The Role of Platelet Activation in the Pathogenesis of Atherothrombosis

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Learning Objectives

• Understand the integral link between platelet aggregation and the coagulation cascade
• Recognize the role of platelet activation and aggregation in atherothrombosis
• Assess the rationale for therapeutic interventions to prevent platelet activation and aggregation

The Role of Platelets in ACS

• Intact endothelium releases antithrombogenic agents (prostacyclin, NO) and expresses CD39, an ectoADPase
• Rupture of coronary plaque exposes thrombogenic subendothelium
• Platelets are recruited to injury site, adhere, and spread
• Platelet adhesion signals the release of platelet agonists, recruitment of nearby platelets, and aggregate formation via GPIIb/IIIa
• Platelet-rich thrombus forms causing partial or total vascular occlusion and resulting in cardiac injury

Platelet Adhesion: The Role of von Willebrand Factor

- Exposure of subendothelium leads to binding of von Willebrand factor (vWF) to collagen within seconds
- Platelets tether to bound vWF and binding to collagen and other matrix proteins via other platelet surface receptors leads to stable adhesion
- Initial adhesion is followed by formation of stable bonds to matrix proteins and platelet activation
- vWF in plasma also has a significant prothrombotic effect by promoting platelet aggregation under high shear stress conditions
- Elevated vWF is a risk factor for acute coronary events


Platelet Adhesion and Aggregation

Platelet Activation

- Multiple potential agonists
- Major agonists include collagen, ADP, thromboxane A₂ (TxA₂) and thrombin
- Tissue factor (TF) released from damaged endothelial wall leads to generation of thrombin
- Generated thrombin binds to the platelet PAR1 receptor and contributes heightened platelet response

Platelet Activation Mechanisms

- Signals generated inside the activated platelet cause GPⅡb/Ⅲa conformational changes that expose fibrinogen or vWF binding sites
  - Phospholipid hydrolysis
  - Protein phosphorylation
  - Increase in platelet intracellular free Ca²⁺
- Binding of fibrinogen (dimeric) and vWF (multimeric) crosslinks adjacent platelets, promoting additional platelet activation and aggregate formation

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### Receptors and Ligands in Platelet Thrombus Formation

<table>
<thead>
<tr>
<th>Phase of response</th>
<th>Substrates, agonists, ligands</th>
<th>Receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initiation</strong></td>
<td>Tethering and adhesion</td>
<td>vWF, GP Ib-IX-V, Collagen, Fibronectin, Fibrinogen, Laminin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GPIb-IX-V, α2β1, GPIV-FcRγ, αIIbβ3, α5β1, α6β1</td>
</tr>
<tr>
<td><strong>Propagation</strong></td>
<td>Activation</td>
<td>α-thrombin, ADP, Thromboxane A2, Epinephrine, PAR1, PAR4, GP Ib-IX-V, P2Y1, P2Y12, TP</td>
</tr>
<tr>
<td></td>
<td>Aggregation</td>
<td>Fibrinogen, vWF, fibronectin, αIIbβ3 (activated)</td>
</tr>
<tr>
<td><strong>Stabilization</strong></td>
<td></td>
<td>P-selectin, Ephrin B1, CD45 ligand, PSGL-1, GPIb-IX-V, other EPH kinases (A4/B1)</td>
</tr>
</tbody>
</table>


### Coagulation

- Not just a cascade of proteolytic reactions as originally proposed
- Coagulation reactions occur as overlapping steps on specific cell surfaces
- Hemostasis requires formation of consolidated platelet-fibrin plug and localization of procoagulant molecules
- Cells in the vasculature play different roles depending on procoagulant and anticoagulant activities
  - Adherent, activated platelets and platelet microparticles support procoagulant activities
  - Vascular endothelial cells maintain anticoagulation (via TFPIs, HS, and TM)


### Coagulation Cascade

- Requires 2 cell types
  - TF-bearing cells
  - Platelets (procoagulant surface and coagulation factors)
- Physical separation of cell types also regulates process until injury occurs
- 3 steps of coagulation
  - Initiation
  - Amplification
  - Propagation

**Initiation of Coagulation**

- Occurs with exposed subendothelium TF or cells that express TF
- Prothrombinase complex is formed on the surface of activated platelets: factor VIIa binds TF, VIIa/TF and activated factor Xa assemble with factor Va
- Thrombin, once formed, amplifies, propagates, and sustains the coagulation response

**Amplification of Coagulation**

- Small amount of thrombin is generated on TF-bearing cells
- Thrombin amplifies coagulation response via several pathways
  - Activates nearby platelets through PAR1 receptor
  - Activates factors V and VIII on platelet surface
  - Releases factor VIII bound to vWF
    - Factor VIIa binds factor IXa and, in conjunction with platelet-bound Xla, forms intrinsic Xase
    - Intrinsic Xase activates factor X even faster than extrinsic Xase

**Propagation of Coagulation**

- Occurs on activated platelets
- Platelet surface is specialized for assembly of Xa/Va and IXa/VIIIa complexes
- Burst of thrombin results from activation of factor X
  - Propagates its own generation
  - Activates platelets via both PAR1 and PAR4
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Thrombin Generated on Platelet Surface Produces Stable Clot

Factors V and XI, fibrinogen, and factor XIII allow for stable clot formation


Role of Platelets in Hemostasis

- Platelet adhesion is the first event in response to vascular injury
- Platelets are activated by collagen and released ADP, but thrombin induces more potent platelet response and procoagulant state
- Effective clot cannot be formed without adequate levels of procoagulant factors
- PAR1 and PAR4 both contribute to full activation of human platelets
- PAR4 requires increased levels of thrombin, likely activated during propagation phase of coagulation


Coagulation

Platelets

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Platelets in ACS

- Antiplatelet therapies target TxA₂, ADP, GPIIb/IIIa, thrombin, collagen, and vWF
- Low amounts of thrombin can initiate both platelet activation and procoagulant activity
- Thrombin-mediated platelet activation can be inhibited by blocking PAR1
- Anticoagulants primarily target thrombin activity and fibrin generation

Coagulation Platelets

- Tissue factor → Factor Xa → Prothrombin → Thrombin
- LMWH, UFH
- Aspirin, Clopidogrel
- GP IIb/IIIa inhibitors
- LMWH, UFH
- Thromboxane A₂ → Fibrinogen cross-linking
- Activated platelets
- Plasmin → Fibrin degradation
- Thrombolytics

Conclusions

- Heightened platelet reactivity facilitates several pathologic processes
  - Infiltration of inflammatory cells into arterial wall contributing to the initiation and progression of atherogenesis
  - Maintenance of prothrombotic environment leading to thrombin generation and fibrin production
  - Platelet deposition and thrombus development in vessel wall following injury
- Redundancy in pathways makes inhibition with a single agent difficult
- Combination therapies have demonstrated reduction in risk of ischemic events

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