



HEMATOLOGY

& LYMPH SYSTEM

Histology

Handout

Number

2

Doctor

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Hematopoietic stem cell transplantation

This is a therapeutic procedure in which normal hematopoietic stem cells (CD34+) from an appropriate donor are transferred to the patient having defective or diseased marrow to re-constitute normal hematopoiesis.

Types of hematopoietic stem cell transplantation (HSCT)

1. Allogenic HSCT

Hematopoietic stem cells are obtained from a donor following HLA (human leukocyte antigen) typing.

2. Autologous HSCT

Stem cells previously collected from the recipient are infused back to the same patient.

Sources of hematopoietic stem cells

1. Peripheral blood stem cells mobilization and harvesting

Although HSCs are known to circulate in peripheral blood, their very small number preclude their use for transplantation. Administration of recombinant hematopoietic growth factor (mainly G-CSF) mobilises HSCs from bone marrow and enhances their subsequent yield from peripheral blood. The yield can be evaluated by mononuclear cell count and CD34+ cells count.

2. Bone marrow harvesting

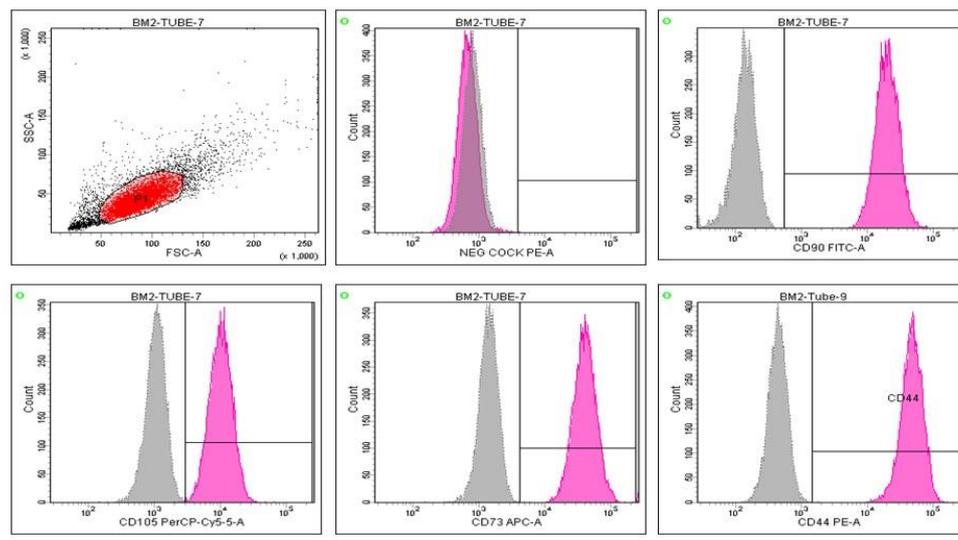
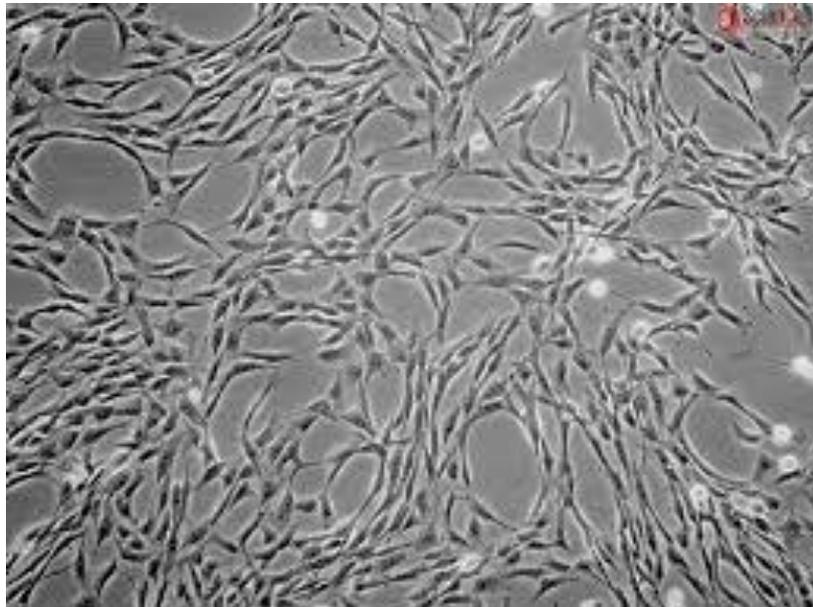
Up to 100 ml of bone marrow can be aspirated from the donor from several sites in the iliac crest. Aspirated marrow is harvested in a bag (or tubes) containing acid citrate dextrose solution.

3. Umbilical cord blood

Umbilical cord blood is an important and readily available source of hematopoietic stem cells (Stem cell banks). However the number of stem cells in the cord blood is limited, and therefore, most of the successful cord blood transplantations have been done in small children.

Normal Bone Marrow Histology (In lab, refer to book chapter) Mesenchymal Stem (Stromal) Cells (MSCs)

Mesenchymal stromal cells are plastic adherent multipotent stem cells that express CD105, CD90, and CD73 but that lack the expression of pan-leukocyte (CD45), endothelial, or primitive hematopoietic (CD34), monocytic (CD14 or CD11b), or B cell (CD79a or CD19) markers, as well as lack the expression of HLA class II (HLA-DR) surface antigen. These cells exhibit a fibroblast-like (spindle) shaped morphology and tri-lineage differentiation into osteoblasts, adipocytes, and chondroblasts. They were first isolated from bone marrow, but have been also identified from multiple sources such as adipose tissue, dental tissues, cord tissue, and others.

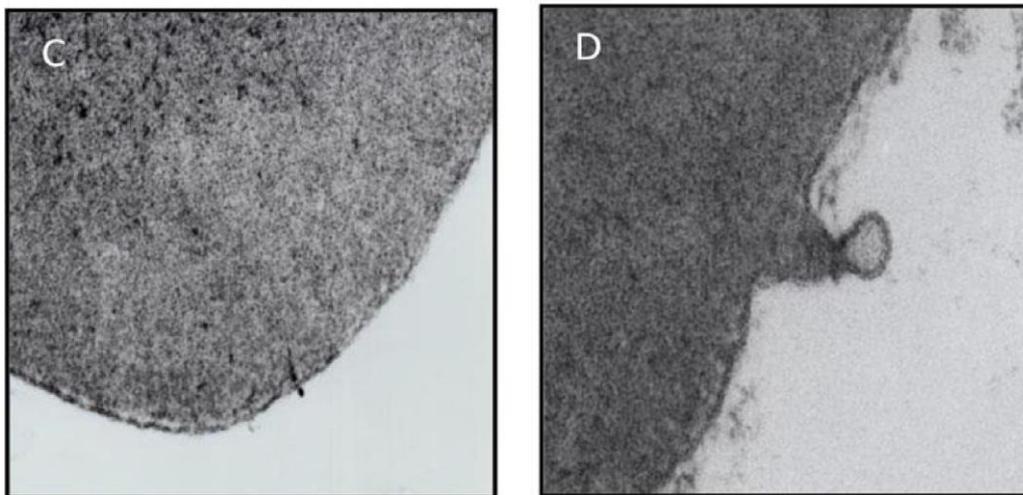


Erythrocytes (Red Blood Cells)

The erythrocytes (which are normally present in the millions) have the important function of transport O₂ and CO₂ throughout the body. The vehicle for this transportation is a protein-iron pigment called **hemoglobin**.

Traditionally, an erythrocyte was viewed as "an outer plasma membrane enclosing hemoglobin and a limited number of enzymes necessary for maintenance of plasma membrane integrity and gas transport function".

However, it has been recently shown that during their lifetime, like all other cell types, red blood cells (RBCs) release both exosomes and plasma membrane derived EVs (ectosomes). RBC exosomes are formed only during the development of RBCs in bone marrow, and are released following the fusion of microvesicular bodies (MVB) with the plasma membrane. On the other hand, RBC EVs are generated during normal aging of RBCs in circulation by budding of the plasma membrane due to complement-mediated calcium influx, followed by vesicle shedding. Interestingly, researchers demonstrated that exosomes from red blood cell units bind to monocytes and induce proinflammatory cytokines, boosting T-cell responses in vitro. (Article).

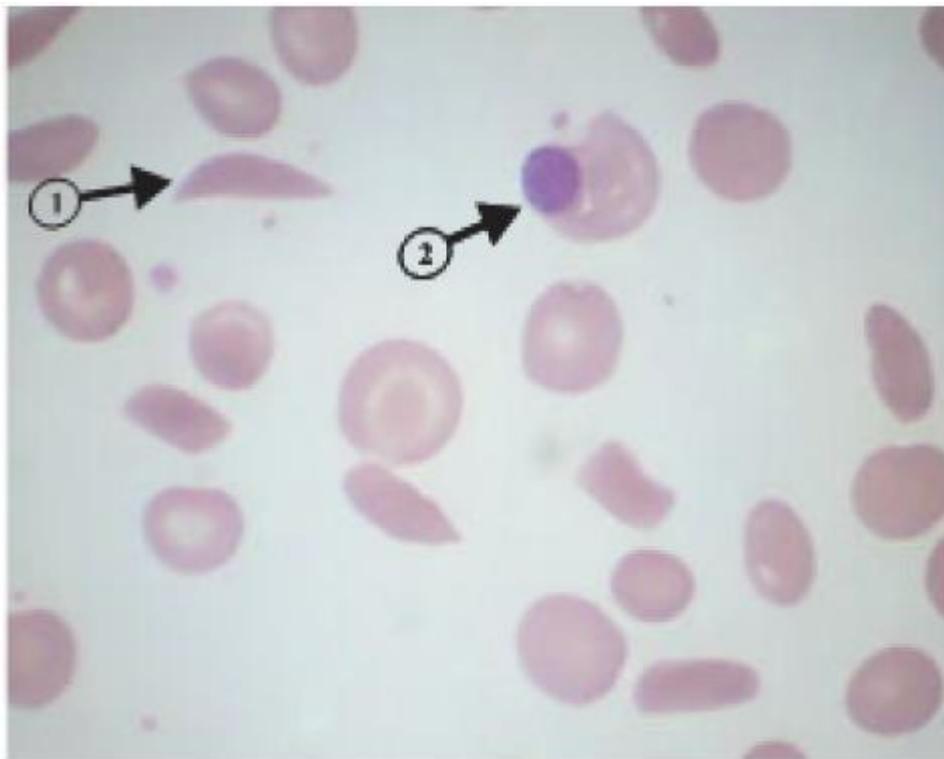


Transmission electron microscopy (TEM) of RBCs showing regular membrane (C) and ectosome release (D)

RBCs normally have a biconcave, disc-like shape, which means their centers are thinner than their edges. Red blood cells are the only human cells without nuclei. Typically, an erythrocyte's diameter is 7-8 μm . The biconcave shape provides around 30% more surface area, compared to spherical form. The larger surface area greatly enhances gas exchange. Additionally, this shape allows

RBCs to "squeeze" themselves through the smallest capillaries (around 3 μm in diameter).

Abnormal RBCs can be named by their morphology. Those that are shaped differently often have difficulty in carrying out their function. For example, sickle cell anemia is a hereditary condition characterized by erythrocytes (RBCs) that are abnormally shaped. They resemble a crescent or sickle. An abnormal hemoglobin found inside these erythrocytes causes sickle-cell anemia in a number of Africans and African-Americans. (Reflection: Is sickle cell always bad?)



1 = Irreversibly sickled red cell; 2 = Nucleated red cell (orthochromic erythroblast)

Another example is spherocytosis, as RBCs assume the shape of spherocytes.

The primary lesion in spherocytosis is loss of membrane surface area, leading to reduced deformability due to defects in certain membrane proteins. Abnormal spherocytes are trapped and destroyed in the spleen and this is the main cause of haemolysis in this disorder.

Erythrocyte Membrane Structure

The erythrocyte membrane consists of two domains, a lipid bilayer and the cytoskeleton. The lipid domain is similar structurally to that found in most mammalian cells. The cytoskeleton differs from what is considered cytoskeleton

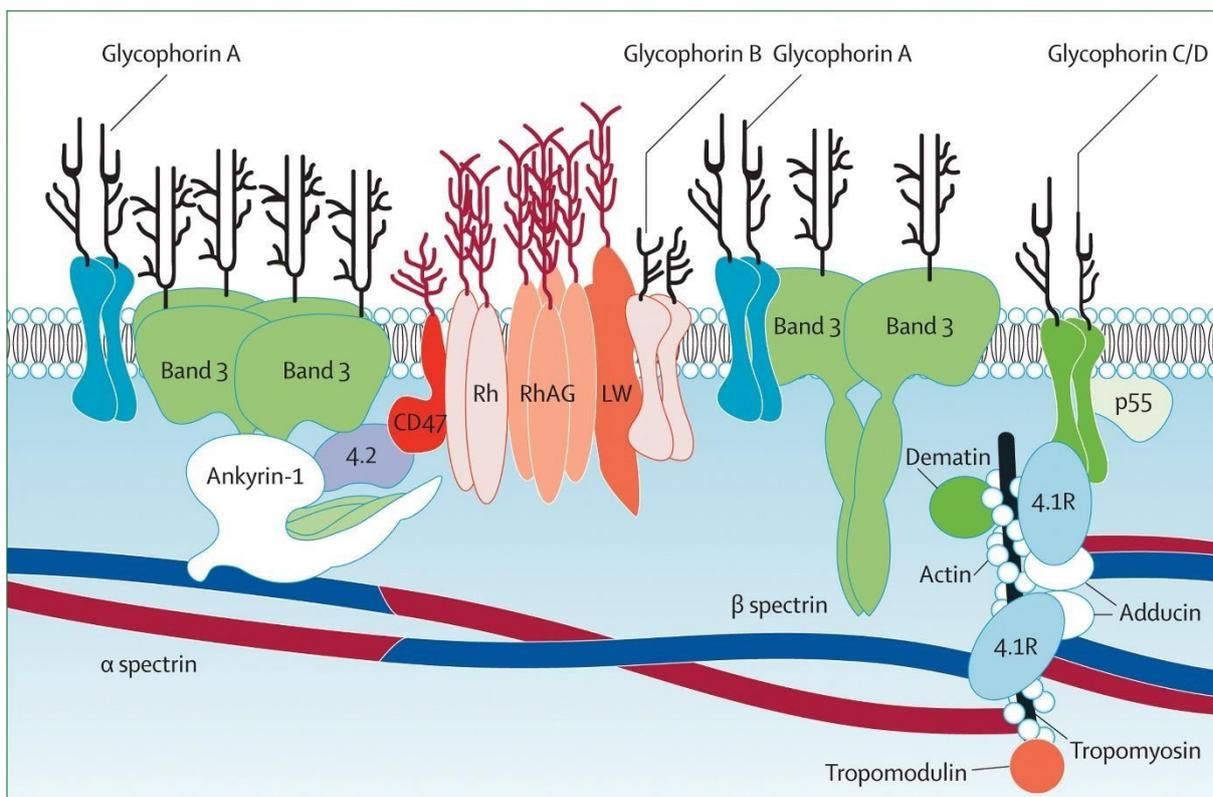
in other cells because it does not contain the structural protein tubulin and is not involved in cell motility or phagocytosis.

Lipid Domain

The lipid domain is composed of nearly equal parts of lipid and protein. The main lipids are cholesterol and phospholipids. Lipid domain fluidity is determined by the molar ratio of cholesterol to phospholipid, degree of unsaturation of phospholipid acyl chains, and phosphatidylcholine to sphingomyelin ratio. Phosphatidylcholine forms highly fluid lipid regions, while sphingomyelin induces rigidity.

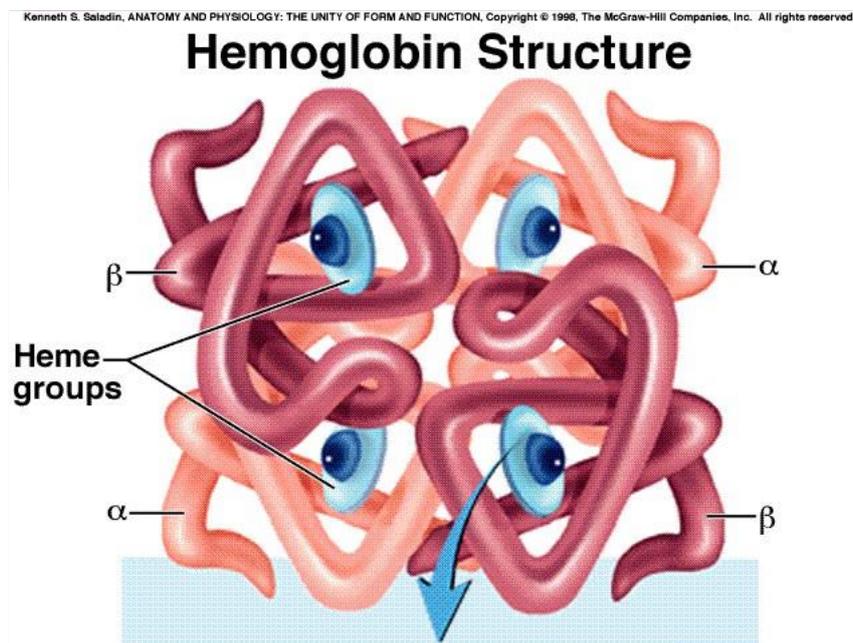
Cytoskeleton

The erythrocyte cytoskeleton consists of several proteins that form a filamentous network under the lipid bilayer. The network is composed of spectrin, ankyrin, actin, and protein 4.1. Cytoskeletal proteins interact with integral proteins and lipids of the bilayer to maintain membrane integrity. The cytoskeleton has an important role in erythrocyte shape, flexibility, and lipid organization.



Hemoglobin

Hemoglobin consists of the protein *globin* and *heme* pigment. Globin consists of two α and two β subunits. Each subunit binds to a heme group. Each heme group bears an atom of iron, which binds reversibly with one molecule of oxygen. So, each hemoglobin molecule carries four molecules of oxygen. On the other hand, carbon monoxide competes with oxygen for heme binding with a much higher affinity. (Smoking and Hct)



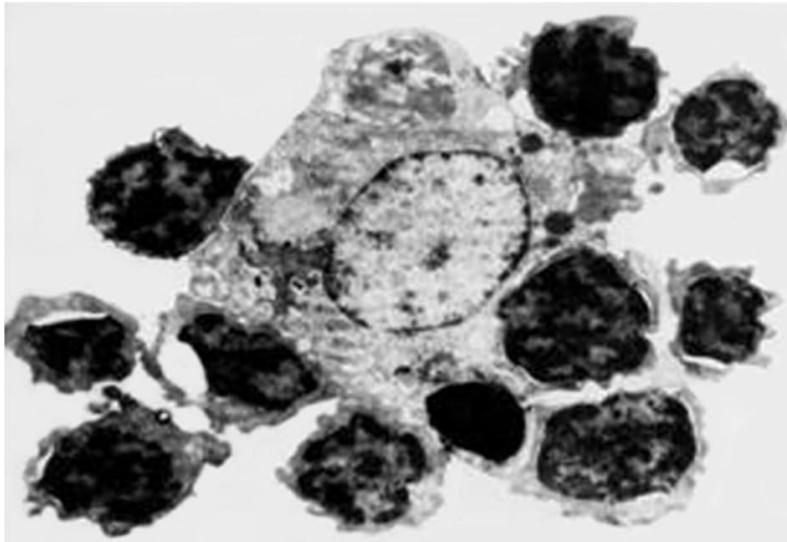
Erythropoiesis

It is the pathway through which an erythrocyte matures from a hemocytoblast (hematopoietic stem cell) into a full-blown erythrocyte. The rate of production is very rapid (estimated at several million new RBCs per second) and a major regulating factor is oxygen. If the body is in a state of hypoxia or lack of oxygen, the kidneys produce a hormone called erythropoietin, which stimulates the red bone marrow to increase the rate of RBC production. This will occur following hemorrhage, or if a person stays for a time at a higher altitude. As a result of the action of erythropoietin, more RBCs will be available to carry oxygen and correct the hypoxic state.

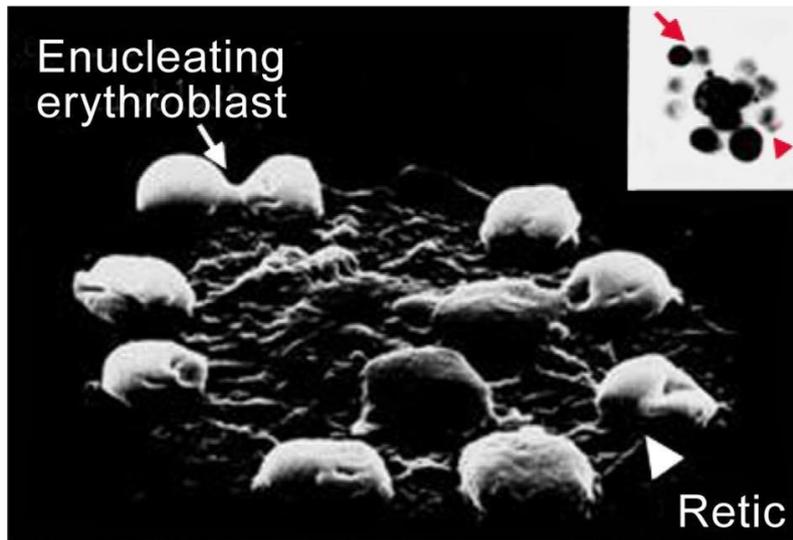
Erythrocyte differentiation takes place in several stages over a period of 1 week. They all take place within the bone marrow (in the adult). Within the bone marrow, erythroid progenitors are found in the form of "islands", called erythroid colonies. An **erythroid colony** is composed of erythroblasts surrounding a central macrophage. The more immature precursors are present close to the macrophage and maturing forms are towards the periphery. The macrophage has dendritic processes which extend between erythroid progenitors, support them, and supply iron for hemoglobin synthesis.

The figure below shows Micrographs of erythroblastic islands. (A) Transmission electron micrograph of an erythroblastic island isolated from bone marrow. Note the extensive cell-cell contact. (B) Scanning electron micrograph of an isolated erythroblastic island. The inset shows an optical microscopic image of the same structure. Note the presence of an enucleating erythroblast (→) and a multilobulated reticulocyte (▶). (C) Confocal immunofluorescence image of an island reconstituted from freshly harvested mouse bone marrow cells stained with erythroid-specific marker (red), macrophage marker (green) and DNAProbe (blue). Central macrophage is indicated by an arrow and a multilobulated reticulocyte by an arrowhead.

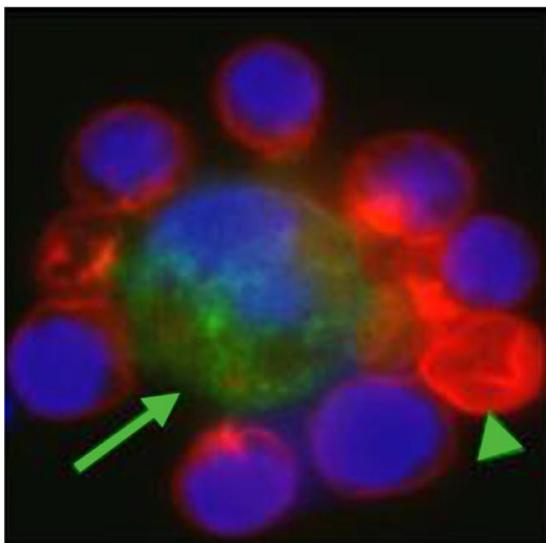
A



B



C



With each stage of erythrocyte differentiation, cell size and nuclear size become smaller and chromatin clumping increases. Color of cytoplasm changes from basophilic to orange-red due to increased accumulation of hemoglobin.

The earliest morphologically identifiable erythroid cell in the bone marrow is the **proerythroblast** (pronormoblast), a large (15-20 μm) cell with a fine, uniform chromatin pattern, one or more nucleoli, and dark blue cytoplasm. The next cell in the maturation process is the **basophilic (early) normoblast**. This cell is smaller in size (12-16 μm) and has a coarser nuclear chromatin with barely visible nucleoli. The cytoplasm is deeply basophilic.

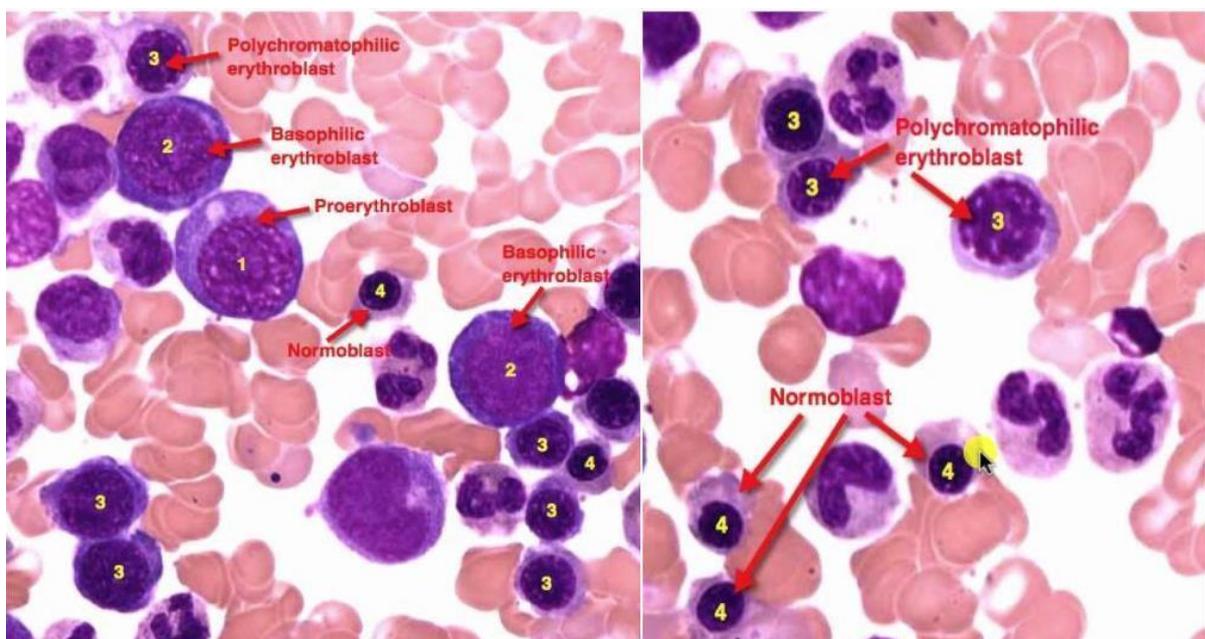
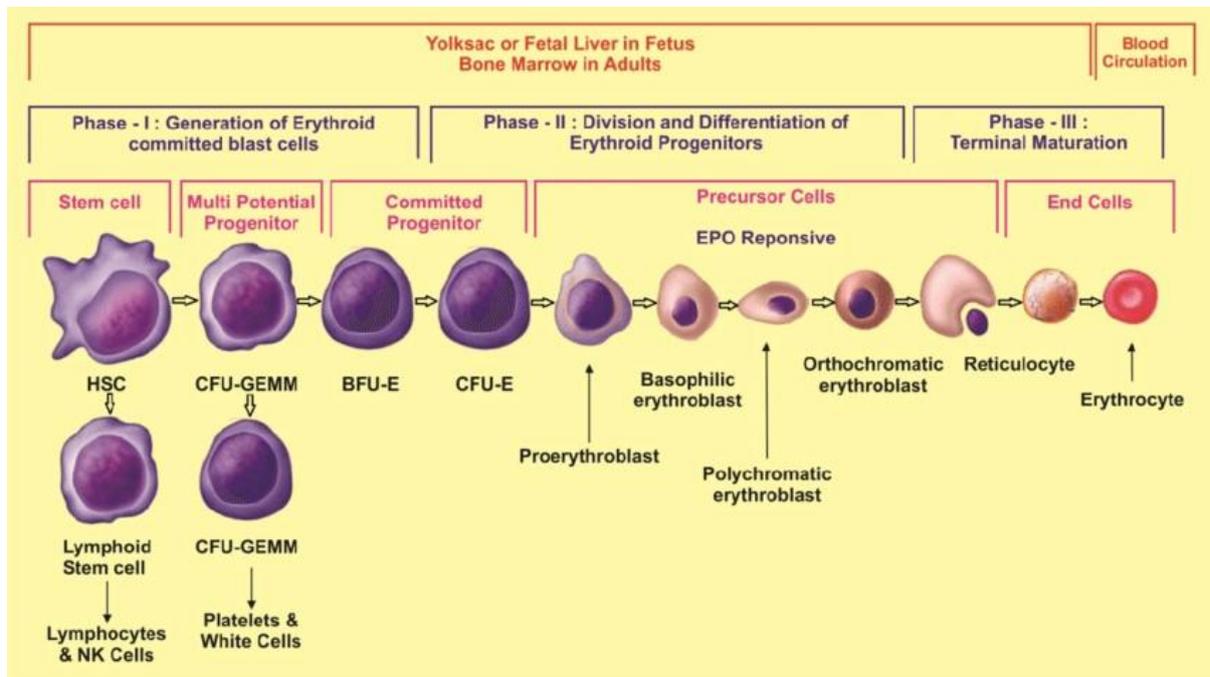
The more differentiated erythroid cell is the **polychromatic (intermediate) normoblast** (size 12-15 μm). The nuclear size is smaller and the chromatin becomes clumped. Polychromasia of cytoplasm results from admixture of blue RNA and pink hemoglobin. This is the last erythroid precursor capable of mitotic division. The **orthochromatic (late) normoblast** is 8 to 12 μm in size. The nucleus is small, dense and pyknotic and commonly eccentrically-located. The cytoplasm stains mostly pink due to hemoglobinisation. It is called orthochromatic because cytoplasmic staining is largely similar to that of erythrocytes.

When the late normoblast ejects its nucleus, it becomes a **reticulocyte**, so called because of a reticular (mesh-like) network of ribosomal RNA visible under a microscope with certain stains such as new methylene blue and Romanowsky stains. The reticulocyte is released into the blood stream, where it then matures 1-2 days later into an erythrocyte. Reticulocytes make up 1% of RBCs in peripheral blood. Large numbers of reticulocytes or normoblasts in the circulating blood mean that the number of mature RBCs is not sufficient to carry the oxygen needed by the body. Such situations include hemorrhage, or when mature RBCs have been destroyed, as in Rh disease of the newborn, and malaria.

In summary, erythropoiesis stages are as follows:

1. Hemocytoblast, or hematopoietic stem cell, which is a multipotent stem cell.
2. Common myeloid progenitor, a multipotent stem cell.
3. Megakaryocyte and erythroid precursor, a committed precursor cell
4. Erythroid progenitor cell, a unipotent stem cell or lineage committed cell
5. Pronormoblast, or proerythroblast

6. Basophilic or early normoblast, also called an erythroblast.
7. Polychromatophilic or intermediate normoblast
8. Orthochromatic or late normoblast
9. Reticulocyte



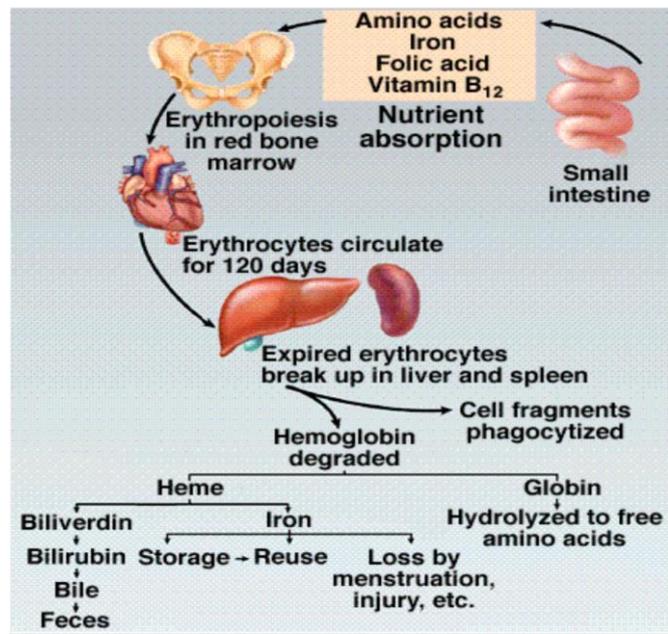
The maturation of red blood cells requires many nutrients. Protein and iron are necessary for the synthesis of hemoglobin and become part of hemoglobin molecules. The vitamins folic acid and B12 are required for DNA synthesis in

the stem cells of the red bone marrow. As these cells undergo mitosis they must continually produce new sets of chromosomes. Vitamin B12 is also called the **extrinsic factor** because its source is external, our food. Parietal cells of the stomach lining produce the **intrinsic factor**, a chemical that combines with the vitamin B12 in food to prevent its digestion and promote its absorption in the small intestines. A deficiency of either vitamin B12 or in the intrinsic factor results in a type of anemia called **pernicious anemia**.

Life span

Red blood cells live for approximately 120 days. As they reach this age they become fragile and are removed from circulation by cells of the **tissue macrophage system** (formerly called the reticuloendothelial or RE system). The organs that contain macrophages are the liver, spleen, and red bone marrow. The old RBCs are phagocytized and digested by macrophages, and the iron they contained is put into the blood to be returned to the red bone marrow to be used for the synthesis of new hemoglobin. If not needed immediately for this purpose, excess iron is stored in the liver. The iron of RBCs is actually recycled over and over again.

Another part of the hemoglobin molecule is the heme portion, which cannot be recycled and is a waste product. The heme is converted to **bilirubin** by macrophages. The liver removes bilirubin from circulation and excretes it into bile; bilirubin is called a bile pigment. Bile is secreted by the liver into the duodenum and passes through the small intestine and colon, so bilirubin is eliminated in feces, and gives feces their characteristic brown color. If bilirubin is not excreted properly, perhaps because of liver disease such as hepatitis, it remains in the blood. This may cause **jaundice**, a condition in which the whites of the eyes appear yellow. This yellow color may also be seen in the skin of light-skinned people.

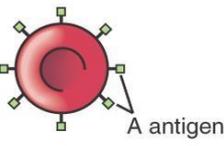
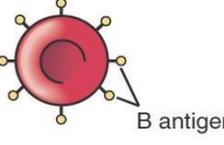
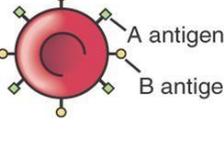


Blood Groups

Human blood is divided into four major different types: **A**, **B**, **Ab**, and **O**. The differences are due to antigens present on the surface of the blood cells.

Antigens are substances that produce an immune reaction by their nature of being perceived as foreign to the body. In response, the body produces substances called **antibodies** that nullify or neutralize the antigens. In blood, these antigens are called **agglutinogens** because their presence can cause the blood to clot.

The antibody is termed an **agglutinin**. For example, type A blood has A antigen, type B has B antigen, type AB has both A and B antigens, and type O has neither A nor B antigens. Following the logic of each of these antigen-antibody reactions, an individual with type AB blood is a **universal recipient**, and an individual with type O blood is a **universal donor**.

Blood Type	Antigen (RBC membrane)	Antibody (plasma)	Can receive blood from	Can donate blood to
A (40%)	 A antigen	Anti-B antibodies 	A, O	A, AB
B (10%)	 B antigen	Anti-A antibodies 	B, O	B, AB
AB (4%)	 A antigen B antigen	No antibodies	A, B, AB, O	AB
O (46%)	 No antigen	Both Anti-A and Anti-B antibodies 	O	O, A, B, AB

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The **Rh factor** is another antigen (often called D) that may be present on RBCs. People whose RBCs have the Rh antigen are Rh positive, those without the antigen are Rh negative. Rh negative people do not have natural antibodies to the Rh antigen, and for them this antigen is foreign. If an Rh negative person receives Rh positive blood by mistake, antibodies will be formed just as they would be to bacteria or viruses.

The Rh factor is important in pregnancy because a mismatch between the fetus and the mother can cause erythroblastosis fetalis, or hemolytic disease of the newborn. In this disorder, a mother with a negative Rh factor will develop antibodies to an RH + fetus during the first pregnancy. If another pregnancy occurs with an Rh + fetus, the antibodies will destroy the fetal blood cells.

Recent Advances

1. Bacterial glycosidases for the production of universal red blood cells
2. Generation of red blood cells from human embryonic/induced pluripotent stem cells for blood transfusion