Transplantation Reviews 33 (2019) 130–136

Contents lists available at ScienceDirect

Transplantation Reviews

journal homepage: www.elsevier.com/locate/trre

Review article

# Regulatory dendritic cells for human organ transplantation

Angus W. Thomson [a,b,*], Diana M. Metes [a,b], Mohamed B. Ezzelarab [a], Dalia Raïch-Regué [c]

[a] Starzl Transplantation Institute, Department of Surgery, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA
[b] Department of Immunology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA
[c] Nephropathies Research Group, IMIM (Hospital del Mar Medical Research Institute), Barcelona, Spain

ARTICLE INFO

*Keywords:*
Dendritic cells
Immune regulation
Transplantation
Therapy

ABSTRACT

Current immunosuppressive (IS) regimens used to prevent organ allograft rejection have well-recognized side effects, that include enhanced risk of infection and certain types of cancer, metabolic disorders, cardiovascular disease, renal complications and failure to control chronic allograft rejection. The life-long dependency of patients on these IS agents reflects their inability to induce donor-specific tolerance. Extensive studies in rodent and non-human primate models have demonstrated the ability of adoptively-transferred regulatory immune cells (either regulatory myeloid cells or regulatory T cells) to promote transplant tolerance. Consequently, there is considerable interest in the potential of regulatory immune cell therapy to allow safe minimization/complete withdrawal of immunosuppression and the promotion of organ transplant tolerance in the clinic. Here, we review the properties of regulatory dendritic cells (DCreg) with a focus on the approaches being taken to generate human DCreg for clinical testing. We also document the early phase clinical trials that are underway to assess DCreg therapy in clinical organ transplantation as well as in autoimmune disorders.



**Contents**



## 1. Introduction

Major successes have been achieved in clinical organ transplantation over the past 50–60 years, resulting from improvements in surgical procedures, histocompatibility testing, organ preservation and especially, the development of safer and more effective anti-rejection drugs. However, current therapies used to prevent graft rejection have well-recognized limitations. Thus, conventional immunosuppressive (IS) agents, including calcineurin inhibitors (CNIs), mycophenolic acid and corticosteroids, that are commonly used in combination, lack

*Abbreviations:* CM, costimulatory molecule; CNI(s), calcineurin inhibitor(s); DC, dendritic cell(s); DCreg, regulatory dendritic cell(s); IS, immunosuppressive; Treg, regulatory T cell(s).

* Corresponding author at: Starzl Transplantation Institute, University of Pittsburgh School of Medicine, 200 Lothrop Street, Biomedical Science Tower W1540, Pittsburgh, PA 15261, USA.
*E-mail address:* thomsonaw@upmc.edu (A.W. Thomson).

https://doi.org/10.1016/j.trre.2019.05.001
0955-470X/

immunological or antigen (Ag) specificity, enhancing the risk of infection and certain types of cancer. They also cause non-immunological side effects that predispose patients to metabolic disorders, cardiovascular disease and renal and other complications. The failure of anti-rejection agents to control chronic allograft rejection, together with their inability to promote donor-specific tolerance, remain major challenges. The mainstay of IS therapy in kidney transplantation is use of CNIs. Whereas these drugs have reduced acute rejection rates, they have failed to improve long-term allograft survival. Moreover, the cardio-metabolic side effects and nephrotoxicity of these agents have shifted clinical investigation to trials of CNI-free IS regimens.

Although introduction of the co-stimulation blocking agent belatacept (a high affinity variant of cytotoxic T cell associated protein 4, that blocks the B7-CD28 T cell co-stimulatory pathway) in renal transplantation has resulted in improved glomerular filtration rates compared with CNIs, there is an increased risk of early, histologically severe, T cell-mediated rejection [1–3]. In addition, the use of depleting antibodies (Abs) such as anti-thymocyte globulin as induction therapy at the time of transplantation has not guaranteed safe withdrawal of CNIs [4,5]. Further, while efforts to induce donor-specific tolerance in renal transplantation using hematopoietic stem cell infusion have provided encouraging results [6–9], many challenges exist in terms of the safety and widespread applicability of this approach. Thus, the important continuing challenge to transplant researchers and clinicians is to identify novel therapeutic strategies that optimize safety alongside improved therapeutic efficacy, with the ultimate goal of achieving drug-free, donor-specific tolerance.

## 2. The promise of regulatory immune cell therapy

Extensive studies in small animal models (reviewed in [10,11]) have demonstrated the ability of regulatory immune cells to promote transplant tolerance. There is also evidence confirming their ability to prolong renal allograft survival in the more clinically-relevant non-human primate (NHP) model [12–14]. Consequently, considerable interest has developed in the potential of adoptive regulatory immune cell therapy to allow safe minimization/complete withdrawal of IS therapy and promotion of organ transplant tolerance in the clinic [15]. Thus, for example, the ONE Study (www.onestudy.org) has coordinated efforts at several centers in Europe and the US to assess the feasibility, safety and preliminary efficacy of regulatory immune cell infusion in living donor renal transplantation. These studies include early phase testing of regulatory innate immune (myeloid) cells (ie regulatory macrophages [Mreg] or regulatory dendritic cells [DCreg]) and regulatory adaptive immune cells (regulatory T cells [Treg]), combined with standard-of-care immunosuppression in adult kidney transplantation.

Here, we provide a background to the development of DCreg therapy in organ transplantation and then focus on the ex vivo generation and assessment of human DCreg for application in clinical organ transplantation.

## 3. Properties of DCreg

DC are professional bone marrow (BM)-derived Ag-presenting cells (APC) of the innate immune system that are distributed ubiquitously throughout the body [16]. Although comparatively rare leukocytes, they can exert profound influences on other immune cells, particularly T lymphocytes, that are crucial mediators of allograft rejection. It is thought that, in the healthy steady state, like naturally-occurring Treg, DC promote self-tolerance [17]. Thus, in the mouse, constitutive loss of DC leads to spontaneous autoimmunity, characterized by autoAb formation, high numbers of T helper (Th) 1 and Th17 cells and inflammatory bowel disease.

DC are well-equipped to link innate and adaptive immunity in response to appropriate signals and can both instigate and control the nature and extent of cell-mediated immune responses [16,18,19], including those to organ allografts [20–24]. While quiescent, circulating and tissue-resident DC are immature APC, that express low levels of cell surface major histocompatibility complex (MHC) gene products and T cell co-stimulatory molecules (CM). They secrete very low levels of Th1 cell-driving IL-12p70, but comparatively high levels of anti-inflammatory cytokines (IL-10; transforming growth factor [TGF]β). Such tolerogenic or regulatory DC (DCreg) may also express high levels (compared with stimulatory DC) of T cell co-inhibitory ligands (e.g. programed death ligand-1 [PD-L1] = B7-H1), a high ratio of PD-L1 to CD86 [25] and death-inducing ligands (e.g. FasL = CD95L) [26,27]. Consequently, they are weak T cell stimulators and inhibit their responses by inducing anergy or apoptosis. In addition, DCreg have the potential to spare, expand or induce de novo generation of Treg [10,28,29]. On the other hand, DC that respond and functionally mature in response to endogenous or exogenous stimuli, such as endogenous alarmins, Toll-like receptor (TLR) ligands, CD40L or pro-inflammatory cytokines, upregulate cell surface MHC class II and CM expression and IL-12 production, becoming potent inducers of T cell proliferation and their effector functions. Transcriptional determinants of tolerogenic and immunogenic states during DC maturation in vitro have been analyzed [30]. It appears from these studies that DC modulate their ability to prime tolerogenic or immunogenic T cells by expressing a core Ag presentation module that is overlaid by distinctive regulatory modules to promote tolerance or immunity. Also of significance, several recent studies have revealed how the Wnt/β-catenin pathway programs DC to regulate the balance between tolerance and immunity [31].

Multiple subsets of freshly-isolated or ex vivo-generated rodent and human DC [32,33], including non-conventional plasmacytoid DC [34] with the ability to regulate immune responses have been described. Their functions include suppression (in small animals) of skin and organ allograft rejection [10], graft-versus-host disease [35],- an adverse outcome of hematopoietic stem cell transplantation, and various experimental autoimmune disorders [36,37]. The most intensively studied DCreg, however, and those that have entered clinical testing in organ transplantation and autoimmune diseases, are conventional, monocyte-derived myeloid lineage DC.

## 4. Inhibition of experimental allograft rejection by DCreg administration

The first reports indicating that DCreg of donor origin could be used to inhibit experimental allograft rejection were published in 1995/1996 [38,39]. Subsequently, there have been numerous reports that donor-derived DCreg or syngeneic/autologous DCreg (the latter either pulsed or not with donor Ag) infused alone, or together with an IS agent(s), can induce indefinite organ allograft survival/donor-specific tolerance in rodents [10,40]. In more recent studies in a pre-clinical, MHC-mismatched NHP renal allograft model (Table 1), Ezzelarab et al. [14] showed that graft survival was prolonged significantly in rhesus macaques given vitamin (Vit) D3- and IL-10-conditioned donor-derived DCreg, one week before transplant. The DCreg were administered in combination with a minimal IS regimen of costimulation blockade and rapamycin. No evidence of host sensitization (generation of donor-specific Abs) was observed. Median graft survival time was also prolonged in this NHP model when autologous DCreg pulsed with donor Ag (cell membrane microvesicles) were infused one day before transplantation [41]. No effect was observed however, with unpulsed autologous DCreg. These important translational studies have demonstrated both the safety and efficacy of a single (donor-derived) DCreg infusion. They have also provided novel mechanistic insights. Thus, infusion of donor-derived DCreg is associated with (i) selective attenuation of anti-donor memory T cell (Tmem) responses, (ii) Eomesodermin$^{lo}$ CTLA4$^{hi}$ alloreactive CD8$^{+}$ Tmem [42] and (iii) maintenance of donor-reactive CD4$^{+}$CTLA4$^{hi}$ T cells with a regulatory phenotype [43]. These observations in NHP have provided a compelling basis for clinical testing of DCreg in organ transplantation.

**Table 1**
Summary of DCreg therapy in NHP renal transplantation.

| Treatment regimen | Results | Reference(s) |
|---|---|---|
| Pre-transplant infusion (day −7) of *donor-derived* DCreg in combination with a minimal immunosuppressive regimen | Safe; no adverse effects<br>Marked prolongation of graft survival<br>No host sensitization<br>Selective attenuation of donor-reactive memory T cells after transplantation,- potential basis of biomarker analysis for monitoring anti-donor memory cell responses | [14,42,43] |
| Pre-transplant infusion (day −1) of *autologous* DCreg pulsed with donor alloAg (microvesicles) in combination with a minimal immunosuppressive regimen | Safe; no adverse effects<br>Prolongation of median graft survival time<br>No host sensitization | [41] |

## 5. Function of donor-derived DCreg in experimental organ transplantation

The therapeutic efficacy of donor-derived DCreg infused one week before transplantation in heart-allografted mice does not appear to depend on the in vivo persistence of intact donor DCreg [44]. Indeed, it has been demonstrated that endogenous DC mediate the effects of the infused DCreg. The donor-derived DCreg are likely killed by host natural killer cells, and the therapeutic effect depends on the function of quiescent, conventional DC in host secondary lymphoid organs (SLO). Thus, as shown by Morelli and colleagues [45], deletion of host DC prevents the therapeutic effect of the donor-derived DCreg. Host DC can acquire donor MHC Ag via the semi-direct pathway of allorecognition by cross-dressing [46,47] that involves acquisition of entire MHC peptide complexes [48] or via the indirect pathway by Ag transfer from the donor DCreg (cross-presentation) [49,50]. A role for donor-derived microvesicles (exosomes) released by the donor DCreg and acquired by host DC [48], may be an advantage, since it amplifies the effect of the infused DCreg. Consequently, host T cell activation is reduced, indirect pathway T cell deletion occurs, $CD4^+$ T cell-B cell help is impaired, and anti-donor Ab production is suppressed [51]. Independence of the immune regulatory effect of donor-derived DCreg on their in vivo persistence following systemic administration, offers a potential advantage over other cell therapy approaches (in particular, the infusion of Treg), the success of which may depend on in vivo persistence/replication/function of the adoptively-transferred cells. Also, DCreg have the important ability to regulate $CD4^+$ and $CD8^+$ Tmem responses [52–55], a major barrier to long-term organ allograft survival in humans.

## 6. Ex vivo generation of human DCreg

There are insufficient DC in peripheral blood to allow their isolation in adequate numbers for human therapeutic application. Human tolerogenic/regulatory DC can however be generated ex vivo, either from fresh or cryopreserved BM precursor cells or, more commonly (as with immunostimulatory DC vaccines used in cancer therapy), circulating blood monocytes, in response to granulocyte-macrophage colony-stimulating factor (GM-CSF) ± IL-4. Usually, one or more pharmacologic/biologic agents are added to the cultures to prevent/stably inhibit DC maturation and enhance their tolerogenicity [56]. These agents include anti-inflammatory cytokines (IL-10; TGF-β), irreversible nuclear factor (NF)κB inhibitors, anti-sense oligonucleotides targeting CM, anti-inflammatory/IS drugs (CNI, rapamycin, mycophenolate mofetil, corticosteroids), VitD3 and cyclic AMP inducers, in particular prostaglandin E2. Alternatively, a combination of VitD3, IL-10 or dexamethasone with a DC stimulatory agent, such as a TLR4 agonist or a proinflammatory cytokine cocktail has been used to generate DCreg [57–59]. Indeed, exposure to dexamethasone and the TLR4 agonist monophosphoryl lipid A (MPLA) can remove disease-associated transcriptional signatures in monocyte-derived DC from rheumatoid arthritis patients while conferring tolerogenic properties [60]. Genetic manipulation has also been employed to generate DCreg by transfer of selected anti-inflammatory genes to DC using viral or non-viral delivery systems [61–63]. Such genetically-engineered (donor-derived) DC can markedly prolong MHC-mismatched organ allograft survival when adoptively transferred to prospective graft recipients [64].

Gene expression profiling studies performed to date (reviewed by Schinnerling [65]) indicate that tolerogenic properties of human monocyte-derived DC emerge as the result of a specific translational program, rather than from retention of an immature state. Further omics studies are needed, however investigation has been hampered by the fact that many different protocols, targeting distinct signaling pathways, are used to generate DCreg.

At present, there is no consensus as to the optimal agent or combination of agents for the ex vivo generation of human DCreg for clinical use. Consequently, T cell unresponsiveness may be mediated by a range of mechanisms. In Table 2, we have summarized the agents used thus far to generate clinical grade human DCreg from blood monocytes and their phenotypic and functional characteristics. Some common characteristics are: (i) low expression of CM molecules (CD40, CD80 and CD86); (ii) low production of pro-inflammatory cytokines (IL-12, IL-23 and tumor necrosis factor [TNF]-α) and high levels of anti-inflammatory cytokines (mainly IL-10); (iii) resistance to further maturation and to becoming immunogenic after exposure to potent pro-inflammatory stimuli; and (iv) the capacity to attenuate or induce hyporesponsiveness in T cells. Some studies have demonstrated that DCreg-primed T cells have a regulatory phenotype and suppressive capacity. Most DCreg generated as a therapy for autoimmune diseases are loaded with disease-specific Ags in order to induce Ag-specific tolerance once injected into the patient. DCreg that are injected locally (i.e. intra-peritoneally in Crohn's disease or intra-articularly in rheumatoid arthritis) may take up and present Ags from the diseased tissue, and therefore may not be loaded with Ag ex vivo. Regulatory myeloid cells generated to induce tolerance in the context of solid organ transplantation are not loaded with Ags, either because DCreg or regulatory macrophages (Mreg) are derived from the donor [25,66–68], or because recipient-derived DCreg may take up donor Ags derived from the transplanted organ in vivo following their infusion [69,70]. Therefore, there are different protocols for generating clinical-grade human DCreg, although these DCreg share many phenotypical and functional characteristics. Within the past few years an initiative has been taken to standardize DCreg manufacturing,- FACTT (action to Focus and Accelerate Cell-based Tolerance-inducing Therapies). It aims to minimize overlap and maximize comparison of tolerogenic DC approaches [71,72]. This is an important step towards the production of standardized and reproducible DCreg for clinical application.

## 7. Early-phase clinical trials of DCreg in renal and liver transplantation

More than 15 years ago, the ability of immature, autologous monocyte-derived DC pulsed with Ag (flu matrix peptide or keyhole limpet hemocyanin) to inhibit Ag-specific effector T cell function in vivo was demonstrated in healthy adult volunteers [73] (Table 3). The potential of DCreg for therapy of autoimmune disorders [36] has been discussed, and early data supporting the safety of autologous DCreg in rheumatoid arthritis, type-1 diabetes and Crohn's disease have been reported [71] (Table 3). The potential of DCreg for the prevention of rejection and promotion of tolerance after clinical solid organ transplantation has also been discussed in recent reviews [74,75], and early phase clinical trials of DCreg in renal or liver transplantation have begun, both in Europe and the US (Table 3).

**Table 2**
Characteristics of human monocyte-derived DCreg (or *Mreg) generated for clinical use.

| Biological agents | Reported properties of DCreg/*Mreg | Ag (if used) | Intended Application | Reference(s) |
|---|---|---|---|---|
| Dexamethasone and vitamin D3 and TLR-agonist (LPS or MPLA) | — MHC Class II high<br>— CD80 and CD86 intermediate<br>— CD40 and CD83 low<br>— IL-10 and TGF-β high<br>— IL-12, IL-23, TNFα low<br>— Capable of CCR7-dependent migration | Synovial fluid containing joint-associated auto-Ag | Rheumatoid Arthritis | [58,82] |
| Antisense oligonucleotides targeting CD40, CD80 and CD86 | — Surface CD40, CD80 and CD86 low<br>— IL-12 and TNFα low | No Ag used | Type 1 Diabetes | [83,84] |
| BAY 11–7082 (NF-κB inhibitor) | — CD40 low<br>— CD86 relatively high<br>— MHC Class II high<br>— Low T cell stimulatory capacity | Four citrullinated peptides | Rheumatoid Arthritis | [85,86] |
| Vitamin D3 and Cytokine cocktail (TNFα, PGE2, IL-1β) | — CD40, CD80 and CD86 low<br>— IL-12 low<br>— IL-10 high<br>— Confer Ag-specific hyporesponsiveness in T cells<br>— Stable and resistant to further stimulation | Seven myelin peptides | Multiple Sclerosis | [57] |
| Dexamethasone and Vitamin A and Cytokine cocktail (IL-1β, IL-6, TNF-α and PGE2) | — CD80, CD83 and HLA-DR low<br>— CD86 high<br>— IL-10 high<br>— IL-12p70, IL-23 not detected<br>— Low T cell stimulatory capacity<br>— Stable and resistant to further stimulation | No Ag used | Crohn's disease | [87,88] |
| IL-10 and Cytokine cocktail (TNFα, PGE2, IL-1β) | — CD80, CD86, CD40 and CD83 low<br>— ILT3 and ILT4 up-regulated<br>— IL-10 very high<br>— IL-13 and IFN-γ low<br>— Low T cell stimulatory capacity<br>— DCreg-primed $CD4^+$<br>— T cells display a regulatory profile and downmodulate T cell responses | No Ag used | N/A | [59,89] |
| Low dose GM-CSF (recipient DCreg) | — CD80, CD86 low<br>— CD83 negative, even after LPS/IFN-γ stimulation<br>— IL-12 very low, IL-10 high | No Ag used | Kidney transplant from living donor | [69,70] |
| M-CSF and IFN-γ (donor *Mreg) | — CD80, CD16 low<br>— CD14, CD86, CD85h, CD258, IDO, DHRS9 positive<br>— convert allogeneic $CD4^+$ T cells to IL-10--producing, $TIGIT^+$ $FoxP3^+$-induced Treg | No Ag used | Kidney transplant from living donor | [66,67] |
| vitamin D3 and IL-10 (donor DCreg) | — CD1c, CD209, CD11c and CCR7 positive<br>— HLA-DR, CD80, CD86 low<br>— PD-L1 high and PD-L1:CD86 ratio elevated (correlated with DCreg function)<br>— Resistance to maturation (LPS; pro--inflammatory cytokines; CD40L)<br>— IL-1β, TNFα, IL-12p70 and IFNγ low; IL-10 high<br>— selectively attenuate anti-donor $CD8^+$ memory T cell responses | No Ag used | Liver transplant or kidney transplant from living donor | [25,68] |

Abbreviations: DHRS9 = dehydrogenase/reductase 9; Foxp3 = forkhead box p3; GM-CSF = granulocyte-macrophage colony-stimulating factor; IDO = indoleamine dioxygenase; ILT = immunoglobulin-like transcript; M-CSF = macrophage colony-stimulating factor; MPLA = monophosphoryl lipid A; PD-L1 = programed death ligand 1; PGE2 = prostaglandin E2; TGFβ = transforming growth factor β; TIGIT = T cell immunoreceptor with immunoglobulin and ITIM (immunoreceptor tyrosine-based inhibition motif) domains; TNF = tumor necrosis factor.

### 7.1. Renal transplantation

Based on the therapeutic effect of autologous DCreg documented in their extensive preceding rodent studies [76–78], investigators at the University of Nantes (France) have infused unpulsed autologous DCreg one day before transplant, in living donor renal transplantation recipients given standard-of-care (SOC) triple drug (azathioprine, steroid, tacrolimus) IS therapy (clinicaltrials.gov identifier: NCT0225055 [70]). A National Institutes of Health (NIH)-supported cell dose escalation trial to test the safety of a single infusion of donor-derived DCreg [79], together with SOC IS: mycophenolic acid (MPA), steroid and tacrolimus, one week before living donor renal transplantation (NCT 0364265) will begin at the University of Pittsburgh (US) in 2019.

### 7.2. Liver transplantation

The possibility that DCreg administration, as a novel adjunct induction therapy, may promote immunological mechanisms conducive to induction of donor-specific T cell hyporesponsiveness (tolerance) and enable early withdrawal of all IS after liver transplantation, carries the potential important advantage of sparing patients the side effects of long-term IS, particularly CNI. Recently, in a multi-center study [80] of early post-transplant IS drug withdrawal (CNI-based therapy; no induction) in liver transplantation, IS minimization starting 12–14 months post-transplant was tolerated by the majority of patients, while complete IS withdrawal was achieved in 13% of those that qualified for the minimization protocol. This degree of success provides a potential

**Table 3**
Early phase clinical testing of human DCreg (or *Mreg).

| Study | Reference(s) |
|---|---|
| Healthy volunteers | |
| In normal humans, adoptive transfer of immature autologous monocyte-derived DCreg inhibits Ag-specific effector T cell function | [73] |
| Autoimmune disease | |
| Early data support the safety of *autologous* (including Ag-pulsed) DCreg administration (s.c./i.d.) in autoimmune disease (rheumatoid arthritis; type-1 diabetes, Crohn's disease) | [58,83,86,88] |
| Organ transplantation | |
| Initial testing of *donor-derived* regulatory macrophage (*Mreg) infusion (a related myeloid cell product) in living donor renal transplant patients has proved safe (NCGT 02085629) | [66] |
| Infusion of *autologous* DCreg 1 day before living donor renal transplantation (NCT03164265) | [74] |
| Infusion of *donor-derived* DCreg 1 week before living donor kidney (NCT03164265) or liver transplantation (NCT03726307) | [68] |

basis for assessing the impact of innovative strategies, including DCreg infusion, aimed at improving the incidence of safe withdrawal of IS therapy and operational tolerance in human liver transplantation.

At the University of Pittsburgh, a first-in-human, single center, open-label, phase I/II study (NCT03164265) to test the safety and preliminary efficacy of a single infusion of donor-derived DCreg in de novo adult living donor liver transplant recipients [68] has been initiated. Patients receive SOC IS (MPA, steroid and tacrolimus), without Ab induction. Good manufacturing practice (GMP) grade DCreg are generated [81] in VitD3 and IL-10 from monocytes obtained by leukapheresis from prospective living organ donors, and infused as induction therapy into their respective recipients, 7 days before transplant. The DCreg dose range (2.5–10 $\times$ $10^6$/kg) corresponds to the range for which both safety and efficacy were demonstrated in the preclinical NHP renal transplant model [14]. A half dose of MPA is administered concomitant with the DCreg infusion and until the time of transplant, to minimize any potential risk of host sensitization. In eligible patients, determined by permissive liver function tests and (at 12 months post-transplant) a permissive liver biopsy, weaning of the remaining IS drug (tacrolimus) begins at 12 months and continues to complete withdrawal by month 18. Follow-up continues for 3 years after the last dose of IS.

**Table 5**
Questions posed by DCreg therapy.

| |
|---|
| Manufacturing (choice of agents(s) to confer tolerogenicity) |
| Cell dosage |
| Timing/frequency of cell infusion |
| Relevance of Ag pulsing |
| Migratory abilities and relevance (depending on the site of administration) |
| Longevity; fate of donor MHC gene products |
| Potential reprogramming under inflammatory conditions |
| 'Appropriate' immunosuppressive agents/regimen |
| Overcoming immunologic memory |
| Comparison with other types of regulatory immune cell therapy (e.g. Treg) |
| Cost |

In addition to DCreg, another type of regulatory myeloid cell-, regulatory macrophages (Mreg), as well as Treg are being evaluated as cellular therapies in clinical renal or liver transplantation. Table 4 lists phase I (safety) or phase I/II (safety and preliminary efficacy) trials of DCreg, Mreg and Treg that have been registered, and the target doses of cells used (single infusion) in renal and liver transplantation. While Mreg are being tested in kidney transplantation, DCreg and Treg are being tested in both kidney and liver transplantation. Many questions are posed and shared by these studies, as indicated in Table 5, in particular: cell dosages, timing of infusion, optimal IS regimen and efficacy. The results of these initial and subsequent trials are awaited with great interest. Experience to date indicates that the cell infusions are safe.

## 8. Conclusions and future prospects

Although there is no current consensus regarding the optimal protocol for ex vivo generation of human clinical grade DCreg, these cell products share many phenotypic and functional characteristics. The initiative to standardize a DCreg manufacturing protocol is an important step in the translation of regulatory immune cell therapy to clinical practice for treatment of autoimmune diseases and organ transplant rejection.

Studies in rodent models have demonstrated the ability of regulatory immune cells to prolong organ renal allograft survival and induce transplant tolerance. Furthermore, recent translational studies in NHP have demonstrated both the safety and efficacy of a single donor-derived DCreg infusion in fully MHC-mismatched renal transplantation, providing a compelling basis for clinical testing of DCreg in organ

**Table 4**
Reported/registered clinical trials of DCreg, Mreg or Treg in kidney or liver transplantation.

| Cell type | Organ K (kidney); L (liver) | Type of trial | Target dose (range) | Trial ID |
|---|---|---|---|---|
| Regulatory DC | | | | |
| Autologous, blood monocyte-derived DCreg | K | Phase I/II | $10^6$/kg | Nantes (ONE STUDY) NCT02252055 |
| Donor blood monocyte-derived DCreg | K | Phase I | 0.5-5 $\times$ $10^6$/kg (dose escalation) | Pittsburgh NCT03164265 |
| Donor blood monocyte-derived DCreg | L | Phase I/II | 2.5-10 $\times$ $10^6$/kg | Pittsburgh NCT03726307 |
| [a]Regulatory macrophages (Mreg) | | | | |
| Donor blood monocyte-derived Mreg | K | Phase I/II | 2.5–7.5 $\times$ $10^6$/kg | Regensburg (ONE STUDY) NCT 02085629 |
| Regulatory T cells | | | | |
| Autologous, polyclonally-expanded Treg | K, L | Phase I/II | 0.5-10 $\times$ $10^6$/kg | Charite (Germany) (ONE STUDY) NCT02371434 (K); KCL-Oxford (ONE STUDY) NCT02129881 (K); KCL NCT02166177 (L) |
| Autologous, polyclonal or donor Ag alloreactive Treg | K | Phase I/II | 400 $\pm$ 100 $\times$ $10^6$ (TOTAL) | UCSF NCT02711826 |
| Autologous, donor Ag-alloreactive Treg | K, L | Phase I | 50-900 $\times$ $10^6$ (TOTAL) | UCSF (ONE STUDY) NCT02244801 (K); UCSF NCT02188719 (L); UCSF NCT02474199(L) |
| Autologous, donor alloreactive 'Treg-enriched' (co-cultured with CoSB) | K, L | Phase I | N/A (K); 0.23–6.37 $\times$ $10^6$/kg (L) | MGH NCT02091232 (ONE STUDY) (K); JAPAN UMIN-000015789 (L) |

KCL, King's College, London; MGH, Massachusetts General Hospital; UCSF, University of California San Francisco.
[a] For a more complete listing of Treg trials, in organ and bone marrow transplantation, see Kawai et al. *Human Immunology* 2018 [90].

transplantation. Early phase clinical trials of DCreg in renal and liver transplantation have begun both in Europe and the US. Preliminary observations have confirmed the safety of DCreg administration. The stage is thus set for evaluation of the efficacy of DCreg in organ transplantation.

## Acknowledgments

The authors' work is supported by National Institutes of Health grants R01 AI118777, U19 AI131453 and U01 AI136779. We thank our colleagues in the laboratory and clinic for valuable discussion and support.

## References

[1] Wojciechowski D, Vincenti F. Current status of costimulatory blockade in renal transplantation. Curr Opin Nephrol Hypertens 2016;25:583–90.
[2] de Graav GN, Baan CC, Clahsen-van Groningen MC, Kraaijeveld R, Dieterich M, Verschoor W, et al. A randomized controlled clinical trial comparing belatacept with tacrolimus after De Novo kidney transplantation. Transplantation 2017;101:2571–81.
[3] Newell KA, Mehta AK, Larsen CP, Stock PG, Farris AB, Mehta SG, et al. Lessons learned: early termination of a randomized trial of calcineurin inhibitor and corticosteroid avoidance using belatacept. Am J Transplant 2017;17:2712–9.
[4] Hricik DE, Formica RN, Nickerson P, Rush D, Fairchild RL, Poggio ED, et al. C. Clinical trials in organ transplantation, adverse outcomes of tacrolimus withdrawal in immune-quiescent kidney transplant recipients. J Am Soc Nephrol 2015;26:3114–22.
[5] Ekberg H, Tedesco-Silva H, Demirbas A, Vitko S, Nashan B, Gurkan A, et al. Reduced exposure to calcineurin inhibitors in renal transplantation. N Engl J Med 2007;357:2562–75.
[6] Kawai T, Leventhal J, Madsen JC, Strober S, Turka LA, Wood KJ. Tolerance: one transplant for life. Transplantation 2014;98:117–21.
[7] Sykes M. Immune tolerance in recipients of combined haploidentical bone marrow and kidney transplantation. Bone Marrow Transplant 2015;50:S82–6.
[8] Strober S. Use of hematopoietic cell transplants to achieve tolerance in patients with solid organ transplants. Blood 2016;127:1539–43.
[9] Chhabra AY, Leventhal J, Merchak AR, Ildstad S. HSCT-based approaches for tolerance induction in renal transplant. Transplantation 2017;101:2682–90.
[10] Morelli AE, Thomson AW. Tolerogenic dendritic cells and the quest for transplant tolerance. Nat Rev Immunol 2007;7:610–21.
[11] Wood KJ, Bushell A, Hester J. Regulatory immune cells in transplantation. Nat Rev Immunol 2012;12:417–30.
[12] Bashuda H, Kimikawa M, Seino K, Kato Y, Ono F, Shimizu A, et al. Renal allograft rejection is prevented by adoptive transfer of anergic T cells in nonhuman primates. J Clin Invest 2005;115:1896–902.
[13] Ma A, Qi S, Song L, Hu Y, Dun H, Massicotte E, et al. Adoptive transfer of CD4+CD25+ regulatory cells combined with low-dose sirolimus and anti-thymocyte globulin delays acute rejection of renal allografts in Cynomolgus monkeys. Int Immunopharmacol 2011;11:618–29.
[14] Ezzelarab MB, Zahorchak AF, Lu L, Morelli AE, Chalasani G, Demetris AJ, et al. Regulatory dendritic cell infusion prolongs kidney allograft survival in nonhuman primates. Am J Transplant 2013;13:1989–2005.
[15] Geissler EK, Hutchinson JA. Cell therapy as a strategy to minimize maintenance immunosuppression in solid organ transplant recipients. Curr Opin Organ Transplant 2013;18:408–15.
[16] Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature 1998;392:245–52.
[17] Ohnmacht C, Pullner A, King SB, Drexler I, Meier S, Brocker T, et al. Constitutive ablation of dendritic cells breaks self-tolerance of CD4 T cells and results in spontaneous fatal autoimmunity. J Exp Med 2009;206:549–59.
[18] Steptoe RJ, Thomson AW. Dendritic cells and tolerance induction. Clin Exp Immunol 1996;105:397–402.
[19] Steinman RM, Hawiger D, Nussenzweig MC. Tolerogenic dendritic cells. Annu Rev Immunol 2003;21:685–711.
[20] Thomson AW, Lu L. Dendritic cells as regulators of immune reactivity: implications for transplantation. Transplantation 1999;68:1–8.
[21] Morelli AE, Hackstein H, Thomson AW. Potential of tolerogenic dendritic cells for transplantation. Semin Immunol 2001;13:323–35.
[22] Coates PT, Thomson AW. Dendritic cells, tolerance induction and transplant outcome. Am J Transplant 2002;2:299–307.
[23] Morelli AE, Thomson AW. Dendritic cells: regulators of alloimmunity and opportunities for tolerance induction. Immunol Rev 2003;196:125–46.
[24] Yamano T, Watanabe S, Hasegawa H, Suzuki T, Abe R, Tahara H, et al. Ex vivo-expanded DCs induce donor-specific central and peripheral tolerance and prolong the acceptance of donor skin grafts. Blood 2011;117:2640–8.
[25] Zahorchak AF, Macedo C, Hamm DE, Butterfield LH, Metes DM, Thomson AW. High PD-L1/CD86 MFI ratio and IL-10 secretion characterize human regulatory dendritic cells generated for clinical testing in organ transplantation. Cell Immunol 2018;323:9–18.
[26] Lu L, Qian S, Hershberger PA, Rudert WA, Lynch DH, Thomson AW. Fas ligand (CD95L) and B7 expression on dendritic cells provide counter-regulatory signals for T cell survival and proliferation. J Immunol 1997;158:5676–84.
[27] Lu L, Thomson AW. Manipulation of dendritic cells for tolerance induction in transplantation and autoimmune disease. Transplantation 2002;73:S19–22.
[28] Huang H, Dawicki W, Zhang X, Town J, Gordon JR. Tolerogenic dendritic cells induce CD4+CD25hiFoxp3+ regulatory T cell differentiation from CD4+CD25-/loFoxp3- effector T cells. J Immunol 2010;185:5003–10.
[29] Raker VK, Domogalla MP, Steinbrink K. Tolerogenic dendritic cells for regulatory T cell induction in man. Front Immunol 2015;6:569.
[30] Vander Lugt B, Riddell J, Khan AA, Hackney JA, Lesch J, DeVoss J, et al. Transcriptional determinants of tolerogenic and immunogenic states during dendritic cell maturation. J Cell Biol 2017;216:779–92.
[31] Swafford D, Manicassamy S. Wnt signaling in dendritic cells: its role in regulation of immunity and tolerance. Discov Med 2015;19:303–10.
[32] Liu YJ. Dendritic cell subsets and lineages, and their functions in innate and adaptive immunity. Cell 2001;106:259–62.
[33] Ueno H, Klechevsky E, Morita R, Aspord C, Cao T, Matsui T, et al. Dendritic cell subsets in health and disease. Immunol Rev 2007;219:118–42.
[34] Rogers NM, Isenberg JS, Thomson AW. Plasmacytoid dendritic cells: no longer an enigma and now key to transplant tolerance? Am J Transplant 2013;13:1125–33.
[35] Stenger EO, Turnquist HR, Mapara MY, Thomson AW. Dendritic cells and regulation of graft-versus-host disease and graft-versus-leukemia activity. Blood 2012;119:5088–103.
[36] Hilkens CM, Isaacs JD, Thomson AW. Development of dendritic cell-based immunotherapy for autoimmunity. Int Rev Immunol 2010;29:156–83.
[37] Thomson AW, Robbins PD. Tolerogenic dendritic cells for autoimmune disease and transplantation. Ann Rheum Dis 2008;67:iii90–96.
[38] Rastellini C, Lu L, Ricordi C, Starzl TE, Rao AS, Thomson AW. Granulocyte/macrophage colony-stimulating factor-stimulated hepatic dendritic cell progenitors prolong pancreatic islet allograft survival. Transplantation 1995;60:1366–70.
[39] Fu F, Li Y, Qian S, Lu L, Chambers F, Starzl TE, et al. Costimulatory molecule-deficient dendritic cell progenitors (MHC class $II^+$, $CD80^{dim}$, $CD86^-$) prolong cardiac allograft survival in nonimmunosuppressed recipients. Transplantation 1996;62:659–65.
[40] Beriou G, Moreau A, Cuturi MC. Tolerogenic dendritic cells: applications for solid organ transplantation. Curr Opin Organ Transplant 2012;17:42–7.
[41] Ezzelarab MB, Raich-Regue D, Lu L, Zahorchak AF, Perez-Gutierrez A, Humar A, et al. Renal allograft survival in nonhuman primates infused with donor antigen-pulsed autologous regulatory dendritic cells. Am J Transplant 2017;17:1476–89.
[42] Ezzelarab MB, Lu L, Guo H, Zahorchak AF, Shufesky WF, Cooper DK, et al. Eomesodermin$^{lo}$ CTLA4$^{hi}$ alloreactive $CD8^+$ memory T cells are associated with prolonged renal transplant survival induced by regulatory dendritic cell infusion in CTLA4 immunoglobulin-treated nonhuman primates. Transplantation 2016;100:91–102.
[43] Ezzelarab MB, Lu L, Shufesky WF, Morelli AE, Thomson AW. Donor-derived regulatory dendritic cell infusion maintains donor-reactive CD4(+)CTLA4(hi) T cells in non-human primate renal allograft recipients treated with CD28 co-stimulation blockade. Front Immunol 2018;9:250.
[44] Divito SJ, Wang Z, Shufesky WJ, Liu Q, Tkacheva OA, Montecalvo A, et al. Endogenous dendritic cells mediate the effects of intravenously injected therapeutic immunosuppressive dendritic cells in transplantation. Blood 2010;116:2694–705.
[45] Wang Z, Divito SJ, Shufesky WJ, Sumpter T, Wang H, Tkacheva OA, et al. Dendritic cell therapies in transplantation revisited: deletion of recipient DCs deters the effect of therapeutic DCs. Am J Transplant 2012;12:1398–408.
[46] Liu Q, Rojas-Canales DM, Divito SJ, Shufesky WJ, Stolz DB, Erdos G, et al. Donor dendritic cell-derived exosomes promote allograft-targeting immune response. J Clin Invest 2016;126:2805–20.
[47] Marino J, Babiker-Mohamed MH, Crosby-Bertorini P, Paster JT, LeGuern C, Germana S, et al. Donor exosomes rather than passenger leukocytes initiate alloreactive T cell responses after transplantation. Sci Immunol 2016;1 pii: aaf8759.
[48] Lindenbergh MFS, Stoorvogel W. Antigen presentation by extracellular vesicles from professional antigen-presenting cells. Annu Rev Immunol 2018;36:435–59.
[49] Zhuang Q, Liu Q, Divito SJ, Zeng Q, Yatim KM, Hughes AD, et al. Graft-infiltrating host dendritic cells play a key role in organ transplant rejection. Nat Commun 2016;7:12623.
[50] Joffre OP, Segura E, Savina A, Amigorena S. Cross-presentation by dendritic cells. Nat Rev Immunol 2012;12:557–69.
[51] Morelli AE, Thomson AW. Orchestration of transplantation tolerance by regulatory dendritic cell therapy or in-situ targeting of dendritic cells. Curr Opin Organ Transplant 2014;19:348–56.
[52] Kenna TJ, Thomas R, Steptoe RJ. Steady-state dendritic cells expressing cognate antigen terminate memory CD8+ T-cell responses. Blood 2008;111:2091–100.
[53] Kenna TJ, Waldie T, McNally A, Thomson M, Yagita H, Thomas R, et al. Targeting antigen to diverse APCs inactivates memory CD8+ T cells without eliciting tissue-destructive effector function. J Immunol 2010;184:598–606.
[54] Nasreen M, Waldie TM, Dixon CM, Steptoe RJ. Steady-state antigen-expressing dendritic cells terminate CD4+ memory T-cell responses. Eur J Immunol 2010;40:2016–25.
[55] Kleijwegt FS, Jansen DT, Teeler J, Joosten AM, Laban S, Nikolic T, et al. Tolerogenic dendritic cells impede priming of naive CD8(+) T cells and deplete memory CD8(+) T cells. Eur J Immunol 2013;43:85–92.
[56] Hackstein H, Thomson AW. Dendritic cells: emerging pharmacological targets of immunosuppressive drugs. Nat Rev Immunol 2004;4:24–35.
[57] Raich-Regue D, Grau-Lopez L, Naranjo-Gomez M, Ramo-Tello C, Pujol-Borrell R, Martinez-Caceres E, et al. Stable antigen-specific T-cell hyporesponsiveness induced by tolerogenic dendritic cells from multiple sclerosis patients. Eur J Immunol 2012;42:771–82.
[58] Bell GM, Anderson AE, Diboll J, Reece R, Eltherington O, Harry RA, et al. Autologous tolerogenic dendritic cells for rheumatoid and inflammatory arthritis. Ann Rheum Dis 2017;76:227–34.

[59] Boks MA, Kager-Groenland JR, Haasjes MS, Zwaginga JJ, van Ham SM, ten Brinke A. IL-10-generated tolerogenic dendritic cells are optimal for functional regulatory T cell induction–a comparative study of human clinical-applicable DC. Clin Immunol 2012;142:332–42.
[60] Garcia-Gonzalez PA, Schinnerling K, Sepulveda-Gutierrez A, Maggi J, Hoyos L, Morales RA, et al. Treatment with dexamethasone and monophosphoryl lipid a removes disease-associated transcriptional signatures in monocyte-derived dendritic cells from rheumatoid arthritis patients and confers tolerogenic features. Front Immunol 2016;7:458.
[61] Takayama T, Nishioka Y, Lu L, Lotze MT, Tahara H, Thomson AW. Retroviral delivery of viral interleukin-10 into myeloid dendritic cells markedly inhibits their allostimulatory activity and promotes the induction of T-cell hyporesponsiveness. Transplantation 1998;66:1567–74.
[62] Lu L, Lee WC, Takayama T, Qian S, Gambotto A, Robbins PD, et al. Genetic engineering of dendritic cells to express immunosuppressive molecules (viral IL-10, TGF-beta, and CTLA4Ig). J Leukoc Biol 1999;66:293–6.
[63] Humbert JM, Halary F. Viral and non-viral methods to genetically modify dendritic cells. Curr Gene Ther 2012;12:127–36.
[64] Bonham CA, Peng L, Liang X, Chen Z, Wang L, Ma L, et al. Marked prolongation of cardiac allograft survival by dendritic cells genetically engineered with NF-kappa B oligodeoxyribonucleotide decoys and adenoviral vectors encoding CTLA4-Ig. J Immunol 2002;169:3382–91.
[65] Schinnerling K, Garcia-Gonzalez P, Aguillon JC. Gene expression profiling of human monocyte-derived dendritic cells - searching for molecular regulators of tolerogenicity. Front Immunol 2015;6:528.
[66] Hutchinson JA, Riquelme P, Sawitzki B, Tomiuk S, Miqueu P, Zuhayra M, et al. Cutting edge: immunological consequences and trafficking of human regulatory macrophages administered to renal transplant recipients. J Immunol 2011;187:2072–8.
[67] Riquelme P, Haarer J, Kammler A, Walter L, Tomiuk S, Ahrens N, et al. TIGIT(+) iTregs elicited by human regulatory macrophages control T cell immunity. Nat Commun 2018;9:2858.
[68] Thomson AW, Humar A, Lakkis FG, Metes DM. Regulatory dendritic cells for promotion of liver transplant operational tolerance: rationale for a clinical trial and accompanying mechanistic studies. Hum Immunol 2018;79:314–21.
[69] Geissler EK. The ONE Study compares cell therapy products in organ transplantation: introduction to a review series on suppressive monocyte-derived cells. Transp Res 2012;1:11.
[70] Moreau A, Alliot-Licht B, Cuturi MC, Blancho G. Tolerogenic dendritic cell therapy in organ transplantation. Transpl Int 2017;30:754–64.
[71] Ten Brinke A, Hilkens CM, Cools N, Geissler EK, Hutchinson JA, Lombardi G, et al. Clinical use of tolerogenic dendritic cells-harmonization approach in European collaborative effort. Mediat Inflamm 2015;2015 471719.
[72] Ten Brinke A, Martinez-Llordella M, Cools N, Hilkens CMU, van Ham SM, Sawitzki B, et al. Ways forward for tolerance-inducing cellular therapies- an AFACTT perspective. Front Immunol 2019;10:181.
[73] Dhodapkar MV, Steinman RM. Antigen-bearing immature dendritic cells induce peptide-specific CD8(+) regulatory T cells in vivo in humans. Blood 2002;100:174–7.
[74] Marin E, Cuturi MC, Moreau A. Tolerogenic dendritic cells in solid organ transplantation: where do we stand? Front Immunol 2018;9:274.
[75] Obregon C, Kumar R, Pascual MA, Vassalli G, Golshayan D. Update on dendritic cell-induced immunological and clinical tolerance. Front Immunol 2017;8:1514.
[76] Beriou G, Peche H, Guillonneau C, Merieau E, Cuturi MC. Donor-specific allograft tolerance by administration of recipient-derived immature dendritic cells and suboptimal immunosuppression. Transplantation 2005;79:969–72.
[77] Peche H, Trinite B, Martinet B, Cuturi MC. Prolongation of heart allograft survival by immature dendritic cells generated from recipient type bone marrow progenitors. Am J Transplant 2005;5:255–67.
[78] Segovia M, Louvet C, Charnet P, Savina A, Tilly G, Gautreau L, et al. Autologous dendritic cells prolong allograft survival through Tmem176b-dependent antigen cross-presentation. Am J Transplant 2014;14:1021–31.
[79] Thomson AW, Zahorchak AF, Ezzelarab MB, Butterfield LH, Lakkis FG, Metes DM. Prospective clinical testing of regulatory dendritic cells in organ transplantation. Front Immunol 2016;7:15.
[80] Shaked A, Feng S, Punch J, Reyes J, Levitsky J, Klintmalm G, et al. Early post-transplant immunosuppression (IS) withdrawal - final outcomes of the ITN030ST AWISH Study. Am J Transplant 2016;16:269.
[81] Zahorchak AF, Perez-Gutierrez A, Ezzelarab MB, Thomson AW. Monocytic myeloid-derived suppressor cells generated from rhesus macaque bone marrow enrich for regulatory T cells. Cell Immunol 2018;329:50–5.
[82] Hilkens CM, Isaacs JD. Tolerogenic dendritic cell therapy for rheumatoid arthritis: where are we now? Clin Exp Immunol 2013;172:148–57.
[83] Giannoukakis N, Phillips B, Finegold D, Harnaha J, Trucco M. Phase I (safety) study of autologous tolerogenic dendritic cells in type 1 diabetic patients. Diabetes Care 2011;34:2026–32.
[84] Phillips BE, Garciafigueroa Y, Engman C, Trucco M, Giannoukakis N. Tolerogenic dendritic cells and T-regulatory cells at the clinical trials crossroad for the treatment of autoimmune disease; emphasis on type 1 diabetes therapy. Front Immunol 2019;10:148.
[85] Thomas R, Street S, Ramnoruth N. Safety and preliminary evidence of efficacy in a phase I clinical trial of autologous tolerizing dendritic cells exposed to citrullinated peptides (Rheumavax) in patients with rheumatoid arthritis. Ann Rheum Dis 2011;70:169.
[86] Benham H, Nel HJ, Law SC, Mehdi AM, Street S, Ramnoruth N, et al. Citrullinated peptide dendritic cell immunotherapy in HLA risk genotype-positive rheumatoid arthritis patients. Sci Transl Med 2015;7 290ra287.
[87] Cabezon R, Ricart E, Espana C, Panes J, Benitez-Ribas D. Gram-negative enterobacteria induce tolerogenic maturation in dexamethasone conditioned dendritic cells. PLoS One 2012;7:e52456.
[88] Jauregui-Amezaga A, Cabezon R, Ramirez-Morros A, Espana C, Rimola J, Bru C, et al. Intraperitoneal administration of autologous tolerogenic dendritic cells for refractory Crohn's disease: a phase I study. J Crohns Colitis 2015;9:1071–8.
[89] Boks MA, Kager-Groenland JR, van Ham SM, ten Brinke A. IL-10/IFNgamma co-expressing CD4(+) T cells induced by IL-10 DC display a regulatory gene profile and downmodulate T cell responses. Clin Immunol 2016;162:91–9.
[90] Kawai K, Uchiyama M, Hester J, Wood K, Issa F. Regulatory T cells for tolerance. Hum Immunol 2018;79:294–303.