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The design of factor VIII (FVIII) molecules with increased specific cofactor activity and half life may help to improve protein replacement therapy as well as gene therapy for hemophilia A. Because FVIII is a neoantigen for the majority of hemophilia patients, modifications of the molecule may not necessarily increase the risk of inhibitor formation. We have previously used canine FVIII for preclinical gene transfer studies. By comparing human and canine FVIII, we demonstrated that canine FVIII has an approximately five-fold higher specific cofactor activity. Characterisation of the structural basis for this difference will be exploited to design human FVIII molecules with increased cofactor activity.

With the exception of the B domain, human and canine FVIII are highly homologous. Therefore, exchange of domains and smaller regions is likely to produce functionally active human/canine hybrid molecules. Site-directed mutagenesis will be used to characterize individual residues

Molecular engineering of coagulation factor VIII

important for functional differences between human and canine FVIII. The resulting human/canine hybrid constructs will be expressed in cell culture and partially purified using ion exchange chromatography. FVIII activity assays will be used together with FVIII-specific enzyme-linked immunosorbent assay to determine the specific activity of hybrid molecules. Recombinant protein and gene transfer into the mouse FVIII/- hemophilia A model will be used to characterize modified FVIII molecules in vivo.

It is expected that these studies will help to define a molecular basis for the increased activity of canine FVIII. The final goal is to construct minimally modified human FVIII molecules with a significantly higher cofactor activity compared to wild-type human FVIII.

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These studies will help to define a molecular basis for the increased activity of canine FVIII over human FVIII

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