Endothelial Cell Injury and Activation Promote the Binding of Anti-Phospholipid Antibodies and Thrombus Formation

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I have no relevant financial relationships to disclose

Evidence Based Medicine


Review on aPL and APS: Levine JS, Ware Branch D and Rauch J. The Antiphospholipid Syndrome. NEJM 2002; 346: 752-763.

Introduction

Anti-Phospholipid Syndrome (APS):
- An autoimmune disease characterized by:
  - the presence of anti-phospholipid antibodies (aPL)
  - the occurrence of clinical events
  - pregnancy morbidity
  - thrombosis

- Some patients with aPL develop clinical events, while others do not

What are the mechanisms responsible for causing aPL-related thrombosis?

Hypothesis

aPL and innate immune activation (through TLR) are both required to produce thrombosis in APS

In Vitro Methods

aPL production by immunization of rabbits with murine β2-glycoprotein I (β2GPI) and complete Freund’s adjuvant
- β2GPI and cardiolipin (CL) binding studies (ELISA)

Endothelial cell (EC) activation
- two cell lines: HUVEC and EOMA
- stimulation with LPS (ligand for toll-like receptor 4 (TLR4))
- measurement of activation (cell-based ELISA)
  - E-selectin
  - P-selectin
  - von Willebrand factor (vWF)

Binding of aPL to activated ECs
- LPS stimulation of ECs + aPL Binding
**Results: aPL**

Characterization of aPL
- Produced in rabbits immunized with murine \( b_2 \)GPI
- Binding of aPL to murine (m) and human (h) \( b_2 \)GPI and CL

**Results: EC activation**

TLR4 stimulation induces EC activation
- E-selectin, vWF and P-selectin expression

**Results: aPL binding to activated ECs**

aPL bind to activated ECs in a dose-dependent manner
- TLR4 activation with LPS

**In Vivo Thrombosis Model**

Ferric chloride (FeCl\(_3\))-induced carotid artery injury

1. Expose carotid
2. Blood flow (BF) sensor
3. Place carotid in sensor
4. Stabilization of BF after manipulation (10min)
5. FeCl\(_3\) application for 3 minutes
6. FeCl\(_3\) removed - acquiring BF data (15 min)

**Results: In Vivo**

Thrombus formation is significantly faster in aPL-treated mice

Vessel occlusion is significantly faster in aPL-treated mice

*\( p<0.05 \) vs Control IgG and Unstimulated Cells
**Results: In Vivo**

Uninjured contralateral carotid arteries: increased expression of P-selectin and vWF

**Conclusions**

Endothelial cell injury and activation promote the binding of aPL and thrombus formation

*In Vitro*

1. TLR4 stimulation → EC activation
   - ↑ E-selectin, P-selectin and vWF expression
   - ↑ aPL binding

*In Vivo*

Endothelial injury/activation + aPL = THROMBOSIS
- Thrombus formation and vessel occlusion are faster in aPL-treated mice
- Increased expression of P-selectin and vWF in uninjured contralateral carotid arteries

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**Perspectives**

A story to complete...

*In Vitro*

1. Other TLRs?
2. Cytokine secretion?
3. Tissue factor (TF) expression on ECs?

*In Vivo*

1. Carotid artery characterization
   - Injured: thrombus size, monocyte infiltration
   - Uninjured: confirmation of EC activation
   - E-selectin, vWF, p-selectin, TF
2. TLR knock-out mice

**In Vivo Thrombosis Model**

- Measurements:
  - Thrombus formation (time and size)
  - Histology of the endothelium
Results: In Vivo

Uninjured contralateral carotid arteries:
- increased expression of P-selectin and vWF

- IgG
- aPL

- P-selectin
- Isotype ctl
- vWF
- Isotype ctl