6 Summary

Antiidiotypic antibodies against a platelet reactive IgG Fab cloned from a combinatorial antibody library of a healthy individual

Since many years, intravenously administered IgG antibodies (IVIG) prepared from plasma of numerous healthy blood donors are successfully used in the therapy of autoimmune thrombocytopenia (AITP) and other autoimmune diseases. IVIG`s mechanism of action seems to be complex and is not clearly clarified yet. Both Fc-receptor dependent effects and immunmodulatory antiidiotypic effects based on the antigen-binding sites, the so called Fab fragments, were discussed to play a role.
Applying phage display and antiidiotypic biopanning with IVIG to investigate the antiidiotypic effects of IVIG on the immune system, the group of PD Dr. Peter Fischer had cloned and characterized many platelet reactive human IgG Fab fragments from patients with AITP for the first time. The platelet reactive Fab fragments originated predominantly from the VH-4 germline gene family. However, the VH germline genes 3-23 and 3-30 represented the most frequent genetic origin of the variable region of the heavy chain regarding the platelet-negative, IVIG selected clones from autoimmune patients and from a healthy individual. Interestingly, the CDR3-region and the light chains seemed to have no influence on the selection by IVIG, likewise there was no accumulation of certain DH- and JH-germline genes. The fact that IVIG preferably bound antibodies originating from the germline gene segments 3-30 and 3-23 independent of a certain disease together with other results suggested a fundamental meaning of these interaction for the regulation of the B-cell repertoire. This led to the hypothesis that IVIG may regulate the B-cell repertoire over a B-cell-superantigen-like-function besides the direct binding of autoantibodies.

In order to get more information about the potential regulating molecules of IVIG and to reach a possible clinical relevance it was the goal of this study to clone the therapeutically relevant molecules from IVIG. For that purpose the IVIG-targets described above and a Fab-Phage-library of a healthy donor (as representative for IVIG) which were already established by the group of PD Dr. Peter Fischer should be used. The structural and functional characterisation of the isolated clones afterwards should made the possible antiidiotypic mechanism of IVIG more clear.

Fab presenting phage clones originating from an IgG library of a healthy individual and isolated by binding to a platelet reactive Fab fragment of the VH 3-30 gene family (LO31) isolated previously with IVIG could be cloned by means of phage display and biopanning technique.
Sequencing of four LO31-binding Fab phage clones, which displayed a different BstNI restriction pattern, revealed the genetic identity of three clones originating from VH4-61 and VL2a2. The fourth clone showed a VH1-02 and VL2a2 germline gene origin.
When sequences were compared at DNA and protein level a great variability of the CDRs as well as framework regions was revealed. The CDRs not only differed in their amino-acid patterns but also partially in their length. Determination of mutation rates from CDRs and framework regions by comparison with germline genes and the relation between replacement

and silent mutations led to the conclusion that mutations of the germline genes were antigen driven.

To characterise the binding properties of the LO31 binding Fab phages, soluble Fab fragments with differing binding specificities and partially differing genetic origin were large-scale produced and purified. Subsequent binding assays demonstrated that isolated Fab phages bound specifically to the Fab fragments by which they had been isolated. Other Fab fragments bound only weak or not at all. Binding to Fc fragment was not detectable. As a result, the isolated Fab fragments did not bind multiple VH3-30 or VH3-23 originating antibodies but were true antiidiotypic antibodies against a single antibody.
In conclusion, two Fab fragments which interact highly specifically/antiidiotypically with a single IVIG-selected Fab were successfully isolated from an IgG library of a healthy individual. They did not represent the postulated subset from IVIG, representing a B cell superantigen, which binds outside of the antigen binding site to multiple B-cell receptors, but refers to antibodies, which - as individual idiotyps – bind rather as Ab2 beta or gamma. The reason may have been the very restricted panning procedures. The isolated Fab fragments might represent antibodies from IVIG with thrombocyte autoantibody-blocking properties and hence inhibit the autoantibody caused destruction of the thrombocytes.