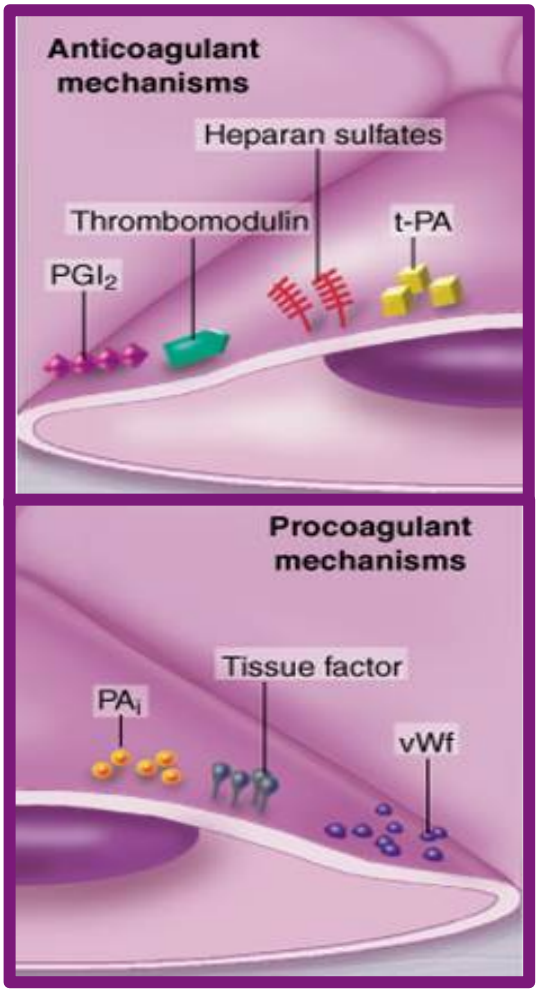
Critical role of the Endothelium in Health and Disease, part 2



Hemostasis

Anticoagulant mechanisms

- Smooth covering to prevent contact activation
- Glycolax layer→negative charge repels platelets and coag factors
- Circulation removes activated products which can lead to thrombosis
- High heparin-like activity in the microvasculature
- Vasodilators also have direct platelet inhibitory effect
- TFPI is synthesized and expressed by endothelium
- Thrombomodulin facilitates removal thrombin
- Thrombomodulin also accelerates activation of proteins C and S
- Synthesize tPA and uPA to activate plasmin→enhanced fibrinolysis

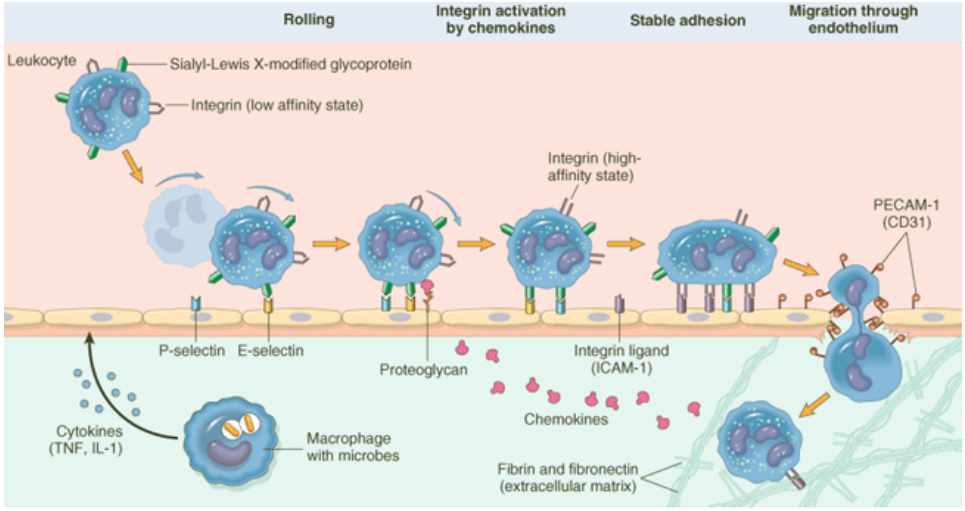
Procoagulant mechanisms

- Destruction of endothelial lining stimulates platelet adherence and aggregation
- Produces vWf which promotes platelet adhesion and thrombus formation
  - Largest multimers are most effective at platelet effects
  - Largest multimers of vWf are stored in Wiebel Palade bodies
  - vWf also prolongs the half life of FVIII in circulation
  - Released in response to histamine, thrombin, complement, cytokines & injury
- Tissue factor embedded in the phospholipid bilayer
  - Not produced in high numbers by normal endothelial cells
  - Induced ex vivo by endotoxin, thrombin, INF-y, IL-1, hypoxia, TNF, bacterial cell walls
  - Production induced in vivo by inflammation
- Serine protease production can be induced in endothelial cells
  - FV induced by mechanical injury
- Consituently express plasminogen activator inhibitor (PAi)
  - inhibits tPA and uPA and inactivates FXIa
  - Upregulated by LPS, IL-1, TNF, and thrombin

Inflammation

- Initiated by chemotactic mediators released from damaged tissues, WBC, endothelial cells, and pathogens

Adhesion molecules



- Selectins are located in the endothelium and leukocytes
- Integrins are located on leukocytes
- Immunoglobulins are located primarily on endothelial cells
- Initially leukocyte rolls along endothelium, mediated by endothelial P selectin and leukocyte L selectin
  - P selectin is mobilized from Weibel-Palade bodies
- Subsequent upregulation of endothelial E selectin allows for margination of leukocytes by slowing their velocity
- Endothelial derived ICAM-1 and VCAM-1 allow for adhesion of leukocytes to endothelial immunoglobulins
  - Upregulation of both ICAM-1 and VCAM-1 with inflammatory cytokines
- Endothelial derived PECAM-1 and ICAM-1 mediate translocation of neutrophils from vasculature to subendothelial space

Role of the vascular endothelium in disease

- Changes to the endothelium occur with many diseases
  - Trauma, hypoxia, malnutrition, microorganisms, chemical agents, and temperature extremes
  - Localized endothelial changes are adaptive, systemic changes may be pathologic
- Hallmarks of diseases leading to systemic inflammation can be related to changes induced by endothelium
  - ↑ vascular permeability→hemoconcentration, hypothension, impaired blood rheology, hypoalbuminemia, and tissue edema
  - Impaired vascular tone→hypotension and inefficient cardiovascular responses
  - Microvascular stasis→increased procoagulant activity and DIC

Endothelial targeted therapies

- Inflammatory cascade
  - Anticytokine and antiadhesive therapy not rewarding
    - Have investigated monoclonal antiendotoxin antibody and anti-TNF-α antibody
    - Levels of inflammatory mediators documented to be decreased with early, high-dose corticosteroid therapy or ibuprofen therapy, but these have not shown efficacy in clinical trials
  - Theories for poor response to anti-inflammatory therapy
    - Agents targeted towards single mediators are ineffective against the complex inflammatory cascade
    - Agents themselves are ineffective
    - Dosing or timing was inadequate
    - Patient populations were too heterogenous
- Inhibition of nitric oxide synthetase
  - Improves blood pressure
  - Risks reduction in capillary flow, increased platelet/leukocyte adhesion, and increased coagulation activation
- Coagulation system
  - Therapies aimed at alterations have yielded favorable results in early clinical trials and variable results in phase III clinical trials (APC, antithrombin)
- Pentoxifylline
  - Impairs leukocyte adherence to the endothelium
  - Increases erythrocyte deformability
  - Prevents activation of coagulation by endotoxins
  - Reduces the direct toxic effects of TNF on the endothelial cell
- Statins
  - Increase cerebral blood flow
  - Attenuates inflammation at the microcirculatory level
  - Inhibition of leukocyte trafficking into inflamed areas
  - Down-regulation of proadhesive cell adhesion molecules
- Intravenous fluids
  - Crystalloids and colloids improve rheology and maintain intravascular volume
  - Colloid molecules larger than the size of the inter-endothelial cell cleft may promote intravascular volume retention in states of increased capillary permeability
- RBC transfusion or HBOC
  - When patient HCT levels drop to precipitous level
- Vasomotor tone manipulation
  - Agents that affect endothelial smooth muscle constriction and dilation
- Hypercoagulability management
  - Anticoagulants and antithrombin replacement

Phase III RCTs of APC for sepsis

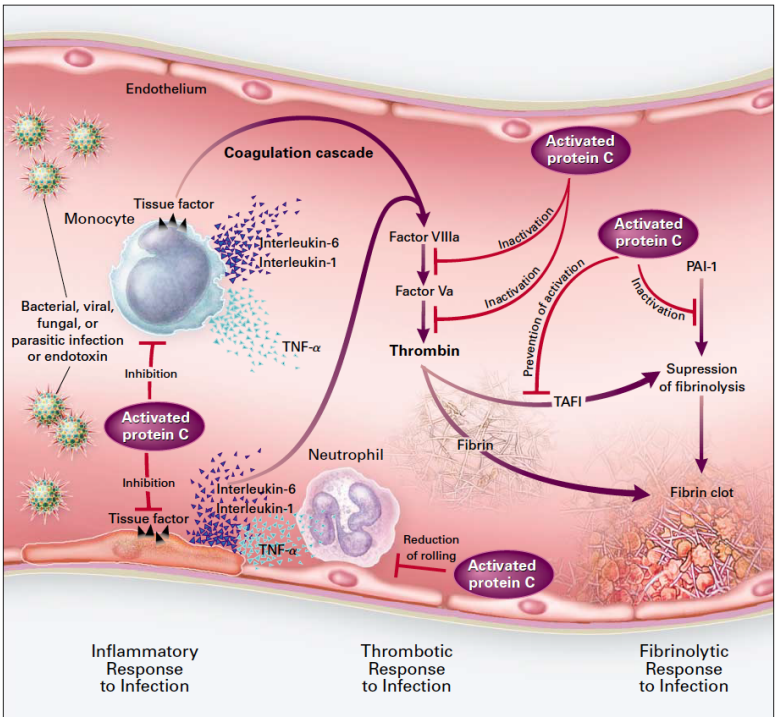
Trial	Survival Benefit?
ADDRESS 2005	No
Dhainaut 2009*	No
PROWESS 2001	Yes
RESOLVE 2007*	No
Raniri 2012	No

*Mortality was not 1° outcome measure

Randomized, phase III trials of APC in Sepsis

Ranieri, et al. Drotrecogin Alfa (Activated) in Adults with Septic Shock, NEJM 366(22); 2012.

- Compared mortality in patients with septic shock receiving APC or placebo
- No difference in 28 or 90 d mortality in all patients
- No difference in 28 or 90 d mortality in patients with protein C deficiency at baseline
- No difference in secondary outcome measures: change in SOFA score at day 7, bleeding events, intracranial bleeding



Dhainaut, et al. Extended Drotrecogin alfa (activated) Treatment in Patients with Prolonged Septic Shock, Int Care Med 35; 2009.

- Assessed patients for time to resolution of vasopressor-dependent hypotension with APC or placebo
- Secondary outcome measures: 28 day all cause mortality, 90 day in hospital mortality, SOFA score, adverse bleeding events
- No difference in primary outcome variable, 28 or 90 day mortality, organ function, PT or bleeding events

ADDRESS trial: Abraham E, et al. Drotrecogin alfa (activated) for adults with severe sepsis and a low risk of death. NEJM 353(13); 2005

- Evaluated patients with severe sepsis and low risk of death defined by low APACHE score or only 1 organ dysfunction at baseline
- Primary endpoint: 28 day mortality between patients receiving APC or placebo
- Secondary measures: in hospital mortality, serious bleeding events
- Subgroups analyzed: APACHE <25, APACHE ≥ 25, single organ dysfunction, multiple organ dysfunction
- No difference in 28 day or in hospital mortality overall or with subgroup analysis
- More serious bleeding events noted with the use of APC
- Was evaluated at 1 year (different paper- CCM 32; 2005) and showed no difference in survival between APC or placebo overall or in subgroups

RESOLVE trial: Nadel S., et al. Drotrecogin alfa (activated) in Children with Severe Sepsis. Lancet 369; 2007.

- Evaluated time to complete organ failure resolution (cardiovascular, respiratory and renal) in children w/ sepsis induced cardiovascular and respiratory dysfunction receiving APC or placebo
- Secondary measures: 28 day mortality and serious bleeding events
- No difference between groups in primary or secondary outcome measures

PROWESS trial: Bernard GR, et al. Efficacy and Safety of Recombinant Human Activated Protein C for Severe Sepsis. NEJM 2001

- Evaluated patients with suspect sepsis and at least 1 organ dysfunction
- Primary endpoint was death from any cause at 28 days
- Subgroups based on APACHE scores, age and protein C activity level
- Reduced risk of death and increased incidence of serious bleeding with the use of APC in all patients
- Reduced risk of death was noted regardless of APACHE score or protein C activity levels

Questions

1. Phase II clinical trials are typically designed to investigate
    a. Drug dosage
    b. Safety of the drug
    c. Efficacy of the drug
    d. Comparison of the drug to standard therapy
2. Thrombomodulin primarily acts upon thrombin to
    a. Enhance procoagulant activity
    b. Inhibit procoagulant activity
    c. Enhance inflammatory activity
    d. Inhibit inflammatory activity
3. The Weibel-Palade bodies store
    a. tPA
    b. Thrombomodulin
    c. Tissue factor
    d. vWf
4. The purported benefit of Activated Protein C in sepsis is
    a. Improved fibrin deposition by enhanced activity of plasminogen activator inhibitor-1
    b. Improved inflammatory responses by enhanced rolling of neutrophils and selectin activity
    c. Improved coagulant activity by enhanced tissue factor mediated cascade activation
    d. Reduction in thrombin formation by inhibition of factors Va and VIIIa
5. Which of the following mediators of inflammatory cell adhesion are derived from endothelium
    a. E selectin
    b. Integrins
    c. L selectin
    d. vWf
6. Pentoxifylline is thought to be of benefit in endothelial injury due to
    a. Impaired leukocyte adherence to the endothelium
    b. Decreased erythrocyte deformability
    c. Improved activation of coagulation by endotoxins
    d. Enhanced toxic effects of TNF on the endothelial cell
7. Statin drugs are throught to be of benefit in endothelial injury due to
    a. Decreased cerebral blood flow
    b. Attenuates inflammation at the microcirculatory level
    c. Improved leukocyte trafficking into inflamed areas
    d. Up-regulation of proadhesive cell adhesion molecules