Contents

• Leukocytes
• The blood types
• Haemolysis

Practical tasks

• Leukogam
• Determination of blood groups of the ABO system
• Determination of the Rhesus system (Rh factor)
• The cross matching test
• Haemolysis
**Types of leukocytes**

- **granulocytes**
  - specific granules (vesicles)
  - lobulated nucleus - polymorphonuclears
    1. neutrophilic 56 - 64%
    2. eosinophilic 1 - 3%
    3. basophilic 0.5 - 1%
- **agranulocytes**
  - do not contain specific granules
  - mononuclear – simple shape nucleus
    4. monocytes 3 - 8%
    5. lymphocytes 24 - 40%

**differential white blood cell count (leukogram)**

- examination of the % of individual types of leukocytes in %
- helps to make diagnosis - individual types of Le are involved in different functions
Neutrophilic granulocytes (56 – 64 % Le)

Properties

• nucleus 1 – 5 segments (lobes)
  - number of segments indicates age of the cell
  - young cells – one segment („stick“)
  - by maturation the number of segments increases

• cytoplasmic granules - purple colour (lysosomes with hydrolytic enzymes)

Function

• professional phagocytes (microphages) – ingest and destroy foreign material
• involved in non-specific immune reactions
• high motility, first line defence – first arrive to the place of invasion (of all WBC)

Eosinophilic granulocytes (1-3% Le)

• dense purple cytoplasmic granules, 2-segment nucleus
• weak ability of phagocytosis

• Function
  - the defence against parasites
  - allergic reactions
Basophilic granulocytes (0.5 – 1% Le)

• dark blue granules in cytoplasm
• two segment nucleus
• release active substances:
  
  histamin – causes vascular dilatation – increases blood flow into areas of tissue damage, facilitates the movement of leucocytes into tissues
  
  heparin – anticoagulant (useful in immune reactions)

Granulocytes - life span

• formation in the bone marrow, mature elements released into blood
• if stimulated (e.g. inflammatory stimuli), they can pass from blood into tissues through the capillary wall
• life span: 4 - 5 days, then die
• if involved into phagocytosis, they die soon afterwards (i.e. earlier than in 4-5 d)
**Monocytes (3 – 8% Le)**

- **largest** blood elements, **kidney-shaped** nucleus

**Life fate**

- in **blood** 10-20 h
- from blood - migrate into **tissues** → here maturate and transform to **macrophages**
  - free macrophages – actively move in tissues
  - fixed macrophages – in the sites of potential invasion of the pathogens
    - e.g. skin (histiocytes), lungs, liver, lymph nodes

**Function:**

- **macrophages** - professional phagocytes (non-specific immunity)
  - antigen presenting cells
    (process foreign material and present to lymphocytes)
Lymphocytes (24 – 40% Le)
- large round nucleus, narrow cytoplasm
- recirculate

Types and functions
- **acquired (specific)** type of immunity
  - T-Ly (produced in thymus)
  - B-Ly (produced in the bone marrow)
- **non specific immunity**
  - NK cells (natural killers)
  - K (killer) cells, LAK cells

Life span: years
Defensive properties of leukocytes

- **chemotaxis** – direction and speed of movement influenced by chemical substances (i.e. produced in the focus of infection)
- **diapedesis** – ability to squeeze and pass through the capillary wall
- **amoeboid motion** – active movement in tissues, the cell projects protoplasmic extensions and follows them
- **adhesivity** – ability to stick to solid surfaces (to receptors in endothelium, bacteria, cells, etc.)
- **phagocytosis** (especially neutrophils and macrophages)

![Diagram of phagocytosis](http://www.gluegrant.org/images/chemotaxis.jpg)
![Diagram of phagocytosis](http://3.bp.blogspot.com/_n8DPzZtYzAQ/TJ3h2MVoIZI/AAAAAAAAAAw/MHjsQTESQ1M/s1600/10.png)
**Immunity**
- capacity to resist foreign substances that tend to damage tissues and organs
  - microorganisms
  - molecules
  - own abnormal cells (cancer cells, infected and old cells)
- function performed by the **immune system**

**Immune system**
- organs positioned throughout the body
  (thymus, lymph nodes, lymphoid tissue in gut, spleen, etc.)
- **white blood cells** - main cells of the immune system

**Immunity**
1. **innate** – non specific
2. **acquired (adaptive)** - specific
   - develops after birth when the body is first attacked by a foreign substances
### 1. Innate immunity (defense mechanisms present from birth)

#### Characteristics

- the immune response is non-specific
  - it is not targeted at a specific agent (bacterium), rather the same to different agents

- early type of immune response

<table>
<thead>
<tr>
<th>Type of leukocyte</th>
<th>Mechanism of innate immunity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Neutrophils</strong> (microphages) and <strong>monocytes</strong> (macrophages)</td>
<td>- phagocytosis</td>
</tr>
<tr>
<td><strong>Eosinophils</strong></td>
<td>- defence against parasites</td>
</tr>
<tr>
<td></td>
<td>- phagocytosis (weaker)</td>
</tr>
<tr>
<td><strong>Basophils</strong></td>
<td>- release histamn – vasodilation and increased permeability of capillaries (WBC can easier reach tissues)</td>
</tr>
<tr>
<td></td>
<td>- release heparin, prevents undesirable haemocoagulation</td>
</tr>
<tr>
<td><strong>NK cells</strong> (lymphocytes)</td>
<td>- recognize absence of “self ” antigens, detect and kill virus infected cells, tumour cells</td>
</tr>
</tbody>
</table>
2. Acquired immunity (developed throughout the life)

A/ Active immunity
- developed in the body after exposure to a foreign antigen (by an infection, vaccination)
  - after first contact with foreign substance a weak immune reaction occurs
  - in next response is strengthened (principle of vaccination)
- active response of a host
- this immunity is permanent

Active immunization - vaccination
- vaccines contain weakened or dead microbes, that trigger an immune response
- active immunity develops

B/ Passive immunization
- transfer of antibodies from exogenous source (e.g. from an immunized donor to a patient, from mother to a newborn via the breastmilk)
- temporary protection (weeks) - no active response of the immune system

http://www.uic.edu/classes/bios/bios100/lectures/memory.jpg
Acquired immunity

- **specific**
  - targeted at foreign material that triggered the response
- therefore **highly effective**
- exhibits **immunological memory** (permanent immunity)
- mediated by **B and T lymphocytes**

**Naive (virgin) cells**
- B and T lymphocytes before they „meet“ the antigen

**Effector cells**
- lymphocytes, that were activated by an antigen (who carry receptors for that specific antigen)

**Memory cells**
- lymphocytes that were once activated can „remember“ the foreign agent
- after repeated contact with that particular antigen they can produce clones directed against the antigen
**Effector cells of acquired immunity**

**B-Lymphocytes**
- formation and maturation in bone marrow
- mediate **humoral type** of immunity

- B-Ly after recognizing the foreign agent - transform into **plasma cells**
  - macrophages – antigen presenting cells (phagocytosis of the foreign material and exposure of the antigens into their cell membranes)
  - activation requires cooperation with T-lymphocytes

- plasma cells produce specific molecules of **antibodies** (immunoglobulins):
  - Ig M  Ig A  Ig G  Ig D  Ig E

- antibodies bind to the foreign agent (e.g. the bacteria) and mark it for destruction (by phagocytosis or by other mechanisms)
T-lymphocytes

- formation in bone marrow, maturation in the thymus

- exhibit cell mediated immunity - directly destroy the target cells (mainly virus infected cells)

• T<sub>c</sub> (cytotoxic) – directly kill foreign cells by releasing substances that attack their cell membranes (make "a hole" in the membrane)

- additional functions of some T-lymphocytes

• T<sub>H</sub> (helper)
  - required for activation of B-Ly (without their cooperation the B-Ly cannot recognize majority of antigens – failure of the immune system)
  - produce interleukins – regulate the immune response

• T<sub>S</sub> (suppressor) - close down the immune response after invading organisms are destroyed and the immune response has achieved its goal

http://www.anselm.edu/homepage/jpitocch/genbio/helperTkill.JPG
Task: Blood smear (Leukogram)

- proportion of individual types of white blood cells in % is assessed
Procedure

- puncture a finger – place a drop of blood on a glass slide
- make a thin blood smear using another glass slide (see picture)
- leave to dry

Staining according to Pappenheim

- place the slide horizontally on a holder
- drop the May-Grünwald stain so that it covers the smear – wait 3 min
- drop distilled water (do not let the stain to float away), wait 1 min
- remove the stain with distilled water
- dilute 15 drops of Giemsa-Romanovski stain in 10 ml of distilled water
- cover the smear with stain, wait 15 – 20 min
- remove the stain, wash the smear with water
- put the smear into a holder in vertical position, leave to dry
• observe in amicroscope with objective 100 (use immersion oil)
• find leukocytes – bright colour (many cells in the background = Ery)
• identify Le types in one visual field
• move the sample, observe next visual fields – move the glass slide in a way shown in the scheme

<table>
<thead>
<tr>
<th>Type</th>
<th>Count</th>
<th>How to recognize them</th>
</tr>
</thead>
</table>
| 1. Ne | ⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔⬔bishop: 2017

Report
- % of individual Le types
- compare your result with normal values
- draw a scheme of a Ne, Ly
Determination of blood groups of the ABO system
Blood groups

Blood type must be considered in:
- transfusions
- transplantations
- gynecology and obstetrics

Major clinical importance (out of all existing blood systems):
1. ABO system
2. Rh system

- in case of ABO / Rh mismatching transfusion – high risk of
  - serious health consequences
  - death

GENERAL RULE: USE MATCHING BLOOD (POSSIBLY THE SAME BLOOD TYPE)
Blood groups in ABO system

– are determined by

• antigens (agglutinogens) A and/or B in the membrane of erythrocytes
• antibodies (agglutinins) anti-A or anti-B in the plasma

Antigen

• a chemical substance in the cell membrane
• determines individual identity (different people – different antigens)
• if a foreign antigen enters a body (e.g. mismatching blood)
  – it is able to trigger production of antibodies
  – it is able to react with antibodies (e.g. anti A + A; anti B + B)
  – reaction with antibodies starts the immune response -the
    foreign cell „marked“ by an antibody is destroyed
• (weak antigens – show only weak or no immune response)
ABO – blood groups

Blood group | Erythrocytes | Agglutinogen | Antigen | Plasma | Agglutinins | antibodies
---|---|---|---|---|---|---
**A** (48%) | ![Diagram](image1) | A | | ![Diagram](image2) | anti B
**B** (9%) | ![Diagram](image3) | B | | ![Diagram](image4) | anti A
**AB** (4%) | ![Diagram](image5) | A,B | | ![Diagram](image6) | not present
**O** (39%) | ![Diagram](image7) | H | | ![Diagram](image8) | anti A, B

Substance H is not an antigen
Determination of a blood group

**Principle**

Blood groups are assessed on the basis of reaction between known diagnostic serum containing antibodies and blood:

- Anti A serum – contains antibodies against agglutinogen A (anti A)
- Anti B serum – contains antibodies against agglutinogen B (anti B)

- If the antibodies in diagnostic serum „find“ antigen, they react with it and cause blood agglutination
- Agglutination = proof that the respective agglutinogen is present in membrane of Ery
- No agglutination – the respective agglutinogen is not present in membrane of Ery

<table>
<thead>
<tr>
<th>Anti A</th>
<th>-</th>
<th>+</th>
<th>Anti B</th>
<th>-</th>
<th>+</th>
<th>Blood group</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td></td>
<td>B</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>AB</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>
Procedure

• take a testcard
• place a drop of **Anti-A** serum into the preprinted area on the testcard
• place a drop of **Anti-B** serum into the preprinted area on the testcard
• puncture a fingertip, wipe the first drop of blood
• place **2 drops of blood** into a pre printed places on the testcards
• take a stick
  – use one end to stir a one blood drop with anti A serum
  – use the other end to stir the second blood drop and anti B serum

• **Result:** observe agglutination

• **Conclusion:** determine the blood group
Blood groups and transfusion

- incompatible blood (mismatched)
  - recipient (patient): A / anti B
  - donor: B / anti A

- compatible (matching) blood
  - recipient (patient): A / anti B
  - donor: A / anti B
## Transfusion of Full Blood

<table>
<thead>
<tr>
<th>Donor</th>
<th>A</th>
<th>B</th>
<th>AB</th>
<th>O</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AB</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>O</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
0 – universal donor?

<table>
<thead>
<tr>
<th>Full blood</th>
<th>0</th>
<th>-</th>
<th>-</th>
<th>-</th>
<th>+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor</td>
<td></td>
<td>A</td>
<td>B</td>
<td>AB</td>
<td>0</td>
</tr>
<tr>
<td>Recipient</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- A
- B
- AB
- 0

Full blood:
- 0
### Transfusion of Erythrocytes

<table>
<thead>
<tr>
<th>Donor</th>
<th>Recipient</th>
<th>A</th>
<th>B</th>
<th>AB</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>+</td>
<td>-</td>
<td></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>-</td>
<td>+</td>
<td></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>AB</td>
<td>-</td>
<td>-</td>
<td></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>0</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Reaction after transfusion of mismatching blood

- the agglutinins are attached to agglutinogens in Er membranes
- this reaction is called **agglutination**
- if agglutination happens aggregates of Er are formed and are visible in the sample

- possible consequences of mismatching transfusion - more or less serious:
  - haemolysis, icterus, immune reaction, **circulatory shock** (breathlessness, pain in chest, nausea, sweating...), kidney failure, death

- symptoms usually occur soon after the transfusion has started – in this case immediately STOP the transfusion

---

**ABO compatibility and transplantation**

- the donated organ should be ABO matching
Blood derivatives

- full blood
  the same blood group

- erythrocytes
  - may be given also to some other blood groups
  - O: universal donor
  - AB: universal recipient

- plasma
  - may be given to some other blood groups
  - AB – universal donor,
  - O – universal recipient
Determination of Rhesus system (Rh factor)
Rh system

1. presence of 3 antigens in the membrane of Er:
   (genetically determined)
   - C or c
   - D or d
   - E or e

   • Rh positivity (Rh⁺) – 85% of population
     - determined by the presence of antigen D
       in the erythrocyte membrane
     - CDE, CDe, cDe, cDE

   • Rh negativity (Rh⁻) – 15% of population
     - d antigen present: CdE, Cde, cde, cdE

   • sometimes the presence of E shows a weak positivity in subjects with d antigen

2. antibodies in Rh system - normally not present
   However!!!
   - D is a strong antigen (all the remaining are weak antigens)
   - if Rh⁺ Er enter blood of a Rh⁻ person, D is recognized as a „foreign“ antigen and production of antibodies is started
**Principle:**
Rh-Factor is assessed on the basis of reaction between known diagnostic serum containing antibodies anti-D and blood

**Procedure:**
- on a glass slide put
  - a drop of anti-D serum
  - a drop of capillary blood
- mix together with a glass stick
- **Result:** observe agglutination (the agglutination may be slow, sometimes it is necessary to wait for 5 minutes)
- **Conclusion:** determine the Rh-factor
Rh factor and transfusion

Rh negat donor → Rh negat patient
• the same blood group - matching

Rh posit donor → Rh posit patient
• the same blood group - matching

Rh negat donor → Rh posit patient
• matching - „d“ does not trigger antibody production
**Rh posit donor ➔ Rh negat recipient**

- Their production can be triggered if Rh+ erythrocytes enter the blood of a Rh- individual (e.g. transfusion of Rh incompatible blood)

**A/ 1st transfusion** – no posttransfusion reaction - no antibodies present in blood of recipient

**B/** Rh+ erythrocytes act as antigen and stimulate production of antibodies against antigen D (within weeks) – the individual becomes sensitized (i.e. antibodies are present in his blood)

**C/ 2nd transfusion of incompatible Rh+ blood** – antibodies react with antigen D, posttransfusion reaction occurs

(“d” does not induce production of antibodies)
Rh\(^+\) father + Rh\(^-\) mother →
A/ Rh\(^-\) fetus (no problem) or
B/ Rh\(^+\) fetus (may be a risk)

1st pregnancy
- blood of the mother and the fetus are separated by placenta that is a barrier for Er
- usually no problems with Rh incompatibility

- in case of complicated birth, accident, etc.
  the Rh\(^+\) erythrocytes of the fetus may enter the blood of the Rh\(^-\) mother

- antibody production against baby’s Er is induced in the mother (even as little as 0,5 ml of blood may start the Ab production)

- antibodies remain in blood of a Rh\(^-\) mother
2nd pregnancy
- problems occur if the 2nd baby is also Rh⁺
- antibodies from mother´s blood enter blood of the fetus through the placenta, attach to baby´s Er
- agglutination and hemolysis of Er of the fetus

Consequences
- hemolytic disease of the newborn: anaemia, hypoxia, icterus-risk of brain damage, death in utero

Next pregnancies – production of antibodies is even more higher (problems in about 3% of 2nd and 10% of 3rd pregnancies)

Treatment and prevention
- anti-D serum latest until 72 hours after termination of the pregnancy (birth, abortion) is given to the mother
- antibodies anti-D from the serum are attached to the Er of baby (in mother´s blood)
- the Er marked by anti-D are destroyed, thus antibody production by the mother´s body is prevented
The crossmatching test (simplified version)
Cross - matching
(major and minor crossmatches)

• Transfusion – general rule:
  „the blood of recipient and donor must be matching (compatible)“

Crossmatching
– assessment of compatibility between blood of donor and recipient
Other blood systems

- About 30 blood systems exist
- Clinically significant:

<table>
<thead>
<tr>
<th>System</th>
<th>MNSs</th>
<th>Kidd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kell (K, k)</td>
<td>MNSs</td>
<td>Kidd</td>
</tr>
<tr>
<td>Lewis (Lewis(^a), Lewis(^b))</td>
<td>Diego</td>
<td>Lutheran, etc.</td>
</tr>
</tbody>
</table>

- may cause incompatibility of donor´s and recipient´s blood despite compatibility in ABO and Rh system
- may cause mother/fetus incompatibility
- may cause posttransfusion reaction in individuals who often receive transfusion
Crossmatching test

- assessment of compatibility between blood of donor and recipient
- blood of both donor and recipient is centrifuged, serum is separated from erythrocytes
- test is done in 2 steps:

1. major crossmatching test:
   serum of recipient is mixed with erythrocytes of donor

2. minor crossmatching test:
   serum of donor and erythrocytes of recipient

Result:
- no agglutination = blood compatible
- agglutination = mismatching blood

Biological test
- when transfusion starts
- give 20 ml of blood, then wait about 2-3 minutes
- repeat 2 more times
- check for symptoms of transfusion reaction
- dyspnea, tachycardia, sweating, low blood pressure, dizziness, etc.
**Principle**
- blood of a donor and a recipient is mixed and the reaction is observed

**Procedure (simplified crossmatching)**
- on a glass slide place
  - a drop of physiological solution into the centre
  - a drop of donor’s blood to one end of the slide
  - a drop of recipients blood to the other end of the slide
- fuse all 3 drops
- read the result after 5 minutes
- if required, investigate microscopically

**Result:** agglutination – yes/no

**Conclusion:** blood matching/mismatching
Question
- the blood group of both a donor and the recipient is A Rh posit
- the cross-matching test shows agglutination
- can you explain this phenomenon?

Question
- will you cause agglutination when giving Rh posit blood to a Rh negat patient?
- please explain
Haemolysis
- destruction of erythrocyte membrane, haemoglobin is released from erythrocyte (e.g. plasma)
  (opaque suspension ⇒ transparent solution)

× osmotic
  - hypertonic solution
  - hypotonic solution

× chemical
  - acids, bases, tensides

× physical
  - mechanic or thermic energy, irrigation

× immunologic
  - transfusion of incompatible blood

× toxic
  - cell lysis caused by enzymes in poison of snakes, wasps, spiders, plants
  - daily approx 1% of Ery do haemolyze – old elements
  - hemolytic anaemia – decreased Hb concentration due to excessive haemolysis
ether and saponine are substances that cause chemical hemolysis

- put physiological solution into 2 tubes (approx ½ of a tube)
- add a few drops (5) of venous blood (with citrate) into both tubes
- add 1 ml ether or 1 small spoon of saponine to 1 tube
- mix well (cca 1 min)
- describe the changes in appearance of the tube content
Topics to study

- Types of leukocytes and their %
- Defensive properties of leukocytes
- Innate and acquired immunity – white blood cells that are involved/mechanisms
- Active/passive immunization
- Blood groups, system ABO – agglutinogens, agglutinins,
- Blood groups, Rh system - antigens
- Minor blood groups and their clinical implications
- Blood groups and transfusion - matching and mismatching blood
- Cross matching test, major and minor crossmatch
- Mother-foetus incompatibility

Next week you will visit a virtual clinical setting. You will face an emergency case in which you will train how to use the physiology knowledge that you studied from books in a clinical situation

- please revise the blood physiology
- it will be useful to know normal values of the blood variables, and causes of main abnormalities