

Small animal models of orthotopic corneal transplantation offer many advantages for the study of immune mechanisms after grafting - not only because of the similar mechanisms of murine and human corneal transplant rejection but also due to the feasibility of the direct observation of the animal without the need to sacrifice it. The purpose of the thesis was to study this model in mouse and rat. We established allotransplantation (BALB/c to C57BL/6 mouse and Wistar Furth to Lewis rat) and concordant xenotransplantation model (rat to mouse; Lewis to BALB/c and Sprague Dawley to BALB/c) and set up grading schemes for the evaluation of the clinical course after grafting.

Initially, we focused on the effect of the suturing technique on the survival of xenografts and on the efficacy of selected immunosuppressants: cyclosporine A, monoclonal antibody against T cells (anti-Thy-1.2) and AMT (a specific inhibitor of inducible nitric oxide synthase, 2-amino-5,6-dihydro-6-methyl-4-H-1,3-thiazine)). The results demonstrate that the suturing technique significantly affects the outcome of transplantation and, importantly, influences the effectiveness of immunosuppressive regimens and therefore must be taken into account when evaluating the efficacy of immunosuppressive drugs.

FTY720 is a novel immunosuppressant with a completely new mechanism of action. It modifies patterns of T cell migration and sequestration in lymph nodes and the thymus. Our results show that treatment with FTY720, even in monotherapy, substantially delays the inflammatory response in a dose dependent manner after corneal concordant xenotransplantation and prevents the necrosis at the graft margin and sloughing of the xenograft, possibly enabling later graft infiltration. However, we have also found that treatment with FTY720 induces a profound reduction in T and B cell expansion and the expression of B cell activation markers (major histocompatibility complex (MHC) class II and CD86) in draining lymph nodes (DLN) with subsequent impairment of late recruitment of inflammatory cells into the graft. We demonstrate that FTY720, used in isolation, is a potent immunosuppressant in the control of xenogenic corneal graft rejection and shows that it may be possible, at least in experimental settings, to develop long-term acceptance of corneal xenografts.

We also demonstrate that FTY720 is effective in corneal allograft rejection with a potential to reverse rejection even after priming. It limits the early corneal infiltration with CD11c⁺ cells and prevents both T and B cell expansion in the DLN with subsequent impairment of the late intragraft recruitment of inflammatory cells. FTY720 also selectively decreases cellularity in the DLN of grafted mice on day 20 after transplantation, suggesting that alloantigen-activated dividing cells can be preferentially affected by the FTY720-treatment.

We show that the reduction of MHC class II expression on lymph node B cells can be induced by FTY720 *in vivo* as well as *in vitro*. However, this effect is limited only to lymph node B cells, since CD11c⁺ or B220⁺ cells from the spleen are not affected. Although FTY720 restricts MHC class II expression on lymph node B cells, additional stimulation with lipopolysaccharide is capable of partially restoring this expression and thus another pathway involved in MHC class II regulation can to some extent operate in the presence of FTY720. In conclusion, FTY720 reduces levels of MHC class II expression on the surface of lymph node B220⁺ cells, which may represent an additional mechanism of FTY720-induced immunosuppression.

Corneal graft rejection is mediated mainly by donor-specific CD4⁺ T cells and the T_H1 response predominates. Redirecting the recipient's immune response from T_H1 towards T_H2 may have a positive effect on the corneal graft outcome. Transduction with AdrIL-4 (alone or in combination with vIL-10) leads to visible attenuation of the iris vessels reaction shortly after the transplantation as well as during rejection. This happens in contrast to control mice or mice with grafts transduced with Adβ-gal or AdvIL-10 alone. Presumably, this reflects an immunosuppressive effect of IL-4 on the inflammatory reaction in the anterior chamber. *Ex vivo* corneal transduction with AdrIL-4 does not delay corneal graft rejection and, in addition, its application is dose dependently associated with increased corneal opacity. This probably occurs because of eosinophil infiltration induced by eotaxin produced by corneal fibroblasts under the influence of IL-4. Combined treatment of IL-4 and vIL-10 is associated with more pronounced corneal opacity, the increased activity of neovascularization; the combination of IL-4 with low titre of vIL-10 shortens the graft survival. To sum up and conclude, the redirection of the local immune response towards T_H2-type does not suffice to delay corneal allograft rejection. Nevertheless, the signs of immune modulations warrant further research. Using small animal models in studies of corneal transplantation significantly extends our possibilities to understand immune mechanisms (and not only those associated with transplantation) and also assists in our efforts to uncover the efficacy and possible mechanisms of action of immunomodulatory drugs or approaches. This thesis demonstrates various possibilities these applications provide.