Molecular determinants of FVIII immunogenicity in hemophilia A
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Reply to: Mannose-sensitive receptors mediate the uptake of factor VIII therapeutics by human dendritic cells

Eszter Herczenik, Simon D. van Haren, Aleksandra Wroblewska, Paul Kaijen, Maartje van den Biggelaar, Alexander B. Meijer, Luisa Martinez-Pomares, Anja ten Brinke and Jan Voorberg

E.H., S.H. & A.W. contributed equally to this work

Correspondence: Mannose-sensitive receptors mediate the uptake of factor VIII therapeutics by human dendritic cells

Yohann Repessé, Suryasarathi Dasgupta, Ana-Maria Navarrete, Sandrine Delignat, Srinivas V. Kaveri, Sébastien Lacroix-Desmazes

To the Editor: Administration of factor VIII (FVIII) to manage bleedings in patients with hemophilia A induces inhibitory anti-FVIII antibodies in a substantial number of patients. The initial step in the development of the naive anti-FVIII immune response is the uptake of the exogenously administered FVIII by dendritic cells (DCs), the professional subset of antigen-presenting cells. The interesting study by Herczenik et al. suggests the implication of a variety of receptors that mediate the internalization of FVIII by DCs.

Here, we wish to emphasize the importance of mannose-bearing residues on different commercially available FVIII therapeutics in endocytosis through mannose-sensitive receptors by human DCs in vitro. We first found that kinetics (data not shown) and dose dependency of FVIII endocytosis by immature DCs were similar whether FVIII was directly labeled or was detected in permeabilized cells by a labeled antibody (Figure 1A). We then compared the endocytosis of different FVIII therapeutics by DCs: 2 recombinant full-length FVIII (Kogenate and Advate) produced in baby hamster kidney or Chine hamster ovary cells, respectively, a recombinant B domain–deleted FVIII (Refacto) produced in Chine hamster ovary cells, and a plasma-derived FVIII (Factane). Kogenate and Advate were endocytosed with identical kinetics and to a greater extent than Refacto and Factane (Figure 1B). The reduced endocytosis of FVIII in the case of the plasma-derived product in our experimental setup is probably due to the presence of von Willebrand factor, as reported earlier. We then confirmed that the endocytosis of the 4 FVIII products results in FVIII presentation to CD4+ T lymphocytes. DCs were incubated with 5 IU/ml of FVIII (a concentration close to that reached in vivo following replacement therapy) in the presence of D9E9, a human FVIII C1 domain–specific T-cell line. All tested FVIII therapeutics induced the secretion of IFN-γ by D9E9 (Figure 1C), demonstrating similar endocytic and intracellular processing of the different products. Furthermore, the incubation of the 4 FVIII therapeutics with DCs in the presence of mannan was accompanied by a reduction in the amount of endocytosed FVIII (reduction of 56% ± 15%, 48% ± 24%, 39% ± 19%, and 76% ± 1% for Kogenate, Advate, Refacto, and Factane, respectively; Figure 1D). The reduction of endocytosis of both Kogenate and Refacto by mannan had been shown to reduce D9E9 activation. Our results indicate that the contribution of mannose-ending sugars to the endocytosis of FVIII by DCs does not differ significantly depending on the plasmatic or recombinant origin of FVIII, or on the presence or absence of the B domain in the recombinant molecule. Interestingly, mannose-sensitive receptors expressed by human monocyte–derived immature DCs are also expressed by splenic and circulating myeloid DCs (data not shown). In the work by Herczenik et al. the reduction of FVIII uptake by DCs by the KM33 IgG was associated with a reduced
immunogenicity of FVIII in vivo. It is not clear whether this effect was due to the quenching of phosphatidylserine–binding moieties of FVIII and due to an ensuing reduction of FVIII aggregation, \(^6\) due to steric hindrance following the binding of the IgG to FVIII, or due to the blocking of a specific, yet unidentified, receptor for FVIII. Together with our observations, the data suggest that the generation of a novel FVIII therapeutic compromised in its capability to interact with DCs upon the removal of selected N-glycosylation sites may be a novel strategy to produce a hemostatically proficient drug with potentially reduced immunogenicity in patients with hemophilia A.

Figure 1. Endocytosis of different commercially available FVIII products by human DCs implicates mannose-sensitive receptors. A. Dose-dependent labeling of DCs following incubation with FVIII. Human monocyte-derived immature DCs were incubated in X-VIVO 15 with FVIII or FITC-labeled FVIII (Kogenate) for 120 minutes at 4 and 37°C, respectively. \(^2\) In the case of nonlabeled FVIII, cells were permeabilized by using 0.1% saponin and FVIII was detected by using the monoclonal anti-FVIII IgG, mAb 77IP52H7 (10 μg/mL), coupled to fluorescein isothiocyanate (FITC). The percentage of labeling at 4°C was subtracted from that measured at 37°C to quantify FVIII endocytosis. B. Dose-dependent endocytosis of therapeutic FVIII concentrates by DCs. C. Activation of the FVIII-specific human CD4\(^+\) T-cell clone D9E9. DCs were generated from healthy blood donors with the MHC II haplotype DRB1*1501/DRB5*01, which matches D9E9. DCs were incubated with D9E9 in the medium alone or with 5 IU/ml of different FVIII preparations, for 20 hours at 37°C. The activation of D9E9 was assessed by measuring IFN-γ in culture supernatants. The amount of IFN-γ produced in the presence of Kogenate was considered as 100%. D. Inhibition of FVIII endocytosis by mannan. DCs were incubated alone or in the presence of mannan (1 mg/ml) for 30 minutes at 37°C before the addition of FVIII for 2 hours at 37°C. Results represent the percentage of inhibition of FVIII endocytosis in the presence of mannan using the condition without mannan as the reference. Error bars indicate SD for 2 to 4 independent experiments.
We thank Joelle Treton (INSERM U662, Hospital Saint-Louis, Paris, France) for providing us with blood from healthy MHC-matched donors. mAb 77IP52H7 and Factane were gifts from LFB. D9E9 and BO2C11 were gifts from J. M. Saint-Remy and M. Jacquemin (CMVB, KUL, Belgium). Kogenate, Helixate, Advate and Refacto were kind gifts from Bayer Healthcare, CSL-Behring, Baxter Bioscience, and Pfizer-Wyeth, respectively.

References


To the Editor

Eszter Herczenik, Simon D. van Haren, Aleksandra Wroblewska, Paul Kaijen, Maartje van den Biggelaar, Alexander B. Meijer, Anja ten Brinke, Jan Voorberg

We first would like to thank Yohann Repessé and his coworkers\(^1\) for their valuable comments regarding our recent article on the uptake of blood coagulation factor VIII (FVIII) by dendritic cells (DCs).\(^2\) In this report, we provided evidence that mannose-sensitive receptors do not play a prominent role in the uptake of FVIII by DCs. In contrast to the recent work of Repessé and coworkers\(^3\) only a very small reduction in FVIII endocytosis was observed on preincubation with mannan.\(^2\) Moreover, siRNA mediated knockdown of the macrophage mannose receptor (CD206) did not influence the endocytosis of FVIII. Conversely, Repessé and coworkers demonstrate that the endocytosis of various therapeutic FVIII preparations such as Kogenate, Advate, Refacto and Factane by DCs is reduced in the presence of mannan.\(^1\) To further address the role of mannose-ending glycans in the contribution to FVIII endocytosis by DCs, we performed experiments by using patient-derived C2 domain–specific T-cell clone 32A-18\(^4,5\) and HLA-matched DCs. Prior to the addition of 100 nM of FVIII for 1 hour, we pretreated the HLA-matched DCs for 15 minutes with mannan. Subsequently, the DCs were combined

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**Figure 1. Proliferation of a FVIII C2 domain–specific CD4\(^+\) T-cell clone is mannan insensitive.** A. Monocytes were isolated from a matched HLA DRB1\(^*\)0101/DRB1\(^*\)1301 and cultured with 1000 U/ml of GM-CSF and 800 U/ml of IL-4 for 4-7 days as described.\(^1\) DCs were first pretreated for 15 minutes with 1 mg/ml of mannan and incubated with 100 nM of FVIII for 1 hour to allow endocytosis. Then the cells were washed twice, resuspended at a concentration of 5×10\(^4\)/100 μl, and combined with 5×10\(^4\)/100 μl of carboxyfluorescein succinimidyl ester–labeled CD4\(^+\) T-cell clone 32A-18, which was derived from a DRB1\(^*\)0101-positive hemophilia A patient.\(^2,3\) After 6 days of culture, the mean fluorescent intensity of the resuspended CD4\(^+\) T cells was measured and the data were analyzed with FlowJo 7.6.4 software (Tree Inc, Ashland, Ore). B. The glycosylation site N2118 in the C1 domain remains distant from the KM33 epitope that includes residues K2092 and F2093. The carbohydrate structure depicted is a core-fucosylated high-mannose structure that was modeled by using the glycam Webserver (http://glycam.crcr.uga.edu/ccrc/). The image was generated by using the PyMOL Molecular Graphics System, Version 1.3, Schrödinger, LLC (New York, NY). FITC, Fluorescein isothiocyanate.
with carboxyfluorescein succinimidyl ester–labeled FVIII-specific T cells. After 6 days, we observed no decrease in fluorescent intensity in the samples in which T cells were added to untreated DCs or DCs treated only with mannan (Figure 1A, upper left and right panels). When DCs were preincubated with FVIII, the fluorescence of the CD4+ T cells decreased (Figure 1A, bottom left panel), which indicates that the majority of the CD4+ T cells proliferated in response to FVIII (Figure 1A). Similarly, we detected comparable CD4+ T-cell responses in the sample that contained DCs that received mannan prior to the addition of FVIII (Figure 1A, bottom right panel). These observations suggest that mannan did not prevent FVIII endocytosis and subsequent peptide presentation to FVIII-specific CD4+ T cells by DCs. We have shown earlier that mAb KM33 prevents the endocytosis of FVIII by DCs and associates with a reduced immunogenicity of FVIII in vivo. 2 We have also demonstrated that KM33 does not block the in vitro binding of FVIII to the mannose receptor. 2 These observations strongly suggest that the mannose receptor does not play a major role in FVIII endocytosis by DCs. We have previously indicated that residues K2092 and F2093 contribute to the binding of KM33 to FVIII (Figure 1B). 6 Mannose-ending glycans are present at N239 and N2118 of FVIII. 7 Analysis of the 3-dimensional structure of FVIII reveals that N2118 is not located in proximity to residues K2092 and F2093 (Figure 1B). We therefore propose that mannan-sensitive receptors do not play a major role in the endocytosis of FVIII by DCs. We are currently addressing which mannan-insensitive mechanisms contribute to the uptake of FVIII by DCs.

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References


