BLOOD PHYSIOLOGY

BY DR. AMEENA NASIR

BLOOD

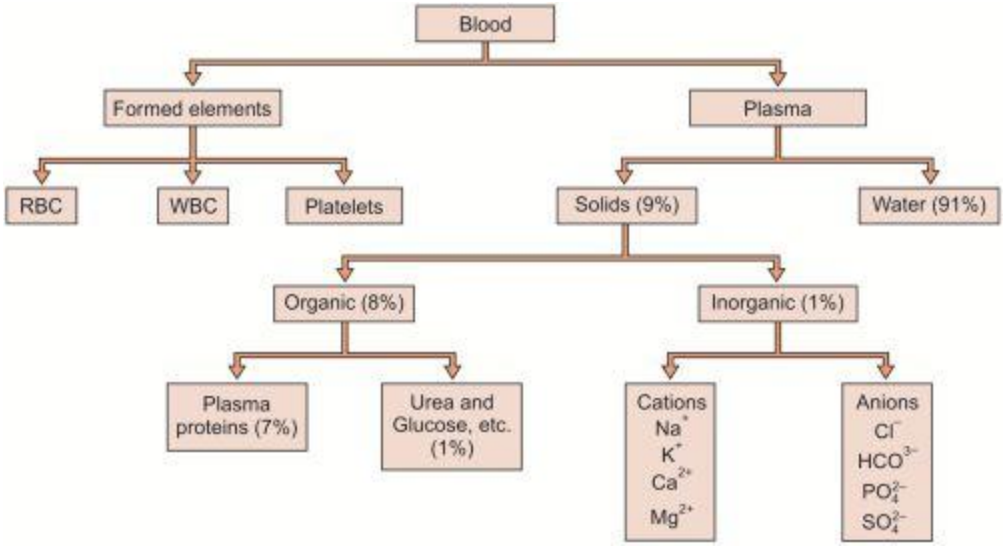
- Volume = 5L
- pH = 7.45
- Specific Gravity = 1.052 – 1.061

FUNCTIONS OF BLOOD

- Provision of nutrients
- Provision of respiratory gases
- Exchange of waste between tissue and vessel
- Carrying vehicle for endocrine hormones
- Act as medium for transport
- Regulation of body temperature
- Regulation of water balance
- Act as buffer

COMPOSITION

- Cellular portion (40 – 45% V/V) → RBCs, WBCs, Platelets
- Liquid portion (50 – 60% V/V) → Plasma



- SERUM: Clear fluid from the clotted blood i.e. plasma without clotting factors

SERUM

- Clear straw colored fluid that oozes out from blood clot
- Fibrinogen is absent and is converted to fibrin during clotting
- Composition is same as plasma except fibrinogen

- Lack clotting factors

PLASMA PROTEINS

- Albumin
- Globulin
- Fibrinogen
- Prothrombin

PROPERTIES	ALBUMIN	GLOBULIN	FIBRINOGEN	PROTHROMBIN
CONCENTRATION	4.5 – 5.7 g/dL	1.5 – 2.5 g/dL	0.2 – 0.4 g/dL	0.01 – 0.02 g/Dl
SOLUBILITY	Water soluble	Insoluble	Insoluble	Insoluble
COAGUBILITY	Heat coagulable	At 70°C	At 56°C	Helps in coagulation
ISOELECTRIC PH	4.7	α – 5.1 β – 5.6 γ – 6	5.8	
PRODUCTION SITE	Liver	Liver except γ-globulin	Liver	Liver

Antibodies are γ-Globulins

ALBUMIN

- Most abundant (50 – 60%)
- Low molecular weight
- Regulation of osmotic pressure (80%)
- Precursor for tissue protein
- Transport carrier for hormones, amino acids
- Maintain pH

GLOBULIN EXAMPLES

- Heptaglobin
- Ceruloplasmin (Protein that binds to copper)
- Antibody (γ-Globulin)
- Carrier lipids
- Transferrin (transport iron)

FUNCTION OF PLASMA PROTEINS

- Immunity
- Osmotic pressure regulation
- Transport of amino acids, hormones
- Maintain viscosity
- Blood clotting

MAINTAIN ESR (ERYTHROCYTE SEDIMENTATION RATE)

1. Fibrinogen (major role in ESR)
2. Globulin
3. Albumin

- **COLLOID OSMOTIC PRESSURE**: The pressure that tends to keep the blood within vessels (28 mmHg)
- **HYDROSTATIC PRESSURE**: The pressure that tends to keep the blood driven away from vessel (repulsive force for H_2O)
- Albumin/Globulin Ratio is 2 : 1
- Osmotic pressure is due to blood solutes
  Oncotic pressure is due to plasma proteins

ERYTHROCYTES

- Biconcave, anucleated
- 7.8 µm mean diameter
- Thickness at center – 1 µm
- Thickness at thickest part – 2.5 µm
- Life span = 120 days
- Flexible cell membrane
- Female Count = 47,00,000 ± 300,000/mm^3
  Male Count = 52,00,000 ± 300,000/mm^3

Why RBCs are less in females?

Ans: Testosterone facilitates erythropoiesis in males thereby increasing RBC count

SITE OF PRODUCTIONS OF RBCs

- BEFORE BIRTH
  - Megaloblastic Stage (early weeks) → Yolksac
  - Hepatic Stage (second trimester) → Liver, spleen, lymphoid tissue
  - Myeloid Stage → Bone Marrow
- AFTER BIRTH
  - Upto 5 Years → Bone Marrow of all bones
  - Till 20 Years → Bone Marrow of long bones except tibia and humerus
  - After 20 Years → Bone Marrow of membranous bones i.e. ribs, sternum, vertebrae, ileum

Erythropoiesis takes 7 days

HEMOPOIESIS

- Origin, development, maturation and processing of blood cells
- Blast → immature cell
  Cyte → Mature cell
- PROCESSION TO MATURITY LEADS TO

- Cell size decrease
- Cytoplasm to nucleus ratio increase
- Nucleoli start disappearing
- Amount of RNA (basophilic) decreases
- Hb starts to appear

STAGE	SIZE	NUCLEUS	CYTOPLASM	Hb
Pro-Erythroblast (Pro-normoblast)	20 µm	Large nucleus Two or more nuclei	Basophilic (RNA)	-short -synthesis don't appear
Basophil Erythroblast (early normoblast)	15 µm	Nucleoli disappear	Basophilic	Start appearing
Polychromatic erythroblast (intermediate normoblast)	10 – 12 µm	Nucleus present	Both stains bcz Hb and RNA present	Start increasing
Orthochromatic erythroblast (Late normoblast)	8 – 10 µm	Inkspot nucleus (Pyknosis) Nuclear extrusion	Almost acidophilic (due to Hb)	Present
Reticulocyte	Larger than mature RBC		Majorly acidophilic due to Hb	Present

RETICULOCYTE

- At Reticulocyte stage, RBC enter into circulation
- Normal Reticulocyte count = less than 1%
  In newborn baby = 2 – 6 %
- Reticulocytes enter into circulation by diapedesis
- Reticulocytes show citric acid cycle and oxidative phosphorylation
- Remain in bone marrow for 1 – 2 days, then enter the blood and lose mitochondria and ribosomes

- SIGNIFICANCEOF RETICULOCYTE COUNT
  The number of reticulocytes in the peripheral blood increases in hemolytic disease of newborn

FACTORS AFFECTING ERYTHROPOIESIS

1. Hypoxia – stimulates erythropoietin
2. Anemia
3. High altitude
4. Cardiac failure
5. Lung disease

ERYTHROPOIETIN

- Hormone
- Glycoprotein

- Molecular weight = 34000
- Site of production:
  1. Kidneys (90%) – Fibroblast cells surrounding tubules in cortex and medukka
  2. Liver (10%)

Stimulus for Erythropoietin

1. Hypoxia in kidney
2. Hypoxia in other organs
3. Epinephrine, norepinephrine, prostaglandins

After stimulus, erythropoietin production takes place within minutes. Erythropoiesis now occurs in 5 days.

Effect of Erythropoietin

1. Production of proerythroblast from hemopoietic stem cells
2. Causes cells to rapidly pass through subsequent stages (act as catalyst)
   Hypoxia → Erythropoietin → Erythropoiesis → Increase in RBCs → Hypoxia Relieved

FACTORS AFFECTING ERYTHROPOIETIN

1. Tissue oxygenation
2. Nutritive factors like Vitamin C, Vitamin B12, Folic acid
3. Growth inducers such as interleukin-3
4. Differentiation inducers – help in Vitamin B_{12} absorption
5. Proteins – supply amino acids
6. Intrinsic factor
7. Extrinsic factor
8. Hormones such as testosterone, ACTH and thyroid hormones
9. Minerals such as iron, cobalt (is a part of Vitamin B_{12},) Cu, Ni, Mn

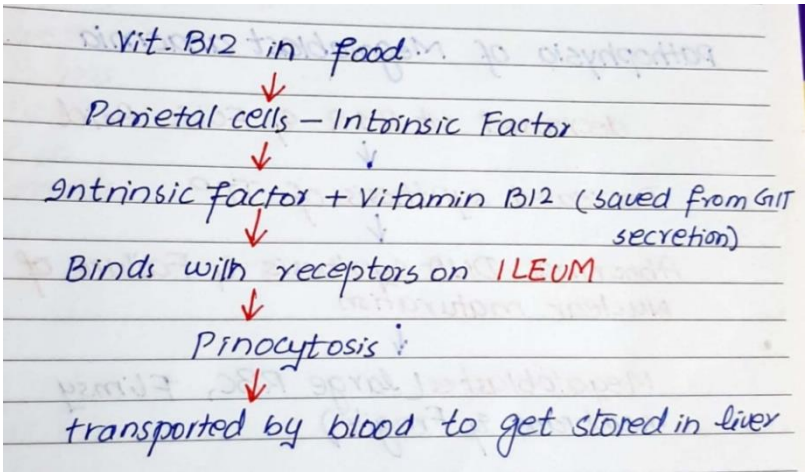
VITAMIN B_{12}

- Important in pyrimidine synthesis (thymidine triphosphate) – involved in cell division of RBCs
- Delay in nuclear maturation and cell division
- Normal requirement per day = 1 – 3 µg
- Storage in liver is 1000 times extra
- Vitamin B_{12} deficiency manifests in 3 – 4 years

CAUSES OF VITAMIN B_{12} DEFICIENCY

1. Dietary
   - Pure strict vegetarians
2. Gastric Factors
   - Gastrectomy
   - Pernicious anemia

ABSORPTION OF VITAMIN B_{12}



PERNICIOUS ANEMIA

Deficiency of intrinsic factor leads to anemia

CAUSES

1. Autoimmunity (most common cause) – Autoimmune antibodies are formed that destroy parietal cells
2. Severe gastritis
3. Ulcer
4. Gastrectomy
5. Sprue

FOLIC ACID

- Involved in DNA methylation
- Deficiency of folate causes megaloblastic anemia

SOURCES OF FOLIC ACID

- Green leafy vegetables
- Bread
- Beef
- Beans
- Pasta
- Rice
- cereals

PATHOPHYSIOLOGY OF MEGALOBLASTIC ANEMIA

Decreased Vitamin B_{12} and Folic acid → Decreased synthesis of TTP → Abnormal DNA synthesis and failure of nuclear maturation → Megaloblasts (large RBC, flimsy membrane and fragile)

MACROCYTE (larger than normal RBC) CAUSES:

1. Alcohol
2. Liver diseases

3. Stomach diseases causing impaired Vitamin B_{12} absorption

VITAMIN C

- Reduces ferric ion to Fe^{+2}
- Helps in iron absorption
- Deficiency causes microcytic anemia

HEMOGLOBIN

- Iron containing coloring matter of RBCs
- 100 ml of blood has 34g of Hb
- Hb level in males = 15 – 17 g/100ml
  Hb level in females – 12 – 14g/100ml
- 1g of Hb binds with 1.34 ml of oxygen
  15g of Hb binds with 20 ml of oxygen
- Oxygen binds loosely and reversibly with hemoglobin
- O_2 forms dative bond with Fe^{+2}
- Oxygen is released in molecular form, not in ionic form
- Hemoglobin act as oxygen buffer

Hb Synthesis

2 Succinyl Co-A + 2 Glycine → Pyrrole

4 Pyrrole → Protoporphyrin IX

Protoporphyrin IX + Fe^{+2} → Heme

Heme + Polypeptide → Hb Chain (α or β)

2 α + 2 β → 1 molecule of Hb (HbA)

1 Hb → 8 atoms of oxygen

1 Hb → 4 molecules of O_2

HEMOGLOBIN CHAINS

- α-chain (having 141 amino acids)
- β-chain (having 146 amino acids)
- γ-chain
- $Delta$-chain

TYPES OF Hb

- HbA (97% of normal adult Hb) → 2 α + 2 β
- HbA_2 (2.5% of normal adult Hb) → 2 α + 2 delta
- HbF → 2 α + 2 γ
- HbS → 2 α + 2 faulty β chain

HbF

- Present in fetal life
- Replaced by HbA immediately after birth
- HbF has more affinity for oxygen than maternal HbA
- Completely replaced in 6 – 10 weeks

IRON

- Total conc. = 4 – 5 g
- Hb - 65%
- Myoglobin – 4%
- Ferritin – 15 – 30%
- Daily Requirement = 30 – 60 mg
- Daily loss of iron is 0.7 mg daily
- Stored form of iron is Fe^{+3}
- Ferritin has ability to bind 4500 Fe/protein
  If iron load increases beyond normal level excess is stored as hemosiderin in liver and heart

Ferritin	Hemosiderin
Main storage form	Smaller storage form
Iron stored in small and dispersed clusters	Stored in large clusters
Seen by electron microscope	Can be seen by ordinary microscope
Fe can be removed quite easily	Fe more difficult to remove

Van Den Bergh Test

- specific test for increased serum bilirubin levels.
- Normal serum gives a negative reaction
- Van Den Bergh reagent = sulfonic acid + $NaNO_2$

ANEMIA

Deficiency of Hb in blood combined with impaired provision of O_2 to tissue

CAUSES

- Too few RBCs
- Too little Hb

SYMPTOMS AND SIGNS

- Weakness
- Fatigue
- Dyspnea (difficulty in breathing)
- Pale skin
- Conjunctiva (transparent membrane on sclera)
- Headache

CLASSIFICATION

1. Blood loss anemia
2. Nutrition deficiency
3. Aplastic anemia
4. Hemolytic anemia
5. Anemia of chronic disease

MORPHOLOGICAL CLASSIFICATION

1. Microcytic anemia
2. Macrocytic anemia
3. Normocytic anemia (less count but normal shape)

MICROCYTIC ANEMIA

- Seen in
  1. Iron deficiency
  2. Thalassemia
  3. Nutritional deficiency (Vitamin C, PLP)
  4. Lead toxicity
- Common features
  MCV < 80 μm^3
  Reason of microcytosis – size reduces to maintain its MHC
- Lab Findings in microcytic anemia
  - Ferritin – low
  - Serum iron – low
  - % saturation – low
  - Serum transferrin levels increase

BLOOD LOSS ANEMIA

- **ACUTE**: normocytic, normochromic
- **CHRONIC**: microcytic, hypochromic
  Chronic blood loss → Person can't absorb enough Fe → Decreased synthesis of Hb → Small RBCs with lesser Hb → Microcytic, hypochromic anemia
- After acute hemorrhage – blood replaces fluid of plasma in 1 – 3 days

APLASTIC ANEMIA

- Bone marrow aplasia
- Hematopoietic stem cells damaged
- Reticulocyte count low

CAUSES

- Radiation
- Chemotherapy
- Idiopathic (unknown causes)
- Toxic chemicals
- Autoimmune disorders

FEATURES OF APLASTIC ANEMIA

1. Low RBC count → anemia
2. Low WBC count → recurrent infections
3. Low platelets → brusing tendency increase

NUTRITIONAL DEFICIENCY ANEMIA

- Vitamin B_{12} deficiency anemia
- Ascorbate deficiency
- Iron deficiency anemia

IRON DEFICIENCY ANEMIA : Low availability of Fe for Hb synthesis

CAUSES

1. Blood loss
2. Increased Fe intake
3. Poor absorption of iron
4. Poor diet in children
5. Breast feeding
6. Pica
7. GIT hemorrhage
8. Increased Fe requirements

FEATURES

- Brittle nails
- Koilonychia (spoon shaped nails)
- Brittle nails

LAB FINDINGS

- Ferritin low
- Serum iron decrease
- % saturation decrease

HEMOLYTIC ANEMIA

- Abnormal RBCs
- Hereditary
- Fragile RBCs
- Sickle cell anemia

EXTRINSIC : Due to external factors causing lysis of cell

EXAMPLE:

- Anemia in erythroblastosis fetalis
- Autoimmune disorders like rheumatic arthritis

INTRINSIC: Intrinsic defect in RBC morphology leads to hemolysius

EXAMPLES:

- Sickle cell anemia
- Hereditary spherocytosis
- Hemoglobinopathies → HbS + HbC
- Thalassemia

HEREDITARY SPHEROCYTOSIS

Inherited defect of RBC cytoskeleton

Spherocytosis → Sphere shaped RBCs → Cant resist compression forces → Rupture → Hemolytic anemia

Sickle-cell anemia – subtype of hemolytic anemia

THALASSEMIA

- Inherited disorder characterized by abnormal hemoglobin
- Decreased Globin → Decreased Hb → Microcytic hypochromic anemia
- **Alpha Thalassemia**: defect in α-chain or absence of α-chain
- **Beta Thalassemia**: defect in β-chain
- **Thalassemia Minor**: symptoms are like iron deficiency anemia
  Defective or decreased synthesis of chain of Hb
- **Thalassemia Major**: complete lack of any one chain of Hb

Anemia	Hb Level (g/dL)
Grade 0	≥ 11.0
Grade 1 (mild)	9.5 – 10.9
Grade 2 (moderate)	8.0 – 9.4
Grade 3 (serious)	6.5 – 7.9
Grade 4 (life threatening)	< 6.5

POLYCYTHEMIA

Increased number of RBC

SECONDARY POLYCYTHEMIA

- RBC count = 6 -7 million/mm^3
- At high altitude
- Physiological phenomenon

POLYCYTHEMIA VERA

- 3Erythemia
- Pathological
- RBC count = 7 – 8 million/mm^3
- Hematocrit = 60 – 70%

- All cell types increase
- Blood volume increase

CAUSE

- Bone marrow damages
- Genetic aberration in hemocytoblastic cells

EFFECT ON CIRCULATORY SYSTEM

- Viscosity increase (10X)
- Entire system get engorged
- Resistance to blood flow increase

COMPLEXION IN POLYCYTHEMIA

Color of skin is due to quantity of blood in skin subpapillary venous plexus

Polycythemia → sluggish flow → large Hb deoxygenation → deoxyhemoglobin masks the red color of oxyhemoglobin → ruddy complexion with bluish tinit (cyanotic complexion)

LEUKOCYTES

- White blood cells
- Mobile units of body's protective system
- Normal value: 4000/µL – 11000/µL

CLASSIFICATION

- Granulocytes - have cytoplasmic granules that contain biologically active molecules
- Agranulocytes

GRANULOCYTES (Polymorphonuclear cells)

- Neutrophils – 62%
- Eiosinophils – 2.3%
- Basophils – 0.4%

AGRANULOCYTES

- Monocytes – 5.3%
- Lymphocytes – 30%

Sites of Leukopoiesis

1. Bone marrow produce granulocyte, monocyte
2. Lymph tissue i.e. spleen, thymus, tonsils etc produce lymphocytes and plasma cells

LIFE SPAN OF WBCs

- Granulocytes
    - In blood: 4 – 8 hrs
    - In tissues: 4 – 5 days

- Monocytes
    - In blood: 10 – 20 hrs
    - In tissues: for months
- Lymphocytes
    - In blood: few hours
    - Continually recycle

PROPERTIES

1. Diapedesis – squeezing of cels
2. Amoeboid – neutrophils and macrophages
3. Chemotaxis – inflammation release cytokines

NEUTROPHILS

- Granules take both stains
- Multi-lobed nucleus
- 10 – 12 µm in diameter
- More lobes of nucleus, more mature it is
- A single neutrophil can phagocytose 3 – 20 bacteria
- Granules have bactericidal component

PROPERTIES	NEUTROPHILS	MACROPHAGES
Types of cells	Mature cells	Derived from monocytes
Site	Blood	Tissues
Power	Less	More power
Bacteria	3 – 20 bacteria	More than 100
Life span	Days (increase in acute infection)	Weeks to months

INFLAMMATION

- Vasodilation
- Increased permeability of capillary
- Increased clotting
- Migration of granulocytes and monocytes to the site of injury
- Swelling

VASODILATION CAUSED BY

- Histamine
- Serotonin
- Bradykinin
- Prostaglandins
- Lymphokines

LINES OF DEFENCE

- FIRST LINE – Tissue macrophages
- SECOND LINE – Macrophage invasion

- THIRD LUNE – Neutrophil invasion
- FOURTH LINE – Granulocyte and monocyte

EOSINOPHILS (pink color)

- Coarse granules
- Stain pink/ red with eosin
- Bilobed nucleus
- 10 – 14 μm in diameter

FUNCTIONS

- Phagocytosis
- Combat parasitic infections e.g. schistosomiasis, trichinosis
- Kills parasites by
    - Hydrolytic enzymes i.e. lysosomes
    - Reactive O_2 species
    - Major basic protein – lavocidal
- Response to allergy
- Termination of inflammatory response reactions
- Eosinophils accumulates and causes
    - Detoxification
    - Phagocytosis
    - Destroys antigen-antibody complexes
    - Prevents spread of inflammation
- Substances released by eosinophils
    - Eosinophil peroxidase
    - Major basic protein
    - Eosinophil cationic protein
    - Eosinophil derived neurotoxin
    - Cytokines

Which granulocytes are phagocytic cells?

1. Eosinophils – weak phagocytes due to smaller size
2. Neutrophils – show phagocytosis

BASOPHILS

- Coarse granules
- Stain purple with methylene blue
- Bilobed nucleus
- 8 – 10 μm in diameter
- 0.4% of WBCs
- Mast cells present in tissue
  Basophils present in circulation
- Mechanism of action of basophil

Allergen → activation of IgE → attachment of IgE with mast cell/basophil → Degranulation of basophils

- Basophils release
  - Histamine
  - Heparin
  - Serotonin
  - Bradykinin
  - Slow releasing substance of anaphylaxis

LEUKOPENIA

- Decrease in number of WBCs
- Less than 4000/mm^3

CAUSES are bone marrow depression due to

- Hypnotics
- Thiauracil
- Chloramphenicol
- Drugs with benzene nuclei – cause aplasia
- Exposure to X-Rays

TREATMENT

- Initially, stem cells in marrow which regenerate bone marrow
- Other options – Transfusion + antibiotic

LEUKOCYTOSIS

- More than 1100/mm^3
- Protective infection
- Leukocytosis – normal WBCs increase in number
  Leukemia – cancer condition in which WBCs increase in number

CAUSES

- Chronic infection – Monocytosis
- Allergy – Eosinophilia
- Viral infections – Lymphocytosis
- Acute bacterial infection – neutrophilic leukocytosis

LEUKEMIA

- Caused by cancerous mutation of myelogenous or lymphogenous cells
- Uncontrolled cancerous production of RBCs
- CAUSES
  - Radiation
  - Chemicals
  - Viruses
  - Genetics

LYMPHOGENOUS LEUKEMIA

- Cancerous production of lymphoid cells
- Sites: Lymphoid nodes, all lymphocytic area
- Acute Lymphoblastic Leukemia
    - Young
    - Adults other than 65 years
- Chronic Lymphocytic leukemia
    - Adults over 55 years

MYELOGENOUS LEUKEMIA

- Cancerous production of myeloid cells
- Neutrophilic leukemia
- Site:
    - Bone marrow
    - Extramedullary tissues e.g. lymph nodes, liver and spleen

ACUTE LEUKEMIA

- Cells are less differentiated
- Provide no protection
- Lead to death within minutes

CHRONIC LEUKEMIA

- More differentiated cells than in acute
- Develop over years

EFFECT ON BODY

1. Metastatic growth of leukemic cells
2. Infections, anemia, thrombocytopenia
3. On bone
4. Excessive use of metabolic substrates by cancerous cell
5. Weakness
6. Death

INNATE IMMUNITY

- Natural resistance by the body
- Does not require prior exposure
- Does not improve after exposure

EXAMPLES

- Phagocytosis by WBCs
- Stomach acid
- Skin

TYPES OF ACQUIRED IMMUNITY

1. Humoral immunity (B-cell)
2. Cell-mediated immunity (T-cell)

PROPERTIES	T-CELL	B-CELL
Origin	Thymus	Bone marrow
Blood	80%	20%
Membrane receptors	TCR	BCR
Function	Cell mediated	Humoral
Diversity	Less (whole cell)	More (antibody)

PREPROCESSING

- Thymus → T cells
- Liver, bone marrow → B cells

MAJOR HISTOCOMPATIBILITY COMPLEX (MHC)

Set of cell surface proteins of acquired immune system to recognize foreign molecules and determine histocompatibility.

HLA – Human Leukocyte Antigen

FUNCTION OF MHC

- Binds antigen of pathogen and display
- Determines compatibility of transplant donor

TYPES OF T-CELLS

- Helper T cells (CD4 and MHC-II)
- Cytotoxic T cells (CD8 and MHC-I)
- Suppressor T cells
- Memory T cells

MHC-I proteins present antigen to cytotoxic T-cells

MHC-II proteins present antigen to helper T-cells

ANTIGEN PRESENTING CELLS

Antigen-presenting cells (APCs) are **a heterogeneous group of immune cells** that mediate the cellular immune response by processing and presenting antigens for recognition by certain lymphocytes such as T cells.

Antigen presenting cells include

- Macrophages (most potent)
- Dendritic cell
- B-lymphocytes

ROLE OF ANTIGEN PRESENTING CELLS

1. Phagocytosis of antigen
2. Fusion of lysosome and phagosome
3. Degradation
4. Fragments of antigen on APC surface
5. Leftover fragments released by exocytosis