



Samenvatting proefschrift Thanyalak Tha-In

'Intravenous Immunoglobulins in liver transplantation: rationale and mechanisms of action'

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Over the last four decades, liver transplantation has evolved from an experimental therapy for a limited number of selected patients to the treatment of choice of patients with end stage liver diseases. This remarkable success is in part attributable to the development of immunosuppressive drug therapy. The introduction of calcineurin inhibitors and other immunosuppressive agents has led to a dramatic reduction in the incidence and the severity of acute rejection, and consequently to a significant improved graft and patient survival during the first post-operative years. Still, the long term outcomes of liver transplantation have not improved in similar degree. Today, the main obstacles to successful long term patient survival are the high incidence of cancer, infection, renal failure, and cardiovascular diseases, due to life long use of immunosuppressive drugs. Currently, the main challenge of transplant physicians is to improve the long term outcomes after liver transplantation by optimising the current immunosuppressive regimen or exploring therapeutic potentials of new immunosuppressive drugs.

The overall aim of this dissertation was to explore ways of reducing long term side effects of immunosuppressive drugs in liver transplant recipients by either optimising the current dosing strategy of cyclosporine, and to investigate the therapeutic potential of alternative immunosuppressive drugs, in particular intravenous immunoglobulins. The basic concept behind our approach is based upon the clinical observation that liver transplant patients treated with anti-viral IVIg as prophylaxis, had a two-until three-fold lower risk of rejection. Based on this finding, we investigated the effect of IVIg on different cell types of the innate and adaptive immune system, searching for the explanation for the beneficial effects of IVIg in hampering the process of cell mediated rejection. Better understanding of the effects of IVIg on the cellular immune



response, may help us to identify the therapeutic potential of IVIg in preventing transplant rejection, which can form a basis to develop a less toxic immunosuppressive regimen after liver transplantation.

In chapter 2, we investigated the effect of cyclosporine dose reduction based on C2 levels, on renal function in long term liver transplant recipients. We concluded that according to current recommendations, cyclosporine overexposure is common among long term liver transplant patients. However, cyclosporine dose reduction based on recommended C2 levels did not result in an improvement in renal function, and was accompanied with a risk of immune activation.

In order to explore the therapeutic potential of other immunosuppressive drugs with less side effects, chapter 3 became the cornerstone of this thesis, as it forms the basis for a concept to investigate the immunomodulatory effects of anti-viral, and non-specific IVIg on the cellular rejection response. In this chapter, the efficacy of anti-viral intravenous immunoglobulins (anti-HBs Ig and anti-CMV Ig) in preventing acute rejection after liver transplantation was assessed in a retrospective analysis, and correlated to their effects on immune cells in vitro. We concluded that anti-HBs Ig protects against acute rejection after liver transplantation, probably by functional inhibition of the two principal immune cells involved in allograft rejection, DC and T cells. The mechanism by which IVIg suppress DC and T-cell function was so far unidentified. In the next chapters, we investigated in depth the immunomodulatory effect of IVIg on immune cells of, both from innate and adaptive origin, which play altogether a pivotal role in the cellular rejection process.

In chapter 4, we compared the suppressive effects of IVIg and the calcineurin inhibitors (CNI), Cyclosporin (CsA) and Tacrolimus (TAC), on human blood derived T cells and DC. We concluded that IVIg have strong suppressive effects on both human T cells and DC, while CNI only inhibit T cells. Therefore, by simultaneous targeting both T cells and DC, we assume that IVIg will be superior in the control of acute rejection and GVHD in comparison to CNI. Since long term IVIg treatment has no significant side effects, inclusion of IVIg in immunosuppressive therapy after transplantation may enable dose reduction of CNI, and thereby contribute to a lower rate of complications. In chapter 5, we focused on the mechanism by which IVIg affects DC function. We found that the decreased T-cell stimulatory capacity of IVIg treated DC was associated with induction of cell death in mature DC. Interestingly, IVIg treatment itself did not induce DC death directly, as the increased death of IVIg treated DC only occurred when cultured together with other immune cells, i.e. NK cells. We showed that dimers or multimers present in IVIg induce ADCC of mature DC by NK cells, which downsizes the antigen presenting pool, and inhibits T-cell priming. In chapter 6, we put a manuscript under review, in which a novel role for NK cells in the setting of co-stimulation blockade was identified.

Furthermore, chapter 7 focus on the mechanisms by which IVIg attenuate T cell responses. We investigated whether IVIg might enhance the suppressive function of CD4+CD25+Foxp3+ regulatory T cells, and if so, whether IVIg could prevent allograft rejection. We demonstrated that through direct binding and activation of regulatory T



cells, IVIg can mediate suppression of allogeneic T cell responses. In vivo, administration of IVIg prevented T cell mediated rejection of fully mismatched skin grafts in immunodeficient mice reconstituted with total CD4+ T cells. Significantly, this IVIg effect was lost when CD4+CD25+ cells were depleted from T-cells which were transferred to the mice, suggesting that IVIg mediate their effect through Tregs. Activation of Tregs in vivo by IVIg treatment may be an attractive option for prophylaxis of transplant rejection, since IVIg administration is safe and has moderate side effects. Importantly, the data underline that the therapeutic potential for IVIg may be broader than today's application in organ transplantation considers. In chapter 8, we discuss recent advances in our understanding of different, mutually non-exclusive mechanisms of action of IVIg on cells of the innate and adaptive immune system. IVIg target the cellular immune compartment at multiple levels, including innate and adaptive immune cells. Although increasing numbers of animal models have provided us with new insights, the reported mechanisms of action of IVIg are either not confirmed in patients or have shown discrepancies. Due to the high costs and the upcoming shortage of IVIg as a consequence of shortage in donated blood plasma, it is important to identify the active components in IVIg in various diseases. IgG dimers and sialylated IgG are promising candidates with encouraging results in experimental models. Still, their efficacy in patients needs to be established. Purification of the anti-inflammatory compound or production of synthetic or biological alternatives of IVIg will enable us to selectively target pathogenic immune activity.

In the setting of organ transplantation, physicians still face the challenge to improve the long term outcomes after transplantation by either optimising current immunosuppressive regimens. or exploring therapeutic potentials of new immunosuppressive drugs. In this thesis, we investigated the effects of IVIg on the different cells of the innate and adaptive immune system, which altogether play a pivotal role in the cellular rejection response. Based on the reported findings from our group and others, we consider IVIg as a promising candidate for prevention and attenuation of the acute cellular rejection process after liver transplantation. Still, clinical trials are first needed to determine optimal dosing strategy, and whether IVIg administration allows reduction or discontinuation of the maintenance immunosuppressive treatment in order to reduce the long term side effects after transplantation.



Conclusions

Chapter 2: Cyclosporine dose reduction based on recommended C2 levels does not result in an improvement in renal function in stable liver transplant patients, and was accompanied with the risk of immune activation, especially in patients on cyclosporine monotherapy.

Chapter 3: Anti-HBs Ig protects against acute rejection after liver transplantation, probably by functional inhibition of the two principal immune cells involved in allograft rejection, i.e. Dendritic Cells and T cells.

Chapter 4: As IVIg can simultaneously target both human T cells and DC, while CNIs only inhibit T cells, inclusion of IVIg in future immunosuppressive therapy after transplantation may enable dose reduction of CNIs, and thereby contribute to a lower rate of complications.

Chapter 5: By influencing the bi-directional interaction between DC and NK cells, IVIg induces ADCC of mature DC by NK cells, and thereby hampers the ability of the innate immunity to trigger proper T-cell activation.

Chapter 6: Antibody-dependent cell-mediated cytotoxicity (ADCC) killing of APC mediated by NK cells may be a pathway by which costimulation blockade induces graft acceptance in a mouse skin transplant model.

Chapter 7: Through immediate binding and activation of regulatory T cells, IVIg can mediate suppression of allogeneic T cell responses, highlighting an additional, important therapeutic potential of IVIg in organ transplantation.

Chapter 8: Due to the high costs and the upcoming shortage of IVIg as a consequence of shortage in donated blood plasma, it is important to identify the active components in IVIg in various diseases. ◀