Physiology of Blood 4

Hemostasis and hemocoagulation
Clinicians view

activation of thrombocytes/coagulation factors

a lot of interesting biochemistry

BLOOD CLOT
Components of hemostasis

- Blood vessels
- Thrombocytes
- Coagulation factors/proteins
Hemostasis

Subendothelial matrix

Hemostatic plug

WBC

PLT

Fibrin

RBC

Endothel

WBC
Vessel Injury

Platelet Activation

Platelet Aggregation

Coagulation Cascade

Tissue Factor

Thrombin

Platelet Plug

Platelet Activation

Vasoconstriction

Clot

Vessel Injury
Endothel

- Integrity of blood vessels protects from blood loss – it contains potent anticoagulative surface.
- Endothel is formed with one continuous layer on basal membrane and so it forms the first barrier against hemostasis and thrombosis.
- Integrity is dependant also on subendothelial and extracellular matrix, that is produced by endothel (e.g. collagen, basal membrane).
- Endothel cells produce substances anticoagulative factors to vascular lumen as well (e.g. heparine).
Endothelial substances produced to subendothel

- basal membrane
- collagen III & IV
- microfibrils
- elastin
- lamillin
- vibronectin
- protease inhibitors
- fibronectin
- mucopolysaccharides
- vWF

These proteins are important in intracellular interactions and in barrier formation (to stop the blood and plasma diffusion into extracellular space)
Endothelial substances produced to blood circulation

- PGI₂
- EDRF
- t-PA
- urokinase
- NO
- glycosaminoglycans
- ATIII/heparin sulfate
- protein kinase/thrombomodulin
- plasminogen activators
- tissue factor
- vWF
- factor V
- inhibitors (PAI-1, ATIII)
- IL₁, TNFα
- endothelin-1
- PAF

These substances improve blood fluidity (reologic characteristics)!!!
Thrombocytes

- Round or oval discs (1-4 μm in diameter).
- No nucleus, they cannot divide
- They have the function characteristics of normal cells
  - contractil elements (actin, myosin and thrombostenin)
  - residue of endoplasmatic reticulum and GA
  - mitochondria and enzymes to create ATP and ADP
  - able to synthesise prostaglandins (thromboxan A2)
  - produce stable factor for fibrin
  - Produce growth factors
- Are formed from megacaryocytes in bone marrow
- Normal value between 150-300,000/ml of blood
Thrombocytes

- membrane contains glycoproteins that decrease the adherence to normal endothel
- however, the adherence to damaged vessels is increased
- membrane contains phospholipids that have activation role in clot formation
- thrombocytes live around 8-12 days
- are degraded from circulation by tissue macrophages in spleen.
Impairment of endothelial cells will expose subendothelial (collagen), which changes the ability of TRO to adhere.

vWF- creates the "bridge" between membrane glycoproteins and TRO.

Collagen and thrombin induce TRO to secrete contents of granules → thromboxan 2 (TXA2) is formed.

TXA2 and ADP released from TRO stimulate TRO to aggregate and the primary hemostatic plug is formed.

Primary hemostatic clot stops bleeding only during the first minute, it is not hemostatis itself.
Thrombocytes activation

1. **Adhesion**
   - GpIIb/IIIa
   - Thrombocytes activation
   - GpIIb/IIIa
   - Platelet
   - Exposed Collagen
   - Endothelium
   - vWF

2. **Aggregation**
   - ADP
   - Adrenaline
   - THROMBIN
   - Platelet
   - GpIIb/IIIa

3. **Collagen**

4. **Endothelium**

5. **Exposed Collagen**
Contents of granules

- Dense granules
  - ATP
  - ADP
  - calcium
  - magnesium
  - serotonín
  - Epinephrine/adrenaline
Contents of granules

- **Hemostatic proteins**
  - fibrinogen
  - factor V
  - vWF
  - plasminogen
  - plasminogen activator inhibitor (PAI-1)
  - $\alpha_2$-antiplasmin

- **Nonhemostatic proteins**
  - $\beta$-thromboglobulin,
  - platelet factor 4
  - platelet derived growth factor (PDGF)
  - albumin
  - fibronectin,
<table>
<thead>
<tr>
<th>Factor No.</th>
<th>Factor name</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Fibrinogen</td>
<td>Fibrin is produced from it</td>
</tr>
<tr>
<td>II</td>
<td>Prothrombin</td>
<td>Creates fibrin</td>
</tr>
<tr>
<td>III</td>
<td>Thromboplastin</td>
<td>Receptor for VIIa</td>
</tr>
<tr>
<td>IV</td>
<td>Calcium</td>
<td>Cofactor</td>
</tr>
<tr>
<td>V</td>
<td>Proaccelerin</td>
<td>Receptor for Xa</td>
</tr>
<tr>
<td>VII</td>
<td>Von Willenbrandt factor</td>
<td>Activates TRO and adhesion</td>
</tr>
<tr>
<td>VIII</td>
<td>Antihemophilic factor</td>
<td>Receptor for IXa</td>
</tr>
<tr>
<td>IX</td>
<td>Christmas factor</td>
<td>Activates factor X</td>
</tr>
<tr>
<td>X</td>
<td>Stuart factor</td>
<td>Activates prothrombin and f VII</td>
</tr>
<tr>
<td>XI</td>
<td>Plasma Thromboplastic Antecedent</td>
<td>Activates factor IX</td>
</tr>
<tr>
<td>XII</td>
<td>Hagen factor</td>
<td>Activates XI</td>
</tr>
<tr>
<td>XIII</td>
<td>Fibrin stabilising factor</td>
<td>Creates fibrin polymers</td>
</tr>
<tr>
<td>XIV</td>
<td>Protein C</td>
<td>Inhibits Va and VIIa</td>
</tr>
</tbody>
</table>
Coagulation factors (by groups)

- **Fibrinogen group: I,V,VIII,XIII**
  - they can be found in TRO, they are consumed during clot formation process

- **Prothrombin group: II,VII,IX,X**
  - vitamin K dependant

- **Contact group: XI, XII, HMWK, prekallikrein**
  - they begin the intrinsic way of coagulation and fibrinolysis
Extrinsic and common pathway

Extrinsic pathway

Injured Cells

VIIa

Tissue Factor

Tissue Trauma

= Calcium & PL complex

*= active serine protease

Common pathway

prothrombin

*thrombin

fibrinogen

fibronogen

Fibrin polymer

XIII

XIIIa

CLOT

V

VIIa

Xa

XIIIa
Intrinsic pathway

- Activated by blood „trauma“ or exposure the collagen of blood vessel wall
- Initiation – activation of factor XII (to XIIa) and phospholipid release from TRO
- Activation of factor XI by factor XIIa.
  - this reaction requires the presence of HMW kininogens and is speed up by prekallikreins
- Activation of factor IX by factor XIa
- Activation of factor X by factor IXa, factor VII, TRO phospholipids and tissue factor
Intrinsic and common pathway

Prothrombin Activator

Common pathway

Intrinsic pathway

Prothrombin

VIII a

VIII

V

VIII a

IX a

IX

X

X a

XI

XI a

XII

prekallikrein

WOUND surface

kininogen (HMWK)

* = active serine protease

= Calcium & PL complex
Thrombin Activation

[Diagram showing the process of thrombin activation involving collagen, von Willebrand factor (vWF), endothelium, platelets, and prothrombin activator complex.]
Intrinsic and extrinsic pathways are common from factor X
Clot formation

- TRO
- ERY
- Fibrin
Anticoagulation and clinics

- **Heparin**
  - stimulates activity of ATIII 100-1000x

- **Kumarins**
  - warfarin will decrease the production of factors formed in liver – II, VII, IX and X
  - competes with vitamin K for binding sites

- **Aspirin (acetylsalicylic acid)**
  - cyclooxygenase inhibitor
  - prevents formation of thromboxan A2 and activation of platelets

- **Calcium-deionising substances**
  - citrate- sodium, ammonium, potassium – will mix with calcium in blood
  - several factors require calcium for activation
Take Home Message

- Hemostasis is always about balance between pro-coagulation and anticoagulation activity.

- Principle of coagulation is the change of prothrombine to thrombine so the thrombine can change fibrinogen to fibrin.
Physiology of blood 4. (Immunity)
Defense layers

Surface defense

Immune response

Inflammation
The levels of defense

Barriers

- Skin
- Mucosa
## Anatomical Barriers - Mechanical Factors

<table>
<thead>
<tr>
<th>System or Organ</th>
<th>Cell type</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>Squamous epithelium</td>
<td>Physical barrier Desquamation</td>
</tr>
<tr>
<td>Mucous Membranes</td>
<td>Non-ciliated epithelium (e.g. GI tract)</td>
<td>Peristalsis</td>
</tr>
<tr>
<td></td>
<td>Ciliated epithelium (e.g. respiratory tract)</td>
<td>Mucociliary elevator</td>
</tr>
<tr>
<td></td>
<td>Epithelium (e.g. nasopharynx)</td>
<td>Flushing action of tears, saliva, mucus, urine</td>
</tr>
</tbody>
</table>
### Anatomical Barriers - Chemical Factors

<table>
<thead>
<tr>
<th>System or Organ</th>
<th>Component</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>Sweat</td>
<td>Anti-microbial fatty acids</td>
</tr>
<tr>
<td>Mucous Membranes</td>
<td>HCl (parietal cells)</td>
<td>Low pH</td>
</tr>
<tr>
<td></td>
<td>Tears and saliva</td>
<td>Lysozyme and phospholipase A</td>
</tr>
<tr>
<td></td>
<td>Defensins (respiratory &amp; GI</td>
<td>Antimicrobial</td>
</tr>
<tr>
<td></td>
<td>tract)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sufactants (lung)</td>
<td>Opsonin</td>
</tr>
</tbody>
</table>
## Anatomical Barriers - Biological Factors

<table>
<thead>
<tr>
<th>System or Organ</th>
<th>Component</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin and mucous membranes</td>
<td>Normal flora</td>
<td>Antimicrobial substances</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Competition for nutrients and colonization</td>
</tr>
</tbody>
</table>
The levels of defence

Non-specific immune response
- Phagocytosis
- NK cells
- Inflammation
- Febrility

If barriers are penetrated

Specific immune response
- Cells immunity
  - Humoral immunity

If not sufficient

Barriers
- Skin
- Mucosa
### Comparison of Innate and Adaptive Immunity

<table>
<thead>
<tr>
<th>Innate Immunity</th>
<th>Adaptive Immunity</th>
</tr>
</thead>
<tbody>
<tr>
<td>- No time lag</td>
<td>- A lag period</td>
</tr>
<tr>
<td>- Not antigen specific</td>
<td>- Antigen specific</td>
</tr>
<tr>
<td>- No memory</td>
<td>- Development of memory</td>
</tr>
</tbody>
</table>
Leukocytes
white blood cells ~ WBC

agranular
- lymphocytes 20 - 25 %
- monocytes 3 - 8%

granular
- basophils .5 - 1%
- neutrophils 60 - 70%
- eosinophils 2 - 4%

T-cell, B-cell, NK Cell
Number per Liter of Blood

- Neutrophils: 1.8-7.7 (59%)
- Eosinophils: 0-0.45 (2.7%)
- Basophils: 0-0.20 (0.5%)

- Leukocytes
  - Granulocytes
    - Neutrophils: 1.8-7.7 (59%)
    - Eosinophils: 0-0.45 (2.7%)
    - Basophils: 0-0.20 (0.5%)
  - Lymphocytes
    - T Cells: 1.0-4.8 (33.7%)
    - B Cells
    - NK Cells: 0-0.80 (4.1%)
  - Monocytes: 0-0.80 (4.1%)

- Total: 4.4-11.3 (100%)

Erythrocytes
- W: 4100-5100
- M: 4520-5900

Platelets: 172-450

W, women; M, men
Practicals

- Coagulation time by Lee-White
- Bleeding time by Duke
- Determination of prothrombine time
- Leukogram