Molecular determinants of FVIII immunogenicity in hemophilia A
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Summary

Hemophilia A is a hereditary X-linked disorder caused by dysfunction or absence of blood coagulation factor VIII. Depending on the severity of the disease, it can be divided into three categories: mild (5-25% of normal plasma levels of FVIII), moderate (1-5%) and severe (<1%) hemophilia A. Patients with severe hemophilia suffer from spontaneous bleeds in their joints, muscles or other locations without preceding trauma. To restore normal coagulation, so-called replacement therapy comprising regular injections of FVIII of either recombinant or plasma-derived origin is used. However, such treatment is frequently compromised by formation of anti-FVIII antibodies, which can rapidly inhibit FVIII function, rendering FVIII infusion therapy ineffective. Non-inhibitory antibodies can also compromise hemophilia treatment by influencing FVIII stability and/or its pharmacokinetics by interfering with binding to its chaperone protein – von Willebrand factor. This thesis described several studies that aim to increase our understanding of the immunological mechanisms related to the development of anti-FVIII antibodies in patients with hemophilia A.

Chapter 1 provides general background information on FVIII, its structure and function. Moreover, we discuss hemophilia A and major complication compromising hemophilia treatment – the development of FVIII-specific, inhibitory antibodies. We focus on how the immune systems deals with FVIII and go through current animal models used to study molecular mechanism underlying anti-FVIII antibody formation. We also review methods applied to induce tolerance to FVIII in animal models for hemophilia A.

In Chapter 2 we summarize recent knowledge on FVIII processing and presentation by antigen-presenting cells (APCs), as well as the diversity of the FVIII-specific T-cell repertoire in mice and humans. Moreover, we discuss possible molecular determinants for FVIII immunogenicity.

In Chapter 3 we study the involvement of candidate receptors in FVIII uptake by dendritic cells (DCs). Furthermore, we explore FVIII residues that mediate endocytosis. We show that upon treatment of DCs with mannan or LRP ligand – receptor associated protein (RAP), only a minor decrease in FVIII internalization is observed. In addition, small interfering RNA–mediated knockdown of LRP, mannose receptor, or DC-SIGN does not prevent FVIII uptake. Binding studies using Fc chimeras reveal that LRP, DC-SIGN and mannose receptor can bind to FVIII; however, no major role for these receptors in FVIII uptake is observed. These findings suggest that FVIII is internalized by an as yet unidentified receptor present on dendritic cells. Pre-incubation of FVIII with A2 domain-targeting monoclonal antibody VK34 does not influence FVIII uptake; however, KM33, recognizing the C1 domain, completely inhibits FVIII endocytosis by both human and murine dendritic cells. Accordingly, anti-FVIII antibody titers were greatly reduced following the pre-administration of KM33 in vivo. This observation reveals that the C1 domain contains a molecular determinant that contributes to the immunogenicity of FVIII.
Chapter 4 follows up on findings described in chapter 3. Here, we create a C1 domain variant (FVIII-R2090A/K2092A/F2093A), which shows only minimal binding to KM33 and retains its chromogenic activity. FVIII-R2090A/K2092A/F2093A displays a strongly reduced internalization by human and mouse dendritic cells, as well as human macrophages. Moreover, we show that mice treated with FVIII-R2090A/K2092A/F2093A have significantly lower anti-FVIII antibody titers and FVIII-specific CD4\(^+\) T cell responses when compared to mice treated with the wild-type FVIII. Together, our observations emphasized the physiological significance of KM33-targeted residues within the C1 domain in the uptake of FVIII by DCs \textit{in vitro} and \textit{in vivo}. Furthermore, we hypothesize that FVIII variants displaying a reduced uptake by antigen-presenting cells provide a novel therapeutic approach to reduce inhibitor development in hemophilia A.

In Chapter 5 we study endocytosis of FVIII-containing immune complexes by APCs. We show that bone marrow-derived murine DCs are able to efficiently take up FVIII pre-complexed with anti-FVIII antibodies (FVIII-IC) in a dose-dependent manner. Moreover, endocytosis of FVIII-IC was 3-6 fold more efficient when compared to equimolar concentrations of soluble FVIII. Uptake of FVIII-IC, but not FVIII alone, could be inhibited with 2.4G2 antibody indicating functional involvement of Fc\( \gamma \)RII/III in this process. These results were confirmed using murine DCs isolated from Fc\( \gamma \)R-deficient mice. Furthermore, enhanced endocytosis of FVIII-IC led to stronger FVIII-specific T cell proliferation as compared to soluble FVIII. Collectively, these data provide further insight into modulation of FVIII endocytosis and subsequent T cell responses in presence of anti-FVIII antibodies.

In Chapter 6 we investigate the detailed (intra)cellular mechanism of FVIII endocytosis. We show that this process is dependent on phosphoinositide-3 kinase and cytoskeleton reorganization. Uptake of FVIII was also significantly inhibited by dextran sulphate, a negatively charged polymer and dimethyl amiloride, an inhibitor of macropinocytosis. Employment of FVIII-targeting monoclonal antibodies further emphasized important role for the C1 domain in this process, but also suggested a potential modulating role for the C2 domain.

Chapter 7 summarizes findings from all other chapters and the results are discussed in the light of current knowledge and studies performed by other investigators.