Immunogenicity of therapeutic factor VIII in patients with mild/moderate hemophilia A

Sébastien Lacroix-Desmazes
Centre de recherche des Cordeliers
INSERM UMRS 1138
Paris, France
Hemophilia A (HA)

- Rare hemorrhagic disorder linked to the X chromosome
- Genetic abnormalities in the gene encoding factor VIII (FVIII)
- Lack of functional FVIII

- Different severities depending on circulating FVIII levels
  
  - <1%  Severe HA (SHA)
  - 1-5%  Mild HA
  - 5-40%  Moderate HA

  MHA >50% of the HA population
Correction or prevention of bleeding

- Intravenous administration of exogenous FVIII
  - Plasma-derived FVIII
  - Recombinant FVIII

- Complication of administration of exogenous FVIII
  - Anti-FVIII IgG
    - Inhibit therapeutic FVIII
    - Occur in 5 to 50% of the patients

Anti-FVIII antibodies or "FVIII Inhibitors"
Immune response to FVIII

Antigen-presenting cell

FVIII

T cell

B cell

Naive FVIII-specific T cell

MHCII

TcR

IgG
Heterogeneity of patients with hemophilia A

Large variety of genetic abnormalities leading to hemophilia A

- Large deletions
- Nonsense mutations
- Intron 22 inversions
- Point deletions
- Missense mutations
- Splice site mutations
- Promoter mutations

Association with phenotype

- Severe hemophilia A
- Mild-Moderate hemophilia A
Correlation between genetic abnormalities and occurrence of FVIII inhibitors

Oldenburg Haemophilia 2002
### Heterogeneity of patients with hemophilia A

<table>
<thead>
<tr>
<th>Large variety of genetic abnormalities leading to hemophilia A</th>
<th>Inhibitor risk</th>
<th>Presence of the FVIII antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large deletions</td>
<td>30-75%</td>
<td>No or partial</td>
</tr>
<tr>
<td>Nonsense mutations</td>
<td>25-45%</td>
<td>No or partial</td>
</tr>
<tr>
<td>Intron 22 inversion</td>
<td>25%</td>
<td>Intracellular*</td>
</tr>
<tr>
<td>Point deletions</td>
<td>5-15%</td>
<td>Yes</td>
</tr>
<tr>
<td>Missense mutations</td>
<td>7%</td>
<td>Yes</td>
</tr>
<tr>
<td>Splice site mutations</td>
<td>&lt;1%</td>
<td>?</td>
</tr>
<tr>
<td>Promoter mutations</td>
<td>&lt;1%</td>
<td>Yes</td>
</tr>
</tbody>
</table>

**Establishment of tolerance to self**

*Pandey Nat Med 2013*
Induction of thymic T-cell tolerance

Gut-associated lymphoid tissue (GALT)
Induction of thymic T-cell tolerance

Developing thymic T cells

Positive selection

Self recognition?

Yes

Self recognition?

No

Elimination

Negative selection

Self recognition?

Yes

Regulatory T cells

No

Effector T cells
Inhibitor-positive MHA patients with circulating FVIII levels

Patient LE
Missense mutation: Arg2150His
Residual FVIII activity: 23%
FVIII Inhibitor: 305 BU/ml

Patient’s endogenous FVIII

Therapeutic FVIII

CD4+ T cells

Jacquemin Blood 2000
Jacquemin Blood 2003
Pratt JTH 2007 Ala2201Pro in the FVIII C2 domain
Yada JTH 2015 Pro1809Leu in the A3 domain
Inhibitor-positive MHA patients with circulating FVIII levels

However, in the majority of the patients => inhibition of both therapeutic and endogenous FVIII
Heterogeneity in inhibitor risk among patients with missense mutations

Position of missense mutations

<table>
<thead>
<tr>
<th>Domain</th>
<th>No inhibitor</th>
<th>Inhibitor</th>
<th>%↑</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>243</td>
<td>5</td>
<td>2.02</td>
<td>0.66–4.64</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>A2</td>
<td>379</td>
<td>32</td>
<td>7.79</td>
<td>5.38–10.81</td>
<td></td>
</tr>
<tr>
<td>A3</td>
<td>283</td>
<td>14</td>
<td>4.71</td>
<td>2.60–7.78</td>
<td></td>
</tr>
<tr>
<td>C1</td>
<td>191</td>
<td>22</td>
<td>10.33</td>
<td>6.59–15.22</td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>154</td>
<td>21</td>
<td>12.00</td>
<td>7.58–17.76</td>
<td></td>
</tr>
<tr>
<td>a1, a2, a3</td>
<td>111</td>
<td>1</td>
<td>0.89</td>
<td>0.02–4.87</td>
<td></td>
</tr>
<tr>
<td>Chain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.087</td>
</tr>
<tr>
<td>Heavy</td>
<td>668</td>
<td>38</td>
<td>5.38</td>
<td>3.84–7.31</td>
<td></td>
</tr>
<tr>
<td>Light</td>
<td>693</td>
<td>57</td>
<td>7.6</td>
<td>5.81–9.731</td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td>1361</td>
<td>95</td>
<td>6.52</td>
<td>5.31–7.92</td>
<td></td>
</tr>
</tbody>
</table>
Heterogeneity in inhibitor risk among patients with missense mutations

Type of change of amino-acid class

Cohort of 712 patients

Intra-class aa change: 1.8%

Inter-class aa change: 5.8%

p=0.039

Schwaab JTH 2013
Heterogeneity in inhibitor risk among patients with missense mutations

On a mutation-dependent basis

<table>
<thead>
<tr>
<th>F8 mutation</th>
<th>No. of patients n (%)*</th>
<th>No. of patients with inhibitor n (%)</th>
<th>Baseline FVIII:C, IU/dL median (min-max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutations in 10 or more patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg531Cys</td>
<td>35 (3.2)</td>
<td>1 (2.9)</td>
<td>8 (2-37)</td>
</tr>
<tr>
<td>Arg593CysII</td>
<td>106 (9.5)</td>
<td>12 (11.3)</td>
<td>17 (5-32)</td>
</tr>
<tr>
<td>Asn618Ser</td>
<td>58 (5.2)</td>
<td>1 (1.7)</td>
<td>24 (8-37)</td>
</tr>
<tr>
<td>Asp2074Gly</td>
<td>11 (1.0)</td>
<td>3 (27.3)</td>
<td>8 (4-14)</td>
</tr>
<tr>
<td>Arg2150His</td>
<td>57 (5.1)</td>
<td>9 (15.8)</td>
<td>7 (2-32)</td>
</tr>
<tr>
<td>Arg2159Cys</td>
<td>21 (1.9)</td>
<td>3 (14.3)</td>
<td>14 (6-29)</td>
</tr>
<tr>
<td>Trp2229Cys</td>
<td>10 (0.9)</td>
<td>5 (50.0)</td>
<td>8 (5-24)</td>
</tr>
<tr>
<td>Mutations in less than 10 patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leu412Phe</td>
<td>5 (0.4)</td>
<td>1 (20.0)</td>
<td>8 (3-14)</td>
</tr>
<tr>
<td>Pro1761Gln</td>
<td>2 (0.2)</td>
<td>1 (50.0)</td>
<td>6 (5-6)</td>
</tr>
<tr>
<td>Phe1775Val</td>
<td>3 (0.3)</td>
<td>2 (66.7)</td>
<td>27 (13-29)</td>
</tr>
<tr>
<td>Arg1781Gly</td>
<td>4 (0.4)</td>
<td>1 (25.0)</td>
<td>8 (6-16)</td>
</tr>
<tr>
<td>Pro1854Leu</td>
<td>4 (0.4)</td>
<td>1 (25.0)</td>
<td>14 (6-25)</td>
</tr>
<tr>
<td>Arg1997Trp</td>
<td>3 (0.3)</td>
<td>2 (66.7)</td>
<td>4 (4-6)</td>
</tr>
<tr>
<td>Phe2101Cys</td>
<td>2 (0.2)</td>
<td>2 (100)</td>
<td>7 (6-7)</td>
</tr>
<tr>
<td>Tyr2105Cys</td>
<td>6 (0.5)</td>
<td>3 (50.0)</td>
<td>19 (12-28)</td>
</tr>
<tr>
<td>Glu2228Asp</td>
<td>3 (0.3)</td>
<td>1 (33.3)</td>
<td>27 (16-36)</td>
</tr>
<tr>
<td>Val2232Ala</td>
<td>1 (0.1)</td>
<td>1 (100.0)</td>
<td>15</td>
</tr>
<tr>
<td>His2309Asp</td>
<td>1 (0.1)</td>
<td>1 (100.0)</td>
<td>2</td>
</tr>
<tr>
<td>Stop2333Cys</td>
<td>1 (0.1)</td>
<td>1 (100.0)</td>
<td>11</td>
</tr>
</tbody>
</table>
Naive FVIII-specific T cell

Therapeutic FVIII

Binding of FVIII peptides to MHC II molecules for immune responses

MHCII

FVIII peptides
Naive FVIII-specific T cell

Therapeutic FVIII

List of mutations leading to MHA

Arg531Cys
Arg593CysII
Asn618Ser
Asp2074Gly
Arg2150His
Arg2159Cys
Trp2229Cys

Mutations in Ies
Leu412Phe
Pro1761Gln
Phe1775Val
Arg1781Gly
Pro1854Leu
Arg1997Trp
Phe2101Cys
Tyr2105Cys
Glu2228Asp
Val2232Ala
His2309Asp

Array of corresponding FVIII peptides

Array of MHCII haplotypes

Binding of FVIII peptides to MHC II molecules for immune responses

% of inhibitors per mutation

1 (2.9)
12 (11.3)
1 (1.7)
3 (27.3)
9 (15.8)
3 (14.3)
5 (50.0)

1 (20.0)
1 (50.0)
2 (66.7)
2 (25.0)
2 (25.0)
2 (66.7)
2 (100)
3 (50.0)
1 (33.3)
1 (100.0)
1 (100.0)
1 (100.0)


**List of mutations leading to MHA**

- Arg531Cys
- Arg593CysII
- Asn618Ser
- Asp2074Gly
- Arg2100Hls
- Arg2199Cys
- Trp2229Cys

**Mutations in Ies**

- Leu412Phe
- Pro1761Gln
- Phe1775Val
- Arg1781Gly
- Pro1854Leu
- Arg1997Trp
- Phe2101Cys
- Tyr2105Cys
- Glu2228Asp
- Val2232Ala
- His2309Asp

**Peptides with high affinity = more inhibitors**

**Peptides with less affinity = less inhibitors**

=> In MHA patients with missense mutations, the strength of binding of the peptides to MHCII molecules plays a role in FVIII immunogenicity.

**% of inhibitors per mutation**

<table>
<thead>
<tr>
<th>Mutation</th>
<th>% of Inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg531Cys</td>
<td>1 (2.9)</td>
</tr>
<tr>
<td>Arg593CysII</td>
<td>12 (11.3)</td>
</tr>
<tr>
<td>Asn618Ser</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td>Asp2074Gly</td>
<td>3 (27.3)</td>
</tr>
<tr>
<td>Arg2100Hls</td>
<td>9 (15.8)</td>
</tr>
<tr>
<td>Arg2199Cys</td>
<td>3 (14.3)</td>
</tr>
<tr>
<td>Trp2229Cys</td>
<td>5 (50.0)</td>
</tr>
</tbody>
</table>

- Leu412Phe   | 1 (20.0)        |
- Pro1761Gln  | 1 (50.0)        |
- Phe1775Val  | 2 (66.7)        |
- Arg1781Gly  | 1 (25.0)        |
- Pro1854Leu  | 2 (25.0)        |
- Arg1997Trp  | 2 (66.7)        |
- Phe2101Cys  | 2 (100.0)       |
- Tyr2105Cys  | 3 (50.0)        |
- Glu2228Asp  | 1 (33.3)        |
- Val2232Ala  | 1 (100.0)       |
- His2309Asp  | 1 (100.0)       |
Conclusion

Therapeutic FVIII

Immune education on self mutated FVIII

Strength of MHC binding

Immune response

Immune tolerance
Conclusions

Patients with mild/moderate hemophilia A are very heterogeneous.

The risk for FVIII inhibitor development is multifactorial.

The type of hemophilia A-causing mutation plays an important role in the inhibitor risk.

Need a better understanding of the repercussion of each individual mutation on:

- FVIII activity
- FVIII structure
- Capacity to induce immune tolerance

Appropriate prevention of FVIII inhibitor development probably relies on individual patient management, and tailor-made therapeutic options.