal endothelium and yet to adhere to injured areas of the vessel wall, especially to injured endothelial cells and even more so to any exposed collagen from deeper in the vessel wall.

- In addition, the membrane contains large amounts of phospholipids that play several activating roles at multiple points in the blood-clotting process.

Thrombopoietin:

- Regulator of platelet production.
- Produced by the liver and kidneys.
- Levels are increased in thrombocytopenia, and reduced in thrombocytosis.
- It increases the no. & rate of maturation of the megakaryocytes.

FUNCTIONS OF PLATELETS



- Platelets assist in **hemostasis, the arrest of bleeding**.
- Serotonin is a potent vasoconstrictor.
- The release of serotonin from thrombocytes, which adhere to the walls of a damaged vessels, is sufficient to close even small arteries.
- Platelets, which come into contact with collagenous fibers in the walls of the vessel (which are not usually exposed to the blood stream), swell, become "sticky" and activate other platelets to undergo the same transformation.
- This cascade of events results in the formation of a platelet plug (or platelet thrombus). Finally, activating substances are released from the damaged vessel walls and from the platelets.
- These substances mediate the conversion of the plasma protein prothrombin into thrombin.
- Thrombin catalyzes the conversion of fibrinogen into fibrin, which polymerizes into fibrils and forms a fibrous net in the arising blood clot.
- Platelets captured in the fibrin net contract leading to clot retraction, which further assists in hemostasis.

GENESIS OF PLATELETS

- The stem cell for platelets is the **hemocytoblast**
- The sequential developmental pathway is as shown.

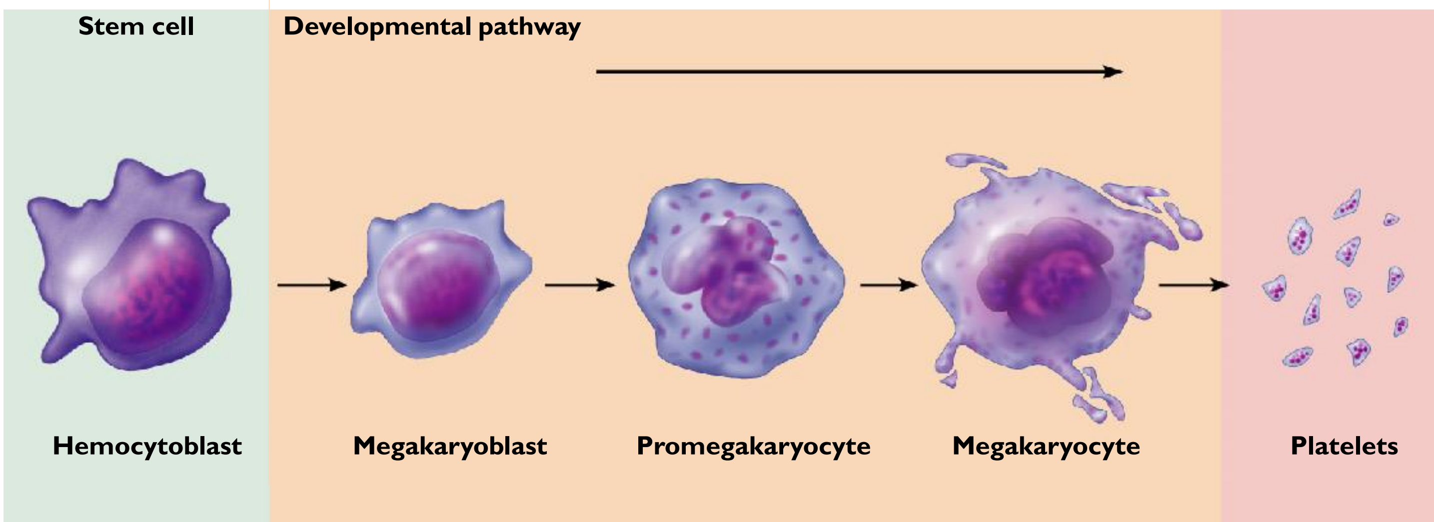
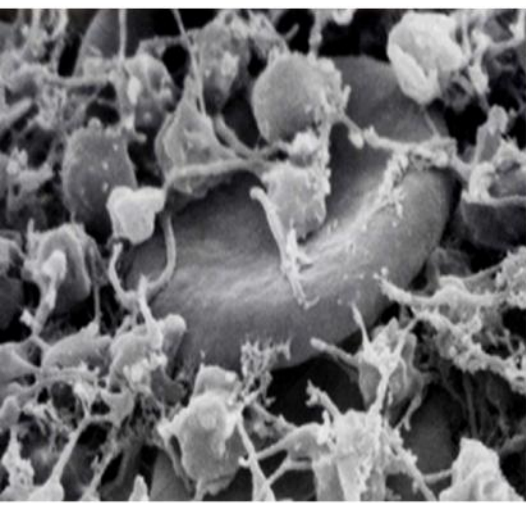
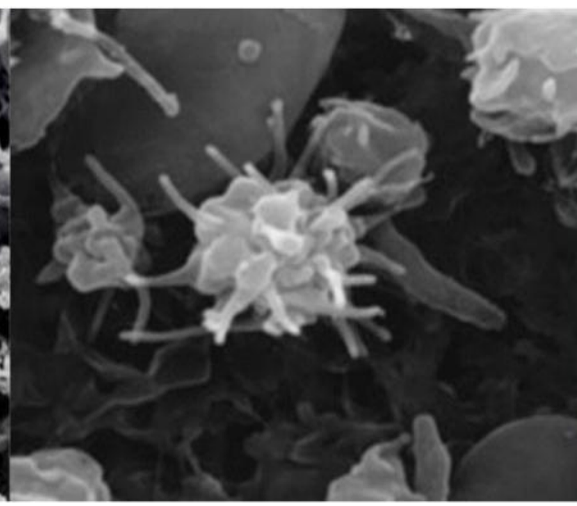


Figure 17.12

PLATELET CIRCULATION

- Normal count is 250,000.
- Normal life span 7-10 days.
- About 1/3 are trapped in the spleen.





PLATELET ADHESION:
BINDING TO COLLAGEN

Platelet activation:
Release of contents, surface receptor expression