Hemostasis and Blood Coagulation

Hemostasis

- Definition: the arrest of bleeding
- Primary hemostasis
  - Formation of platelet plugs
  - Blood vessels (endothelium)
- Secondary hemostasis
  - Formation of fibrin through the coagulation cascade
- Tertiary hemostasis
  - Formation of plasmin for the breakdown of the clot
Extracellular Matrix Proteins

- **Fibronectin**
  - An important protein in terms of cell adhesion and matrix stability
  - Has binding sites (e.g., RGD) for integrins, as well as other ECM molecules such as collagen and fibrin

- **Laminin**
  - A large group of cross-shaped glycoproteins
  - Serves primarily a structural role in basement membranes, anchoring cells to the basal lamina
  - Important in cell migration, especially in neuron outgrowth

- **Collagen**
  - A large family of proteins that represent the majority of proteins in mammalian tissue (~25%)
  - Many different types of collagen: fibrillar, fibril-associated, and network forming collagen types
Overview of Hemostasis

1-2 sec  
1. Platelet adhesion  
ADP, TxA₂

10-20 sec  
2. Platelet aggregation/activation  
Microparticles release

1-3 min  
3. Plug formation  
Fibrin formation

3-5 min  
4. Consolidation  
Retraction

5-10 min  
5. Fibrin stabilization

Global Scheme of Hemostasis

Platelet system  Vascular system  Coagulation system

Injured vessel  →  Tissue factor  
Collagen  →  Intrinsic clotting  
Platelet adhesion  →  Thrombin

Platelet activation  ←  Vasoconstriction

Platelet aggregation  ↓  Fibrin

Permanent fibrin-platelet plug
Balance Between Hemostasis, Bleeding and Thrombosis

- Coagulation
- ↑Fibrinolysis
- ↑Coag. inhibitors
- □Primary hemostasis

Bleeding disorder    Normal hemostasis    Thrombotic disorder

Coagulation Cascade

[Diagram of the Coagulation Cascade]

[Diagram showing the intrinsic and extrinsic systems of the coagulation cascade, including factors and their interactions, such as factor XII, factor XI, factor VIII, and fibrin formation.]

[Diagram illustrating the role of kallikrein and prekallikrein in the cascade.]
Activation of Coagulation Pathway

• Intrinsic pathway
  – Takes place in the absence of Ca2+
  – Initiated by the exposure of blood to material surface
    (subendothelium, polymers) and negatively charged surfaces
    (glass, clay)
    • fXII adheres to negatively charged surfaces and undergoes
      conformational changes
    • Highly susceptible to cleavage by trace amount of fXIIa

• Extrinsic pathway
  – Thromboplastin (Tissue Factor)
    • Transmembrane protein
    • Found in high levels in brain, lung and placenta
    • Found in blood vessel intima
  – fVII

Clotting Factors

<table>
<thead>
<tr>
<th>Clotting Factor</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Fibrinogen</td>
</tr>
<tr>
<td>II</td>
<td>Prothrombin</td>
</tr>
<tr>
<td>III</td>
<td>Thromboplastin</td>
</tr>
<tr>
<td>IV</td>
<td>Calcium</td>
</tr>
<tr>
<td>V</td>
<td>Labile factor</td>
</tr>
<tr>
<td>VI</td>
<td>Proconvertin</td>
</tr>
<tr>
<td>VIII</td>
<td>Anti-hemophilic factor (AHF)</td>
</tr>
<tr>
<td>IX</td>
<td>Christmas factor</td>
</tr>
<tr>
<td>X</td>
<td>Stuart-Prower factor</td>
</tr>
<tr>
<td>XI</td>
<td>Plasma thromboplastin antecendent (PTA)</td>
</tr>
<tr>
<td>XII</td>
<td>Hageman factor</td>
</tr>
<tr>
<td>XIII</td>
<td>Fibrin stabilizing factor</td>
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</tbody>
</table>
Classification of Clotting Factors

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Transglutaminase</th>
<th>Serine protease</th>
<th>Cofactor</th>
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<tbody>
<tr>
<td>Fibrinogen</td>
<td>Factor XIII</td>
<td>Factor XII</td>
<td>High MW Kinnogen</td>
</tr>
<tr>
<td>Prekallekrein</td>
<td>Factor VIII</td>
<td>Factor IX</td>
<td>Tissue factor</td>
</tr>
<tr>
<td>Factor XI</td>
<td>Factor V</td>
<td>Factor X</td>
<td></td>
</tr>
<tr>
<td>Factor IX</td>
<td>Tissue factor</td>
<td>Factor VII</td>
<td></td>
</tr>
<tr>
<td>Factor X</td>
<td></td>
<td>Factor VII</td>
<td></td>
</tr>
<tr>
<td>Factor II</td>
<td></td>
<td>Protein C.S</td>
<td></td>
</tr>
</tbody>
</table>

Fibrin Assembly

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Fibrinolysis: Breakdown of Fibrin Clots

Control Mechanisms

- Required to prevent massive clotting throughout body
- Blood flow reduces local concentration of activated factors
- Rate increases with surface area
- Natural coagulation inhibitors and feedback proteins remove activated proteins
  - antithrombin; protein C system; heparin; thrombomodulin
- Fibrinolysis
  - Activation of plasmin from plasminogen; catalyzed by plasminogen activators such as tissue plasminogen activator (tPA) and urokinase
  - Plasmin in turn cleaves fibrin into different degradation products
Thrombin can bind to……

Prothrombotic
• Factor XIII
• Factor V
• Fibrinogen
• Platelet
• Endothelial cell
Anti-thrombotic (or Profibrolytic)
• Antithrombin III
• Thrombomodulin/Protein C

Platelets
• Non-nucleated, disk-shaped cells having a diameter 3-4 \( \mu \text{m} \)
• Functions
  – Bleeding arrest and stabilize blood clots by
    • catalyzing thrombin production from prothrombin
    • forming rapid assembly
• 3 types of cytoplasmic storage granules whose contents are released upon platelet activation
  – α granules: heparin binding proteins, platelet factor 4 (PF4), β-thromboglobulin, platelet derived growth factor (PDGF), coagulation proteins such as fibrinogen, von Willebrand factor, factor V and factor VIII, and ECM proteins such as fibronectin
  – Dense granules: adenosine diphosphate (ADP), serotonin, and Ca2+
  – Lysosomal granules: enzymes such as acid hydrolase
• Activated by ADP, thrombin, fibrinogen binding etc
• Adhesion, aggregation and activation
Platelet Adhesion, Aggregation and Activation

- Adhere to both artificial (implants) and natural surfaces (ECM, vascular injuries)
- Interaction of surface receptors
  - GPIIb/IIIa (most abundant) to RGD of fibrinogen, fibronectin, vitronectin, von Willebrand factor etc
  - GP Ib to von Willebrand factor
  - GPII/III to collagen
  - Mediated by Ca2+
- Aggregation through the binding with “bridging molecules” such as fibrinogen
- Recruitment of more platelets by cytokines and other mediators such as thromboxane A2, arachidonic acid and epinephrine
- Activation
  - Initiation of contractile processes lead to shape change; from discoid to pseudopodium formation
  - Release of granule contents
    - ADP, thrombin: platelet activators
    - TxA2: recruitment of more platelets
    - PF4, βTG, Ca2+

Platelet Activation

PS: pseudopodium
Markers of Platelet Activation

- **Release products**
  - Platelet factor 4 (PF4)
  - Beta thromboglobulin
  - thrombospandin

- **Neo-surface expression of Neo-epitopes**
  - P-selectin
  - Thrombospandin bound to GPIV
  - Fibronectin bound to GPIIa-Ic
  - Fibrinogen bound to GPIIb-IIIa
  - Neo-epitopes on GPIIb-IIIa

Platelet-Fibrinogen Bridging
Effect of Blood Flow on Endothelial Cell Shape

Functions of Endothelial Cell

- A semipermeable barrier for the transfer of substances between blood and surrounding tissues
- Mediation of vascular repair processes
- Processing of antigen immunity
- Maintenance of thromboresistance
- Synthesis of mediators that regulate interactions between vessel wall and blood components
  - Factor VIII, von Willebrand’s factor
  - Fibronectin
  - Collagen
  - Thrombomodulin
  - Tissue plasminogen activator (tPA)
  - Plasminogen activation inhibitor (PAI-1)
Thromboresistance of Endothelial Cell

- Antiplatelet
  - Prostacyclin (PGI\(_2\)): inhibits platelet adhesion and aggregation
  - Degradation of ADP, a platelet activator

- Anticoagulant
  - Thrombomodulin
    - Uptake, inactivation and clearance of thrombin
    - Participate in Protein C activation
  - Heparan sulfate
    - Antithrombin III binding

- Profibrinolytic
  - Plasminogen activators: plasmin production for fibrin degradation (e.g., tissue plasminogen activator (tPA))
Consequences of Endothelial Disruption

- Vasoconstriction
- Platelet adhesion to subendothelium
  - Aggregation and activation
- Initiation of coagulation
  - Coagulation factor released from endothelium
    - fV, fVIII, von Willebrand factor, tissue factor
- Fibrinolysis follows the release of tissue plasminogen (tPA) from endothelium

Stimuli that Change Endothelium to Procoagulant State

- Endotoxin
- Cytokines
  - TNFα, IL-1
- Thrombin
- Others
  - Substrates
Prothrombotic Roles of Endothelium

- **Procoagulant**
  - Increase procoagulant (vWF/fVIII)
  - Decrease inhibitors
- **Anti-fibrinolytic**
  - Increase plasminogen activator inhibitor (PAI-1)
  - Decrease tissue plasminogen activator (tPA)
- **Pro-platelet activating**
  - Platelet activating factor
- **Increase leukocyte adhesion**
Antithrombotic Agents

- Anticoagulants
  - Heparin
  - Coumadin
  - Hirudins (produced by the leech)
  - Sodium citrate
  - P-PACK (D-phenylalanyl-L-prolyl-L-arginychloromethyl-ketone)
  - Recombinant proteins of thrombomodulin, antithrombin III, tissue factor pathway inhibitor

- Antiplatelet agents
  - Inhibitors of prostaglandin and thromboxane synthesis (e.g., aspirin, ibuprofen)
  - Decrease the availability of intracellular Ca2+
  - Block platelet aggregation (e.g., anti-GPIIb-IIIa antibodies)
  - Act on biological membrane

Mechanism of Heparin Action

1. Heparin binding to antithrombin III
2. Conformational changes of antithrombin III
3. Enhances binding with thrombin (TAT complex)
   (Antithrombin III neutralizes thrombin, fIXa, fXa, fXla and fXIIa by forming a 1:1 complex whose formation occurs slowly in the absence of heparin)
4. Reuse of heparin
Warfarin (Coumadin) Therapy

- Vitamin K exists in two forms:
  - K1: from leafy vegetables and oils
  - K2: form bacteria of gut
- Absorbed in the presence of bile salts
- No significant body storage
- Necessary for full function of coagulation factors (II, VII, IX, X, Pro C)

Weblinks

- Complement system
  www-medlib.med.utah.edu/webPath/INFLHTML/Infl080.html
  www-medlib.med.utah.edu/webPath/INFLHTML/Infl081.html
- Diapedesis
  www-medlib.med.utah.edu/webPath/INFLHTML/Infl070.html
  www.cat.cc.md.us/courses/bio141/lecguide/unit1/prostruct/cytogp/diaped1.html
- Histology of white blood cells
  www-medlib.med.utah.edu/webPath/INFLHTML/Infl073.html
- Coagulation cascade
  www.mhhe.com/biosci/esp/2002_general/Esp/folder_structure/tr/m1/s7/trm1s7_3.htm