Chapter 1

Introduction and aims of the thesis
Kidney transplantation, success and challenges

The history of organ transplantation as a feasible therapy for end-stage diseases began in 1954 with the kidney transplantation between identical twins performed by Murray et al. It was the perfect immunological match between donor and recipient that ensured the success of this transplantation, as the attempts to transplant allografts during the following decade failed constantly\(^1\). Introduction of immunosuppressive therapy, especially of cyclosporine in the 1980’s, the advances in HLA matching\(^2\), organ procurement and preservation\(^3\) and standardization of surgical techniques have progressively transformed kidney transplantation into a reliable clinical procedure. Nowadays, kidney transplantation is the therapy of choice for patients with end-stage renal disease\(^4\), with a graft survival rate of about 90% at 1 year post-transplantation\(^5\).

The short-term success of renal engraftment is determined mainly by the capacity to cope with delayed graft function (DGF) and acute (and hyperacute) rejection episodes. The frequency of DGF of cadaver renal transplants is higher (2-50%) than of kidneys from living donors (4-10%)\(^6\), reflecting the pivotal role played by ischemia-reperfusion (I/R) injury in the pathogenesis of DGF. Whereas hyperacute rejection is sporadic nowadays, up to 50% of the patients receiving a renal graft experience one or more acute rejection (AR) episodes. Despite the “optimal” immunosuppressive therapy, half of the transplanted kidneys stop functioning within 10 years after engraftment\(^7\). The major cause of chronic graft loss is chronic allograft nephropathy (CAN)\(^8\). CAN is a consequence of both immune factors, such as AR episodes, and non-immune factors, such as I/R injury during transplantation, hyperlipidemia, hypertension, proteinuria and immunosuppressive drug toxicity\(^9;10\). CAN is characterized by a progressive decline in renal function, associated with proteinuria and hypertension, and histopathologically by mononuclear cell infiltration, tubulo-interstitial fibrosis and vascular obliteration. To date, there is no efficacious way to prevent or treat CAN. Several strategies, such as calcineurin inhibitors withdrawal\(^11\) or inhibition of the renin-angiotensin system\(^12\) may slow-down progression of injury in CAN. The clinical condition of the transplanted patients is even further complicated by the serious side-effects of sustained systemic immunosuppressive therapy, including development of malignancies\(^13\), opportunistic infections\(^14;15\), cardiovascular disorders\(^16\) and other drug-specific side effects. Thus, the search for a therapeutic approach that efficiently and selectively inhibits the alloantigen immune response or induces tolerance to alloantigens, prevents I/R injury and CAN, and is free of major side-effects is still ongoing.

This thesis focuses on two topics: (1) the potential of gene therapy with Interleukin-13, an immunomodulatory and cytoprotective cytokine, to mitigate renal I/R injury and acute kidney transplant rejection and (2) the potential of (local) immunosuppressive gene therapy in kidney transplantation.

Ischemia-reperfusion injury

I/R injury is a major determinant of both the short- and long-term outcome of kidney transplantation. On short-term, the damage inflicted onto the donor kidney during ischemia and reperfusion may lead to DGF, with reduced glomerular filtration rate (GFR) and oliguria early after transplantation\(^17\). Besides, I/R injury increases the expression of MHC
(HLA in humans) class II\textsuperscript{18} as well as adhesion molecule expression\textsuperscript{18,19} in the kidney, rendering the graft more prone to acute rejection. In long-term perspective, I/R injury is an independent risk factor for development of CAN\textsuperscript{20}. Thus, prevention or attenuation of I/R injury is expected to diminish the risk of both acute and chronic dysfunction of the grafted organ (Figure).

Both experimental and clinical studies have contributed to the understanding of the pathophysiology of I/R injury. Already during the ischemic period, renal damage occurs, depending largely on the duration of ischemia and whether the organ is preserved at body temperature or at lower temperatures ("warm" vs. "cold" ischemia). During ischemia, shortage of oxygen and nutrients causes a rapid depletion of ATP, a switch from aerobic metabolism to anaerobic glycolysis, mitochondrial damage and, in severe ischemia, cell death\textsuperscript{6}. Restoration of blood flow may avert cells from apoptosis or necrosis; however it also produces additional damage, which is referred to as "reperfusion injury". The pathophysiology of I/R injury involves primarily vasomotor alterations\textsuperscript{21}, cell death\textsuperscript{22}, oxidant stress\textsuperscript{23} and inflammation\textsuperscript{24}. The main players in the onset of I/R injury are the tubular epithelial cells (TECs) and the vasculature, especially the endothelial cells. The most affected by I/R injury are the TECs, especially proximal TECs. In severe I/R injury, large areas of both necrotic as well as apoptotic cells are found especially in outer medulla. Reduction in GFR early after I/R has been attributed to endothelial injury\textsuperscript{25}, and vasoconstriction due to changes in the vasoreactivity and predominance of vasoconstrictive (such as endothelin-1\textsuperscript{26}) over vasodilator factors (such as endothelial-derived nitric oxide\textsuperscript{25}; see also chapter 7). Severe proximal tubular damage may also contribute to increased vascular resistance through back-leak of filtrate\textsuperscript{21}. Besides, activation of endothelial cells with expression of adhesion molecules triggers inflammatory cell infiltration, first neutrophils, then macrophages and to a lesser degree T cells\textsuperscript{28}. Inflammatory cells amplify the initial injury through mechanical obstruction of the microvessels and production and release of ROS, proteases, chemokines and cytokines (see also chapter 4 and 5 for details). Complement activation also contributes to I/R injury\textsuperscript{27}. In addition, injured TECs express chemokines and pro-inflammatory cytokines, such as TNF-alpha and MCP-1, which stimulate further damage. Though mild I/R injury is in principle a reversible phenomenon, severe I/R injury is followed by defective repair\textsuperscript{28} and contributes to development of long-term fibrosis and CAN. Incriminated in occurrence of fibrosis after severe I/R are a persistent, low-level inflammation\textsuperscript{29}, a rarefaction of peritubular cappillaries\textsuperscript{30} after I/R and a lack of balance between extracellular matrix production and proteases activity\textsuperscript{29}.

Experimental strategies aimed at inhibiting or preventing I/R injury have developed alongside the understanding of the pathophysiology of I/R. Preventive strategies focus on organ procurement, preservation techniques and use of anti-oxidant, anti-inflammatory, vasodilator agents or growth factors\textsuperscript{6}. However, the clinical benefit from using such drugs remains to be clarified. The therapeutic approach for DGF secondary to I/R injury relies still on dialysis.
Acute rejection

Acute rejection (AR) is primarily a cell-mediated immune response of the recipient against the alloantigens present on the renal graft. AR is a serious post-transplantation complication, affecting up to 50% of the patients receiving a renal graft and it is the most important risk factors for development of CAN (Figure). In humans, AR occurs within days to weeks after engraftment. As an AR episode may develop displaying only non-specific symptoms or even without symptoms, close monitoring, early diagnosis and prompt therapeutic intervention are essential for salvaging the renal graft. The risk of acute rejection is determined mostly by the HLA mismatches between donor and recipient, yet I/R injury, cardio-vascular comorbidity, donor and recipient age and CMV mismatching play also a role herein. The outcome of acute rejection is still predicted best by the extent of abnormalities found in histological examination of a graft biopsy.

AR is characterized by interstitial inflammatory infiltrates, tubulitis and various degrees of arteritis (Banff classification). The inflammatory infiltrates are composed of T cells (both CD4+ as well as CD8+ cells), macrophages, neutrophils, natural killer cells and B cells. AR is triggered by the recognition of the HLA antigens and the other alloantigens exposed on the TECs, endothelium and mesangial cells of the graft by the T lymphocytes of the recipient. CD8+ T cells, which are predominantly killer T cells, recognize antigens presented by HLA class I molecules. Once activated, they release perforin and granzyme B into the target cell and destroy it. Interaction with antigen presenting cells and antigen recognition via HLA class II is insufficient to activate fully the CD4+ T cells. A second (co-stimulatory) signal, such as CD28-B7-1/2, CD40-CD154 or PD1-PD-L1/2, is required. After activation,
CD4+ T cells differentiate into T helper (Th) type 1 or 2 cells, which further modulate the activation of macrophages, B cells and some CD8+ cells. Th1 and Th2 are defined by production of various cytokines which have in general opposite effects. Th1 cells produce “pro-inflammatory” cytokines, including IFN-γ, IL-2, TNF-α, whereas Th2 cells produce IL-4, IL-10, IL-13, which are generally regarded as “anti-inflammatory” cytokines. The balance between the two types of cells/cytokines may modulate the immune response during AR. Recent studies have highlighted the importance of regulatory T cells, which are a specialized subpopulation of foxp3+ T cells that are able to modulate the immune response towards tolerance, in determining the fate of the graft during and after an AR episode. Resident renal cells are also active players during AR. They can function as “non-professional” antigen presenting cells. Besides, they produce cytokines and chemokines, such as TNF-alpha, MCP-1, RANTES, hence contributing to perpetuation of the inflammatory response. The advances in the immunosuppressive drugs and regimens have considerably improved prevention and treatment of acute rejection episodes. The most used immunosuppressive agents are cyclosporine, tacrolimus and sirolimus, glucocorticosteroids, azathioprine, mycophenolat mofetil, anti-T cell antibodies and anti-IL-2 antibodies. They target T cell activation, cytokine production (especially IL-2) and clonal expansion. Also, new pharmacological agents interfering with co-stimulatory pathways, such as CTLA4Ig have been approved for clinical use. Unfortunately, the immunosuppressive drugs do not have solely beneficial effects for the transplanted patients. Immunosuppressive therapy, as currently available, suppresses non-selectively all immune responses, including those directed against bacteria, fungi, viruses and tumor cells. As a consequence, systemic immunosuppression in transplanted patients is associated with major side effects, including serious infections and malignancies. The current strategy to attenuate these life-threatening side effects is the use of combined immunosuppressive regimens. However, local instead of generalized immunosuppression may improve graft survival and quality of life of the transplanted patients.

**Interleukin-13**

Interleukin (IL)-13 was first described in 1993 by Minty et al. as a “lymphokine regulating inflammatory and immune processes”. IL-13 pertains to the family of Th2 cytokines and is structurally and functionally related with IL-4. IL-13 displays various effects, depending on the type of the pathological process in which it is involved and the cell type on which it acts. IL-13 is generally seen as an anti-inflammatory molecule, able to alleviate acute and chronic inflammatory processes. However, IL-13 has been involved in the pathogenesis of asthma and of several other eosinophil-mediated and allergic diseases. Recent studies in liver demonstrated both endogenous as well as exogenous IL-13 to be protective against I/R injury through a direct, anti-oxidant and anti-apoptotic effect, as well as an anti-inflammatory effect. In addition, IL-13 has regulatory functions in antigen-specific processes. IL-13 (but not IL-4) was shown to be essential for induction of tolerance to foreign antigens in newborn mice. Also in mice, IL-13 had additive effects with anti-IL-12 antibodies and allogeneic dendritic cells on prolonging skin allograft survival. In a recent study by Skapenko et al., a critical role of IL-13 (and IL-4) in extra-thymic generation of foxp3+ regulatory T cells was demonstrated. Yet, the role played by endogenous IL-13 in
rejection of transplanted kidney is not clear. IL-13 acts via a receptor which is partially common with IL-4 receptor. The complex IL-4Ra/IL-13Ra1 mediates most of the IL-13 effects, whereas IL-13Ra2 may function as both a decoy as well as a signaling receptor. Data on the expression of IL-13 receptors in normal and diseased kidney is limited. In a study by van den Berg et al., glomerular expression of IL-13 receptors was described.

**Gene therapy in kidney transplantation**

Gene therapy is a modality of treating diseases through delivery of specific genes to target cells. Gene transfer approaches may provide valuable strategies for virtually all the problems currently faced by patients and doctors in the field of kidney transplantation. First and most importantly, gene therapy holds the potential to induce tolerance or local immunosuppression while circumventing systemic side-effects. In a study by Benigni et al., adenovirus-delivered CTLA4Ig, which is a blocker of co-stimulatory pathway CD28-B7, prevented AR, prolonged survival and induced temporary unresponsiveness to alloantigens. Furthermore, when long-lasting vectors are employed, local immunosuppression through gene therapy may prevent development of CAN. Using an adeno-associated virus as vector, Tomasoni et al. demonstrated CTLA4Ig gene therapy to protect the renal allograft from chronic rejection. Second, gene therapy may be instrumental in expanding the donor pool and increasing the quality of the grafts, especially of those from non-heart beating donors. Blydt-Hansen et al. showed that adenovirus-mediated transduction of donor kidneys with HO-1 during 24 hour-preservation improved renal function and morphology and prolonged survival of isografts. Third, gene therapy with anti-inflammatory molecules (such as decoy NFkB or ICAM-1) may ameliorate I/R injury in both living-related as well as deceased donor kidneys.

The determinant factors for a successful gene therapy are an efficient vector, a well-established technique of vector delivery, lack of side-effects and selection of appropriate gene(s). Current gene therapy approaches make use of non-viral (naked plasmids, enhanced naked plasmids, liposomes and haemaglutinating virus of Japan (HVJ) liposomes), viral (adenovirus, adeno-associated virus, retrovirus and lentivirus) and cellular (mesangial cells and inflammatory cells) vectors. The various approaches and methods employed in studies on gene therapy in kidney transplantation in particular and in renal diseases in general are described in detail in chapter 2. The most used vector for gene therapy in both experimental and clinical studies are adenoviruses. Advantages of adenoviruses over the other vectors include a relative high efficiency and easiness of production on large scale. In addition, adenoviruses retain their infectivity at low temperature, which allows transduction of the kidney during hypothermic preservation of the graft. As transduction efficacy of adenovirus depends largely on the presence of its natural receptor, coxsackie adenovirus receptor (CAR), and as CAR expression is scarce in several organs, including human kidneys, strategies to re-target the adenovirus to different cell entry pathways, such as a Arg-Gly-Asp (RGD)-modification of the fiber knob, have been developed. RGD peptide binds to integrins (mostly αvβ3 and αvβ5) and thereby virus internalization is achieved. Integrins are abundantly expressed in the kidney. As RGD-modification of adenovirus was shown to increase its infectiousness in several organs in which integrins are present at high levels, we
employed an RGD-modified adenovirus in our studies as a strategy to achieve high transduction efficacy, allowing functional studies.

IL-13 is an appealing candidate for gene therapy in kidney transplantation, as it combines anti-oxidant and anti-apoptotic properties with anti-inflammatory and immunomodulatory effects and hence may mitigate both I/R injury as well as acute rejection, the two most common short-term complications of the kidney transplantation.

**Aims of the thesis**

The current thesis studies the effects of gene therapy with IL-13 on renal I/R injury and AR. As vector for gene delivery, we use an RGD-modified adenovirus. The effects of both systemic as well as local IL-13 gene therapies are investigated. **Chapter 2** gives an overview of the gene therapy approaches in renal diseases in general and in kidney transplantation in particular. In **chapter 3** we characterize the RGD-modified adenovirus as vector for gene delivery into the transplanted kidney. In **chapter 4** we study the sequence of molecular and morphological changes in rat kidneys after warm I/R injury. Several markers of damage and potential therapeutic targets are described. **Chapter 5** focuses on the protective effects of adenovirus-mediated systemic gene therapy with IL-13 against I/R injury in the kidney. Short- as well as long-term effects on renal damage and inflammation are investigated. Based on the data from chapters 3 and 5, we address in **chapter 6** the question whether IL-13 can prevent AR of the transplanted kidney and we compare in this respect systemic and local gene therapy. As reduced endothelial derived NO is incriminated as an important mediator of early injury during I/R injury, we investigate in **chapter 7** the expression of eNOS during hibernation, which is a model of repetitive renal I/R devoid of damage. **Chapter 8** gives a summary of the current thesis and sheds light on the future perspectives of gene therapy for transplanted kidney.
Chapter 1

References


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