

Differential White Blood Cells

White blood cells (WBCs) are also called leukocytes, they defend against diseases. Leukocytes develop from hemocytoblasts (stem cells) in the bone marrow in response to certain hormones. Once WBCs are fully matured they enter the blood stream and blood can transport them to the sites of infection.

Normally, five types of WBCs are found in circulating blood. **They differ in size, the composition of the cytoplasm, the shape of the nucleus, and their staining characteristics.** Some types of leukocytes have granular cytoplasm and they make up a group called **granulocytes**; whereas others lack cytoplasmic granules and are called **agranulocytes**.

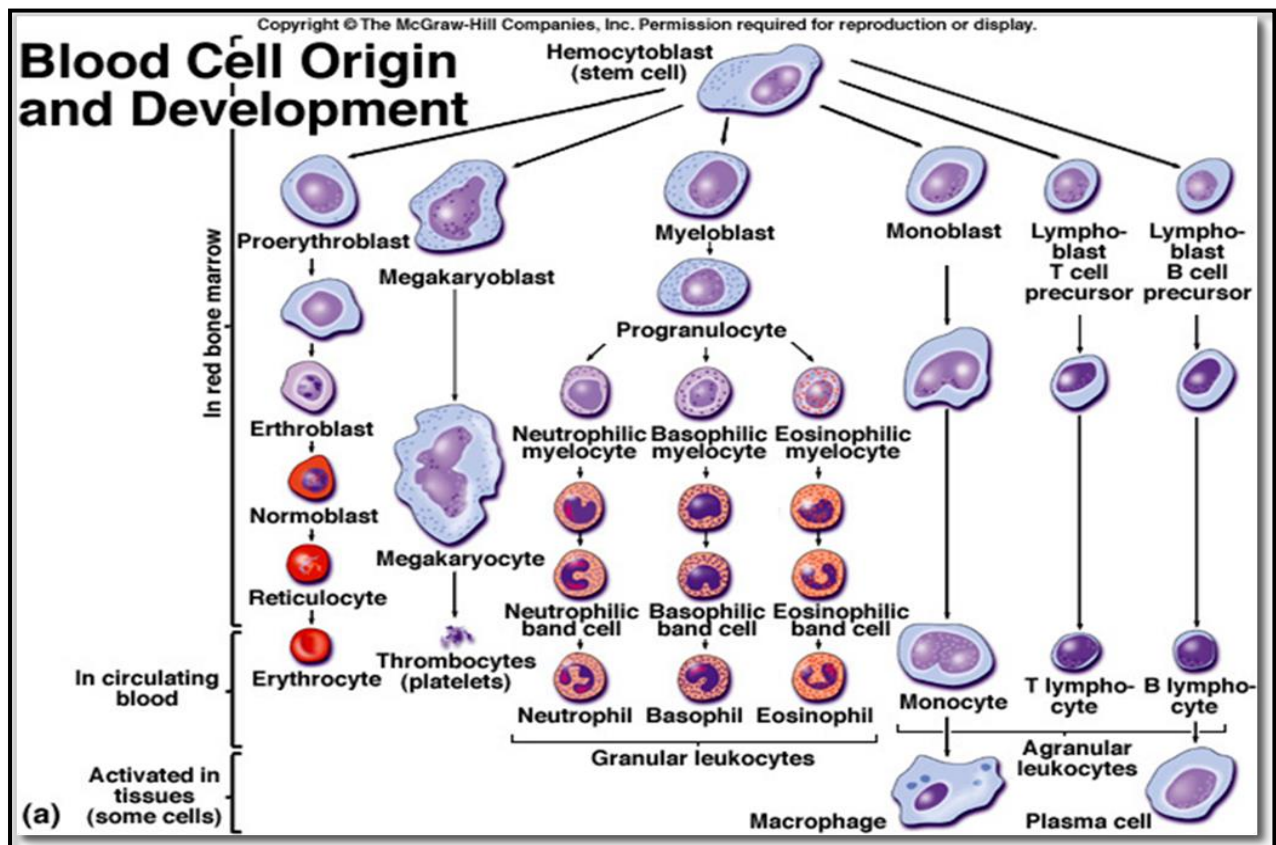
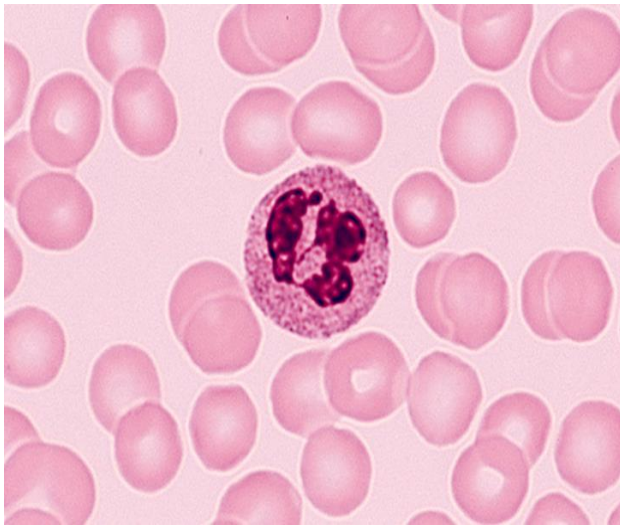


Figure (1): the origins of the white blood cells (WBCs)

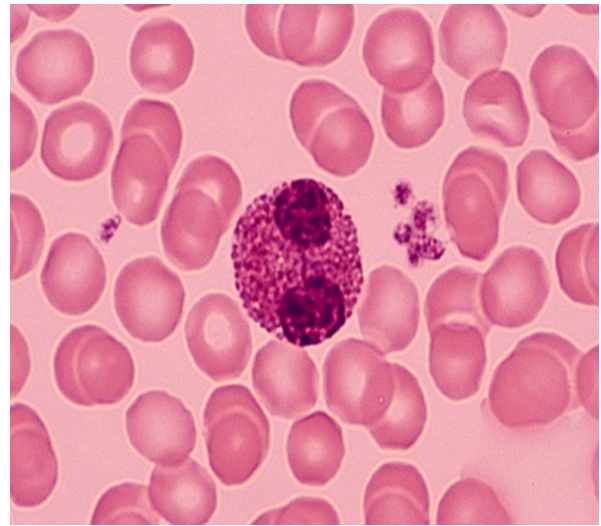
1. Granulated WBCs:

They are of three types: (Neutrophil, Eosinophil and Basophil) and they are described in the below table:

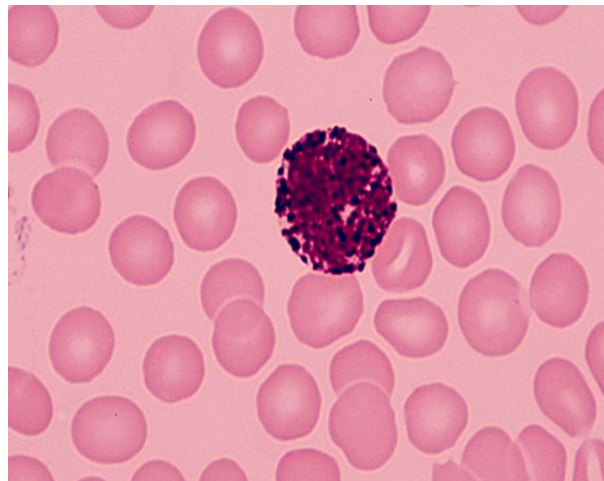
#	Neutrophil	Eosinophil	Basophil
1-	Have fine granulated cytoplasm	Coarse granulated cytoplasm	Very coarse granulated cytoplasm
2-	Lobulated nucleus (2-5) lobes	Lobulated nucleus (2 lobes)	Lobulated nucleus (2 lobes)
3-	Granules stain purple in color in acid-base stains	Granules stain bright red in color	Granules stain deep blue in color
4-	Accounts for about 50 – 70% of leukocytes	Accounts for about 1 – 3% of leukocytes	Accounts for about less than 1% of leukocytes
5-	Fully mature and specialized neutrophils are called microphages	Release the enzyme (histaminase) that combat the effect of histamine and other inflammatory mediators	Release histamine to promote inflammation and heparin to inhibit blood clotting
6-	They are the first line of defense in microbial invasion and capable of phagocytosis	Active during moderate allergic reactions and parasitic worm infection and also capable of phagocytosis	Have little function as phagocytes but they prevent intracellular coagulation due to the heparin content
7-	Increase in the number called (Neutrophilia) and occurs in: <ul style="list-style-type: none"> - Acute inflammation (gout) - Acute bacterial infection 	Increase in the number called (eosinophilia) and occurs in: <ul style="list-style-type: none"> - Parasitic infection - Allergic reaction and asthma 	Increase in the number called (basophilia) and occurs in: <ul style="list-style-type: none"> - TB infection - Hypothyroidism - Ulcerative colitis
8-	Decrease in number called (Neutropenia)and occurs in: <ul style="list-style-type: none"> - Viral infection - Parasitic infection (malaria) 	Decrease in number called (eosinopenia) and occurs in: <ul style="list-style-type: none"> - Cushing’s syndrome - Acromegaly 	Decrease in number called (basopenia) and occurs in: <ul style="list-style-type: none"> - Hyperthyroidism - Cushing’s syndrome



Neutrophil



Eosinophil



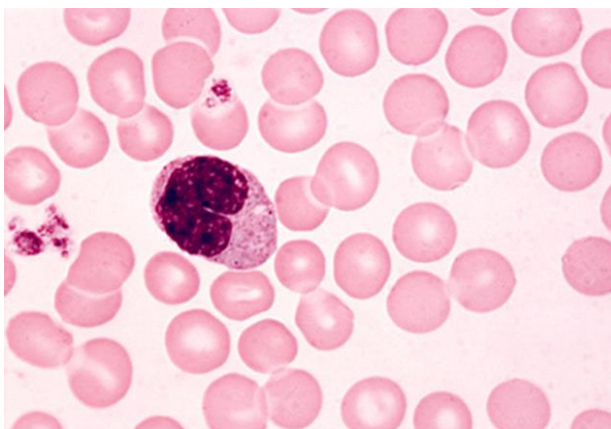
Basophil

2. Agranulated WBCs:

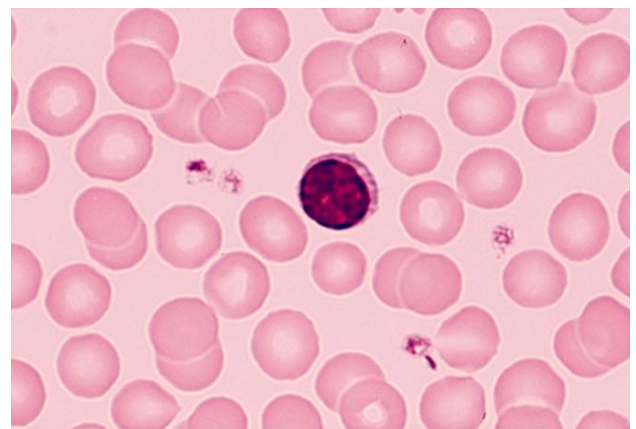
They are of two types (Monocytes and Lymphocytes) and their characteristics are described below:

#	Monocytes	Lymphocytes
1-	Not granulated cytoplasm, stains light blue	Not granulated cytoplasm, stains light blue
2-	Single nucleus, not lobulated and it is a kidney - shaped like. Nucleus stains purple	Single nucleus, not lobulated. The nucleus is round and takes up most of the cell leaving a small amount of cytoplasm surrounding the nucleus, stains purple
3-	Largest cells	Smallest cells

4-	Accounts for 2 - 8% of the leukocytes	Accounts for 20 - 40% of the leukocytes
5-	Specialized monocytes leave the blood stream and localize in different organs, then they are called macrophages	They are of two types: <ul style="list-style-type: none"> - T lymphocytes: originate from bone marrow and mature in the thymus and important for cellular immunity - B lymphocytes: originates from bone marrow and mature in bone marrow and they secrete antibodies
6-	They are the first line of defense in chronic infection, and they are capable of phagocytosis. They secrete anti-viral substance called interferon and inflammatory substance called interleukin 1	Important in cellular immunity and antibodies production
7-	Increase in the number called (monocytosis) and occurs in cases like: <ul style="list-style-type: none"> - Chronic ulcerative colitis - Bacterial infection like tuberculosis (TB) 	Increase in the number called (lymphocytosis) and occurs in cases like: <ul style="list-style-type: none"> - Viral disease like mumps and chicken pox - Crohn's disease
8-	Decrease in number called (monocytopenia) and occurs in cases like: <ul style="list-style-type: none"> - Hairy cell leukemia - Prednisolone treatment 	Decrease in number called (lymphocytopenia) and occurs in cases like: <ul style="list-style-type: none"> - Malignancies - Aplastic anemia



Monocytes



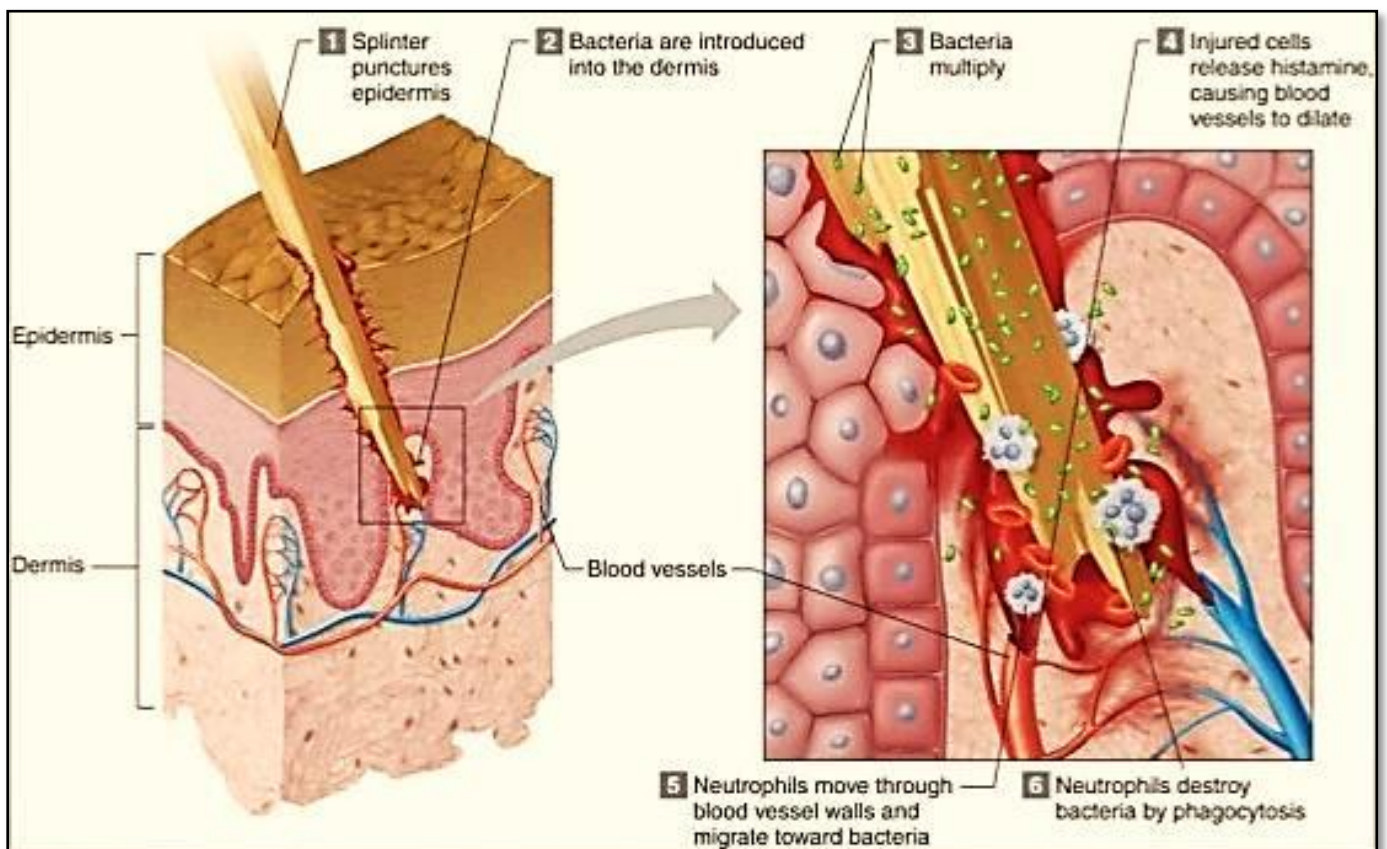
Lymphocytes

As mentioned previously, mature monocytes are specialized and tend to localize in different organs and they are called (macrophages), these include:

- **Histiocytes** in connective tissue
- **Microglia** in the brain
- **Kupffer cells** in the liver
- **Alveolar macrophages** in the lungs
- **Sinus - lining macrophages (reticular cells)** in the spleen, lymph nodes and thymus gland
- **Mesangial cells** in the glomerulus of nephrons in the kidney
- **Osteoclasts** in bones

How do Leukocytes fight the infection?

- Splinter puncture the epidermis
- Bacteria are introduced into the dermis
- Bacteria multiply
- Injured cells release histamine, causing blood vessels to dilate
- Neutrophils move through blood vessel wall and migrate toward bacteria
- Neutrophils destroy bacteria by phagocytosis



Objective of the Experiment:

1. To learn how to prepare a proper, uniform and thin blood film
2. use the correct type of stain and how to make the staining of the blood film
3. to be able to examine the stained blood film under the microscope in order to view the different types of WBCs and count them

Procedure:

1. Making the blood film:

- Place a small drop of blood close to one end of a clean slide that is on a flat surface.
- With the thumb and forefinger of the right hand, hold the end of a second slide (the "spreader") against the surface of the first slide at an angle of 30-45 degrees. Slowly back the spreader into the blood drop
- Draw the spreader back to contact the drop of blood, allowing the blood to pool at the angle between the two slides.
- Gently push the spreader slide at a moderate speed forward, spreading the drop of blood out into a thin film.
- The end of the smear (the feathered edge) should be smooth and even.

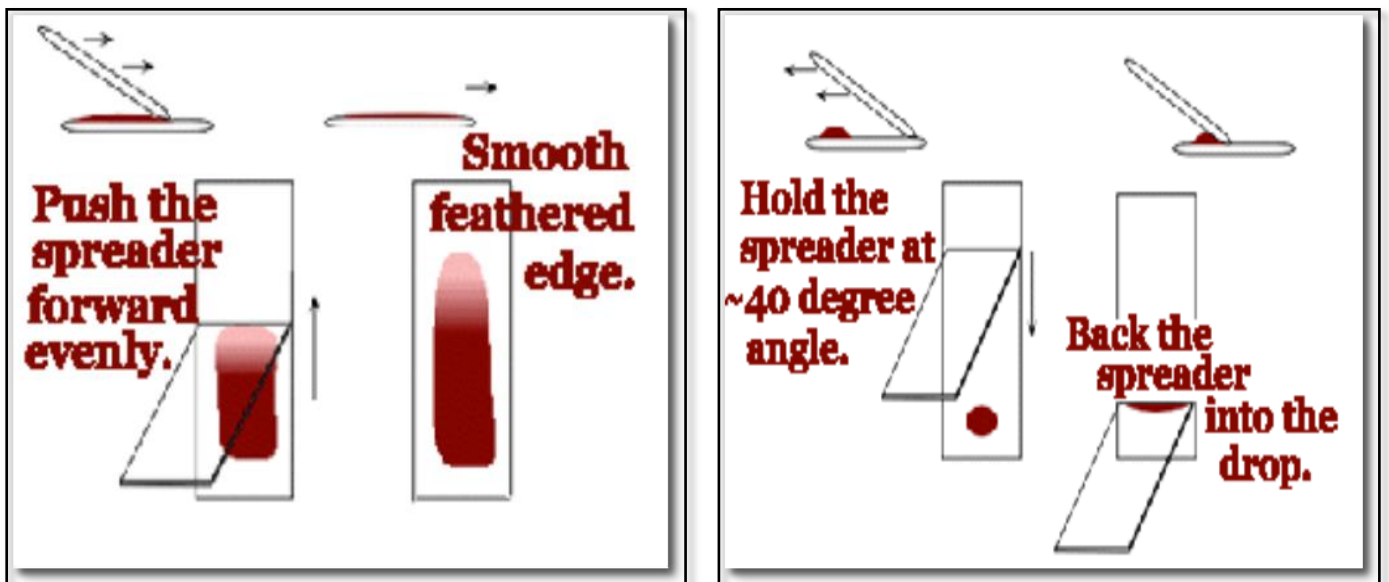


Figure (2): making blood film process

Note: The blood films (smears) are stained for hematology observations with a special stain called Giemsa stain or Wright's stain. This staining applies a mixture of basic stains (Methylene blue and Azur 2) and acid stains (Eosin) in order to visualize nuclei, cytoplasm and cytoplasmic granules in various types of blood cells.

2. Staining process:

- The thin smear of blood is left to air dry.
- Overflow the smear with Leishman stain for 1-5 min.
- Add a double amount of distilled water and mix the stain by blowing the fluid.
- Leave the mixture on the slide for 10-15 min.
- Wash off by dipping the slide in a beaker filled with DW to remove the extra liquid stain.
- Stand slide on end, and let dry in air.
- Examination

Note: in this experiment (Leishman stain) powder was used to stain the different WBCs, which consists of:

1. Methylene blue:

- Basic stain
- Has blue – purple color
- Stains nuclei, ribosomes and rough endoplasmic reticulum

2. Eosin:

- Acidic stain
- Pink – red color
- Stains most of cytoplasmic proteins

Note: solution of Leishman stain consists of the followings:

1. Leishman stain powder for staining the blood film
2. Methanol as a solvent for the stain powder and a fixative agent for the cells
3. Glycerin to facilitate the entrance of the stain inside the cells

3. Counting of WBCs Process:

The dry and stained film is examined without a cover slide under low power then under oil immersion objective. The condenser should be raised and the diaphragm only opened sufficiently to give adequate illumination.

The film should be traversed using the mechanical stage systematically. Every cell seen should be recorded in a table under the following headings: Neutrophil, Eosinophil, Basophil, Monocyte and Lymphocytes. It should be noted that the white cells tend to accumulate at the edges of the film and in the tail.