Selection and characterization of single-chain variable antibody fragments (scFvs) specific for anti-FVIII antibodies

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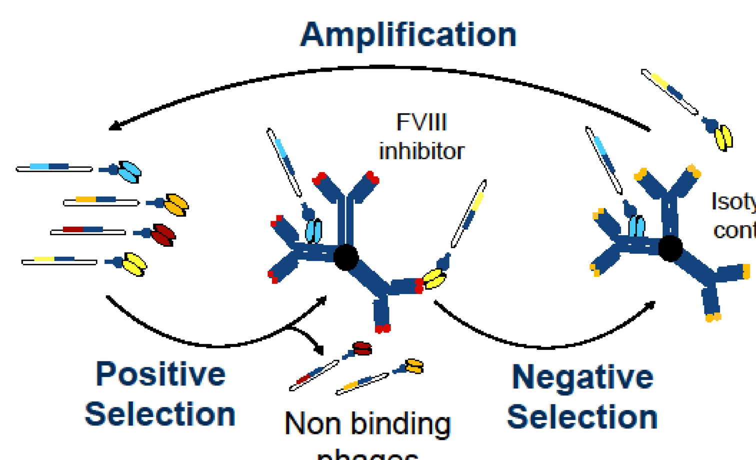
Introduction

The development of inhibitory allo-antibodies against coagulation factor VIII (FVIII) is currently the most serious complication for hemophilia A patients, who undergo FVIII replacement therapy. Non-hemophiliacs can also spontaneously develop inhibitory auto-antibodies to FVIII, which results in a condition called acquired hemophilia A. One mechanism to counteract the allo- or autoimmune response to FVIII seems to involve the development of anti-idiotypic antibodies in the affected patients (1).

The aim of this project is to elucidate the capacity of single chain variable antibody fragments (scFvs) for neutralization of inhibitory anti-FVIII antibodies (FVIII inhibitors). Phage display technology was applied to select scFvs for strongly inhibitory murine monoclonal anti-FVIII antibodies (mAbs) from synthetic libraries. As the majority of inhibitory mAbs are directed against the A2 or C2 domain of FVIII, anti-A2 and anti-C2 mAbs were used as targets. Affinity selection led to the identification of several specific, potential anti-idiotypic scFvs, which were further characterized.

Isolation of FVIII inhibitor-specific scFvs



Schematic representation of the selection of scFvs from synthetic phage displayed libraries. ScFvs were selected for an anti-A2 or an anti-C2 mAb (positive selection). Bound phages subsequently underwent negative selection on the respective isotype control. After three rounds of selections individual phage clones were analyzed for binding to the FVIII inhibitors.

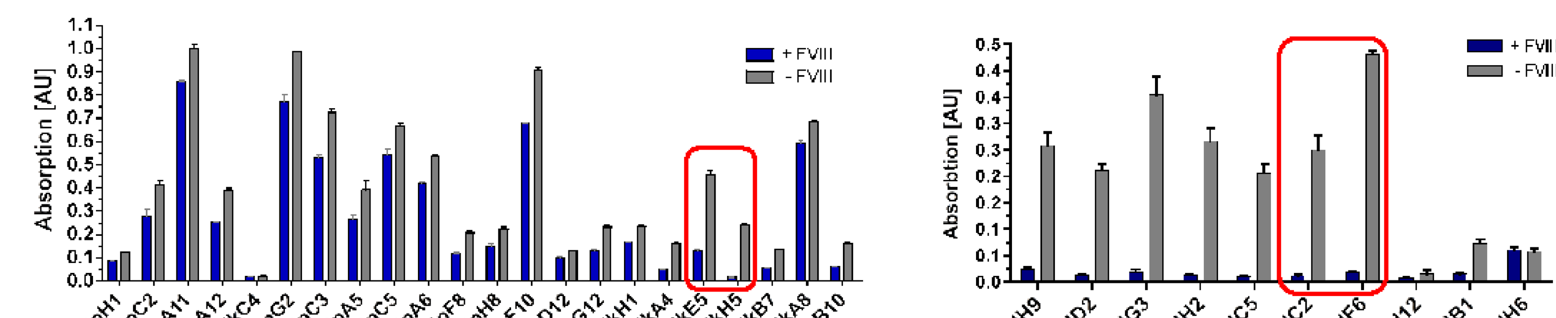
Identification of scFvs specific for FVIII inhibitors

Affinity selection for an anti-A2 and an anti-C2 mAb resulted in the isolation of several scFvs, which bound to the respective mAb but not the isotype control.

Anti-A2 or anti-C2 mAb and the respective isotype control were coated onto ELISA plates. Plates were blocked and subsequently incubated with phages overnight at 4 C. Binding of phages was detected using HRP-conjugated anti-M13 antibody and absorption was measured at 492 nm and 620 nm (reference).

FVIII and scFvs compete for binding to FVIII inhibitors

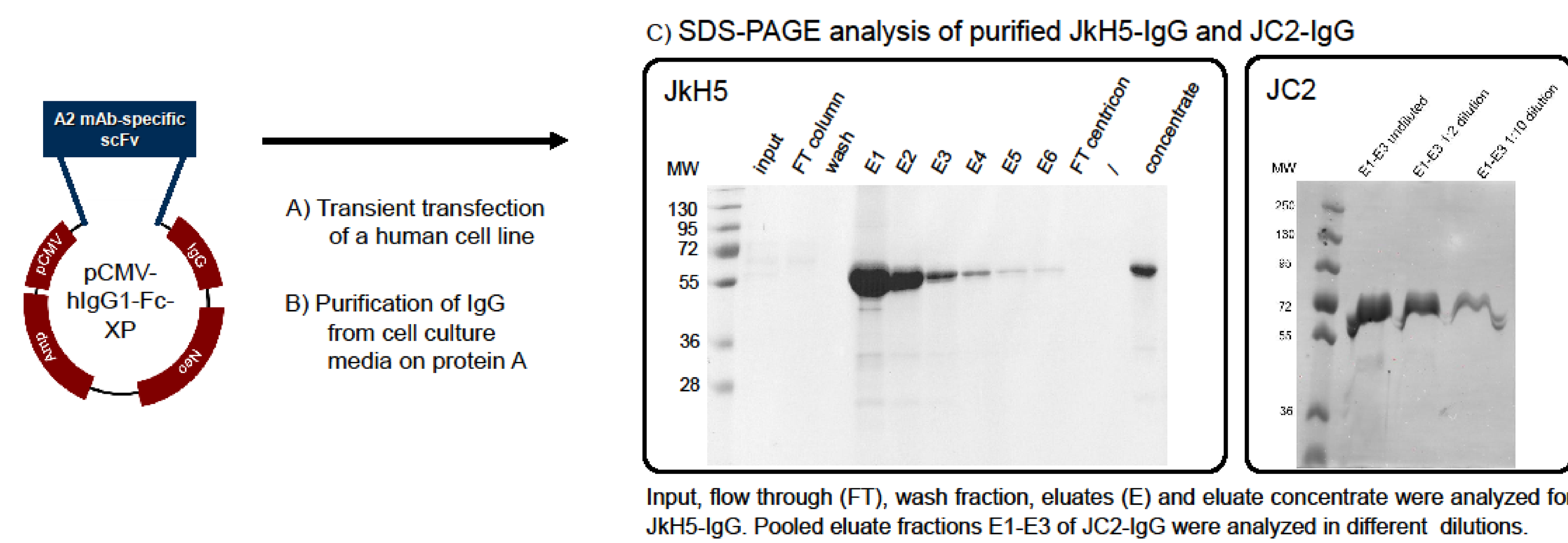
Binding of several scFvs to anti-A2 or anti-C2 mAb was strongly reduced in the presence of FVIII, suggesting that the binding site is part of the mAb paratope. Four unique scFvs (red boxes) were selected for further characterization as scFv-IgG.



Anti-A2 or anti-C2 mAb was immobilized on plates. Wells were preincubated with FVIII or buffer for 30 min at 37 C. Phages were added for 2 h at 37 C. Binding of phages was detected with HRP-conjugated anti-M13 antibody and absorption was measured at 492 nm and 620 nm (reference).

Cloning and production of scFv-IgG proteins

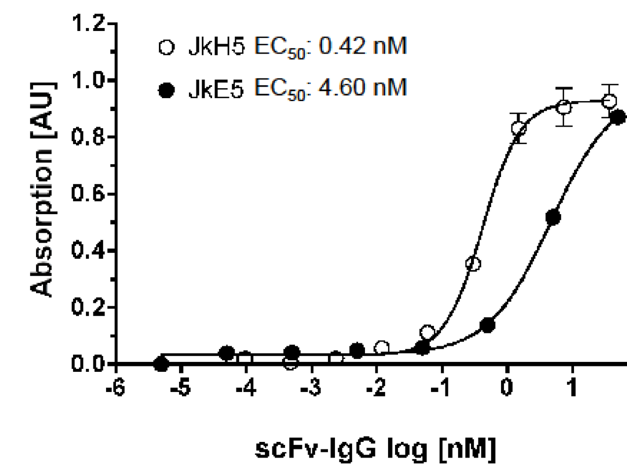
ScFvs were fused to the Fc region of human IgG1, transiently expressed in a human cell line, and purified by protein A chromatography (2).



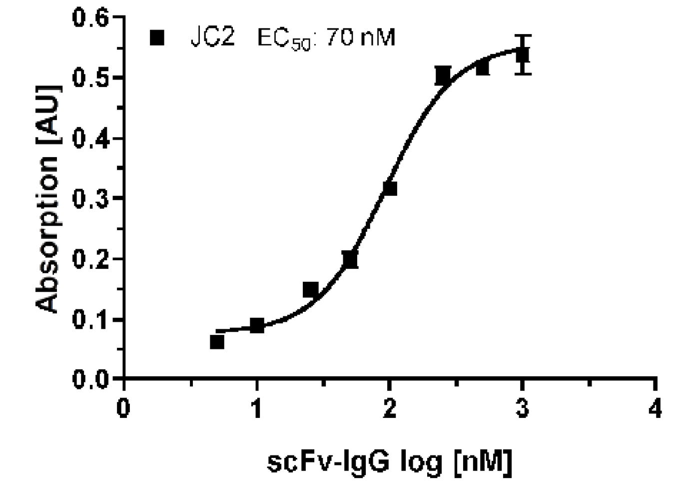
Input, flow through (FT), wash fraction, eluates (E) and eluate concentrate were analyzed for JkH5-IgG. Pooled eluate fractions E1-E3 of JC2-IgG were analyzed in different dilutions.

Affinity of scFv-IgG proteins

The affinity of JkH5-IgG for the anti-A2 mAb is ten times higher than the affinity of JkE5-IgG.



JC2-IgG binds to the anti-C2 mAb in the nanomolar range.

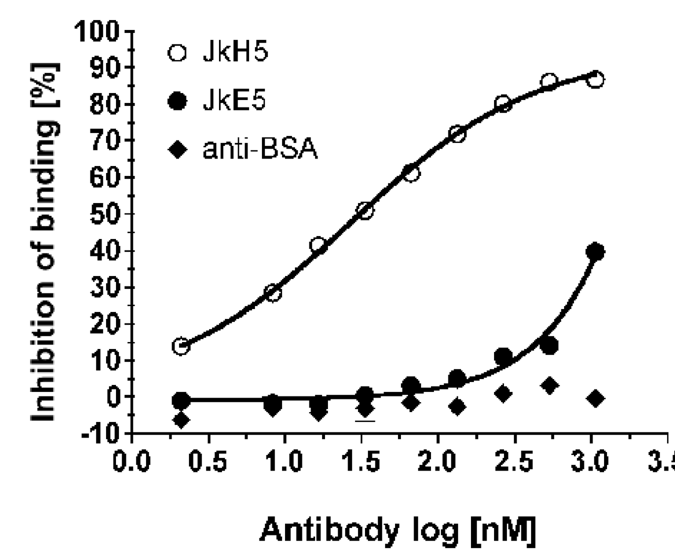


Anti-A2 or anti-C2 mAb was immobilized on plates. Wells were incubated with defined concentrations of mAb-specific scFv-IgGs for 2 h at room temperature. Binding of antibodies was detected with HRP-conjugated anti-human IgG antibody and absorption was measured at 492 nm and 620 nm (reference).

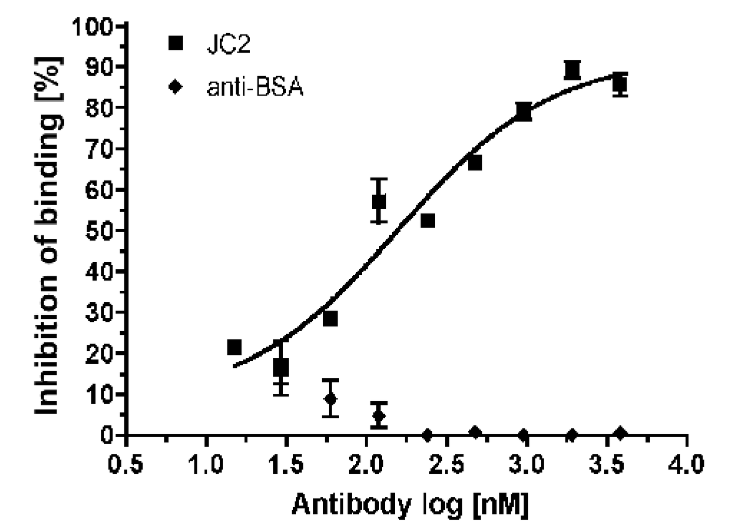
ScFv-IgGs compete with FVIII inhibitors for FVIII binding

Binding of anti-FVIII mAbs to FVIII is inhibited by scFv-IgGs in a concentration-dependent manner.

JkH5-IgG inhibits binding of anti-A2 mAb to FVIII with an EC_{50} of ~ 30 nM.



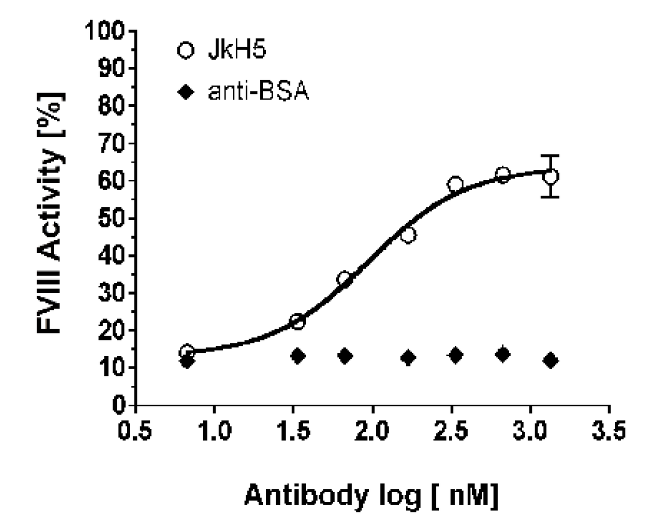
JC2-IgG blocks binding of anti-C2 mAb to FVIII with an EC_{50} of ~ 160 nM.



FVIII was immobilized on plates. Anti-A2 or anti-C2 mAb was preincubated with different concentrations of scFv-IgGs for 1 h at 37 C. Samples were incubated with FVIII for 2 h at room temperature. Bindings of the anti-A2 or anti-C2 mAb were detected with HRP-conjugated anti-mouse antibody and absorption was measured at 492 nm and 620 nm (reference).

In vitro neutralization capacity of scFv-IgGs

JkH5-IgG partially restores FVIII activity in the presence of the inhibitory anti-A2 mAb with an EC_{50} of ~100 nM.



Prior to neutralization the amount of anti-A2 mAb necessary to inhibit more than 80% of 1 U/ml FVIII was determined. This amount of anti-A2 mAb was incubated with different concentrations of scFv-IgGs for 1 h at 37 C. FVIII was added and samples were incubated for 30 min at 37 C. FVIII activity was measured in a one stage clotting assay on a BCT.

Summary

- Synthetic scFv phage display libraries can be used to select FVIII inhibitor-specific antibody fragments
- ScFv-IgGs bind to FVIII inhibitors with nanomolar affinities
- ScFv-IgGs compete with FVIII inhibitors for binding to FVIII
- The anti-A2 mAb-specific JkH5-IgG is able to neutralize the FVIII inhibitor in a concentration dependent manner

Outlook

- Further *in vitro* characterization of the anti-C2 mAb-specific scFv-IgGs
- Cross-reactivity tests of scFv-IgGs for other mAbs and patient derived FVIII inhibitors
- Analysis of *in vivo* neutralization capacity of scFv-IgGs in hemophilic mice
- Selection and characterization of scFvs specific for human FVIII inhibitors

References

(1) Gilles, J. G., B. G. Desqueper, H. Lenk, J. Vermylen, and J. M. Saint-Remy. 1996. Neutralizing antiidiotypic antibodies to factor VIII inhibitors after desensitization in patients with hemophilia A. *J Clin Invest* 97: 1382-1388.

(2) Schirrmann, T., and K. Büssow. 2010. Transient Production of scFv-Fc Fusion Proteins in Mammalian Cells. *Antibody Engineering* 387-400.

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