EDO UNIVERSITY IYAMHO
FACULTY OF BASIC MEDICAL SCIENCES
DEPARTMENT OF PHYSIOLOGY

PHS 212 Body Fluid and Blood Physiology

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General overview of lecture: The essence of this course is to develop basic understanding of the functions of blood and body fluid compartments. Knowledge from this course will enable the student develop management technique and understanding of basic clinical skills when dealing with cases related to blood diseases

Prerequisites: Students should be familiar with the definition of body fluids and body fluid compartments. Regulation of body fluid volumes. Physiological variation of body fluid volumes. Techniques for quantifying various body fluid volumes. Blood: Functions of blood and classification of blood cells. Erythropoiesis. Haematological indices. Hemoglobin genotype and Blood groups. Immunology and cell defense. Hemostasis

Learning outcomes: At the completion of this course, students are expected to:

i. know the different body fluids compartments
ii. understand the components/classification of blood and their functions
iii. explain the genesis of haematopoiesis and blood disorders
iv. fully understand haemoglobin and its disorders and the role of iron metabolism
v. explain inflammation and role of leucocytes in disease conditions
vi. understand the concept of Immunity, Hypersensitivity and Blood typing
vii. explain platelets aggregation and coagulation

Assignments: In this course, there will be six (6) individual assignments and three (3) group assignments. Among these assignments will be Term Papers that will include Presentations. Besides this, there will also be written continuous assessment test to be written three weeks to the start of University General Examination.

Grading: Grading will be assigned as follows: five per cent (5%) of total score to individual assignments; five per cent (5%) to Group Assignments; twenty per cent (20%) to Continuous Assessment Test; seventy per cent (70%) will be assigned to the final Examination

Textbook: The recommended textbook for this class are as stated:

Title: *Textbook of Medical Physiology*
Authors: Guyton, A.C. and Hall, J.E.
Publisher: Elsevier Inc. 13TH Edition
ISBN 0-7216-0240-1

Title: *Human Physiology*
Authors: Stuart, I.F.
Publisher: The McGraw-Hill Companies, Inc, 12th Edition
ISBN 978–0–07–337811–4
MHID 0–07–337811–9

Title: *Ganong's Review of Medical Physiology*
Author: Barrett, K. E., Boitano, S., Barman, S. M. and Brooks, H. L.
Publisher: The McGraw-Hill Companies, Inc, 23rd Edition
ISBN: 978-0-07-160568-7
MHID: 0-07-160568-1

Title: *Essentials of Medical Physiology*
Author: Sembulingam, K. and Sembulingam, P.
Publisher: Jaypee Brothers Medical Publishers (P) Ltd
ISBN 978-93-5025-936-8

Main Lecture: Below is a description of the contents. The order as it is been arranged here may be changed so as to accommodate the materials needed for better understanding of the course.

Introduction to Blood Physiology

Blood is a tissue in fluid form. It is regarded as a tissue because it contains different cells floating in a fluid called plasma. The different cells of blood floating in the plasma include erythrocytes also called red blood cells, leucocytes also called white blood cells and thrombocytes also called platelets. The normal total circulating blood volume is about 8% of

the body weight in a normal 70kg man. The volume of blood is slightly higher in males. Blood is slightly alkaline and its pH in normal conditions is 7.4.

Functions of Blood

Blood carries different functions amongst which are outlined as follow below:

a. **Transportation:** Blood helps to transport different substances from site of production to where they are needed. These substances could be nutrients, gases, hormones, waste metabolites etc
    i. Nutrient: Nutrients such as glucose, amino acids and fatty acids are absorbed from the gastrointestinal tract into blood stream, here they are transported to tissues
    ii. Gases: Gases such as oxygen and carbo dioxide and transported in the blood. Oxygen is absorbed from the lungs and transported to tissues via blood while carbon dioxide is transported from tissues to the lungs
    iii. Hormones: Testosterone, insulin and gonadotropins are examples of hormones produced from endocrine gland and transported via blood to target organs
    iv. Waste metabolites: Waste products such as urea, ammonia, creatinine are produced from organs/tissues and released into blood where they are transported to the kidney for elimination.

b. **Thermoregulation**: Blood conducts heat from core body temperature generated from integral body organs like heart, lungs, kidney etc, to peripheral body organs, in this way regulate or distribute heat in the body evenly (i.e. the balance between heat loss and heat gain in the body)

c. **Maintenance of Vascular Integrity:** Special proteins and platelets in blood help to maintain or repair damage blood vessels when ruptured (blood clot). The clotting mechanism protects against blood loss when vessels are damaged
d. **Defense**: The major defense mechanism in the body is in the blood. White blood cells fight against pathogens
e. **Buffer Mechanism**: Blood also regulate the acidity and alkalinity to normal pH of 7.4. Examples of buffer substances in blood include the enzyme carbonic anhydrase, bicarbonate ion, HPO4, and other proteins such as haemoglobin.

PLASMA

Plasma is a straw-colored, slightly yellow, clear liquid part of blood. It contains about 92% of water and the remaining 8% is solids. The solids are the organic and the inorganic substances. Under normal condition is plasma makes up 55% of total blood volume, the other 45% of blood is made of cellular elements called packed cell volume or haematocrit.

The organic solid of plasma include the following:

i. Plasma proteins: Examples of plasma proteins are Albumin, Globulin and Fibrinogen. Fibrinogen is what makes the difference between plasma and serum which is a clear fluid found oozing out after clotting has taken place. **Serum = Plasma – Fibrinogen**
ii. Enzymes: Examples of enzymes include, carbonic anhydrase, alanine aminotransferase,
iii. Hormones: Examples of hormones include estrogen, glucagon, growth hormone etc
iv. Waste Products: Examples include urea, creatinine, uric acid etc
v. Gases: Oxygen, Carbon dioxide, nitrogen are example of gases in plasma
vi. Nutrients: Examples include glucose, amino acids, cholesterol etc

The inorganic solid of plasma are the electrolytes such as sodium ion, potassium ion, magnesium, calcium, chloride ion etc

RED BLOOD CELLS

Red Blood Cell is also known as erythrocytes, red cells, hematids. Its main function is to transport oxygen and carbon dioxide. The cytoplasm of erythrocytes is rich in haemoglobin, an iron containing molecule that binds which oxygen and its responsible for the red coloration of erythrocytes.

Erythrocytes are non-nucleated, flattened, biconcave discs about 7μm in diameter and 2.2μm thick. Their unique shape aids their function of transporting oxygen as it provides an increased surface area through which gas can diffuse. The membrane of erythrocyte is elastic allowing the red blood cell to meander or squeeze through small capillaries. Erythrocytes is higher in males compare to women; in male it is about 4.7 to 5.5 million cells per cubic millimetre while for female it is about 4.5 million to 5 million cells per cubic millimetre.

Lifespan of Red Blood Cells

Erythrocytes have an average lifespan of 90 to 120 days, after which the old RBCs are destroyed by the reticuloendothelial system especially in the spleen. The membrane of the old RBC becomes fragile and no longer flexible, hence they are destroyed while trying to squeeze through small capillaries within the spleen.

Erythropoiesis

Erythropoiesis is simply the process of production of red blood cells. It is the process of generaton, growth, development and maturation of erythrocytes. While erythropoiesis is involve in red blood cell production, Hemopoiesis or hematopoiesis is defined as process for the production of blood cells, i.e. it involves erythropoiesis, leucopoiesis and thrombopoiesis.

A Site of Erythropoiesis

For easy understanding, the site of red blood cell production is divided into two Phases: Fetal and Post Fetal Phase

Fetal Stage

In fetal life stage, the erythropoiesis occurs in three stages:

1. Mesoblastic Stage

During the first few weeks of life, red blood cells which are primitive and nucleated are produced from the mesenchyme of yolk sac.

2. Hepatic Stage

From third month of gestation or mid trimester, liver is the main organ that produces RBCs. Spleen and lymphoid nodes can also produce red blood cell at this stage.

3. Myeloid Stage

Towards the end of pregnancy, the bone marrow of all bones solely produces red blood cells. This continues even after birth.

After Birth

The bone marrow of all bones produces red blood cells until a person is 5 years old. Gradually towards 20 years of age, only bone marrow of long and membraneous bones can produce

red blood cells except for the proximal portions of the humeri and tibiae. Beyond 20 years till old age and death, only bone marrow of membraneous bones such as the vertebrae, sternum, ribs, and ilia produce red blood cells. Even in these bones, the marrow becomes less productive as age increases. The reason for the decrease in bone marrow from all bones to selected bones of the body is because the bone marrow becomes quite fatty and produces no more red blood cells.

B Genesis of Red Blood Cell Production

Stem cells are the primary cells capable of self-renewal and differentiating into specialized cells. Hemopoietic stem cells otherwise Pleuripotent Hematopoietic Stem Cells (PHSC) are the primitive cells in the bone marrow, which give rise to the blood cells. The PHSC originally are uncommitted stem cells, they are regarded as uncommitted because the blood cells they give rise to is unknown, but the stem cell differentiate into committed stem cells.

N/B: 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 differentiate into any of

   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.

Since we are under the subject red blood cells, explanations will dwell on CFU-E, i.e. the committed stem cell for erythropoiesis is CFU-E, for leucopoiesis it is CFU-GM while for thrombopoiesis is CFU-M.

<u>Stages of Erythropoiesis</u>

Colony Forming Unit Erythrocyte (CFU-E) differentiates into different primitive cells which are

i. Proerythroblast
ii. Basophil erythoblast
iii. Polychromatophil erythroblast.
iv. Orthchromatic erythoblast
v. Reticulocyte
vi. Erythrocyte.

As the progenitor cells of RBC undergo maturation, they become smaller in size

1.Proerythroblast

Proerythroblast or megaloblast or rubriblast, is the first cell derived from CFU-E. It is very large in size with a diameter of about 20 μm. Its nucleus has two or more nucleoli and a chromatin network . Proerythroblast does not contain haemoglobin but its synthesis starts at this stage. The proerythroblast undergoes mitosis and differentiates to form basophil erythoblast

2. Basophil erythroblast

Also called early normoblast, it is little smaller than proerythroblast with a diameter of about 15 μm. In the nucleus, the nucleoli disappear while the chromatin network becomes dense. Basophil erythroblast is rich in RNA which reacts with basic dye to give a blue coloration, hence the name basophil erythoblast. This cell undergoes mitosis and differentiates into polychromatophil erythroblast.

3. Polychromatophil Erythroblast

The cell is smaller than the basophil erythroblast with a diameter of 10 to 12 μ. The nucleus is still present. But, the chromatin network shows further condensation. The hemoglobin starts appearing to give a red color. Cytoplasm is still basophilic, meaning it also gives a blue color. Now, because of the two colors (red and blue), this cell is called polychromophil erythroblast (poly = more than one; chromo = color; phil = to like). This cell undergoes mitosis and differentiates into Orthchromatic erythoblast

4. Orthchromatic erythoblast

The diameter of the cell decreases further to about 8 to 10 μ. Nucleus becomes very small with very much condensed chromatin network. The synthesis of haemoglobin continues and so increases number. The RNA is absent, hence the cell is not basic. Therefore

Orthchromatic erythoblast has one color. Again Orthchromatic erythoblast undergoes mitosis and differentiates to become reticulocyte, but first it loses or expels its nucleus. The process by which nucleus disappears is called pyknosis.

5. Reticulocyte

Reticulocyte is slightly larger than matured erythrocytes. The cytoplasm contains the remnants of Golgi apparatus, mitochondria, and a few other cytoplasmic organelles which form reticular network, hence the name.

N/B: The main difference between Orthchromatic erythoblast and reticulocyte is the absence of nucleus

During this reticulocyte stage, the cells pass from the bone marrow into the blood capillaries by diapedesis (squeezing through the pores of the capillary membrane). The reticulocyte slightly less than 1% of total RBCs.

6. Matured Erythrocyte

In the blood stream, the 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 cytoplasm of matured RBC largely contains hemoglobin and there is absence of nucleus. The growth and development of proerythroblast to matured erythrocyte takes an average of seven days. It requires 5 days for the formation of reticulocytes from proerythroblast. Reticulocyte takes 2 more days to become the matured RBC.

<u>Factors That Regulate Erythropoiesis</u>

Several factors are known to regulate (speed or slow down) the rate of production of red blood cells. These factors include

i. Tissue oxygenation
ii. Hematopoietic growth and differentiation inducers
iii. Nutrients
iv. Vitamins.

1 Tissue Oxygenation

Any condition that reduces the rate of oxygen diffusion or transport to tissues inevitably increases the rate of red blood cell production. For example, when a person has abnormal

low red blood cells concentration, the bone marrow begins to produce large quantities of red blood cells. Examples of factors that can impede on oxygen transport to tissues include

i. Low blood volume
ii. Anemia
iii. Low hemoglobin
iv. Poor blood flow
v. Pulmonary disease

At very high altitudes, where the quantity of oxygen in the air is greatly decreased, insufficient oxygen is transported to the tissues and red cell production is greatly increased.

2. Hemopoietic Growth Factors

Hemopoietic growth factors such as growth inducers and differentiation inducers cause the development and differentiation of primitive red blood cells respectively. Their examples are the interleukins and stem cell factor. Generally these factors induce the proliferation of PHSCs. Interleukins involved in erythropoiesis: Interleukin-3 (IL-3), Interleukin-6 (IL-6) and Interleukin-11 (IL-11). These hemopoietic growth factors act directly on the primitive red blood cells.

3. Vitamins

Vitamins are also contribute to the process of erythropoiesis. Two very important Vitamins necessary for maturation of red blood cells include Vitamin B12 (Cyanocobalamin) and Folic Acid. Both of these are essential for the synthesis of DNA because each, in a different way, is required for the formation of thymidine triphosphate, one of the essential building blocks of DNA. Therefore, lack of either vitamin B12 or folic acid causes abnormal and diminished DNA and, consequently, failure of nuclear maturation and cell division. Therefore, it is said that deficiency of either vitamin B12 or folic acid causes maturation failure in the process of erythropoiesis.

Vitamin B12 (Cyanocobalamin)

One common cause of failure in red blood cell maturation is failure in the absorption of vitamin B12 from the gastrointestinal tract's small intestine. The parietal cells of gastric mucosa secrete a glycoprotein called intrinsic factor, which combines with vitamin B12 in food and makes the B12 available for absorption by the small intestine.

The intrinsic factor role is to protect the Vitamin B12 from destruction from gastric (stomach) acid; and aid absorption of Vitamin B12 into the blood. When there is *Atrophic Gastric Mucosa*, the gastric mucosa fails to produce intrinsic factor, hence Vitamin B12 is destroyed by gastric acid. This often occurs in the disease pernicious anemia.

N/B: Deficiency of intrinsic factor also occurs in cases of Severe gastritis, Ulcer and Gastrectomy.

Once vitamin B12 has been absorbed from the gastrointestinal tract, it is first stored in large quantities in the liver and then released slowly as needed by the bone marrow.

Folic Acid (Pteroylglutamic Acid).

Folic acid is found in green vegetables, some fruits, and meats (especially liver). Note that it is easily destroyed during cooking. Also, people with gastrointestinal absorption abnormalities, especially in the small intestinal (a disease like sprue), experience serious difficulty in absorbing both folic acid and vitamin B12. In the absence of folic acid, the synthesis of DNA decreases causing failure of erythrocyte maturation. Anemia due to folic acid deficiency is called megaloblastic anemia.

4. Hormones

Several hormones also play important role in production of red blood cells, these include erythropoietin, thyroxine, growth hormone, testosterone, estrogen and cortisol.

Erythropoietin

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

About 95 % of Erythropoietin is secreted by peritubular capillaries of kidney, 4% is produced from the liver while 1% is produced in the brain. The main stimulant for production and secretion of erythropoietin is Hypoxia (abnormal low concentration of oxygen in blood).

Erythropoietin indirectly acts on red blood cells, i.e. it causes the formation of hemopoietic growth factors, which causes formation and release of new RBCs into circulation. Production of erythropoietin increases the rate of red blood cell production, so instead of the normal 7 days of erythropoiesism, it takes 4 to 5 days.

Erythropoietin promotes the following processes:

a. Production of proerythroblasts from CFU-E of the bone marrow
b. Development of proerythroblasts into reticulocyte through the earlier discussed stages
c. Release of matured erythrocytes into blood. Even some reticulocytes (more than 1%) are released along with matured RBCs.

Thyroxine, growth hormone and testosterone accelerates the process of erythropoiesis at many levels while estrogen are known for reducing red blood cell production.

Hemoglobin

It is the oxygen-carrying pigment in the red blood. It is responsible for the red coloration of erythrocytes. Haemoglobin has two functions. These are buffer function and transportation of gases.

A Transportation of Respiratory Gases

Hemoglobin transports Oxygen from the lungs to tissues and Carbon dioxide from tissues to lungs.

1. Transport of Oxygen

When oxygen binds with hemoglobin, it results in the formation of oxyhemoglobin. Oxyhemoglobin is an unstable compound and the combination is reversible, i.e. when more oxygen is available, it combines with hemoglobin and whenever oxygen is required, hemoglobin can release oxygen readily. When oxygen is released from oxyhemoglobin, it is called reduced hemoglobin or ferrohemoglobin.

2. Transport of Carbon Dioxide

When carbon dioxide binds with hemoglobin, carbhemoglobin is formed. It is also an unstable compound and the combination is reversible, i.e. the carbon dioxide can be released from this compound.

B. Buffer Action

Hemoglobin acts as a buffer and plays an important role in acid base balance

Structure of Hemoglobin

Hemoglobin is made of two molecules; the heme portion and globin portion.

The heme is made up of Iron and Protoporphyrin IX. Iron is normally in ferrous state (Fe^{2+}). In some abnormal conditions, the iron is converted into ferric (Fe^{3+}) state, which is a stable form. Oxygen binds to iron in ferrous state but not in ferric state. Protoporphyrin IX is the pigment part of heme, formed by four pyrrole rings (tetrapyrrole).

Globin contains four polypeptide chains. These are alpha, beta, delta and gamma polypeptide chains. Among the four polypeptide chains, two pairs of polypeptide chains form the globin of haemoglobin.

In normal adult human haemoglobin (haemoglobin A), the two polypeptides are called α chains, each of which contains 141 amino acid residues, and β chains, each of which contains 146 amino acid residues. Thus, hemoglobin A is designated $\alpha_2\beta_2$. Fetal Hemoglobin (HbF) structure is similar to that of haemoglobin A except that the β chains are replaced by γ chains.There for Fetal Hemoglobin is made of two pairs of polypeptides of α chains and γ chains.

Destruction of Hemoglobin

After the lifespan of 90 to 120 days, the RBC is destroyed in the reticuloendothelial system, particularly in spleen and the hemoglobin is released into plasma. Soon, the hemoglobin is degraded in the reticuloendothelial cells and split into globin and heme. The globin is reused while the heme is further broken down into iron and Protoporphyrin IX. The Iron is stored while the Protoporphyrin IX is converted to biliverdin. Biliverdin is converted to bilirubin by the enzyme Bilirubin reductase.

Iron Metabolism

Iron is an important electrolye and very essential component of haemoglobin involved in oxygen transport. Iron is important for the formation of hemoglobin and myoglobin (the oxygen carrying protein of muscle). Iron is also necessary for the formation of other substances like cytochrome, cytochrome oxidase, peroxidase and catalase.

Absorption of Iron

Iron in diet is usually in ferric (Fe^{3+}) form but converted into ferrous form (Fe^{2+}) by ferric reductase. It is absorbed mainly through the enterocytes of the small intestine by pinocytosis and transported into the blood. Bile aids the absorption of iron.

Transport of Iron

Immediately after absorption into blood, iron combines with a β-globulin called apotransferrin (secreted by liver through bile) resulting in the formation of transferrin. And iron is transported in blood in the form of transferrin.

Storage Of Iron

Iron is stored in large quantities in reticuloendothelial cells and liver cells. In other cells also it is stored in small quantities. Transferrin dissociates to form iron and apoferritin, iron is then actively transported into the cytoplasm of the cell, and stored as ferritin in large amount. When large ferritin are stored, a saturated level is reached, subsequent storage of iron will be in form of hemosiderin which is in small quantity of iron.

Disorders of Red Blood Cells

Anemia

Anemia is the abnormal decrease in red blood cell, haemoglobin and packed cell volume. Some types of anemia and their physiologic causes are the following

1. Hemorrhagic Anemia

This is anemia due to hemorrhage or blood loss. It is of two types; acute and chronic hemorrhagic anemia.

Acute hemorrhage

Acute hemorrhage refers to sudden loss of a large quantity of blood. A typical example is blood loss during an accident

Chronic hemorrhage

It refers to gradual loss of blood over a long period of time. It occurs in conditions like peptic ulcer, purpura, hemophilia and menorrhagia.

2. Hemolytic Anemia

Hemolysis is the destruction of RBCs. Anemia due to excessive hemolysis which is not compensated by increased RBC production is called hemolytic anemia. It is classified into two types: Extrinsic hemolytic anemia and Intrinsic hemolytic anemia.

Extrinsic hemolytic anemia: It is the type of anemia caused by destruction of RBCs by external factors. Factors such as antibodies, chemicals (lead, coal and tar) and drugs (penicillin, antimalarial drugs and sulfa drugs) are external agents to the body and are capable of hemolyzing red blood cells. Also diseases such as Renal disorder, Liver failure , autoimmune disorders (rheumatoid arthritis and ulcerative colitis) etc

Intrinsic hemolytic anemia: This type of anemia caused by destruction of RBCs due to defection of red blood cells. Most of the intrinsic haemolytic anemia are hereditary and short lived, it includes hemoglobinopathies (such sickle cell and thalassemias), spherocytosis etc.

Sickle cell anemia

Sickle cell anemia is a blood disorder characterized by sickle shaped erythrocytes. It is due to due to the abnormal haemoglobin called hemoglobin S. In sickle cell haemoglobin α-chains are normal and but the abnormality is in the β - chains. Sickle cell anemia occurs when two abnormal genes (one from each parent) are inherited.

In sickle cell anemia, which is present in 0.3 to 1.0 percent of West African, when the haemoglobin S is exposed to low concentrations of oxygen, it precipitates into long crystals inside the red blood cell. These crystals elongate the cell and give it the appearance of a sickle rather than a biconcave disc. The precipitated haemoglobin also damages the cell membrane, resulting in hemolysis, so the cells become highly fragile, leading to serious anemia

Thalassemia

Thalassemia is also inherited and a disorder due to abnormal haemoglobin . it is also called Mediterranean anemia. Thalassemia is of two types: α-thalassemia and β - thalassemia.

In normal hemoglobin, number of α and β polypeptide chains is equal. In thalassemia, the number of amino acids in these chains become imbalanced because of defective synthesis of globin genes. In α-Thalassemia the number of amino acids in α – chains are less, absent

or abnormal while the amino acids in βchains are in excess. The reverse is the case for β-Thalassemia

3. Nutrition Deficiency Anemia

This is anemia due to deficiency of a nutrients required for normal erythropoiesis. The common nutrients necessary for erythropoiesis include iron, proteins and vitamins (Vitamin B12 and folic acid). Examples of nutrition deficiency anemia are:

Iron deficiency anemia

Iron deficiency anemia is the most common type of anemia. It develops due to inadequate availability of iron for hemoglobin synthesis. RBCs are microcytic and hypochromic.

Pernicious anemia

This is anemia due to deficiency of vitamin B12. It is also called Addison's anemia. It is due the presence of gastric atrophy which is caused by autoimmune destruction of parietal cells that secretes intrinsic factors. The gastric atrophy decreases the production of intrinsic factor and leads to poor absorption of vitamin B12, which is the maturation factor for RBC.

Megaloblastic anemia

This is anemia due to deficiency of folic acid. Folic acid is another red blood cell maturation factor. In folic acid defiency, DNA synthesis in red blood cell becomes defective, and so the nucleus remains immature. The RBCs are megaloblastic and hypochromic.

4. Aplastic Anemia

This is anemia due to disorder of bone marrow. Under normal circumstances, the bone marrow produces red blood cells, and as age progress, the bone marrow becomes fatty. In pathological conditions, bone marrow is destroyed leading to Aplastic anemia. Bone marrow disorder occurs in the following conditions: repeated exposure to radiations (Xray or gamma ray); presence of bacterial toxins, quinine etc, presence of diseases such as Tuberculosis, Viral infections (hepatitis and HIV infections).

5. Idiopathic Anemia

This is anemia of unknown cause.

Polycythemia

1. Secondary Polycythemia.

Hypoxia is the main stimulant for erythropoiesis. When tissues are deprived of oxygen either because of low atmospheric oxygen in cases of high altitude or because of failure of oxygen delivery to the tissues, such as in cardiac failure or lung disease, large quantities of red blood cells are subsequently produced. This condition is called secondary polycythemia or physiologic polycythemia, and the red cell count commonly rises to 6 to 7 million/mm^3, and haematocrit becomes about 30 percent above normal.

2. Polycythemia Vera (Erythremia).

While secondary polycythemia is physiologic, Polycythemia is pathologic. In this condition the haematocrit level rises to about 60 to 70 percent. Polycythemia vera is caused by a genetic aberration in the PHSC that produce the blood cells. Under normal conditions, the stem cells stop producing red blood cells in the event of too many erythrocytes, but for Polycythemia vera, stem cell continue the production of red blood cells in a manner similar to cancer.

WHITE BLOOD CELLS

These are otherwise called leucocytes. Their main function is to fight against pathogens such as bacteria, fungi, virus, toxins etc. Compared to RBCs, the WBCs are larger in size but lesser in number. Leucocytes are also irregular in shape, and nucleated.

Types of White Blood Cells

There are five types of White Blood Cells. These are:

i. Neutrophils
ii. Basophils
iii. Eosinophils
iv. Monocytes
v. Lymphocytes

Classification of White Blood Cells

There are two classifications of White Blood Cells. Classification based on number of nuclear lobes and classification based on presence of granules in cytoplasm

1. Classification Based on Number of Nuclear Lobes

Under this classification, there are two types of White Blood Cells, these are the polymorphonuclear and monomorphonuclear. Polymorphonuclear are white blood cells whose nucleus are divided into more than one lobes, these include Neutrophils, Basophils and Eosinophils. Monomorphonuclear are white blood cells whose nucleus are not divided into lobes, these include monocytes and lymphocytes.

2. Classification Based on the Presence of Granules in Cytoplasm

White Blo

od Cells are classified into two, these are granulocytes and agranulocytes. Granulocytes are white blood cells that have granules in their cytoplasm, these include neutrophils, basophils and eosinophils. Agranulocytes do not have granules in their cytoplasm and they are monocytes and lymphocytes.

Morphology of White Blood Cells

Neutrophils

Neutrophils have fine or small granules in the cytoplasm. The granules react to acidic and basic stains hence the name neutro from neutral. When stained with Leishman's stain (which contains acidic eosin and basic methylene blue) the granules appear violet or dark pink in color. Nucleus is multilobed, having between 3 to lobes. The number of lobes in the nucleus depends upon the age of cell.

Eosinophils

Eosinophils have coarse (larger) granules in the cytoplasm. It reacts with acidic eosin in Leischman's stain to give a reddish color. Nucleus is bilobed and spectacle-shaped.

Basophils

Basophils just like eosinophils also have coarse granules in the cytoplasm, and their nucleus is bilobed. The granules stain purple blue with basic methylene blue.

Monocytes

Monocytes are the largest leukocytes. The cytoplasm is clear without granules. Nucleus is round, oval and horseshoe shaped, bean shaped or kidney shaped. Nucleus is placed either in the center of the cell or pushed to one side and a large amount of cytoplasm is seen.

Lymphocytes

Like monocytes, the lymphocytes also do not have granules in the cytoplasm. Nucleus is oval, bean-shaped or kidney-shaped. Nucleus occupies the whole of the cytoplasm. A rim of cytoplasm may or may not be seen.

Genesis of White Blood Cells (Leucopopoiesis)

The process involved in the development and production of leucocytes is known as leucopoiesis. Like erythrocytes, leucocytes are produced from the bone marrow, starting off from the progenitor cells, the PHSC – Pleuripotent Hematopoietic Stem Cells. The PHSC are usually not committed hence they different into committed stems. The committed stem for white blood cells are of two types, they are the myeloblast or the myeloid stem cells or Colony Forming Unit – Granulocytes and Monocytes and the Lymphoid Stem Cell (LSC) or Lymphoblast. Myeloloid Stem Cells eventually give rise to granulocytes (neutrophils, basophils and eosinophils) and monocytes, while the Lymphoid Stem Cells give rise to Lymphocytes.

Production of Granulocytes and Monocytes

Granulocytes and Monocytes are produced from the myelocytic lineage. For the purpose of proper understanding, neutrophils will be used as an example for the production of granulocytes. There two stages involved in describing production of granulocytes, these are the mitotic stage and the postmitotic stage.

Mitotic Stage

During this stage, the noticeable change in granulocytes are the change in digestive enzymes and the ability to undergo mitosis during differentiation. In this case, Myeloblast different into promyelocytes. Granules in the cytoplasm of promyelocytes do not react with Leischman's stain and hence their types cannot be recognized. The granules contain hydrolytic enzymes which can only digest nutrients such as carbohydrate, protein and lipid. The promyelocyte differentiates into neutrophil myelocytes or basophil myelocyte or eosinophil myelocytes. The

granules of these myelocytes react with Leischman's stain hence the possibility to recognize neutrophil myelocyte from basophil myelocyte and eosinophil myelocyte. Again the granules contain proteolytic enzymes which have the ability to digest or destroy pathogens.

Post-Mitotic Stage

In post-mitotic stage, the changes observed are the division of nucleus into loves and the absence of mitosis. Neutrophil Myelocyte becomes Young Neutrophil Meta-myelocyte when indentation appears in the nucleus. This further differentiates to form Band Neutrophil Meta-myelocyte when the indentation deepens making the nucleus look like a sausage. The Band Neutrophil Meta-myelocyte differentiates into matured Neutrophils, in this case the indentation deepens again so that the nucleus is separated into lobes held by chromatin thread.

Regulation of Leukopoiesis

A variety of cytokines stimulate different stages of leukocyte development. The cytokines known as multipotent growth factor-1, interleukin-1, and interleukin-3 have general effects, stimulating the development of different types of white blood cells. Granulocyte colony-stimulating factor (G-CSF) acts in a highly specific manner to stimulate the development of neutrophils, whereas granulocyte-monocyte colony-stimulating factor (GM-CSF) stimulates the development of monocytes and eosinophils.

IMMUNITY

Immunity is the ability and capacity of the body to resist pathogenic agents and the entry of bacteria, virus, toxic substances, etc.

Types of Immunity

Immunity is of two types
I. Innate immunity.
II. Acquired immunity.

Innate Immunity Or Non-Specific Immunity

Innate immunity is also known as non-specific Immunity or natural immunity, it is the inborn capacity of the body to resist pathogens. It serves as the first line of defence against diseases. It is not specific to any pathogen. Examples include:

i. Lysozymes in Saliva
ii. Phagocytic cells such as macrophages and granulocytes
iii. Gastric acid of the Stomach
iv. Resistance of the skin to invasion by organisms.
v. Presence in the blood of certain chemical compounds in the blood, compounds such as basic polypeptides, complement complex system, natural killer cells

Acquired Immunity Or Specific Immunity

Acquired immunity, also called adaptive immunity or specific immunity is the resistance developed in the body against any pathogens, vaccines and even transplanted tissues. Lymphocytes are responsible for the development of these two types of immunity. It is of two types:

1. Cellular immunity or T-Cell Immunity

2. Humoral immunity or B-Cell Immunity

T Lymphocytes

T lymphocytes are processed in thymus and they control cell mediated immunity. They are of four types:

1. Helper T cells or inducer T cells.

2. Cytotoxic T cells or killer T cells.

3. Suppressor T cells or Regulatory T Cells

4. Memory T cells.

B Lymphocytes

B lymphocytes were first discovered in the bursa of Fabricius in birds, hence the name B lymphocytes. Bursa is absent in mammals and the processing of B lymphocytes takes place

in liver (during fetal life) and bone marrow (after birth). There are two types of B Lymphocytes; They include Plasma cells and Memory cells. Plasma cells produce and secrete antibodies

Antibodies

The antibodies are gamma globulins called immunoglobulins abbreviated as Ig). They make up about 20 per cent of all the plasma proteins. All antibodies are composed of two light and two heavy polypeptide chains. Where the light and heavy chains form pairs is the variable portion of antibody while the remainder of each chain is called the constant portion. The variable portion is different for each specificity of antibody, and it is this portion that attaches specifically to a particular type of antigen.

Antibodies have five types. These include:

1. IgA (Ig alpha)

2. IgD (Ig delta)

3. IgE (Ig epsilon)

4. IgG (Ig gamma)

5. IgM (Ig mu).

Among these antibodies, IgG forms 75% of the antibodies in the body. IgA is found in secretions such as semen, saliva, breast milk etc

Mechanism of Actions of Antibodies

Antibodies protect the body and destroy pathogens through two different mechanism. These are

1. By direct actions

2. Indirect Actions (Through complement system).

1. Direct Actions of Antibodies

Antibodies directly inactivate the invading organism by

i. Agglutination: Antibodies bind to the surface of pathogens and hold them together to form clumps

ii. Precipitation: In this case antibodies bind to soluble antigens like tetanus toxin and then convert them into insoluble forms
iii. Neutralization: During this, the antibodies cover the toxic sites of antigenic products.
iv. Lysis: Antibodies rupture the cell membrane of the organisms and then destroy them.

Indirect Actions of Antibodies (Complement System)

Complement Complex is a system of plasma enzymes, which are identified by numbers from C1 to C9, B and D. These enzymes are in inactive form and are activated in two ways:

a. Classical pathway

b. Alternate pathway.

In a cascade reaction the inactive plasma enzymes become activated and form new active enzymes. These active enzymes cause the following

i. Opsonization: Activation of neutrophils and macrophages to engulf the bacteria.
ii. Lysis: Destruction of bacteria by rupturing the cell membrane.
iii. Chemotaxis: Attraction of leukocytes to the site of antigen-antibody reaction.
iv. Agglutination: Clumping of foreign bodies like RBCs or bacteria.
v. Neutralization: Covering the toxic sites of antigenic products.
vi. Activation of mast cells and basophils, which liberate histamine: Histamine dilates the blood vessels and increases capillary permeability.

<u>Antigens</u>

Antigens are proteins which induce immune reactions in the body. Antigens are of two types, these are:

i. Autoantigens or self antigens, these are present on the body's own cells, for example agglutinogens found on the surface of RBCs.
ii. Foreign antigen s or non-self antigens that enter the body from outside, and found in foreign agents such as pathogens, toxins, chemical etc.

<u>Antigen-Presenting Cells</u>

Antigen-presenting cells are immune cells that induce immune reaction simply by presenting antigen to helper T cells. Antigen-presenting cells are of three types: these are Macrophages, Dendritic cells and B-lymphocytes.

Mechanism of Antigen-presenting Cells

When invading foreign organisms are engulfed (phagocytosis) or trapped by macrophages or dendritic cells respectively, the antigen from these pathogens is broken into small peptide products. These antigenic peptide products are carried to the surface of the antigen-presenting cells by Major Histocompartibility Complex (MHC).

MHC molecules in human beings are divided into two types:

1. Class I MHC molecule: It is found on every cell in human body except red blood cells. Antigens held by this Class I MHC molecule react against cytotoxic t-cells
2. Class II MHC molecule: It is found on the antigen presenting cells. It is responsible for presenting the exogenous antigens (antigens of bacteria or viruses which are engulfed by antigen-presenting cells) to helper T cells

Passive Immunity

Active Immunity have previously being discussed. The innate and acquired immunity make up the active immunity. In active immunity, a person is induced with immunity that will last a life time following exposure to pathogens and toxins, passive immunity on the other hand is produced without challenging the immune system of the body. It is a 'borrowed' immunity. It is induced by administration of serum or gamma globulins from a person who is already immunized (affected by the disease) to a non-immune person.

Passive immunization is acquired either naturally or artificially.

Passive Natural Immunization

Example of passive natural immunization, is antibodies transferred from a mother to child. Before birth, immunity is transferred from mother to the fetus in the form of maternal antibodies (mainly IgG) through placenta. After birth, the antibodies (IgA) are transferred through breast milk.

Passive Artificial Immunization

During passive artificial immunization, antibodies are developed by injecting previously prepared antibodies using serum from humans or animals. Antibodies are obtained from the persons affected by the disease or from animals. The serum containing the antibody

(antiserum) is administered to people who have developed the disease (therapeutic). It is also used as a prophylactic measure.

Hypersensitivity and Allergy

Hypersensitivity, also known as allergy, is an exaggerated immune reaction. It is a pathological reaction of the immune system to external antigens – allergens, which exist normally in the environment (pollens, molds, animals, foods, insect stings, etc). It is of four types

Hypersensitivity type 1

This type of reaction is also called immediate hypersensitivity. It makes use of IgE antibody. In the first exposure to an allergen, IgE is produced, presumably under the stimulation of T-Helper type 2. These IgE is bound to the Fc receptor on mast cell. The mast cell is 'sensitized' against the allergen. Subsequent exposure to allergen results in degranulation of the mast cell, releasing its mediators, namely cytokines, vasoactive amines and eicosanoids - prostaglandin and leukotrines) These mediators cause vasodilation, increased vasopermeability, tissue damage and smooth muscle contraction. This is called early phase reaction.Clinical example include: Asthma and anaphylaxis

Hypersensitivity type 2

This type of hypersensitivity is also called antibody mediated hypersensitivity.IgM and IgG against self-antigens (autoantibodies) of the extracellular matrix or cell surface triggers the classical pathway of complement activation. Clinical example include: Autoimmune hemolytic anemia, Goodpasture's Syndrome etc

Hypersensitivity type 3

This is also called immune-complex mediated hypersensitivty. In this case, the antibodies that is formed against pathogens forms immune-complex that is deposited in tissues. This may also activate the complement system or become target of phagocytes, causing damage to the vessel wall and ultimately the organ itself. Clinical example include: Post-streptococcal vasculitis, post-streptococcal glomerulonephritis, Systemic lupus erythematosus

Hypersensitivity type 4

This is also called the T-cell mediated hypersensitivity or delayed Hypersensitivity. T-cell mediated cytotoxicity (mediated by CD8+ T cells) - in this reaction, T-cytotoxic cell specific for autoantigen on host cells may directly kill these cells. Another mechanism for Type 4 Hypersensitivity is that Antigent Presenting Cells activate Helper and Cytotoxic T cells, causing them to secrete cytokines. This cytokines recruits and activates phagocytes, as well as inducing local inflammation. Ultimately, tissue injury occurs. Clinical examples include Contact dermatitis, rheumatoid arthritis, mantoux test

BLOOD GROUPS

There are certain antigens on the surfaces of red blood cells. These antigens are known as agglutinogens. These agglutinogens react against incompartible antibodies known as agglutinins. There are about 30 commonly occurring antigens, but most are weak so are only used in paternity tests. Two are of great importance, they are the ABO system and Rhesus

ABO system:

There two types of agglutinogens, agglutinogen A and agglutinogen B. Based on the presence or absence of agglutinogen A and agglutinogen B, blood is divided into four groups:

1. 'A' group
2. 'B' group
3. 'AB' group
4. 'O' group.

Blood having antigen A belongs to 'A' group. This blood has β-antibody in the serum. Blood with antigen B and α-antibody belongs to 'B' group. If both the antigens are present, blood group is called 'AB' group and serum of this group does not contain any antibody. If both antigens are absent, the blood group is called 'O' group and both α and β antibodies are present in the serum.

ABO blood type

Genotype	Blood type	Agglutinogen (antigen)	Agglutinin (antibody)
OO	O	-	Anti A & Anti B

OA/AA	A	A	Anti B
OB/BB	B	B	Anti A
AB	AB	A & B	-

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The associated anti-A antibodies and anti-B antibodies are usually IgM antibodies, which are usually produced in the first years of life by sensitization to environmental substances such as food, bacteria and viruses

In case of mismatch in transfusion, the following occur: RBCs clump together (agglutinate) as a result of agglutinins attaching to RBCs. The clumps block small vessels. Physical distortion or attack by phagocytic WBCs destroy membranes of agglutinated cells. Hemoglobin is released into plasma.

RH system:

The difference between ABO and Rhesus system, is the spontaneous reaction of agglutinogen with agglutinins which occurs in ABO and almost never in Rhesus system. There are 6 agglutinogens in Rhesus system, these are C, D, E, c, d, e (each of which is called an Rh factor). Rh D and d is the most important. If Rh D is present – it is called Rh+ (because it is considerably more antigenic than the others), if it is absent, Rh d will be present and hence Rh negative

Rh immune response – transfusion reactions: When Rh+ is injected in Rh− person, anti-Rh+ antibodies develop slowly (up to 2–4 months). When the antibodies do develop they agglutinate with the antigen on Rh+ RBC forming clumps which are hemolyzed by macrophages. Therefore a transfusion reaction does occur but is mild. On subsequent transfusions however, reactions are more severe because Rh+ already exists in blood. A typical case is in erythroblastosis fetalis. If rh negative is injected in a rhesus positive person reaction will not occur.

Erythroblastosis fetalis (Hemolytic disease of newborn)

It is characterized by agglutination of RBCs and subsequent phagocytosis leading to hemolytic anemia. It also occurs mostly in Rh− mothers bearing an Rh+ child; the mother

develops anti-Rh+ agglutinins from exposure to fetus's Rh+ antigen; these agglutinins then diffuse through the placenta into the fetus and cause RBC agglutination.

Incidence rises progressively with subsequent pregnancies.

Prevention: administration of Rh immunoglobulin globin (an anti-D antibody) to the expectant mother at 28–30 weeks of gestation, to prevent sensitization of mother to D antigen of fetus (usually administered after first pregnancy and before second to kill first fetus's cells that remained in mother's circulation).

HEMOSTASIS

Hemostasis is one important homeostatic mechanism of the human body. It prevents loss of blood when a blood vessel is teared. When a blood vessel is injured, a number of physiological mechanisms are activated that promote hemostasis, or the cessation of bleeding (hemo = blood; stasis = standing).

Breakage of the endothelial lining of a vessel exposes collagen proteins from the subendothelial connective tissue to the blood. This initiates three separate, but overlapping, hemostatic mechanisms: (1) vasoconstriction, (2) the formation of a platelet plug, and (3) formation of blood clot by the production of a web of fibrin proteins that penetrates and surrounds the platelet plug.

Vasoconstriction

Once the blood vessel has been cut or ruptured, the smooth muscle of the vessel begins to contract reducing immediately the blood flow. The reduction in blood flow may last for minutes or even hours. This contraction is caused by:

i. Local myogenic contraction (spasm) which is initiated by direct damage to the wall of the blood vessel
ii. Local humoral factors from the damaged tissues and the platelets (for example, thromboxane A and serotonin)
iii. Nervous reflexes which are initiated by pain or other nerve impulses that originate in the site of the damage or surrounding tissues.

The greater the damage of the blood vessel, the greater the degree of the spasm or vasoconstriction

Formation of a Platelet Plug

Many small vascular holes develop during the day throughout the human body. These small holes or any other minor cuts are often sealed by a platelet plug rather than a blood clot.

Platelets

Platelets are also called thrombocytes, they form part of the cellular elements of blood. Platelets are small and non-nucleated cells. They are smaller than red blood cells. They have several shapes which include spherical or rod-shaped, oval or disk-shaped, dumbbell shape, comma shape, cigar shape or any other unusual shape. The normal concentration of platelets in the blood is between 150,000 and 300,000 per microliter.

Platelets have many functional characteristics which include

i. actin and myosin molecules, which are contractile proteins that can cause the platelets to contract;
ii. endoplasmic reticulum and the Golgi apparatus that synthesize various enzymes and especially store large quantities of calcium ions;
iii. mitochondria and enzyme systems that are capable of forming adenosine triphosphate (ATP) and adenosine diphosphate (ADP);
iv. enzyme systems that synthesize prostaglandins, which are local hormones that cause many vascular and other local tissue reactions;
v. fibrin-stabilizing factor
vi. a growth factor

The whole mechanism of the formation of the platelet plug is based on important functions of the platelets. Once the platelets come in contact with the collagen fibres present in the vascular wall they instantly alter their own characteristics. The platelets start to swell

They take irregular forms with many irradiating pseudopodes projecting from their surfaces. Their contractile proteins (actin, myosin and thrombosthenin) contract resulting in the release of granules that contain several actice factors. The platelets become sticky and therefore are able to adhere to the collaged present in the damaged vascular tissue and to a protein called con Willebrand factor that has leaked into the damaged tissue from the plasma. They secrete large amounts of Adenosine Diphospate (ADP). The enzymes of platelets form thromboxane A. ADP and thromboxane A activate nearby platelets and those additional sticky platelets adhere to the original activated platelets. The damaged blood vessel wall manages to activate successively increasing numbers of platelets and those platelets in turn, attract more

additional platelets resulting in the formation of a platelet plug. Initially, the platelet plug is loose but it is successful in stopping the blood loss from a minor vascular opening. During the blood coagulation process fibrin fibres form which attach tightly to the platelets making in an unyielding plug.

Formation of Blood Clot:

If the platelet plug is not enough to stop the bleeding, the third stage of hemostasis begins: the formation of a blood clot. First, blood changes from a liquid to a gel. At least 12 substances called clotting factors take part in a series of chemical reactions (cascade reactions). These clotting factors are in an inactive state and must be activated to bring about blood clotting. Generally, the three stages involved in clotting formation are: formation of prothrombin activator, conversion of prothrombin into thrombin and finally thrombin is converted to fibrin

The thirteen known clotting factors are:

i. Fibrinogen (Factor 1)
ii. Prothrombin (Factor 2)
iii. Thromboplastin (Factor 3)
iv. Calcium (Factor 4)
v. Proaccelerin or Labile Factor (Factor 5)
vi. Stable Factor (Factor 6)
vii. Antihemophilic Factor (Factor 8)
viii. Christmas Factor (Factor 9)
ix. Stuart - Power Factor (Factor 10)
x. Plasma Thrombin antecedent (Factor 11)
xi. Hegman Factor (Factor 12)
xii. Fibrin Stabilising Factor (Factor 13)

Formation of Prothrombin Activator: When blood vessels are damaged, vessels and nearby platelets are stimulated to release a substance called prothrombin activator,

Conversion of Prothrombin to Thrombin. Prothrombin activator activates the conversion of prothrombin, a plasma protein, into an enzyme called thrombin. This reaction requires calcium ions.

Thrombin: Thrombin facilitates the conversion of a soluble plasma protein called fibrinogen into long insoluble fibers or threads of the protein fibrin.

Fibrin: Fibrinogen is cleaved by thrombin to form its active form, "fibrin." Fibrin threads wind around the platelet plug at the damaged area of the blood vessel, forming an interlocking network of fibers and a framework for the clot. This net of fibers traps and helps hold platelets, blood cells and other molecules tight to the site of injury, functioning as the initial clot. This temporary fibrin clot can form in less than a minute, and usually does a good job of reducing the blood flow. Next, platelets in the clot begin to shrink, tightening the clot and drawing together the vessel walls. Usually, this whole process of clot formation and tightening takes less than a half hour.

The conversion of fibrinogen into fibrin may occur via either of two pathways. Blood left in a test tube will clot without the addition of any external chemicals; the pathway that produces this clot is thus called the intrinsic pathway. Damaged tissues, however, release a chemical that initiates a “shortcut” to the formation of fibrin. Because this chemical is not part of blood, the shorter pathway is called the extrinsic pathway.

The intrinsic pathway is initiated by the exposure of plasma to a negatively charged surface, such as that provided by collagen at the site of a wound or by the glass of a test tube. This contact pathway activates a plasma protein called factor XII, which is a protein-digesting enzyme (a protease). Active factor XII in turn activates another clotting factor, which activates yet another. The plasma clotting factors are numbered in order of their discovery, which does not reflect the actual sequence of reactions.

The next steps in the sequence require the presence of Ca 2 + and phospholipids, the latter provided by platelets. These steps result in the conversion of an inactive glycoprotein, called prothrombin, into the active enzyme thrombin.