TTP, Sickle Cell Disease, and the role of von Willebrand factor

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Disclosures

No conflicts to disclose.
Thrombotic Thrombocytopenic Purpura

1. Thrombocytopenia
2. Microangiopathic hemolytic anemia
3. No alternative explanation
4. Systemic microvascular platelet thrombi
TTP: Pathophysiology

• Presence of ultra-large forms of von Willebrand factor (ULVWF) that clump platelets in the microcirculation and on the vessel walls

• Generally caused by failure of ULVWF processing
VWF Multimer Synthesis

Synthesis From mRNA 2813 aa

Signal Peptide Cleavage

Dimerization C-terminal
VWF multimer gel
Thus far, the only known reason for ADAMTS13’s existence is to cleave VWF.

TTP: congenital ADAMTS13 absence or inhibitory antibody
ULVWF strands self-associate to form long endothelial bound strings.

Dong JF, et al., Blood, 2002

Bar = 100 um
ULVWF is not only “ultra-large” it is “ultra-sticky”
Individual Bond Strengths Between GP Ib and Different forms of von Willebrand Factor

A1 Domains in ULVWF are in the Active Conformation

* * *

Conformation of Highly active VWF

* * *

Conformation of “Peaceful” VWF
Llama nanobody AU/VWFα-11 detects active VWF

A novel nanobody that detects the gain-of-function phenotype of von Willebrand factor in ADAMTS13 deficiency and von Willebrand disease type 2B

Janine J. J. Hulstein, Philip G. de Groot, Karen Silence, Agnès Veyradier, Rob Fijnheer, and Peter J. Lenting

Blood. 2005;106:3035-3042
Elevated AU/VWFα-11 nanobody binding in:

- TTP
- Type 2B VWD
- Anti-phospholipid syndrome
- HELLP syndrome
- Malaria
Role of VWF and ADAMTS13 in Sickle Cell Disease
Sickle Cell Disease

β-globin

Glutamate

Hemoglobin A

mutant β-globin

Valine

Hemoglobin S
Sickle cell/Endothelial Adhesion under Shear Stress

![Diagram showing erythrocyte adhesion with and without anti-VWF (20 µg/ml).]
ULVWF or USVWF in the Plasma of Sickle Cell Patients?
Sickle Cell Anemia Patients: VWF Multimer Gel
Nanobody Binding

Principle of VWF “Activation Factor”

Highly active VWF

“Peaceful” VWF

Activation factor = Slope 2 / Slope 1

Sickle Cell patient
Slope 2
Pooled Plasma
Slope 1

[VWF] nM
Total and Activated VWF in Sickle Cell Patients

VWF Antigen

Sickle Cell Anemia Patients
Total and Activated VWF in Sickle Cell Patients
Total active VWF correlates with the extent of hemolysis in SCD

$p = 0.007$
VWF Oxidation in Sickle Cell Disease?
VWF methionine oxidation by hypochlorous acid (HOCl) inhibits VWF cleavage by ADAMTS13 and increases VWF adhesiveness.

Oxidative modification of von Willebrand factor by neutrophil oxidants inhibits its cleavage by ADAMTS13.

Shear stress-induced unfolding of VWF accelerates oxidation of key methionine residues in the A1A2A3 region.
Functional Effect of VWF Oxidation

Platelet Agglutination

Nanobody Binding

Time (min)

Platelet Agglutination (%)

Oxidized

Control

Nanobody binding

VWF (nM)
VWF Oxidation in SCD Plasma

- Oxidized M1385
- Oxidized M1606

Relative Abundance

Time (min)

Normal

Sickle
Investigating mechanisms of microangiopathies using in vitro microvessels
Artificial Microvessel Fabrication

Injection Molding:

Top Piece:
- PDMS mold
- Inlet
- Outlet

Bottom Piece:
- Collagen gel

Assembled Device:
- Embedded microchannel
- Inlet
- Outlet

Endothelial Cell seeding:
- Collagen gel pre-seeded with perivascular cells
- Endothelial cells

1 Hour

Attached endothelial cells

- Multi-cellular proximity
- 3D luminal geometry
- Laminar flow
- Extracellular matrix
- Controlled microenvironment

Ying Zheng, Ph.D.
Engineering microvessels - Structure

Day 14

Lumen: Endothelial cells

1% collagen

Lumen

PECAM1
Nuclei

100 µm

Zheng et al., PNAS, (109) 9342, 2012
**Structure and barrier function**

**After 10 minutes flow**

FITC-Dextran (70 kDa): $K \approx 1.3 \times 10^{-6} \text{ cm/s}$

Fluorescein (330 Da): $K \approx 8.0 \times 10^{-5} \text{ cm/s}$
Whole blood perfusion of vessel
Platelets: CD41a labeled
Control, unstimulated
PMA-stimulated
After 15 minutes of whole blood perfusion

Blood – microvessel interaction

Non-stimulated vessels

Stimulated vessels

PECAM1
CD41a
Nuclei
CD45

Endothelium
Platelets
monocytes

Endothelium
leukocytes
Platelets
Stimulated endothelium
Endothelial Response to Stimuli

PECAM1
VWF
Nuclei

200 µm

2 µm
Artificial Vessels – 3D geometry

VWF secretion (confocal z-stack)
Plasma perfusion: Normal vs TTP

Normal Plasma

TTP Plasma

String length (µm)

Control
ADAMTS13
Normal Plasma
TTP Plasma
Platelet accumulation on endothelial VWF

mlG: control
A10: inhibited ADAMTS13
A10 + AK2: inhibited ADAMTS13 + blocked GPIb
Erythrocytes accumulate on endothelial-bound VWF strands
Inhibition of platelet binding almost completely eliminates RBC binding.
Leukocytes also accumulate on platelet-VWF strings

Whole blood perfusion, ADAMTS13 inhibited
Effect of vessel geometry on VWF self-association

Flow direction

Cross-section at center of vessel

PECAM1
VWF
Nuclei
Self-associated VWF can form strands of enormous length.
Fluid-phase VWF attaching to transluminal fiber
Effect of HDL/apoA-1 on VWF self-association on endothelial cells
Macromolecules in boiled plasma prevent VWF binding to surfaces.
SDS-PAGE of Boiled Plasma

- α1 acid glycoprotein (AGP)
- Apo A-1
- Prealbumin

Molecular weights:
- 115 kD
- 82 kD
- 64 kD
- 49 kD
- 37 kD
- 26 kD
- 15 kD
Effect of HDL on VWF-platelet strings

- No HDL
- HDL - stimulation
- HDL - perfusion
- HDL - stimulation and perfusion
Effect of HDL on VWF-platelet strings

Strings (normalized to control)

Control  HDL Perf  HDL Stim  HDL Stim-Perf
Microvessel activation then perfusion of Apo-A1
Total VWF
Apo-A1
Fluid phase VWF
Nuclei
Total VWF

Apo-A1

Fluid phase VWF

Nuclei
Decreased levels of HDL/apoA1 in:

- TTP
- Severe malaria
- SCD crisis
- Sepsis
Hyperadhesive VWF and ApoA1 in TTP and Sepsis
Summary

VWF is involved in many microvascular diseases, including TTP and SCD.

In sickle cell disease, high levels of highly active VWF are associated with an increased hemolytic rate, possibly caused by VWF oxidation.

VWF:
- From the vessel wall self-associates to form thicker, more adhesive strands.
- From plasma can associate with strands from the vessel wall.
- HDL and apoA1 both modulate VWF self-association.
Apheresis implications

• Exchange plasma in addition to RBC for SCD patients?

• Consider role of HDL/apoA1 in plasma used for plasma exchange for microangiopathies
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