Modulated rat dendritic cells in renal transplantation models : immune regulation and graft outcome

Stax, A.M.

CHAPTER 1

General Introduction

Cees van Kooten[1], Annelein M. Stax[1], Andrea M. Woltman[2], Kyra A. Gelderman[1]

[1] Dept of Nephrology, Leiden University Medical Center Leiden, the Netherlands
[2] Dept of Gastroenterology and Hepatology, Erasmus Medical Center, Rotterdam, the Netherlands

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Renal transplantation

Renal transplantation is currently the treatment of choice for patients with end stage organ failure. The majority of the recipients receive genetically non-identical donor-organs (allografts). Most of these transplants would be rejected if patients would not receive immunosuppressive treatment. Various types of rejection can be distinguished, including hyperacute rejection, which occurs immediately after establishment of the blood supply, acute rejection, which arises early after transplantation (mostly within the first 6 months) or chronic rejection, which takes place after more than 6 months. To prevent rejection of transplanted allogeneic organs, it is necessary to apply immunosuppressive therapy. Traditionally immunosuppressive drugs have been mostly directed against T cells. Over the years, powerful combinations of drugs have been developed, resulting in strongly improved short-term survival increases [1]. In contrast to the improved 1-year survival rates, less progress have been made to improve long-term survival. In addition, patients treated with immunosuppressive medication suffer from various side effects, including malignancy and infections, due to the non-specificity of this type of medication [2]. The development of novel therapeutic strategies is therefore important to improve long-term transplant survival.

Immunology of transplantation

The immune system distinguishes self from non-self and has the capacity to either maintain tolerance or to induce immunity to eliminate infectious agents and to minimize tissue damage. Since transplanted organs are recognized as non-self, immunity arises in most cases after transplantation leading to allograft rejection. Although various components of the immune system and a wide array of effector mechanisms are involved, most attention has been paid to the role of T cells.

Activation of alloreactive T cells

Activation of T cells requires a variety of signals to determine the antigen specificity, the strength of the response and the type of the response. The first signal is triggered by the interaction between the T cell receptor (TCR) and a MHC/peptide complex on antigen presenting cells (APC). Simultaneously, a second signal can be delivered when CD28, which is constitutively expressed by naïve T cells, binds to the co-stimulatory B7 molecules (CD80 and CD86), present on activated APC. In addition, a third signal is provided by proinflammatory cytokines, such as IL-12, which are produced by activated APC [3, 4]. This activation process is rapidly amplified when co-stimulation via the CD40-CD40L pathway takes place, due to elevated expression levels of MHC, B7 molecules and increased cytokine production. Together these signals lead to full activation of naïve T cells, resulting in proliferation and secretion of various cytokines, such as IL-2 and IFN-γ (**Fig. 1**) [5, 6]. Subsequent to T cell priming, the T cell displays an increasing transcription and expression of cytotoxic T lymphocyte antigen (CTLA)-4 on the cell surface. This molecule has a higher affinity for B7 molecules than CD28 and has been shown to exert a suppressive effect on T cell activation [7]. More recent

studies demonstrate the presence of additional co-stimulatory molecules, which are involved in “positive” or “negative” signaling. Many of these receptor-ligand pairs belong to the CD28-B7 or CD40-CD40L families. These interactions point to a role of APC in the regulation of T cell activation [8-10].

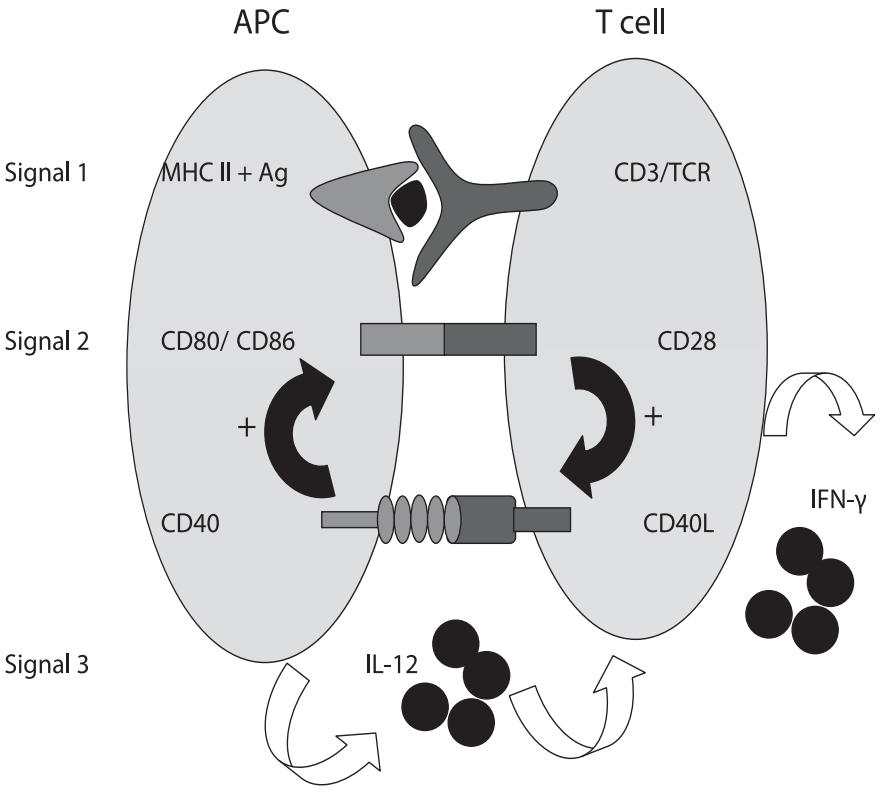

Figure 1. Signals involved in T cell activation.
Three signals are necessary to activate T cells. These signals are provided by matured antigen presenting cells (APC) and include presentation of foreign peptides to naïve T cells in the context of MHC molecules (signal 1). The second signal comprises the interaction between B7 molecules (CD80 and CD86), expressed by mature APC, and CD28, which is constitutively expressed on naïve T cells. Together with the secreted proinflammatory cytokines, such as IL-12 (signal 3), this will result in T cell activation and involves upregulation of CD40L. Subsequently, CD40L interacts with CD40, expressed by mature APC, and leads to an enhanced maturation status of the APC as shown by higher levels of MHC and B7 molecule expression together with elevated levels of proinflammatory cytokine secretion. Consequently, T cells become fully activated and produce high levels of cytokines, such as IFN-γ.

Dendritic cells in allograft rejection

One of the most potent APC are dendritic cells (DC). These are bone marrow-derived cells that populate all lymphoid- as well as non-lymphoid organs, including the kidney. They have a central role in immune regulation, ranging from tolerance induction and the prevention of autoimmunity to the induction of anti-tumor immunity and the protection against infectious agents. Although DC are a heterogeneous group of cells that represent differences in origin, anatomic location, cell surface phenotype, and function, they all have potent antigen presenting capacity to stimulate naive, memory,

effector and/or regulatory T cells (**Fig 2**). Therefore, DC serve as an essential link between innate and adaptive immune responses [11, 12].
Immature DC have specialized capacities for internalisation and processing of self or non-self antigens. DC express several Toll like receptors (TLR), which detect self and non-self products. In response to a broad spectrum of pathogen-associated molecular patterns (PAMPs) these TLR activate different signaling pathways, leading to maturation of DC [13]. Consequently, DC migrate towards secondary lymphoid organs and along the way, lose their capacity to capture and process antigens while gaining the capacity to stimulate T cells. Efficient T cell stimulation by DC requires antigen presentation to T cells, upregulation of co-stimulatory molecules and production of proinflammatory cytokines. Upon interaction with antigen-specific T cells, DC maturation is further ehanced via engagement of CD40 with CD40L, expressed by these activated T cells [14, 15]. In transplantation settings, both donor passenger DC and recipient DC can cause

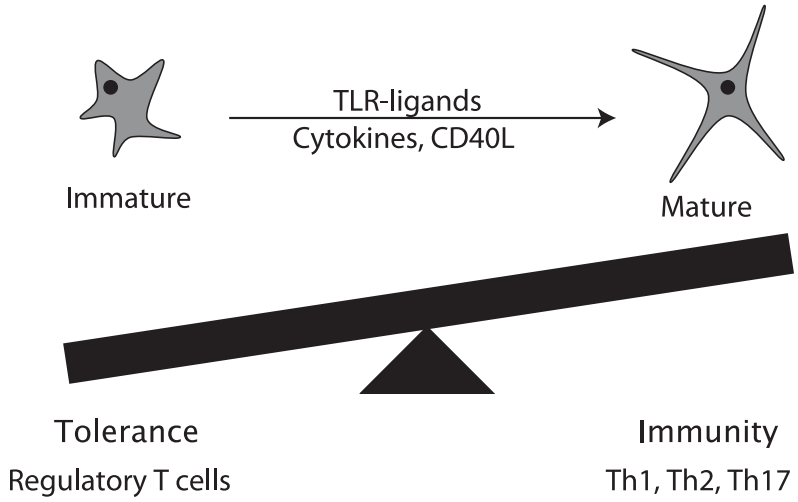

Figure 2. Immune responses induced by DC.
Immature DC express low levels of MHC and co-stimulatory molecules and have the capacity to induce tolerogenic responses. In the presence of danger signals, such as TLR ligands (either PAMPs or endogenous danger signals), certain cytokines or CD40L, immature DC will mature. Mature DC express high levels of MHC and co-stimulatory molecules and produce proinflammatory cytokines. In addition, mature DC have the capacity to induce immunogenic responses.

activation of alloreactive T cells, however this occurs via different routes of recognition. Recipient T cells either recognize the foreign MHC molecule on donor DC, or recognize foreign MHC peptides presented by recipient MHC molecules on recipients DC, known as the direct or indirect pathway of T cell activation, respectively (**Fig. 3**).
The type of T cell response is determined by the cytokines produced by activated DC [15, 16]. DC involved in specific allo-immune responses are programmed to produce high levels of IL-12, which subsequently induces $CD4^+$ T helper 1 cells (Th1). These Th1 secrete IFN-γ and promote cellular mechanisms of immunity through activation of macrophages, NK cells and $CD8^+$ T cells. Although most of the allo-responses are regulated via Th1, also Th2 can be involved in allograft rejection. Th2 provide help to B cells to produce allo-antibodies, which subsequently can mediating rejection [17-19].

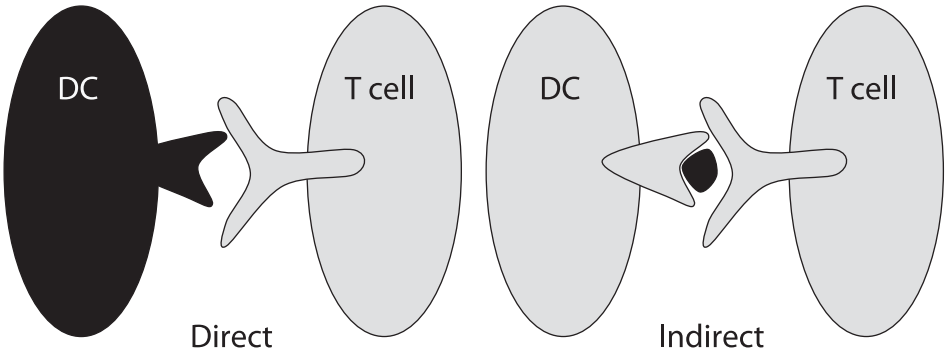

Figure 3. Antigen recognition by recipient T cells.
Recipient T cells can recognize donor antigen via direct or indirect presentation. In case of direct presentation recipient T cells recognize the full MHC molecule on donor APC (black), whereas via indirect presentation recipient APC (gray) present donor antigens (black) on recipient MHC molecules.ect presentation.

Next to Th1 and Th2, another T helper cell has been described recently. This “novel” T helper cell (Th17) has been shown to be important in the pathogenesis of autoimmunity and involves cytokines including IL-23 and IL-17 [20, 21]. Although increased levels of IL-17 have been detected in renal tissue derived from rejected biopsies [22], the role of Th17 cells in transplantation immunity is still unclear. Along with Th1 cells, Th17 cells may mediate allograft rejection, and their role may be more important when Th1 responses are suppressed (**Fig. 4**) [23].
Beside T cells involved in immunogenic responses, regulatory T cells (Treg) have been shown to be important negative regulators of immune responses. They have been identified as being CD4 positive with high levels of cell-surface expression of CD25. Various Treg subsets can be distinguished, including the naturally occurring Treg, which develop in the thymus, and the inducible Treg, which are generated in the periphery under various tolerogenic conditions. The latter subset can be classified in Tr1 and Th3 cells. Tr1 cells secrete IL-10 and TGF-β and are generated by antigenic stimulation in the presence of IL-10 [24], whereas Th3 cells are identified after the induction of oral tolerance and secrete TGF-β [25]. The suppressive capacity of Treg towards effector T cells plays a major role in immunopathology. Absence or dysfunction of Treg has been correlated with autoimmunity [26, 27], whereas their presence has been associated with tolerance as shown in transplantation models [28, 29].

Experimental models of transplantation

Experimental models of transplantation have been studied widely. These models differ in many aspects, including the donor-recipient strain combinations, the transplanted organ and treatments. There seems to be a hierarchy in the immunogenicity of transplanted organs, ranging from low levels of alloresponse in liver transplantation and increasing levels in kidney, heart, skin and bone marrow transplantations [30]. In addition, it has been proposed that transplantations performed in rats are more stringent than in

mice. Even within one species, some strains raise vigorous alloreactive responses and reject most grafts quickly, while in other strains grafts are accepted more rapidly. Consequently, treatments are more effective in models that are associated with less immunogenicity [30].

Kidney transplantation is preferentially studied in rats. The technical procedure in mouse models is very demanding resulting in low survival rates, whereas a higher survival rate is achieved when rats are used. To study various types of kidney rejection different models have been used. Chronic rejection has been examined in the strain combination Fisher (F344, $RT1^{lv1}$) to Lewis (LEW, $RT1^{l}$). Renal transplantation from donor F344 rats to recipients LEW rats demonstrates acute rejection episodes followed by chronic lesions, which ultimately result in kidney failure. In the reverse strain combination, a LEW kidney transplanted into a F344 recipient, acute rejection episodes can be observed, but lesions spontaneously resolve with maintenance of renal function [31, 32]. Other strain combinations, such as Brown Norway (BN, $RT1^{n}$) to LEW or Dark Agouti (DA, $RT1^{a}$) to LEW, demonstrate acute allograft rejection within 7 days [33, 34].

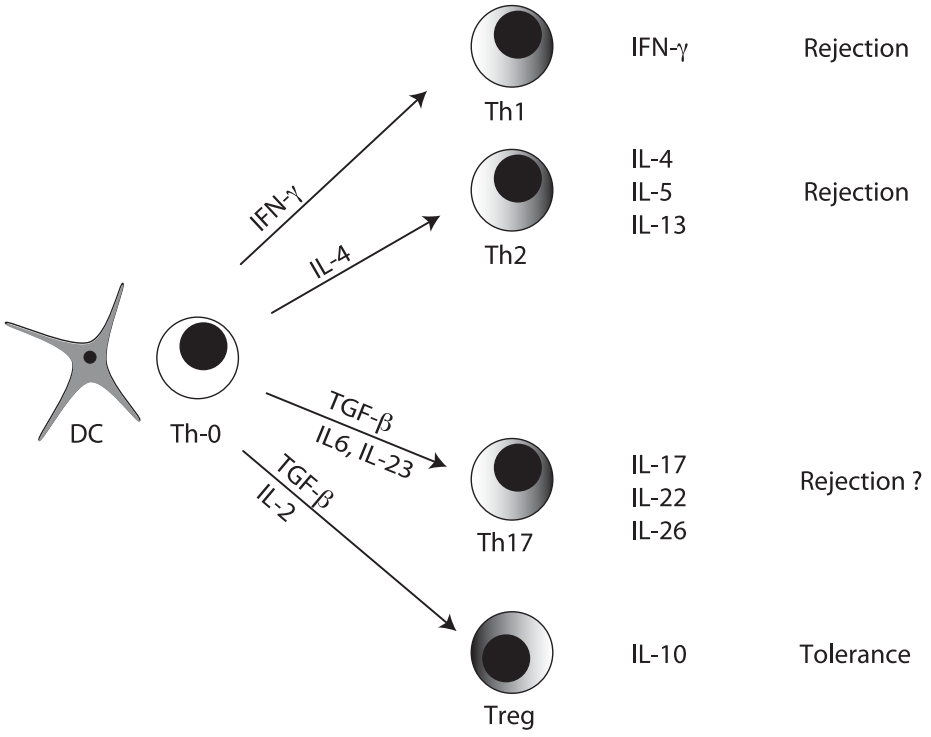

Figure 4. T cell lineage commitment in human.
Naïve T cells develop into T cell lineages defined by their cytokine profile when stimulated by antigen presenting cells (APC). Th1 cell development is induced when APC secrete IL-12 and the cells are characterized by their IFN-γ production. Th2 cells require IL-4 and upon activation produce IL-4, IL-5 and IL-13. Both Th1 and Th2 cells play a role in the graft rejection process. TGF-β is critical for the development of both Th17 and Treg cells. In conjunction with IL-6 or IL-23 inflammatory Th17 cells develop, while Treg development requires IL-2. Treg have been shown to induce tolerance, but the role of Th17 cells in transplantation settings is still unknown.

Depletion of $CD4^+$ cells, using RIB-5/2, prolonged allogeneic kidney survival in a DA to LEW model [35, 36], implying that interference with the immune response in a fully mismatched model can prolong graft survival.

Transplantation Therapies

The introduction of efficient and strong immunosuppressive strategies has significantly improved the short-term survival of allogeneic organs. However, this has come with the price that patients treated with these immunosuppressive drugs suffer from various side effects, including an increased rate of malignancy and infections. It is therefore necessary to develop more specific therapies, in which the ultimate goal is to induce donor-specific tolerance. At present, the various strategies that have been implemented are aimed to control allospecific T cells, including lymphocyte depletion, blockade of co-stimulatory molecules or cell-based therapies.

Therapeutic targeting of co-stimulatory molecules

The molecular identification and characterization of co-stimulatory molecules has provided new tools to target co-stimulatory molecules in rodents and in primates. Since co-stimulatory molecules are essential to induce full T cell activation and T cell activation is a crucial feature of graft rejection, various studies elucidated the effect of co-stimulatory blockade on graft survival. The most extensively studied interactions are CD40-CD40L and CD28-B7 [37, 38]. In rat models, rejection of various solid organs was prevented, and even donor-specific tolerance was induced, when either one of these interactions was blocked [39-41]. More recently it was shown that blocking one of the co-stimulatory pathways indeed resulted in prevention of acute rejection. However, histological signs of chronic rejection were still found at a later stage. Yet, blocking both pathways simultaneously resulted in 50% reduction of chronic rejection in these recipients [42, 43]. In clinical settings, the use of anti-CD40L antibodies in autoimmune disease and transplantation was terminated due to an unanticipated, elevated incidence of thrombo-embolic complications [44, 45]. In contrast, preliminary data from clinical trials using an optimized version of CTLA4-Ig (belatacept or LEA29Y) treatment, demonstrated improved renal function and reduced chronic allograft nephropathy at 1 year without thrombotic complications [46-48].

Donor-specific transfusion

Donor-derived blood transfusions (donor-specific transfusion, DST) have been shown to positively influence survival of allogeneic organs, at least under some conditions [49, 50]. Studies in rodents and non-human primates have demonstrated that a combination of DST and anti-CD40L blocking antibody (Ab), or DST, anti-CD40L blocking Ab and Sirolimus (immunosuppression) synergistically enhanced the survival rate of allogeneic kidneys or pancreatic islets [51, 52]. However, blood transfusions also hold the risk of sensitization and the mechanisms underlying the blood transfusion effect are incompletely understood. Studies in several rodent models indicate that clonal deletion or anergy may play an essential role in silencing the alloreactive T cells [53]. In addition,

rodent and human studies suggest that $CD4^+CD25^+$ regulatory T cells are induced after DST treatment and play a role in graft survival. In the latter studies, it has been shown that only in the case of HLA-DR matched-blood transfusion, these regulatory T cells are induced, whereas when mismatched-blood is administered regulatory T cells are not induced. Survival of allogeneic kidneys in this study was significantly higher in the matched-transfusion recipients compared to mismatched-transfusion recipients [54, 55].

Regulatory T cells

Both human and rodent studies have demonstrated that Treg contain the capacity to suppress effector T cells [29, 56, 57], making Treg an interesting target for cell-based therapy. Clinical application of Treg cells in transplantation settings can be realized by promoting the development of Treg in vivo by administration of immunosuppressive drugs, either alone or together with alloantigens. For example, mycophenolate mofetil has been shown to promote and increase the frequency of $CD4^+CD25^+$ Treg in mice and to induce transplantation tolerance when combined with 1α, 25-dihydroxyvitamin D3 [58]. Another approach is to isolate Treg cells from the recipient and expand these in vitro [59-61]. Infusion of these expanded $CD4^+CD25^+$ Treg has been shown to inhibit graft-versus-host disease [62], and prolong allogeneic skin and heart survival in irradiated mice [63]. To apply Treg in the clinic one has to confirm that they maintain regulatory activity after expansion ex vivo. Many other properties will also need to be examined, including the capacity of expanded Treg cells to survive and migrate appropriately in vivo when re-introduced into the transplant recipient. All of these factors will require careful evaluation in relevant models before such clonally expanded Treg can be used to treat transplant recipients effectively and safely [28, 29].

Tolerogenic dendritic cells

The broad range of powerful immune stimulatory as well as regulatory functions, has made DC targets for vaccine development strategies. This includes cellular vaccination for treatment of cancer or infectious diseases, as well as “negative vaccination” for the treatment of autoimmune diseases and prevention of allograft rejections. The latter can be accomplished by inhibiting the immunostimulatory capacity of DC or more importantly, used to exploit tolerogenic DC to specifically silence immune responses.

Since only low numbers of DC can be isolated from blood, researchers are required to make use of DC progenitor cells isolated from either blood or bone marrow and to differentiate these progenitor cells into DC in vitro. Generation of human-derived DC is realized by culturing monocytes, isolated from blood, in the presence of granulocyte monocyte colony stimulating factor (GM-CSF) and IL-4. Studies on rodent DC make use of bone marrow (BM) cells, which are cultured in the presence of GM-CSF and in some cases also IL-4, to generate DC.

Both human monocyte-derived DC (Mo-DC) and rodent BM-derived DC (BM-DC) have been shown to express MHC and B7 molecules and upon maturation they have the capacity to induce T cell stimulation. Phenotypically, there are some differences in marker expression, Mo-DC are characterised by the expression of CD1a and DC-SIGN [64, 65], murine BM-DC express CD11c [66] and no typical DC marker has been

determined for rat BM-DC, although OX62 has been shown to be expressed by some DC subsets [67]. Modulation of these generated DC will lead to tolerogenic properties as will be described in the next section.

Mixed chimerism

A powerful strategy to induce transplant tolerance is the induction of mixed chimerism. This is a situation where the immune system of donor and recipient are coexisting, thereby actively inducing central and peripheral tolerance. To induce mixed chimerism, recipients are pretreated with T cell depleting antibodies or with co-stimulatory blocking antibodies along with a sublethal dose of total body irradiation. This treatment results in the presence of hematopoietic cells from both the recipient and the donor in the thymus and will consequently delete both host-reactive and donor-reactive T cells, resulting in a peripheral T cell repertoire that is tolerant toward the donor and the host. Studies performed in experimental models have demonstrated that these treatments resulting in mixed chimerism can prolong graft survival [68]. However, this approach to induce transplant tolerance is highly toxic and therefore prohibited as a standard procedure in the clinic. Nevertheless, recently 2 studies described the induction of mixed chimerism in patients receiving a kidney and hematopoietic stem-cell transplantation [69, 70]. Another recent study described the induction of mixed chimerism in a patient who received a completely mismatched liver in the absence of stem-cell infusion. In this patient lymphopenia was detected, most likely caused by a viral infection, which persisted for a half year. In the mean time, passenger leukocytes from the graft largely replaced the recipient's leukocytes [71]. The allografts in patients described in the 3 studies have maintained good function for up to 5 years in the absence of immunosuppressive treatment. These studies demonstrate that tolerance induction in these patients was either caused by the stem cell co-transplantation or by replacement of recipient's leukocytes by donor passenger leukocytes due to lymphopenia and clearly show the power of mixed chimerism.

Generation and application of tolerogenic dendritic cells

In vitro generation and characterization of tolerogenic dendritic cells

One approach to promote the tolerogenicity of DC is to suppress their maturation by using anti-inflammatory cytokines or pharmacological agents or by using genetically engineered DC expressing immunosuppressive molecules, as recently reviewed by several groups [72-75]. One class of agents which has shown promising effects on prevention of DC maturation and which is widely applicable are glucocorticoids (GC). GC are among the most potent immunosuppressive and anti-inflammatory drugs currently available and are effective in the treatment of both Th1 and Th2 associated inflammatory diseases, including allograft rejection, rheumatoid arthritis and asthma [76]. The therapeutic effects of GC were initially ascribed to the strong inhibitory effect on T cells. At the moment, however, it is obvious that also antigen presenting cells (APC) are strongly affected by GC. In clinical practice, various derivatives of GC are used and, as far as we know, there are no differences in the functional effects on

DC between the different compounds, although the in vivo efficacy might be different. Most experimental studies have used dexamethasone (Dex), but other GC have shown similar effects.
A consistent finding has been the inhibitory effect of GC on the development of immature DC from monocytes or bone marrow precursors. The GC-treated human monocytes retain a monocyte/macrophage phenotype with high CD14 expression and no expression of CD1a, a typical DC marker, but also lack expression of CD68, a typical macrophage marker [77-79]. However, these cells do express the DC marker DC-SIGN [80]. Upon stimulation of Dexamethasone-treated DC (DexDC), these cells were strongly hampered in their upregulation of co-stimulatory and MHC molecules [77, 79] (**Fig. 5**). This reduced expression was observed with different modes of activation, including proinflammatory cytokines, LPS or CD40L and cannot be explained by a reduced receptor expression.
Next to reduced levels of co-stimulatory molecules, it was demonstrated that activated DexDC secrete reduced levels of proinflammatory cytokines, including IL-6, TNF-α, IL-1β, and IL-12 [77, 79, 81, 82]. Importantly, the same conditions result in an increased production of the anti-inflammatory cytokine IL-10. It is thought that the balance between IL-10 and IL-12 is important for the outcome of T cell activation. As a consequence, DexDC are poor stimulators of allogeneic T cells [77, 79]. Moreover, T cells recovered from this primary stimulation showed a hyporesponsiveness upon secondary challenge. Hyporesponsiveness was observed for proliferative responses, but especially IFN-γ production was strongly suppressed [80]. This points towards a role for cytokines produced by activated DC, since these are as a determining factor for the development of different functional T cell subsets.

In vivo use of immature dendritic cells

Phenotypically, iDC express low levels of co-stimulatory molecules and do not produce cytokines. Consequently, these cells are involved in the generation of tolerance. In transplantation research, iDC have been used for cell-based therapies to prolong allograft survival or even more importantly to induce donor-specific tolerance. Mice pretreated with donor-derived immature DC showed prolonged allograft survival in models of pancreatic islet and heart transplantation [83-85]. Similarly, the regulatory role of DC was shown by adoptive transfer of allopeptide-loaded recipient-derived lymphoid and myeloid DC that were able to prolong cardiac and islet allograft survival in rat transplantation models [86, 87].

In vivo use of Dexamethason-treated dendritic cells

In view of the need to control DC maturation, and the fear that use of immature DC could result in further maturation after administration, these cells have been treated with several of the modulating agents as described above, including Dex. The effect of DexDC has been studied in transplantation models such as the fully mismatched mice combination of C57BL/6 to Balb/c. Pretreatment of recipients with 10^6 Dex treated D1 cells (a DC cell line of C57BL/6 origin) resulted in prolonged skin graft survival from 17 to 35 days, whereas third party skins were rejected with the same speed

[88]. DC used for treatment were treated with Dex from day 6 onwards, whereas LPS was included at day 7 for the last 48 hours. A similar model and approach was used in a heart transplantation model. Pretreatment with alternatively activated LPS-Dex DC generated from donor bone marrow prolonged heart allograft survival from 10 to 20 days [89]. This prolongation was not observed when Dex-DC were used without LPS activation. The requirement for activation of the tolerogenic DC to obtain optimal effects was also demonstrated with other modes of DC modulation (IL-10 + TGF-β)

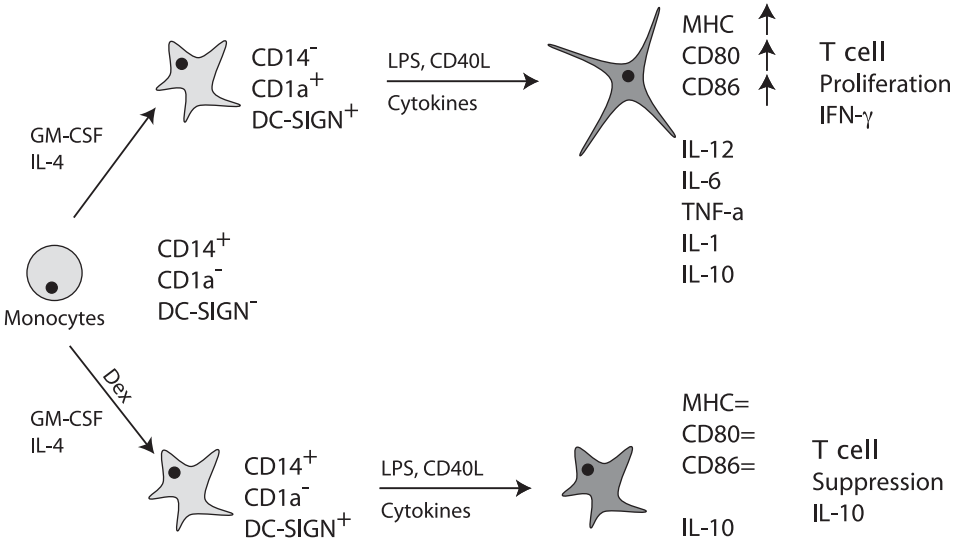

Figure 5. Phenotypic analysis of control DC versus dexamethasone-treated DC.
Human-derived monocytes cultured in the presence of GM-CSF and IL-4 differentiate into immature DC, expressing CD1a and DC-SIGN. Upon maturation signals, including LPS or CD40L, DC upregulate CD80, CD86 and MHC molecule expression levels and secrete IL-1, IL-6, IL-10, IL-12 and TNF-α. In addition, these mature DC have the capacity to induce T cell proliferation and IFN-γ production. In contrast, adding dexamethasone to the human-derived monocytes culture results in DC-SIGN expressing cells, which lack expression of the macrophage marker CD68. Stimulation of these cells with LPS or CD40L does not result in an upregulation of co-stimulatory or MHC molecules. In addition, compared to control DC, a reduced production of proinflammatory cytokines and an elevated level of IL-10 production can be observed. These matured Dex-DC suppress T cell proliferation and IFN-γ production, but induce IL-10 production by the T cells.

[90]. Interestingly, heart allograft prolongation was also obtained when immature DC were combined with Dex treatment in vivo. However, this was only successful when Dex was added after, but not before, the transplantation procedure [89]. In contrast, administration of LPS-Dex-DC of donor origin was not able to prevent rejection of fully mismatched or haploidentical stem cells [91].
In rats tolerogenic DC have been investigated in a August (AUG, RT1^c) to Lewis model. In this model, indefinite renal graft survival was induced using a DC vaccination strategy. In this case, F1 DC (LEW x AUG) were administered to LEW recipients in combination with CTLA4-Ig treatment 10 days before transplantation and recipients were treated with CsA (10 mg/kg/day) for the first 10 days after transplantation. This conditioning resulted in the development of T cell anergy and induction of allo-specific,

self-restricted Treg and a completely normal histology at day 100. Importantly, these results could not be observed when donor DC, treated and applied in the same way, or when mature non-Dex-treated F1 DC were used [92].

Scope of the thesis

The scope of this thesis is to obtain more insight into the effect of dexamethasone-modulated DC in rat kidney transplantation models. Some studies have demonstrated that immature or DexDC have the capacity to prolong allogeneic skin, heart or pancreatic islet survival in murine models. Kidney transplantation in mouse models are very demanding microsurgical procedures, and even in experienced hands only show survival rates between 40 and 70%. Kidney transplantations are therefore preferable performed in rat models. This requires the development of rat specific tools and methods.

To elucidate the effect of tolerogenic DC on allograft survival in rat kidney transplantation models, we assessed the optimal culture conditions to generate bone marrow-derived DC of rat origin. **Chapter 2** describes the phenotype and function of these DC and demonstrates the difference in cytokine production by DC after lipopolysaccharide (LPS) and CD40L stimulation.

Chapter 3 and 4 focus on the effect of tolerogenic donor-derived DC in transplantation models. First, we characterised the tolerogenic properties of DexDC in vitro and demonstrated that DexDC are maturation-resistant. Subsequently, we determined the effect of donor-derived LPS-stimulated DexDC in fully mismatched kidney transplantation models (BN to LEW and DA to LEW) on graft survival and histology (**chapter 3**). In addition, we examined the regulation of the recipient's immune response (**chapter 4**). Although treatment of recipients with LPS-DexDC induced no prolonged graft survival in the examined models, the clear donor-specific T cell hyporesponse and reduced number of infiltrating $CD8^+$ T cells into the graft, demonstrates the regulatory capacity of LPS-DexDC. We think that cell-based therapy can be improved when LPS-DexDC treatment is combined with co-stimulatory blocking antibodies. Co-stimulatory blocking antibodies specifically targeting rat antigens are not widely available. In **chapter 5** we describe the generation and characterisation of a novel anti-rat CD40L blocking antibody, providing new tools for the development of therapeutic strategies to induce long-term allograft survival.

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