Many Viruses Can Affect Clotting Proteins and Cells

Herpes simplex virus 1 and 2
Dengue Virus
Cytomegalovirus
Dengue virus
Immunodeficiency viruses 1 and 2
Hepatitis C virus
Influenza virus A and B
Varicella zoster virus
Epstein-Barr virus
Kaposi sarcoma virus
Ebola virus
and others

Today’s Objectives

1) To learn about clotting triggered on viruses
2) To learn that viruses bind and alter platelets
3) To understand how viruses exploit hemostasis
4) To review hemostasis
The physiological *CONTROL* of bleeding

**Hemostasis** Definition

**Platelets**

**Coagulation**

**Herpes simplex (HSV)**

**Dengue (DENV)**

A Few Coagulation Proteases and Cofactors

- TF
- VIIa
- Xa
- IIa
- Va
- Xa
- FIBRIN CLOT

*initiation*

*common*

*amplification*

**A Few Coagulation Proteases and Cofactors**

**Tissue Factor** and **Protease Activated Receptor 2**

**Protease-Activated Receptors (PARs)**

- Four PARs exist: PAR1, 2, 3 and 4
- Many proteases specifically trigger PARs (e.g. FVIIa, FXa, thrombin, kallikrein, plasmin, activated protein C, etc.)
- Proteases may be presented by cofactors (e.g. TF)
- Many cell types express PARs
Protease-Activated Receptor Ubiquity

Tissue and Cells
- Airway: Epithelium, Trachea, Fibroblasts, Smooth Muscle cells
- Blood: Platelets
- Bone: Osteoblasts
- Connective Tissue: Synovial fibroblasts
- Cardiovascular System: Vascular smooth muscle cells, endothelial cells, myocytes
- Exocrine glands: Salivary cells, parotid, sublingual cells
- Immune System: Monocytes, mast cells, T cells, macrophages
- Intestine: Smooth muscle cells
- Kidney: Glomerular epithelial cells, glomerular mesangial cells
- Nervous System: Glia, astrocytes, neurons
- Pancreas: Duct epithelial cells
- Skeletal Muscle: Myocytes
- Stomach: Smooth muscle cells
- Uterus: Circular muscle cells

(partial list!)

Non-Traditional Roles for Clotting Proteases in Pathology

- Cancer: Versteeg et al., Blood 2008
- Pain Perception: Lam and Schmidt, Pain 2011
- Wound Repair: Xu et al., Mol. Med. 2010

(partial list!)

TF/FVIIa in Ebola Infection

- Ebola causes hemorrhagic fever
- Ebola causes TF expression in leukocytes
- Inhibition of TF/FVIIa complex by NAPC2 inhibited virus infection in primate model

Geisbert et al., Lancet. 2003
Herpes Simplex Virus: Our Model

Herpes Virus Links to Vascular Disease

Cultured Cells
- procoagulant, etc. (Visser, 1988 and many others)
- monocyte adhesion (Etingin, 1990)
- SMC proliferation (Spär, 1996)
- oxLDL binding (Zhou, 1996)

Animals
- thrombosis and atherosclerosis (Fabricant, 1983 and others)

Clinical
- restenosis after angioplasty (Wu, 1992 and others)
- increased death after heart attack (Siscovick, 2000)
- arterial thickening (Epstein, 2000 and others)
- viral genome/antigen in plaque (Benditt, 1983 and others)

HSV Section Goals
- Show activation of clotting enzymes by the virus
- Identify coagulation cofactors on the virus
- Show in vitro cofactor/protease roles in infection
- Demonstrate in vivo cofactor/host roles in infection
Herpes Viruses Bypass Cells to Initiate Blood Clotting

<table>
<thead>
<tr>
<th>Virus</th>
<th>FXa</th>
<th>FXa-H</th>
<th>FX/H</th>
<th>VIIa</th>
<th>Ca²⁺</th>
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<tbody>
<tr>
<td>HSV1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CMV</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>HSV2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Factor X is Activated on Herpes Viruses

HSV Section Goals

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Tissue Factor and aPL on Herpes Viruses

Immuno-Gold EM
Endogenous Tissue Factor
Added Factor V

CMV
HSV
HSV2

Endogenous Tissue Factor Initiates Clotting:
But is not Alone

Virus-Encoded gC-Deficient HSV1 to Evaluate a Role in FX Activation
gC Contributes to Factor X Activation on HSV1

FX Binding to HSV1 is Enhanced by gC

A Novel Virus with Restricted Host Protein: TF-Deficient
gC Enhances TF-Dependent FX Activation on the Virus Surface

<table>
<thead>
<tr>
<th>FX Activation (mOD/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
</tr>
<tr>
<td>0</td>
</tr>
</tbody>
</table>

FVIIa (nM)

(100 nM FX)

TF+/gC+
TF+/gC-
TF-/gC+
TF-/gC-

Center for Blood Research

Show activation of clotting enzymes by the virus
Identify coagulation cofactors on the virus
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Demonstrate in vivo cofactor/host roles in infection

HSV Section Goals

Cytopathic Plaque assays:
Thrombin Enhances HSV1 Infection

No Thrombin
Infected Endothelial Cells
+ Thrombin

Center for Blood Research
Thrombin FVIIa, FXa and Plasmin Enhance In Vitro Infection

In Vitro, IIa Mediates Infection by PAR1 But, Xa, VIIa, Plasmin by PAR2

Viral TF Enhances In Vitro Xa- and VIIa-Mediated Infection
HSV Section Goals

- Show activation of clotting enzymes by the virus
- Identify coagulation cofactors on the virus
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Our Patients

TF on HSV1 Enhances Infectious Virus Production In Vivo

[Bar graph showing infectious virus production in different tissues with TF+ and TF- virus]
TF on HSV1 Enhances Virus Number In Vivo

Anti-TF on HSV1 Inhibits Infectious Virus Production In Vivo

Clotting Proteins on the Virus Modulate Infection

- Host- and virus-encoded
- Many proteases activated
- Clotting and fibrinolysis
- Fibrinolysis may explain weak HSV1 link to CVD risk
Part 2

Viral Impact on Hemostasis: Heinous Herpes, Diabolical Dengue

Why Study DENV?

One of the most prevalent pathogenic viruses: With no vaccine

Annual Global Incidence
Total Infections: ~390 million
Mild Illness: ~100 million
Severe Illness: >2 million
Fatalities: >22,000
Asymptomatic: 10^6 viruses per mL

Quiz: What are these?
Meet *Aedes aegypti*: The Dengue Virus Vector

Dengue Virus: The World’s Most Prevalent Arbovirus

Severe DENV Pathology: Hemorrhagic Fever/Shock Syndrome
Why Else Study DENV?

To extend our “host envelope protein model of infection” from Herpesvirus to Flavivirus

**Table:**

<table>
<thead>
<tr>
<th>% Clotting Time</th>
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<tbody>
<tr>
<td>0</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>20</td>
</tr>
<tr>
<td>30</td>
</tr>
<tr>
<td>40</td>
</tr>
<tr>
<td>&gt;50</td>
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</table>

**Graph:**

- FX deficient plasma +10% deficient factor
- FVII deficient plasma

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Flavivirus Infection Mechanism

- Binding: DC-SIGN, αVβ3, ?
- Decapsidation
- Ribosomes
- Nucleus
- 5'-3' (+)ssRNA
- H2N- -CO₂H
- Polyprotein
- Host translational template recruitment
- Exosome: Maturation
- Golgi: Translation
- NS5
- 3'-5'
- NS5
- NS1
- Structural
- Non-structural

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DENV Project Goals

- Quantify Dengue virus-platelet binding
- Characterize Dengue virus receptors on platelets
  - Evaluate replication of Dengue virus (+)ssRNA genome by platelets
  - Evaluate the Fate of Dengue virus in cellular blood products
Controversial Platelet-DENV Interaction (Antibody-Dependent Enhancement?)

Saturable DENV-Platelet Binding

Antigenic Confirmation of DENV-Platelet Binding
DENV Project Goals

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DC-SIGN / HSP-Dependent DENV-Platelet Binding

<table>
<thead>
<tr>
<th>Temperature</th>
<th>DENV1</th>
<th>DENV2</th>
<th>DENV3</th>
<th>DENV4</th>
</tr>
</thead>
<tbody>
<tr>
<td>4°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37°C</td>
<td></td>
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</tr>
</tbody>
</table>

Bound DENV (genome copies x 10^5)
Fate of DENV2 without Viable Platelets

DENV Genome is Replicated by Platelets

DENV-Encoded NS1 Translation by Platelets
DENV Project Goals

- Quantify Dengue virus-platelet binding
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Stored Platelet Units
Stabilize and Replicate DENV

Stored RBC Units Also
Stabilize and Replicate DENV
DENV Gene Replication Specificity

Primary Platelet-DENV Interaction

• Indicates novel platelet permissiveness for DENV replication and pathogenesis
• Highlights need for pathogen reduction implementation
• Do platelets harbor other RNA viruses and contribute to the infection cycle?