A Novel Coagulation Factor VIII with Reduced Immunogenicity

Karina Winterling[1], William Martin[2], Anne S. De Groot[2], Jens Daufenbach[1], Steffen Kistner[1]

[1] Biotest AG, Dreieich, Germany; [2] EpiVax, Inc., Providence, USA

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INTRODUCTION

Up to 30 % of Hemophilia A patients develop inhibitory antibodies against the therapeutic coagulation Factor VIII (FVIII). The development of a deimmunized FVIII is thus an unmet high medical need. Although improved recombinant FVIII (rFVIII) products evolved within the last years, the immunogenicity of rFVIII has not been solved . A deimmunized FVIII could reduce the probability of inhibitor development, providing a safer therapy and preventing immune tolerance induction therapies. Thus, we aimed for the development of a deimmunized FVIII molecule by modifying MHC Class I presentation, leading to a functional, but less immunogenic molecule.

MATERIALS & METHODS

Using Epivax' *in silico* tools, the B-Domain-truncated FVIII sequence (unmodified FVIII) was analyzed and FVIII-unique peptides predicted to bind with high affinity to the MHC class II were identified. Amino acid substitutions were incorporated in several screening rounds in order to reduce MHC II presentation without altering FVIII regions important for activity, folding and binding. Testing for functional and structural similarity of the deimmunized rFVIII to unmodified control FVIII and approved products was performed. Reduced immunogenicity was confirmed by an *in vitro* DC-T cell-assay with cells derived from healthy donors.

CONCLUSION

Based on *in silico* predictions a deimmunized FVIII comprising 19 amino acid substitutions was developed. This FVIII-19M is fully active and was shown to be less immunogenic compared with an unmodified FVIII in an *in vitro* DC-T cell-assay. Thus, this molecule provides the potential to reduce the risk of inhibitor development in hemophilia A patients, especially preventing the burden of immune tolerance inductions.

DEIMMUNIZATION OF FVIII

The incorporation of 57 amino acid substitutions was experimentally tested, starting with single amino acid substitutions, followed by combination in clusters, finally leading to a combination of all functional substitutions (Fig. 1). The main readout was functionality of the FVIII variants.

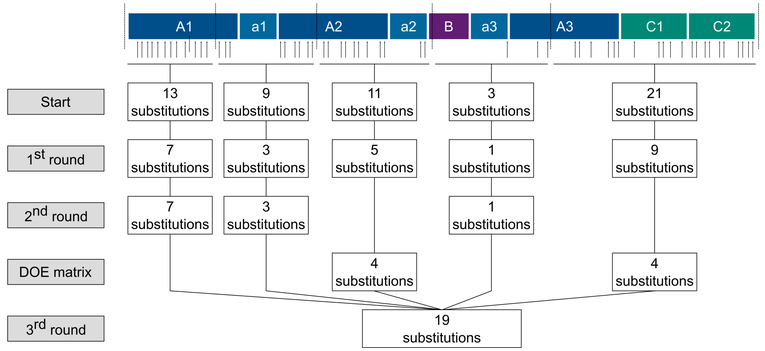


Fig. 1 Deimmunization of FVIII. The arrows indicate the position of the 57 single substitutions. The substitutions are clustered in regions to facilitate the experiments. The remaining substitutions of each screening round are indicated. Finally, 19 substitutions were incorporated throughout the FVIII sequence.

The calculated immunogenicity score of FVIII-19M is -10.55 compared with a score of 7.01 for the unmodified FVIII (Fig. 2). A negative immunogenicity score corresponds to a lower amount of immunogenic epitopes compared with a protein with a random sequence. This indicates that FVIII-19M would be better tolerated by the immune system than the unmodified FVIII.

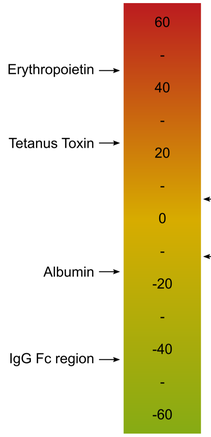
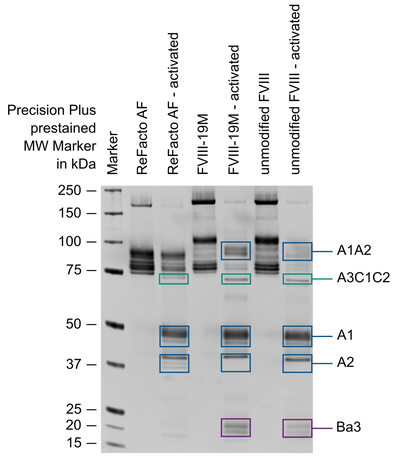


Fig. 2 Immunogenicity scale. The score indicates the calculated immunogenicity of a protein in relation to a protein with a randomized sequence whose score is set to 0.

CHARACTERIZATION OF DEIMMUNIZED FVIII



FVIII-19M revealed the expected cleavage pattern of an activated FVIII, indicating that the deimmunization does not interfere with the activity of the molecule. This supports the results from the activity assays performed during the deimmunization. The high amount of single chain (170 kDa) is due to the production cell line and does not affect the activity.

Fig. 3 FVIII products activated by thrombin. Each product was applied in its non-activated and activated form. In the non-activated form the typical bands for the single chain, heavy chain and light chain were detectable. After thrombin cleavage additional bands for FVIII A1, A2, A1A2, Ba3 and A3C1C2 domains were detectable.

For the immunogenicity test, DCs and $CD4^+$ T cells were co-cultivated in the presence of either FVIII-19M or the unmodified FVIII. The T cell fractions were depleted of CD4+CD25+ regulatory T cells, in order to use cells from healthy donors. Proliferation of the T cells was analyzed and was found to be reduced when FVIII-19M was applied compared with the unmodified FVIII.

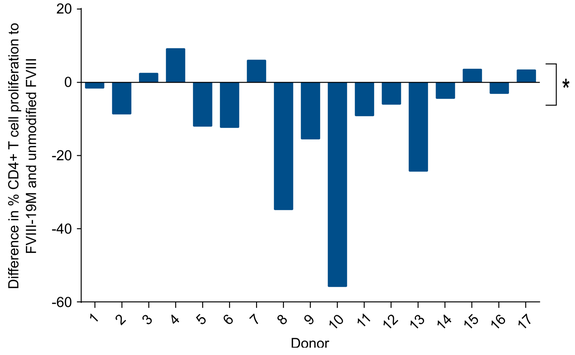


Fig. 4 Difference between CD4+ T cell proliferation against DCs stimulated with IL-Mix plus FVIII-19M and DCs stimulated with IL-Mix plus unmodified FVIII. The bars below 0 indicate a reduced CD4+ T cell response to FVIII-19M. The lower CD4+ T cell response to FVIII-19M compared with unmodified FVIII is significant using the Wilcoxon test ($p = 0.027$).