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Regulatory dendritic cells: profiling, targeting and therapeutic application

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Abstract

Purpose of review—There is currently increased focus on improved understanding of how dendritic cell (DC) tolerogenicity is determined and maintained, and on their therapeutic potential. We review recent progress in profiling of regulatory DC (DCreg), innovative approaches to enhancing DC tolerogenicity in situ, ex vivo-generation of DCreg and initial clinical testing of these cells in organ transplantation.

Recent findings—“Omics” studies indicate that the distinctive properties of DCreg are the result of a specific transcriptional program characterized by activation of tolerance-enhancing genes, rather than the retention of an immature state. In situ DC-directed targeting of nanovesicles bearing immune regulatory molecules can trigger in vivo expansion of Ag-specific regulatory cells. Innovative approaches to ex vivo modification of DC to enhance their regulatory function and capacity to migrate to secondary lymphoid organs have been described. Cross-dressing (with donor MHC molecules) of graft-infiltrating host DC that regulate anti-donor T cell responses has been implicated in “spontaneous” liver transplant tolerance. Clinical trials of DCreg therapy have begun in living donor renal and liver transplantation.

Summary—Further definition of molecules that can be targeted to promote the function and stability of DCreg in vivo may lead to standardization of DCreg manufacturing for therapeutic application.

Keywords

Dendritic cells; immune regulation; tolerance; therapy

Introduction

Dendritic cells (DC) are a heterogeneous group of bone marrow (BM)-derived professional antigen (Ag)-presenting cells (APC) that promote self-tolerance in the healthy steady-state [1] and regulate innate and adaptive immunity [2,3]. There is therefore a strong rationale for

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targeting DC in situ or for using DC as cellular therapies to improve long-term outcomes in organ transplantation. Challenges to optimizing these approaches induce (i) the diversity and plasticity of DC, (ii) incomplete understanding of the molecular pathways that induce a stable, enduring regulatory profile, (iii) establishing optimal strategies for selective targeting of DC in vivo, (iv) ex vivo generation of regulatory DC (DCreg), (v) combination of DCreg therapy with appropriate immunosuppressive (IS) agents, and clinical trial design.

Human DC comprise multiple subsets, i.e. CD141⁺ (cDC1) and CD1c⁺ (cDC2) myeloid cells that originate from a common DC precursor [4], inflammatory DC that differentiate from monocytes under inflammatory conditions and non-conventional CD123⁺ plasmacytoid DC [5,6]. Indeed, multiple subsets of rodent and human DC [7–9] with the ability to regulate (in rodents) organ transplant rejection and graft-versus-host disease following hematopoietic stem cell transplantation [10–12] have been characterized. Recently, transcription factors that differentially guide DC subset development have been reviewed [13] and transcriptional determinants of tolerogenic and immunogenic states during DC maturation described [14]. Mechanisms that underlie the roles of DC as inducers of peripheral tolerance have also been reviewed [15], as have methods and protocols for the ex vivo generation of human DCreg and their mechanisms of tolerance induction [16]. Some of these protocols have been incorporated into clinical trials, but with no current consensus regarding the optimal approach [17]. Herein, we consider recent developments in DCreg profiling, targeting and use of DCreg as therapeutic agents in transplantation.

Transcriptional profiling/signatures of DCreg

Intracellular pathways and transcriptional regulators that determine DCreg differentiation and function are poorly understood and few attempts have been made to better comprehend DCreg biology based on transcriptomic or proteomic (“omics”) profiles. Studies to date (reviewed by Schinnerling et al [18]) indicate that tolerogenic properties of DC emerge as the result of a specific transcriptional program rather than from the retention of an immature state. The transcription factor interferon regulatory factor 4 promotes regulatory T cell (Treg) generation by augmenting expression of genes required for Ag presentation, together with those that promote T cell tolerance [14]. Further omics studies are needed to define which molecules induce a regulatory profile and can therefore be targeted to render DC tolerogenic and enhance their stability, longevity and resistance to pro-inflammatory stimuli for therapeutic application. Investigation has been hampered by the fact that many different protocols that target distinct signaling pathways are used to generate DCreg, e.g. use of IL-10, transforming growth factor (TGF) β , vitamin (Vit)D3 or dexamethasone (Dex), or a combination of IL-10 or Dex with a stimulatory agent, such as the Toll-like receptor (TLR) 4 agonist bacterial lipopolysaccharide or CD40L. Recently, Garcia-Gonzalez et al [19,20] examined the transcriptional profile of human monocyte-derived DCreg generated with Dex and activated with the clinically-used TLR4 agonist monophosphoryl lipid A (MPLA). Dex and MPLA jointly induced a distinct transcriptional profile in DC characterized by the activation of tolerance-associated genes and suppression of inflammatory genes, conferring the potential to regulate immune responses. The pathways that showed greatest regulation were those involving cellular chemotactic responses, cell-cell signaling and interaction, as well as zinc and reactive oxygen species metabolism, favoring the recruitment and

proliferation of Treg, while suppressing effector T cell responses. This and other recent innovative strategies to promote DCreg function discussed below are depicted in Fig. 1.

In vivo function of infused and endogenous DCreg in organ transplantation tolerance

The therapeutic effect of pre-transplant infusion of donor-derived DCreg in heart-allografted mice does not appear to depend on the in vivo persistence of intact donor DCreg [21] that are likely killed by host natural killer (NK) cells, but on the function of quiescent, conventional host DC in secondary lymphoid organs (SLO). Indeed, as shown by Morelli and colleagues [22], deletion of host DC prevents the therapeutic effect of donor-derived DCreg. Host DC acquire donor MHC Ag via the direct pathway of allorecognition by cross-dressing [23,24] or via the indirect pathway by Ag transfer from the donor DCreg (cross-presentation) [25] (Fig. 2). A role for donor-derived microvesicles (exosomes) released by the donor DCreg and acquired by host DC may be an advantage, since it amplifies the effect of the infused DCreg. Consequently, T cell activation is reduced, indirect pathway T cell deletion occurs, CD4⁺ T cell-B cell help is impaired, and anti-donor antibody (Ab) production is suppressed [26]. Independence of the immune regulatory effect of donor-derived DCreg on their persistence following systemic administration offers a potential advantage over other cell therapy approaches (e.g. 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⁺ memory T cell (Tmem) responses [27–30], a major barrier to long-term allograft survival in humans.

In both preclinical (mouse) and clinical studies, total lymphoid irradiation and anti-thymocyte globulin administration promotes hematopoietic cell chimerism and tolerance in organ transplant recipients. In studying the mechanistic basis of these effects, Hongo et al [31] have established that CD8⁺ DC are likely responsible for the initiation of tolerance induction since they ingest apoptotic cells and suppress anti-donor T cell responses via mechanisms including indoleamine dioxygenase production. Moreover, reciprocal interactions between CD8⁺ DC and invariant NKT cells were required for tolerance induction.

The role of cross-dressing of host DC in organ transplant tolerance

Cross-dressing has only recently been implicated in the instigation of transplant tolerance. Thus, Ono et al [32] have investigated the role of graft-infiltrating, cross-dressed DC in mouse fully MHC-mismatched liver transplant tolerance that occurs without use of IS agents [33]. While donor interstitial DC diminished rapidly following liver transplantation, they were replaced in the graft by host DC that peaked on postoperative day 7 and persisted indefinitely. About 60% of these recipient DC displayed donor MHC class I, indicating cross-dressing. By contrast, only a very minor fraction (0–2%) of cross-dressed DC (CD-DC) was evident in the spleen. CD-DC sorted from the liver grafts expressed much higher levels of the T cell coinhibitory molecule programmed death ligand 1 (PD-L1) and high levels of anti-inflammatory IL-10 compared with graft non-CD-DC. Concomitantly, high incidences of programmed death protein 1 (PD-1)^{hi} T cell immunoglobulin and mucin domain

containing-3 (TIM-3)⁺ exhausted graft-infiltrating CD8⁺ T cells were observed. Importantly, unlike non-CD-DC, the CD-DC did not stimulate proliferation of allogeneic T cells, but markedly suppressed anti-donor host T cell proliferation. CD-DC were much less evident in allografts from donors lacking the transmembrane adaptor protein DNAX-activating protein of 12kDa that are rejected acutely [34]. These findings suggest that graft-infiltrating PD-L1^{hi} CD-DC play a key role in the regulation of alloimmunity and induction of liver transplant tolerance.

Cross-dressing of host DC has also been implicated in allospecific tolerance in a different context. Thus, maternal hematopoietic microchimerism, that has been linked to the development of allospecific tolerance, has been shown recently [35] to be associated with membrane alloAg acquisition by host DC. In the mouse model examined, cross-dressing was associated with enhanced expression of PD-L1 on myeloid DC and reduced presentation of allopeptide + self-MHC complexes together with increased PD-L1 on plasmacytoid DC that was associated with PD-L1-dependent CD4⁺ T cell anergy. Thus, acquisition of exosomes bearing alloAg by host DC may provide an important link between microchimerism and the induction of tolerance.

Innovative approaches to in situ targeting of DC

Prolonged ischemia enhances DC maturation and potentiates adaptive immunity. In the post-ischemic period, CD4⁺ T cells recruited to the liver are closely associated with hepatic DC. Using 2-photon intravital microscopy together with confocal microscopy, Funken et al have shown [36] that in situ targeting of hepatic DC with a VitD analog (paricalcitol) attenuates their maturation, promotes their tolerogenicity and ameliorates CD4⁺ Th1 cell responses post-ischemia. This beneficial effect was abolished by blocking DC-T cell interactions mediated by the cell surface glycoprotein CD44. In a recent report, Zheng et al [37] found that injected exosomes derived from DC could attenuate ischemia-reperfusion injury by modulating the balance between Treg and Th17 cells.

Important pathways and molecular mechanisms that regulate peripheral tolerance are being uncovered by combining methods that target delivery of defined T cell Ags to DC in vivo with genetic modification of the DC [15]. These approaches are determining the roles of specific immunomodulatory pathways and different DC subsets in maintaining immunological tolerance. In a recent example, Reeves et al [38] have shown that APC-targeted expression of proinsulin converts insulin-specific CD8⁺ T cell priming to tolerance in autoimmune-prone, non-obese diabetic mice. This shift in T cell priming to tolerance exemplifies the tolerogenic capacity of autoAg expression by APC in situ and the ability to overcome defects in pathways controlling peripheral tolerance.

Nanoparticle-based drug delivery systems that enable directed, cell type-specific targeting in vivo in combination with delivery of multiple drugs in a single formulation have emerged as a promising approach to DC-based immunotherapy. Receptors that have been used to target DC include DEC205 (CD205), CD11c, DC-specific intracellular adhesion molecule-3-grabbing non-integrin (DC-SIGN), the mannose receptor, Fc receptors and CD40. While most approaches have focused on induction of immunity in the context of tumor

immunotherapy, nanoparticle delivery is also a promising approach to induce DC with tolerogenic capacity. Thus, polymeric synthetic nanoparticles ('nanocarriers') that target DC induce stable Ag (ovalbumin)-specific tolerance by delivery of rapamycin [39]. Recently, biodegradable porous silicon nanoparticles displaying anti-DC-SIGN and loaded with rapamycin were shown [40] to target and be taken up more efficiently (compared with isotype-coated nanoparticles) by myeloid DC in human blood. Moreover, DC pre-conditioning with the rapamycin-loaded nanoparticles suppressed allogeneic T cell proliferation. Notably, Clemente-Casares et al [41] have shown that systemic delivery of nanoparticles coated with autoAg bound to MHC II molecules triggers in vivo expansion of Ag-specific type-1 Treg that, in turn, inhibit autoAg-loaded APCs and drive differentiation of regulatory B cells in experimental autoimmune disease.

Ex vivo generation of DCreg

Myeloid DC with tolerogenic properties can be generated readily from BM precursors or blood monocytes in response to granulocyte-macrophage colony-stimulating factor $\pm$ IL-4, with addition of one or more pharmacologic/biologic agents that stably inhibit their maturation and promote their tolerogenicity [42]. These agents include anti-inflammatory cytokines (IL-10; TGF β), anti-sense oligonucleotides targeting costimulatory molecules, anti-inflammatory/IS drugs (calcineurin inhibitors [CNI], rapamycin, mycophenolate mofetil [MMF], corticosteroids), VitD3 and cyclic AMP inducers, in particular prostaglandin E2. Currently, there is no consensus as to the optimal agent(s) or combination of agents to use for generation of clinical grade DCreg. Phenotypic characteristics of DCreg include low levels of MHC class I and II and T cell costimulatory molecule (CD80; CD86) expression and enhanced expression of T cell co-inhibitory (e.g. PD-L1) and death-inducing ligands (e.g. FasL). Their functional characteristics include low secretion of bioactive IL-12p70 and comparatively high levels of anti-inflammatory cytokine (IL-10; TGF β) production. In addition, DCreg can expand or induce the de novo generation of Treg [10,43,44].

An important consideration regarding generation of DCreg is their ability to migrate to SLO. Genetic modification of DC to enhance their tolerogenic function and promote their in vivo migration to SLO has been documented [45–47]. In a more recent study, Dong et al [48] co-transfected donor-derived immature DC with an adenovirus bearing the chemokine receptor type 7 (CCR7) gene and a small interfering RNA targeting the nuclear factor- κ B transcription factor subunit RelB to concurrently overexpress CCR7 and downregulate RelB expression. The co-transfected cells displayed enhanced migratory ability, resistance to apoptosis and enhanced ability (compared with CCR7 or RelB knockdown alone) to upregulate Treg and improve mouse skin allograft survival, suggesting that this might be a promising approach in skin (including vascularized composite allograft) transplantation. In a related study, Xin et al [49] reported that overexpression of CCR7 and BTLA (B and T lymphocyte attenuator, an Ig superfamily coinhibitory receptor similar structurally to PD-1 and CTLA4) in immature mouse DC enhanced their tolerogenic function and migration to the SLO chemokine CCL19.

The potential of donor-derived DCreg generated from (murine) induced pluripotent stem cells (iPSC) to function as therapeutic cellular vaccines to generate Treg and induce donor-

specific tolerance has been examined recently by Cai et al [50]. Mouse heart allografts were accepted permanently in a donor-specific manner, accompanied by differentiation of donor-specific CD4⁺ Treg, illustrating the promising potential of iPSC-DCreg.

DCreg subsets for therapeutic application

Human IL-10-modulated DC (IL-10 DC) [51] that potently induce Treg [52] have been the focus of research on DCreg for many years. A recent report by Steinbrink and colleagues [53] identified two populations of human monocyte-derived IL-10 DC with tolerogenic properties, - CD83^{hi} CCR7⁺ IL-10 DC and CD83^{lo} CCR7⁻ IL-10 DC. The CD83^{hi} CCR7⁺ IL-10 DC generated more potently suppressive CD4⁺ Treg, exhibited efficient CCR7-directed migration (towards the SLO chemokine CCL21) and retained stable function under inflammatory conditions, thus satisfying important criteria for prospective clinical application.

Donor-versus recipient-derived DCreg for therapy of graft rejection

The first reports suggesting that DCreg of donor origin could be used to inhibit allograft rejection appeared in 1995/1996 [54,55]. Subsequently, many reports have shown that donor-derived DCreg or syngeneic/autologous DCreg (either pulsed or unpulsed with donor Ag) infused alone or together with an IS agent(s) can induce indefinite organ allograft survival/donor-specific tolerance in rodents [10,56]. In more recent studies in a pre-clinical, MHC-mismatched non-human primate (NHP) renal allograft model, graft survival was prolonged significantly in rhesus macaques given VitD3- and IL-10-conditioned DCreg one week before transplant, in combination with a minimal IS regimen of costimulation blockade and rapamycin [57]. No evidence of host sensitization (donor-specific Abs) was observed. Median graft survival time was also prolonged in this model when autologous DCreg pulsed with donor Ag (cell membrane microvesicles) were infused a day before transplantation [58]. These important translational studies demonstrate both the safety and efficacy of a single (donor-derived) DCreg infusion. They also provide novel mechanistic insights. Thus, infusion of donor-derived DCreg is associated with selective attenuation of anti-donor Tmem responses, Eomesodermin^{lo} CTLA4^{hi} alloreactive CD8⁺ Tmem [59] and maintenance of donor-reactive CD4⁺CTLA4^{hi} T cells with a regulatory phenotype [60]. These observations in NHP provide a compelling basis for clinical testing of DCreg in organ transplantation.

Innovative approaches to combination therapy

Many pharmacologic and biologic agents promote DC tolerogenicity in rodents [42]. Following observations that exosomes derived from immature donor DC (immDex) prolonged experimental graft survival and that their effects were associated with Treg, Ma et al [61] combined the exosomes with donor Ag-specific Treg and achieved tolerance without IS therapy in a rat liver allograft model. The immDex amplified Treg in vivo most likely through binding/fusing with host DC and being presented by these DC, which could be augmented by IL-2 administration. Also, in a rat liver allograft model, combined infusion of donor-derived immature DC and CD4⁺ Treg, 7 days before transplantation, was more

effective than either regulatory cell population alone and appeared to maintain a feedback loop between the DCreg and Treg in vivo [62].

Clinical trials of DCreg therapy in renal and liver transplantation

The potential of DCreg for the prevention of rejection after clinical solid organ transplantation has been discussed extensively in recent reviews [17,63]. Clinical trials of DCreg therapy in renal or liver transplantation have begun in Europe and the US. Autologous DCreg infusion, 1 day before transplant, is under examination at the University of Nantes, France, in live donor renal transplantation with standard of care (SOC) triple drug IS (azathioprine, steroid, tacrolimus) ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02250555) Identifier: NCT02250555 [64]). An NIH-supported clinical trial to test the safety of donor-derived DCreg infusion in living donor renal transplantation will begin at the University of Pittsburgh, US in 2018 [65].

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 of early post-transplant IS drug withdrawal (CNI-based therapy; no induction) in liver transplantation [66], 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 basis for assessment of 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 [67] has been initiated. Patients receive SOC IS (MMF, steroid and tacrolimus), without Ab induction. GMP grade DCreg are generated [68] in VitD3 and IL-10 from monocytes obtained by leukapheresis from prospective living donors and infused as induction therapy into their respective recipients, 7 days before transplant. The DCreg dose range ($2.5\text{--}10 \times 10^6/\text{kg}$) corresponds to the range for which both safety and efficacy were demonstrated in the preclinical NHP renal transplant model [57]. A half dose of MMF is administered concomitant with DCreg infusion and until the time of transplant to minimize any potential risk of sensitization. In eligible patients, determined by permissive liver function tests and (at 12 months post-transplant) a permissive liver biopsy, IS drug weaning begins at month 6 and continues through month 18. Follow-up continues for 3 years after the last dose of IS.

CONCLUSIONS AND FUTURE PROSPECTS

Omics studies are better defining the molecules that enhance the tolerogenic phenotype, stability and longevity of DCreg and their resistance to proinflammatory stimuli. This is likely to instruct improved and more standardized design of protocols for generation of

clinical grade DCreg for therapeutic application. Strategies that target DC selectively in situ to enhance their immune regulatory function and that enhance migration of DCreg to SLO show promise for potential therapeutic application. Acquisition of donor MHC by host DC (cross-dressing) and the immune regulatory function of these cross-dressed DC in allograft recipients in relation to development of transplant tolerance is a key topic for future mechanistic studies. Clinical trials of DCreg in organ transplantation have been instigated and will provide valuable insights into the value of these novel regulatory immune cells for improved outcomes in organ transplantation.

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Figure 1. Recent approaches to targeting/modification of DC to promote tolerance.

(a) Activation of dexamethasone (Dex)-generated DCreg with the Toll-like receptor 4 ligand MPLA (monophosphoryl lipid A) is associated with upregulation of tolerance-associated genes and downregulation of inflammatory genes. (b) Rapamycin-loaded silicon nanoparticles displaying anti-DC-SIGN are taken up by myeloid DC that, in turn, suppress allogeneic T cell proliferation. (c) Overexpression of the chemokine receptor type 7 (CCR7) and BTLA (B and T lymphocyte attenuator) enhances immature mouse DC migration and tolerogenic function. (d) Infusion of donor-derived DCreg generated from induced pluripotent stem cells (iPSC) is associated with prolonged allograft survival. (e) IL-10 induces a CD83^{hi} CCR7⁺ human DCreg subset that exhibits efficient CCR7-directed migration and generates potentially suppressive CD4⁺ Treg.

Figure 2. Acquisition of donor Ag may underly host DCreg regulation of alloimmunity and promotion of tolerance.

(a) Cross-presentation of processed exogenous donor Ag (peptide) and endogenous MHC I by quiescent host DCs and (b) cross-dressing of host DCs to express exogenous Ag and exogenous MHC I/II, together with high levels of programmed death ligand 1 (PD-L1) and IL-10 may play key roles in the regulation of alloimmunity and the promotion of tolerance. For further information and discussion, see refs [22,25,32].