Development of inhibitors in hemophilia A: An illustrated review

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Abstract
This illustrated review focuses on the development of inhibitors in patients with congenital hemophilia, which is the most serious treatment-related complication in these patients. Hemophilia A (HA) is an inherited X-linked bleeding disorder affecting 1:5000-10,000 newborn males worldwide. It results from the deficiency of coagulation factor VIII (FVIII), due to mutation(s) in its coding gene (F8). Treatment requires administration of FVIII-containing products either on demand or as prophylaxis, which can induce inhibitor development in 20%-35% of patients. Inhibitors are alloantibodies that neutralize the procoagulant activity of exogenous FVIII. During the initial administration of FVIII-containing products, patients with HA can develop a proinflammatory immune response with synthesis of anti-FVIII IgG1, which has no FVIII inhibitory activity. However, in patients with inhibitors, immune response shifts toward an anti-inflammatory/regulatory pattern favoring the synthesis of anti-FVIII IgG4 antibodies. Patients with inhibitors present with bleeding episodes that are difficult to control, and they have reduced response to FVIII replacement. Currently, immune tolerance induction is the available treatment for eradication of persistent high-titer inhibitors. Despite the clinical relevance, the immunological mechanisms for inhibitor development in patients with HA remains unexplained.

Keywords
antibody, factor VIII, hemophilia, immune response, inhibitor

Essentials
- Hemophilia A (HA) is a bleeding disorder caused by the deficiency of coagulation factor VIII (FVIII).
- The main treatment-related complication in patients with HA is the development of inhibitor.
- Inhibitors are alloantibodies that neutralize the procoagulant activity of infused FVIII.
- The reasons why only 20%-30% of the patients with HA develop inhibitors remain a challenge.
Hemophilia A (HA) is an inherited X-linked bleeding disorder caused by the deficiency of coagulation FVIII due to mutations in \( F8 \). HA affects 1:5000 - 10 000 newborn males worldwide.

The main determinant of the bleeding phenotype is the residual level of FVIII:

- \( \text{FVIII} < 1\%: \) SEVERE
- \( \text{FVIII} 1\%-5\%: \) MODERATE
- \( \text{FVIII} > 5\%-40\%: \) MILD

The most common bleeding sites are: joint, muscles and soft tissues.

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**Factor VIII - Gene and protein**

a) Structure of factor VIII gene (\( F8 \));

b) Transcription of messenger RNA, with 2 noncoding regions (5´UTR and 3´UTR);

c) Primary structure of FVIII, representing the domains and the breakpoints in acidic regions a1, a2, and a3;

d) Inactive FVIII associated with von Willebrand factor. Acidic regions a1, a2, and a3 contain interaction sites recognized by FX and thrombin;

e) Protein activation after thrombin cleavage and dissociation of von Willebrand factor.
Treatment of hemophilia A

The type of FVIII concentrate can be:

- PLASMA-DERIVED (pdFVIII)
- RECOMBINANT (rFVIII)

Inhibitors in hemophilia A

Neutralizing alloantibodies (inhibitors) are the main treatment-related complication in patients with severe HA.5-6

Inhibitors bind FVIII epitopes, neutralizing the therapeutic activity of the infused protein, leading to bleeding that is difficult to control.7,11

Cumulative incidence of inhibitor development in patients with HA is 20%-35%.

During initial administration of FVIII-containing products, the immune system develop a pro-inflammatory response which involves synthesis of antibodies against FVIII.7-10

In about 95% of patients with HA who develop inhibitors, it occurs within the first 75 exposure days to FVIII replacement.12
An inhibitor is confirmed when there is an antibody titer above 0.6 Bethesda Unit (BU)/mL in two consecutive plasma measurements. Then, inhibitor is classified as:

**High-titer inhibitors:**
- Titer ≥5 BU/mL

**Low-titer inhibitors:**
- Titer <5 BU/mL

### Risk factors for inhibitor development

- **Mutations in genes related to immune response**
- **Intensity of FVIII infusions**
- **Family history of inhibitors**
- **Younger age at first exposure to FVIII**
- **Type of FVIII concentrate used (pdFVIII or rFVIII)**
- **Type of F8 mutation**

### Immunology of inhibitors in hemophilia A

#### Part I: Central tolerance for the deficient protein

Immature T cells enter the thymus and interact with thymic epithelial cells (TECs)

As a result of their genetic mutation, some patients with HA are unable to express large portions or any amount of FVIII. These patients are not able to eliminate high-affinity FVIII-reactive T cells. These T cells will then populate peripheral tissues and may induce the development of inhibitors when patients are treated with FVIII.
These interactions result in a clonal expansion of the active T cells which migrate to the B-lymphocyte follicles in the spleen.
Immunology of inhibitors in hemophilia A
Part III: Primary response\textsuperscript{6,15-17}.

Follicular B lymphocytes that have been primed by infused FVIII and have internalized FVIII through the BCR present FVIII peptides via class II HLA on the membrane surface in the B lymphocyte follicle.

Interaction between activated T cells and B lymphocytes through TCR-HLA, co-stimulatory molecules, and cytokine stimulation triggers the differentiation of follicular B lymphocytes.

Figure legend:
- BCR, B-cell receptor
- TCR, T-cell receptor
- HLA II, class II human leukocyte antigen
- IL, Interleukin
- ILR, Interleukin receptor

Follicular B lymphocyte can differentiate into:
- Plasma cells
  - Involved in long-lasting antibody production
- Memory B lymphocytes
  - Involved in long-lasting antibody production
Memory B lymphocytes can develop a faster and stronger immune response against FVIII than naive B lymphocytes, with production of high-affinity neutralizing antibodies.\textsuperscript{15}

Anti-FVIII antibodies, mostly IgG1, IgM, and IgA, have been reported in healthy individuals. Anti-FVIII IgG4 is predominantly found in patients with HA who developed inhibitors, especially those with high titer.\textsuperscript{17-19}
Immune tolerance induction (ITI) is the unique available treatment for eradication of persistent high-titer inhibitors in HA. ITI is effective in 60%-80% of treated patients with HA.

However, ITI is a demanding and high-cost treatment and requires frequent infusions of FVIII for months to years.

Final Remarks
Inhibitor development is the main complication of HA, affecting about 30% of patients. ITI can eradicate inhibitors but is costly and not successful for all patients. Furthermore, inhibitors can recur. Therefore, a better understanding of biological mechanisms, epidemiology, and risk factors for inhibitor development is needed.
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