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Tolerogenic Dendritic Cells in Organ Transplantation

Jordi Ochando[1,2], **Farideh Ordikhani**[1], **Stefan Jordan**[1], **Peter Boros**[1], **Angus W. Thomson**[3]

[1]Department of Oncological Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA

[2]Immunología de Trasplantes, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Madrid, Spain

[3]Starzl Transplantation Institute, Department of Surgery and Department of Immunology, University of Pittsburgh School of Medicine, Pittsburgh, PA USA

Abstract

Dendritic cells (DCs) are specialized cells of the innate immune system that are characterized by their ability to take up, process and present antigens (Ag) to effector T cells. They are derived from DC precursors produced in the bone marrow. Different DC subsets have been described according to lineage-specific transcription factors required for their development and function. Functionally, DCs are responsible for inducing Ag-specific immune responses that mediate organ transplant rejection. Consequently, to prevent anti-donor immune responses, therapeutic strategies have been directed towards the inhibition of DC activation. In addition however, an extensive body of pre-clinical research, using transplant models in rodents and non-human primates, has established a central role of DCs in the negative regulation of alloimmune responses. As a result, DCs have been employed as cell-based immunotherapy in early phase I/II clinical trials in organ transplantation. Together with in vivo targeting through use of myeloid cell-specific nanobiologics, DC manipulation represents a promising approach for the induction of transplantation tolerance. In this review, we summarize fundamental characteristics of DCs and their roles in promotion of central and peripheral tolerance. We also discuss their clinical application to promote improved long-term outcomes in organ transplantation.

Keywords

Dendritic cells; immune tolerance; organ transplantation

Basic principles

Dendritic cells (DCs) were first identified and characterized by Steinman and Cohn in 1973–4 [1, 2]. These cells are uniquely specialized in antigen (Ag) uptake, processing and presentation, with the ability to stimulate T cell proliferation in mixed leukocyte reactions (MLR) more potently than other Ag-presenting cells (APC) [3]. They link innate and adaptive immune responses [4]. DCs are derived from committed DC precursors (pre-DCs)

Corresponding authors: Jordi Ochando: Jordi.ochando@mssm.edu or Angus W. Thomson: thomsonaw@upmc.edu.
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in the bone marrow (BM) and comprise different subsets, according to their ontogeny, tissue distribution, phenotype and function.

The main conventional DC (cDC) subsets include cDC1 and cDC2, that are defined by lineage-specific transcription factors, such as interferon regulatory factor (IRF)8, basic leucine zipper ATF-like transcription factor 3 (BATF3) and inhibitor of DNA binding 2 (ID2) (cDC1) and IRF4 and zinc finger E-box binding homeobox 2 (ZEB2) (cDC2). In addition, cell surface phenotypic markers may be used to characterize cDC1 (X-C motif chemokine receptor 1 [XCR1] and C-type lectin domain family 9 member A [Clec9a]) and cDC2 (CD172). Development of a separate subset, non-conventional plasmacytoid DCs (pDCs), depends on the transcription factor E2–2. pDCs are characterized phenotypically by the absence of myeloid Ags and the expression of CD123 (IL-3Rα). cDCs are located in lymphoid and non-lymphoid tissues and are known primarily for presenting Ags through major histocompatibility complex class II (MHC-II) and MHC-I via cross-presentation [5]. pDCs also reside in lymphoid and peripheral organs and secrete high amounts of type I interferon (IFN) upon viral infection [6].

It remains unclear whether monocyte-derived cells constitute a DC subset. Monocyte-derived cells express classical DC markers, such as CD11c and MHC-II under inflammatory conditions, and are capable of inducing T cell proliferation in vitro. Consequently, monocyte-derived cells were classified initially as monocyte-derived DCs on the basis of limited phenotypic markers and in vitro functional properties. However, while cDCs and pDCs derive from a common DC precursor (CDP) and depend on FMS-like tyrosine kinase 3 (FLT3) for their development, monocyte-derived cells arise from common monocyte progenitors and develop in response to colony-stimulating factors 1 and 2 (CSF1/2). Therefore, a recently proposed classification [7] suggests that monocyte-derived cells represent a different cell type, with overlapping DC functions.

Besides Ag capture, processing and presentation that induce T cell priming in response to non-self [8, 9], an essential role of DC subsets is to coordinate an adequate physiological response to preserve self-tolerance [10]. Removal of DCs in transgenic CD11c-CRE mice results in the development of spontaneous autoimmunity [11]. In the context of organ transplantation, depletion of CD11c-expressing myeloid cells can lead to prolonged allograft survival, suggesting that the absence of DCs prevents an efficient immune response to the transplanted organ [12]. While removal of DCs represents a potential therapeutic methodology for the induction of immune tolerance, protective immunity against infections may be compromised using myeloid cell-specific depletional approaches. As a general view, anti-donor immune responses are mediated by mature DCs expressing high levels of MHC and costimulatory molecules (CM) under inflammatory conditions, whereas immune tolerance is induced by immature, tolerogenic DCs (tolDCs). Therefore, generation of tolDCs with or without loading of donor Ag, represents a clinically applicable approach for the induction of indefinite allograft survival in comparison with procedures that deplete stimulatory DCs.

Mechanisms by which tolDCs regulate immunity

TolDCs subvert effector T cell responses via distinct mechanisms, that include the induction of T cell anergy and clonal deletion due to inadequate expression of cell surface CM [13] (Figure 1). TolDCs also induce apoptosis in naïve and memory T cells via the Fas (CD95)/FasL pathway and by elevated expression of indoleamine 2,3-dioxygenase (IDO) [14, 15]. Another important function of tolDCs is their ability to promote the induction and expansion of different subsets of regulatory lymphocytes that, in turn, promote peripheral tolerance. These regulatory cells include classical $CD4^{+}CD25^{hi}$ forkhead box p3 ($Foxp3^{+}$) Tregs [16], $LAG\text{-}3^{+}CD49b^{+}CD25^{+}Foxp3^{+/-}$ T regulatory type 1 (Tr1) cells [17], $CD8^{+}$ Tregs [18], regulatory B cells (Bregs) [19], and IFNγ-producing double-negative ($CD3^{+}CD4^{-}CD8^{-}$) T cells, in both mice and humans [20, 21]. TolDCs also contribute to the development of tolerance by increased expression and release of immunomodulatory molecules. These include programed death ligand (PD-L) 1, PD-L2, human leukocyte Ag-G (HLA-G), and tumor necrosis factor (TNF)-related apoptosis-inducing ligands. Other immunosuppressive (IS) factors include IL-10, transforming growth factor beta (TGFβ), IL-27, and nitric oxide (NO) [22–25]. Heme-oxygenase (HO-1) has been shown to confer tolerogenic properties to DCs [26]. HO-1 is a rate-limiting enzyme that degrades free heme in biliverdin, carbon monoxide (CO) and Fe^{++}, which have several anti-inflammatory and tolerogenic actions [27]. Expression of HO-1 has been shown to be a mechanism of action of tolerogenic DCs in organ transplantation [28]. These suggest that tolDCs employ different mechanisms to facilitate tolerance induction through distinct immune regulatory pathways.

Regulatory role of DC-derived exosomes

More recently, a unique, although not cell-specific mechanism by which DCs modulate the alloimmune response has been described. Exosomes are membrane nanovesicles with a uniform shape and size described originally in the 1980s, produced by a variety of cells, such as DCs, T and B lymphocytes and macrophages [29]. While their biological function is not fully understood [30], recent findings suggest that exosomes act as non-cellular vehicles to transfer molecules between cells under homeostatic [31] and pathological conditions [32]. Exosomes display a specific pattern of molecules on their surface that reflects the type and state of activation of the cell of origin. In the case of DCs and other professional APCs, this may include MHC molecules, T cell CM, as well as adhesion molecules, indicating that DC-derived exosomes function as Ag-presenting nanovesicles (< 100 nm) [33]. While it is becoming clear that DC-derived exosomes bearing MHC molecules are effective intercellular communicators and provide activating signals that promote anti-donor immune responses [34, 35], donor-derived exosomes also participate in the induction and maintenance of peripheral T cell tolerance [36].

The tolerogenic function of exosomes was demonstrated initially in experimental oral tolerance in which exosomes released by the intestinal epithelium of rats fed with a model Ag induced specific tolerance when injected into naïve recipients [37, 38]. Around the same time, it was demonstrated that presentation of donor MHC Ags by BM-derived DC exosomes prolonged heart allograft survival in rats when administered before transplantation [39]. Interestingly, the combination of BM-derived exosomes with short-term

desoxypergualin analog treatment induced Ag-specific tolerance to the graft [40]. It remains unclear whether exosomes derived from cDCs or pDCs may be better able to modulate immune reactivity to favor tolerance. However, it has been demonstrated recently that tolerance associated with microchimerism may be induced by cross-dressed cDCs and pDCs that acquire donor exosomes and upregulate immune regulatory molecules, such as PD-L1 and prolong allograft survival [41]. Moreover, spontaneous liver transplant tolerance in mice is associated with cross-dressing of host cDCs within the allograft. These cross-dressed DCs exhibit elevated levels of PD-L1 and IL-10 and markedly inhibit anti-donor T cell responses, concomitant with senescence of $PD1^+$ $TIM3^+$ graft-infiltrating effector T cells [42]. Based on their important roles in regulation of the alloresponse, DCs are potential targets for manipulation to achieve prolonged graft survival and transplantation tolerance. Approaches that have been used to target DCs in situ to promote transplant tolerance and its immune regulatory effects are summarized in Table 1.

Nanoparticle-based modulation of DCs in vivo

Current clinical organ transplant management requires continuous, and typically, life-long IS drug administration. Common anti-rejection agents, including steroids and the IS pro-drugs, cyclosporine, tacrolimus and rapamycin, modulate various immune cell types non-specifically. This results in generalized IS, with associated risks of cancer development and infection [43]. Engineering nanoparticles (NP) for modulating innate immune responses in organ transplantation represents a valuable tool to avoid these side effects [44]. The potential benefits of in vivo NP-based therapeutics include improved pharmacokinetics, increased bioavailability of IS drugs, specific biodistribution to minimize systemic toxicity, protection of therapeutic molecules from enzymatic and chemical degradation, and co-delivery of multiple therapeutic agents [45–47]. While tolDC may ingest and process peptides and tolerogenic molecules in vitro without an nano-envelopment, material composition, size, shape, charge, and hydrophobicity of NP are some of the key parameters that affect the delivery of therapeutic agents to tolDC in vivo.

The use of NP for therapeutic drug delivery represents a unique approach to deliver Ags and immune modulatory agents to APCs in vivo [44], which capture and phagocytose virus-like particles in the range 50–1000 nm [45]. Delivery of Ags to APCs has been achieved through the use of monoclonal antibodies (mAbs) specific for DC receptors [48]. In this respect, development of drug-loaded NP that express mAbs on their surface represents a promising approach to deliver large immune modulatory molecules to specific APC subsets [49, 50].

Another approach to induce tolDCs is to engineer NP that provide Ag to harness the natural tolerogenic process. The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that induces tolDCs that express low levels of surface MHC and CM, and promote T cell anergy and Treg development [51]. In an elegant study, Tsai et al [52] demonstrated that stimulation of self-Ag-specific $CD8^+$ T cells with iron oxide NP conjugated with disease-relevant peptide-MHC complexes resulted in expansion of autoregulatory memory-like T cells, and consequent suppression of autoreactive $CD8^+$ T cell activation through killing of autoAg-presenting APCs. However, delivery of NP containing only Ags in an inflammatory microenvironment may augment the immune response. One suggested strategy to

circumvent this problem is to develop NP that concurrently deliver encapsulated Ags and IS therapeutics, to recruit and modulate DCs toward a tolerogenic phenotype. The co-delivery of 2-(1′H-indole-3′-carbonyl)-thiazole-4-carboxylic acid methyl ester (an endogenous AHR ligand) and a T cell epitope from myelin oligodendrocyte glycoprotein (MOG)35–55 by gold NP has shown promising results in the induction of tolDCs and expansion of Tregs to suppress autoimmunity [53]. The co-administration of MHC class I Ag and apoptosis-inducing anti-Fas mAb with magnetic beads has also resulted in selective depletion of Ag-specific T cells in a murine allogeneic skin transplant model [54].

Nanocarrier-based approaches to promotion of transplant tolerance are summarized in Table 2. Transplanted mice have been treated with NP-encapsulated IS drugs, including tacrolimus, rapamycin, and mycophenolic acid, that have superior efficacy in terms of inhibitory effects on DC maturation when compared to soluble drugs [55, 56]. Recently, high-density lipoprotein (HDL) NP have been tested in transplant models. These natural, small NP exert an immune protective function through macrophage targeting [57, 58]. HDL-NPs interact preferentially with receptors that are highly expressed on myeloid cells, including ATP-binding cassette receptor A1 and scavenger receptor type B-1 [59]. This allows for targeting of the innate immune system to prevent development of graft-reactive immune responses by encapsulating rapamycin, an IS drug used in organ transplantation since 1991 [60, 61]. Besides T cell suppression and the induction of Treg, rapamycin treatment induces tolDC [61]. However, its poor water solubility and low bioavailability compromise its systemic use [62].

In recent work, we engineered a rapamycin HDL nanobiologic termed mTOR inhibitor (i)-HDL for the induction of organ transplant acceptance. In this study, specific myeloid-derived cell targeting allowed downregulation of the innate immune response through inhibition of pro-inflammatory mediators and CM, such as TNF-α, IL-6 and CD40, which resulted in organ transplant acceptance [63]. In a separate study, polymeric NP containing rapamycin and Ag induced durable Ag-specific immune tolerance [64]. Mechanistically, these NP were shown to generate tolDC, expand Tregs, and inhibit effector T cell activation [64, 65], suggesting that Ag-specific immune tolerance may be achieved through use of NP loaded with donor Ags.

Besides their use as drug nanocarriers, NP can help visualize and monitor events within transplanted organs. Thus, NP have been used to visualize APCs *in vivo*, and to assess their number, migration and functional state [66–68]. Using different NP designs and suitable detection methods, it may be possible to obtain diagnostic and prognostic information and to evaluate treatment efficacy in transplant patients [44].

DCs as cellular therapeutic agents in transplantation models

Several approaches have been adopted to generate tolDC of donor or host origin that have been adoptively-transferred to experimental allograft recipients. Their in vivo fate and function, including the role of host DCs in mediating the immune regulatory function of donor-derived (d-d) tolDCs have also been examined [24, 69, 70].

(i) Generation and testing of d-d tolDC

The concept that tolDCs might be used in transplantation as suppressors of allograft rejection was first examined >20 years ago [71, 72]. In these reports, Thomson and colleagues showed that pancreatic islet or cardiac allograft survival was prolonged when recipient animals were pre-treated iv with d-d DC progenitors expressing MHC-II, but low levels of CM [72]. These cells induced alloAg-specific T cell anergy in vitro [73]. In contrast, transfer of d-d mature DC expressing high levels of CD80 and CD86 stimulated T cell proliferation and accelerated heart allograft rejection.

Since these early studies, numerous protocols have been used to generate donor- or recipient-derived tolDCs that have been tested extensively in transplant models [24, 74–76] (Table 3). Lutz et al [77] generated d-d DCs with an immature phenotype from BM progenitors using low concentrations of GM-CSF. Compared to mature DCs generated in the presence of high concentrations of GM-CSF or GM-CSF plus IL-4, these immature DCs were weak stimulators of allogeneic and peptide-specific T cell responses, but were more effective in the presentation of native protein. Interestingly, the immature DC were resistant to maturation under inflammatory conditions, such as exposure to bacterial lipopolysaccharide (LPS), TNFα or anti-CD40 mAb, and did not increase expression of surface CM. They induced T cell unresponsiveness in vitro and in vivo, and prolonged haplotype-specific cardiac allograft survival. However, administration of in vitro-generated immature DCs had to occur at a specific time-point before transplantation (7, but not 3, 14 or 28 days pre-transplant was effective), indicating specific kinetics for tolerance induction by tolDCs. Importantly, homing to secondary lymphoid organs was found to be required to elicit the beneficial effects of ex vivo-generated tolerogenic d-d DCs on graft survival [78]. This represents a challenge for the infusion of tolDCs, since immature DCs express the chemokine receptor CCR5 that guides their migration to peripheral tissues, while CCR7 expression (by mature DCs) is required for homing to secondary lymphoid organs. A solution might be the use of semi-mature DCs that can be generated in the presence of corticosteroids. Emmer et al [79] cultured DCs in the presence of dexamethasone (dex) and matured these cells using LPS. They upregulated CD40, but expression of MHC-II and CD86 remained low. Moreover, production of pro-inflammatory IL-12 was much lower compared to mature DCs, while IL-10 production was unaffected, leading to an increased IL-10/IL-12 ratio for cells generated with dex LPS. After infusion of d-d DCs exposed to dex and LPS, responder T cells of the recipients showed donor-specific hyporesponsiveness, while fully-mismatched heart allograft survival was prolonged.

Since DC maturation depends on activation of the NFκβ pathway, Li et al [80] silenced RelB,- the primary NFκβ protein involved in DC maturation using small inhibitory (si) RNA. DC maturation was arrested, with reduced expression of MHC-II and CM, while d-d RelB-silenced DCs inhibited MLR and prevented heart allograft rejection.

(ii) Generation and testing of host-derived tolDCs

The mammalian target of rapamycin (mTOR) pathway represents an interesting target for generation of stable, maturation-resistant tolDC. When pulsed with donor alloAg and administered a week before transplant, together with a short course of rapamycin, they

promote graft infiltration by alloAg-specific Tregs and indefinite heart graft survival [61, 81].

Garrovillo et al [82] showed that intrathymic or systemic administration of immunodominant allopeptide-pulsed host thymic DCs 7 days before transplant, combined with transient anti-lymphocyte serum, resulted in permanent, donor-specific rat heart allograft survival. These results were reproduced in a more clinically-relevant model using iv injection of peptide-pulsed host BM-derived DCs. In addition, Cuturi and colleagues have studied extensively the influence of host-BM-derived tolDCs unpulsed with donor Ags (thus unable to induce host sensitization) on rodent organ allograft survival [20, 83–85]. They have shown that, in conjunction with minimal IS therapy (including use of a deoxyspergualin analog/NFKB inhibitor or anti-CD3 Ab), iv infusion of these host-derived tolDC capable of cross-presentation of donor alloAg, a day before transplant induces donor-specific Tregs and prolongs graft survival in a donor-specific fashion. It is important to highlight that, while some common immunosuppressants negatively affect the induction of Treg, IS therapy with deoxyspergualin analogs promote tolerance induction through a self-maintaining regulatory loop between TolDC and Treg [86]. Strategies using host-derived tolDC, whether or not they are pulsed with donor alloAgs, can potentially be generalized to deceased donor organ or composite tissue allotransplantation [87, 88].

(iii) Genetic modification of tolDCs

Besides exposure to pharmaceutical agents or si RNA for the generation of stable tolDCs, genetic engineering of DCs to express immunoregulatory surface molecules or cytokines has been explored. Thus, for example, BM-derived DC transfected with Fas ligand (FasL) to augment their capacity to induce apoptosis in Fas^+ cells [89] inhibited T cell proliferation in MLR and induced hyporesponsiveness to alloAg *in vivo*. Moreover, infusion of d-d FasL-transfected DCs prolonged MHC-mismatched allograft survival.

Bonham et al [90] engineered d-d DCs to secrete cytotoxic T lymphocyte Ag 4 (CTLA4-Ig), a potent costimulation-blocking agent. These cells promoted apoptosis of activated T cells and when infused 7 days before transplant, prolonged mouse heart allograft survival. Interestingly, to prevent maturation of DCs after infection with the transducing adenoviral vector, the authors used double-stranded “decoy” oligodeoxyribonucleotides with binding sites for NFκβ, demonstrating that NFκβ antisense decoys, in conjunction with recombinant adenoviral vectors, represented a successful strategy to avoid DC maturation during the genetic engineering process.

(iv) Fate of adoptively-transferred tolDCs, and the role of host DCs in mediating the effect of d-d tolDCs

Adoptively-transferred tolDCs have been tracked by immunohistochemical staining, or fluorochrome- or radio-labeling. Host-derived, rapamycin-conditioned tolDCs labeled with PKH-67 and infused i.v. home to T cell areas of mouse secondary lymphoid tissue [61], whereas i.v.-infused indium-111-tagged tolerogenic allopeptide-primed autologous rat DC home to the spleen and liver, but not the thymus [82]. Hill et al [20] further showed that i.v.-injected PKH-26-lableled autologous tolDC established close contact with double negative T

cells in spleens of rats that became tolerant to donor allografts. Yamano et al [91] observed that FITC-labeled d-d tolDC generated from mouse BM in Flt3L (but not GM-CSF) reached the thymus and spleen (but not lymph nodes) after iv injection. These cells induced both central and peripheral tolerance to donor MHC Ags and prolonged survival of donor skin grafts in NK cell-depleted and costimulation blockade-treated recipients.

While transferred d-d tolDCs may interact directly with anti-donor T cells, inducing anergy, deletion and regulation, endogenous host DC are thought to play an important role in their immunoregulatory effects [92]. Thus, in mice, infused d-d tolDCs are thought to undergo NK cell-mediated cell death and to be reprocessed by recipient DCs for presentation of donor Ag to $CD4^+$ T cells, increasing the number of Tregs. In this concept, therapeutic donor-derived DC function as Ag-transporting cells rather than APCs to prolong allograft survival. Hence, modulating the recipient DC compartment as described above, is an alternative strategy to prolong graft survival, potentially more effectively [70, 75, 84, 93, 94].

(v) TolDCs in non-human primate (NHP) transplant studies

Pre-clinical testing of tolDCs in transplantation has been extended to NHP models. Pre-transplant (day −7) infusion of tolDC generated from donor blood monocytes in the presence of vitamin D3 and IL-10, together with minimal IS therapy (rapamycin and CTLA4Ig), was shown to prolong subsequent MHC mis-matched kidney allograft survival in rhesus macaques [95]. The rhesus d-d tolDCs expressed low MHC-II and CM, but high levels of PD-L1, and were resistant to maturation in response to pro-inflammatory cytokines. No adverse events were associated with their infusion. DC treatment reduced memory/Treg ratios in the graft recipients. More recently, the same group has addressed the influence of CTLA4-Ig on expression of the transcription factor Eomes by memory T cells in their NHP renal transplant model. The results showed that prolonged renal allograft survival achieved with d-d tolDC infusion was associated with $Eomes^{lo}$ $CTLA4^{hi}$ donor-reactive $CD8^+$ suppressive memory T cells [96].

Of note, generation and infusion of tolDCs might not always be required to exhibit the potential of tolDCs after organ transplantation. It was shown [97] that ligation of the vitamin D receptor on DCs with 1,25-dihydroxyvitamin D(3) (VitD3) reduced expression of CM on DCs, as well as IL-12 expression and increased expression of IL-10, promoting a persistent state of DC immaturity. Adorini et al [98] treated fully-mismatched islet allografts briefly with VitD3 before transplantation. This conditioning treatment increased the percentage of $CD4^+CD25^+$ Tregs in spleen and draining lymph nodes and protected 100% of recipients from rejection.

Testing of tolDCs in clinical organ transplantation

The potential of tolDCs as a novel, adjunct induction therapy for prevention of rejection and promotion of clinical transplant tolerance has been discussed extensively in recent reviews [75, 76, 99, 100] and is an emerging approach to reduce dependence on pharmacologic IS [76, 101]. Early phase clinical trials of tolDCs in renal or liver transplantation have begun, both in Europe and the US (Table 4). Based on the therapeutic efficacy of autologous tolDCs

documented in their earlier rodent allograft studies [83–85], investigators at the University of Nantes (France) have conducted a phase 1/2 (feasibility/safety) trial under the umbrella of the European consortium “The ONE Study” (www.onestudy.org), of unpulsed (no donor alloAg), autologous tolDCs, infused one day before transplant, into living donor renal transplant recipients given standard-of-care (SOC) triple drug (mycophenolic acid [MPA], steroid, tacrolimus) IS therapy (clinicaltrials.gov identifier: [69]). In this trial, the autologous, monocyte-derived tolDCs are generated in low concentration GM-CSF. The investigators postulate that following their infusion, they migrate to the graft where they capture and process d-d Ag leading to Ag-specific regulation of the host response. They also consider that use of recipient-derived tolDCs (compared with d-d tolDCs) is associated with a lower perceived risk of host sensitization, absence of NK cell-mediated killing of the infused tolDC, and suitability for application in both living- and deceased-donor transplantation. At the University of Pittsburgh (US) on the other hand, a National Institutes of Health (NIH)-supported cell dose escalation trial to test the safety of a single infusion of donor monocyte-derived tolDCs administered one week before living donor renal transplantation (Table 4) [96], together with SOC IS (MPA, steroid and tacrolimus) (), will commence in 2019. The rationale for this alternative approach, based on the extensive rodent and NHP studies, is that although the allogeneic d-d cells may not survive very long, their products are acquired by quiescent host DCs in secondary lymphoid tissue that mediate the tolerogenic effects of the infused tolDCs [92, 102].

A first-in-human, single center, open-label, phase I/II study () to test the safety and preliminary efficacy of a single infusion of d-d tolDCs in de novo adult living donor liver transplant recipients [101] has been initiated at the University of Pittsburgh. Patients receive SOC IS (MPA, steroid and tacrolimus), without Ab induction. Good manufacturing practice (GMP) grade tolDCs are generated [103] in VitD3 and IL-10 from monocytes obtained by leukapheresis from prospective living organ donors, and infused as induction therapy into their respective recipients, one week before transplant. The tolDC dose range ($2.5–10 \times 10^6$/kg) corresponds to the range for which both safety and efficacy were established in the preclinical NHP renal transplant model [95]. A half dose of MPA is administered concomitant with the tolDC infusion and until the time of transplant, to minimize any low 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 24. Follow-up continues for 3 years after the last dose of IS.

Therapeutic potential of DC-derived exosomes

Exosomes derived from immature donor DCs presenting MHC-Ag complexes prolong heart allograft survival in rats, with decreased anti-donor $CD4^+$ T cell responses [39, 40] (Table 1). Similar results have been obtained using exosomes from immature BM-derived DC in a rat intestinal transplant model, in which graft prolongation was associated with an increase in Tregs [104]. Since DC-derived exosomes exhibit immune regulatory properties in an Ag-specific manner, efforts are being made to produce and characterize clinical grade (cGMP) exosomes, that may be used as therapeutic agents [105]. As discussed above, the role of exosomes in development of tolerance versus immunity depends on the surface

characteristics of the vesicles and the type and stage of activation of the cells that secrete the exosomes [106]. Additionally, the microenvironment in which the exosome interaction occurs affects the outcome of the immune response: exosomes acting in a tolerogenic milieu promote tolerance [107]. This suggests that d-d exosomes bearing MHC molecules impact the effectiveness of the immune response against non-self MHC molecules, in both vascularized and non-vascularized transplant models. Exosome-derived immune regulation may occur in secondary lymphoid tissues where cross-dressed recipient DCs present donor MHC to naïve T cells, or in the donor organ where graft-infiltrating recipient DCs acquire donor exosomes to regulate memory T cell responses. A better understanding of the regulatory interactions between DC-derived microvesicles and immune effector cells [108] will open new possibilities for optimizing and using these nanovesicles synergistically in combination with current IS agents for the induction of donor-specific immune tolerance in organ transplantation [109].

Conclusions, challenges and future prospects

Cell therapy using tolDCs of donor or host origin, or targeting of DCs *in situ* to promote their tolerogenicity represent emerging approaches to reduce the use of systemic pharmacologic IS in transplant patients and to promote donor-specific tolerance [44, 76, 101]. BM-derived DCs generated with GM-CSF and exhibiting immunoregulatory properties prolong allograft survival following their adoptive transfer into transplant recipients [72, 77]. These cells express DC-specific markers, including CD11c (N418) and 33D1 [110, 111]. Since 33D1 is also known as DC inhibitory receptor 2 (DCIR2), its ability to regulate Ag processing and T cell activation has been evaluated using a chimeric 33D1 mAb bearing ovalbumin (OVA). Interestingly, Ag delivered via 33D1 mAb elicited no detectable CD8 T cell responses in vitro [112]. In vivo dose-response experiments confirmed that Ag-specific CD8 T cell cell expansion after 33D1-OVA treatment was modest. This suggests that $CD8^{-}CD33D1^{+}$ (CLEC4A4/DCIR2) DCs, that correspond to cDC2, might be the main DC subset that contributes to development of tolDC. Indeed, recent reports are consistent with this hypothesis, and demonstrate that DCIR2 cDC2 promote Ag-specific activation and proliferative expansion of naturally-occurring $Foxp3^{+}$ Tregs and tolerance [113, 114]. However, cDC2 are also specialized in $CD4^{+}$ T cell stimulation [112, 115]. Besides, Ab targeting to DEC205 (cDC1) but not DCIR2, contributes to peripheral tolerance through the development of induced $Foxp3^{+}$ Tregs under inflammatory conditions [48, 116].

Together with data showing that cDC1 contribute to homeostatic tolerance under steady-state conditions [117, 118], it remains unclear whether tolDCs represent a specific DC subset or a functional state of any particular DC subset. While strong data demonstrate that differentiation into cDC1 or cDC2 is determined within the BM at the common DC progenitor stage [119], it seems that either cDC1 or cDC2 can present Ag in vivo in a tolerogenic or immunogenic fashion [120]. The quest to identify and develop FLT3-dependent [121, 122], clinical grade human tolDCs for the induction of transplantation tolerance is ongoing [24].

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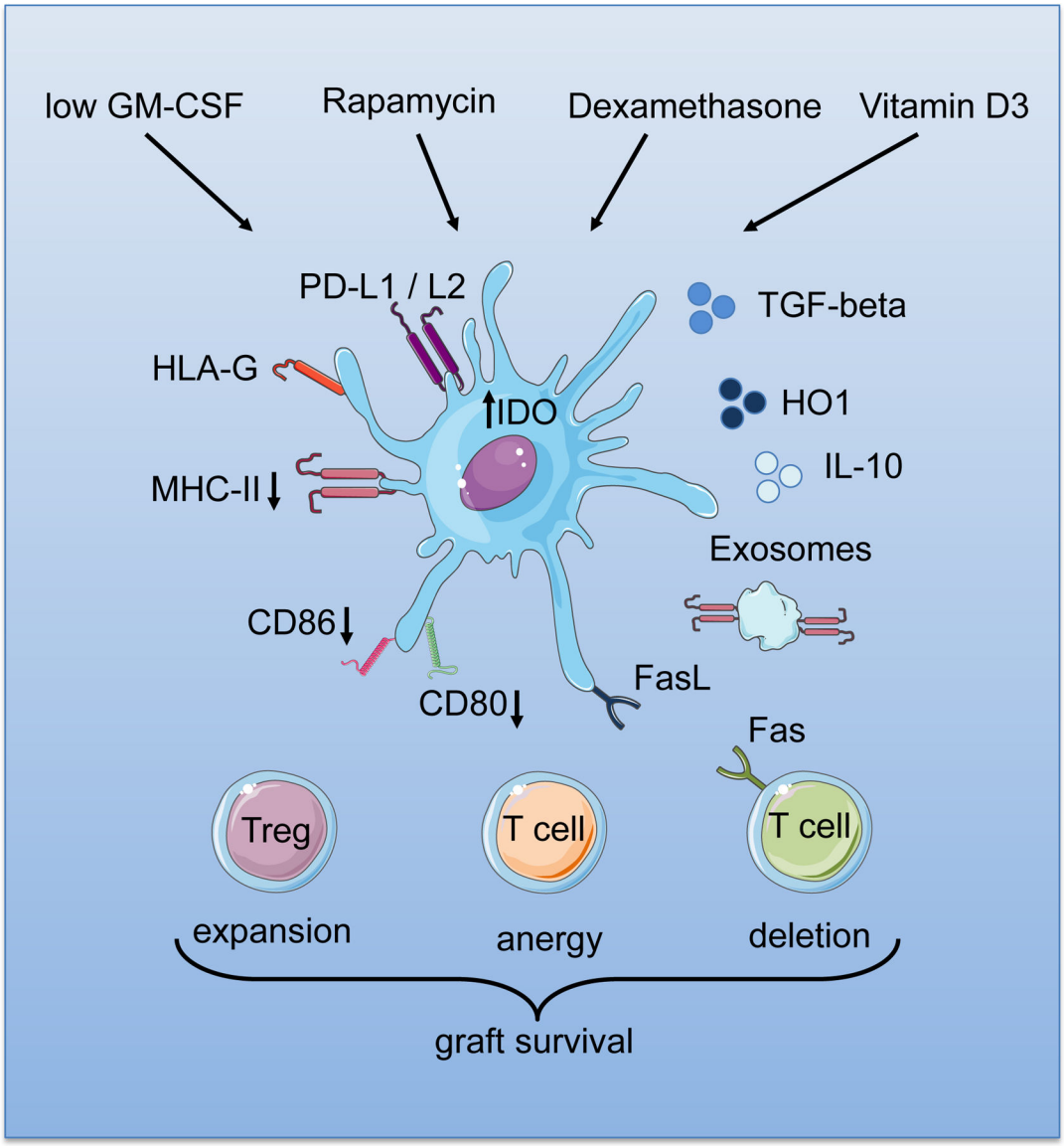

Figure 1.

Conditioning factors that promote the generation of tolerogenic DC (TolDC), their cell surface characteristics, and products that regulate alloreactive T cell responses and promote graft survival/transplant tolerance.

Table 1

Targeting of DC in situ to promote (transplant) tolerance

Method	Species	Protocol	Effect	Refs
Vesicles				
Apoptotic cell vesicles	Mouse	i.v. injection of donor splenocytes in early apoptosis alone or with αCD154 mAb, 7 days before heart transplant	Donor specific deletion of indirectly alloreactive T cells; increase in alloreactive T regs	[123–125]
Immature donor DC-derived exosomes	Mouse	i.v. injection before or after heart transplant plus low close rapamycin	Donor-specific tolerance	[109]
	Rat	Pre-transplant (heart) infusion of donor BM-derived exosomes in fully MHC-mismatched recipients	Prolongation of graft survival; decreased anti-donor T cell responses; increased anti-donor MHC II alloAb production	[39]
	Rat	Post-transplant infusion (x2) combined with deoxyspergualin analogue	Donor-specific tolerance; suppression of chronic rejection	[40]
	Rat	Caudal injection on d −7, 0 and 7 in relation to allogeneic liver transplantation ± exogenous donor-specific Tregs	Indefinite graft survival with exosome/Treg combination	[126]
Antibody				
mAb directed against DC surface Ags (lectin-like receptors)	Mouse	Ag coupled to anti-CD205 mAb	Ag-specific CD8 T cell deletional tolerance	[117]
	Mouse	Pretreatment with anti-33D1 (DCIR2) conj. with $H2k^d$ monomer in combination with αCD8-depleting Ab	Prevention of CD4 indirect alloresponses and IgG against partially MHC I-mismatched skin grafts ($B6.K^d$)	[127]
	Rhesus monkey	i.v. MD-3 anti-ICAM Ab combined with low dose rapamycin and αCD154	Long-term survival of pig xenoislets	[128]
	Humanized mouse	MD-3 mAb before transplant	Xenospecific T cell tolerance; prevention of xenoislet rejection	[128]
Anti-DC-A5GPR† mAb	Cynomolgus monkey	i.d. immunization with Ag fused to anti-DC-ASGPRAb every 5–6 w after flu virus	Ag-specific, IL-10 producing Tregs in vivo	[129]
Myeloid cell-specific nanobiologics*	Mouse	Post-transplant treatment of heart allograft recipients	Indefinite graft survival with expansion of $CD4^+$ Tregs	[63]

*mTORi HDL treatment + CD40-TRAF6-specific nanobiologic (TRAF6i-HDL);

†DC-ASGPR = DC-asialoglycoprotein

Table 2

Nanocarrier-based approaches to mediate transplant tolerance

Nanocarrier	Drug/Agent	Model	Effect	Reference
PLGA-NPs	Anti-CD3	*In vivo*	Prolongation of mouse heart allograft survival increased intragraft and draining lymph node Treg depletion	[49]
PLGA-MPs	H-2Kb-Ig dimer and anti-Fas mAb	*In vivo*	Prolongation of mouse skin allograft survival and depletion of Ag-specific CD8 T cells	[130]
PLGA-NPs	Rapamycin	*In vitro*	Secretion of high levels of TGF-β and very low levels of IL-10 and IL-12 by DCs	[56]
MPEG-PLA-NPs	Tacrolimus	*In vivo*	Prolongation of rat liver transplant survival	[131]
PEG-*bl*-PPS Micelle	Rapamycin and Tacrolimus	*In vivo*	Prolongation of mouse skin allograft survival	[132]
PLG-NPs	Donor Ag	*In vivo*	Induction of transplant tolerance in fully MHC-mismatched mouse allogeneic islet transplantation	[133]
PLGA-NPs	Rapamycin	*In vitro*	Downregulation of ICAM-1 and maintenance of an immunosuppressive cytokine milieu for DCs	[134]
PLGA-NPs	Mycophenolic acid	*In vivo*	Prolongation of mouse skin allograft survival	[135]
HDL-NPs	CD40-TRAF6 inhibitory and Rapamycin	*In vivo*	Prevention of alloreactive $CD8^+$ T cell-mediated immunity and promotion of tolerogenic Treg cell expansion	[63]
PLGA-NPs	Either protein or peptide Ags and rapamycin	*In vivo*	Inhibition of Ag-specific $CD4^+$ and $CD8^+$ T cells and B cell activation while inducing Ag-specific Tregs and Bregs	[64]

Abbreviations: HDL: high-density lipoprotein; MPEG-PLA: poly(ethyleneglycol)-poly(D,L-lactide); NPs: nanoparticles; PEG-*bl*-PPS: poly(ethylene glycol)-*bl*-poly(propylene sulfide); PLG: poly(lactide-co-glycolide); PLGA, polylactic-co-glycolic acid; TRAF6; tumor necrosis factor receptor associated factor 6

Table 3

Promotion of indefinite organ allograft[†] survival in rodents by adoptive transfer of tolDC

DC	Species	DC treatment	Additional host treatment	Route of injection (day, d)	MST	Ref
Donor-derived tolDC						
MoDC	Rat	GM-CSF	TLI; ATG	iv (d14/15)	>160d	[136]
BMDC	Mouse	GM-CSF + IL-4 or TGFβ	Anti-CD40L mAb	iv (d −7)	>100d (40%)	[137]
BMDC	Mouse	Low GM-CSF	None	iv (d −7)	>100d	[77]
BMDC	Mouse	BM-CSF + IL-4 + NFκβ ODN + rAd CTLA4Ig	None	iv (d −7)	>100d (40%)	[90]
BMDC	Rat	Low GM-CSF + IL-4	ALS	iv (d −7)	>200d[§] (50%)	[138]
BMDC	Mouse	Low GM-CSF	Anti-CD54 mAb + CTLA4Ig	iv (d −7)	100d	[139]
BMDC	Rat (kidney)	GM-CSF + IL-4 + dexamethasone	CTLA4Ig (x1) + cyclosporine	iv (d −10)	>100d	[140]
BMDC	Rat (liver)	GM-CSF + IL-4	host Tregs	iv (d −7) (both tolDC and Treg)	22d (tolDC); 30d (Treg); 42d (tolDC + Treg) vs. 8d (control)	[141]
Spleen DC	Mouse (skin)	Flt3L	Cyclophosphamide + T cell-depleted donor BM cells	iv (d 0)	>100d	[142]
BMDC	Mouse (skin)	Flt3L	CTLA4Ig + anti-CD40L; anti-NK1.1Ab	iv (d −10)	51d (tolDC) vs. 15d (conrol)	[91]
Recipient-derived tolDC						
BMDC	Rat	GM-CSF + IL-4 + donor MHC I peptide (RT1.Au)	ALS	it (d −7)	>150d	[143]
BMDC	Rat	GM-CSF + IL-4 + donor MHC I peptide (RT1.Au)	ALS	iv (d −7)	>200d	[82]
BMDC	Mouse	GM-CSF + IL-4 + RAPA + donor cell lysate	None	iv (X3) (d −10, −3, 0)	>100d	[61]
BMDC	Rat	GM-CSF + IL-4	LF 15–0195[*]	iv	>100d	[83]
BMDC	Mouse	GM-CSF + IL-4	NFκβ ODN + donor-derived lysate	iv	>100d (33%)	[144]
BMDC	Rat	Low GM-CSF + IL-4	None	iv	>100d (20%)	[84]

[†]Heart allografts unless otherwise specified;

[*]Deoxyspergaulin derivative;

ALS: anti-lymphocyte serum; ATG: anti-thymocyte globulin; BMDC: bone marrow-derived dendritic cells; Flt3L: fms-like tyrosine kinase 3 ligand; i.t: intra-thymic; iv: intravenous; MoDC: monocyte-derived DC; MST: mean survival time; ODN: oligodeoxyribonucleotides; rAd: recombinant adenovirus; RAPA: rapamycin; TLI: total lymphoid irradiation; Tregs: regulatory T cells

For more exhaustive review of the influence of adoptively-transferred tolDC an organ allograft survival in rodents, see references [24, 69, 70, 94, 145, 146].

Table 4

Registered clinical trials of tolDCreg or regulatory macrophages in living donor kidney or liver transplantation

Cell type*	Organ transplant K (kidney); L (liver)	Type of trial	Target cell dose (range)	Trial ID	Recruitment status (# patients)
TolDC					
Autologous, blood monocyte-derived tolDC	K	Phase I/II	10^6/kg	University of Nantes (ONE STUDY)	Completed (11)
Donor blood monocyte-derived tolDC	K	Phase I	0.5–5×10^6/kg (dose escalation)	University of Pittsburgh	Recruiting (14)
Donor blood monocyte-derived tolDC	L	Phase I/II	2.5–10×10^6/kg	University of Pittsburgh	Recruiting (14)
***Regulatory macrophages (Mreg)**					
Donor blood monocyte-derived regulatory macrophages	K	Phase I/II	2.5–7.5×10^6/kg	University of Regensburg (ONE STUDY)	Terminated (8)

*Administered before transplantation

In each instance, immunosuppressive therapy comprises prednisone, MPA, and tacrolimus