Pathophysiology of the Immune Thrombocytopenias

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Outline of talk:

1. Crash course on immunity, T cells, autoimmunity and platelets.

Reviews of the Literature since 2004.

1. Pathophysiology of immune thrombocytopenia (ITP).
2. Pathophysiology of alloimmune thrombocytopenia (NAITP).
3. Pathophysiology of heparin induced thrombocytopenia (HIT).
4. Pathophysiology of autoimmune neutropenia.
5. Pathophysiology of platelet MHC alloimmunity.
The Immune System:

The principal mechanisms of innate and adaptive immunity.

Innate immunity:
- Microbe
- Epithelial barriers
- Phagocytes
- Complement
- NK cells

Adaptive immunity:
- B lymphocytes
- Antibodies
- T lymphocytes
- Effector T cells

Time after infection:
- Hours: 0, 6, 12
- Days: 1, 3, 5

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T cell Recognition:

- Binds 8-10mers
- Expressed on most nucleated cells and platelets
- Presents endogenous proteins to CD8+ T cells

- Binds 13-25mers
- Expressed on APCs, Macs, B cells, activated T cells
- Presents exogenous proteins to CD4+ T cells
T cell Activation:

Mature Dendritic cell APC

Signal 1

MHC II TCR

Signal 2

B7 CD28

Activated T_H cell

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T cell activation is regulated by signals derived from the TCR/CD3/CD4 complex and the CD40L and CD28/CTLA-4 co-stimulatory molecules:
Cytokine Regulation via Th1/Th2 Balance:

- Th1
  - IL-12
  - IL-18
  - IL-10 & TGF-β
  - Low affinity Between TCR and APC
  - Low [Antigen]

- Th2
  - IL-4
  - IL-10 & TGF-β
  - IFN-γ
  - High affinity Between TCR and APC
  - High [Antigen]

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Th1 T cells and Th2 T cells can downregulate each other by the cytokines produced. IFNγ and IL-12 downregulate Th2 cells while IL-10 inhibits Th1 cells.

T regulatory cells (Tregs) CD4+CD25+Fox3+ mediate suppression by cell-cell contact and cytokines (IL-10 and TGF).

Regulatory T cell deficiencies may result in autoimmune diseases or allergic responses.
Autoimmunity:

- Immune reactivity against self.

- Generally classed into systemic (e.g. SLE, RA, GVHD) and organ-specific (e.g. MS, IDDM, IBD, ITP etc.).
Venn Diagram: Requirements for the Development of Autoimmune Disease

Nature Immunology, 2001
Organ specific (sp) Autoimmunity:

• Deficiency of central and/or peripheral tolerance induction mechanisms
  • failure to eliminate or deactivate self reactive lymphocytes.
T Cell Development: and potential for autoimmunity:

Central Tolerance

Peripheral Tolerance

Molecular mimicry
Abnormal antigen expression
T helper 1/T helper 2 imbalance
Cytokine Milieu
Costimulation/activation faults

Abnormal release of autoreactive T cells

Thymus

Selected T cells

Bone marrow

T cell precursors
A crash course on platelets:

- Resting
- Activated

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Platelets:

- Anucleated cell fragments pinched off from megakaryocytes in bone marrow.
- Important in preventing blood loss:
  - Platelet plug
  - Promote formation and contraction of clots.

Normal range: 150-400x10^9/L
Platelet adhesion and aggregation

Platelet activation

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How platelets interact with innate immune mechanisms
Linking platelets to Immunity by Phenotype:

**Acquired**
- CD80 (B7)
- CD86 (B7)
- Plasma proteins
- MHC Class I
- Allo. MHC Ag

**Activation-induced**
- PAF
- PF4
- 5-HT
- IL1-β
- TGF-β
- PDGF
- RANTES
- TLR 1, 2, 3, 6, 7, 9
- TLR 4
- CD40L
- CD40
- CD40L
- MHC Class I
- TLR 9
- GPIbIX (CD42)
- GPIIbIIIa (CD41)

**Constitutive**
- CD14
- MHC Class II
- CD80 (B7)
- CD86
- MHC class I
- Allo. MHC Ag
- TGF-β
- CD40L
- CD40
- TLR 1, 2, 3, 6, 7, 9
- TLR 4
- TREM
- CD40

Innate Immunity
- Pro- and Anti-Inflammatory effects
- Innate Immunity
- Immune costimulation
- Endothelial interactions

Temporary functional capabilities??

Auto./Allo. Ag (platelet-specific)
Platelets and CD40/CD40L (132 papers since 1998):

**CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells**  *Nature, 1998*

Volker Henn†, Joseph R. Slupsy‡, Michael Gräfe§, Ioannis Anagnostopoulos‡, Reinhold Förster‡, Gert Müller-Berghaus‡ & Richard A. Kroczek

* Molecular Immunology, Robert Koch-Institute, 13353 Berlin, Germany
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† These authors contributed equally to this work.

**Enhanced Levels of Soluble and Membrane-Bound CD40 Ligand in Patients With Unstable Angina**

Possible Reflection of T Lymphocyte and Platelet Involvement in the Pathogenesis of Acute Coronary Syndromes

Phil Aufrüst, MD, PhD; Fredrik Müller, MD, PhD; Thor Ueland, BS; Trude Berget, MD; Elisei Aaser, MD; Anne Brunsvig, BS; Nils Olof Solum, PhD; Kolbjorn Forfang, MD, PhD; Steig S. Froland, MD, PhD; Lars Gulstad, MD, PhD

**Role of Platelet P-Selectin and CD40 Ligand in the Induction of Monocytic Tissue Factor Expression**

Eva Lindmark, Taavo Teno, Agneta Siegbahn

**Arteriosclerosis Thromb Vas Biol, 2000**

**HEMOSTASIS, THROMBOSIS, AND VASCULAR BIOLOGY**

The inflammatory action of CD40 ligand (CD154) expressed on activated human platelets is temporally limited by coexpressed CD40

*Blood, 2001*

Volker Henn, Sabine Steinbach, Kerstin Büchner, Peter Presek, and Richard A. Kroczek

**Brief report**

Increased soluble and platelet-associated CD40 ligand in essential thrombocythemia and reactive thrombocytosis

*Blood, 2002*

Jean-François Viallard, Anne Solanilla, Bruno Gauthier, Cécile Contin, Julie Déchanet, Christophe Grosset, Jean-François Moreau, Vincent Prelon, Pequita Norden, Jean-Luc Pellegrin, Alan T. Norden, and Jean Ripoche

**CD40 Is Constitutively Expressed on Platelets and Provides a Novel Mechanism for Platelet Activation**

*J Immunol, 2004*

David P. Inwald, Alison McDowall, Mark J. Peters, Robin E. Callard, Nigel J. Klenk

**Cutting Edge: T Cells Trigger CD40-Dependent Platelet Activation and Granular RANTES Release: A Novel Pathway for Immune Response Amplification***

Silvio Danese,* Carol de la Motte,† Brenda M. Rivera Reyes, * Miquel Sans,* Alan D. Levine,* and Claudio Fiacchii‡*

*J Immunol, 2004*

**Cooperation between platelet-derived CD154 and CD4<sup>+</sup> T cells for enhanced germinal center formation**

*J Leuk Biol, 2005*

Bennett D. Elzey,* Julieann F. Grant,†‡ Haley W. Sinn,* Bernhard Niesswandt,§ Thomas J. Waldschmidt,†‡ and Timothy L. Ratliff,*†‡,§,∥,¶,†,‡

**Platelet-derived or soluble CD154 induces vascularized allograft rejection independent of cell-bound CD154**

*J Clin Invest, 2006*

He Xu, Xiaojie Zhang, Roslyn B. Mannon, and Allan D. Kirk
Platelet can regulate innate immune mechanisms in many ways:

Direct

Indirect

Thrombosis
- Tissue factor

Differentiation to macrophages

Activation
- TNF-α
- IL-8

Proteolysis
- uPA/uPAR
- MMPs

Chemotaxis
- MCP-1
- MIP-1α

Adhesion
- Mac-1
- VLA-4

Endothelial ligands of Mac-1:
- α<sub>1b</sub>β<sub>3</sub>/fibrinogen, GPIIbα, or JAM-3

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Immune thrombocytopenic purpura (ITP)
ITP, pathogenesis:

Autoantibodies

Platelet → Increased RES Destruction

Harrington et al, J Lab Clin Med 38:1, 1951
Acute ITP:

Good example of molecular mimicry.

Cross reactivity of anti-viral antibodies with normal platelet epitopes.

Acute ITP, Molecular Mimicry:

Infectious Agents
- VZV
- HIV

Platelet
Are there other immune mechanisms where infectious agents and platelets interact and potentially affect T cells?

In other immune-mediated diseases, the answer is yes!


What about in acute ITP?
Toll-like receptors:

Akira J. Biol. Chem. 2003 278:38105–38108

www.angelfire.com/ut/johnsnote/index.htm
Recent Literature: Platelet–TLR (16 papers)

Platelets Express TLR that is Functional:

**MURINE PLATELETS EXPRESS TOLL LIKE RECEPTOR 2: A POTENTIAL REGULATOR OF INNATE AND ADAPTIVE IMMUNITY**

*J. Semple, R. Aslam, J. Freedman*  
*Platelets, 2004*

*St. Michael's Hospital, Toronto, ON, Canada*

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**Expression of Toll-like receptors on human platelets**

*Rio Shiraki*¹, Nobutaka Inoue*², Satoru Kawasaki*³, Asumi Takei*³, Makoto Kadotani*³, Yoshio Ohnishi*³, Junya Ejiri*⁴, Seiichi Kobayashi*⁵, Ken-ichi Hirata*⁴, Seinosuke Kawashima*⁴, Mitsuhiro Yokoyama*⁴

*ImmunoBiology*

**Blood, 2005**

Platelets express functional Toll-like receptor-4

*Graciela Andonegui, Steven M. Kerfoot, Kelly McNagny, Kirsten V. J. Ebbert, Kamala D. Patel, and Paul Kubes*

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**Platelet Toll-like receptor expression modulates lipopolysaccharide-induced thrombocytopenia and tumor necrosis factor-α production in vivo**


*ImmunoBiology, Hemostasis, Thrombosis, and Vascular Biology*

**Blood, 2006**

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**Brief report**

Platelet-bound lipopolysaccharide enhances Fc receptor–mediated phagocytosis of IgG-opsonized platelets

*John W. Semple,¹,⁶ Rukhsana Aslam,⁵,⁶ Michael Kim,⁴ Edwin R. Speck,⁵,⁶ and John Freedman¹,⁵,⁶*

**Blood, 2007**
How do platelets and LPS interact with monocytes?

RBC (couldn't find a picture with a plt and macrophage)

macrophage

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ITP, pathogenesis:

Autoantibodies

Platelet → Increased RES Destruction

Harrington et al, J Lab Clin Med 38:1, 1951
Platelet-bound LPS Synergizes with autoantibodies to enhance platelet phagocytosis:
Platelet-TLR-induced RES activation:

Potential for:
- Altered processing/presentation to T cells:
- Autoreactive T cell activation

Infection

Platelet

TLR4

LPS

RES Activation

APC
Platelet-TLR-induced RES activation:

Enhanced Phagocytosis:
Altered processing/presentation to T cells:
Autoreactive T cell activation

Infection

TLR4

Platelet

RES Activation

Synergism

APC

M

LPS
Is there in vivo Proof? Perhaps Alan’s mouse model:

Anti-GPllbllla Antibody

1 day

Thrombocytopenia (IVlg effective in raising platelet counts)

IVlg inhibits reticuloendothelial system function and ameliorates murine passive-immune thrombocytopenia independent of anti-idiotypic reactivity

Andrew R. Crow,1,2 Seng Song,1 John W. Semple,1 John Freedman1,2 and Alan H. Lazarus1,2
In vivo Proof (Bazin et al Infect Immunity 2007):

Anti-GPIIbIIIa Antibody + 1 ng LPS

1 day

Profound (prolonged) Thrombocytopenia (IVIg not effective)
These results suggest that infectious agents in combination with anti-platelet antibodies can significantly affect platelet destruction in vivo.

They may explain why thrombocytopenia worsens in some patients with ITP during infections (adding LPS) and, alternatively, resolves in other patients with ITP who are treated with bacterial eradication therapy e.g. H. pylori (removing LPS).
Pathophysiology of Chronic ITP:

History of T cell involvement in ITP:

Enhanced MIF production (1970’s) (3)

PBMC blastogenesis against platelets (1970 -1980’s) (17)

PBMC IL-2 production against platelets (1991) (10)

Anti-platelet T cells clones (1993) (10)

Th1 cytokine bias during active disease (1996) (15)

HLA association (Japanese) (1998) (3)

GPIIIA peptide mapping (1998) (9)

Fas/FasL abnormalities (2000) (6)

Cell-mediated cytotoxicity against platelets (2003) (3)

Depressed CD4+CD25+Fox3+ T regulatory cells (2003) (7)

Genomics: IFN-upregulated genes in ITP (2007) (1)
Recent Literature: T cells and ITP

1. Ling Y et al. Circulating dendritic cells subsets and CD4+Foxp3+ regulatory T cells in adult patients with chronic ITP before and after treatment with high-dose dexamethasone. Eur J Haematol, Published online: 10-Aug-2007 doi: 10.1111/j.1600-0609.2007.00917.x
Mapping T cell epitopes in ITP:

Peripheral blood of 4 adults with chronic ITP. 

Spleen cells from 4/7 children with chronic ITP. 
These results suggest that the T cell anti-GPIIIa reactivity patterns are different in children and adults with chronic ITP.

Thus, peptide therapies can be developed but need to be tailored to the particular disorder.
A cellular immunologist (reductionist) view of haematology and all the IgG antibodies that everyone is talking about.

If there is an IgG antibody, there is a T cell.
If there is somatic mutation, there is a T cell.
What can B cells tell us about T cells in ITP?:

Antibody proof that chronic ITP is a T cell initiated disease.


What about antigen presenting cells and platelets?
Recent Literature:
Platelets/APC/NK(T) cells and ITP (Innate)


Suggests that a novel therapeutic targeting cell mediated immunity may benefit some ITP patients.
Platelet lysis (%)

Control
Active ITP
ITP in remission

MAIN CONCLUSION (early):

1. ITP is associated with many different T cell abnormalities and these defects are not only responsible for IgG anti-platelet autoantibody production but in some cases (40%), the direct destruction of platelets.

2. The T cell abnormalities in ITP also appear to be related to or perhaps caused by a variety of innate immune events and cells e.g. infections, macrophages/dendritic cells (antigen presenting cells) and/or the platelets themselves.

3. Based on the recent literature, it appears that ITP may be aggrevated because CD4+ regulatory T cell disturbances and more research is required to better understand these events

4. This will allow the development of peptide antigen-specific immunotherapies.

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Neonatal Alloimmune thrombocytopenia (NAIT)
NAITP:

**HPA 1a**

Leucine

**HPA 1b**

Proline

IgG anti-HPA 1a antibody response

What are the T cell epitopes that are responsible for this Response?
Suggested that a T cell epitope for anti-HPA 1a antibody production may actually be part of the antibody epitope (e.g. around the leucine residue).


A novel murine model of fetal and neonatal alloimmune thrombocytopenia: response to intravenous IgG therapy

Heyu Ni, Pingguo Chen, Christopher M. Spring, Ebrahim Sayeh, John W. Semple, Alan H. Lazarus, Richard O. Hynes, and John Freedman
Heparin-induced thrombocytopenia (HIT):

- Complexes of heparin (GAG) and PF-4 molecules form
- IgG binds to the PF-4/heparin complex
- IgG binds to the PF-4/heparin complex activates via the Fc receptor
- Fc stimulation leads to the generation of procoagulant-rich microparticles

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Platelet MHC alloimmunization:

**Direct Allorecognition**
- Donor APC
- Recipient CD4+ T\(_H\) cell
- Recipient B cell

**Indirect Allorecognition**
- Donor Platelets
- Recipient APC
- Recipient CD4+ T\(_H\) cell


Septic lung injury and perhaps TRALI:

Yet another function of platelet TLR4 expression:

Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood

Stephen R Clark¹,6, Adrienne C Ma¹,6, Samantha A Tavener¹, Braedon McDonald¹, Zahra Goodarzi¹, Margaret M Kelly¹,2, Kamala D Patel¹,3, Subhadeep Chakrabarti¹,3, Erin McAvoy¹, Gary D Sinclair²,³, Elizabeth M Keys², Emma Allen-Vercoe⁴, Rebekah DeVinney⁴, Christopher J Doig⁵, Francis H Y Green² & Paul Kubes¹
Netting bacteria in sepsis

Constantin Urban & Arturo Zychlinsky

Platelets act as intermediaries in the pathogenesis of sepsis—sensing bacteria and signaling neutrophils to release fibrous traps that remove bacteria from the bloodstream. This response may also contribute to tissue injury (pages 463–469).

Figure 1 Platelets activate neutrophils in sepsis. (a) At low levels of LPS, platelets and neutrophils are in circulation. (b) When LPS levels increase, LPS binds to TLR4 on platelets. Platelets, in turn, activate adherent neutrophils to form NETs. More neutrophils are recruited to the bloodstream. (c) The release of NETs results in bacterial trapping, but also in tissue damage.
The immune thrombocytopenias are caused by platelets initially being recognized by the innate immune system. These recognition events are critical to whether adaptive autoimmune processes e.g. anti-platelet autoantibodies will be stimulated.

Platelets express a variety of pro- and anti-inflammatory molecules that links them with the innate immune system.

Some of the pro-inflammatory molecules expressed on platelets e.g. TLR4, at least, enable the cells to very quickly bind infectious agents and present them to the innate immune system.

Thus, platelets perhaps have a critical role in mediating their own immunological fate within a host.
Questions?

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