

Correspondence

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

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.¹ 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 labeled² or was detected in permeabilized cells by a labeled antibody¹ (Fig 1, A). 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 Chinese hamster ovary cells, respectively, a recombinant B domain-deleted FVIII (Refacto) produced in Chinese 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 (Fig 1, B). 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.³ 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.⁴ All tested FVIII therapeutics induced the secretion of IFN- γ by D9E9 (Fig 1, C), 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% $\pm$ 15%, 48% $\pm$ 24%, 39% $\pm$ 19%, and 76% $\pm$ 1% for Kogenate, Advate, Refacto, and Factane, respectively; Fig 1, D). The reduction of endocytosis of both Kogenate and Refacto by mannan had been shown to reduce D9E9 activation.²

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 DCs⁵ are also expressed by splenic and circulating myeloid DCs (data not shown). In the work by Herczenik et al.,¹ 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 phosphatidyl-serine-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.

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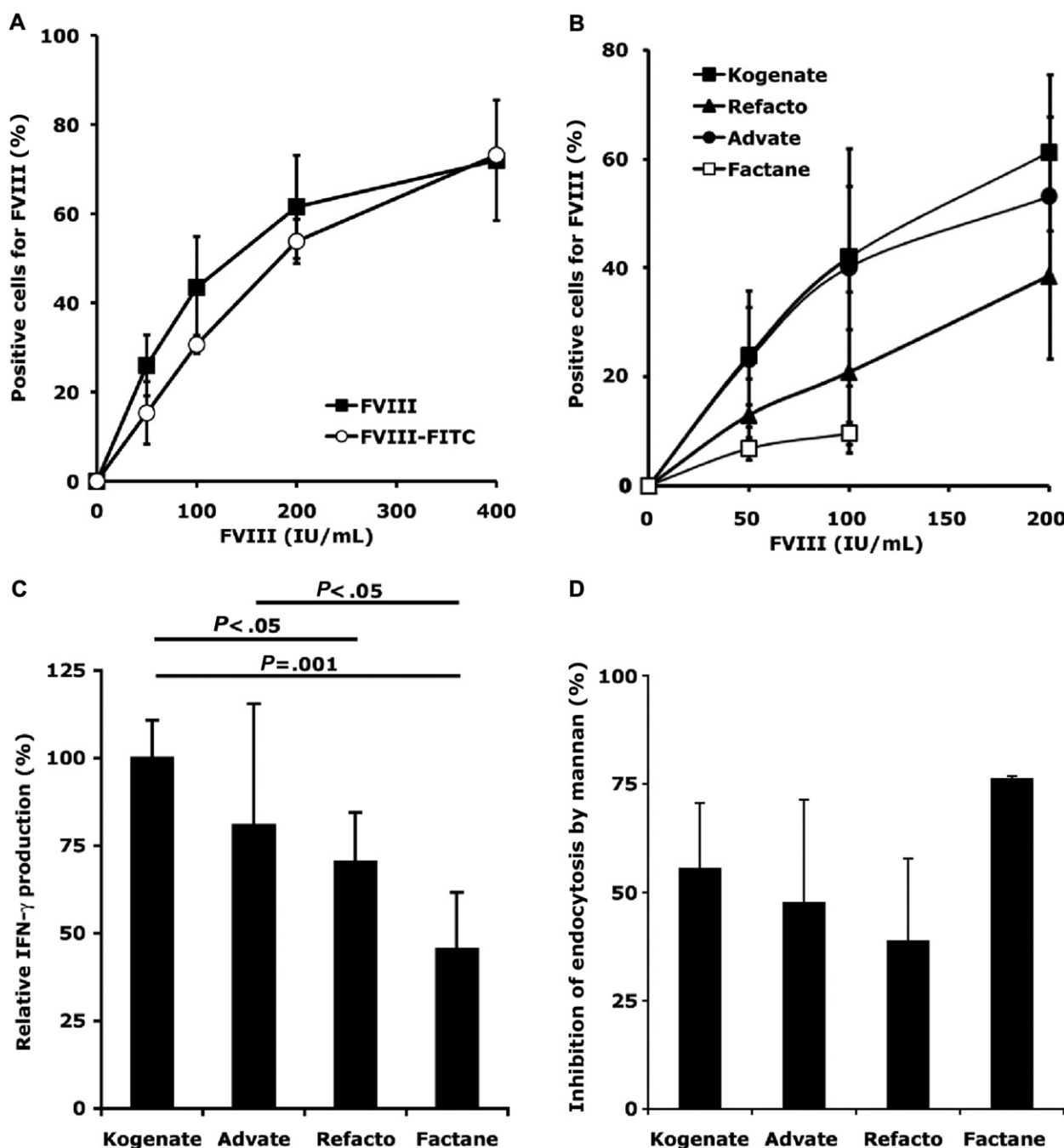


FIG 1. Endocytosis of different commercially available FVIII products by human DCs implicates mannose-sensitive receptors. **A**, Dose-dependent labeling of DCs following incubation with FVIII. Human monocyte-derived immature DCs were incubated in X-VIVO¹⁵ with FVIII or FITC-labeled FVIII (Kogenate) 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.