Keeping Things Fluid:
The Delicate Balance Between Bleeding and Clotting

6/11/13

Andrew D. Leavitt, MD

BIOGRAPHY:

Andrew D. Leavitt, MD is a Professor with appointments in the Department of Laboratory Medicine and the Department of Medicine (Hematology). He received his MD from Harvard Medical School, and completed his internal medicine residency training at The University of Michigan. He then came to UCSF in 1988 to begin his Hematology Fellowship training and was appointed to the UCSF faculty in 1992. Dr. Leavitt is the Medical Director for the Adult Blood and Marrow Transplant (BMT) Laboratory, and Assistant Medical director for the UCSF Blood Bank. He is also Directs the Non-malignant Hematology section within the UCSF Department of Medicine, and is the Medical Director of the UCSF Adult Hemophilia Treatment Center, a federally funded center for patients with congenital bleeding disorders. He sees patients in the UCSF hematology clinic, and provides hematology consultation for patients in the hospital. Among his various teaching responsibilities, he is the director for the UCSF School of Medicine 2nd year Hematology course. His past research has focused on megakaryocyte development and platelet function, and his current laboratory research focuses on improving hematopoietic stem cell options for patients in need of a bone marrow transplant. He has served on the editorial boards for Blood, the leading professional journal in hematology, and the Journal of Clinical Investigation.
Keeping Things Fluid: The Delicate Balance Between Bleeding and Clotting

Andrew D. Leavitt, MD
UCSF Mini Medical School
June 11, 2013

Hemostasis: General Points

- Purpose: To keep blood where it belongs
- Intricate interplay involving:
  1. Vessel wall
  2. Platelets
  3. Coagulation cascade
- Platelets form the initial plug
- "Long term" goal = cross-linked fibrin mesh (i.e., make "the clot")

The General Plan for This Evening

- The Physiology of Hemostasis
- Bleeding Disorders
- Thrombotic Disorders

Blood Vessels
Endothelial Cells - The Magic Surface

If You Were To Design A System To Plug The Leaks

Megakaryocyte undergoing proplatelet formation
560 images @ 10 minute intervals

Keeping it fluid reflects the amazing properties of the endothelial cells.

Megakaryocyte undergoing proplatelet formation
560 images @ 10 minute intervals
The Adhesive Anatomy of a Platelet

Adhesive ligands
- vWF: high flow/sheer
- Collagen: low flow

Aspirin
NSAIDs
Plavix

Three Steps in platelet plug formation
1. Adherence
2. Shape change/spreading
3. Secretion/recruitment
4. Binding/aggregation

Formation of the Fibrin Mesh (THE CLOT)

The Mighty Clotting Cascade

Test Tube Coagulation Cascade

Moving from the Cellular (Platelets)
to the Humoral (Clotting factors)
Coagulation: General Overview Points

- The cascade design provides exponential amplification (enzymes)
- Factors V & VIII are actually co-factors
- Prothrombinase complex formation is key (F-X & F-V)
- Calcium & phospholipid* surfaces are essential at some steps
- Calcium removal is therefore an effective anticoagulant
- Goal = cross-linked fibrin meshwork (THE CLOT)
- We have multiple systems to "put on the brakes"

*provided by the platelet

The Coagulation Cascade – “Y” do I have to learn this?

Coagulation in You and Me

Coagulation in patients/in vivo

The Two Faces of Thrombin (IIa)

*Two of the many faces of thrombin
**Thrombin and Its Receptor – A Great Story**

Ligand

Receptor

On a cell surface

Occupied Receptor

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**Thrombin and Protease Activated Receptors (PARs)**

**Activation of PAR1 by thrombin**

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**Coagulation Cascade – 3 Ways to put on the Brakes**

1. **Protein C & S**
   - Protein C is activated (APC) by thrombin at site of injury
   - Protein C cleaves/inactivates Va and VIIIa (the co-factors)
   - Protein S serves as co-factor for APC
   - Factor V Leiden (APC resistant)*

2. **Antithrombin (AT; ATIII)**
   - inactivates many active factors (IIa, VIIa, IXa, Xa)
   - heparin accelerates AT activity (espec. IIa & Xa)

3. **Tissue Factor Pathway Inhibitor**
   - inhibits Xa and VIIa

*Deficiencies can lead to hypercoagulable* states

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**Coagulation cascade in patients/in vivo**

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**The Fibrinolytic System**

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*Image credits: [Schwartz, Coughlin, Journal of Thrombosis and Haemostasis, 2005]*
Fibrinogen $\xrightarrow{\text{Fibrin}} \text{Cross-linked Fibrin} \xrightarrow{\text{Cleaved Fibrin}}$

The Coagulation Cascade – Basic Laboratory Tests

SO YOU THINK YOU MIGHT HAVE A TENDENCY TO BLEED..

The Coagulation Cascade – Basic Laboratory Tests

Activated Partial Thromboplastin Time (aPTT)

Prothrombin Time (PT, INR)

MIXING STUDY: Assay for specific factors

SO YOU THINK YOU MIGHT HAVE A TENDENCY TO BLEED..

Now We Shift From Physiology to the Pathology

We will Discuss Three Pathologic States

- A clotting factor problem
- A platelet-related problem
- A thrombosis problem

Hemophilia -
Long Appreciated But Only Recently Understood

2nd Century AD: Rabbinical (Talmud) writing: males did not need to be circumcised if 2 brothers had exsanguinated from the procedure

1803 Philadelphia physician Dr. Otto: reported a hereditary bleeding problem affecting boys, & traced founder to woman who settled in Plymouth NH in 1720.

1828 The term ‘hemophilia’ first used in a medical report: The University of Zurich

The more recent understanding involves UCSF and the bay area... but more on that later

So you have a vascular defect in need of repair

1. Plts ADHERE: collagen & vWF
2. Spread/shape change
3. Thrombin - ACTIV/SECRET (ADP, TxA2)
4. RECRUITMENT & AGGREGATION
5. Fibrin meshwork strengthens plt plug
6. Cross-linking (F XIII) for long term (>48h) clot strength

Real-time thrombus formation in a mouse

Laser injury 60 sec. Movie

Platelets
Fibrin
Tissue factor
Hemophilia – From England Outward: “The Royal Disease”

Queen Victoria (1837-1901) – Family Tree

Prince Albert

Married

The Russian
Tsar Nicholas

Died of
Brain
Bleed

Only Men
Are Affected

Died of Brain Bleed
Like Grandfather

A VERY BRIEF Genetics Primer

Normal Karyotype

Humans:
22 pairs of autosomes
1 set of sex chromosomes
XY = male
XX = female

Hemophilia is due to
a mutation on the “X” chromosome

Hemophilia – 20th Century History

1930s:
Clearly shown that a protein (a ‘factor’) and not a blood cell (platelets) could correct the bleeding problem.

1950s & 60s:
-Still using whole blood or fresh plasma to treat bleeds.
-1965: cryoprecipitate shown to contain F-VIII so use as therapy ended high-volume whole plasma infusions

1970s:
Freeze-dried powder factor concentrates revolutionizes treatment
Allows for at home self-infusion, great independence and employment

1980s:
HIV devastates the hemophilia community due to pooling of plasma
Late 1980s: ‘safe’ products – treated & filtered; then recombinant

Hemophilia – A Little Clinical Info

<table>
<thead>
<tr>
<th>Type</th>
<th>VIII level</th>
<th>% of cases</th>
<th>Bleeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe</td>
<td>&lt;1%</td>
<td>50-60%</td>
<td>Frequent spontaneous Prolonged post surgery</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Prolonged post trauma</td>
</tr>
<tr>
<td>Moderate</td>
<td>1-5%</td>
<td>~10%</td>
<td>Occasional spontaneous Excessive post trauma</td>
</tr>
<tr>
<td>Mild</td>
<td>6-49%</td>
<td>30-40%</td>
<td>Rare spontaneous Excessive post surgery</td>
</tr>
</tbody>
</table>

Solving the mystery of Severe Hemophilia

A Story of Gene Duplication and Lack of a Dancing Partner

DNA  RNA  PROTEIN

The Welcome Trust
Solving the mystery of Severe Hemophilia
A Story of Gene Duplication and Lack of a Dancing Partner

Lakhich et al Nature Genetics 5:23641, 1993

Lest You Think Scientists Lack a Sense of Humor

Dr. Gitschier's Famous View of the X-chromosome, once published in Science

von Willebrand Disease - 1926

Erik von Willebrand

- 5 year-old girl and extended family
- Aland (Aoland) Islands
- Mucocutaneous and not joint
- Not x-linked (autosomal)
- Most common inherited bleeding ~1:100 (but many subclinical)

von Willebrand Disease: Some Fun Facts

- A protein problem, but functionally a platelet problem
- Mucocutaneous
  - Epistaxis (nose bleeds)
  - Menorrhagia (heavy menstrual bleeding)
- Different types:
  - Mild to severe
  - Different size multimers
  - Too few is most common (quantitative)
  - Can be a specific binding problem (qualitative)
- Estrogens increase levels
  - Can first manifest post-partum

von Willebrand Factor and Hemostasis

Let's Shift From Too Much Flow To Too Little Flow

Thrombosis or Clotting Problem

- Venous Thrombosis
- Venous Thromboembolism
- Deep Venous Thrombosis (DVT)
- Pulmonary Embolism (PE)

Hemostasis = normal physiology; Thrombosis = pathology

Venous Thrombosis – Risk Factors

- Stasis – long plane flight, paralysis, immobilized
- Smoking
- Birth control pills, pregnancy
- Excess weight/obesity
- Injury/surgery
- Congenital predisposition (Thrombophilia) (i.e., Protein C; Protein S; Antithrombin; Factor V Leiden)
- Acquired predisposition (auto-immune states; cancer)
- Increasing age
- Prior DVT or PE

Provoked vs Not Provoked

Venous Thromboembolism (VTE) – Best Estimates for the United States

- Best estimates from CDC: 300,000 – 600,000 DVT/PE per year in US
- 60,000 – 100,000 American die from VTE each year
  10-30% die within 1 month
  Sudden death is the presenting symptom in ~25% with PE
- 1/3-1/2 of patients with DVT have a long-term consequence in the leg ‘post-phlebitic syndrome’ – pain, swelling, discoloration
- 5-8% are thought to have some form of thrombophilia

Venous Thrombosis

1856 – Published “Virchow’s Triad”

- Stasis
- Endothelial Injury
- Hypercoagulability

Venous Thromboembolism (VTE)

General, Non-specific Findings

- Deep Vein Thrombosis
  - Pain
  - Swelling
  - Redness
  - Warmth
  - Tenderness
  - Typically unilateral

- Pulmonary Embolism
  - Chest Pain (pleuritic)
  - Short of breath
  - Cough up blood
  - Fast Heart Rate
  - Light headed

Image from Wikipedia.com

Rudolph Virchow
German Pathologist
1821 – 1902

http://www.cdc.gov/ncbddd/dvt/data.html
Leg Ultrasound to Diagnose DVT

CT images courtesy of V.K. Agarwal (UCSF)

Computed Tomography Pulmonary Arteriogram

CT images courtesy of V.K. Agarwal (UCSF)

Filters to Prevent DVT from Becoming a PE

http://www.sironavascular.com/?_escaped_fragment_=dvtthrombophlebitis/c1ie9

Anticoagulants: aka, Blood Thinners

Thank You For Your Attention!
Questions Please

Colorized Scanning EM of a blood clot – John Weisel, U. Penn