

Module 7 Introduction to Hematology

The present module details the components of blood plasma cell (RBC, WBC, and platelets). We will see how these cells are different from other cells which we have studied till now. Further it also describes Haemopoiesis, erythropoiesis and leucopoiesis.

Lecture 1 Components of blood plasma cell

The blood plasma is mainly composed of red blood corpuscles (RBC), white blood corpuscles (WBC) and platelets. These cell types are different from most of the cells you have come across.

RBC

The first person to describe red blood cells or erythrocytes was a Dutch biologist Jan Swammerdam, in 1658. Anton van Leeuwenhoek provided further descriptions of the RBCs in 1674. Red blood cells are the most common type of blood cell (about 4-6 millions/mm³). Its role is to deliver oxygen to the body tissues. A typical human mature erythrocyte is 6–8 µm in diameter and 2 µm thick. The RBCs mature in the bone marrow. In humans, mature red blood cells are flexible biconcave disks that lack a cell nucleus and most organelles as mitochondria, Golgi apparatus and endoplasmic reticulum. The shape of the red blood cells become sickle shaped in the disease called sickle-cell anaemia. The mean life of erythrocytes is about 120 days. When they come to the terminal end of their life, they are retained by the spleen where they are phagocytosed by macrophages. They take up oxygen in the lungs or gills and release it while squeezing through the body's capillaries. This role is accomplished by haemoglobin which is an iron-containing biomolecule that can bind oxygen and is responsible for the blood's red color. Haemoglobin structure was discovered in 1959 through X-ray crystallography by Dr. Max Perutz who unraveled the structure of hemoglobin. Hemoglobin in the erythrocytes also carries carbon dioxide back from the tissues which is transported back to the pulmonary capillaries of the lungs as bicarbonate dissolved in the blood plasma. Other than hemoglobin, the cell membrane of red blood cells play many roles that regulate surface deformability, flexibility, adhesion to other cells and immune recognition. The red blood cell membrane is composed of 3 layers which are the glycocalyx on the exterior (rich in carbohydrates) and the lipid bilayer which comprising of transmembrane proteins.

WBC

White blood cells (WBCs) or Leukocytes confer immunity to organisms. The density of the leukocytes in the blood has been reported to be 5000-7000 /mm³. They are of two types namely granulocytes (cytoplasm having granules) and agranulocytes (cytoplasm lacking granules). Further, granulocytes can be distinguished into neutrophil, eosinophil and basophil. The granules in the granulocyte lineage have different affinity towards neutral, acid or basic stains giving the cytoplasm different colors. The agranulocytes are lymphocytes and monocytes. The proportion of each type of leucocyte along with their primary function in blood is listed in Table 1.

Table 1: Proportions of leukocyte present in the blood

Leukocyte type	Percentage proportion %	Function
Granulocyte		
Neutrophil	50 - 70	Phagocytosing bacteria
Eosinophil	2 - 4	Attack parasites and phagocytose antigen-antibody complexes.
Basophil	0.5 - 1	Secrete anti-coagulant and vasodilatory substances as histamines and serotonin
Agranulocyte		
Lymphocyte	20 - 40	Main constituent of the immune system
Monocyte	3 - 8	Replenish resident macrophages and dendritic cells

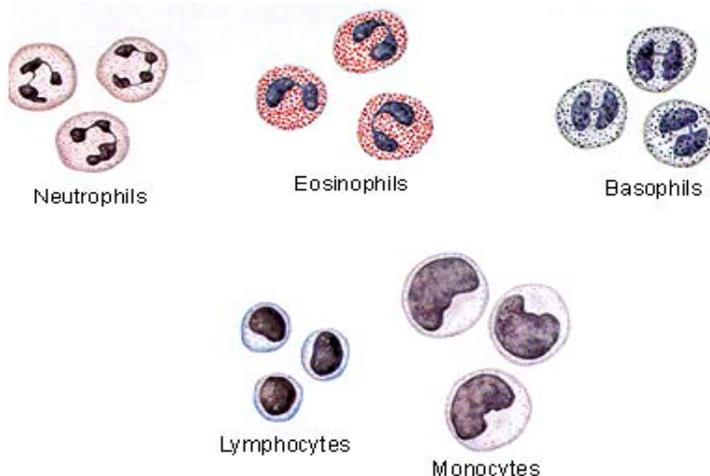


Figure 1: Different types of Leucocytes

Neutrophils: The neutrophils have a diameter of 12-15 μm . Their nucleus is divided into 2 - 5 lobes connected by a fine nuclear strand or filament. The cytoplasm is transparent due to the presence of small granules. Interestingly in the nucleus of the neutrophil from females, we can observe a Barr body which is the inactivated X chromosome (Figure 1).

Eosinophils: The eosinophils are quite rare in the blood. They have the same size as the neutrophils and generally have bilobed nucleus. The cytoplasm is granular which stains pink to orange with acidic dyes (Figure 1).

Basophils: Basophils are the rarest leukocytes having a diameter of 9-10 μm . Their cytoplasm is granular which stains dark purple with basic dyes. Their nucleus is bi or tri-lobed.

Lymphocytes: Lymphocytes are 8-10 μm in diameter and generally they are smaller than the other leukocytes (Figure 1). The cytoplasm is transparent. The nucleus is round and large occupying most of the cellular space. With respect to the amount of cytoplasm, lymphocytes are divided into small, medium and large. The lymphocytes are the main constituents of the immune system which is a defense against the attack of pathogenic micro-organisms such as viruses, bacteria, fungi and protista. Lymphocytes yield antibodies and arrange them on their membrane. An antibody is a molecule able to bind itself to molecules of a complementary shape called antigens, and recognize them. Lymphocytes can be further divided into B and T cells and the natural killer cells. The natural killer cells are characterized by their cytotoxic activity. They kill viruses, bacteria, infected and neoplastic cells and also regulate the production of other hematic cells such as erythrocytes and granulocytes.

Monocytes: Monocytes are the largest leukocytes having the diameter of 16-20 μm (Figure 1). They have a horseshoe-shaped nucleus with a transparent cytoplasm. Most monocytes are the precursors of macrophages which are larger blood cells. In the presence of an inflammation site, monocytes quickly migrate from the blood vessel and start an intense phagocytory activity. They produce substances which have defensive functions such as lysozyme, interferons and other substances which modulate the functionality of other cells. Macrophages cooperate in the immune defense. They expose molecules of digested bodies on the membrane and present them to more specialized cells, such as B and T lymphocytes.

Platelets

Platelets or thrombocytes are only about 20% of the diameter of red blood cells and are the most numerous cell of the blood. Their diameter is about 2-3 μm ; hence they are much smaller than erythrocytes. Their density in the blood is 200000-300000 / mm^3 and a normal platelet count in a healthy individual is between 150,000 and 450,000 per μl (microlitre) of blood $(150-450)\times 10^9/\text{L}$. Platelets are not only the smallest blood cell, they are also the lightest. Therefore they are pushed out from the center of flowing blood to the wall of the blood vessel. There they roll along the surface of the vessel wall, which is lined by cells called endothelium. The endothelium is a very special surface, like Teflon, that prevents anything from sticking to it. However when there is an injury or cut, and the endothelial layer is broken, the tough fibers that surround a blood vessel are exposed to the liquid flowing blood. It is the platelets that react first to injury. The tough fibers surrounding the vessel wall, like an envelope, attract platelets like a magnet, stimulate the shape change that is shown in the pictures above, and platelets then clump onto these fibers, providing the initial seal to prevent bleeding, the leak of red blood cells and plasma through the vessel injury. Their function is to stop the loss of blood from wounds (hematostasis). They do so by releasing factors like serotonin which reduce the diameter of lesioned vessels and slow down the blood flux, the fibrin which trap cells and forms the clotting. Even if platelets appear roundish in shape, they are not real cells and with Giemsa stain they have an intense purple color. Platelets are produced in the bone marrow from cells known as megakaryocytes by the process of fragmentation which results in the release of over 1,000 platelets per megakaryocyte. The dominant hormone controlling megakaryocyte development is thrombopoietin.

Functions of Platelet:

When there is a wound, platelets aggregate using the protein fibrinogen and von Willebrand factor (vWF) as a connecting agent. The most abundant platelet aggregation receptor is glycoprotein IIb/IIIa which is a calcium-dependent receptor for fibrinogen, fibronectin, vitronectin, thrombospondin vWF. Activated platelets adhere with the help of glycoprotein Ia, to the collagen that is exposed by endothelial damage. Aggregation and adhesion act together to form the platelet plug. Platelet aggregation is stimulated by ADP, thromboxane, and α_2 receptor-activation, but inhibited by other inflammatory products

like PGI₂ and PGD₂. Other role of platelets includes modulation of inflammatory processes by interacting with leukocytes and by secreting cytokines, chemokines, and other inflammatory mediators. They Platelets also secrete platelet-derived growth factor (PDGF). Diseases like thrombocytopenia and thrombocytosis may present with coagulation problems due to low platelet counts which increase bleeding risks.

Lecture 2 Haemopoiesis, erythropoiesis and leucopoiesis

Haemopoiesis

Haemopoiesis or haematopoiesis is the process of formation of new blood cellular components. It has been estimated that in an adult human, approximately 10^{11} – 10^{12} new blood cells are produced daily in order to maintain steady state levels in the peripheral circulation. The mother cells from which the progeny daughter blood cells are generated are known as haematopoietic stem cells. In an embryo yolk sac is the main site of haemopoiesis whereas in human the basic sites where haemopoiesis occurs are the bone marrow (femur and tibia in infants; pelvis, cranium, vertebrae, and sternum of adults), liver, spleen and lymph nodes (Table 1). In other vertebrates haemopoiesis occurs in loose stroma of connective tissue of the gut, spleen, kidney or ovaries.

Table 1: Sites of Haemopoiesis in humans

Stage	Sites
Fetus	0–2 months (yolk sac) 2–7 months (liver, spleen) 5–9 months (bone marrow)
Infants	Bone marrow
Adults	Vertebrae, ribs, sternum, skull, sacrum and pelvis, proximal ends of femur

The process of haemopoiesis

Pluripotent stem cells with the capability of self renewal, in the bone marrow known as the haemopoiesis mother cell give rise to the separate blood cell lineages. This haematopoietic stem cell is rare, perhaps 1 in every 20 million nucleated cells in bone marrow. Figure 1 illustrates the bone marrow pluripotent stem cell and the cell lines that arise from it. Cell differentiation occurs from a committed progenitor haematopoietic stem cell and one stem cell is capable of producing about 10^6 mature blood cells after 20 cell divisions. The process leads to division of stem cells and commitment of each cell to differentiate into one of the different blood progenitor cells. The cell lineage chosen by the progenitor cells is a matter both chance and on the external stimuli received by progenitor cells. Internal transcription factors like PU.1 commits cells to the myeloid lineage whereas GATA-1 leads to erythropoietic and megakaryocytic differentiation. The proliferation and differentiation of haematopoietic progenitor cells and the function of mature blood cells is in turn regulated by glycoprotein hormones like Granulocyte colony

stimulating factor or G-CSF. The growth factors may cause cell proliferation but can also stimulate differentiation, maturation, prevent apoptosis and affect the function of mature cells. The other growth factors that act at various levels of haemopoiesis are interleukin (IL-1 and IL-3); macrophage colony-stimulating factor; stem cell factor; and tumour necrosis factor (Table 2).

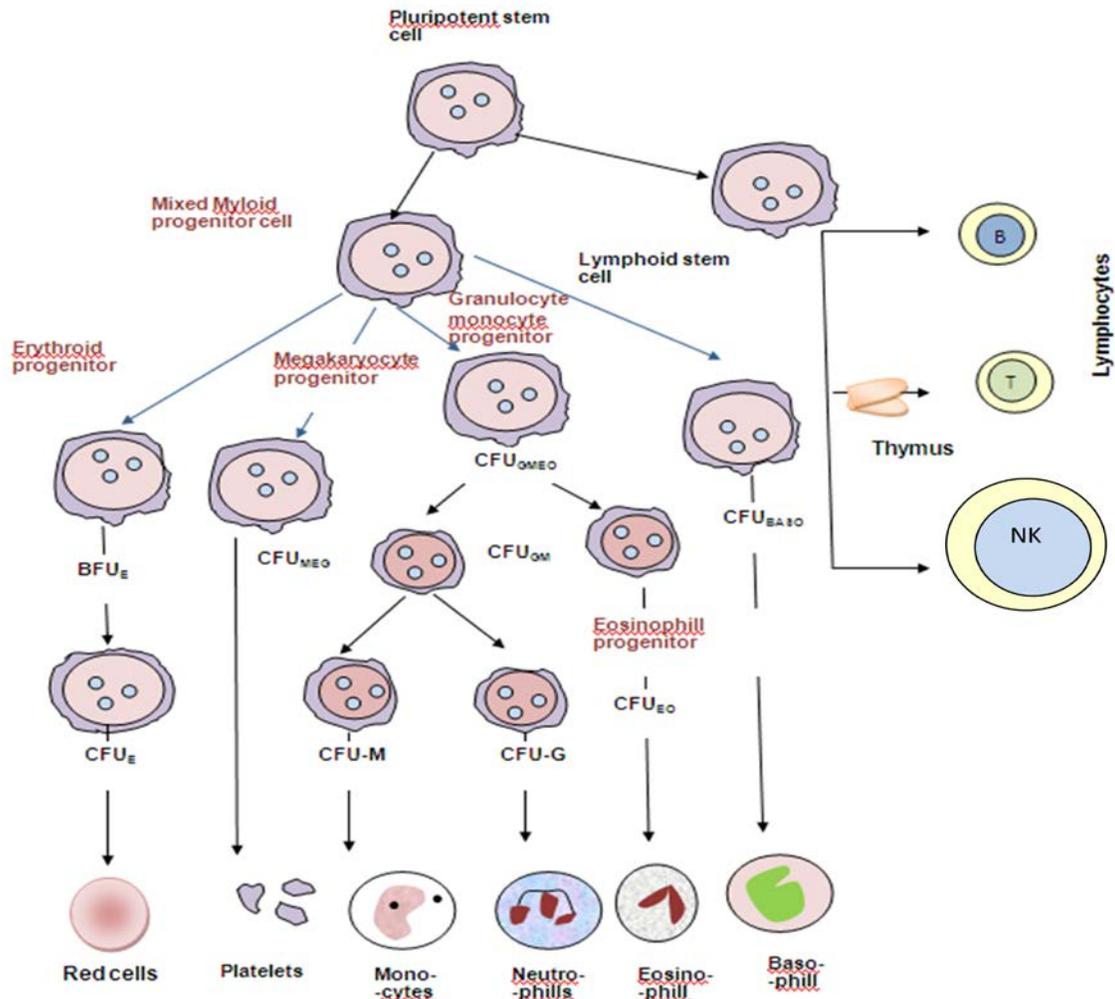


Figure 1: Diagrammatic representation of the bone marrow pluripotent stem cell and the cell lines that arise from it. Various progenitor cells can be identified by culture in semi-solid medium by the type of colony they form. Baso, basophil; BFU, burst-forming unit; CFU, colony-forming unit; E, erythroid; Eo, eosinophil; GM, granulocyte, monocyte; Meg, megakaryocyte; NK, natural killer cell (Hoffbrand et al. 2011).

Table 2 Growth factors in haemopoiesis

Acts On	Growth factor type
stromal cells	IL-1, TNF
pluripotential stem cells	SCF, Flt-L
multipotential progenitor cells	IL-3, GM-CSF, IL-6, G-CSF, Thrombopoietin
committed progenitor cells	G-CSF, M-CSF, IL-5, Thrombopoietin

Legend: Flt-L, Flt ligand; G- and GM-CSF, granulocyte and granulocyte–macrophage colony-stimulating factor; IL, interleukin; M-CSF, macrophage colony-stimulating factor; SCF, stem cell factor; TNF, tumour necrosis factor.

Growth factor receptors and signal transduction

The biological effects of growth factors are mediated through specific receptors on target progenitor cells. Receptors like granulocyte macrophage colony-stimulating factor GMCSF-R are from the haematopoietin receptor superfamily which possesses the capacity to dimerize after binding their ligand. This results in cascade of intracellular signal transduction pathways of which the three major ones are the Janus associated kinase or JAK/STAT, the mitogen activated protein (MAP) kinase and the phosphatidylinositol 3 (PI3) kinase pathways (see Figure 2). The JAK proteins are tyrosine-specific protein kinases that associate with the intracellular domains of the growth factor receptors. A growth factor molecule binds simultaneously to the extracellular domains of two or three receptor molecules, resulting in their aggregation. JAKs then phosphorylate members of the signal STAT family of transcription factors resulting in their dimerization and translocation from the cell cytoplasm across the nuclear membrane to the cell nucleus where specific genes are transcribed. JAK also activates the MAPK pathway which is in turn controlled by Ras. Different domains of the intracellular receptor protein may signal for the different processes (e.g. proliferation or suppression of apoptosis) mediated by growth factors. Other growth factors like SCF, Flt-3L and macrophage colony-stimulating factor (M-CSF) bind to receptors that have an extracellular immunoglobulin-like domain linked. Growth factor binding results in dimerization of these receptors and consequent activation of the tyrosine kinase domain. Phosphorylation of tyrosine residues in the receptor itself generates binding sites for signalling proteins which initiate complex cascades of biochemical events resulting in changes in gene expression, cell proliferation and prevention of apoptosis.

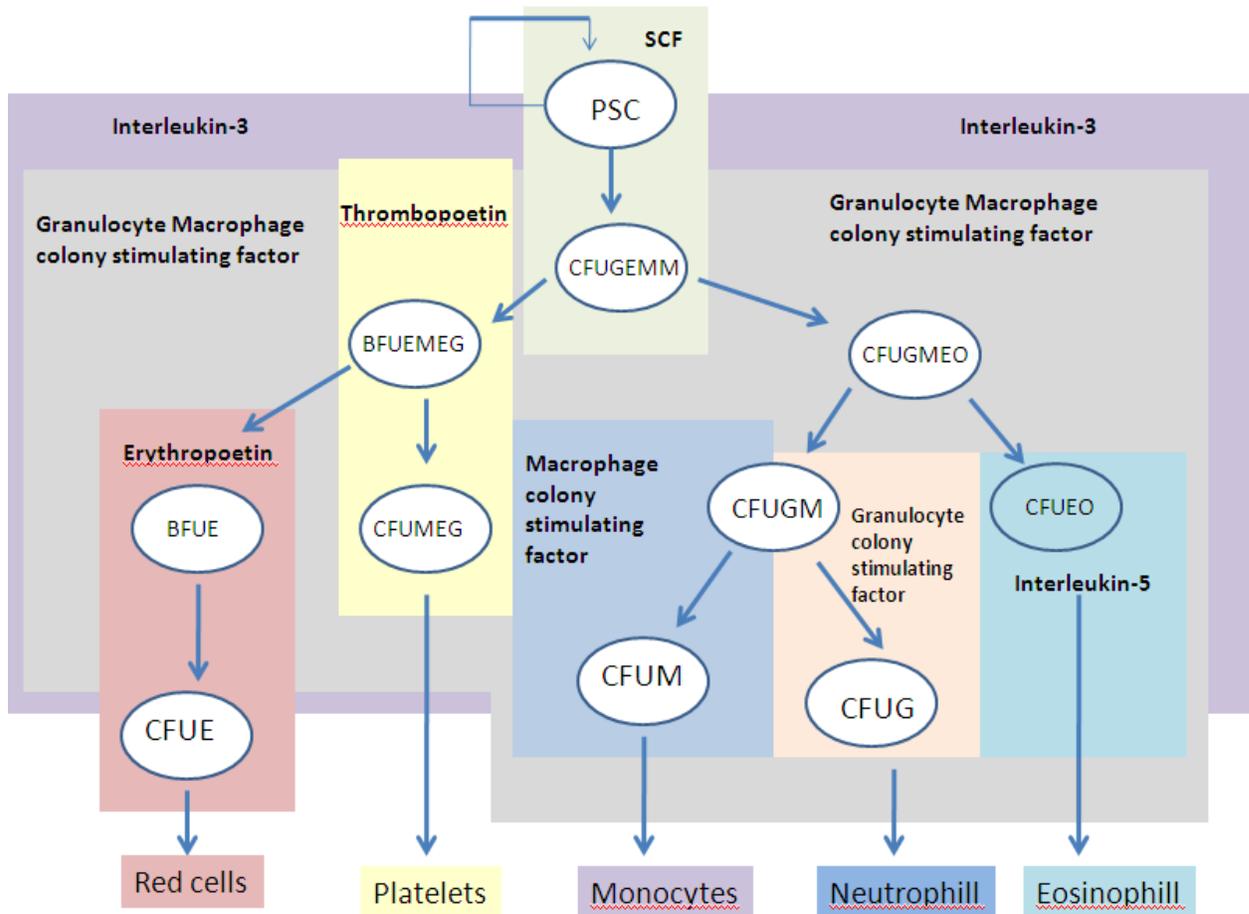


Figure 2: Schematic representation of the role of growth factors in normal haemopoiesis. PSC, pluripotent stem cell; SCF, stem cell factor. For other abbreviations see Figure 1.

Erythropoiesis

Erythropoiesis is the name for the process which leads to the formation of red blood cells (RBCs) or more properly termed as the erythrocytes. The normal life span of RBC's is about 120 days, thus new erythrocytes need to be formed. The overall process occurs in five days and the bone marrow is the site for the production of RBCs. A condition known as hypoxia which is shortage in RBC's oxygen carrying capacity leads to the release of the growth factor erythropoietin. Other growth factors which are released are IL-1, IL-4, IL-6, IL-11, IL-12, and SCF. Furthermore, Insulin, Growth hormone, and steroid hormones are very crucial in RBC production. EPO acts on precursor RBC cells which are Burst Forming Unit-Erythroid (BFUE) and Colony Forming Unit-Erythroid cells (CFUE) leading to their proliferation. The scheme given below summarizes the process of erythropoiesis. During sudden hypoxia due to massive blood loss the entire aforesaid process takes place in three days.

↓ **O₂ tension** → ↑ **EPO** → ↑ **RBC's precursors (BFU-E and CFU-E)** → ↑ **differentiation & proliferation** → ↑ **mature RBC's release in 5 days.**

They are six morphologically identifiable stages in erythroid differentiation which can be visualized under the microscope using Romanowsky (or Geimsa) stained slides. The different stages are namely:

a. **Pronormoblasts:** These cells makes up about 1-2% of all nucleated cells in the bone marrow. The cytoplasm is very basophilic, i.e., has very dark blue color.

b. **Basophilic normoblasts:** These cells constitutes up to 4% of all nucleated cells in the bone marrow. Under the microscope the cytoplasm shows deep blue color.

c. **Polychromatophilic normoblasts:** These cells makes up to 10-20% of all nucleated cells in the bone marrow. The cytoplasm varies in color due to the synthesis of hemoglobin, which leads to a wide range of colors consisting of a mixture of gray, blue, mauve, and/or violet.

d. **Orthochromic normoblasts:** The cytoplasm of these cells has a resultant color of pale grayish-blue-violet due to the presence of hemoglobin

e. **Reticulocytes:** The retics appear slightly larger than normal erythrocytes, with a varying degree of color. The cytoplasm may be irregular and might have inclusions known as “basophilic stippling”, which are the residual RNA remaining in the cells.

f. **The mature erythrocyte (RBC):** The erythrocyte has a diameter of about 7 μ and width of about 2 μ . The cell lacks nucleus, and mitochondria.

Leucopoiesis

Leucopoiesis is the process by which white blood cells or lymphocytes (B-cells and T-cells) are produced and developed from the lymphoid progenitor cells, it is also known as leukocytopoiesis or lymphopoiesis. Lymphocytes are formed in the six constituents of the lymphomyeloid complex (LMC) which are namely the bone marrow, thymus, lymph nodes, subepithelial lymphoid tissue, spleen, connective tissue (including blood). The existence of specific markers on the lymphocyte membrane (CD-antigens) has enabled the differentiation of lymphocytic subpopulations. The largest number of the lymphocytes in the peripheral blood belongs to the subpopulations of the mature T-cells. A considerable smaller number of the lymphocytes belong to mature B-cells. The precursors of T- and B-cells are of the least number. Lymphoblast is the earliest

morphologically recognizable cell of the lymphocytic lineage. During the lymphocytopoiesis, three developing cell forms can be seen. This process mainly comprises the formation of functional antigen receptors of the T-cells in the thymus and the ability to form and secrete immunoglobulins by the B cells in the bone marrow. Leucopoiesis also results in development of natural killer cells (NKC). The process starts with the primitive reticular cell, which on activation develops into cytoplasmic basophilia and finally becomes a lymphoblast. A series of cell divisions (6-8 cell divisions) results in reduction in the amount of cytoplasm leading to the development of small lymphocyte.

B-cell development

B cell development occurs through several stages, each stage representing a change in the genome content at the antibody. When the B cell fails in any step of the maturation process, it will die by a mechanism called apoptosis. B cell leucopoiesis is dependent on the integration of extracellular stimuli by transcription factors that specify hematopoietic progenitors to differentiation into highly-specialized effector B-cells. The B cell factor-1 or Ebf1 is expressed in the early stages of the B cell lineage and in the stromal cells of the bone marrow. Ebf1 functions in a complex regulatory network with other transcription factors to establish the B cell program. B cell membrane receptors evolve and change throughout the B cell life span. Examples of such receptors are the TACI, BCMA and BAFF-R which are present on both immature B cells and mature B cells. CD20 is expressed on all stages of B cell development except the first and last; it is present from pre-B cells through memory cells, but not on either pre-pro-B cells or plasma cells. Figure 3 illustrates the stages of B-cell development.

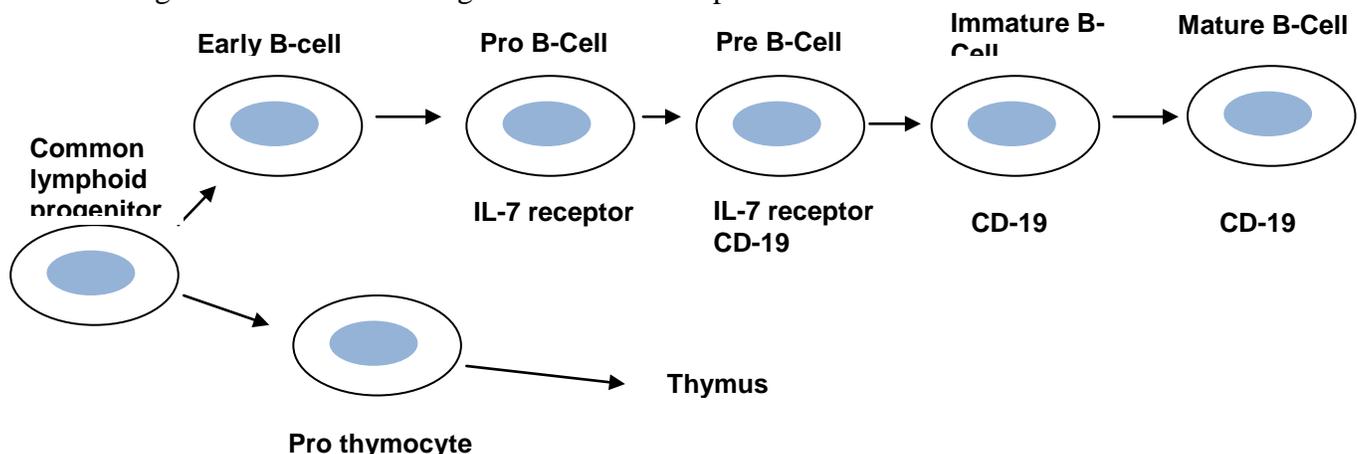


Figure 3: B cell developmental stages.

T-cell leucopoiesis

T cells are formed in bone marrow and then they migrate to the cortex of the thymus to undergo maturation in an antigen-free environment for about one week. About 2-4% of the T cells succeed to mature and the other 96-98% of T cells undergoes apoptosis and is phagocytosed by macrophages in the thymus. This process is termed as thymus education wherein T-cells capable of recognizing self antigens undergo apoptosis.

The mature forms of T-cells are:

1. T-helper: Activates other cells such as B cells and macrophages.
2. T-cytotoxic: Kills virally infected cells.
3. T-memory: Remembers previously encountered antigens.
4. T-suppressor cells: Moderates the immune response of other leukocytes.

Interesting facts:

1. Blood makes up around 7% of the weight of a human body which is about 5 liters.
2. Red blood cells develop in bone marrow and circulate in the body for around 120 days.
3. Mature RBC lacks nucleus, mitochondria, Golgi apparatus and endoplasmic reticulum
4. In some vertebrates, haematopoiesis can occur wherever there is a loose stroma of connective tissue and slow blood supply, such as the gut, spleen, kidney or ovaries.
5. Approximately 10¹³ new myeloid cells (all blood cells excluding lymphocytes) are produced each day.
6. Vitamin B12 (as well as B6 and folic acid) are crucial for the development of RBC's, as they are required for protein synthesis
7. Hemophilia A is the X-linked genetic disease in which the individual does not produce factor VIII and so is more susceptible to severe hemorrhages.
8. In Medicine anticoagulants like heparin are used in surgeries in which tissue injuries made by the surgical act could trigger undesirable systemic blood clotting.
9. Macrophages breakdown Hb to biliverdin and iron.

Questions:

- Q1. What are granulocytes and agranulocytes?
- Q2. Enumerate the structure of lymphocytes?
- Q3. How does platelet aggregation occur?
- Q4. Explain the process of haemopoiesis?
- Q5. What is Erythropoiesis?
- Q6. What are the functions of B cell and T cell?

Further Readings

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