Biochemistry of blood cells and coagulation.

Bruno Sopko
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• Introduction
• Blood Plasma
• Metabolism of Erythrocytes
• Metabolism of White Cells
  – Phagocytic cells
  – Basophils and mast cells
  – Lymphocytes
• Biochemistry of Platelets/Blood Coagulation
• Literature
Introduction

• Functions
• Blood Composition
Introduction – Functions I.

• Respiratory
  – CO₂ transport from tissues to lungs
  – O₂ transport from lungs to tissues

  Nutrition
  – Transports nutrients from digestion system to tissues

• Excretory
  – Transports waste from tissues to kidneys (urea, uric acid, water, salts etc.)
Introduction – Functions II.

• Regulatory
  – Water content in the tissues
  – Distribution of the regulatory compounds (hormons etc.)

• Body Temperature
  – Water has high heat capacity (heat accumulation)
  – Heat spreading from one source (cooling, warming)

• Protective
  – Antibodies, antitoxins, white blood cells
Introduction – Blood Composition

• 8% of the body weight (5–6 L)

• Suspension of cells in carrier fluid
  – 45% cells 55% plasma

• Plasma
• Red Cells
• White cells
• Platelets
Hematopoietic tree

- Bone marrow
  - pluripotent hematopoietic stem cell
  - common lymphoid progenitor
  - common myeloid progenitor
  - granulocyte/macrophage progenitor
  - megakaryocyte/erythrocyte progenitor

- Blood
  - Granulocytes (or polymorphonuclear leukocytes)
    - neutrophil
    - eosinophil
    - basophil
    - unknown precursor
    - monocyte
    - immature dendritic cell
  - platelets
  - erythrocyte

- Effector cells
  - B cell
  - T cell
  - plasma cell
  - activated T cell

- Tissues
  - mast cell
  - macrophage
  - immature dendritic cell

- Lymph nodes
  - mature dendritic cell
Erythrocytes

5.2 \times 10^6 \text{ (men)}; 4.6 \times 10^6 \text{ women cells/ml}
The spectrin molecules form a mesh-like pattern that is anchored to the membrane by ankyrin molecules (ellipses). The basic shape is hexagonal (shaded).
Erythrocytes - Hemoglobin
BPG

\[ 4 \text{O}_2 + \text{Hb}_T \cdot \text{BPG} \]

\[ \text{Hb}_R \cdot (\text{O}_2)_4 + \text{H}^+ + \text{BPG} \]

Deoxyhemoglobin T form

BPG stabilizes T form

O$_2$ bond weakens association

O$_2$ affinity 70 times higher than in T form

Oxyhemoglobin R form
Hemoglobin saturation curves:
Erythrocytes - Metabolism

pH dependent!
Haemoglobin autooxidation

- $O_2$ binds $Fe^{2+}$ - an intermediate structure - an electron is delocalized between the iron ion and the $O_2$

- the side effect - every so often a molecule of oxyhaemoglobin undergoes decomposition and release superoxide

$$\text{Hem} - Fe^{2+} - O_2 \leftrightarrow \text{Hem} - Fe^{3+} - O_2\cdot^-$$

- 3% of the haemoglobin undergoes oxidation every day

- Methaemoglobin ($Fe^{3+}$) is unable to bind $O_2$ (methaemoglobin reductase)
Glutathione synthesis
The pentose phosphate pathway in erythrocytes

• Generates NADPH - reduction of glutathione (eliminates $H_2O_2$ formed in erythrocytes)

**Clinical aspect:**

• **Glucose-6-phosphate dehydrogenase deficiency**
  – Causes hemolytic anemia (decreased production of NADPH - reduced protection against oxidative stress - haemoglobin oxidation and Heinz bodies formation, membrane lipid peroxidation and hemolysis)
  – Hemolytic crises are evoked by drugs (primaquine, sulphonamide antibiotics) and foods (broad beans)
  – The most common enzyme deficiency disease in the world (100 million people)
Haemoglobinopathies

**Haemoglobinopathy**
- abnormal structure of the haemoglobin (mutation)
- large number of haemoglobin mutations, a fraction has deleterious effects
- sickling, change in O$_2$ affinity, heme loss or dissociation of tetramer
- **haemoglobin M and S**, and **thalassemias**

**Haemoglobin M**
- replacement of the histidine (E8 or F7) in α or β-chain by the tyrosine
- the iron in the heme group is in the Fe$^{3+}$ state (methaemoglobin) stabilized by the tyrosine
- methaemoglobin can not bind oxygen

**Thalassemias**
- genetic defects- α or β-chains are not produced (α or β-thalassemia)

**Haemoglobin S (sickle-cell)**
- Causes a sickle-cell anemia
- Erythrocytes adopt an elongated sickle shape due to the aggregation of haemoglobin S
Hemoglobin switching:

Chromosome 16

\[ \text{HS40} \quad \zeta \quad \alpha_2 \quad \alpha_1 \quad 3' \]

Chromosome 11

\[ \text{LCR} \quad \epsilon \quad G_y \quad A_y \quad \delta \quad \beta \quad 3' \]

Embryo: \( \zeta_2 \epsilon_2 = \text{Gower 1} \)

\( \zeta_2 \gamma_2 = \text{Portland} \)

\( \alpha_2 \epsilon_2 = \text{Gower 2} \)

Fetus: \( \alpha_2 \gamma_2 = \text{HbF} \)

Adult: \( \alpha_2 \gamma_2 = \text{HbF} \)

\( \alpha_2 \delta_2 = A_2 \)

\( \alpha_2 \beta_2 = A \)

![Graph showing hemoglobin switching over prenatal and postnatal ages](image)
Degradation of Heme
Iron cycle
Glycated haemoglobin (HbA1)

- formed by hemoglobin's exposure to high plasma levels of glucose

- non-enzymatic glycolysation (glycation)- sugar bonding to a protein

- normal level HbA1- 5%; a buildup of HbA1- increased glucose concentration

- the HbA1 level is proportional to average blood glucose concentration over previous weeks; in individuals with poorly controlled diabetes, increases in the quantities of these glycated haemoglobins are noted (patients monitoring)

\[ \text{Sugar} - \text{CHO} + \text{NH}_2 - \text{CH}_2 - \text{Protein} \]

\[ \downarrow \]

\[ \text{Sugar} - \text{CH} = \text{N} - \text{CH}_2 - \text{Protein} \]

*Amadori reaction*

\[ \downarrow \]

\[ \text{Sugar} - \text{CH}_2 - \text{NH} - \text{CH}_2 - \text{Protein} \]

*Glycosylated protein*

*Schiff base*
# White Blood cells

<table>
<thead>
<tr>
<th>Type</th>
<th>Microscopic Appearance</th>
<th>Diagram</th>
<th>Approx. % in adults</th>
<th>Diameter (µm)</th>
<th>Main targets</th>
<th>Nucleus</th>
<th>Granules</th>
<th>Lifetime</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophil</td>
<td></td>
<td><img src="image" alt="Neutrophil" /></td>
<td>54–62%[8]</td>
<td>10–12</td>
<td>- <strong>bacteria</strong>&lt;br&gt;- <strong>fungi</strong></td>
<td>multilobed</td>
<td>fine, faintly pink (H&amp;E Stain)</td>
<td>6 hours–few days (&lt;i&gt;days in soleum&lt;/i&gt; and other tissue)</td>
</tr>
<tr>
<td>Eosinophil</td>
<td></td>
<td><img src="image" alt="Eosinophil" /></td>
<td>1–6%</td>
<td>10–12</td>
<td>- <strong>larval parasites</strong>&lt;br&gt;- modulate allergic inflammatory responses</td>
<td>bi-lobed</td>
<td>full of pink-orange (H&amp;E Stain)</td>
<td>8–12 days (circulate for 4–5 hours)</td>
</tr>
<tr>
<td>Basophil</td>
<td></td>
<td><img src="image" alt="Basophil" /></td>
<td>&lt;1%</td>
<td>12–16</td>
<td>- release histamine for inflammatory responses</td>
<td>bi-lobed or tri-lobed</td>
<td>large blue</td>
<td>a few hours to a few days</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td></td>
<td><img src="image" alt="Lymphocyte" /></td>
<td>28–33%[8]</td>
<td>7–8</td>
<td>- B cells: releases antibodies and assists activation of T cells&lt;br&gt;- T cells:&lt;br&gt;  - CD4+ Th (T helper) cells: activate and regulate T and B cells&lt;br&gt;  - CD8+ cytotoxic T cells: virus-infected and tumor cells&lt;br&gt;  - γδ T cells:&lt;br&gt;  - Regulatory (suppressor) T cells: Returns the functioning of the immune system to normal operation after infection, prevents autoimmunity&lt;br&gt;  - Natural killer cells: virus-infected and tumor cells.</td>
<td>deeply staining, eccentric</td>
<td>NK-cells and Cytotoxic (CD8+) T-cells</td>
<td>years for memory cells, weeks for all else</td>
</tr>
<tr>
<td>Monocyte</td>
<td></td>
<td><img src="image" alt="Monocyte" /></td>
<td>2–10%[7]</td>
<td>7.72–9.99[7]</td>
<td>Monocytes migrate from the bloodstream to other tissues and differentiate into tissue resident macrophages, Kupffer cells in the liver.</td>
<td>kidney shaped</td>
<td>none</td>
<td>hours to days</td>
</tr>
<tr>
<td>Macrophage</td>
<td></td>
<td><img src="image" alt="Macrophage" /></td>
<td>21 (human)[8]</td>
<td>21</td>
<td>Is a monocyte derivative. Phagocytosis (engulfment and digestion) of cellular debris and pathogens, and stimulation of lymphocytes and other immune cells that respond to the pathogen.</td>
<td>none</td>
<td>none</td>
<td>activated; days immune; months to years</td>
</tr>
<tr>
<td>Dendritic cells</td>
<td></td>
<td><img src="image" alt="Dendritic cells" /></td>
<td></td>
<td></td>
<td>Is a monocyte derivative. Main function is as an antigen-presenting cell (APC) that activates T lymphocytes.</td>
<td>none</td>
<td>none</td>
<td>similar to macrophages</td>
</tr>
</tbody>
</table>
White Blood cells

• Phagocytic cells
• Basophils and mast cells
• Lymphocytes
Regulations

• Many functions of leukocytes are regulated by monomeric GTP-binding proteins, e.g. Rac, Rho:
  ➢ activation of NADHP oxidase
  ➢ chemotaxis
  ➢ phagocytosis
  ➢ fusion of phagosome with granules

• Rho and Rac are able to modulate the assembly of actin filaments, which plays a role in the processes listed above
Phagocytic cells

• Introduction

• Granulocytes
  – Neutrophils – most abundant
  – Eosinophils
  – Basophils

• Monocytes

• Macrophages – rise by differentiation of monocytes in tissues
Degradation of the ingested particle

1) Activation of NADPH oxidase

2) Production of NO by nitric oxide synthase

3) Fusion of phagosome with lysosomes of the phagocytic cell that contain bactericidal substances and hydrolytic enzymes (often with acidic pH$_{opt}$)
NADPH-oxidase

- Protein complex of neutrophils, eosinophils, monocytes, macrophages

\[
\text{NADPH} + 2 \text{O}_2 \rightarrow \text{NADP}^+ + \text{H}^+ + 2 \text{O}_2^- \quad \text{superoxide anion}
\]

\[
\downarrow
\]

\[
2 \text{O}_2^- + 2 \text{H}^+ \rightarrow \text{O}_2 + \text{H}_2\text{O}_2
\]

- \( \text{H}_2\text{O}_2 \) can damage bacteria directly or after conversion to \( \text{OH}\bullet \):

\[
\text{H}_2\text{O}_2 + \text{M}^+ \rightarrow \text{OH}\bullet + \text{OH}^- + \text{M}^{2+} \quad (\text{M}; \text{metal})
\]
NADPH-oxidase

- Activation: by association of the components localized in cytosol with cytochrome \( b_{558} \) in the membrane; electrons from cytosolic NADPH are – via FAD and cytochrome – transferred to oxygen
NADPH-oxidase

plasma membrane

outside  inside

\[ \text{NADPH} \rightarrow \text{O}_2 \rightarrow \text{O}_2^- \rightarrow \text{NADP}^+ \]

phagosome

fusion with lysosomes
Myeloperoxidase

- Present in granules of neutrophils and monocytes, but not macrophages!

- Significant portion of $H_2O_2$ (produced by dismutation of $O_2$· generated by NADPH oxidase) is used by myeloperoxidase to oxidize $Cl^-$ to $HClO$

- $HClO$ is highly reactive, able to oxidize biomolecules; it also provides toxic chlorine gas:
  \[ HClO + H^+ + Cl^- \rightarrow Cl_2 + H_2O \]

- $HClO$ also reacts with $O_2$·· yielding $OH$·:
  \[ HClO + O_2$·· \rightarrow O_2 + OH$\cdot + Cl^- \]
Chronic granulomatous disease

- Caused by a deficiency of one of the NADPH oxidase subunits

- Superoxide and the other reactive oxygen species are not produced

- Severe infections that are very hard to treat – e.g.:
  - *Burkholderia cepacea* causes pneumonia
  - *Aspergillus* causes intractable pneumonia, septicaemia; can lead to death

- Treatment: antibiotics, antifungal agents
Nitric oxide production

- Mainly by inducible nitric oxide synthase (iNOS) of macrophages which is induced by cytokines (INF-γ, TNF) or bacterial lipopolysaccharide:

\[
\text{Arg} \quad \xrightarrow{\text{NADPH} + \text{O}_2} \quad \text{citrulline}
\]

- NO• can kill bacteria directly (e.g. by inhibition of the respiratory chain) or indirectly: by reaction with O₂•⁻, generating peroxynitrite ONOO⁻ which attacks Fe-S proteins and essential –SH groups, inactivates enzymes...
NADPH oxidase and NO

• NADPH oxidase is effective mainly in degradation of extracellular pathogens (*Salmonella*, *Staphylococcus*, *Streptococcus pyogenes*)...*neutrophils*

  X

• NO serves mainly to kill the intracellular parasites (*Listeria*, *Brucella*, *Candida albicans*)...*macrophages*
Granulocytes - introduction

- All of them are phagocytes
- Contain several types of granules
  - Primary granules – participating in phagocytosis
  - Secondary granules – releasing cytotoxic and immune response mediators (defensins, cathepsins etc.)
- After phagocytosis is accomplished, the respiratory burst occurs
Neutrophils

- 40 – 65 % of white blood cells
- Must be activated
- React mostly with *opsonised* cells
- Mediate other immune response (eicosanoids, cytokines)
Neutrophils

Main targets are bacteria

- **Myeloperoxidase**

- **Lysozyme** – cleaves glycosidic bonds in peptidoglycan of the bacterial (primarily G+) cell walls

- **Defensins** – cationic peptides (Arg) with $M_r$ of 3.5-6 kDa; interact with anionic lipids of bacterial membrane and make pores in it; can also inhibit synthesis of DNA and proteins

- **Hydrolases**, e.g. **elastase** – serine protease: can damage bacteria and cleave virulence factors, but also cause harm to host tissues (cleaves the proteins of extracellular matrix, too)
Eosinophils

Main targets are eucaryotic Parasites

- ROS production

- Contain eosinophil peroxidase
  - similar to myeloperoxidase, but prefers Br\(^-\) as a substrate (instead of Cl\(^-\)), thus generating HBrO (instead of HClO)

- proteases

- Granules contain Major Basic Protein, cytotoxic to parasites

- Release of histamine
Eosinophils

Stimulation
- Tissue injury
- Infections
- Allergens
- Allografts
- Tumors

Secretion
- Cytotoxic granules: EPO, MBP, ECP, EDN
- Cytokines: IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-16, IL-18, TGF-α/β, GM-CSF, TNF-α, INF-γ
- Chemokines: Eotaxin-1, RANTES, MIP-1α
- Lipid mediators: Leukotrienes, platelet activating factor
- Neuro mediators: Substance P, NGF, VIP

T cell communication
- Antigen presentation (T cell activation)
- MHC II, CD80, CD86
- T cell polarization
- IDO, KYN

Mast cell activation
- MBP
Basophils

- Mainly immune response mediator (histamine and serotonin)
- Contain IgE receptors, once activated, degranulate
- Responsible for allergic symptoms
- Activate synthesis of eicosanoids; leukotrienes are potent bronchoconstrictors, stimulate chemotaxis and leukocyte activation
Histamine

- Produced by histidine decarboxylation:

\[
\text{Histidine} \xrightarrow{\text{histidine decarboxylase (PLP dependent)}} \text{Histamine}
\]

- Causes vasodilation and bronchoconstriction $\Rightarrow$ helps to eliminate parasites (cough, peristalsis, enhanced production of mucus)
Atopy

- IgE recognizing allergens (from pollen, food...) are produced and bind to IgE receptors of basophils (mast cells). Next exposure to the allergen can lead to release of histamine and heparin and synthesis of eicosanoids

- **Local symptoms** occur: allergic rhinitis, asthma, conjunctivitis

- If the allergen enters bloodstream, it can cause a massive degranulation of basophils (mast cells) $\Rightarrow$ increase in vascular permeability, decrease in blood pressure $\Rightarrow$ pulmonary oedema, ischemia... **anaphylactic shock**

- Treatment: antihistamines – block histamine receptors
TABLE 1. Specific activities of various proteases in uninfected macrophage and amastigote lysates

<table>
<thead>
<tr>
<th>Enzyme activity</th>
<th>Sp act (µmol or arbitrary unit/10⁶ cells per h) in:</th>
<th>Sp act (µmol or arbitrary unit/mg of protein per h) in:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Macrophages</td>
<td>Amastigotes</td>
</tr>
<tr>
<td>Cathepsin B</td>
<td>3.90 ± 0.27 (4)</td>
<td>0.013 ± 0.002 (4)</td>
</tr>
<tr>
<td>Cathepsin H</td>
<td>0.058 ± 0.005 (4)</td>
<td>0.0003 (1)</td>
</tr>
<tr>
<td>DPP I</td>
<td>8.30 ± 1.96 (6)</td>
<td>0.013 ± 0.002 (7)</td>
</tr>
<tr>
<td>DPP II</td>
<td>2.14 ± 0.25 (4)</td>
<td>0 (2)</td>
</tr>
<tr>
<td>Cathepsin D</td>
<td>0.33 ± 0.07 (3)</td>
<td>0.007 ± 0.001 (2)</td>
</tr>
</tbody>
</table>
Lyphocytes

- T cells
- B cells
- NK cells
Fixed leukocytes

• Histiocytes
• Dendritic Cells
• Mast Cells
• Microglia
• Kupffer Cells (liver)
Blood coagulation - platelets

• Non nucleated

• Granulated
  – electron-dense granules, which contain calcium, adenosine diphosphate (ADP), adenosine triphosphate (ATP), and serotonin
  – granule, which contains a heparin antagonist (heparin interferes with blood clotting; see biochemical comments), platelet-derived growth factor, -thromboglobulin, fibrinogen, von Willebrand factor (vWF), and other clotting factors
  – the lysosomal granule, which contains hydrolytic enzymes
Platelets

- Form blood clots, act as vasoconstrictors
- Participate in defence against infections, e.g.: they suppress the growth of *Plasmodium falciparum* (infectious agent that causes malaria)
- Generate $\text{O}_2^\cdot$ and $\text{H}_2\text{O}_2$ that may synergize with pro-aggregatory stimuli
- Contain thromboxan A synthase that catalyzes conversion of prosta-glandin H$_2$ to thromboxan A$_2$:

$$\text{PGH}_2 \rightarrow \text{TXA}_2$$

$\text{TXA}_2$ – promotes platelet aggregation and vasoconstriction
Platelets

• Platelet-Activating Factor (PAF)
• Platelet-Derived Growth Factor (PDGF)
Platelet-Activating Factor

- Mainly juxtacrine and paracrine signalling via GPCR
- Promotes platelet aggregation
- Induces activation of leukocytes, adhesion, chemotaxis, cytokine production, causes vasodilation and bronchoconstriction
- Mediates interplay between thrombotic and inflammatory cascades
- BUT: it is also suspected of contributing to allergy, anaphylactic shock...
- It is produced also by endothelial cells, monocytes, granulocytes...
Platelet-Derived Growth Factor (PDGF)

• Dimeric protein, 3 isoforms

• Receptors: tyrosine kinases – expressed on fibroblasts, glia, smooth muscle cells, leukocytes…. 

• Effects:
  – Proliferation
  – Chemotaxis
  – cytoskeletal rearrangements
  – differentiation of certain types of cells (e.g. in CNS)
  – ⇒ participates in wound healing, capillary formation, embryonic and postnatal development!

• BUT: probably also plays a role in pathogenesis (some tumours)
## Blood Coagulation

<table>
<thead>
<tr>
<th>Coagulation Factors</th>
<th>Function/Active Form</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Factor</strong></td>
<td><strong>Descriptive Name</strong></td>
</tr>
<tr>
<td>I</td>
<td>Fibrinogen</td>
</tr>
<tr>
<td>II</td>
<td>Prothrombin</td>
</tr>
<tr>
<td>III</td>
<td>Tissue factor</td>
</tr>
<tr>
<td>IV</td>
<td>Ca²⁺</td>
</tr>
<tr>
<td>V</td>
<td>Proaccelerin, labile factor</td>
</tr>
<tr>
<td>VII</td>
<td>Proconvertin</td>
</tr>
<tr>
<td>VIII</td>
<td>Antihemophilia factor A</td>
</tr>
<tr>
<td>IX</td>
<td>Antihemophilia factor B, Christmas factor</td>
</tr>
<tr>
<td>X</td>
<td>Stuart-Prower factor</td>
</tr>
<tr>
<td>XI</td>
<td>Plasma thromboplastin antecedent</td>
</tr>
<tr>
<td>XII</td>
<td>Hageman (contact) factor</td>
</tr>
<tr>
<td>XIII</td>
<td>Fibrin stabilizing factor</td>
</tr>
<tr>
<td></td>
<td>Prekallikrein</td>
</tr>
<tr>
<td></td>
<td>High-molecular-weight kininogen</td>
</tr>
</tbody>
</table>

### Regulatory Proteins

<table>
<thead>
<tr>
<th>****</th>
<th><strong>Thrombomodulin</strong></th>
<th>****</th>
</tr>
</thead>
<tbody>
<tr>
<td>****</td>
<td><strong>Protein C</strong></td>
<td>Activated by thrombomodulin-bound thrombin; is a serine protease</td>
</tr>
<tr>
<td>****</td>
<td><strong>Protein S</strong></td>
<td>cofactor; binds activated protein C</td>
</tr>
</tbody>
</table>
Classic-test tube coagulation cascade

Since 1961
Coagulation cascade *in vivo*

- Tissue Factor + VII
- PL
- IX
- IXa
- PL Ca^{++}
- VIIIa
- X
- IXa
- Prothrombin (II)
- Xa
- PL
- Va
- Thrombin (IIa)
- XIII
- Fibrinogen (I)
- Fibrin (weak)
- Fibrin (strong)
- Xla
- XI
A. N-terminal regions of α,β chains of two triple helices held together with disulfide bonds

Fibrinogen → Site of thrombin attack

α,β,γ tripeptide → γ-chains held together by a disulfide bond

B. Thrombin + H₂O → Fibrinopeptides

Fibrinogen → Fibrin monomer → Aggregation

Soft clot of fibrin

Fibrin cloth formation
Blood Coagulation Cascade

Transamidation by factor XIIIa / transglutaminase
Blood Coagulation – Vitamin K
Blood Coagulation - termination
Blood Coagulation - Fibrinolysis

Liver uptake: tPA

vessel lumen: Plasminogen

PAI-1: tPA

Endothelial Cells

Clot dissolution: Plasmin

α2-antiplasmin: Plasmin
Blood Coagulation - Fibrinolysis
Blood Coagulation - Fibrinolysis
Literature

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