



Dr  
**Rainer  
Schwaab**

In patients with severe hemophilia A the most significant complication of factor VIII (FVIII) replacement therapy is the occurrence of FVIII inhibitory antibodies. These antibodies are of polyclonal origin and bind to different sites (epitopes) of FVIII. The inhibitor epitopes occurring most frequently in patients are thought to lie on domains A2, A3 and C2 (Fijnvandraat et al., 2003), and great efforts have been made to characterize their precise location. Information of this kind is crucial for the rational development of therapies that can avoid or overcome the immune response to FVIII.

To date, most epitope locations have been determined by binding studies using murine or human monoclonal antibodies with peptides generated by phage display or synthesized chemically. Our approach differs from these methods. We will first determine the accurate location of an epitope directly from an immune complex of FVIII with an antibody using the epitope

## Design and validation of a mimotope for blocking a FVIII-inhibitor antibody

excision method. In this method, FVIII bound to the antibody is subjected to limited proteolysis, and the epitope, protected from digestion by the bound antibody, is then dissociated from the latter and identified using mass spectrometry. For method development we plan to use the murine monoclonal antibody ESH8 as a model. ESH8 is relatively well characterized and binds to a region on the C2-domain (Villard et al., 2002; Scandella et al., 1995). This region is of particular clinical interest because it is a major target of FVIII-antibodies in patients.

We will then try to design a mimotope, i.e. a peptide that mimicks the epitope and thus blocks the FVIII-binding site of a FVIII-antibody. In contrast to ongoing studies, we will use a new computational (in silico) method based on evolutionary optimization algorithms to find candidate peptides likely to bind to the FVIII-antibody. As input for our algorithms we will use the X-ray structure of the C2-domain (Pratt et al., 1999) and the epitope determined experimentally by epitope excision. The most promising candidates will be synthesized and their capability to bind ESH8 in presence of factor VIII will be tested. The best peptides will be further optimized in silico and theoretical results will be again tested in vitro. After several rounds of in silico optimization and in vitro testing we expect to have generated a mimotope that binds ESH8 with high affinity. Finally, this mimotope will be tested against human antibodies suspected to target the same region of the C2-domain.

Two groups will collaborate in the proposed project to cover all the required expertise. The groups of Daniel Hoffmann and Martin Zabe-Kühn of the Center of Advanced European Studies and Research (Caesar) will collaborate with the group of Rainer Schwaab at the Institute of Experimental Hematology and Transfusion Medicine. Both groups are located in Bonn, Germany.

“

*Information on the location of inhibitor epitopes is crucial for the rational development of therapies that can avoid or overcome the immune response to FVIII*

”

Institute for Experimental Hematology and Transfusion Medicine  
Germany