

Lecture 5: Erythropoiesis

■ DEFINITION

Erythropoiesis is the process of the origin, development and maturation of erythrocytes. Hemopoiesis or hematopoiesis is the process of origin, development and maturation of all the blood cells.

■ SITE OF ERYTHROPOIESIS

■ IN FETAL LIFE

In fetal life, the erythropoiesis occurs in three stages:

1. *Mesoblastic Stage*

During the first two months of intrauterine life, the RBCs are produced from **mesenchyme** of yolk sac.

2. *Hepatic Stage*

From third month of intrauterine life, **liver** is the main organ that produces RBCs. **Spleen** and **lymphoid organs** are also involved in erythropoiesis.

3. *Myeloid Stage*

During the last three months of intrauterine life, the RBCs are produced from red **bone marrow** and **liver**.

■ IN NEWBORN BABIES, CHILDREN AND ADULTS

In newborn babies, growing children and adults, RBCs are produced only from the red bone marrow.

1. *Up to the age of 20 years*: RBCs are produced from red bone marrow of all bones (**long bones** and all the **flat bones**).
2. *After the age of 20 years*: RBCs are produced from **membranous bones** like vertebra, sternum, ribs, scapula, iliac bones and skull bones and from the ends of long bones. After 20 years of age, the shaft of the long bones becomes yellow bone marrow because of fat deposition and loses the erythropoietic function.

In adults, liver and spleen may produce the blood cells if the bone marrow is destroyed or fibrosed. Collectively bone marrow is almost equal to liver in size and weight. It is also as active as liver. Though bone marrow is the site of production of all blood cells, comparatively 75% of the bone marrow is involved in the production of leukocytes and only 25% is involved in the production of erythrocytes.

But still, the leukocytes are less in number than the erythrocytes, the ratio being 1:500. This is mainly because of the lifespan of these cells. Lifespan of erythrocytes is 120 days whereas the lifespan of leukocytes is very

short ranging from one to ten days. So the leukocytes need larger production than erythrocytes to maintain the required number.

■ PROCESS OF ERYTHROPOIESIS

■ STEM CELLS

Stem cells are the primary cells capable of self-renewal and differentiating into specialized cells (Chapter 1). Hemopoietic stem cells are the primitive cells in the bone marrow, which give rise to the blood cells.

Hemopoietic stem cells in the bone marrow are called uncommitted pluripotent hemopoietic stem cells (PHSC). PHSC is defined as a cell that can give rise to all types of blood cells. In early stages, the PHSC are not designed to form a particular type of blood cell. And it is also not possible to determine the blood cell to be developed from these cells: hence, the name uncommitted PHSC (Fig. 1). In adults, only a few number of these cells are present. But the best source of these cells is the umbilical cord blood.

When the cells are designed to form a particular type of blood cell, the uncommitted PHSCs are called committed PHSCs. Committed PHSC is defined as a cell, which is restricted to give rise to one group of blood cells.

Committed PHSCs are of two types:

1. Lymphoid stem cells (LSC) which give rise to lymphocytes and natural killer (NK) cells
2. Colony forming blastocytes, which give rise to myeloid cells. Myeloid cells are the blood cells other than lymphocytes. When grown in cultures, these cells form colonies hence the name colony forming blastocytes.

Different units of colony forming cells are:

- i. Colony forming unit-erythrocytes (CFU-E) – Cells of this unit develop into erythrocytes
- ii. Colony forming unit-granulocytes/monocytes (CFU-GM) – These cells give rise to granulocytes (neutrophils, basophils and eosinophils) and monocytes
- iii. Colony forming unit-megakaryocytes (CFU-M) – Platelets are developed from these cells.

■ CHANGES DURING ERYTHROPOIESIS

Cells of CFU-E pass through different stages and finally become the matured RBCs. During these stages four important changes are noticed.

1. Reduction in size of the cell (from the diameter of 25 to 7.2 μ)
2. Disappearance of nucleoli and nucleus

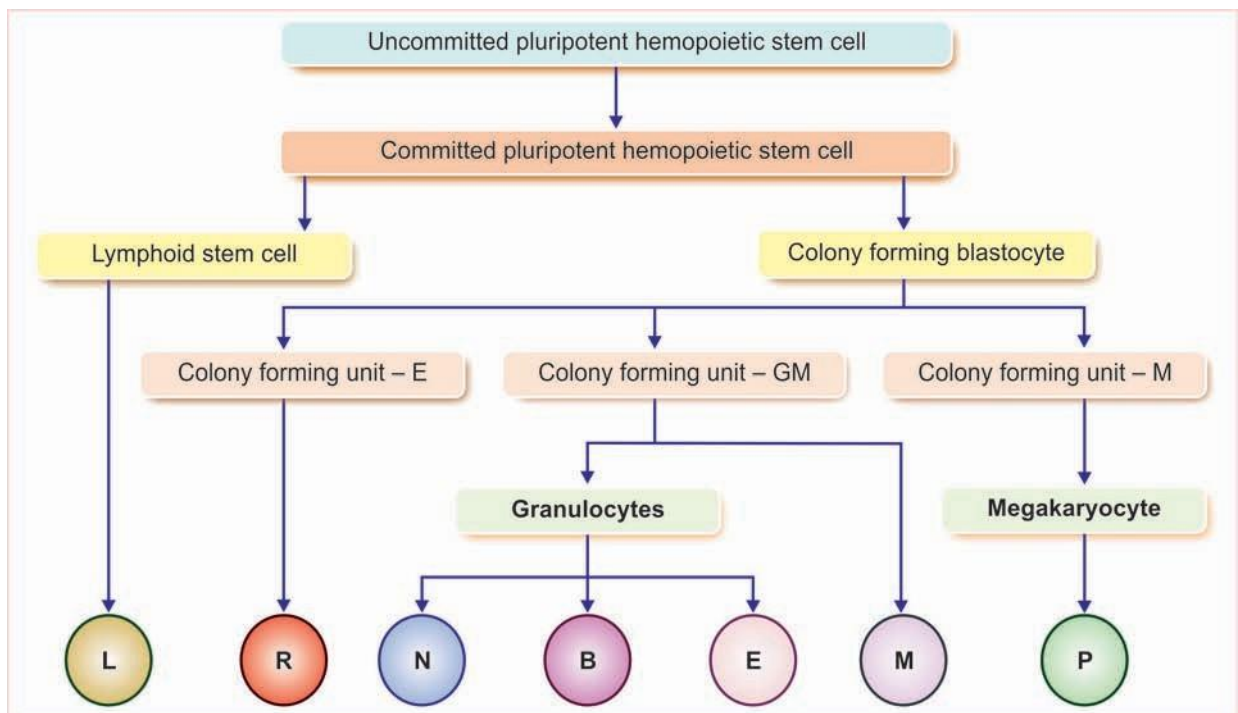


FIGURE 1: Stem cells. L = Lymphocyte, R = Red blood cell, N = Neutrophil, B = Basophil, E = Eosinophil, M = Monocyte, P = Platelet.

3. Appearance of hemoglobin
4. Change in the staining properties of the cytoplasm.

4. Late normoblast
5. Reticulocyte
6. Matured erythrocyte.

■ STAGES OF ERYTHROPOIESIS

Various stages between CFU-E cells and matured RBCs are (Fig. 2):

1. Proerythroblast
2. Early normoblast
3. Intermediate normoblast.

1. Proerythroblast (Megaloblast)

Proerythroblast or megaloblast is the first cell derived from CFU-E. It is very large in size with a diameter of about 20 μ . Its nucleus is large and occupies the cell almost completely. The nucleus has two or more nucleoli

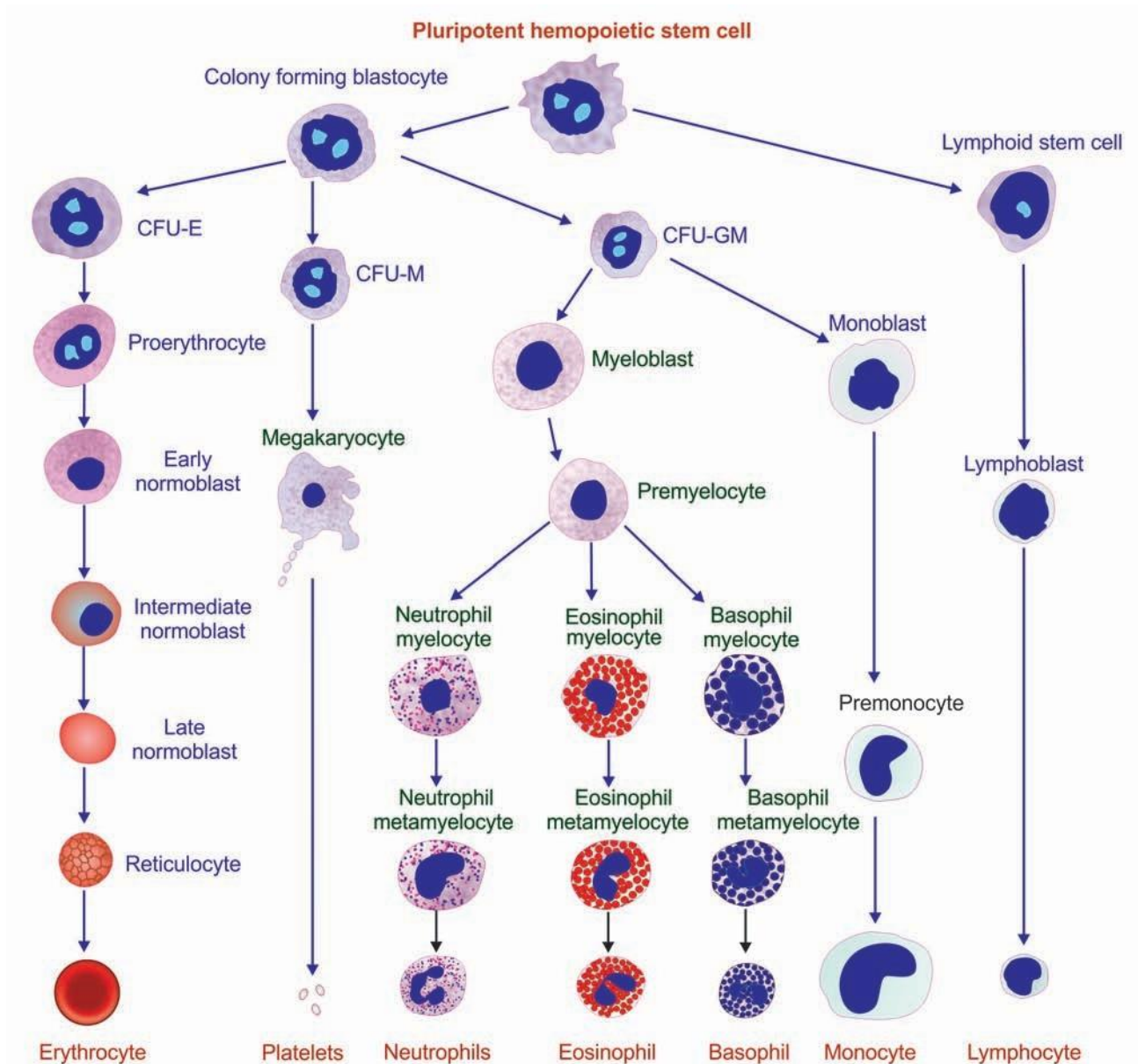


FIGURE 2: Stages of erythropoiesis. CFU-E = Colony forming unit-erythrocyte, CFU-M = Colony forming unit-megakaryocyte, CFU-GM = Colony forming unit-granulocyte/monocyte.

and a reticular network. Proerythroblast does not contain hemoglobin. The cytoplasm is basophilic in nature. Proerythroblast multiplies several times and finally forms the cell of next stage called early normoblast. Synthesis of hemoglobin starts in this stage. However, appearance of hemoglobin occurs only in intermediate normoblast.

2. Early Normoblast

The early normoblast is little smaller than proerythroblast with a diameter of about 15 μ . In the nucleus, the nucleoli disappear. Condensation of chromatin network occurs. The condensed network becomes dense. The cytoplasm is basophilic in nature. So, this cell is also called **basophilic erythroblast**. This cell develops into next stage called intermediate normoblast.

3. Intermediate Normoblast

Cell is smaller than the early normoblast with a diameter of 10 to 12 μ . The nucleus is still present. But, the chromatin network shows further condensation. The hemoglobin starts appearing.

Cytoplasm is already basophilic. Now, because of the presence of hemoglobin, it stains with both acidic as well as basic stains. So this cell is called polychromophilic or **polychromatic erythroblast**. This cell develops into next stage called late normoblast.

4. Late Normoblast

Diameter of the cell decreases further to about 8 to 10 μ . Nucleus becomes very small with very much condensed chromatin network and it is known as ink-spot nucleus.

Quantity of hemoglobin increases. And the cytoplasm becomes almost acidophilic. So, the cell is now called orthochromic erythroblast. In the final stage of late normoblast just before it passes to next stage, the nucleus disintegrates and disappears. The process by which nucleus disappears is called **pyknosis**. The final remnant is extruded from the cell. Late normoblast develops into the next stage called reticulocyte.

5. Reticulocyte

Reticulocyte is otherwise known as immature RBC. It is slightly larger than matured RBC. The cytoplasm contains the reticular network or reticulum, which is formed by remnants of disintegrated organelles. Due to the reticular network, the cell is called reticulocyte. The reticulum of reticulocyte stains with supravital stain.

In newborn babies, the reticulocyte count is 2% to 6% of RBCs, i.e. 2 to 6 reticulocytes are present for every 100 RBCs. The number of reticulocytes decreases

during the first week after birth. Later, the reticulocyte count remains constant at or below 1% of RBCs. The number increases whenever production and release of RBCs increase.

Reticulocyte is basophilic due to the presence of remnants of disintegrated Golgi apparatus, mitochondria and other organelles of cytoplasm. During this stage, the cells enter the blood capillaries through capillary membrane from site of production by diapedesis. Important events during erythropoiesis is given in Table 1

6. Matured Erythrocyte

Reticular network disappears and the cell becomes the matured RBC and attains the biconcave shape. The cell decreases in size to 7.2 μ diameter. The matured RBC is with hemoglobin but without nucleus.

It requires 7 days for the development and maturation of RBC from proerythroblast. It requires 5 days up to the stage of reticulocyte. Reticulocyte takes 2 more days to become the matured RBC.

TABLE 1: Important events during erythropoiesis

Stage of erythropoiesis	Important event
Proerythroblast	Synthesis of hemoglobin starts
Early normoblast	Nucleoli disappear
Intermediate normoblast	Hemoglobin starts appearing
Late normoblast	Nucleus disappears
Reticulocyte	Reticulum is formed. Cell enters capillary from site of production
Matured RBC	Reticulum disappears Cell attains biconcavity

■ FACTORS NECESSARY FOR ERYTHROPOIESIS

Development and maturation of erythrocytes require variety of factors, which are classified into three categories:

1. General factors
2. Maturation factors
3. Factors necessary for hemoglobin formation.

■ GENERAL FACTORS

General factors necessary for erythropoiesis are:

- i. Erythropoietin
- ii. Thyroxine
- iii. Hemopoietic growth factors
- iv. Vitamins.

i. Erythropoietin

Most important general factor for erythropoiesis is the hormone called erythropoietin. It is also called hemopoietin or erythrocyte stimulating factor.

Chemistry

Erythropoietin is a glycoprotein with 165 amino acids.

Source of secretion

Major quantity of erythropoietin is secreted by peritubular capillaries of kidney. A small quantity is also secreted from liver and brain.

Stimulant for secretion

Hypoxia is the stimulant for the secretion of erythropoietin.

Actions of erythropoietin

Erythropoietin causes formation and release of new RBCs into circulation. After secretion, it takes 4 to 5 days to show the action.

Erythropoietin promotes the following processes:

- Production of proerythroblasts from CFU-E of the bone marrow
- Development of proerythroblasts into matured RBCs through the several stages – early normoblast, intermediate normoblast, late normoblast and reticulocyte
- Release of matured erythrocytes into blood. Even some reticulocytes (immature erythrocytes) are released along with matured RBCs.

Blood level of erythropoietin increases in anemia.

ii. Thyroxine

Being a general metabolic hormone, thyroxine accelerates the process of erythropoiesis at many levels. So, hyperthyroidism and polycythemia are common.

iii. Hemopoietic Growth Factors

Hemopoietic growth factors or growth inducers are the interleukins and stem cell factor (steel factor). Generally these factors induce the proliferation of PHSCs. Interleukins (IL) are glycoproteins, which belong to the cytokines family.

Interleukins involved in erythropoiesis:

- Interleukin-3 (IL-3) secreted by T-cells
- Interleukin-6 (IL-6) secreted by T-cells, endothelial cells and macrophages
- Interleukin-11 (IL-11) secreted by osteoblast.

iv. Vitamins

Some vitamins are also necessary for the process of erythropoiesis. Deficiency of these vitamins cause anemia associated with other disorders.

Vitamins necessary for erythropoiesis:

- Vitamin B:** Its deficiency causes anemia and pellagra (disease characterized by skin lesions, diarrhea, weakness, nervousness and dementia).
- Vitamin C:** Its deficiency causes anemia and scurvy (ancient disease characterized by impaired collagen synthesis resulting in rough skin, bleeding gum, loosening of teeth, poor wound healing, bone pain, lethargy and emotional changes).
- Vitamin D:** Its deficiency causes anemia and rickets (bone disease –).
- Vitamin E:** Its deficiency leads to anemia and malnutrition.

■ MATURATION FACTORS

Vitamin B12, intrinsic factor and folic acid are necessary for the maturation of RBCs.

1. Vitamin B12 (Cyanocobalamin)

Vitamin B12 is the maturation factor necessary for erythropoiesis.

Source

Vitamin B12 is called **extrinsic factor** since it is obtained mostly from diet. Its absorption from intestine requires the presence of **intrinsic factor of Castle**. Vitamin B12 is stored mostly in liver and in small quantity in muscle. When necessary, it is transported to the bone marrow to promote maturation of RBCs. It is also produced in the large intestine by the intestinal flora.

Action

Vitamin B12 is essential for synthesis of DNA in RBCs. Its deficiency leads to failure in maturation of the cell and reduction in the cell division. Also, the cells are larger with fragile and weak cell membrane resulting in macrocytic anemia.

Deficiency of vitamin B12 causes **pernicious anemia**. So, vitamin B12 is called antipernicious factor.

2. Intrinsic Factor of Castle

Intrinsic factor of castle is produced in gastric mucosa by the parietal cells of the gastric glands. It is essential

for the absorption of vitamin B12 from intestine. In the absence of intrinsic factor, vitamin B12 is not absorbed from intestine. This leads to pernicious anemia.

Deficiency of intrinsic factor occurs in:

- i. Severe gastritis
- ii. Ulcer
- iii. Gastrectomy.

Hematinic principle

Hematinic principle is the principle thought to be produced by the action of intrinsic factor on extrinsic factor. It is also called or **antianemia principle**. It is a maturation factor.

3. Folic Acid

Folic acid is also essential for maturation. It is required for the synthesis of DNA. In the absence of folic acid, the synthesis of DNA decreases causing failure of maturation. This leads to anemia in which the cells are larger and appear in megaloblastic (proerythroblastic) stage. And, anemia due to folic acid deficiency is called **megaloblastic anemia**.

■ FACTORS NECESSARY FOR HEMOGLOBIN FORMATION

Various materials are essential for the formation of hemoglobin in the RBCs. Deficiency of these substances decreases the production of hemoglobin leading to anemia.

Such factors are:

1. *First class proteins and amino acids*: Proteins of high biological value are essential for the formation of hemoglobin. Amino acids derived from these proteins are required for the synthesis of protein part of hemoglobin, i.e. the globin.
2. *Iron*: Necessary for the formation of heme part of the hemoglobin.
3. *Copper*: Necessary for the absorption of iron from the gastrointestinal tract.
4. *Cobalt and nickel*: These metals are essential for the utilization of iron during hemoglobin formation.
5. *Vitamins*: Vitamin C, riboflavin, nicotinic acid and pyridoxine are also essential for the formation of hemoglobin.

Reference:

Essentials of Medical Physiology Sixth
Edition K Sembulingam PhD and Prema
Sembulingam PhD