Red Blood Cells (Erythrocytes)

Szilvia Benkő, PhD
Red Blood Cells (Erythrocytes)

- Main characteristics of erythrocytes
- Differentiation and maturation of erythrocytes
- Regulation of erythrocyte development
- Clinical aspects
- Blood groups (ABO, RhD)
Properties of Red Blood Cells (Erythrocytes)

1. Concentration in blood is 4-6 million/cubic mm (4-6 T/l)
   1. Gender differences
   2. High individual variability

2. Morphology: biconcave discs
   1. Large surface area
   2. Enables cells to bend in small capillaries

3. Main characteristics: reduced cell
   1. No nucleus → cannot reproduce (average life span: 120 days)
   2. No mitochondria → no metabolism
   3. No ribosomes → no protein synthesis

4. Function: transport hemoglobin (280 million hemoglobin molecules/cell)
   1. Contain high concentration of carbonic anhydrase
   2. Contain high concentration of HCO_3^-/Cl^- pump
No mitochondria

GLUT1

Red blood cells

Blood sugar level!!

Glycolysis: in the cytosol

Tricarboxylic acid (TCA) cycle: in the mitochondria

C6H12O6 + 6O2 + 32 ADP3- + 32 Pi2 → 6CO2 + 6H2O + 32 ATP4- + 32 OH
Properties of Hemoglobin

1. Structure
   1. Quaternary structure: \((\alpha_2\beta_2)\) (Foetal: \(\alpha_2\gamma_2\))
   2. Each subunit is: 1 Heme + 1 globin
   3. Each heme contains 1 iron (2+ ↔ 3+)

Heme: porphine derivative, contains an iron (Fe2+ - ferro!)
Properties of Hemoglobin

1. Structure
   1. Quaternary structure: \((\alpha_2\beta_2)\)
   2. Each subunit is: 1 Heme + 1 globin
   3. Each heme contains 1 iron \((2+ \leftrightarrow 3+)\)

2. Function
   1. Oxygen binding and transport
   2. CO\(_2\) binding and transport

3. Hemoglobin levels
   1. infants: 140-200 g/l
   2. Adult males: 140-180 g/l
   3. Adult females: 120-160 g/l

Heme: porfirine derivative, contains an iron (Fe\(2+\) - ferro!)
Adults: mainly in the flat bones such as hip bone, breast bone, skull, ribs, vertebrae and shoulder blades, and in the "spongy" material at the proximal ends of the long bones femur and humerus.

Embryo: yolk sac, liver
Development of Red Blood Cells (Erythrocytes)

- proliferate
- self-renewal

Multipotential hematopoietic stem cell (HSC)

Common myeloid progenitor (CMP)
- Erythrocyte
- Mast cell
- Myeloblast

Common lymphoid progenitor (CLP)
- Small lymphocyte
- Natural killer cell
- B lymphocyte
- T lymphocyte
- Monocyte
- Plasma cell
- Macrophage

Megakaryocyte

Thrombocytes
Erythropoiesis
Formation of erythrocytes

1. Location of erythropoiesis: Bone marrow
2. Takes 7 days
3. Rate of Erythropoiesis: 2.5 million RBC/second

- Adenylate cyclase activity drops
- Metabolism turns to anaerobic
- ATPase activity stops
- Passive transport instead of active transport
- Decreased transferrin receptor expression
- No iron uptake

Multipotent Stem cell

Proerythroblast

Early erythroblast

Late erythroblast

Normoblast

Reticulocyte

Erythrocyte

Phase 1: ribosome synthesis
Phase 2: hemoglobin accumulation
Phase 3: ejection of nucleus
Young reticulocyte
Extruded nucleus
Enucleating erythroblast
Early erythroblast
Macrophage
Nuclear phagocytosis
Late erythroblast

removing membrane MHC I

reticular (mesh-like) ribosomal RNS
Factors determining the rate of erythropoiesis

1. Erythropoietin
2. Iron
3. Vitamin $B_{12}$
4. Nutritional state of the body
Erythropoietic factors 1: Erythropoietin
The feed-back regulation of erythropoietin secretion

Erythropoietin: produced by the kidney

- ACTH
- steroids
- Androgen

Erythropoiesis (Hb synthesis)

- Anemia
  - Hystotoxic effects
  - Reduced blood flow
  - Decreased O₂ saturation in blood
  - Increased O₂ demand (Thyreotoxicosis)

Hypoxia (in the kidney)

Reduced O₂ demand (hypothyreosis)

Increased erythropoietin secretion (transcriptional regulation-mRNA)

Estrogen
Erythropoietic factors 2: Iron

1. Mixture of Fe²⁺ & Fe³⁺ is ingested
2. HCl converts Fe³⁺ to Fe²⁺
3. Fe²⁺ binds to gastroferritin
4. Gastroferritin transports iron to small intestine
5. Iron is absorbed from GI tract to blood plasma
6. Iron binds to transferrin in blood
7. Iron binds to apoferritin in liver and in enterocytes, and stored as ferritin
8. Iron is distributed to tissues

Total body iron in human adult: 3-4g. 2/3 of it is incorporated into the heme of erythrocytes.

Fe³⁺ → Fe²⁺

Fe²⁺ absorption in duodenum and jejunum

Fe²⁺ binding to gastroferritin
Erythropoietic factors 3: Vitamin B$_{12}$ (cobalamine)

- water soluble
- corrinoid ring
- similar to porfirin
- only some bacteria and protozoa are able to synthesize
  (importance of colon bacteria!)

- plants do not contain (VEGETARIANS!)
- meat, LIVER, egg, milk
- nicotine reduces the absorption (smoking!)

- daily requirement: 1-2ug
- stored in liver (3-6 years)
Erythropoietic factors 3: Vitamin B$_{12}$ (cobalamine)

- **R-protein** (from saliva and stomach)
- **Intrinsic factor** (from stomach)
- **Transcobalamin II**

**Pancrease protease**

**dimers**

**distal ileum**

Transports into and out of the blood stream goes through *the enterocytes by receptor-mediated endocytosis (recognizes dimers!)*
folic acid (folate, vitamin B\textsubscript{9})

RNA [Uracil] \{protein synthesis\} \rightarrow DNA (Thymine) \{Cell division\}

Adenine

Thymine (dTMP)
Complete Blood Count (CBC)

- Determination of the number of red blood cell count
- One of the most routinely performed clinical tests. Different disorders can have dramatic effect on the total number or relative proportions of blood cells
- Can be determined only from blood samples taken from big veins
- Can be performed manually (hemocytometer) or with electric counter
## Complete Blood Count (CBC)

<table>
<thead>
<tr>
<th></th>
<th>Percentage</th>
<th>Cell number/mm$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WHITE BLOOD CELLS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>leukocytes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>neutrophil granulocytes</td>
<td>40 – 74</td>
<td>1.9 – 8 x 10$^9$</td>
</tr>
<tr>
<td>eosinophil granulocytes</td>
<td>0.1 – 5</td>
<td>0.01 – 0.6 x 10$^9$</td>
</tr>
<tr>
<td>basophil granulocytes</td>
<td>0.1 – 1.5</td>
<td>0.01 – 0.2 x 10$^9$</td>
</tr>
<tr>
<td>lymphocytes</td>
<td>19 – 41</td>
<td>0.9 – 4.4 x 10$^9$</td>
</tr>
<tr>
<td>monocytes</td>
<td>3.4 – 9</td>
<td>0.16 – 0.9 x 10$^9$</td>
</tr>
<tr>
<td><strong>RED BLOOD CELLS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>erithrocytes</strong></td>
<td></td>
<td>4.2 – 6.1 x 10$^{12}$</td>
</tr>
<tr>
<td><strong>PLATELETS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>thrombocytes</td>
<td></td>
<td>150-400 x 10$^9$</td>
</tr>
</tbody>
</table>
**Hematocrit**

**Definition:**
Percentage of whole blood occupied by packed red blood cells

**Normal values:**
- Males: 46% (40-54)
- Females: 42% (37-47)

**Determination:**
centrifuging a blood sample, so that formed elements come out of suspension

**Indicates:**
- Anemia (low Hct)
- Polycythemia / dehydration (high Hct)

\[
\frac{36 \text{ mm}}{80 \text{ mm}} \times 100 = 45\%
\]
DEFINITIONS

*Haematocrit:* volume of cells / total volume ~0.37 - 0.52

*MCV* (mean corpuscular volume): haematokrit / RBC count  
\[ 80 - 100 \text{ fl } (10^{-15}) \]

*MCH* (mean corpuscular hemoglobin): Hb concentration / RBC count  
\[ 27 - 31 \text{ pg/RBC} \]

*Stain index:* Hb% / RBC%  
Hb\%= Hb concentration actual / Hb concentration standard  
RBCt \%= RBC count actual / RBC count standard

Fl= 1 normochrom
Fl<1 hypochrom (pl. iron deficiency anemia)
Fl>1 hyperchrom (pl. B_{12} vitamin deficiency anemia)
Pathologic aspects of RBC number (pathophysiology)

- Increased RBC: **Polycythemia**

- Decreased RBC: **Anemia**
  - Decreased erythropoiesis
    - Iron deficiency
    - B$_{12}$ deficiency
    - Aplastic anemia / bone marrow diseases

- Increased erythrolysis
  - Hemolysis
  - Hepatosplenomegaly

- Bleeding
  - Acute (injury)
  - Chronic (GI bleeding)
  - Menstruation
  - Pregnancy / delivery
Polycythemia

- Elevated hematocrit with a normal blood volume

- Pathomechanism:
  - Primary:
    Due to uncontrolled erythropoiesis in bone marrow (Polycythemia Rubra Vera) or cancer
  - Secondary:
    Caused by pathologically increased erythropoietin production in the kidney (i.e. hypoxia due to reduced local perfusion)

- Impact on circulation:
  increased hematocrit and TPR (Total Peripheral Resistance)

- Therapy:
  - Traditional (only historical aspects):
    removing/diluting blood
  - Current:
    elimination of the pathologic stimuli and/or suppression of erythropoiesis
Clinical classification of anemias (based on chromic index)

1. Hypochromic (iron dependent or hypochromic microcyter anemia)
2. Hyperchromic (pernicious anemia)
3. Normochromic (aplastic anemia)
4. Other types
Anemia 1: Hypochromic microcyter anemia

- Pathomechanism: **Iron deficiency**
  - Iron deficit or malabsorption
  - Increased iron requirement (pregnancy, lactation, rapid growth phase in children)

- Symptoms: unspecific

- Lab. Findings:
  - Decreased HCT, Hgb, MCV (mean corpuscular volume) – cell size, MCH (mean corpuscular hemoglobin) – amount of Hgb,
  - Low ferritin and iron level in serum
  - Increased Total Iron Binding Capacity (TIBC) in the blood - transferrin

- Therapy: Oral iron supplement
  (food: Total body iron in human adult: 3-4g. 2/3 of it is incorporated into the heme of erythrocytes.)
Anemia 2: Hyperchromic (megaloblastic)

- Pathomechanism:
  - $B_{12}$ (very rarely folic acid) **deficiency**
  - Intrinsic factor deficiency (there is NO primary $B_{12}$ deficiency)
    - Primary
    - Secondary (hypacidity, anacidity, alcoholism, tumor.. etc)

- Symptoms:
  - Neurological: neuritis, dementia
  - Non specific: weight loss, pale and dry skin, diarrhea, soreness of the tongue, tendency for hemolysis

- Lab. Findings:
  - Decreased (sometime extremely low) RBC number, HCT, Hgb
  - Increased MCV, MCH
  - In peripheral smear: Macrocytes

- Therapy:
  - Parenteral (!!!!!!) $B_{12}$ supplement
  - Folic acid, iron, Vitamin C……
Anemia 3-4: 
Normochromic and other types

- **Hemorrhagic**: Acute or chronic blood loss (GI/urinary tract .. etc)

- **Hemolytic**: Life span of RBCs is reduced
  - Hemoglobin abnormalities
  - Bacterial/viral infections, autoimmune disease
  - Toxins

- **Aplastic**: Reduced hemopoiesis due to bone marrow disease
  - Tumor/immune disease
  - Destruction of bone marrow by infection, radiation, drugs or toxins

- **Sickle cell anemia**: Mutation in the gene for the β chain of hemoglobin
  - Due to the mutation Hgb link together forming stiff rods at low $O_2$ of pH → sickle cell formation
  - increased malaria resistance

- **Thalassemia**
  - reduced rate of synthesis or no synthesis of one of the globin chains due to mutation (deletion) in regulatory region of Hgb genes
  - High incidence in the Mediterranean region
  - increased malaria resistance
Blood groups
Common blood group systems

1. ABO
2. Rh
3. MNS (M+ & N+)
4. Kell (K+ & K-)
5. Lewis (Le^a & Le^b)
6. ....... etc
Terminology

- **Blood type (blood group):** A classification of blood based on the presence or absence of inherited antigenic substances (proteins, carbohydrates, glycoproteins, or glycolipids) on the surface of red blood cells.

- **Agglutination:** clumping red blood cells as a result of mixing of samples from incompatible blood groups (precipitation, coagulation)

- **Agglutinin:** a substance that causes particles to coagulate to form a thickened mass *(antibody)*

- **Agglutinogen:** a substance that, acting as an *(antigen)*, stimulates the production of agglutinin

- **Transfusion:** It is the most frequent type of organ transplantation
AB0 system

- **H-gene:**
  Codes for \textit{H-transferase}, puts \textit{fucose} on galactose
  it is \textit{common} in each blood type
  fucose is a requirement

- **A-gene:**
  Codes for a specific transferase to put \textit{N-acetyl-galactosamine} to galactose

- **B-gene:**
  Codes for a specific transferase to put \textit{galactose} to galactose

- **0-gene**
  Codes for an inactive „enzyme”, no additional carbohydrate on galactose

**Phenotype (genotype)**
- A (AA or A0)
- B (BB or B0)
- AB (AB)
- 0 (0)

\textit{Codominant inheritance}
Structures of the ABO blood group antigens

Defined by specific enzymes inherited co-dominant genes (Mendelian rules)

- **Fuc** - Fucose
- **GalNAc** - N acetyl-galactosamine
- **Gal** - Galactose
- **Glu** - Glucose

RBC
<table>
<thead>
<tr>
<th>RECIPIENT blood type (serum - antibody!!)</th>
<th>DONOR blood type (RBC - antigen!!)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>O</strong></td>
<td><strong>O</strong></td>
</tr>
<tr>
<td><img src="image1" alt="Image of hemolyzed RBCs" /></td>
<td><img src="image2" alt="Image of hemolyzed RBCs" /></td>
</tr>
<tr>
<td><strong>A</strong></td>
<td><strong>A</strong></td>
</tr>
<tr>
<td><img src="image3" alt="Image of RBCs in hemolyzed state" /></td>
<td><img src="image4" alt="Image of RBCs in hemolyzed state" /></td>
</tr>
<tr>
<td><strong>B</strong></td>
<td><strong>B</strong></td>
</tr>
<tr>
<td><img src="image5" alt="Image of RBCs in hemolyzed state" /></td>
<td><img src="image6" alt="Image of RBCs in hemolyzed state" /></td>
</tr>
<tr>
<td><strong>AB</strong></td>
<td><strong>AB</strong></td>
</tr>
<tr>
<td><img src="image7" alt="Image of RBCs in hemolyzed state" /></td>
<td><img src="image8" alt="Image of RBCs in hemolyzed state" /></td>
</tr>
</tbody>
</table>

- haemolysis
- kidney failure
- death
Rh system
Two pair of genes with multiple allele combination

Antibody production is induced only when an RhD-RBC enters the blood stream!!!
(difference from AB0-system)

PROBLEM: - Rh- gets Rh+ blood
- pregnancy
Rh phenotypes

1. Rh+: characterized serologically by their strong reactivity with monoclonal anti-D antibody.

2. Rh-: absence of reaction with anti-D antibody.
   - C or E antigens in Rh- negative person can still cause mild transfusion reaction

3. Many transitional forms between Rh+ and Rh- were reported:
   - Weak D
   - Partial D (D mosaic)
   - D epitope expressed on RhCE
   - Etc.....
Agglutinins of the Rh system

1. No anti-D antibodies are found in the plasma of a Rh- person’s plasma prior to transfusion of Rh+ RBCs

2. Production of anti-D antibody is proportional with the number of transfusions with Rh+ blood/RBCs

3. Anti-D antibody (IgG) can diffuse through placenta causing RBC agglutination/hemolysis (Erythroblastosis fetalis)
**Rh blood groups**

**Rh+**: D antigen / no anti-D antibody

**Rh-**: no D antigen / originally no anti-D antibody
- produced at **first** immune response
- can not penetrate placenta

- produced at **second** immune response
- can penetrate placenta
Transfusion rules

- Whole blood is very rarely used for transfusion
- Only the same AB0/Rh type blood can be used for transfusion
- **No Universal donor or Universal acceptor**