

Correspondence

Reply

To the Editor:

We first would like to thank Yohann Repessé and his co-workers¹ 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 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 (Fig 1, A, upper left and right panels). When DCs were preincubated with FVIII, the fluorescence of the CD4⁺ T cells decreased (Fig 1, A, bottom left panel), which indicates that the majority of the CD4⁺ T cells proliferated in response to FVIII (Fig 1, A). Similarly, we detected comparable CD4⁺ T-cell responses in the sample that contained DCs that received mannan prior to the addition of FVIII (Fig 1, A, bottom right panel). These observations suggest that mannann 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 FVIII⁶ (Fig 1, B). 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 (Fig 1, B). We therefore propose that mannann-sensitive receptors do not play a major role in the endocytosis of FVIII by DCs. We are currently addressing which mannann-insensitive mechanisms contribute to the uptake of FVIII by DCs.

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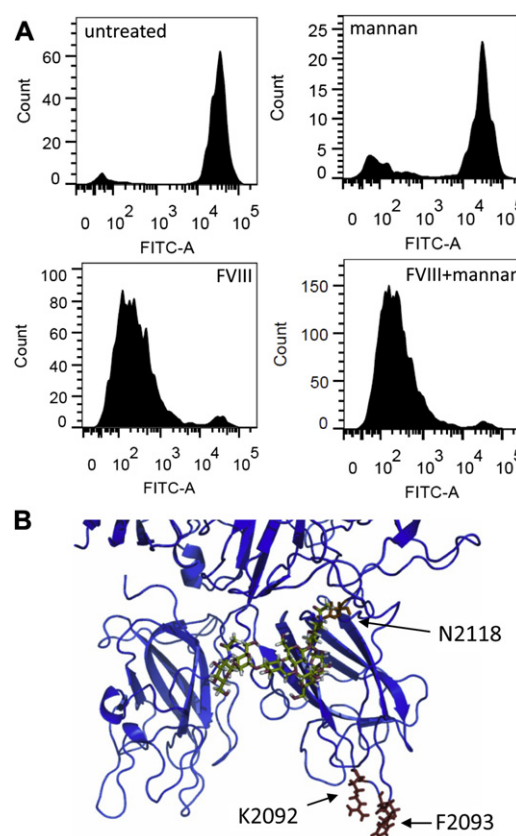


FIG 1. Proliferation of a FVIII C2 domain-specific CD4⁺ T-cell clone is mannan insensitive. **A**, Monocytes were isolated from a matched HLA DRB1*0101/DRB1*1301 and cultured with 1000 U/mL of GM-CSF and 800 U/mL of IL-4 for 4-7 days as described.¹ DCs were first pretreated for 15 minutes with 1 mg/mL 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 $5 \times 10^3/100 \mu\text{L}$, and combined with $5 \times 10^4/100 \mu\text{L}$ 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 was 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.ccruc.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.

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