Molecular determinants of FVIII immunogenicity in hemophilia A
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Dangerous liaisons: how the immune system deals with factor VIII

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Abstract

Only a fraction of hemophilia A patients develops a neutralizing antibody (inhibitor) response to therapeutic infusions of factor VIII (FVIII). Our present understanding of the underlying causes of the immunogenicity of this protein is limited. In the past few years, insights into the uptake and processing of FVIII by antigen presenting cells (APCs) have expanded significantly. While the mechanism of endocytosis remains unclear, current data indicate that FVIII enters APCs via its C1 domain. Its subsequent processing within endolysosomes allows for presentation of a heterogeneous collection of FVIII peptides on MHC class II, and this peptide-MHC class II complex may then be recognized by cognate effector CD4+ T cells, leading to anti-FVIII antibody production. Here we aim to summarize recent knowledge gained on FVIII processing and presentation by APCs, as well as the diversity of the FVIII-specific T-cell repertoire in mice and men. Moreover, we discuss possible factors that can drive FVIII immunogenicity. We believe that increasing understanding of immune recognition of FVIII and the cellular mechanisms of anti-FVIII antibody production will lead to novel therapeutic approaches to prevent inhibitor formation in patients with hemophilia A.
Introduction
Professional APCs such as dendritic cells (DCs), macrophages and B cells endocytose proteins that are degraded along the endocytic pathway and then presented on MHC class II molecules to CD4⁺ T cells. Apart from internalized foreign antigens, many peptides presented on MHC class II are derived from proteins residing in intracellular compartments that are sampled by autophagy. Peptides from proteins degraded in the endosome are loaded on MHC class II in the so-called MHC class II peptide-loading compartment and subsequently transported to the plasma membrane (Figure 1). In DCs, MHC class II expression at the cell surface increases following their maturation, which also results in up-regulation of co-stimulatory molecules required for activation of naive CD4⁺ T cells. The complex mechanisms involved in antigen presentation and their coordinated interplay with pattern-recognition receptors of the innate immune system have been exploited by modern medicine, e.g. by harnessing individuals with pre-existing immunity (neutralizing antibodies) against incoming microbial challenges. ¹,² A growing appreciation of the skewing of immune responses by pattern recognition receptors of the innate immune system has advanced the field of rational design of vaccines. ¹,² Undesired immune responses have been observed following repeated administration of a number of therapeutic proteins, including anti-TNF-alpha antibodies such as adalimumab or infliximab, coagulation factors VIII and IX and erythropoietin. ³ In view of the ubiquitous presentation of self- and non-self peptides on MHC class II, one can easily appreciate that peptides derived from biotherapeutic proteins are also presented on MHC class II. However, it is more difficult to envision how biotherapeutics, in the absence of immunologic “danger signals”, would induce activation of APCs thereby promoting the up-regulation of co-stimulatory molecules required for activation of naïve CD4⁺ T cells.

This review will explore the issue of neutralizing anti-drug antibodies in the context of current knowledge of blood coagulation factor VIII (FVIII) immunogenicity. Approximately 25% of patients with the severe form of the X-linked bleeding disorder hemophilia A (defined by FVIII pro-coagulant activity < 1% normal) develop an immune response resulting in the formation of neutralizing anti-FVIII antibodies. A number of recent reviews have summarized genetic and non-genetic treatment-related risk factors that contribute to inhibitor formation in hemophilia A. ⁴-⁷ Guidelines for treatment of hemophilia A patients with inhibitors have been provided in a recent paper by Kempton and White. ⁸ Inhibitor formation as well as somatic hypermutations and subclass switching of anti-FVIII antibodies are considered to be CD4⁺ T-cell dependent processes in both hemophilic mice ⁹-¹¹ and hemophilia A patients. ¹²-¹⁷ Activation of FVIII-specific T cells is preceded by the uptake of FVIII by antigen-presenting cells (APCs) and the subsequent presentation of FVIII peptides on MHC class II molecules on the surface of these APCs. ¹⁸ HLA alleles DRB1*15 and DQB1*0602 have been suggested to correlate with increased risk for inhibitor development in hemophilia patients. ⁵,¹⁹ However, the association between MHC class II molecules and FVIII antibody formation is not strong, and this reflects a central and intriguing aspect of anti-FVIII immune
responses: a large majority of hemophilia A patients achieve functional immune tolerance to FVIII, either following their initial infusions or after eradicating an inhibitor response via immune tolerance induction (intensive FVIII therapy) or immunosuppression. The promiscuity of FVIII peptides in terms of binding to different HLA alleles, and the huge diversity in HLA types when compared to the number of hemophilia patients included in earlier studies investigating the role of HLA in inhibitor risks, also make the establishment of statistically significant associations with individual HLA types challenging.

This review focuses on the most recent data available on FVIII endocytosis and processing by APCs as well as presentation of FVIII-specific peptides to T cells. We discuss new insights into mechanisms of FVIII endocytosis by DCs in humans and mice and describe recent investigations into what determines the immunogenicity of FVIII. We also summarize efforts made to interfere with immune recognition of FVIII and with events subsequent to FVIII uptake by APCs that influence anti-FVIII antibody formation. Finally, we discuss investigations into the presentation of FVIII-specific peptides and the diversity and functionality of the subsequent FVIII-specific T-cell repertoire.

Endocytosis and processing of FVIII by APCs

Factor VIII is internalized by APCs such as DCs and macrophages, where it is processed efficiently into peptides, some of which may then be presented on MHC class II molecules at the cell surface. If a given MHC class II-peptide is then recognized by a T-cell receptor on an effector T cell, as can happen in hemophilia A patients who do not have pre-existing immune tolerance to FVIII.
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Factor VIII, this can lead to subsequent activation of Factor VIII-specific T and B cells and the production of antibodies. Inhibitory anti-Factor VIII antibodies interfere with its pro-coagulant function, e.g. by blocking its interaction with thrombin-activated platelet membranes and microparticles derived from these membranes that expose negatively-charged phosphatidylserine on their surface. The complexity of the B-cell responses to Factor VIII has been described in two recent reviews. Here we focus on critical determinants on Factor VIII involved in its uptake by APCs. Endocytosis of Factor VIII by APCs requires bivalent cations, as it can be inhibited by EDTA. The macrophage mannose receptor has been suggested to be an important interaction partner for Factor VIII. Mannose-ending glycans are linked to residues in the heavy chain (N239) and in the light chain (N2118). Blockage with mannan leads to partial inhibition of Factor VIII endocytosis by immature human DCs. Furthermore, strongly reduced proliferation of Factor VIII-specific human CD4+ T-cell clone D9E9 was seen after pulsing DCs with Factor VIII in the presence of mannan. In a more recent report, the same authors concluded that mannan did not block the uptake of Factor VIII by murine bone-marrow derived DCs. In an independent study, Herczenik and co-workers demonstrated that mannan did not block the uptake of Factor VIII by human or murine DCs, and siRNA-mediated knock-down of mannose receptor expression on DCs did not affect Factor VIII endocytosis. These recent studies imply that additional receptors can contribute to endocytosis of Factor VIII by DCs.

Low-density lipoprotein receptor-related protein (LRP), a broadly expressed scavenger molecule, has been implicated in Factor VIII clearance. Lysine 2092 and phenylalanine 2093 in Factor VIII have been shown to play a role in its binding to LRP. Moreover, it has been suggested that disruption of Factor VIII-LRP binding could potentially lead to a prolonged half-life of Factor VIII in vivo. However, LRP has not been implicated in the uptake of Factor VIII by DCs. Factor VIII binds with high affinity to negatively-charged phospholipid membranes, but lactadherin, which competes with Factor VIII for binding to these membranes, does not block Factor VIII endocytosis by DCs. Therefore, PS-rich membranes do not seem to play a significant role in Factor VIII uptake. The monoclonal antibody KM33, which targets the Factor VIII C1 domain, was recently shown to inhibit Factor VIII endocytosis by human as well as murine DCs (Figure 2A). Moreover, in vivo administration of KM33 prevented neutralizing antibody production against Factor VIII. The in vitro and in vivo inhibitory effect of KM33 suggests that this antibody targets an epitope on the Factor VIII surface that is essential for its uptake by APCs. KM33 also interferes with binding of Factor VIII to LRP, although, as mentioned above, this receptor does not seem to be involved in Factor VIII endocytosis, most probably due to its low affinity binding of Factor VIII. Factor VIII residues Arg2090, Lys2092 and Phe2093 (Figure 2B) have been implicated in KM33 binding to Factor VIII. Moreover, infusions of Factor VIII variant proteins with alanine substitutions at these three positions in Factor VIII-deficient mice led to reduced T- and B-cell responses as compared to wild type Factor VIII. Taken together, these findings suggest that an as-yet-unidentified cellular component that interacts with an exposed loop in the Factor VIII C1 domain promotes endocytosis of Factor VIII by APCs.
Modulating the effect of von Willebrand factor on uptake of FVIII by APCs

FVIII circulates in plasma as a large glycoprotein complexed with its multimeric chaperone molecule, von Willebrand Factor (VWF). VWF protects FVIII from premature activation as well as degradation or inactivation by circulating proteases. It also regulates FVIII catabolism and transports it to sites of injury. A number of studies performed using mouse models of hemophilia A have indicated that pre-incubation of recombinant FVIII with VWF leads to a reduction in titers of inhibitory anti-FVIII antibodies following FVIII infusions. However, results of these studies should be interpreted carefully since human VWF is also immunogenic in mice. Immune responses to infused human VWF may result in lower anti-FVIII antibody titers simply due to antigenic competition. In vitro studies performed with human APCs clearly showed that VWF protects FVIII from endocytosis by these cells and leads to decreased FVIII-specific T-cell proliferation (Figure 2A). These experimental results have relevance to epidemiological studies that have suggested a reduced prevalence of inhibitors in hemophilia A patients treated with VWF-containing FVIII concentrates. However, this clinical observation was not reproduced in a larger cohort study, which concluded that the risk of inhibitor development is similar for plasma-derived and recombinant FVIII. Moreover, patients with hemophilia A have normal levels of circulating VWF. Due to the high on-rate of the FVIII-VWF complex, one would expect that infused FVIII associates rapidly with endogenous VWF. Randomized, prospective clinical studies, such as the RES.I.ST (Randomization study of First Time Immune Tolerance Induction in Patients with Severe Type A Hemophilia with Inhibitor at High Risk of Failure: Comparison of Induction of Immune Tolerance With Factor VIII Concentrates With or Without Von Willebrand Factor) and SIPPET (Survey of Inhibitors in Plasma-Product Exposed Toddlers) studies, are addressing potential benefit of VWF containing concentrates for hemophilia A treatment.

On the immunogenicity of bio-engineered FVIII derivatives

PEGylation is a common method to reduce the immunogenicity and antigenicity of protein therapeutics. Site-specific PEGylation through engineered free cysteines in the heavy and light chains of FVIII has prolonged its half-life in animal models of hemophilia A. This PEGylated FVIII variant showed diminished endocytosis by human monocyte-DCs, followed by significantly reduced proliferation and production of IFNγ by FVIII-specific T cells. Moreover, studies performed in hemophilic mice, rats and rabbits showed a lower incidence of anti-FVIII antibodies in animals infused with PEGylated FVIII as compared to its recombinant non-PEGylated counterpart. On the other hand, a recent report by van Helden et al. indicated that PEGylation of FVIII could also result in a FVIII protein that expresses higher immunogenicity in hemophilic mouse models. Also, incorporation of PEGylated lipids into complexes of FVIII and phosphatidylinositol resulted in increased immunogenicity following intravenous infusion in a murine hemophilia A model. Genetic fusions of FVIII to the Fc fragment of IgG1 have been utilized to prolong the half-life of FVIII. Prolongation of therapeutic protein-Fc fusion half-lives requires effective complex formation between the Fc portion of IgG1
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and FcRn in the early endosome; recycling of endosomes re-exposes IgG1-Fc to physiological pH at the cell surface, resulting in its dissociation from FcRn. FcRn is also expressed on APCs. Therefore, internalization of FVIII-Fc fusion proteins by APCs most likely proceeds via a different mechanism compared to endocytosis of unmodified FVIII, which may be followed by FVIII peptide presentation on the MHC class II and subsequent T-cell stimulation. In this respect it is interesting to note that retroviral transduction of B-cell blasts with Ig-fusions harboring the A2 and C2 domains of FVIII can restore tolerance in hemophilia A mice with pre-existing inhibitors. Initial data on the immunogenicity of FVIII-Fc proteins in murine hemophilia A models has recently been reported in two meeting abstracts. These initial reports suggested a slightly reduced immunogenicity of the FVIII-Fc fusions, as compared to a B-domain deleted FVIII, when they were administered at therapeutic dosages (50 – 100 IU/kg). Infusion of supraphysiological dosages (250 IU/kg), however, resulted in significantly higher inhibitor titres when compared to B-domain deleted FVIII. Additional studies are needed to shed light on the immunogenicity of FVIII-Fc fusions and other bio-engineered FVIII derivatives, including the delivery methods and dosages.

Activation of the immune system by FVIII: looking for the “danger signals”.

Activation of naive CD4+ T cells requires presentation of antigenic peptides by mature DCs in the context of MHC class II molecules and co-stimulatory signals. Although FVIII is endocytosed efficiently, it is still not clear what sometimes prompts the immune system to raise a high titre antibody response against it, especially since it is administered intravenously, which is a normally tolerogenic route, in the absence of any adjuvant. Pfistershammer and colleagues demonstrated that neither FVIII alone, in its native circulating or thrombin-activated from, nor FVIII in complex with VWF, present danger signals to the immune system. Cytokine expression profiles, cellular maturation markers and the ability of FVIII to stimulate autologous or allogenic T cells did not

Figure 2. Modulation of FVIII endocytosis by antigen-presenting cells. A. Interference of FVIII uptake by antigen-presenting cells. C1 domain-directed monoclonal anti-FVIII antibody KM33 as well as the FVIII chaperone molecule, VWF, abolish uptake of FVIII by DCs. Mutations in the C1 domain were shown to diminish FVIII endocytosis by both DCs and macrophages. B. Three-dimensional structure of FVIII highlighting sites important for its recognition by antigen-presenting cells. Left panel provides an overview of the domain organization of the crystallized FVIII protein. The right panel shows a close-up view of the C1 and C2 domains with residues Arg2090, Lys2092 and Phe2093 highlighted in red. Models were based on FVIII crystal structure (PDB code 3cdz) and prepared using PyMol imaging software.
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FVIII exerts its pro-coagulant effect at a critical control point in the coagulation cascade, and coagulation itself has been shown to be a hallmark of sepsis as well as viral hemorrhagic fevers. The administration of anticoagulants, such as recombinant activated protein C, significantly reduces mortality in patients with severe sepsis. Coagulation induces a pro-inflammatory response: thrombin and other coagulation factors can directly activate endothelial cells, platelets and white blood cells via protease-activated receptors (PARs). Moreover, DCs are the primary site in the lymphatic compartment that intersects inflammation and coagulation. PAR1 signalling and cross-talk between PAR1 and sphingosine 1-phosphate (S1P) activates DCs and promotes systemic inflammation. Pro-coagulant activity of FVIII leads to thrombin generation, which can potentially act as a “danger signal” and thus contribute to FVIII immunogenicity. Skupsky and colleagues demonstrated that co-injections of FVIII and OVA caused mice to mount an immune response to the latter, which when injected alone is poorly immunogenic. Furthermore, co-administration of OVA and thrombin was sufficient to provoke an immune response to OVA. Anticoagulant warfarin as well as the direct thrombin inhibitor hirudin also significantly reduced B- and T-cell responses to FVIII. Moreover, heat inactivation of FVIII, which reduces the number of B-cell epitopes but preserves the linear T-cell epitopes, also resulted in reduced immunogenicity of FVIII following infusions into mice. It is interesting to speculate that heat-treated FVIII may also be recognized and therefore endocytosed less efficiently by DCs, the most potent APCs in the primary immune response induction and/or by memory B cells, which mediate recall responses to FVIII after initial antibody development. However, heat treatment (pasteurization) may also increase the immunogenicity of FVIII, as was found when patients were treated with one lot of a heat-pasteurized product. In a recent study, FVIII mutants defective in pro-coagulant activity were tested for their ability to raise an immune response in FVIII- and FVIII/VWF-deficient mice. FVIII R372A/R1689A was inactive due to substitutions at thrombin and factor X proteolytic activation sites, whereas the other variant tested, FVIII V634M, although cleaved by thrombin, displayed less than 1% of the pro-coagulant activity of FVIII wild-type. Various injection protocols demonstrated that FVIII wild-type and V634M were equally immunogenic, independently from FVIII dosages and mouse models used. FVIII R372A/R1689A was slightly less immunogenic than the other two variants tested, however this could be potentially explained by its limited release from VWF. These recent experimental results suggest that the pro-coagulant function of FVIII may not be the major determinant of its immunogenicity.

We anticipate that bystander bacterial or viral challenges can provide co-stimulatory signals to provoke immune responses against FVIII. High-intensity FVIII treatment due to excessive bleeding episodes has also been linked to inhibitor development. Intensive treatment may allow FVIII to compete more efficiently with other antigens for uptake by APCs, resulting in a more efficient...
presentation of FVIII derived peptides to CD4+ T cells. The reported association between intensity of treatment and inhibitor development has influenced clinical risk assessment and decision-making regarding initial FVIII exposures. Many clinicians now consider the potential benefits of beginning early prophylaxis and of delaying FVIII replacement therapy, when possible, in the face of clinical conditions that could potentially lead to activation of the immune system. ⁶⁹

Presentation of FVIII-specific peptides by APCs and subsequent T-cell response

Recently, a plethora of new data have emerged regarding the presentation of FVIII peptides by APCs to T cells, both in humans and mice, using a broad spectrum of tools including mass spectrometry, humanized mouse models and bioinformatics. Activation of T-helper cells is dependent on the proper presentation of antigen-derived peptides on MHC class II molecules expressed on APCs. Van Haren and colleagues ²⁰ explored the repertoire of FVIII peptides presented on MHC class II molecules on human monocyte-derived DCs from four HLA-typed healthy donors using mass spectrometry. Thirty-two core peptides were identified, among which many promiscuous epitopes appeared, whereas others were presented in a donor-specific manner. Interestingly, a number of peptides identified in this study were previously reported as immunodominant T-cell epitopes. ¹³,⁷⁰,⁷¹

Many studies of the FVIII-specific T-cell repertoire have been carried out using mouse models of hemophilia A, e.g. immunodominant T-cell epitopes in FVIII were identified using E16-KO mice. ⁷² Regardless of the route of FVIII administration (subcutaneous or intravenous), T cells recognized peptides corresponding to residues 2191-2220 in the FVIII C2 domain, while none of the C1 domain peptides induced T-cell proliferation. This same FVIII region interacts with VWF and activated phospholipid membranes. More recently, the development of a humanized hemophilic E17 HLA-DRB1*1501 mouse model has been described. ⁷³,⁷⁴ Humanized mice have been utilized to study the regulation of HLA class II-restricted immune responses to various antigens and they are highly suitable for in vivo research into the mechanistic basis of human diseases associated with activation of CD4+ T cells. ⁷⁵ HLA-DRB1*1501 was selected due to a strong connection between this haplotype and many immunologic diseases, as well as a previously noted link between inhibitor incidence and DRB1*1501 in patients with severe hemophilia A. ⁵,¹⁹,⁷⁷ Despite some obvious limitations, such new models can be used to analyze the differences in FVIII-specific T-cell repertoire between mice and men.

Steinitz and colleagues ⁷⁴ recently utilized these humanized hemophilic mice to analyze FVIII peptides presented by HLA-DRB1*1501 (Table 1). Eight different immunodominant regions were identified, and subsequent in vitro binding assays also demonstrated that most of these epitopes were promiscuous. Interestingly, although the application route did not alter the FVIII-specific T-cell repertoire, it did influence the incidence of a neutralizing antibody response to FVIII. A subset of DRB1*1501 mice did not respond to FVIII infusions following intravenous administration, which might have been due to induction of peripheral tolerance.
in these animals. However, when FVIII was co-injected with LPS, all of the mice responded and developed inhibitory antibodies. In the future, this new hemophilia mouse model, complemented with models expressing alternative MHC class II molecules, could serve to unravel the complex mechanisms that induce immune responses or tolerance to infused FVIII.

As an alternative to mass spectrometry or humanized mice models, Moise and colleagues have used bioinformatic tools to predict HLA-DR epitopes in the C2 domain of FVIII. Furthermore, they validated these predictions using HLA binding assays and by immunization studies using various hemophilic mouse models. Reding and co-workers pioneered the analysis of CD4+ T-cell responses in patients with hemophilia A. By using overlapping synthetic peptides, they identified potential CD4+ T-cell epitopes in the A3 and C2 domains of FVIII. More recently, T-cell epitopes in the A2, C1 and C2 domains of mild hemophilia A patients with and without clinically significant inhibitors have been identified by isolation and characterization of T-cell clones and polyclonal lines that recognize peptides containing the wild-type FVIII sequence at the site of the hemophilic missense mutation (at FVIII residues 593, 2150 or 2201). Interestingly, the results of these studies indicate that the wild-type sequence as present in the infused FVIII contains a neo-epitope that arises due to single amino acid mismatch with endogenously expressed FVIII. Based on these observations we hypothesize that inhibitor formation in mild hemophilia A is induced following presentation of a FVIII peptide containing the wild-type (infused) sequence that overlaps the missense site, in a patient with an HLA type that can bind to this peptide. In other words, the neo-epitope occurs at the single region in FVIII to which a mild hemophilia A patient would not have developed immune tolerance through clonal deletion or anergic pathways in utero. The number of potential T-cell epitopes increases significantly in severe hemophilia A, as these patients do not express or circulate a full-length, functional FVIII protein. Accordingly, the inhibitor incidence is higher in severe than in mild or moderate severity hemophilia A. Currently known FVIII-derived peptides that either contain potential T-cell epitopes (due to their presentation by human or humanized mouse model APCs), or that contain T-cell epitopes (confirmed on the basis of human or humanized mouse model T-cell responses) are summarized in Table 1.

A potentially clinically relevant situation analogous to inhibitor development in mild hemophilia A exists, in which infused FVIII may provoke immune responses due to single amino acid differences with the patient’s hemophilic FVIII. Human genome sequencing projects, as well as sequencing studies of the gene encoding FVIII, are identifying an increasing number of non-synonymous single nucleotide polymorphisms in FVIII. It is conceivable that when some hemophilia A patients, including those who produce, and hence are tolerant to, a dysfunctional hemophilic FVIII are infused with therapeutic FVIII, the wild-type sequence at the mismatch site could provoke a T-cell response leading to inhibitor formation. Potential T-cell epitopes must bind effectively to MHC class II receptors in order
for T-cell stimulation to occur. The 32 core FVIII peptides recently shown to bind human MHC class II did not include peptides with amino acids residues (R484H, R776G, D1241E and M2238V) encoded by four recently identified ns-SNPs in the F8 gene. Peptide-MHC binding assays, however, indicate that these regions (if presented as naturally processed peptides in vivo) could bind effectively to several class II receptors. Future studies should reveal whether CD4 T-cell responses arise in some hemophilia A patients as a result of exposure to these mismatched FVIII sequences.

### Table 1. FVIII-derived peptides presented on MHC class II molecules

Peptides have been divided into two categories: (1) reported to be presented by antigen-presenting cells and (2) described as T-cell epitopes. Light and dark bars indicate peptides that meet the criteria for one or two of these categories, respectively. Detailed information regarding HLA binding of individual peptides can be found in papers by van Haren et al., Moise et al. and Steinitz et al.

<table>
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<th>FVIII domains</th>
<th>Residues</th>
<th>Sequence</th>
<th>Presented by APCs</th>
<th>Described T-cell epitope</th>
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<td>C2 (2174-2332)</td>
<td>2191-2220</td>
<td>TASSYTMNFATWPSKARHLHLQGRSNAWR</td>
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<td>14, 20, 72, 78, 81</td>
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<td>2254-2269</td>
<td>SMYVKEFLISQSDG</td>
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This review has discussed recent clinical and basic science investigations that together illustrate the heterogeneity of the CD4+ T-cell repertoire in anti-FVIII immune responses. Promiscuously presented FVIII peptides have so far been identified in the FVIII A2, A3 and C1 domains. In vitro peptide-HLA class II binding studies, however, suggest that additional FVIII-derived derived peptides might also be presented by multiple MHC class II alleles. Current research efforts are being directed towards better understanding the immunologic mechanisms leading to inhibitor development versus immune tolerance to FVIII, and on utilizing this information to improve patient outcomes. For example, it may be possible to design less immunogenic FVIII proteins targeted to patients with higher-risk HLA and hemophilia genotypes by modifying anchor residues crucial for MHC class II binding, in order to abrogate the presentation of immunodominant peptides. However, alteration of multiple residues would likely be required in order to significantly reduce the immunogenicity of FVIII. Successful outcome of this approach is critically dependent on the diversity and hierarchy of CD4+ T-cell responses in hemophilia A patients. We expect that continuing research into the basis of FVIII immunogenicity, and into novel approaches to promote immune tolerance to infused FVIII, will translate eventually into new therapies to improve patient outcomes.
References

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