BLOOD CELLS

HEMOPOIESIS

ERYTHROCYTES
  Erythropoiesis
  Role of iron
  Characteristics of the erythrocyte
    Dimension, number, composition, lifespan
  Blood groups

HEMOGLOBIN
  Synthesis
  Compounds
  Dissociation curve

TRANSPORT OF THE OXYGEN IN THE BLOOD
TRANSPORT OF CARBON DIOXIDE IN THE BLOOD

LEUCOCYTES

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  Eosinophils
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  Monocytes
  Lymphocytes
    T cells
    B cells
    The role of the lymphocytes in the defense of the body
    Immunity

PLATELETS

HEMOSTASIS

COAGULATION

FIBRINOLYSIS
BLOOD CELLS (formed elements)

There are many types of formed elements in the blood

- red blood cells (erythrocytes)
- white blood cells (leucocytes)

The production of formed elements is called hemopoiesis

Before birth, hemopoiesis occurs primarily in the liver and spleen, but some cells develop in the thymus, lymph nodes and red bone marrow.

After birth most production is limited to red bone marrow in specific region, but some white blood cells are produced in the lymphoid tissue.

All types of formed elements develop from a single multipotential cell called stem cell or hemocytoblast

Seven different cells, each controlled by a specific growth factor, develop from the stem cell.
When a stem cell divides, one of the daughters remains stem cell and the other becomes a precursor cell (progenitor cell, committed cell) either a lymphoid or a myeloid cell.

These cells continue to mature into various blood cells.
ERYTHROCYTES (red blood cells)

In adults erythrocytes are formed in the red bone marrow in a process called erythropoiesis.

Erythropoiesis is controlled by erythropoietin a hormone synthesized by kidney in response to decreased oxygen in the blood. Erythropoietin stimulates the bone marrow to produce more red blood cells.

An example is the increase of the number of erythrocytes at high altitude.
There are several steps from stem cell to adult red cell:

In the process an important role plays the iron.

All body cells need iron for energy production and cellular growth and proliferation. In the red blood cell the iron is a component of hemoglobin, the main transporter of oxygen.

The human body contains only 3-4 g of iron. From diet we obtain 10-20 mg daily and absorb 1 mg in the duodenum and upper jejunum. Entering the bloodstream the iron is transported by a globulin transferrin.

75% of absorbed iron is bound to hemoglobin. 10-20% goes into a storage pool as ferritin. There is no physiologic mechanism for excretion.
Daily Diet contains 10-20 mg iron

Absorb 1-2 mg iron/day

TRANSFERRIN (transports iron)

Lose 1-2 mg iron/day from desquamation of epithelia

75%

10-20%

5-15%

Other Processes

FERRITIN (stores iron in liver & heart)

Hemoglobin/Erythropoiesis

No Physiologic Excretion Mechanism
The best source of iron is lean red meat. Iron can also be found in chicken, turkey, eggs, and cereals.
The normal serum iron values

men         65-175 µg/100 ml
women       50-170
children    50-120
clinical symptoms in iron deficiency
30% of global population

- Lethargy
- Fatigue
- Malaise/depression
- Angina
- Impaired cognition
- Impaired immune system
- Anorexia
- Intolerance to cold
- Endocrine/metabolic abnormalities
- Cardiorespiratory disturbances
- Gastrointestinal disturbances
- Tendency toward bleeding
- Reduced exercise tolerance
- Weakness
- Shortness of breath
- Exertional chest pain
- Impaired concentration
- Impaired libido/impotence
- Insomnia
- Headache
- Pallor
- Neuromuscular disturbances
- Cutaneous disturbances
- Musculoskeletal symptoms
- Pruritus
In addition to iron for a normal erythropoiesis are necessary vitamin B12, folate (vitamin B9) and copper.

**Vitamin B12**

Food sources of vitamin B12:
- Eggs
- Meat
- Poultry
- Shellfish
- Milk
- Milk products

**Vitamin B9 (Folate)**

Food sources of folate include:
- Beans and legumes
- Citrus fruits and juices
- Whole grains
- Dark green leafy vegetables
- Poultry
- Pork
- Shellfish
- Liver
Spinach is rich in folate and iron but the absorption of iron is hampered by the presence of oxalate.

Ceruloplasmin is a multicopperoxidase, synthesized and secreted in the plasma by the liver, that helps the iron oxidation \((Fe^{2+} \rightarrow Fe^{3+})\) and fixation to transferrin.
The erythrocytes have a characteristic form of a disc with a diameter of 7 micrometers and a thickness of 2 micrometers.

The volume 85 $\mu m^3$
The surface 125 $\mu m^2$
their total surface 3000 $m^2$
The number of erythrocytes is 4-6 millions/mm\(^3\) or 4-6.10\(^{12}\)/L.

There are physiologic variations of their number according:

- **age** (greater in newborns)
- **sex** (greater in males)
- **altitude** (greater at high altitudes)

The reduced number of erythrocytes is a characteristic of anemias.

**Poliglobulina** is characterized by a higher number of erythrocytes.

The aspect of erythrocytes can be studied on blood smears using an optic microscope or on special preparations using scanning electron microscope.

Modification of the normal size and shape of the erythrocytes can be observed in different categories of anemia.

- **Anisocytosis** - variable size of erythrocytes
- **Poikilocytosis** - variable shape of erythrocytes
aspect of normal erythrocytes

optic microscopy

scanning electron microscopy
anisocytosis

Poikilocytic red cells (elliptocytes¹, schistocytes², target cells³, tear drop⁴, spherocytes⁵ & hypochromic⁶) usually present in Thalassemia Major.
Erythrocytes are highly deformable due to the visco-elastic properties of their membrane. This is important for the normal circulation of blood in small vessels.

The erythrocyte composition:
- 57% water
- 33% hemoglobin (30 pg)
- 7% lipids
- 3% sugars

An important role in the metabolic activity of erythrocytes play carbonic anhydrase and 2-3 diphosphoglycerate.
Lifespan 120 days
Young erythrocytes are called reticulocytes
5-15/1000 erythrocytes

A proportion of 1% of erythrocytes are destroyed daily

The destruction is made by agglutination and hemolysis (rupture of the cell membrane)
On the surface of erythrocytes exist antigenic determinants (antigens) that react with some antibodies (agglutinins).

Based on the types of antigenic determinants and agglutinins, Landsteiner classified the 4 main blood groups 0, A, B and AB.
Later was discovered the Rh antigen that is present in the erythrocytes of 85% of white population (Rh +) or over 96% in colored population.

Rh antigen has many components C,c,D,d,E,e

The most powerful is D antigen, the presence of D antigen or a combination of 2 antigens (one being D) conferring the status of Rh+.

The presence of the other antigens C,E,c,d,e confers the status of Rh-

Persons Rh- are at risk in 2 cases:

- a second transfusion with Rh+ blood
- a mother Rh- with a fetus Rh+

In both cases the Rh- persons develop anti D antibodies that react with Rh+ erythrocytes causing their destruction.

The blood transfusion is permitted only with Rh and ABO compatible blood.
In order to prevent this situation the mother have to receive Rh immune globulin that destroys the Rh+ erythrocytes that entered in her bloodstream before she was able to produce her own antibodies.
HEMOGLOBIN (Hb) is a red colored conjugated protein made-up of globin and heme.

Globin consists of 4 polypeptide chains alfa, beta, gamma and delta synthesized in ribosomes.

According to the kind of polypeptide chains there are 3 types of hemoglobin:
- A1  2 alfa, 2 beta
- A2  2 alfa, 2 delta
- F    2 alfa, 2 gamma
The synthesis of polypeptide chains is different in fetus and adult.

The process is controlled by genetic mechanisms.
Disorders of these mechanisms cause modifications of the structure of polypeptidic chains and as a consequence modifications of the hemoglobin structure, its capacity to carry oxygen and of the shape of erythrocytes.

The disorders are known as hemoglobinopaties. There are 2 major types of hemoglobinopaties:
- Thalasemia
- Sickle cell disease
Thalasemia is caused by deficiencies in the production of globin chains. The suppression of beta chains is followed by the presence of hemoglobin A2 and F, anemia due to a marked destruction of erythrocytes and an inefficient erythropoiesis.

The disease is inherited and is present especially in the mediterranean zone.
Sickle cell disease is caused by abnormalities of the structure of globin chains, where an aminoacid is replaced by another one.

In the beta chain, the glutamic acid (position 6) is replaced by valine forming a special hemoglobin called S (sickle). The erythrocytes are sickle shaped and their resistance is reduced.
Heme is a tetrapyrolic structure, synthesized in mitochondria, that binds iron facilitating the oxygen transport.

The combination of globin with heme occurs in the cytoplasm of the red blood cells. Each erythrocyte contains 300 million Hb molecules. The total amount of Hb in the human blood is ~15 g/100 mL. 1 g of Hb can combine with 1.34 mL O₂
Red blood cells destruction occurs primarily in the spleen, liver and bone marrow by rupture of the cell membrane.

The Hb is degraded to heme and globin that are recycled.

The heme is degraded to iron and biliverdin. Iron is recycled and biliverdin is reduced to bilirubin that is bound to albumin forming a complex albumin-unconjugated bilirubin.

In the liver this complex is broken and bilirubin is recombined with glucuronic acid to form conjugated bilirubin that is excreted into the bile.

In the small intestine the conjugated bilirubin is transformed in stercobilinogen and stercobilin.

Part of them is excreted and the remainder is reabsorbed into the circulation and recycled in the liver or excreted by the kidneys as urobilinogen and urobilin.
Total amount of bilirubin in the blood
0.8 mg/100 mL
When the quantity is over 2mg/100 mL the skin and conjunctiva are colored in yellow a characteristic of icterus or jaundice

The jaundice is produced by:
- **excessive hemolysis** (prehepatic)
- **hepatic diseases** (hepatic)
- **obstacles on biliary tract** (posthepatic)
Icterus

Schematic Representation of Normal Bilirubin Metabolism in the Liver

Increased Bilirubin Production Beyond the Liver's Capacity to Conjugate It

Normal

Prehepatic

Decreased Bilirubin Uptake by Liver Cells

Obstruction Somewhere in the Biliary Network (Intrahepatic or Extrahepatic)

Hepatic

Posthepatic
The compounds of hemoglobin:
- oxyhemoglobin $O_2Hb$
- reduced hemoglobin $HHb$
- carbamino-hemoglobin $CO_2Hb$
- carboxyhemoglobin $COHb$
- methemoglobin $metHb$
- sulfhemoglobin $SHb$

OxyHb is a labile combination of Hb with oxygen, each Hb molecule being able to bind up to 4 oxygen molecules. Represents the major form for oxygen transport in the arterial blood.
HHb is the reduced form of Hb when a molecule of oxygen is lost (deoxyHb).

\[ \text{O}_2\text{Hb} \leftrightarrow \text{HHb} + \text{O}_2 + \text{K}^+ \]

HHb is present in the venous blood in a quantity of \(~3 \text{ g/100 mL}\).

Values greater than 4-5 g/100 mL produce a bluish coloration of the lips and fingers known as cyanosis.

Low oxygen levels in the blood cause the lips, fingers, and toes to look blue (cyanotic)
The labile combination of Hb with CO₂ constitutes carbaminoHb, one of the form of CO₂ transport in the blood.

MetHb is the result of oxidation of iron in the Hb molecule from ferrous state (Fe²⁺) to ferric state (Fe³⁺) and cannot bind oxygen.

Up to 3% of total Hb is converted to metHb even in normals. Values greater than normal contribute to the apparition of cyanosis.

The combination of Hb with CO results in production of carboxyHb, a more stable form because of the great affinity of Hb for CO. In normals the concentration of carboxyHb in blood is under 1%.

People who live in the cities have a higher level of carboxyHb and those who smoke have concentrations of carboxyHb of ≈ 5%.

When carboxyHb reach concentrations of 10% judgement is impaired, at 50% unconsciousness is a real risk and death can occur.

To withdraw the CO from the combination with Hb the administration of O₂ is necessary.
possible sources of CO in the house

dangerous substances for smokers
A characteristic of CO poisoning is the redish coloration of the skin.

SulfHb is a stable combination of Hb with sulfur that cannot carry O$_2$. The apparition of such combination is possible when Hb is exposed to some drugs (phenacetin, sulfonamides).
There is a special relationship between Hb and $O_2$ according its partial pressure.

![OxyHemoglobin Dissociation Curve](image)

The total amount of oxygen in the arterial blood is $\approx 20$ mL/100 mL.

The saturation of Hb in the arterial blood is $\approx 98 \%$ because some venous blood enters in the arterial blood.

There are factors that influence the dissociation curve of oxyHb.
A shift to right of the curve means more oxygen available for the tissues. A shift to left means more oxygen bound to the Hb.
Hb plays an important role as a buffer when binds or loses oxygen.
In the arterial blood at pH 7.4 O$_2$Hb has a negative charge (weak acid) and binds base (K$^+$)

The reaction is inverse in the venous blood.

$$O_2Hb \leftrightarrow HHb + O_2 + K^+$$

One mol of Hb can bind 0.6 – 0.7 mEq H$^+$

$$CO_2 + H_2O \leftrightarrow HCO_3^- \leftrightarrow H^+ + CO_3^{2-}$$
Transport of oxygen in the blood.

A small part of $O_2$ (0.3 mL%) is dissolved in the plasma.
The most important part of $O_2$ is bound to the Hb.

1 g Hb binds 1.34 mL $O_2$
15 g Hb/100 mL bind 20 mL $O_2$
In 1 L of blood there are 200 mL $O_2$
In 5 L of blood there is 1 L $O_2$

A greater quantity of $O_2$ is obtained by the increase of the cardiac output (normally 5 L/min) and not by the increase of the quantity of $O_2$ bound to Hb.
- Transport of CO₂ in the blood.

CO₂ is more soluble than O₂ that is why in the plasma there is dissolved a quantity of 2.9 mL CO₂/100 mL (7-8%).
The remaining quantity is both in plasma and red blood cells as:
- HCO₃⁻ (80%)
- carbamino forms (especially carbaminoHb 5-10%)

The transfer of O₂ and CO₂ in the tissues

Metabolic activity of the tissues produces a great amount of CO₂ that enters in the blood according the pressure gradient.
In the plasma the CO₂ exists as:
- dissolved
- carbamino forms with plasma proteins
- HCO₃⁻ and HCO₃Na

A part of dissolved CO₂ enters the red blood cells where is coupled with Hb forming carbaminoHb.
The O₂ lost by oxyHb enters the plasma and
then goes to the tissues.

Another part of CO$_2$ reacts with water, in the presence of an enzyme (carbonic anhydrase CA) that facilitates the process, forming HCO$_3^-$H.

CA

\[
\text{HCO}_3^- \leftrightarrow \text{HCO}_3^{-} + \text{H}^+
\]

A great part of HCO$_3^-$ migrates in the plasma in exchange with Cl$^-$ (chloride shift).

As a result of these reactions in the 100 mL of arterial blood there are 19 volumes of O$_2$ and 48 volumes of CO$_2$.

In the venous blood there are 14 volumes of O$_2$ and 52 volumes of CO$_2$. 
LEUCOCYTES (white blood cells)

Leucocytes are nucleated blood cells involved in the defense mechanisms of the body. They can be classified according different criteria:

the form of nucleus:
- polymorphonuclear cells (PMN)
- mononuclear cells

the presence of granulations:
- granulocytes
- agranulocytes

their functions:
- phagocytes
- immunocytes

The most used classification is according morphologic criteria based on the microscopic aspects of leucocytes studied on stained blood smears:

**polymorphonuclear cells** or **granulocytes**
(neutrophils, eosinophils, basophils)

**mononuclear cells** or **agranulocytes**
(monocytes, lymphocytes)

neutrophils, eosinophils, bazophils and monocytes have a common origin and are phagocytes

lymphocytes are immunocytes
The number of leucocytes in the normal blood

\[6000 - 8000 / \text{mm}^3\]

The formation of leucocytes take place in the bone marrow from a stem cell, similar to that for erythrocytes, and is called leucopoiesis.
Leucopoiesis is stimulated by several growth factors or colony stimulating factors (CSF) acting on the different cell lines.

These factors belong to the group of cytokines and are also known as interleukins (IL).

<table>
<thead>
<tr>
<th>Hematopoietic growth factors and their target cells</th>
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<tbody>
<tr>
<td>Type of growth factor</td>
</tr>
<tr>
<td>---------------------------------------------------</td>
</tr>
<tr>
<td>Erythropoietin (EPO)</td>
</tr>
<tr>
<td>Granulocyte colony-stimulating factor (G-CSF)</td>
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<tr>
<td>Interleukin-4</td>
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<tr>
<td>Interleukin-7</td>
</tr>
<tr>
<td>Interleukin-3</td>
</tr>
<tr>
<td>Granulocyte-macrophage colony-stimulating factor (GM-CSF)</td>
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<tr>
<td>Interleukin-5</td>
</tr>
<tr>
<td>Interleukin-2</td>
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<tr>
<td>Interleukin-6</td>
</tr>
<tr>
<td>Thrombopoietin</td>
</tr>
<tr>
<td>Macrophage colony-stimulating factor (M-CSF)</td>
</tr>
</tbody>
</table>
The granulocytes (PMN) have lysosomal granules in their cytoplasm and a lobed nucleus. There are 3 types of PMN according the affinity of the granules for different stains.

NEUTROPHILS (60% of the white cells) take the name from the staining properties of their cytoplasmic lysosomal granules. They have no special affinity for either acidic or basic stains but are stained mildly by both. Nuclei of mature neutrophils are elongated and pinched into several lobes. Diameter 12 micrometers.
Neutrophils, like the other granulocytes, arise in the bone marrow from myeloblasts and pass through several steps.

Neutrophils are involved in engulfing and destroying foreign invaders, like bacteria (search and destroy) using the powerful enzymes of the lysosomal granules. They play an important role in the inflammation being attracted by the substances liberated in the tissues by the intruders.

The activation of neutrophils is aided by IL1.
EOSINOPHILS (3 % of white blood cells) have granulations which stain red with acidic stains such as eosin.

Eosinophils are involved in allergies and increase in number during parasitic worm infections.
BASOPHILS (1% of white blood cells) have granulations which stain blue with basic stains.

Basophils produce heparin that prevents the blood clotting and are involved in the inflammation and allergies (bronchial asthma) due to the substances (histamin) that contract smooth muscles of the airways.
The white cells without visible granules are known as agranulocytes: monocytes and lymphocytes.

MONOCYTES (6% of white cells) are the largest of the leucocytes, diameter 20 micrometers, with a large unlobed kidney-bean or bent horseshoe shape nucleus.

Monocytes interact with lymphocytes to recognize and destroy foreign bodies. When stimulated monocytes are transformed in macrophages, giant cells with a diameter of 200 micrometers, powerful phagocytic cells.
Monocytes produce several substances with important roles in the immune response of the body (interleukins, interferons, growth factors, toxic radicals) prepare and present the antigens to the lymphocytes simultaneously with the major histocompatibility complex (MHC)
LYMPHOCYTES (30% of the white cells) are small cells, diameter 7-8 micrometers, with a round nucleus surrounded by a thin rim of cytoplasm.

Lymphocytes play a crucial role in the immune response.
There are 2 types of lymphocytes:
- T lymphocytes or T cells
- B lymphocytes or B cells

Both types are produced in the bone marrow and can be differentiated only on the basis of their specific markers (CD) and not on the basis of their morphology.
T cells are so called because they mature in the thymus and are instructed to recognize different antigens presented by the antigen presenting cells.

There are several types of T cells:

- **T helper/inducer cells** that have a CD4 marker and stimulate the activity of B cells, cytotoxic T cells and suppressor T cells.
- **T amplifier cells** have also a CD4 marker and stimulate the activity of cytotoxic T cells and that of natural killer T cells.
- **T suppressor cells** have a CD5 marker and reduce the activity of killer T cells.
- **T cytotoxic or killer cells** have a CD8 marker and are able to kill all cells recognized as foreign or non-self (cancer cells, virus infected cells, transplanted cells) secreting specific substances (perforins, tumor necrose factor, interferons).
- **T natural killer cells** are considered front line soldiers that kill immediately any non-self cell, even without the action of B or other T lymphocytes.

B cells remain in the bone marrow for maturation and when stimulated are transformed in plasmocytes, cells able to secrete antibodies, immunoglobulins that react with specific antigens.
The study of the proportion of different types of leucocytes is made on a stained blood smear using optic or electron microscopes or automate devices with special laser scatter detectors.
Electron micrograph showing an erythrocyte, a platelet and a leucocyte

Automate classification of leucocytes using a laser scatter detector
The percentage of different types of leucocytes constitutes the leucocytic formula or leucogram.

- **N**eutrophils: 60%
- **L**ymphocytes: 30%
- **M**onocytes: 6%
- **E**osinophils: 3%
- **B**asophils: 1%

To memorize easier:

Never Let Monkeys Eat Bananas
The role of leucocytes in the defense of the body

There are 3 lines of defense against pathogens (bacteria, fungi, viruses).

The first line of defense is represented by the surface coverage.

The skin acts as a physical barrier and defeat most pathogens, since they are not able to penetrate and enter easily the body at this level.

The leaks in the protective barrier:
- digestive openings
- reproductive openings
- respiratory openings
- sensory organs

At the level of mucous membranes there are cilia, hairs, sticky mucus and substances (lysozime, hydrochloric acid, acidity of vagina, urine) that stop or destroy pathogens.
The second line of defense is represented by a non-specific, immediate response involving phagocytes and some proteins. Neutrophiles, eosinophils, basophils and monocytes-macrophages (big eaters) engage in phagocytosis.

There are several proteins involved in the non-specific response.

Interferons (alpha, beta, gamma) are produced when a cell is infected by a virus and offer to other cells around a protection against the virus or against other viruses. The resistance offered by interferons is short termed.
Complement proteins are produced by the liver and after being activated they coat the surface of the microbe (opsonization) making it far easier for macrophage to identify and engulf the microbe. Activated complement proteins (membrane attack complex MAC) can form holes in the microbe cell membrane causing the death of the microbe.
Properdin interacts with microbes or fungi and together with activated complement proteins destroys them.

Lisozyme is an enzyme (present in saliva, tears) which destroys the cell membrane of some bacteria.

Reactive C protein is a globulin that activates the complement proteins, facilitates the opsonization and the phagocytosis.

PHAGOCYTOSIS is the process of engulfing of pathogens by some leucocytes.
Phases of the phagocytosis

1. Chemotaxis and adherence of microbe to phagocyte.
2. Ingestion of microbe by phagocyte.
3. Formation of a phagosome.
4. Fusion of the phagosome with a lysosome to form a phagolysosome.
5. Digestion of ingested microbe by enzymes.
6. Formation of residual body containing indigestible material.

[a] Phases of phagocytosis

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**INFLAMMATORY RESPONSE** is a major part of the non-specific defense system and is activated by any damage of the tissues produced by microbes, insects bites or physical agents.

Different effector cells secrete several mediators involved in the processes of defense of the body.

<table>
<thead>
<tr>
<th>Effector cells</th>
<th>Mediators</th>
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<tbody>
<tr>
<td>Mast cells</td>
<td>histamine&lt;br&gt;leukotrien C4&lt;br&gt;prostaglandin E2&lt;br&gt;tryptase</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>leukotriene&lt;br&gt;prostaglandin E2&lt;br&gt;ECP, EDN, MBP, MPO&lt;br&gt;TGF-β, TNF-α,&lt;br&gt;IL-5, IL-4</td>
</tr>
<tr>
<td>T-cell</td>
<td>IL-4, IL-5, IL-13, IL-10</td>
</tr>
<tr>
<td>Basophil</td>
<td>IL-13, IL-4, Histamin</td>
</tr>
</tbody>
</table>

ECP - eosinophil cationic protein  
MBP - maltose binding protein,  
TGFβ - transforming growth factor  
IL – interleukin  
EDN - eosinophil derived neurotoxin  
MPO - myeloperoxidase  
TNF α - tumor necrosis factor
Fazes of the inflammatory response

1. Bacteria and other pathogens enter wound.

2. Platelets from blood release blood-clotting proteins at wound site.

3. Mast cells secrete factors that mediate vasodilation and vascular constriction. Delivery of blood, plasma, and cells to injured area increases.

4. Neutrophils secrete factors that kill and degrade pathogens.

5. Neutrophils and macrophages remove pathogens by phagocytosis.

6. Macrophages secrete hormones called cytokines that attract immune system cells to the site and activate cells involved in tissue repair.

7. Inflammatory response continues until the foreign material is eliminated and the wound is repaired.
Classical signs of inflammation: **calor** (heat), **rubor** (redness), **dolor** (pain) and **tumor** (swelling) are known from antiquity, their explanation being made today on the basis of physiological phenomena.
The third line of defense is represented by the specific response of the immune system represented by lymphocytes.

Immunological specificity and memory involve 3 events:
- recognition of a specific invader
- repeated cell divisions that form a huge lymphocyte population
- differentiation into subpopulations of effector and memory cells

Each type of virus, bacteria or other foreign body has molecular markers which make it unique.

Host lymphocytes can recognize self proteins or self by comparison with foreign proteins or non self.

When a non self body is detected lymphocytes (T cells and B cells) are stimulated and are transformed in effector cells and memory cells.

The process is aided by helper T cells that secrete chemicals which promote large number of effector and memory cells.

Cytotoxic T cells eliminate infected body cells and tumor cells and B cells produce antibodies.
For the recognition of foreign bodies these are engulfed and prepared to be presented by antigen presenting cells (macrophage, dendritic cell) to the T cells that recognize the specific antigen.

**MHC** (major histocompatibility complex) **proteins** belong to the **HLA** (human leucocyte antigen) **system** that is encoded by genes being unique for an individual (self). These proteins present the possible antigen to the B and T cells.
B and T cells receptors must be able to recognize these molecules and compare them with the fragments of foreign proteins (non-self).

There are different sorts of MHC (HLA): class I and class II MHC.
Class I MHC presents antigens found within the cell to the cytotoxic T cells (CD8)
Class II MHC presents antigens found outside the cell to the helper T Cells (CD4).
IMMUNITY represents the system that defends the body against pathogens (bacteria, viruses, fungi, protozoa, prions).

Immune tolerance is the process by which B and T cells ignore the body own tissues considered as self.

There is an innate immunity (unspecific response) and an acquired immunity (specific adaptive response), both divided in humoral and cellular.
Types of immunity

Vaccines, best way to prevent infectious diseases, consist of killed or modified microbes, parts of microbes that are able to induce an immune response of the host person.
Antigen is a substance (protein, polysaccharide or even small molecules known as haptenes attached to bigger carrier molecules) that induced the formation of antibodies because it is recognized by the immune system as non self and a threat.

Antibodies are formed against a small part of the antigen the so called epitope.

Antibodies are plasma proteins (gamma globulins) classified as immunoglobulins in several classes. They are produced by plasma cells derived from stimulated B cells.

<table>
<thead>
<tr>
<th>Table 2. Gamma Globulins</th>
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<tbody>
<tr>
<td>IgA</td>
</tr>
<tr>
<td>IgE</td>
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<tr>
<td>IgG</td>
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<tr>
<td>IgM</td>
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</table>
The structural scheme of the terminal part of an antibody
Immunoglobulins

The natural antibodies, like the blood groups agglutinins (alpha and beta), belong to the IgM family.

Coupling a plasma cell and a cancerous cell in experimental conditions is obtained a hybridoma secreting the so called monoclonal antibodies.
There are 2 forms of immunity or immune response: humoral and cellular.
The HUMORAL IMMUNE RESPONSE is based on the production of antibodies by the plasma cells derived from stimulated B lymphocytes.

Antibodies can destroy antigens directly or by activation of the complement proteins.
The humoral response is implied in allergies. Allergies are considered as exaggerated responses in the case of the second encounter with an antigen. This is followed by an enhanced production of IgE that binds to mastocytes and bazophils that secrete histamine and leucotrienes, active substances that act especially on smooth muscles.

![Diagram of allergic response]

- The first time the allergy-prone person runs across an allergen such as ragweed,
  - he or she makes large amounts of ragweed IgE antibody.
  - These IgE molecules attach themselves to mast cells.
  - The second time that person has a brush with ragweed,
  - the IgE-primed mast cell will release its powerful chemicals,
  - and the person will suffer the wheezing and/or sneezing, runny nose, watery eyes, and itching of allergy.
Allergic reactions can occur immediately (minutes) or after several hours (late phase response).
Examples of allergic reactions: urticaria, allergic rhinitis (hay fever), bronchial asthma.

Urticaria

Hay fever
Bronchial asthma

Anaphylaxis represents a grave, life threatening form of allergy.
Figure 1. Mechanisms of mediator release in anaphylaxis. NSAIDs, nonsteroidal anti-inflammatory drugs.
The CELLULAR IMMUNE RESPONSE is based on the action of T lymphocytes stimulated after their contact with antigen presenting cells.

AUTOIMMUNITY or autoimmune response is possible when lymphocytes are unleashed against the own body cells that are recognized as foreign or non self and destroyed.
AIDS (acquired immune deficiency syndrome) is characterized by a severely depressed immune system caused by HIV (human immunodeficiency virus) that destroys T helper cells and impairs the ability to resist opportunistic infections.

Opportunistic infections such as pneumocystosis or malignancies such as Kaposi’s sarcoma can signal the final stage of HIV infection, AIDS.
The most common methods of transmission of HIV are:

- Unprotected sex with an infected partner
- Sharing needles with infected person

Almost eliminated as risk factors for HIV transmission are:

- Transmission from infected mother to fetus
- Infection from blood products
PLATELETS or THROMBOCYTES are anucleate, disk-shaped fragments (2-3 micrometers) from a large bone marrow-derived cells called megacaryocytes.

Their number 150 000 – 300 000/ mm³ with a lifespan of 8-10 days.
Platelets have a complex structure.
The dense bodies contain ADP, Ca^{2+} and serotonin, the alpha granules contain von Willebrand Factor (vWF), thrombin, thrombospondin and growth factors.

On their surface there are glycoproteins (GP I,II,III,IV each with a and b subdivisions) acting as receptors for several substances (collagen, ADP, serotonin, vWF).

The main function of platelets is to scan the vascular system and respond to endothelial damage by tethering to the site of injury or lesion. Thus it is an essential component of the clotting system.

Platelet adhesion on damaged area of a blood vessel occurs in seconds.
When platelets adhere at the site of injury a process of activation occurs.

Activation is followed by:

- exocytosis of dense granules and alpha granules
- activation of the membrane enzyme phospholipase A2 leading to the formation of thromboxane A2 (TXA2)
- change in shape to more amorphous form with projecting fingers
- adhesion of platelets to one another and to collagen under the endothelium forming platelet plug (white clot)
- on the surface of activated platelets the receptors for vWF and fibrinogen promote the binding to collagen and the adhesion of platelets to one another
- coagulation reactions are promoted
- platelet activation together with coagulation reactions form the red clot
Injury to lining of vessel exposes collagen fibers; platelets adhere

Platelet plug forms

Fibrin clot with trapped red blood cells

Collagen fibers

Platelets

Fibrin

Platelets release chemicals that make nearby platelets sticky

PF₃ from platelets and tissue factor from damaged tissue cells

Calcium and other clotting factors in blood plasma

Coagulation

Formation of prothrombin activator

Prothrombin

Thrombin

Fibrinogen (soluble)

Fibrin (insoluble)
Pathways of platelet activation

Thickness of line indicates strength of activator

Thrombus formation

Break in blood vessel

Collagen

Fibrinogen

Fibrin

TXA2

ADP

VWF

Growth factors, etc.

Dense granules

Alpha granules

VWF

Platelet

GP IIb/IIIa

ReoPro

Aspirin

Thienopyridine

Heparin/LMWH

Direct thrombin inhibitor
The adhesion of platelets is reversible but after their activation a process of irreversible aggregation occurs.

The aggregation is mediated by GP IIb/IIIa receptors that bind various adhesion proteins, especially fibrinogen. Fibrin strengthens the structure allowing continued platelet aggregation, thrombus formation and growth.

The platelets are intimately involved in the successive phases of atherosclerosis that are triggered by damage of the endothelial lining of the arterial wall.

ATHEROSCLEROSIS is the process in which deposits of fatty substances, cholesterol, cellular waste products, calcium and other substances buildup in the inner lining of an artery.
Atherosclerotic plaque
This buildup called plaque can grow large enough to reduce blood flow through an artery. Activated platelets attached to an atherosclerotic lesion may influence plaque progression interacting with monocytes and lymphocytes and releasing growth factors. The rupture of plaque cause blood clots that can block blood flow with severe consequences depending on the site of blocked artery (heart, brain).

Causes of damage to the arterial wall:

- high blood cholesterol
- cigarette smoking
- high blood pressure
- diabetes
- obesity
- physical inactivity

Modulation of platelet's activity with some chemicals can help the prevention of arterial wall damage.
Disorders of platelet number or quality cause spontaneous bleedings.

Thrombocytopenia (reduced number of platelets) allow blood to seep into the tissues evidenced by purple blotches (purpura).

Glanzmann's thrombasthenia poor quality of platelets due to a hereditary disorder in the production of GP IIb/IIIa that cause bleedings from mucosal tissues and excessive bleeding following skin damage.
Purpura

Glanzmann's thrombastenia
HEMOSTASIS. COAGULATION. FIBRINOLYSIS

Hemostasis is a complex physiologic process aiming at the protection from bleeding and the preservation of regular blood flow.

The process of hemostasis occurs in 4 phases:

- vascular constriction
- intervention of platelets
- formation of fibrin mesh
- dissolution of the clot

Vascular constriction is induced by pain (caused by the injury) as well as by release of vasoconstrictive substances (serotonin) by platelets.

Intervention of platelets (adhesion, activation, aggregation) plays an important role in the minor ruptures of the small vessels and capillaries.

Vascular constriction and the intervention of platelets constitutes the primary hemostasis.

Secondary hemostasis is represented by coagulation of blood (formation of fibrin mesh) followed by fibrinolysis (dissolution of the clot).
Hemostasis
COAGULATION or blood clotting is an enzymatic process during which fibrinogen is transformed in fibrin that forms a mesh entrapping the blood cells.
Coagulation is the result of the intervention of several factors known as clotting factors indicated by roman numerals.

**CLOTTING FACTORS**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>Factor I</td>
<td>Fibrinogen</td>
</tr>
<tr>
<td>Factor II</td>
<td>Prothrombin</td>
</tr>
<tr>
<td>Factor III</td>
<td>Tissue Thromboplastin</td>
</tr>
<tr>
<td>Factor IV</td>
<td>Calcium Ions</td>
</tr>
<tr>
<td>Factor V</td>
<td>Labile Factor</td>
</tr>
<tr>
<td>Factor VII</td>
<td>Stable Factor</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>Antihemophilic Factor</td>
</tr>
</tbody>
</table>
| Factor IX | Christmas Factor, or  
Plasma Thromboplastin 
Component (PTC) |
| Factor X | Stuart-Prower Factor                 |
| Factor XI | Plasma Thromboplastin Antecedent (PTA) |
| Factor XII| Hageman Factor                      |
| Factor XIII| Fibrin Stabilizing Factor           |

The majority of these factors are glycoproteins (globulins) formed in the liver. For the synthesis of the factors II, VII, IX and X is necessary the presence of K vitamin.
Coagulation cascade is initiated by two pathways:
- the contact activation pathway (intrinsic)
- the tissue factor pathway (extrinsic)

Both activate the final common pathway of factor X, thrombin and fibrin.

Thrombin is the most important constituent of the coagulation cascade in terms of its feedback activation roles.

The pathways are a series of reaction in which an inactive enzyme precursor (serineprotease) and its cofactor are activated to become active component that then catalyze the next reaction in the cascade.
Coagulation cascade

HK - high molecular weight Kininogen, PK - pre Kallicrein, PL - phospholipids
TF – tissue factor
After a short period the clot retracts due to the intervention of platelet's contractile proteins attached to the fibrin filaments.

ANTICOAGULANTS are substances that hamper the process of blood coagulation.

Heparin produced in the liver and lung (basophils and mastocytes) inhibits the activity of thrombin. It is a fast acting but short-lived drug used in acute problems.

Antithrombin III inhibits activated factors II, IX, X, XI, XII, kallicrein, plasmin and probably factor VII. Its activity is accelerated by interaction with heparin.

Proteins C and S produced in the liver in the presence of K vitamin inhibit activated clotting factors V and VIII and facilitate the intravascular clot lysis.

These substances are considered natural anticoagulants.
There are also extrinsic anticoagulants like:

Coumadin (dicoumarol, warfarin) is a slow-acting drug taken orally for long term control of blood coagulation. Inhibits the K vitamin required for the synthesis of clotting factors II, VII, IX and X.

Citrates bind up (chelate) calcium inhibiting formation of clots. They are used in long term blood storage.

Hirudin (obtained from leach saliva) and serpent's venoms have anticoagulant activity blocking the action of thrombin.
FIBRINOLYSIS is the process of destruction of clot by the lysis of fibrin filaments as a consequence of the action of a protease called plasmin.
Fibrin degradation products are 4 fibrinopeptides called E,D,X,Y.

Streptokinase, an enzyme produced by streptococcus bacteria that activates fibrinolysis, is used in medical practice to dissolve intravascular clots.

Aprotinin (Trasylol), a protein obtained from bovine lungs that inhibits fibrinolysis, is used to reduce or prevent excessive blood loss during major surgery.

The hemostatic and fibrinolytic systems are well balanced thanks to the cooperation of activating and suppressing enzymes.

Disorders of these systems can lead to an increased risk for bleeding (hemorrhage) and/or clotting (thrombosis).
Hemophilia, an inherited disorder that produce excessive bleeding, is caused by a deficiency of any of the clotting factors, in 80% of cases F VIII.
Disseminated intravascular coagulation (DIC) is produced after massive tissue injury, sepsis, pregnancy complications, snake bites, cancer or liver diseases.

Anticoagulant and fibrinolytic systems are activated simultaneously and overwhelmed leading to disseminated microthrombi and tissue ischemia, consumption of platelets, coagulation factors and natural anticoagulants with severe bleeding.

Disseminated intravascular coagulation
Arterial or venous thrombosis can lead to serious complications as a consequence of the obstruction or mobilization of thrombi in the blood vessels (embolism).
In cases of thrombosis is possible to desobstruct the vessels by thrombolysis using some substances that can dissolve the clot or by mechanical removal of obstruction with special catheters (angioplasty).
Balloon angioplasty

Stent angioplasty