The Role of von Willebrand Factor in Mediating Factor VIII Immunogenicity

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Background

- Clinical epidemiology studies
  - SIPPET 1.87-fold increase with rFVIII products
  - CANAL
  - RODIN
  - French Study

- Experimental/biological studies
  - to explain why there’s a difference in immunogenicity
General Comments on a Biological Explanation for Differential Immunogenicity

Given –

1. Length of time to show difference in immunogenicity (20 yrs +)
2. Immunogenicity difference (1.87-fold), is not binary (yes/no)

Biological Explanation will likely be -

- Relatively subtle
- Complex

Why Might Recombinant FVIII be More Immunogenic?

- Increased protein aggregates
- Different post-translational modifications
  - eg, different glycans
- Lacking supplemental plasma immunodulatory proteins
  - eg. TGFβ, IL-10 etc.
- Reduced VWF binding – limiting an immunomodulatory effect of VWF
The Factor VIII-VWF Association

<table>
<thead>
<tr>
<th>FVIII</th>
<th>VWF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endothelial cells</td>
<td>Endothelial cells/Megakaryocytes</td>
</tr>
<tr>
<td>200 ng/mL</td>
<td>10 µg/mL</td>
</tr>
<tr>
<td>1 nM</td>
<td>35 nM</td>
</tr>
</tbody>
</table>

The Factor VIII – VWF Association

Kd - 0.2 nmol/L

At saturation: Stoichiometry 1 FVIII: 50 VWF

2-5%

Established VWF-FVIII Associations

- Synthesized together in some endothelial cells

- VWF influence dominant on FVIII clearance

Presentation Summary

1. Brief review of 5 experimental reports
   
   Pfistershammer et al. Thromb Haemost 2006
   Qadura et al. Blood 2009
   Sorvillo et al Haematologica 2015

2. Proposal of a novel hypothesis implicating VWF as an immunomodulatory influence for FVIII inhibitor development
Recombinant factor VIII and factor VIII von Willebrand factor complex do not present danger signals for human dendritic cells

Pfistershammer et al. Thromb Haemost. 2006

Neither rFVIII nor rFVIII-VWF complexes result in Immature Dendritic Cell Activation

rFVIII

rFVIII+VWF

Immature Monocyte-derived Dendritic Cells

NO

significant changes in

• Cytokine expression
• iDC maturation
• T cell activation

Pfistershammer et al. Thromb Haemost. 2006
Recombinant and plasma-derived factor VIII products induce distinct splenic cytokine environments in hemophilia A mice


**Factor VIII “Immunization” Protocol**

4 weekly IV infusions

2 IU (80 U/kg: ~400 ngs) FVIII

1 week later, test for anti-FVIII immune response

Qadura et al. Blood 2009

Anti-Human FVIII Neutralizing Antibody Titers

A) $r_{FVIII} =$ Full length FVIII
$p_{dFVIII} =$ 1:1 VWF:FVIII ratio

B) N = 8-10
* $P < 0.01$

Anti-Human FVIII Antibody Titers

Qadura et al. Blood 2009
Anti-Human VWF Antibody Response in F8 KO Mice

Qadura et al. Blood 2009

HBSS (N=24 mice), rFVIII (N=24 mice), pdFVIII (N=24 mice)
24 hrs
Splenocyte Isolation: pools of 6 spleens = 1 biological replicate (total of 4 biological replicates per condition)
Purify CD11c⁺ Dendritic Cells
Isolate RNA
Microarray (1100 genes)
Each biological sample tested on 2 different arrays to generate a biological and technical read
Confirmatory QRT-PCR
Each biological sample run in quadruplicate

Demonstration of Differential Splenic Dendritic Cell Transcript Generation Following rFVIII or pdFVIII infusion
### Differential Immune Response Gene Expression

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>rFVIII/pdFVIII Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ccl2</td>
<td>0.25</td>
</tr>
<tr>
<td>Cxcl1</td>
<td>0.39</td>
</tr>
<tr>
<td>Cxcl2</td>
<td>0.41</td>
</tr>
<tr>
<td>Hspb1</td>
<td>0.44</td>
</tr>
<tr>
<td>Hspa1a</td>
<td>0.45</td>
</tr>
<tr>
<td>Il6</td>
<td>0.46</td>
</tr>
<tr>
<td>Jun</td>
<td>0.47</td>
</tr>
<tr>
<td>Gdf15</td>
<td>0.47</td>
</tr>
<tr>
<td>Egr1</td>
<td>0.47</td>
</tr>
<tr>
<td>Plk2</td>
<td>0.48</td>
</tr>
<tr>
<td>Egr2</td>
<td>0.49</td>
</tr>
<tr>
<td>Itga2b</td>
<td>2.01</td>
</tr>
<tr>
<td>Cxcl4</td>
<td>2.10</td>
</tr>
<tr>
<td>Ltf</td>
<td>2.17</td>
</tr>
<tr>
<td>Ppbp</td>
<td>2.41</td>
</tr>
<tr>
<td>Camp</td>
<td>2.72</td>
</tr>
</tbody>
</table>

**Consistent 2-fold difference**

- 4 biological replicates
- 6 technical replicates
- qRT-PCR x 4

16 immune response transcripts

- Ccl2 – chemokine ligand 2
- Heat shock protein 1
- Lactotransferrin

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**VWF protects FVIII from endocytosis by dendritic cells and subsequent presentation to immune effectors**

Dasgupta et al Blood 2007
VWF reduces FVIII endocytosis by DCs and the consequent presentation to FVIII-specific T cells

Dasgupta et al Blood 2007

Immunoprotective effect of von Willebrand factor towards therapeutic factor VIII in experimental haemophilia A

Levels of anti-FVIII IgG in F8 KO mice after injection of FVIII (empty circles) or FVIII with a 50-molar excess of purified VWF (filled circles)

Von Willebrand factor binds to the surface of dendritic cells and modulates peptide presentation of factor VIII

Nicoletta Sorvillo et al. Haematologica 2015 [Epub ahead of print]
Binding of VWF to iDCs assessed by confocal microscopy.

DC-SIGN  
VWF  
Merge

Sorvillo et al. Haematologica 2015 [Epub ahead of print]
International Conference on Inhibitors in Hemophilia A • Milan, Italy, 4-5 March 2016
VWF Peptides are only presented by Immature DCs when delivered with FVIII

Von Willebrand factor binds to the surface of dendritic cells and modulates peptide presentation of factor VIII

Conclusions

- VWF binds to the surface of immature DCs but is not internalized
- VWF reduces uptake of FVIII by immature DCs
- Different FVIII peptides were presented with VWF/FVIII vs FVIII alone
- VWF requires FVIII for the presentation of any VWF peptides by immature DCs
Questions Posed by the Reported Experimental Observations

1. If FVIII presentation is inhibited by VWF, how is FVIII tolerance generated?

2. Are monocyte-derived immature DCs a good candidate for FVIII antigen presentation?

3. Why doesn't the patient's own intrinsic VWF fulfill these functions with rFVIII?
Questions Posed by the Reported Experimental Observations

1. If FVIII presentation is inhibited by VWF, how is FVIII tolerance generated?

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Clearance Receptors for the VWF-FVIII

- SCARA5
- Interstitial Fibroblast ? endothelium

Derived from (Casari et al. 2013)

What are the Antigen Presenting Cells for VWF–VWF?

Candidate antigen presenting cells for VWF-FVIII

- **Spleen**
  - Marginal zone macrophages
  - Metalophilic macrophages
  - Marginal zone B cells
  - Dendritic cells

- **Liver**
  - Kupffer cells
  - Dendritic cells
  - Sinusoidal endothelial cells
Co-Localization of FVIII with Splenic Marginal Zone Macrophages (MARCO +ve)

PBS Infusion

30 mins post-FVIII Infusion

Does the absence of VWF change this localization?  
Data representative of at least 2 biological replicates  
Jesse Lai, Unpublished data

rFVIII Presentation in a naïve Hemophilia A Patient

rFVIII

APC

MHC II

rFVIII is the only source of foreign peptides

CD4+ T Cell

Tolerance already established for intrinsic (self) VWF and other plasma proteins

No competition for rFVIII Immunogenicity
Contrast with pdFVIII-VWF and Naïve PUP Responses

pd-FVIII - Variably tolerant

pd-VWF - have "self" VWF – therefore tolerant?

Many other plasma proteins - have "self" proteins therefore tolerant

Heterogeneity of pdVWF-FVIII

However,

pdVWF and FVIII are polymorphic proteins

and

pdFVIII concentrates derive from 1,000s of donors
**VWF Genomic Variability in 1000 Genomes**  
(Wang et al. JTH 2013)

2,728 SNVs

- 94% intronic
- 6% exonic (152)

91 of 152 exonic SNPs - non-synonymous (AA substitutions)

Minor allele frequencies: 0.05 – 3.3%  
(but pdFVIII lots ~>10,000 donors)

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**Complexity and diversity of F8 genetic variations in the 1000 genomes**  
(Li et al. JTH 2015)

3,030 SNVs

- 2.18% exonic (85/3,030)

56/85 variants are missense substitutions

Again, minor allele frequencies are low
Heterogeneity of pdFVIII Concentrates

Therefore ….

Infusion of pdFVIII involves exposure to

91 variant forms of VWF
&
56 variant forms of FVIII

pdFVIII Presentation in a naïve Hemophilia A Patient

FVIII immunogenicity is "distracted"/"diluted" by antigen competition from many pdFVIII-derived VWF and FVIII variant proteins
Conclusion

There is increasing evidence that the immune response to rFVIII is different to that for pdFVIII.

However, this difference has only emerged after >25 years of clinical use of rFVIII, and there is still not consensus about the significance of these recent findings.

In light of the importance of FVIII inhibitor development, further studies of the epidemiology of FVIII immunogenicity are required to confirm recent reports, and basic science experiments are needed to provide a biological basis that would support an apparent differential immunogenicity associated with rFVIII.