Summary

The goal of my work was to enhance the knowledge on Lewis Y binding antibodies and their interaction with the antigen for the development of Lewis Y-specific therapeutics using advanced antibody technologies including anti-idiotypic antibodies. The Lewis Y carbohydrate antigen is overexpressed in 60-90% of cancers of epithelial origin, which makes it a target of interest for therapy. Immunotherapeutics are promising candidates for future clinical applications. Several antibodies have been developed against Lewis Y, but most of them cross-react with related carbohydrate structures.

The specificity determination of the four Nemod Lewis Y binding antibodies showed that one (A70-C/C8) bound exclusively to the Lewis Y antigen, whereas the three others revealed cross-reactivities to related carbohydrate structures. A chimeric mouse-human IgG was successfully generated from the IgM antibody A70-C/C8 and this chimeric antibody (cIgG CC8) proved to be as specific towards Lewis Y, as the parent antibody. Starting from two mouse monoclonal antibodies two new chimeric antibodies were created by rearrangement of their chains. As a result new chimeric antibodies (AA9/CC8) were generated as IgM as well as IgG. These new antibodies were found to have two important features: (1) Exclusive recognition of Lewis Y and (2) enhanced reactivity to Lewis Y. Approximately a factor of 10 was estimated for the cIgG AA9/CC8 when compared to the cIgG CC8. The antibodies were evaluated in ELISA, flow cytometry, immunocytology, and surface plasmon resonance. The data generated show that the antibody cIgG AA9/CC8 is the most promising candidate for further development and optimising.

A thorough investigation of the antigens recognised by the Nemod antibody A70-A/A9 revealed two related carbohydrate structures, Lewis Y and Lewis b, and surprisingly the histone H1. This is the first real hint to a naturally occurring immunological mimicry between carbohydrate structures and a naturally occurring protein. Anti-histone antibodies are often found in patients suffering from the auto-immune disease systemic lupus erythematosus (SLE). The mimicry identified here suggests a possible explanation for the cross-reaction of some SLE auto-antibodies to the surface of cancer cells.

Generally carbohydrates are weak immunogens whereas it is possible to generate a strong anti-carbohydrate response by vaccination with protein mimics, e.g. anti-idiotypic antibodies. Anti-idiotypic antibodies have so far been generated by a tedious process involving immunising mice and generation of hybridomas. We developed an improved phage display selection strategy that after only a few rounds of selection generated clones with the wanted specificity from naïve scFv-phage libraries. Only two rounds of selection using specific elution with the antigen and a proteolytic helper phage ensured that maximum diversity was retained in the selected clones. It could be also shown that an anti-idiotypic scFv generated with this new technique was able to induce an anti-carbohydrate response in mice. This technique is promising as a new tool for the selection of anti-idiotypic antibodies and surrogate molecules. Furthermore, it could be demonstrated that the fusion of DI of the phage protein p3 to the scFvs, previously shown to be a mediator of activity, also provides good adjuvant properties and is a new method for inducing immunogenic responses. Results of basic research presented here enhance the knowledge for the development of tailored immunotherapeutics.