Cell-based Coagulation

Coagulation System Overview
And Cell-Based Coagulation

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Your Interactive Hemostasis Resource
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Coagulation Summary

• Platelets
• Plasma-based cascade
• Coagulation controls
• Fibrinolysis
• Cell-based coagulation
• Virtues and limitations

3 YO Boy: Chronic Joint Pain

A 3 year-old boy experienced painful joints. His hematologist ordered a prothrombin time (PT), activated partial thromboplastin time (PTT), and platelet count.

Results:

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>RI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet count</td>
<td>324,000/uL</td>
<td>150–400,000/uL</td>
</tr>
<tr>
<td>PT</td>
<td>12.9 sec</td>
<td>12.6–14.6 sec</td>
</tr>
<tr>
<td>PTT</td>
<td>67 sec</td>
<td>25–35 sec</td>
</tr>
</tbody>
</table>

What are the Possibilities?

• Liver disease, vitamin K deficiency, renal disease?
  – No symptoms, normal diet, PT normal
  – Liver enzymes normal
• Lupus anticoagulant?
  – Unlikely in child
• Inherited single factor deficiency

PT and PTT Test Results
in Inherited Coagulopathies

<table>
<thead>
<tr>
<th>Factor</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIII</td>
<td>12%</td>
</tr>
<tr>
<td>IX</td>
<td>95%</td>
</tr>
<tr>
<td>XI</td>
<td>108%</td>
</tr>
<tr>
<td>RI</td>
<td>50–150%</td>
</tr>
</tbody>
</table>

• Implications of factor VIII deficiency: hemophilia A
• The mild form accounts for late onset and mild symptoms
• Therapy: RICE, DDAVP, tranexamic acid, FVIII concentrate
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Plasma-Based Coagulation Model 1964–2004


Intact intimae suppress hemostasis:
- Plasminogen activator inhibitor -1
- Tissue factor pathway inhibitor
- Tissue plasminogen activator
- Negative surface charge
- Prostacyclin (PGI₂)
- Thrombomodulin
- Heparan sulfate
- ADPase (CD39)
- Nitric oxide
- Urokinase
- SMC
- FB
- EC
- RBC
- PLT
- WBC

Injured Endothelial Cells Become Hemostatic

- Integrins
  - α5β1: fibronectin
  - α6β1: laminin
  - αvβ3: vitronectin
- Chemokines:
  - MIP-1α
  - MCP-1
  - IL-8
- Adhesion Molecules:
  - ICAM-1
  - ICAM-2
  - VCAM-1
  - PECAM
  - P-selectin

Trauma to Vessel Exposes Collagen and Tissue Factor

- Intact intimae suppress hemostasis:
  - Plasminogen activator inhibitor -1
  - Tissue factor pathway inhibitor
  - Tissue plasminogen activator
  - Negative surface charge
  - Prostacyclin (PGI₂)
  - Thrombomodulin
  - Heparan sulfate
  - ADPase (CD39)
  - Nitric oxide
  - Urokinase

Tissue Factor “Extrinsic” Pathway

Access to tissue factor (TF) on smooth muscle cells and fibroblasts in injured vessel wall activates VII. In inflammation, TF appears on endothelial cells and monocytes.

By Shape:
- SMC
- FB
- EC
- RBC
- PLT
- WBC

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**Tissue Factor and VIIa Activate IX of the “Common” Pathway**

- **By Shape:**
  - **TF:** Tissue Factor
  - **VIIa:** Factor VIIa
  - **IX:** Factor IX
  - **X:** Factor X
  - **VII:** Factor VII

**Common Pathway**

- **Xa binds VIIIa, platelet phosphatidyl serine, and Ca^{++} to form “tenase,” which activates factor X**

**Thrombin Cleaves Fibrinogen**

- **Fibrinopeptides A and B**

**Fibrin Polymerization and Crosslinking**

- **XIa stabilizes fibrin polymer by forming intermolecular γ-dimers between neighboring γ-chain glutamine and lysine**

**Intrinsic Pathway**

- **XIa binds VIIIa, platelet phosphatidyl serine, and Ca^{++} to form the “tenase” complex that activates X**

**Initiation:** vascular injury exposes fibroblast (FB) tissue factor (TF)

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In Vitro Contact Activation

- XIa binds HMWK (Fitzgerald factor) and PK (Fletcher factor) to activate factor XI
- Negatively charged particles or surfaces activate XII
- Intrinsinc pathway proceeds from XIa

Vitamin K

- Necessary for normal activity of prothrombin (II) and factors VII, IX, and X
- Also necessary for normal activity of control proteins C, S, and Z
- Mediates γ-carboxylation of glutamic acid
- Necessary for Ca++ fixation to phospholipid
- Affected by oral anticoagulants

γ-carboxylation of Glutamic Acid

- Vitamin K
- Necessary for γ-carboxylation of glutamic acid
- Involved in the formation of factor X and prothrombin

Thrombin Properties

- THR (IIa)
- Properties of thrombin include:
  - clotting activity
  - ability to activate other clotting factors
  - interaction with fibrinogen and fibrin

Procoagulant Concentrations and Their Plasma Half-lives

<table>
<thead>
<tr>
<th>Factor</th>
<th>Category</th>
<th>Half-life</th>
<th>Plasma Level</th>
<th>Hemostatic Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen (I)</td>
<td>Substrate</td>
<td>4 days</td>
<td>280 mg/dL</td>
<td>50 mg/dL</td>
</tr>
<tr>
<td>Prothrombin (II)</td>
<td>Protease</td>
<td>60 hours</td>
<td>1300 µg/mL</td>
<td>20%</td>
</tr>
<tr>
<td>V</td>
<td>Collator</td>
<td>16 hours</td>
<td>680 µg/mL</td>
<td>25%</td>
</tr>
<tr>
<td>VII</td>
<td>Protease</td>
<td>6 hours</td>
<td>120 µg/mL</td>
<td>20%</td>
</tr>
<tr>
<td>VIII</td>
<td>Collator</td>
<td>12 hours</td>
<td>0.24 µg/mL</td>
<td>30%</td>
</tr>
<tr>
<td>IX</td>
<td>Protease</td>
<td>24 hours</td>
<td>5 µg/mL</td>
<td>30%</td>
</tr>
<tr>
<td>X</td>
<td>Protease</td>
<td>30 hours</td>
<td>10 µg/mL</td>
<td>25%</td>
</tr>
<tr>
<td>XI</td>
<td>Protease</td>
<td>2-3 days</td>
<td>6 µg/mL</td>
<td>25%</td>
</tr>
<tr>
<td>XIII</td>
<td>Tranegulaminase</td>
<td>7-10 days</td>
<td>290 µg/mL</td>
<td>2-3%</td>
</tr>
<tr>
<td>VWF</td>
<td>Collator</td>
<td>30 hours</td>
<td>6 µg/mL</td>
<td>50%</td>
</tr>
</tbody>
</table>

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The Platelet Clot or “White” Clot
- Composed of platelets and von Willebrand factor
- The endpoint of “primary” hemostasis
- Complete hemostasis in invertebrates and lower vertebrates

The Fibrin Clot or “Red” Clot
- Composed of platelets, fibrin, and RBCs
- The endpoint of “secondary” hemostasis
- Complete hemostasis in higher vertebrates

Platelet Adhesion Properties
- Platelets bind vessel wall via VWF and fibrin
  - Platelet receptors GP Ia/IIa, IV, and VI bind intimal collagen
  - Platelet receptors GP Ib/V/IX and GP IIb/IIIa adhere to VWF and fibrin
  - GP IIb/IIIa supports platelet aggregation
- Platelets bind other adhesive proteins: thrombospondin, fibronectin
- Fibrin binds platelet interior actin: clot retraction

A 7 Year-old Girl: Elective Surgery
A healthy 7 year-old girl was scheduled for elective outpatient surgery. The surgeon ordered a screening platelet count, PT, and PTT. She had experienced no bleeding.

Results:

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet count</td>
<td>237,000/uL</td>
</tr>
<tr>
<td>PT</td>
<td>13.5 sec</td>
</tr>
<tr>
<td>PTT (APTT)</td>
<td>47 sec</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixing study patient/control</td>
<td>47 sec</td>
</tr>
<tr>
<td>Mixing study 2h incubated control</td>
<td>34 sec</td>
</tr>
<tr>
<td>Mixing study 2h incubated PTT 1:1</td>
<td>36.5 sec</td>
</tr>
<tr>
<td>Mixing study PTT RI</td>
<td>25–35 sec</td>
</tr>
</tbody>
</table>

What are the Possibilities?

- Lupus anticoagulant inhibitor?
  - No: immediate correction within 10% of control
- Specific inhibitor?
  - No: 2h correction to within 10% of 2h control
- Liver disease, vitamin K deficiency, renal?
  - No symptoms, PT normal, liver enzymes normal
- Inherited single factor deficiency?
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PT and PTT Test Results in Inherited Coagulopathies

<table>
<thead>
<tr>
<th>PT</th>
<th>PTT</th>
<th>Congenital Single Factor Deficiency (Hemophilia)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long</td>
<td>Normal</td>
<td>VII</td>
</tr>
<tr>
<td>Long</td>
<td>Long</td>
<td>X, V, II, and fibrinogen</td>
</tr>
<tr>
<td>Normal</td>
<td>Long</td>
<td>VIII, IX, XI</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Contact factors: XII, prekallikrein, high MW kininogen</td>
</tr>
</tbody>
</table>

1. PT and PTT prolonged when fibrinogen is < 100 mg/dL.
2. Contact factor deficiencies affect PTT results, but do not cause bleeding.

A 7 Year-old Girl: Elective Surgery Factor Assay Results

<table>
<thead>
<tr>
<th>Intrinsic Pathway Factor</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor XI</td>
<td>123%</td>
</tr>
<tr>
<td>Factor XII</td>
<td>37%</td>
</tr>
<tr>
<td>PK</td>
<td>97%</td>
</tr>
<tr>
<td>HMWK</td>
<td>89%</td>
</tr>
<tr>
<td>RI</td>
<td>50–150%</td>
</tr>
</tbody>
</table>

- Bleeding implications of factor XII deficiency: none
- Incidence: 3%

True or False

1. Coagulation is triggered by exposure to tissue factor.
2. Coagulation is also triggered by the in vivo activation of factor XII.
3. Factor VIII deficiency causes bleeding.
4. Coagulation can occur without platelet phosphatidylserine.

Antithrombin

- Plasma antithrombin activity is upregulated by endothelial cell heparan sulfate, a long-chain glycosaminoglycan
- Therapeutic heparin upregulates plasma antithrombin 1000-2000×
- Antithrombin is a serine protease inhibitor (SERPIN) that neutralizes thrombin and Xa

Glycosaminoglycan

- Linear heteropolysaccharide
  - Disaccharide repeating unit
  - Chondroitin sulfate
  - Dermatan sulfate
  - Keratan sulfate
  - Heparan sulfate
  - Hyaluronic acid
  - Heparin

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Unfractionated Heparin Binds Antithrombin to Thrombin

Unfractionated Heparin Binds Antithrombin to Xa

The Protein C Control Pathway

• Protein C activated by thrombomodulin-bound thrombin
• Becomes serine protease specific for Va and VIIIa
• Forms cell-surface complex specific for Va, VIIIa

Protein C Control Pathway
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Virtues of the Plasma Coagulation System Model

- It models coagulation as a series of amplifying proteolytic reactions
  - Each protease cleaves and activates the subsequent substrate zymogen in the series
- It recognizes the participation of platelet anionic phospholipids, mainly phosphatidylserine
  - Inert although essential assembly site
- It models the screening tests PT and PTT as corresponding to the “extrinsic” and “intrinsic” systems

Limitations of the Plasma Coagulation System

- If there is a separate tissue factor pathway, why doesn’t VIIa/TF activate enough X to compensate for a lack of factor VIII or IX in hemophilia?
- If VIII or IX deficiency both cause severe bleeding, why is XI deficiency bleeding mild and variable?
- Why is no fibrin generated when the platelet count is less than 10,000/uL?
- Why does aspirin reduce thrombin formation?

The Answer

- There is no “extrinsic,” “common,” or “intrinsic” pathway
- There is no plasma coagulation
- Coagulation occurs only under the control of cells

True or False

1. Antithrombin deficiency confers a risk of thrombosis.
2. Protein S excess confers a risk of hemorrhage.
3. Deep vein thrombosis is common in 30-year-olds.
4. Though thrombin is procoagulant, it activates protein C on endothelial cells.

The Cell-Based Coagulation System 2001-Present


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Overlapping Coagulation Phases

Initiation: Tissue Factor Bearing Cell

- TF constitutive on fibroblast, smooth muscle cell, or induced on monocytes or endothelial cells
- Exposure of TF cleaves VII → VIIa and binds TF/VIIa
- TF/VIIa cleaves X → Xa; Xa cleaves and binds V → Va
- Xa/Va cleaves prothrombin (Pro, II) → thrombin (Thr, IIa)
- TF/VIIa also cleaves IX → IXa
  - With no VIII around, IXa cannot function
- Free initiation proteases are bound by TFPI, ZPI or AT
- Occurs away from injury site and outside of vessels

Initiation: TF-bearing Cell

- Thrombin (IIa) in direct “hand-off” from TF-bearing cells escapes AT and binds nearby COAT platelets
  - Triggers release of VIII/VWF and V from α-granules
  - Cleaves and activates VII → VIIa and V → Va
  - Cleaves VIII from VIII/WF
  - Cleaves and activates XI from plasma and PLT α-granules → XIa
- IXa from TF-bearing cells binds VIIa on COAT platelets
- Xa from TF-bearing cells binds Va on COAT platelets
- XIa binds COAT platelet membranes
  - Cleaves and activates IX from plasma/platelets → IXa

Amplification: COAT Platelet

- Amplification begins at injury
- Platelets adhere to injury site collagen and VWF
- Platelets contact thrombin-producing tissue factor-bearing cells and become partially activated
- Called “collagen and thrombin-stimulated” (COAT) platelets

Amplification: COAT Platelet

- Tissue factor-bearing fibroblast delivers IIa, IXa, and Xa

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Propagation: COAT Platelet

- COAT platelet continues to be activated by thrombin, collagen and VWF
- Tenase and prothrombinase complexes now assemble on platelet surface
  - TF/VIIa and Xa/Va are the extrinsic mechanism
  - Xa and IXa/VIIIa are intrinsic
- The pathways now act at the platelet surface to produce high-volume thrombin
- Fibrin polymerization and stabilization

Cell Localization

- Tissue factor has to be on a cell that supports prothrombinase activity (Va/Xa)
  - Fibroblasts, smooth muscle cells, also macrophages, monocytes, and endothelial cells when induced
- Malignant cells that make TF but don’t support prothrombinase activity are not as effective

Coagulation Control: Endothelial Cells

- Thrombomodulin (TM): binds thrombin and activates protein C → APC
- Endothelial protein C receptor (EPCR-1) binds APC to surface
- APC binds protein S, inactivates Va and VIIIa
- Heparan-like glycosaminoglycans activate AT
- Cell-surface ADPase neutralizes platelet ADP

Cell Localization: Platelets

- No TF on platelets, so they have to adjoin TF bearing cells
- No factor VII or Vlla receptor site on platelets
- Thrombin cleaves protease activated receptor (PAR)
- COAT activation moves phosphatidylserine to outer leaflet to support “tenase” and “prothrombinase”
- GP Ib/IX binds VWF, COAT platelets adhere to injury sites
- Glycoprotein Ib/IIa binds fibrinogen and VWF
- Platelet provides surface receptors for VIIIa, Va, IX, X, XI
- Subsequent non-COAT platelet layer damps the reaction

Virtues of the Cellular Coagulation System

- If there is a separate tissue factor pathway, why can’t the activation of factor X by VIIa/TF compensate for a lack of factor VIII or IX in hemophiliacs?
  - Because the activation of X by VIIa/TF occurs on the wrong cell—the fibroblast
  - Free Xa is inhibited by AT and TFPI—it does not diffuse to a platelet
- If VIII or IX deficiency both cause severe bleeding, why is XI deficiency bleeding variable?
  - Free IXa can transfer to the platelet as AT has less effect
  - Free IXa on platelet bypasses need for factor XI
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Thrombosis and Cellular Coagulation

- If thrombosis is hemostasis that occurs on endothelial cells, therapy could target the vulnerable endothelial cells
- Aspirin effect on platelets also slows coagulation
- Thrombocytopenia also slows coagulation

Fibrinolysis: Fibrin Degradation

- PAI-1, TPA, and plasmin bind fibrin polymers through “kringle” structures
- Plasmin cleaves fibrin at selected lysine and arginine residues
- TAFIa cleaves fibrin C-terminal lysines that plasmin requires for their cofactor activity

Kringle Adhesion

Fibrinolysis

Wound Healing

- Fibrin attaches to platelet actin, clot retracts
- Neutrophils that first appear are replaced by macrophages
- Macrophages secrete vascular endothelial cell growth factor (VEGF), triggers neovascularization
- Platelets secrete platelet derived growth factor that attracts and stimulates fibroblasts and smooth muscle cells

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