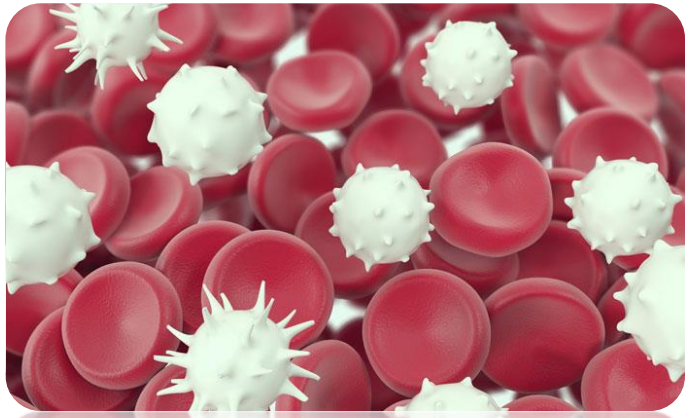
University of Diyala/ College of Medicine
Department of Physiology
Physiology Lab

WBC Blood Cells (WBCs)/Leukocytes Count

Dr. Asmaa Abbas Ajwad



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Outlines

- Introduction
- Aims of WBCs Count Experiment
- Methods and Procedure
- Calculations
- Some Medical Considerations

Introduction

- Acting together, WBCs provide our body with powerful defenses against tumor and viral, fungal, bacterial, and parasitic infections.
- WBCs (Leukocytes) are round shaped, nucleated cells. They are found throughout the body including the ***blood*** and ***lymphatic system***.
- **The normal level of WBCs** under basal condition of complete physical and mental rest is ***4500-11000 cell/ mm3***. A condition with WBCs level more than 11000 mm3 is called **leukocytosis.** The opposite condition, **leukopenia**, is an abnormally low WBCs count.
- WBCs make up approximately **1%** of the blood volume in a healthy adult.
- The lifespan of WBCs varies from few days to few weeks.
- The name "***White Blood Cell***" derives from the fact that after centrifugation of a blood sample, the WBCs are found in the Buffy coat, a thin layer of nucleated cells between the sedimented RBCs and the blood plasma, which is typically ***white in color***. The scientific term ***leukocyte*** directly reflects this description, derived from Greek ***leuko - white***, and ***cyte - cell***.
- ***Leucopoiesis*** is the process of WBCs/leucocytes production.

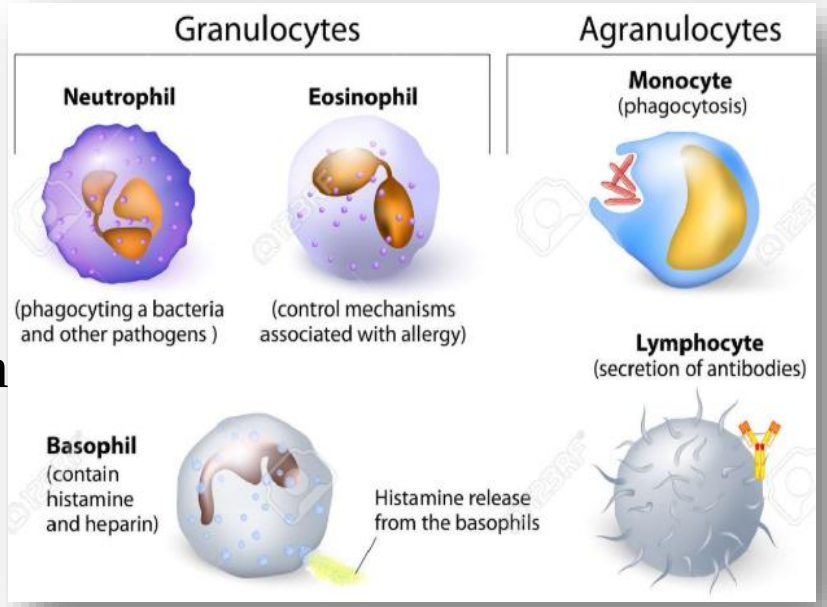
WBCs Classification

- WBCs are classified by several ways. One type of classification is ***based on the presence or absence of specific staining granules*** in the cytoplasm. Accordingly, WBCs are divided into :

A. Granulocytes: they are 10-15 µm in diameter. According to the nature of their specific staining granules, they are subdivided into: ***neutrophils*** (granules stain with acidic and basic dyes), ***eosinophils*** (granules stain with acidic dyes), and ***basophils*** (granules stain with basic dyes). Their nuclei are lobulated . They are also called ***polymorphonuclear leukocytes***.

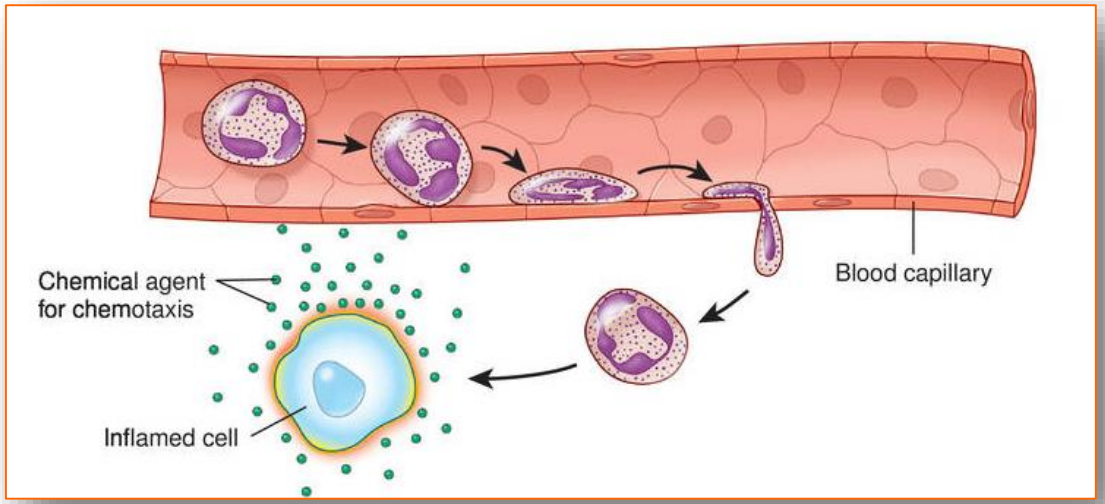
B. Non- granulocytes (Agranulocytes):

They include ***lymphocytes*** (6-16 µm in diameter) and monocytes (15-20 µm in diameter). While the ***lymphocyte*** has a large nucleus and scanty cytoplasm, monocyte on the other side has abundant cytoplasm and kidney shaped or round nucleus.



Properties of WBCs: Diapedesis

- ✓ **Diapedesis** is defined as the power by which WBCs can move outside the vascular system to gain rapid access to the site of the infection. It is present in ***neutrophils*** and ***monocytes***.
- ✓ It starts by coming close to the margin of the capillary, send pseudopodia to the wall of the capillary, squeeze its self to the outside, then by the use of the pseudopodia, it will have an amoeboid movement to the infection site.



Diapedesis Property of WBCs

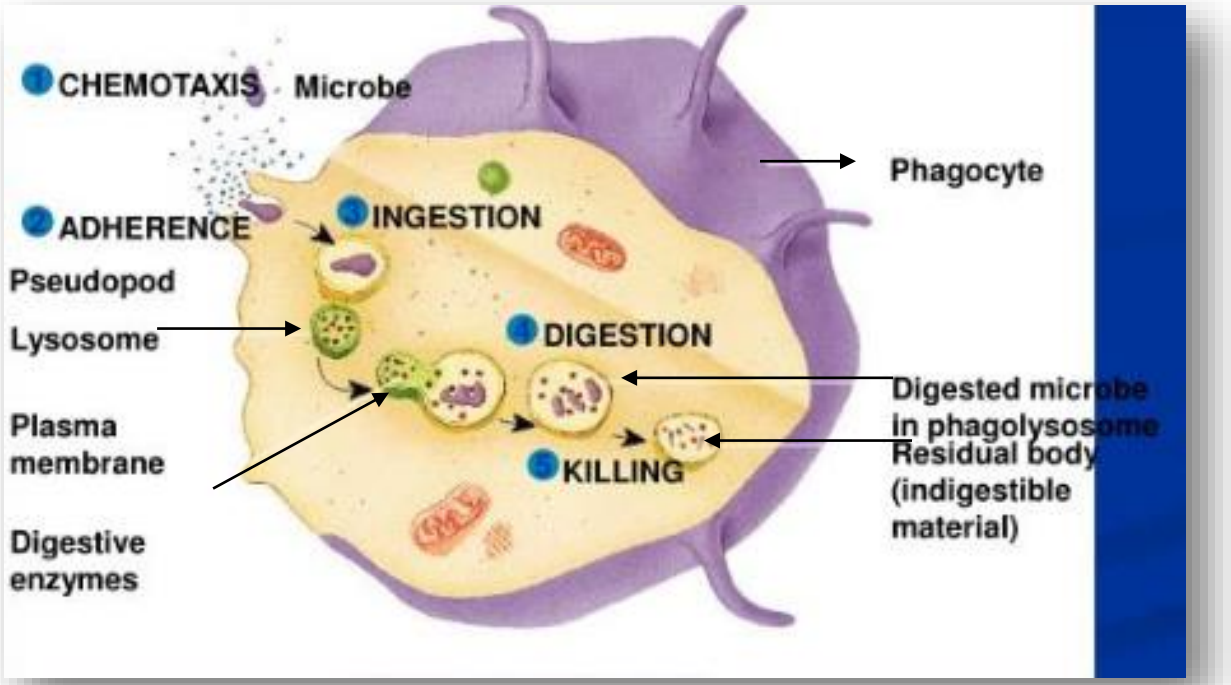
Properties of WBCs: Phagocytosis

Invasion of the body by bacteria will lead to the process of inflammation, whereby the bone marrow will start to form large quantities of neutrophils that will migrate to the site of infection and start the process of ***phagocytosis, so:***

- ✓ ***Phagocytosis*** is the process of swallowing foreign antigens by neutrophils and monocytes.
- ✓ It begins by being attracted by foreign chemical substances or the complement factor C5a.
- ✓ Grnayolcytes from the blood attach themselves to the inner walls of the blood vessels (***margination***) , from which they then escape and move towards the site of attack (***migration***) where they surround and ingest the invaders by ***endocytosis***.
- ✓ ***Endocytosis:*** engulfing the foreign body leading to the formation of endocytes. After that, endocytes will fuse with lysosomes leading to degranulation of lysosomes (all granulocytes contain myeloperoxidase which catalyze the formation of substances that kill bacteria).
- ✓ Sometimes degranulation occurs outside the cell membrane because the release of granules occurs before closure of endocytes.

Properties of WBCs: Phagocytosis

- ✓ In addition to the degranulation, there is an increase in the oxygen uptake, metabolism, and production of hydrogen peroxide and superoxide which all lead to killing of bacteria. Finally, lytic enzymes are released to digest the waste products



Phagocytosis Property of WBCs

Aims of WBC Count Experiment

1. WBCs count is part of the complete blood count (CBC) test that is used to evaluate the overall health and detect a wide range of disorders such as anemia, infection and leukemia.

2. To know whether or not subjects/patients are suffering from Leukocytosis (the increase in the no. of WBCs to more than 11000/mm3) or Leucopenia (the decrease in the no. of WBCs to less than 4000 or 1500 /mm3)

3. To learn how to use the manual method in the lab to get the number of white blood cells.

Principle of WBC Count Experiment

- A Very large number of WBCs are present in the blood specimen. Practically, counting this much amount of WBCs directly under the microscope is highly impossible. So, the WBCs are counted by using a special type of chamber known as ***Hemocytometer*** or ***Neubauer's*** chamber(as in RBCs count experiment).

- For this, the blood specimen is diluted (usually in 1:20 ratio) with the help of WBCs diluting fluid (commonly the Turk's Fluid) which preserves the WBCs and Lysis the RBCs. The Turk's fluid is isotonic to the WBC and does not cause any damage to it.

- After diluting the specimen, the content is charged on Hemocytometer / Neubauer's chamber and the cells are counted in the areas specific for WBCs count.

Manual RBC Count Materials and Instruments

- ***White blood cell count*** is the total number of leukocytes in a volume of blood, expressed as thousands/µl.
- As with the RBCs, the WBCs count experiment can be done by ***manual method*** or by ***automated cell counters***. **In our today lab, we will use the Manual Method**.
- **Manual WBCs count materials include:**

1. Anticoagulated whole blood (using EDTA or heparin as an anticoagulant) or capillary blood can be used.
2. **Turk's solution** (diluting fluid) composed of:

• Glacial acetic acid	2.00 ml
• Gentian Violet (1% w/v)	1.00 gm
(it stains the WBCs nuclei so it can be easily seen)	
• Distilled water	97.00 ml

3. WBC pipette which is composed of stem and mixing chamber with a white bead that facilitates the mixing of blood with the diluting fluid (also it used to differentiate WBC pipette from the RBC one).

Manual WBCs Count Materials and Instruments

4. Haemocytometer / “Neubauer” chamber is the counting chamber with a coverslip.
5. Microscope
6. Lancet, Alcohol 70%, and Cotton.

WBC Diluting Fluid

Microscope

WBC Pipette

Neubauer Chamber

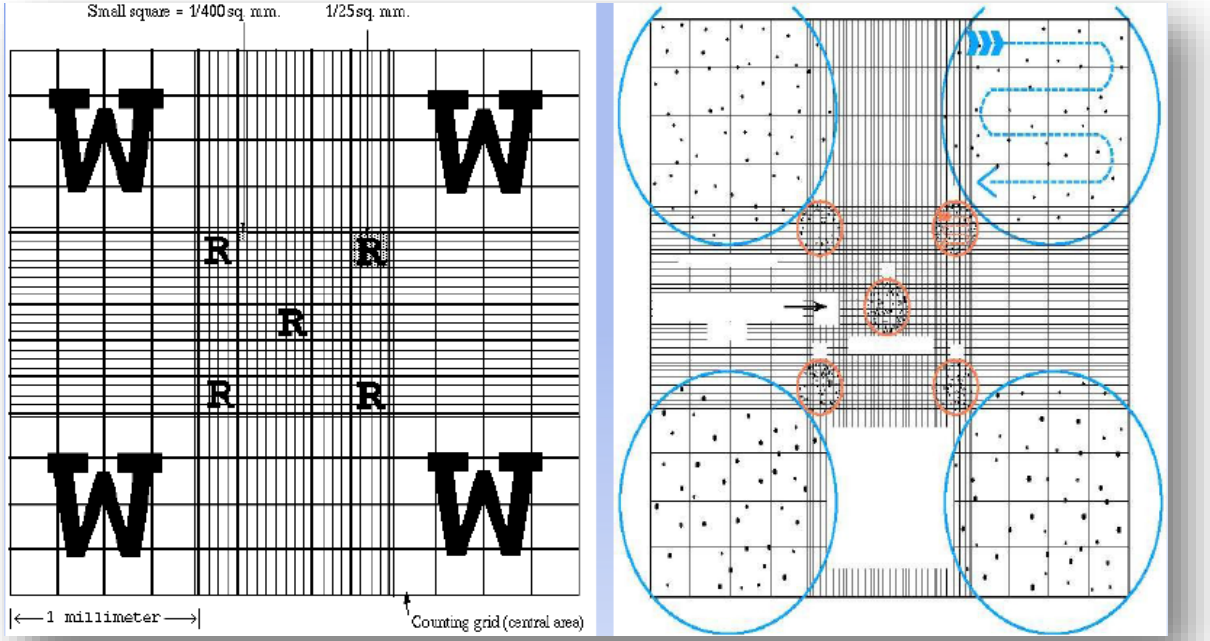
Alcohol Pad

Procedure

- The counting chamber and the coverslip are cleaned and the coverslip is placed on the lateral bars across the middle of the counting chamber. Remember to double check that you get only one coverslip not two and do not press the coverslip over the platform to avoid its break.
- Wipe your partner's finger with cotton soaked with alcohol and allow it to dry. With a sterilized disposable lancet , make a small prick on the finger tip. When a drop of reasonable size gets collected , hold the WBC pipette slightly tilted from the vertical position , apply its tip to the drop, and aspirate blood to 0.5 mark.
- Wipe off any blood adhering to its outer side. If the blood gets beyond 0.5 mark, tap the tip gently till the blood is exactly at the 0.5 mark. Never allow the blood to clot inside the pipette , if this happened then you have to blow the sample out , clean the pipette, and start over again.
- Aspirate the diluting fluid to 11 mark, thus making ***1: 20*** dilution of blood.
- Hold the pipette horizontally and shake it with both hands between index finger and thumb for 2-3 min.
- Blow out quarter of the contents to remove the pure diluting fluid in the stem.
- Hold the pipette 45° and touch its tip gently on the surface of the counting platform where it projects beyond the coverslip and a small amount will be drawn under the coverslip.

Procedure

- Place the Neubauer chamber on the stage of the microscope and leave it for 2-3 min to allow the blood cells to get settled.
- Under 10x magnifications, scan to ensure even distribution of the cells. WBCs are counted in all four large squares of WBCs starting in the upper left large corner square. Move to the upper right corner square, bottom right corner square, and end in the bottom left corner square.
- Count all cells that touch any of the upper and left lines, do not count any cell that touches a lower or right line.



How to count and calculate White blood cells

- ✓ Count the number of WBCs (N) in 64 small squares that are located in the 4 large size squares at the corners.
- ✓ ***Number of WBCs/mm3(µl)of whole blood =***

No. of WBCs counted X Dilution /Area counted X Depth

where, **Dilution**=20 (any one remembers how we get the diluting factor?);

- **Area counted**=4x1sq.mm=4sq.mm;
- **Depth** =0.1mm(constant)

Thus;

No.of WBCs / mm3 of whole blood=No.of cells counted * 20 / (4 * 0.1)

=No.of cells counted * 50

WBCs Count Variation with Age and Pregnancy

Table 1. White Blood Cell Count Variation with Age and Pregnancy

Patient characteristic	*Normal total leukocyte count*
Newborn infant	13,000 to 38,000 per mm^3 (13.0 to 38.0 × 10^9 per L)
Infant two weeks of age	5,000 to 20,000 per mm^3 (5.0 to 20.0 × 10^9 per L)
Adult	4,500 to 11,000 per mm^3 (4.5 to 11.0 × 10^9 per L)
Pregnant female (third trimester)	5,800 to 13,200 per mm^3 (5.8 to 13.2 × 10^9 per L)

Information from reference 3.

https://www.aafp.org/afp/2015/1201/p1004.pdf

Sources of Errors

- ***Failure to:*** have required blood volume, mix well, discard the first 3-4 drops, and properly charge the counting chamber.
- Not wiping off excess blood from outside of the pipette after filling with sample.
- Overfilling or underfilling the chamber of the hemocytometer. ***Be patient. "It will take practice and may require multiple attempts to completely and accurately fill the hemacytometer chamber".***
- Not allowing cells to settle completely prior to counting.

Sources of Errors

- Moist or unclean pipette or chamber and Air bubbles in the counting chamber .
- Slowness in doing the procedural steps which may lead to formation of blood clot.
- Too little or too much diluting fluid.
- Mistakes in counting or calculations.

Some Medical Consideration: Leukocytosis

- A total WBCs count above 11.000 cells/mm3 is referred to as **leukocytosis.** Leukocytosis generally indicates that a bacterial or viral infection is stewing in the body. It is usually due to:

✓ Bacterial infection such as appendicitis, tonsillitis, ulcers and urinary tract infection

✓ Leukemia

✓ Pregnancy

✓ Hemolytic disease of newborn

✓ Following exercise and emotional stress

✓ Cigarette smoking

Some Medical Consideration: Leukopenia

- **Leukopenia** is a condition characterized by a decreased number of white cells in the blood, which is usually due to:

✓ Viral disease such as measles and infectious hepatitis

✓ Some bacterial infections such as typhoid fever, brucellosis, and typhus fever

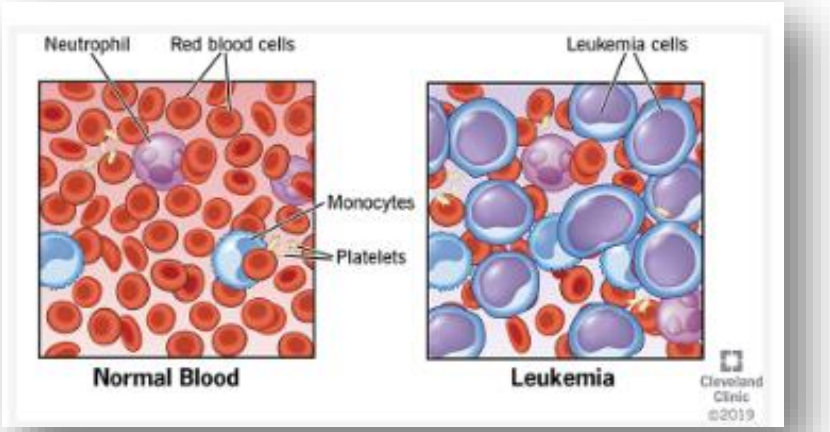
✓ Rheumatoid arthritis

✓ Systemic Lupus Erythematosis

✓ Certain drugs such as such as corticosteroids, radio therapy and chemotherapy.

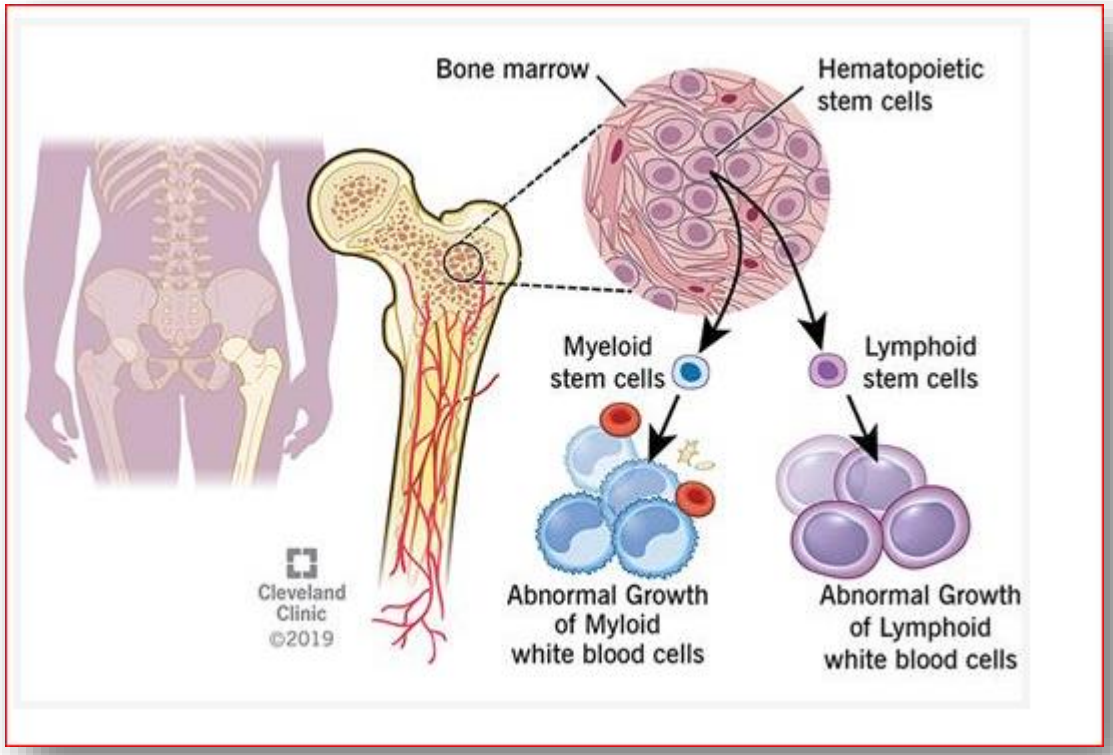
Some Medical Consideration: Leukemia

- ***Leukemia*** is a cancer of the blood and bone marrow. **In simple terms, cancer is defined as the uncontrolled growth of abnormal cells.** Cancer can develop anywhere in the body. **In leukemia, this rapid, out-of-control growth of abnormal cells takes place in the bone marrow.** These abnormal cells then spill into the bloodstream. Unlike other cancers, leukemia generally doesn't form into a mass (tumor) that can be seen in imaging tests, such as X-rays.
- There are many types of leukemia. Some are more common in children; others are more common in adults. Treatment depends on the type of leukemia and some other factors.



How does leukemia develop?

As stem cells in bone marrow begin to divide and multiply, they develop into all the needed types of blood cells. In patients with leukemia, cell growth goes "haywire," and there is a rapid growth of abnormal white blood cells.



To finish up let's go over the most interesting facts about WBCs

10 Interesting WBCs Facts

Location

- Do you know the location of white blood cells? They are spreading around the body. The lymphatic and blood system also have white blood cells.

Nuclie

- Nuclei can be found in all types of white blood cells. The platelets and red blood cells do not have nuclei.

Categories

- There are two primary standards used to categorize the WBCs. The first one is seen from the cell division lineage, which includes lymphoid cells and myeloid cells. The second is seen from the structure, which can be agranulocytes or granulocytes

Primary Types

- WBCs are divided in five primary types. They are monocytes, lymphocytes, basophils, eosinophils and neutrophils. The functional and physical characteristics will determine the differences of each type.

Subtypes

- The types of white blood cells can be divided based on the subtypes. The NK cells, T cells and B cells are the subtypes of lymphocytes.

10 Interesting WBCs Facts

Indicator of Disease

- One indicator of disease can be seen from the number of WBCs. The healthy adult will have 1% of WBCs of the total blood volume.

The Highest Number

- The highest number of WBCs is neutrophils. It makes up around 60 to 70 percent of the WBCs in the body.

Eosinophils

- Eosinophils have a low amount in WBCs. It only accounts for 2 until 4 percent. During the menstruation, the number of Eosinophils is fluctuated.

Function of Eosinophils

- The function of Eosinophils is to handle the parasitic infections.

Basophils

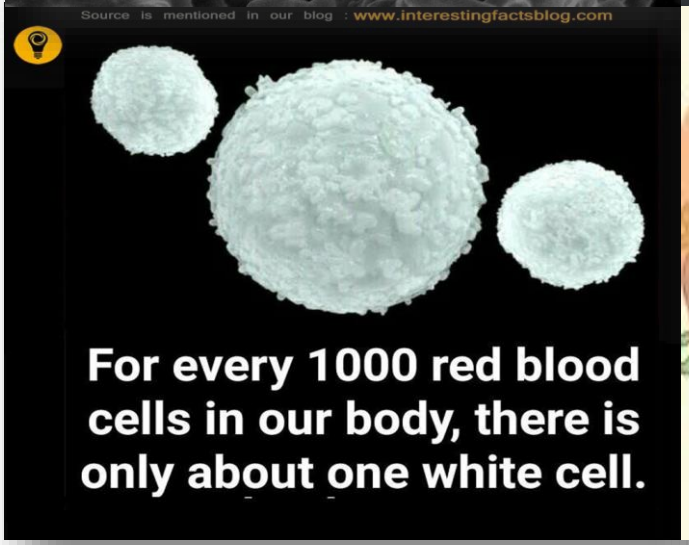
- The type of white blood cell, which handles the antigen and allergic reaction, is Basophils.

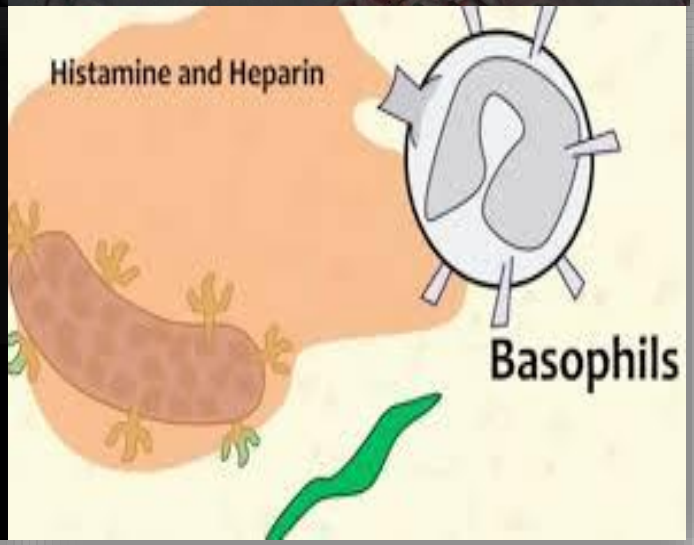
COOL FACTS

- One might think of them as immunity cells that are constantly fighting to keep us safe during the "good fight".
- Have different kinds of shapes and don't change shape when multiplied

COOL FACTS (2)

- WBCs are irregular in shape, but have a nucleus and an outer buffer coat.
- their lifespan is 4-30 days depending on body
- The WBC count goes up significantly when there is any infection





Thanks for Your Attention