

## HEMATOPOIETIC SYSTEM I: PERIPHERAL BLOOD

**READING:** Gartner and Hiatt, pages 89-101

### LEARNING OBJECTIVES:

1. Describe the composition of normal peripheral blood, including the relative quantities of the blood cells.
2. Identify normal peripheral blood cells.
3. Describe the function and "life cycle" of normal peripheral blood cells.
4. List the components of the CBC (complete blood count).

**Key Words:** Erythrocytes, reticulocytes, neutrophils, eosinophils, basophils, monocytes, lymphocytes, platelets, CBC

### I. Composition.

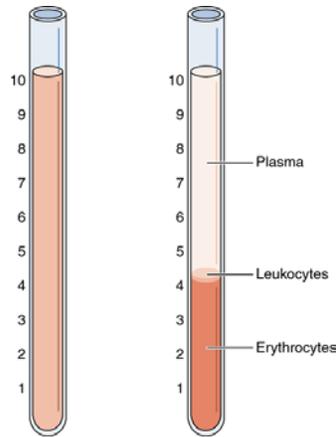
Blood is a fluid connective tissue constituting about 7% of our total body weight (about 5 liters in the human). The primary components are:

**A. Plasma:** The liquid in which peripheral blood cells are suspended. Composed of water, electrolytes such as  $\text{Na}^+$  and  $\text{Cl}$ , (0.9%), 7% plasma proteins (such as albumin, fibrinogen, globulins), hormones, fats, amino acids, vitamins carbohydrates, lipoproteins as well as other substances. The normal plasma volume is 40 ml/kg of body weight.

### B. Formed Elements (blood cells):

1. **Erythrocytes (red blood cells or rbc):** occupy about 40-45% of the total blood volume or 30 ml/kg body weight.
2. **Leukocytes (white blood cells or wbc) and Platelets:** together make up about 1-2% of the total blood volume.

Freshly drawn blood is a red fluid (specific gravity of 1.052-1.064). It forms a jelly-like mass if allowed to clot. The clot is formed by the conversion of fibrinogen to fibrin which forms a meshwork that entraps the formed elements. If the gel is centrifuged, cell free serum can be obtained. If an anticoagulant is added, blood can be sedimented into three distinct layers due to different densities of elements:  $\text{rbc} > \text{wbc} > \text{plasma}$ . The lowermost layer (45% of the blood volume) consists of erythrocytes. Above this region is a grayish white layer (buffy coat) which represents the platelets, lymphocytes and granulocytes (about 1-2% volume). Plasma is the top layer.



## II. Morphology and Function of the Blood Cells

### A. Erythrocytes (mature red blood cells)

#### 1. Cytology

The mature human erythrocyte measures about 7-8  $\mu\text{m}$  in diameter and appears as an anucleate, acidophilic cell. In addition to the nucleus, the mature erythrocyte also loses its Golgi apparatus, centrioles, ER and most of its mitochondria.

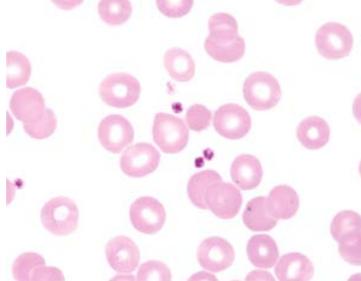
- a. The bulk of the cytoplasm (90-95% of dry weight) consists of the iron-carrying pigment **hemoglobin**. Very few organelles are present. The measurement of the hemoglobin concentration in whole blood, referred to simply as “hemoglobin”, involves lysing the erythrocytes, producing an evenly distributed solution of hemoglobin in the sample. The normal range for hemoglobin is age- and sex-dependent, with men having higher values than women, and adults having higher values than children (except newborns, who have the highest values of all). At Memorial Hermann Hospital, the normal range for young female adults is 12-16 g/dL; for young male adults it is 14-18 g/dL.

The **hematocrit** is a measure of the total volume of the erythrocytes relative to the total volume of whole blood in a sample. The result is a proportion, often expressed as a percent. The normal range is 37-47 % for women, and 42-52% for men.

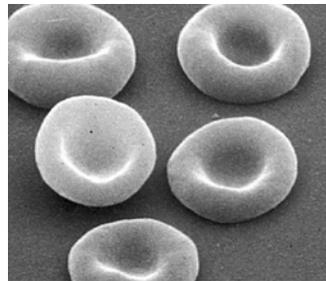
#### b. Shape

A normal red blood cell is a **biconcave disk** to achieve a maximum surface area to cytoplasmic volume ratio. The surface area of an erythrocyte is calculated to be 128  $\mu\text{m}^2$ . Thus, the average person has 3840  $\text{m}^2$  of RBC membrane area for respiratory exchange. The cell is enclosed in a typical fluid, bilayered cell membrane which is flexible and elastic enough to allow the cell to move through capillaries. Cells frequently assume a cup shape in capillaries. Peripheral membrane proteins, spectrin and actin, serve a cytoskeletal function.

Peripheral blood: red blood cells (air dried, Wright stain)



Scanning electron micrograph of red blood cells



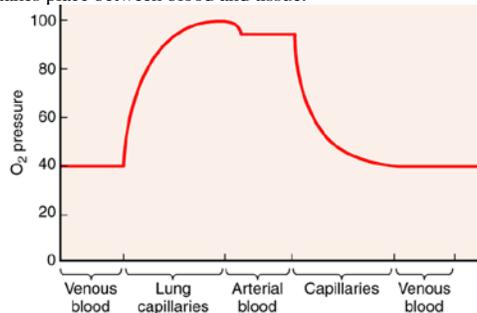
- b. **Life span:** The mature erythrocyte has a life span of approximately 120 days in the

circulation. As red blood cells age, the surface area decreases relative to cytoplasmic volume resulting in a sphere form which is more rigid and is ultimately trapped in splenic cords.

### c. Function

To provide an environment for the iron-containing respiratory pigment, heme, which is complexed to two alpha and two beta globin chains comprising the hemoglobin molecule. The **major physiologic role of hemoglobin is oxygen and CO<sub>2</sub> transport**. The erythrocyte also contains enzymes that participate in the glycolytic and hexose monophosphate biochemical pathways. Under normal circumstances, red blood cells never leave the circulatory system.

Blood O<sub>2</sub> content in each type of blood vessel. The amount of O<sub>2</sub> is highest in arteries and lung capillaries; it decreases in tissue capillaries, where exchange takes place between blood and tissue.



## 2. Frequency (number)

The erythrocyte count, also referred to as the “RBCs”, simply involves counting the number of RBCs per unit volume of whole blood. This is done by an automated instrument. Male = 4.7-6.1 x 10<sup>6</sup> cells/mm<sup>3</sup>; Female = 4.2-5.4 x 10<sup>6</sup> cells/mm<sup>3</sup>.

## 3. Red cell indices

### a. Mean corpuscular volume (MCV)

This is the mean volume of all the erythrocytes counted in the sample. The value is expressed in volume units, in this case very small ones – femtoliters (fL, 10<sup>-15</sup> liter). The normal range is 80-94 fL. The formula for the calculation in general terms is

$$\text{MCV} = \frac{\text{hematocrit}}{\text{RBC count}}$$

When the MCV is low, the red blood cells are said to be **microcytic**, when high, **macrocytic**. **Normocytic** refers to blood with a normal MCV. Keep in mind that the MCV measures only average cell volume. The MCV can be normal while the individual red cells vary wildly in volume from one cell to the next. Such an abnormal variation in cell volume is called **anisocytosis**. The degree of anisocytosis is measured by the automated cell counter and reported as red cell distribution width (RDW). The normal range for the RDW is 11.5-14.5%.

### b. Mean corpuscular hemoglobin (MCH)

The MCH represents the mean mass of hemoglobin in the RBC and is expressed in the mass unit, pictograms (pg, 10<sup>-12</sup> gram). The value is determined by the formula,

$$\text{MCH} = \frac{\text{hemoglobin}}{\text{RBC count}}$$

The normal range is 27-31 pg. Since small cells have less hemoglobin than large cells, variation in the MCH tends to track along with that of the MCV.

### c. Mean corpuscular hemoglobin concentration (MCHC)

This is the mean concentration of hemoglobin in the red cell. The formula is:

$$\text{MCHC} = \frac{\text{hemoglobin}}{\text{hematocrit}}$$

Cells with normal, high, and low MCHC are referred to as normochromic, hyperchromic, and hypochromic, respectively. These terms will be important in anemia classification.

#### 4. **Reticulocytes**

These are **less mature erythrocytes**. Red blood cells released from the bone marrow into the peripheral circulation are considered reticulocytes for about **2 days**.

##### a. **Cytology**

Reticulocytes are slightly larger than the older RBCs and have slightly more RNA, giving them a slightly bluish staining quality (referred to as polychromasia). Reticulocytes can be identified by staining with a supravital dye that stains the RNA.

##### b. **Frequency**

Normally make up 1-2% of red cells; after approximately 1-2 days in peripheral blood they are indistinguishable from mature blood cells. Reticulocytes are increased in number when the bone marrow is producing and releasing them faster, e.g., in response to blood loss. When something is wrong with the bone marrow, such as leukemia or metastatic cancer, the bone marrow will not be able to respond appropriately.

#### 5. **Anemia**

##### a. **Definition**

A significant reduction in the total body erythrocyte mass, measured as a reduction in the RBC count, the hemoglobin, and the hematocrit. Anemia exists when the hemoglobin is less than 12 g/dL or the hematocrit is less than 37%.

##### b. **Classification**

Anemias can be classified by cytometric schemes (microcytic, macrocytic, or normocytic), erythrokinetic schemes (those that take into account the rates of RBC production and destruction), and biochemical/molecular schemes (those that consider the etiology of the anemia at the molecular level). Anemias will be studied in detail in the MSII year.

### B. **White Blood Cells**

White blood cells include granulocytes (neutrophils, eosinophils and basophils), lymphocytes and monocytes. In the CBC, a total number of white blood cells is given (the **WBC count**, white blood cell count) and, if ordered, a “**differential**” count or “diff”. The differential indicates the proportion of the total number of white blood cells represented by each of five types of WBC (the neutrophils, eosinophils, basophils, lymphocytes and monocytes) as a percentage. An absolute number of each of these cell types is also given.

#### 1. **Neutrophils (neutrophilic granulocytes):**

##### a. **Cytology**

The mature neutrophil measures approximately 12-15  $\mu\text{m}$  diameter in a smear. It is characterized by a prominent nucleus segmented into 2-5 lobes joined by extremely fine nuclear strands. **Most numerous of the white blood cells in an adult** (lymphocytes are more

numerous in young children). Slightly less mature neutrophils (bands) have a non-segmented or band-shaped nucleus. Rarely, earlier forms such as metamyelocytes may be seen in the PB.

### Granules

Cytoplasm contains two major types of granules:

#### 1) **Specific (secondary) granules**

Most numerous type; small with a pinkish hue (fairly “neutral” staining), so not easily seen, collagenase, lactoferrin, bacteriocidal phagocytins and lysozyme.

#### 2) **Azurophilic (primary) granules**

- These are less numerous, larger, and stain reddish purple. They are most commonly found in the promyelocyte stage of granulocyte precursors and are not specific for neutrophils. Promyelocytes are found in the bone marrow, but not normally in the peripheral blood. They are primary lysosomes which contain myeloperoxidase and acid hydrolases characteristic of lysosomes.

Cytoplasm also contains glycogen and numerous filaments and microtubules (function in movement).

Neutrophils which have just been released from the bone marrow often have a band-shaped or horseshoe-shaped nucleus and are known as band neutrophils or just “**bands**”.

In females the inactivated X chromosome can appear as a nuclear appendage (Barr body).

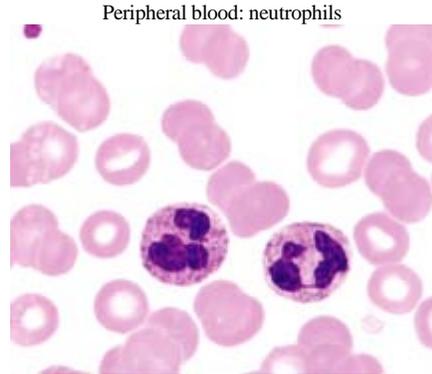
### c. **Frequency**

The white cells of peripheral blood are generally 50-75% neutrophils; about 3-5% are band forms. **Half-life in blood is only 6-8 hrs, 1-4 day lifespan.** Neutrophils move from the peripheral circulation into the tissues in response to infection or tissue damage (e.g., a myocardial infarct).

### d. **Functions**

- **Neutrophils are the first line of defense against microorganisms, especially bacteria.** They are **active phagocytes**. Particles are taken up in a vacuole, the pH is lowered by a membrane proton pump, then the azurophilic granules fuse and empty. Killing and digestion occur. Activated oxygen species also form and kill microorganisms. Lysozyme destroys cell wall of gram-positive bacteria.
- Neutrophils undergo chemotaxis. They are attracted by devitalized tissue, bacteria, foreign bodies, and complement components.
- Phagocytosis can be non-specific but will be enhanced if the body has made specific antibodies due to previous exposure. **Blood borne antibody (IgG) binds to surface antigen or bacteria, and then complement C<sub>3</sub>b binds (process called opsonization), facilitating phagocytosis.**
- The bone marrow will release more neutrophils in response to stimuli such as

**bacterial infection, tissue necrosis, etc. (See next lecture)**



## 2. Eosinophils

### a. Cytology

The mature eosinophil is about the same size as the neutrophil; about 12-15  $\mu\text{m}$  in diameter in dry smears. The eosinophil generally contains a bilobed nucleus; however, three to four lobes are noted occasionally. The cytoplasm has characteristics similar to the neutrophil with the notable exception that the specific granules are refractile and stain bright orange (eosinophilic) with the Romanowsky stain. These granules are somewhat unique because by electron microscopy they can be seen to contain a **dense filamentous core of major basic protein (MBP)**. These structures are known as crystalloids. The matrix of the granules contains lysosomal enzymes, and in particular has a high content of peroxidase, arylsulfatase, acid phosphatase, RNase and cathepsin.

### b. Frequency

The peripheral blood contains about 1-3% eosinophils. The number of eosinophils is high in individuals with allergies or parasitic infections. **Also circulates about 8 hours.**

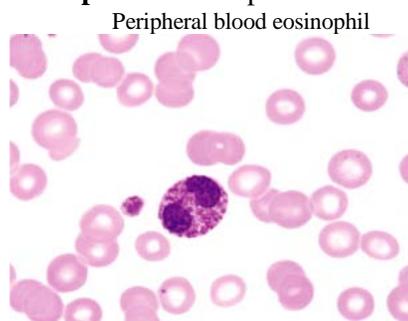
### c. Functions

The function of eosinophils is not entirely clear, but they are **increased in some parasitic infections, in allergic responses to a variety of stimuli including pollen, some drug reactions.**

### d. Chemotaxis

These cells are known to phagocytose antigen-antibody complexes and inactivate mediators of inflammation such as leukotrienes.

### e. Major basic protein. Kills parasitic worms.



### 3. Basophils

#### a. Cytology

The mature basophil measures 9-10  $\mu\text{m}$  in diameter in smears and has a nucleus containing 2-3 lobes which are often difficult to see because of the **large, dark-staining specific granules**.

The cytoplasm contains numerous metachromatic granules of varying size that stain a deep purple color with the usual stains used for peripheral blood smears. These granules contain high concentrations of heparin (an anticoagulant), a number of vasodilating agents which cause increased vascular permeability such as histamine, slow reacting substance of anaphylaxis (leukotriene), serotonin, and eosinophil chemotactic factor.

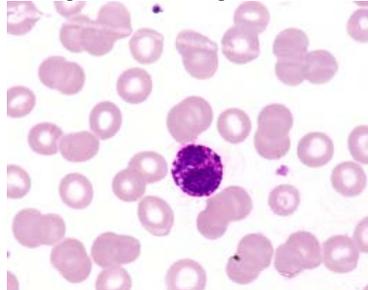
#### b. Frequency

The peripheral blood contains 0.5-1% basophils, and thus basophils are the least numerous granulocyte and least numerous white blood cell in the peripheral blood. Basophils are similar to mast cells but not identical. (Mast cells are seen in the bone marrow and other tissues, but not the normal peripheral blood.)

#### c. Function

Although the basophil possesses phagocytic capabilities, it is mainly the secretory cell which **mediates the hypersensitivity reaction**. Basophils (and mast cells) can bind IgE antibody, and, when subsequently exposed to the corresponding antigen, release vasoactive substances leading to hypersensitivity reactions.

Peripheral blood: basophil



#### d. Summary

**Neutrophils, eosinophils and basophils** are the three types of blood cells referred to as **granulocytes**. They have a segmented nucleus in the mature stage and granules in the cytoplasm.

### 4. Monocytes

#### a. Cytology

The mature monocyte measures 12-15  $\mu\text{m}$  in diameter in blood smears and represents a circulating cell derived from bone marrow promonocytes. They spend about a week in blood and migrate into tissue where they become tissue macrophages or histiocytes. They are the largest cell in the peripheral blood.

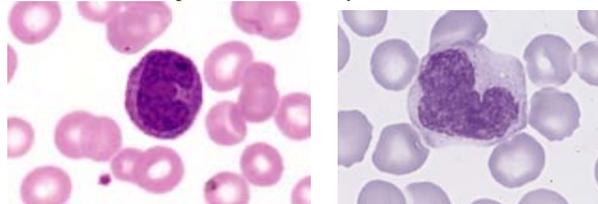
They contain a fairly large nucleus which is ovoid, kidney- or horseshoe-shaped, often located in an eccentric position. Nucleoli are sometimes seen.

The cytoplasm is pale and may contain fine azurophilic granules (lysosomes), small amounts of RER, free ribosomes, polyribosomes, and a well-developed Golgi apparatus (making lysosomes). Pinocytotic (clear) vacuoles are frequently seen in cytoplasm.

Cell membrane has many finger-like microvilli.

Peripheral blood has 3-8% monocytes. Life span 5-8 days.

Peripheral blood: monocytes



#### b. Function

Monocytes belong to the monocuclear-phagocyte system. These cells leave the blood and enter the tissue, differentiating into macrophages or tissue histiocytes. **They ingest (phagocytize) and remove particulate matter, tissue debris, and infectious agents.** Histiocytes/macrophages are present in many tissues, but are particularly numerous in the bone marrow, liver and spleen.

Interact with lymphocytes and play essential role with antigen interaction of immunocompetent cells.

### 5. Lymphocytes

#### a. Cytology

Mature lymphocytes show a spectrum of sizes ranging from small lymphocytes (6-8 microns in diameter) to larger forms (10-12 microns in diameter or even higher).

They contain a single, deeply-stained, spherical nucleus which can have an slight indentation. The chromatin in the nucleus is condensed into coarse clumps. The nucleus is surrounded by a thin rim of lightly basophilic cytoplasm in the smaller types. The cytoplasm is more abundant in the larger lymphocytes.

The cytoplasm may contain a few nonspecific granules (lysosomes), a few mitochondria, many ribosomes, and a Golgi apparatus.

Two major subtypes of usually morphologically indistinguishable lymphocytes can be identified (by lifespan, function, surface receptors, site of differentiation) – T cells and B cells. A third type of lymphocyte is referred to as natural killer (NK) cells.

Peripheral blood: lymphocyte



## b. Function

**Lymphocytes are an important part of the immune system.**

### T-lymphocytes

Processed by the thymus and participate in cell-mediated immunity (such as graft rejection). Some T-cells (cytotoxic) can elaborate cytotoxic agents. They can make lymphokines (including interferon, macrophage migration inhibitor factor, chemotactic factors for basophils). There are also memory T-cells that survive for years or decades.

### B-lymphocytes

(Bursa derived) - these cells play a central role in humoral or antibody-mediated immunity. Some divide and differentiate into plasma cells in tissue. Some are memory cells. They are immunologically characterized by immunoglobulin on their cell membrane.

#### Plasma cells

Derive from B-lymphocytes. They have an eccentrically placed nucleus, contain abundant amounts of RER whose cisternae can be filled with antibody, and have a well-developed Golgi. **Plasma cells produce antibodies (immunoglobulins).**

#### NK cells

NK cells lack the surface antigens of T and B cells and do not require prior stimulation to attack virus-infected cells or tumor cells.

## c. Frequency

About 20-50% of peripheral blood white blood cells are lymphocytes. Of these, about 80% are T-cells, 15% B-cells, 5% NK cells. Plasma cells are not normally seen in the peripheral blood but are present in the bone marrow and other tissues.

## d. Summary

The **monocytes and lymphocytes** are referred to as **mononuclear white blood cells** because their nuclei are not segmented (as opposed to the granulocytes which contain segmented nuclei). They do not contain prominent granules in their cytoplasm, in contrast to the granulocytes discussed earlier.

The WBC count and differential and absolute counts are reported as part of the CBC and will look something like this:

WBC	6000/ $\mu$ L	
Neutrophils	62%	3720/ $\mu$ L
Lymphocytes	25%	1500/ $\mu$ L
Monocytes	8%	480/ $\mu$ L
Eosionphils	3%	180/ $\mu$ L
Basophils	2%	120/ $\mu$ L

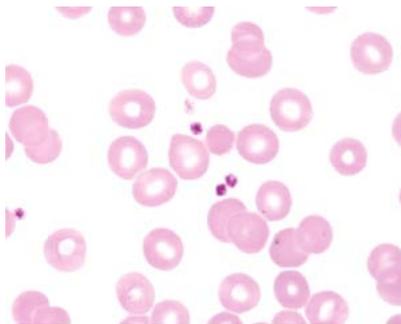
## C. Platelets

### 1. Structure

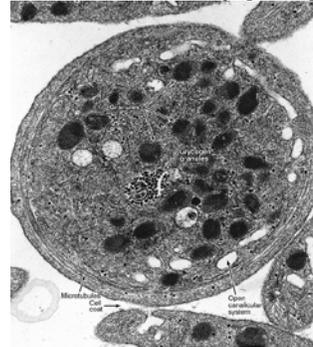
Non-nucleated flat, biconvex, round or ovoid disks (2-5  $\mu\text{m}$  diameter); **derived from bone marrow megakaryocytes.**

- a. Contain pale-blue peripheral hyalomere with a system of invaginating channels (open canalicular system) connecting with the platelet membrane, a system of electron-dense tubules, a marginal bundle of 10-15 circumferential microtubules to maintain the disk shape, and actin and myosin. When platelets become activated, the microtubular system “squeezes” the granules to the center of the platelet, the contents are released, and the coagulation system is triggered (see below).
- b. Contain thicker central purple granulomere containing several kinds of granules. Alpha granules are azurophilic and contain fibrinogen, platelet-derived growth factor, etc. Dense granules contain calcium, pyrophosphate, ADP and ATP. They also store serotonin. Small vesicles (lambda granules) contain lysosomal enzymes.

Peripheral blood: platelets (and RBCs)



Transmission electron micrograph of a platelet



### 2. Functions

Platelets are involved in **hemostasis** (stopping bleeding). Platelets aggregate on damaged endothelium and exposed collagen, release contents of alpha and dense granules, **promote the coagulation cascade** involving plasma factors **to form a blood clot**. The formation of the platelet plug is referred to as “primary hemostasis”. The clotting process is good to limit hemorrhage but can be life threatening on the wall of a coronary artery (coronary thrombosis) or when breaking free from veins of extremities and going to lungs to cause pulmonary embolism. **A deficiency of platelets is called thrombocytopenia.** Thrombocytopenia may result from a number of causes, such as immune destruction by antibodies directed against platelets, bone marrow infiltration by metastatic cancer, or primary malignancy of the bone marrow. **Thrombocytopenia may result in bruising or bleeding.**

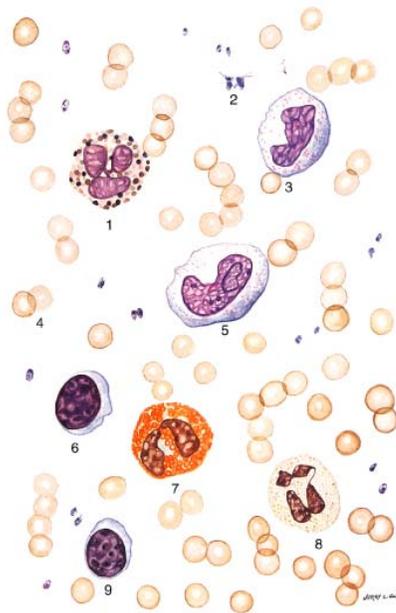
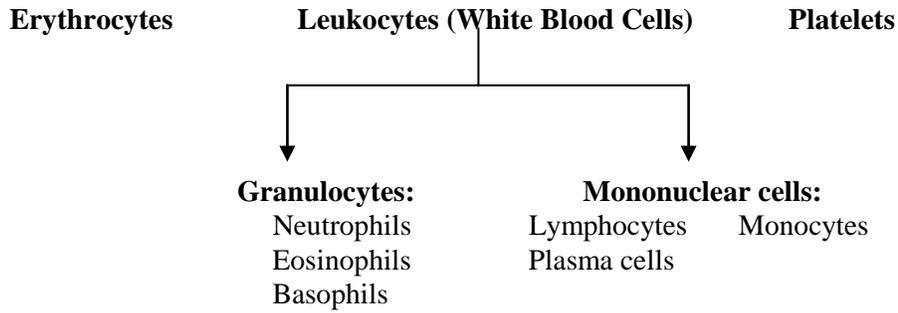
### 3. Frequency

Blood contains 150,000-350,000/ $\text{mm}^3$ . Life span is 7-10 days. The platelet count is typically reported with the CBC (“CBC with diff and platelets”).

## III. The CBC (Complete Blood Count)

- A. The CBC is a very common laboratory test. A great deal of information can be obtained from evaluating the patient’s red blood cells, white blood cells, and platelets. You can see if the patient is anemic, if they are likely to have a viral or bacterial infection, and if they are likely to have bleeding due to thrombocytopenia.

## Normal Peripheral Blood Cells



1.Basophil 2.Platelets 3.Monocyte 4.Erythrocytes 5.Monocyte 6.Lymphocyte 7.Eosinophil 8.Neutrophil 9.Lymphocyte

### Products and Functions of the Blood Cells

Cell Type	Main Products or Components	Main Functions
Erythrocyte	Hemoglobin	CO <sub>2</sub> and O <sub>2</sub> transport
Leukocytes		
Neutrophil	Specific granules and modified lysosomes (azurophilic granules)	Phagocytosis of bacteria
Eosinophil	Specific granules, pharmacologically active substances	Defense against parasitic helminths; modulation of inflammatory processes
Basophil	Specific granules containing histamine and heparin	Release of histamine and other inflammation mediators

Monocyte	Granules with lysosomal enzymes	Generation of monoclear-phagocyte system cells in tissues; phagocytosis and digestion of protozoa- and virus-infected and senescent cells
B lymphocyte (Plasma cell)	Immunoglobulins	Generation of antibody-producing terminal cells (plasma cells)
T lymphocyte	Substances that kill cells, substances that control the activity of other leukocytes (interleukins)	Killing of virus-infected cells
Natural killer cell	Attacks virus-infected cells and cancer cells without previous stimulation	Killing of some tumor and virus-infected cells
Platelet	Blood-clotting substances	Clotting of blood

## HEMATOPOIETIC SYSTEM II: BONE MARROW

### LEARNING OBJECTIVES:

1. List the types of hematopoietic tissues.
2. Describe the development of hematopoiesis throughout the life of the individual.
3. Describe the developmental stages of erythroid and myeloid cells.
4. Identify the other types of cells in the bone marrow, i.e. megakaryocytes, lymphocytes, plasma cells, macrophages, adipocytes.
5. Describe the factors which control hematopoiesis, e.g. growth factors such as erythropoietin, GM-CSF.

**Key Words:** blood cell renewal, hematopoiesis, bone marrow, erythropoiesis, granulopoiesis, megakaryopoiesis, growth factors.

### D. Introduction

Bone marrow is a richly vascularized connective tissue which specializes in production of all the formed elements of blood. Bone marrow is the most rapidly replicative tissue in the body.

## II. Blood Cell Renewal

### A. The problem

The different blood cells have finite and often brief life spans and must be renewed to maintain appropriate circulating levels. The process of renewal is known as hematopoiesis (hemopoiesis).

#### 1. Influences on hematopoiesis

- Infection, hemorrhage, tumors and other factors may induce increases or decreases in the level of hematopoiesis in one or more of the blood cell lines.
2. Several growth factors and other products affect the proliferation and differentiation of hematopoietic cells. Different factors are involved in erythropoiesis, granulopoiesis, etc. (See table below)

### B. Hematopoietic tissues

Tissues which actively or potentially produce blood cells are hematopoietic tissues.

#### 1. Types of hematopoietic tissues

##### a. Myeloid tissues

Blood forming tissues identified as the "red marrow" of bones. The amount and location of red marrow is different in the fetus and the adult. (See below)

##### b. Lymphoid tissues

Some lymphocytes of peripheral blood arise from proliferation within lymphoid tissues. Lymphoid tissues are elements of the larger lymphatic system (lymph nodes, tonsils,

Peyer's patches, etc.) These will be addressed in another Histology lecture. (Please note, the lymphocyte "stem cell" is located in bone marrow.)

### C. Hematopoiesis in Utero:

#### 1. Embryo and fetus (intrauterine development)

Hematopoiesis in the developing organism is a dynamic process which involves a variety of tissue sites. The different tissues serve as major blood cell producers at different gestational ages. Furthermore, gestational hematopoiesis occurs in three major waves or phases and a few minor ones as well. The three phases are mesoblastic, hepatic and myeloid.

GESTATIONAL AGE	PHASE	LOCATION
2 weeks - 2 months	Mesoblastic	Wall of yolk sac
6 weeks - birth	Hepatic	Liver
2 1/2 month - birth	Myeloid	Bone Marrow

**TABLE 13.1 GESTATIONAL HEMATOPOIESIS**

##### a. Mesoblastic phase

Mesoblastic phase extends from two weeks to two months. Hematopoiesis begins in the yolk sac wall where small nests of blood cell production can be visualized. These nests are referred to as "blood islands".

##### b. Hepatic phase

Islands of blood cell development occur within the liver parenchyma from 6 weeks to birth. Although the liver is the dominant site of hematopoiesis for the first half of gestation, blood cell production also occurs at lesser intensity within the spleen.

##### c. Myeloid phase

Hematopoiesis within bone marrow begins in the clavicle at about 2 1/2 months. Activity continues to rise until myeloid tissue becomes the major site of hematopoiesis in the latter half of gestation. Myeloid hematopoiesis is centered in most bone marrow (red marrow) during intrauterine life.

#### 2. Birth

At birth, the myeloid phase predominates in hematopoiesis and occurs in the medullary cavities of long bones. As a child grows, the long bones assume the weight bearing function and the marrow becomes yellow. The site of hematopoiesis subsequently shifts to the red marrow of the axial skeleton. (In case you haven't had the axial skeleton yet, it consists of skull, vertebral column, ribs, sternum, shoulder girdle and pelvic girdle).

#### 3. Adults

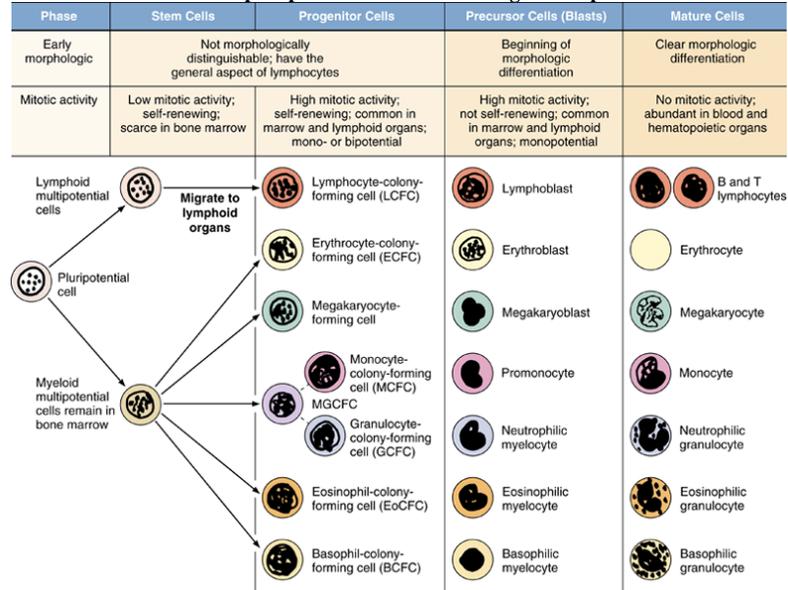
Under normal conditions, hematopoiesis resides in red marrow of the axial skeleton. However, under certain conditions, the spleen, liver and yellow marrow (converts to red) can resume hematopoietic activity in the adult.

### II. Stem Cells, Growth Factors, and Differentiation

All blood cell types arise from common or **pluripotential** stem cells. Some of the daughter cells will become specific, irreversibly differentiated cell types, and other daughter cells remain stem cells. These cells proliferate and form one cell lineage that will become lymphocytes (lymphoid

cells) and another lineage that will form the myeloid cells that develop in bone marrow (granulocytes, monocytes, erythrocytes, and megakaryocytes). Early in their development, lymphoid cells migrate from the bone marrow to the thymus, lymph nodes, spleen, and other lymphoid tissues. Lymphoid tissue will be covered in more detail in other lectures.

**Differentiation of pluripotent stem cells during hematopoiesis**



### III. Red Marrow

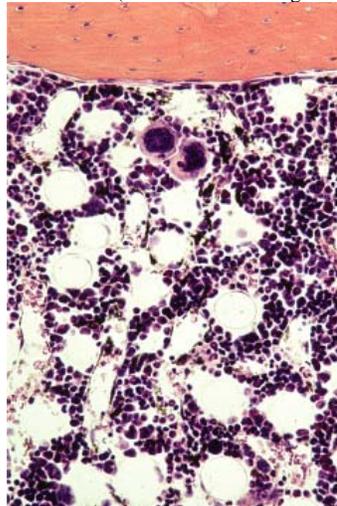
#### A. Histology

Red marrow is composed of a network of vascular sinuses (specialized blood vessels) situated throughout a sponge-like network of hematopoietic cells. Sinuses are interposed between arteries and veins and contain, essentially, peripheral blood. Red Marrow is organized into sacs (alveoli) within which hematopoietic cells develop in nests or foci. Reticular cells and reticular fibers form a supportive framework for the developing blood cells and sinuses.

##### 1. Location

In red marrow, cells of the RBC line develop near the sinus wall. Each RBC nest has an associated macrophage which contains iron. Cells of the granulocyte series (eosinophilic, neutrophilic, and basophilic) develop away from the sinus. Megakaryocytes (cells producing platelets) also develop next to sinuses.

Bone Marrow (H&E stained histologic section)



## IV. Developmental stages of Blood Cells

### A. Erythropoiesis (RBCs)

Process of formation and differentiation of RBCs. (See diagram below and on page 97)

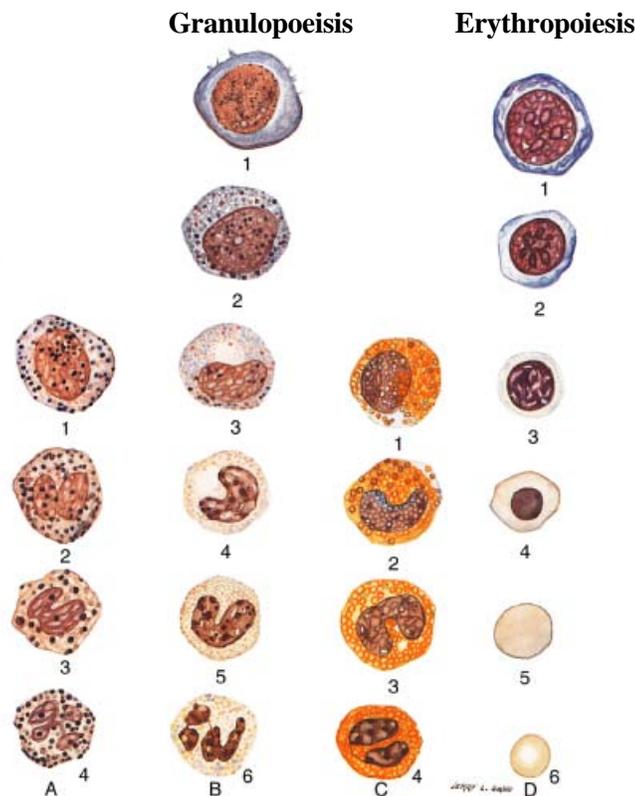
1. **Proerythroblast** - divides.
2. **Basophilic erythroblast** - divides
3. **Polychromatophilic erythroblast** - divides.
4. **Orthochromic erythroblast** - no division.
5. **Reticulocyte** - no division.
6. **Erythrocyte** - no division.
7. **Control of erythropoiesis**

Development from a basophilic normoblast to a mature erythrocyte involves 16-32 cell divisions and takes approximately one week. RBC formation is under the control of a kidney-produced hormone known as **erythropoietin**. Dietary iron, Vitamin B<sub>12</sub> and folic acid are required for the normal production of RBCs.

### B. Granulopoiesis

Process of formation and differentiation of granulocytes.

1. The following are stages in granulopoiesis
  - a. **Myeloblast** - Divides.
  - b. **Promyelocyte** - Divides.
  - c. **Neutrophilic, eosinophilic, or basophilic myelocyte** - Divides.
  - d. **Neutrophilic, eosinophilic, or basophilic metamyelocyte** - No division.
  - e. **Neutrophilic, eosinophilic, or basophilic band** - No division.
  - f. **Segmented neutrophil, eosinophil, or basophil** - No division.



## 2. Control of granulopoiesis

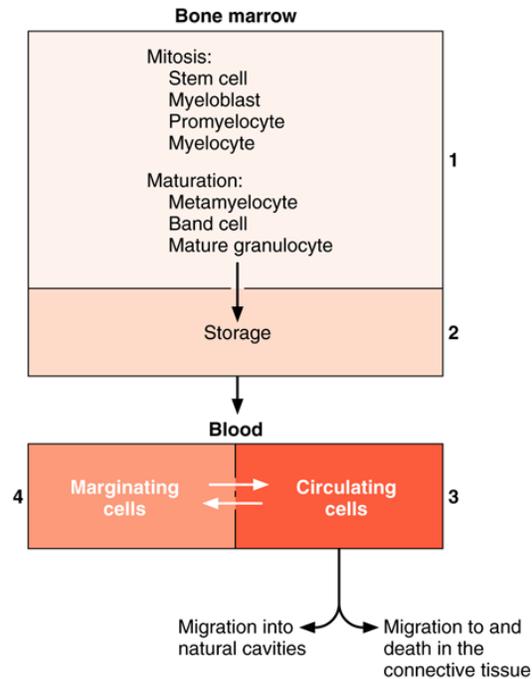
Granulopoiesis is stimulated by CSF (**colony stimulating factors**), substances released by the macrophage/monocyte community, such as G-CSF or GM-CSF. One stimulus for CSF release is increased cell migration into tissues, such as would occur in a bacterial infection.

## 3. Kinetics of Neutrophil Production

The total time taken for a myeloblast to emerge as a mature neutrophil in the circulation is about 11 days. Under normal circumstances, five mitotic divisions occur in the myeloblast, promyelocyte, and neutrophilic myelocyte stages of development. Neutrophils pass through several functionally and anatomically defined compartments:

- a. The medullary formation compartment can be subdivided into a mitotic compartment and a maturation compartment. This compartment is in the bone marrow.
- b. The medullary storage compartment acts as a buffer system, capable of releasing large numbers of mature neutrophils upon demand. This compartment is in the bone marrow.
- c. The circulating compartment consists of neutrophils suspended in plasma and circulating in blood vessels. This compartment is not in the bone marrow, but in the blood.
- d. The marginating compartment is composed of neutrophils present in blood but not circulating. These neutrophils are in capillaries and are temporarily excluded from the circulation by vasoconstriction, or, especially in the lungs, may be at the periphery of vessels, adhering to the endothelium, and not in the main blood stream.

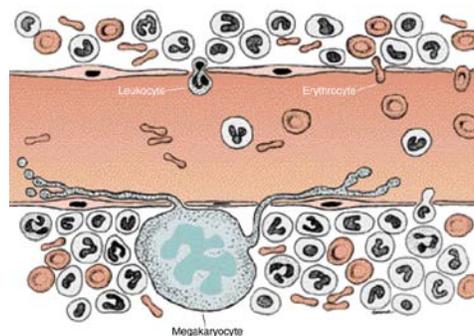
The marginating and circulating compartments are of about equal size, and there is a constant interchange of cells between them. **This is important because in the case of an acute bacterial infection, neutrophils can be recruited quickly from the marginating pool, rather than waiting for new neutrophils to be formed and/or released from the bone marrow.**



### C. Thrombopoiesis

Process of formation of platelets.

1. **Megakaryoblast** - Large cell with a big nucleus (4n) situated close to sinuses.
2. **Promegakaryocyte** – Larger than a megakaryoblast, this cell contains a voluminous polyploid nucleus (8n). Develops platelet demarcation channels.
3. **Megakaryocyte** - A giant cell with a large polyploid nucleus (32n-64n). Platelet demarcation channels very distinct. A massive protrusion of the cell penetrates a sinus and fragments into platelets.
4. **Thrombocyte (platelet)** -The final end product which circulates in the peripheral blood. Responsible for primary hemostasis (clot formation).
5. **Control of thrombopoiesis** - A serum glycoprotein, known as **thrombopoietin**, has been demonstrated. Thrombocytopenia stimulates megakaryocytes and platelet production.



### D. Other red marrow cells

1. **Macrophages**

These cells are the tissue counterparts of monocytes of the peripheral blood. In the bone marrow, they function in phagocytosis and iron storage. (Sometimes we refer to macrophages as histiocytes).

**2. Structural cells**

These include fat cells (adipocytes), reticular cells, and endothelial cells.

**3. Lymphocytes**

A few lymphocytes and plasma cells are usually present in the bone marrow.

**4. Mast cells**

**HEMATOLOGY LAB SLIDE 44 – BLOOD SMEAR**

**IN THIS LAB YOU NEED TO USE OIL. ONE DROP ON THE SLIDE ONLY AND SHIFT TO THE 100X MAGNIFICATION LENS**

Go from one edge of the smear to the other and count the different cell types and see how close you come to the normal range.

- Normal adult range (Of peripheral white cells)
- Neutrophils 50-75% (3-5% stab or band form)
- Lymphocytes 20-50% (80% T, 15% B, 5% Null)
- Monocytes 3-8%
- Eosinophils 1-3%
- Basophils 0.5-1%

The differential count is an excellent diagnostic tool for diagnosis, In viral infections there is predominance of lymphocytes, in bacterial infections it is neutrophils. Disease progress can also be followed such as myeloproliferative disorders, infectious mononucleosis and such.

**SLIDE 45 – BONE MARROW ASPIRATE**

- On this slide you will be looking for members of the granulocytic and erythroid cell lines. The marrow is obtained by inserting a needle through cortical bone and withdrawing the aspirate, a procedure that is often very painful.
- Look for members of the erythropoietic series from basophilic normoblast through to erythrocyte and for members of the granulocyte series from promyelocyte to neutrophil. Identify a blast cell and a Megakaryocyte