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REVIEW

Regulatory dendritic cell therapy in organ transplantation

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Summary

Dendritic cells (DCs) are uniquely well equipped antigen (Ag)-presenting cells. Their classic function was thought to be that of potent initiators of innate and adaptive immunity to infectious organisms and other Ags (including transplanted organs). Evidence has emerged, however, that DCs have a central and crucial role in determining the fate of immune responses toward either immunity or tolerance. This dichotomous function of DCs, coupled with their remarkable plasticity, renders them attractive therapeutic targets for immune modulation. In transplantation, much recent work has focused on the ability of DCs to silence immune reactivity in an Ag-specific manner in the hope of preventing rejection and diminishing reliance on potentially harmful immunosuppressive agents. Experimental strategies have included *in vivo* targeting of DCs, as well as *ex vivo* generation of regulatory (or tolerogenic) DCs with subsequent reinfusion (i.e. cell therapy). Different approaches to 'program' DC toward tolerogenic properties include genetic (transgene insertion), biologic (differential culture conditions, anti-inflammatory cytokine exposure) and pharmacologic manipulation. Recent data suggest a promising role for pharmacologic treatment as a means of generating potent regulatory DCs and have further stimulated speculation regarding their potential clinical application. Herein, we discuss evidence that the potential of regulatory DC therapy is considerable and that there are compelling reasons to evaluate it in the setting of organ transplantation in the near future.

Introduction: clinical need, tolerance, and regulatory DCs

The transplantation of organs has expanded greatly over the last five decades with an ever-increasing number of patients with an end-organ failure benefiting from kidney, liver, heart, and lung transplantation. Improvements in surgical techniques and ancillary care, as well as adoption of the multi-drug immunosuppressive regimens now widely employed in most organ transplant recipients, have led to dramatically improved short-term (1–3 year) patient and graft survival rates, with 1-year graft survival approaching or exceeding 80% for many organ systems (e.g. kidney, intestine, liver, pancreas, heart, and lung; data from http://www.ustransplant.org). However, despite these dramatic improvements in short-term graft survival, as well as a significant reduction in acute rejection rates, little improvement has been made in long-term graft attrition [1,2]. Although there are nonimmune factors that contribute to late graft loss (i.e. ischemia reperfusion, infection, drug-specific toxicities, hypertension, and dyslipidemia [2]), allo-immunity leading to chronic rejection plays a dominant role in rejection of most organs, i.e. the kidney, heart, and lung [2]. In the US, the renal allograft population, although in need of improved outcomes, has a 1-year graft survival in excess of 90%

and 5-year graft survival in excess of 79% (http://www.ustransplant.org). Patient populations in particular need of improved outcomes are thoracic transplant recipients (heart and lung). While 5-year graft survival of heart transplant recipients is slightly less than that of live-donor kidney recipients (71% compared with 79%), patient survival is much more discrepant (72% for heart recipients and 90% for living-donor kidney recipients) because, in part, of the availability of dialysis for kidney recipients with failed grafts, while there is no such replacement therapy for heart recipients with failing grafts short of a re-transplant. In addition, chronic rejection in heart transplant recipients is frequently silent, leading to sudden death from ischemia. Lung transplant recipients suffer more. In the US, 1-year patient survival approximates 80%, while 5-year survival is approximately 45% (http://www.ustransplant.org) with recipients also suffering from a high rate of infectious complications (the leading cause of death in the first three years following transplantation) and immunosuppression-related drug toxicities.

Since the seminal work of Billingham *et al.* in 1953 [3], transplant researchers have aspired to achieve the 'holy grail' of tolerance – perhaps best defined as the lack of a destructive immune response against a graft in the absence of chronic immunosuppression (with retention of generalized immune competence). Achievement of this lofty goal would not only alleviate the burden of chronic, nonspecific immune depression and drug-related toxicities, but also greatly alleviate the problem of late graft loss (or at least that portion because of chronic rejection resulting from alloimmunity). Following the studies of Medawar's group utilizing donor strain hematopoietic cells to induce tolerance, tremendous effort, especially in recent years, has been expended to develop models of tolerance induction and define their mechanisms with the hope of clinical translation [4,5]. However, the excellent short-term outcomes achieved with conventional multidrug immunosuppression – particularly for the kidney – have made it ethically difficult to initiate clinical tolerance trials, particularly when the approach involves a dramatic departure from standard clinical immunosuppression.

Among the strategies being evaluated to modify recipient antidonor immunologic responses in the setting of organ transplantation, much interest has focused on the potential of dendritic cells (DCs) [6,7]. Recent results in experimental models that have examined the impact of infusion of DCs conditioned to be regulatory (or 'tolerogenic') DCs have fueled this enthusiasm, suggesting that regulatory DCs can drive *in vivo* generation of regulatory T cells (Treg), thus promoting robust peripheral tolerance [8]. The potential for clinical application of this approach is supported by ongoing clinical trials utilizing DC vaccines for tumor immunotherapy [9], as well as the fact that regulatory DC therapy in transplantation could be used in (and perhaps benefit from) the setting of conventional immunosuppression. In this review, we discuss DC biology and recent developments in the generation of regulatory DCs, as well as the hurdles for their application in clinical transplantation. Much of the discussion is generally applicable to live donor organ transplantation, but the potential of regulatory DCs to affect deceased donor transplant outcome is also considered. We provide a rationale for regulatory DC therapy and a proposed strategy for its implementation in the clinic.

Immunobiology of DCs

Dendritic cell are rare, ubiquitously distributed migratory leukocytes, derived from $CD34^+$ stem cells. In the normal steady state, DCs are present as 'immature' antigen (Ag)-presenting cells (APC) in the interstitium of nonlymphoid/peripheral tissues, including the commonly transplanted organs (liver, heart, lung, kidney, pancreas, and skin). Under these conditions and when freshly isolated, they express few surface major histocompatibility complex (MHC) and accessory (intercellular adhesion/costimulatory) molecules (CD40, CD54 [ICAM-1], CD80 [B7–1], or CD86 [B7–2]) and are at best, poor stimulators of naïve T cells. These immature DCs, however, are extremely well equipped, both for Ag capture and for efficient loading of foreign Ag fragments onto MHC class II molecules for export to the cell surface. It is now evident that DCs also phagocytose and process dying (apoptotic) cells [10]. This capacity to phagocytose apoptotic bodies appears to be restricted to the immature stage of DC development [11,12] and may be a potential means by which DCs maintain peripheral self-tolerance under steady-state conditions (see below). Integral to their function as sentinels of the immune system, DCs possess several other important properties, including (i) the ability to convey Ag from peripheral sites to T-cell areas of secondary lymphoid organs (where primary immune responses are initiated); (ii) in their mature form, they are potent stimulators of naïve $CD4^+$ and $CD8^+$ T cells; and (iii) they are able to 'cross-prime' or 'cross-tolerize' T cells [13,14] to either self-proteins or alloAgs. Furthermore, immature DCs can acquire Ag from living hematopoietic cells for cross-presentation to cytotoxic T lymphocytes [15].

Dendritic cell maturation is essential for these APCs to stimulate T-cell responses. Maturation is stimulated by microbial products [e.g. exogenous 'danger' signals, including bacterial lipopolysaccharide (LPS), unmethylated cytosine poly-guanine (CpG) motifs and double-stranded RNA], pro-inflammatory cytokines [granulocyte/macrophage-colony-stimulating factor (GM-CSF), interleukin (IL)-1β, tumor necrosis factor (TNF)-α and interferon

(IFN)-α], cyclooxygenase metabolites, and CD40 ligand (L) (e.g. on activated platelets and T cells). Maturation is promoted by several nominal endogenous mediators following necrotic cell death or ischemia/reperfusion injury, such as high mobility group box 1 [16–18], heat shock proteins [19–21], purine metabolites, including uric acid [22] and adenosine triphosphate [23], and the S100 family of molecules [24]. In addition to CD40L, these endogenous mediators may be of great importance in the setting of the ischemic and inflammatory injury sustained at the time of organ transplantation that ultimately leads to maturation of DCs (see below). Further, nuclear translocation of the transcription factor nuclear factor κB induced by signaling through TNF receptor (R) family members [e.g. TNFR, CD40, and TNF-related activation-induced cytokine (TRANCE)/receptor activator of NFκB (RANK)] and ligation of toll-like receptors (TLR) are two other mechanisms that trigger maturation of DCs. Mature DCs are potent stimulators of naïve and memory T cells. Expression of CC chemokine receptor (CCR) 7 by activated DCs facilitates their trafficking to T-cell areas of secondary lymphoid tissues in response to the CCR7 Ls, CCL19, and CCL21.

DCs and transplant rejection

Following transplantation, recognition of alloAg (primarily the MHC) by recipient T cells can occur via direct and/or indirect pathways [25]. While DCs are central to both pathways, the roles of donor versus recipient-derived DCs are discrete. The direct pathway involves interaction (and stimulation) of recipient T cells directly with intact MHC molecules on the surface of donor APC transferred with the graft (i.e. 'passenger leukocytes'). The high frequency of recipient T cells capable of responding in this capacity (between 1% and 7% of the T-cell repertoire [26]) is explained by studies suggesting that T cells with a memory phenotype represent the greater proportion of T cells capable of recognizing allo-MHC in a direct fashion. Such T cells are specific for self-MHC (with allo-MHC peptide) but cross-react with allo-MHC directly. Classic passenger leukocyte depletion experiments performed in the 70s and 80s indicated that graft-resident donor DCs were the predominant APC population responsible for recipient T-cell activation via this pathway [27–29]. DCs trafficking from heart or skin allografts to host secondary lymphoid tissue were implicated as the principal instigators of graft rejection [30,31]. Current thought suggests that graft-resident donor DCs receive maturation signals from the inflammatory environment in the graft following transplantation (pro-inflammatory cytokines and other 'danger' signals discussed above) after which they mature and migrate to draining lymph nodes. Once in the T-cell-rich area of secondary lymphoid organs, they initiate primary and cross-reactive memory responses via the direct pathway that are responsible for graft rejection. On the other hand, Starzl *et al.* (1992) observed persistent, donor-derived leukocytes (including DC) in lymphoid and nonlymphoid tissues of long-surviving stable human organ graft recipients, including patients off all immunosuppression [32,33]. This led to speculation that donor-derived DC could play a role in the induction/maintenance of organ transplant tolerance [34].

Recipient DCs participate in activation of T cells via the indirect pathway. The indirect pathway involves processing and presentation of allo-MHC peptides on recipient MHC to T cells in an MHC-restricted fashion. The proportion of T cells capable of responding to allopeptide in this fashion is significantly less than that via the direct pathway [6,35] and more closely mimics acquired immunity to environmental pathogens. Allosensitization via the indirect pathway results from migration of recipient immature DC to the graft with subsequent acquisition of donor MHC (by mechanisms described above). In the presence of 'danger' signals in the graft, these DCs mature, traffic to secondary lymphoid organs, and initiate primary immune responses.

Experimental data suggest that both the direct and the indirect pathways are involved in allograft rejection, although the relative contributions of each have been debated for some time [25]. However, based on animal and human work, a paradigm has evolved whereby the direct pathway is thought to mediate early (several months) alloimmune responses, whereas the indirect pathway is the primary contributor to ongoing late alloresponses [25]. Evidence supporting this paradigm in human renal [36], cardiac [37,38], and lung transplant [39,40] recipients has demonstrated that direct alloreactivity diminishes with time from transplant, whereas the indirect pathway response increases over time, with increased frequencies of T cells with indirect allo-specificities. These studies suggest that the indirect pathway plays a central role in the development of chronic rejection. With emerging evidence that humoral immunity plays an important role in chronic rejection [41] and as elicited antibody (Ab) responses require $CD4^+$ T-cell help, this is further evidence of indirect pathway involvement in chronic rejection. As discussed, recipient DCs are central to the development of indirect alloreactivity and are uniquely well equipped to activate (and perhaps to tolerize) both $CD4^+$ T cells through MHC class II as well as cross-prime $CD8^+$ T cells through MHC class I molecules. As such, tolerance-enhancing strategies utilizing recipient-derived (as opposed to donor-derived) regulatory DCs may be more successful in controlling long-term alloreactivity (discussed below).

DCs and tolerance

The role of DCs in central tolerance and induction/maintenance of peripheral self-tolerance is now well recognized. A role for DCs in tolerance induction was first demonstrated in the context of intrathymic self-tolerance, where DCs were shown to be integral to negative selection in the thymus [42,43]. Subsequent work demonstrated that intrathymic injection of allo-DCs (minor lymphocyte-stimulating locus-incompatible spleen or thymic DCs) in neonatal mice can induce tolerance (via T-cell clonal anergy) [44]. Similar results have been reported in bone marrow (BM) chimeric and transgenic mice [45,46]. Tolerance exhibited following intrathymic inoculation of alloAg appears to be dependent on thymic DC [47], and, indeed, BM-derived host MDC pulsed with allopeptide and injected intrathymically can induce organ or pancreatic islet transplant tolerance in antilymphocyte serum-conditioned hosts ([48,49] and Table 2).

Dendritic cells also play an important role in the induction and maintenance of peripheral tolerance. Evidence suggests that the presentation of peripherally derived Ag by DC within secondary lymphoid tissue is not only effective for T-cell priming, but, under steady-state conditions, is also effective for the induction of T-cell tolerance to self-Ag expressed exclusively by peripheral (extralymphoid) tissues. Steinman *et al.* [14,50] have suggested that the presentation of newly exposed or -expressed 'self Ags' by immature DCs to Ag-specific T cells in the absence of danger signals results in tolerance. This hypothesis is supported by data demonstrating that the interaction of Ag-specific T cells with DCs expressing low levels (or no) costimulatory molecules leads to anergy/apoptosis of the T cell [51] or to generation of Treg cells [52]. Further evidence supporting this concept has come from experiments targeting DCs *in vivo* in the steady state with exogenous Ag. Under steady-state conditions in mice, Hawiger *et al.* [53] targeted low doses of the hen egg lysozyme (HEL) peptide to DC utilizing an Ab to the DC receptor CD205 (DEC205). Transgenic $CD4^+$ HEL peptide-specific T cells underwent several rounds of division and then were entirely deleted. Subsequent rechallenge with Ag in conjunction with an immune adjuvant demonstrated that the adoptively transferred HEL peptide-specific T cells had been tolerized.

Recent work has demonstrated that apoptotic cells injected intravenously are efficiently captured by DCs, whose pro-inflammatory function is undermined [6,54]. Several groups have targeted DCs in the steady state with apoptotic cells in an attempt to promote Ag-specific unresponsiveness. This work has demonstrated that uptake of apoptotic cells by DCs does not induce inflammation or maturation of DC and that DCs are able to process and present apoptotic cell-derived peptides [55,56]. Indeed, a recent report by Morelli *et al.* [10] indicates that delivery of apoptotic donor leukocytes 7 days prior to cardiac transplantation in mice, in the absence of immunosuppression, significantly prolongs graft survival and induces deficient activation, transient proliferation and subsequent deletion of adoptively transferred, allospecific transgenic $CD4^+$ T cells. These interesting data suggest that targeting of DCs *in vivo* with apoptotic donor leukocytes under steady-state conditions might be an alternative to *ex vivo* generation of regulatory DCs for cell therapy in transplantation. Such a strategy, however, would require access to donor leukocytes with subsequent cell administration under steady-state conditions several days (7 days in results reported thus far) prior to transplantation. This approach is applicable to living donor transplantation.

Regulatory DCs for cell therapy in organ transplantation

A large body of evidence has accumulated in recent years regarding the ability of DCs administered as cell therapy to diminish alloreactivity or promote tolerance in the setting of transplantation [6]. Although various subsets of DCs are being examined in experimental models, we will focus our discussion in this review on classic myeloid (or monocyte-derived) DCs. The biological principles underlying this approach for myeloid DCs are based mainly on those discussed above for the role of DCs in induction and maintenance of peripheral tolerance. It is clear, however, that DCs can regulate immune reactivity by a variety of mechanisms (as reviewed in [6,57,58]). Indeed, the role(s) of DCs in immune regulation (i.e. expansion/induction of Treg cells, such as $CD4^+CD25^+$ T cells or Tr1 cells that make IL-10) and in anergy/deletion has both been postulated to be important, and likely not mutually exclusive mechanisms via, which DCs exert their tolerogenicity. Initial studies of regulatory DC therapy focused on *ex vivo* generation of immature myeloid DCs from donor bone marrow under specific cell culture conditions. Subsequent work has demonstrated the feasibility of genetic or pharmacologic approaches to enhance and maintain the tolerogenicity of either *ex vivo*-generated donor or recipient DCs, as well as explored the potential of 'alternatively activated' DCs. The various approaches are discussed below.

Generation of regulatory DCs utilizing cell culture conditions

The first demonstration of *in vitro* generation of regulatory DCs was by Lu *et al.* in 1995 [59]. They generated

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immature DCs from murine BM *in vitro* utilizing GM-CSF. These *in vitro*-generated myeloid DCs were phenotypically immature (costimulatory molecule-deficient) and induced Ag-specific hyporesponsiveness in allogeneic T cells *in vitro*. A subsequent study demonstrated that recipient injection with these costimulatory molecule-deficient, donor-derived DCs, 7 days prior to transplantation in a fully MHC-mismatched, murine cardiac transplantation model – without pharmacologic or biologic immunosuppressive cover – resulted in significant prolongation of graft survival compared with untreated recipients [60]. Since these seminal experiments, the goal has been to optimize DC manipulation and/or delivery to maximize their Ag-specific, tolerogenic potential. Much attention has also been given to determining the ideal DC 'subset' for alloAg-specific prolongation of graft survival; however, the most successful models to date (>100 d cardiac allograft survival) have utilized classic myeloid DCs.

Therapeutic strategies with regulatory DCs have included the use of donor or recipient DCs, with or without short-term immunosuppression or other biological agents (Tables 1 and 2). Donor DC therapies most frequently involve the targeting of co-stimulatory molecule expression or interactions with their T-cell-expressed Ls, through generation of immature DCs [61–64] that are then administered with or without monoclonal (m) Ab or CTLA4 (cytotoxic T-lymphocyte-associated Ag 4)-immunoglobulin (Ig)-targeting of key co-stimulatory molecules expressed by mature/activated DCs [63,65–67] (Table 1). In cardiac allograft models, the addition of co-stimulation blockade to donor DC therapy results in a striking synergistic effect, with >100-day prolongation of graft survival (Table 1). To directly address the issue of chronic vascular rejection, we have evaluated the influence of immature donor DCs administered in conjunction with anti-CD40L (CD154) mAb in a murine aortic

Table 1. Promotion of indefinite heart graft survival by donor dendritic cells (DC).

DC Source	Species	DC treatment	Additional treatment	Route of injection	MST	Refs
MoDC	Rat	Granulocyte/macrophage -colony-stimulating factor (GM-CSF)		i.v.	>160 days	[64]
BMDC	Mouse	GM-CSF+GFβ	Anti-CD40L mAb	i.v.	>100 days (40%)	[63]
BMDC	Mouse	Low GM-CSF		i.v.	>100 days	[62]
BMDC	Mouse	NF-κB + rAd CTLA4Ig		i.v.	>100 days (40%)	[65]
BMDC	Rat	GM-CSF + IL-4	ALS	i.v.	>200 days† (50%)	[66]
BMDC	Mouse	Low GM-CSF	Anti-CD54 mAb + CTLA4Ig	i.v.	>100 days*	[67]

*Secondary challenge: cardiac allograft recipients were tested with skin grafts 30 days after heart transplantation, regardless of rejection. All 3rd party grafts were rejected; approximately 50% of donor skin grafts were accepted in anti-CD54 + CTLA4Ig-treated recipients.
†Secondary challenge post-100 days: 2nd donor heart accepted; 3rd party hearts rejected.
ALS: antilymphocyte serum; BMDC: bone marrow-derived dendritic cells; i.v.: intravenous; MoDC: monocyte-derived DC; MST: mean survival time; 'blank': none.

Table 2. Promotion of indefinite heart graft survival by recipient DCs.

DC Source	Species	DC treatment	Additional treatment	Route of injection	MST	Refs
BMDC	Rat	Donor MHC I peptide (RT1.Au)	ALS	i.t.	>150 days*	[49]
BMDC	Rat	Donor MHC I peptide (RT1.Au)	ALS	i.v.	>200 days*	[70]
BMDC	Mouse	RAPA + donor cell lysate		i.v. (×3)	>100 days	[69]
BMDC	Rat	GM-CSF + IL-4	LF 15–0195†	i.v.	>100 days	[72]
BMDC	Mouse	GM-CSF + IL-4	NFκB ODN + donor-derived cell lysate	i.v.	>100 days (33%)	[71]
BMDC	Rat	Low GM-CSF + IL-4		i.v.	>100 days (20%)	[68]

*Secondary challenge post-100 days: 2nd donor heart accepted; 3rd party rejected.
†Deoxyspergaulin derivative.
ALS: antilymphocyte serum; BMDC: bone marrow-derived dendritic cells; i.t.: intra-thymic; i.v.: intravenous; ODN: oligodeoxyribonucleotides; RAPA: rapamycin; 'blank': none.

allograft model [61]. Immature donor DCs were administered on days −7, 0, 4 and 10, with or without anti-CD40L mAb. While either treatment alone resulted in diminished intimal cell proliferation compared with untreated animals, the combination resulted in near complete inhibition of vascular sclerosis (Fig. 1). This effect was associated with significant reductions in T cell (direct pathway) and humoral immunity to donor.

Strategies utilizing recipient DCs have also included generation of immature DCs [68,69], as well as enhanced targeting of the indirect pathway through pulsing of recipient DCs with donor peptide or donor cell lysate [49,69–71], with or without additional pharmacologic or biologic treatment (Table 2). The theoretical advantage of use of recipient DCs to impact the indirect pathway could have significant benefit in counteracting the major cause of late graft failure in human organ transplantation – chronic rejection. In addition, recipient DCs are more readily available (at least in the setting of deceased donor transplantation) than donor DCs. Recently, Beriou *et al.* have utilized this approach and demonstrated that donor-specific, indefinite heart graft survival can be achieved in rats given *ex vivo*-generated, recipient-derived, regulatory (immature) DCs, together with only a short course of perioperative LF 15–0195 (a deoxyspergualin derivative) to control the direct pathway. While this effect implies induction of indirect pathway regulation, the mechanism(s) that underlies graft prolongation has yet to be elucidated [72]. A recent human study adds further support for this approach in humans. Thus, Dhodapkar *et al.*

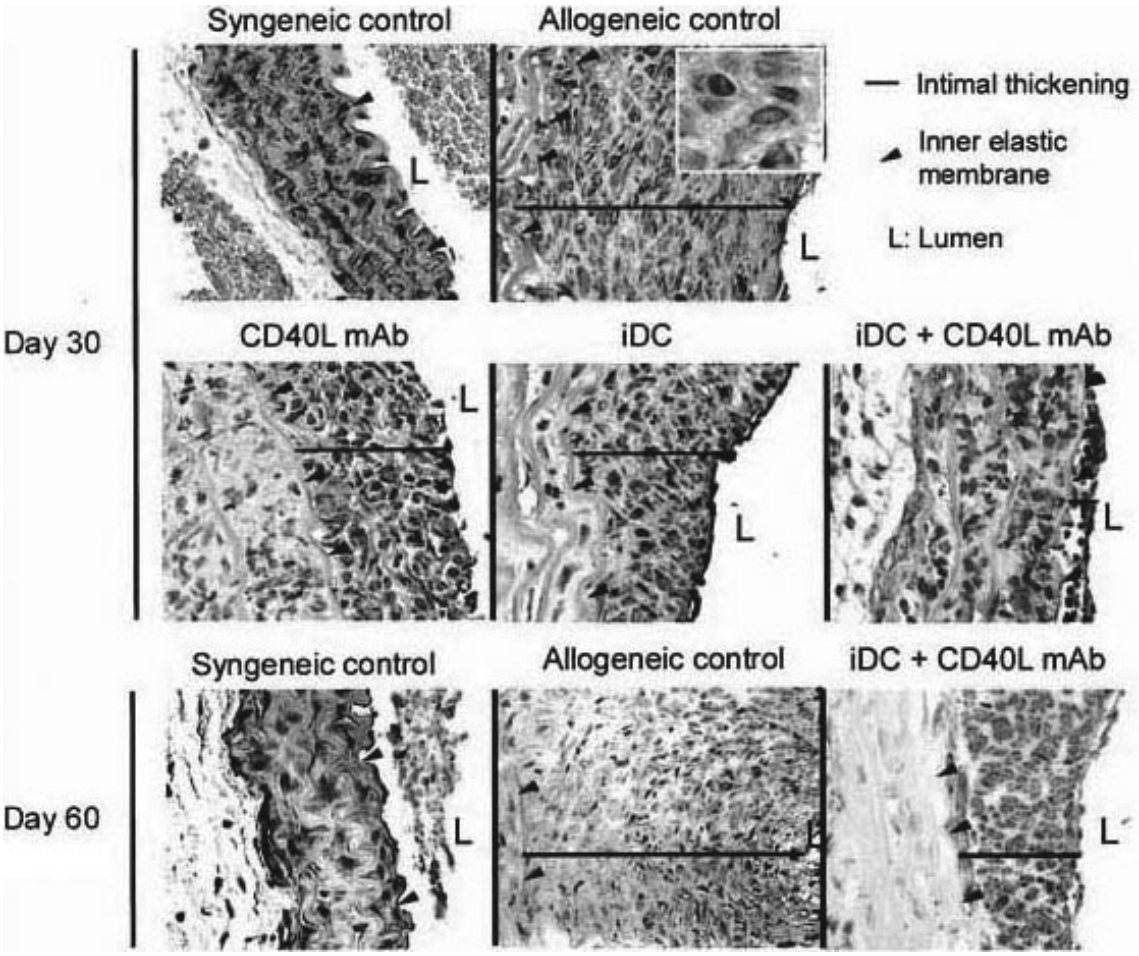

Figure 1 Immature DC + anti-CD40L monoclonal antibody (mAb) dramatically reduces transplant vasculopathy. View of aortic allografts of recipients treated with immature donor DC (iDC) or anti-CD40L mAb, either alone or in combination. Sections were stained for α-smooth muscle (αsmA) actin-positive cells by immunohistochemistry and intimal thickness was measured. Whereas moderate reductions in αsmA actin-positive cells are evident with either treatment alone compared with untreated controls at day 30, proliferation is almost completely inhibited by combination of donor iDC and anti-CD40L mAb. This suppressive effect of the combination therapy persisted on day 60. Data obtained from normal control aortas and syngeneic aortic grafts are also shown. Results are mean ± 1 SD from groups of four to six animals per group. ABC immunoperoxidase, counterstained with H&E (magnification × 200). Reprinted with permission from Wang *et al*. Transplantation 2003; 76: 562.

demonstrated that a single s.c. injection of immature autologous myeloid DCs pulsed with influenza matrix peptide in two healthy human volunteers led to inhibition of MP-specific $CD8^+$ T-cell effector function, as evidenced by diminished IFN-γ production and cytolytic function [73]. This was associated with detection of IL-10-producing $CD8^+$ Treg cells. Silencing of $CD8^+$ T-cell effectors was specific for MP, as cytomegalovirus-specific $CD8^+$ T effector cells were unaffected. This important study demonstrates that human immature DCs administered in the absence of danger signals promote the development of Treg cells capable of regulating immunity in an Ag-specific manner and provide further impetus for assessment of this approach in human organ transplantation.

In transplantation, one of the potential difficulties associated with the administration of immature, regulatory DCs is that, in the context of the danger signals present following surgical trauma and ischemia-reperfusion injury, the administered DCs may mature and accelerate graft rejection or, at least, be unable to diminish alloresponses. This potential difficulty would be overcome if the immature, regulatory DCs were administered sufficiently in advance of transplantation, such that their tolerogenic effect would be achieved by the time of transplantation (the approach taken in most experimental animal models). Such an approach is possible in live donor transplantation, but not in the deceased donor setting (including the majority of thoracic organ transplants – with the exception of the relatively small numbers of living donor lobar [lung] transplants). In this regard, several strategies have been evaluated to develop maturation-resistant DCs, with perhaps the most robust being pharmacologic treatment *in vitro*.

Manipulation of DCs to generate regulatory DCs

One potential solution to the problem of the inflammatory environment and the risk of DC maturation is to manipulate DCs *in vitro* to produce maturation-resistant, immature DCs or 'alternatively activated' DCs with stable tolerogenic properties. Strategies aimed at this goal have utilized various biologic agents [including ultraviolet B radiation, the cytokines IL-10, TGF-β, and the chimeric fusion protein CTLA4-Ig and pharmacologic agents (including corticosteroids, cyclosporine, rapamycin, mycophenolate mofetil, vitamin D3, and prostaglandin E2)] to confer tolerogenic properties on DCs ([74] and [75] and Tables 3 and 4). Of these various strategies, pharmacologic manipulation stands out as a safe, often predictable and clinically applicable option. To date, many conventional immunosuppressants and a wide variety of other pharmacologic agents with known immunoregulatory effects have been investigated for their impact on DC generation and function (reviewed in [74,75]; for a compilation of recent reports, see also Table 4). Cyclosporine (CsA) has been shown to inhibit maturation and allostimulatory capacity of mouse myeloid DCs, by inhibiting NF-κB translocation. CsA also impairs IL-6 and -12 production by DCs, and DC-triggered production of IFN-γ, IL-2 and IL-4 by T cells in the bi-directional DC-T-cell system [76]. By contrast, human monocyte-derived DCs appear resistant to the inhibitory effects of CsA on DC maturation and allostimulatory capacity. Similarly, another calcineurin inhibitor, FK506 (tacrolimus) has been reported to have heterogenous effects on DC maturation, depending on the stimuli used to trigger DC maturation [74]. FK506, however, consistently

Table 3. Effect(s) of biologic agents on DCs.

Agent	Species	DC subset	DC phenotype	T-cell stim	DC/T-cell cytokines	DC depletion	Refs
Alemtuzumab/campath	Hu	MoDC/pDC/ MDC		0		50–100%	[88–90]
ALG	Hu	MoDC		↓		25–50%	[78]
ATG	Hu	MDC/ pDC				100%	[78,91]
CD200Ig/anti-CD200R	M	pDC/BMDC		↓	IDO		[92,93]
CMR-44*	Hu	LC		↓		>97%	[94]
CTLA4Ig	Hu/M	$CD8\alpha^+$DC/pDC/$CD19^+$DC		↓	IDO		[95–97]
IL-10	Hu/M	MoDC/BMDC/MDC†	↓CM, MHC I and II	↓	↓IL-1β, IL-6, IL-12, TNFα		[98]
TGF-β	Hu/M	MoDC/BMDC/ MDC	↓CM, MHC II	↓			[99]
		LC	↑CM, MHC II	↑			

*Mouse IgM mAb reactive to human DC.

†Immature DCs only; mature DCs exposed to IL-10 retain a stable phenotype.

↑, enhancement/upregulation; ↓, suppression/reduction; 0, no major effect; ALG, antilymphocyte globulin; ATG, antithymocyte globulin; BMDC, bone marrow-derived dendritic cells; CM, classic co-stimulatory molecules; Hu, human; IDO, indoleamine 2,3-dioxygenase; LC, Langerhans cells; MDC, myeloid dendritic cells; MoDC, monocyte-derived DC; M, mouse; pDC, plasmacytoid DC; 'blank': not tested.

Table 4. Newly reported effect(s) of pharmacologic agents on DCs*.

Agent	Species	DC subset	DC Ag uptake	DC phenotype	DC/T-cell cytokines	T-cell stim	Refs
Cyclosporine A	Hu	MDC pDC	+	↓CD80, CD83, MHCII	↑IL-4 ↓IFNγ	↓	[100,101]
Cilomilast†	M	BMDC			↓TNFα, IL-12	0	[102]
Dexamethasone	Hu/M	PB/CB MoDC/ pDC	+	↑CM, CD14, CD123, MHC II ↓CD83	↑IL-4, IL-10, TNFα, IL-6 ↓IL-2, IFNγ⇔ IFNα	↓	[101,103,104]
FK778‡	Hu	MoDC		↓CM, CD83, MHC II	↓IL-12	↓	[105]
FTY720	Hu/M	MoDC/BMDC	0	0	↑ IL-10 ↓IL-12	↓/0	[106,107]
JM34§	Hu	MDC		↓CD83, MHC II	↑ IL-10 ↓IL-2	↓	[108]
Rapamycin	M	BMDC	+	↓CM, MHC II	↓IFNγ, IL-2 TNFα, IL-12	↓	[69,102]
Resveratrol¶	M	BMDC	+	↓CM, MHC II	↓IL-2, IL-12	↓	[109]
Sanglifehrin A	Hu	MoDC	–	0		0	[110]
Tacrolimus	Hu	MoDC		↓CM, CD83, MHC II	↑IL-4 ↓IFNγ	↓	[101]
Tetrahydro-4-aminobiopterin**	M	BMDC		↓MHC II	↓Th1	↓	[111]
Triptolide††	Hu	MoDC	+	↓CM, MHC II ↑ CD14		↓	[112]
Vincristine‡‡	Hu	MoDC		0	↑IL-10 ↓IL-12	↓	[113]
Vitamin D_3 and analogues	Hu/M	MoDC/BMDC		↓CM, MHC II ↑↑ CD40			[114,115]

*Not intended to be comprehensive – updated from reviews by Hackstein and Thomson [74] and Adorini *et al.* [75] and contains only recently published information.

†Oral selective phosphodiesterase IV inhibitor.

‡Leflunomide-metabolite derivative.

§Carboxamide compound.

¶Natural polyphenol found in grapes and grape products.

**Tetrahydrobiopterin analog, inhibitor of all nitric oxide synthase isoenzymes.

††Purified diterpene triepoxide from the traditional Chinese herb *Tripterygium wilfordi.*

‡‡Antineoplastic cancer therapeutic agent.

+ or ↑, enhancement/upregulation; – or ↓, suppression/reduction; 0 or ⇔, no major effect.

BMDC, bone marrow-derived dendritic cells; CB, cord blood; CM, classic costimulatory molecules; Hu, human; M, mouse; MDC, myeloid dendritic cells; MoDC, monocyte-derived DC; PB, peripheral blood; pDC, plasmacytoid DC; 'blank': not tested.

inhibits T-cell allostimulatory capacity of both mouse and human DCs, irrespective of their maturation status [74]. Glucocorticoids inhibit LPS- or CD40L-induced DC maturation and DC production of IL-12 and TNFα. DCs exposed to dexamethasone fail to prime Th1 cells efficiently, and repeated stimulation of T cells with these DCs generates IL-10-producing Treg cells. Hackstein *et al.* [69,77] have recently shown that rapamycin (RAPA) inhibits mouse BM-derived DC maturation and T-cell stimulatory capacity both *in vitro* and *in vivo*. RAPA-treated DCs are poor producers of the inflammatory cytokines IL-12 and TNFα, and render T cells hyporesponsiveness to further donor-specific Ag stimulation when infused into mice. These effects of RAPA on DCs appear to be partially related to downregulation of surface IL-4 receptor expression. Similar changes in phenotype and function have been reported regarding RAPA-treated human monocyte-derived DCs [78]. Also, in recent work, we have demonstrated that RAPA treatment does not block alloAg uptake by DCs nor impair their *in vivo* homing to T-cell areas of secondary lymphoid tissue [69]. Furthermore, a single infusion of RAPA-treated, donor-splenocyte lysate-pulsed DCs results in significant prolongation of murine cardiac allograft survival – an effect that was augmented by a short, post-transplant course of immunosuppression (FK506). Significantly, repeated infusion of RAPA-treated, donor-lysate-pulsed DCs lead to indefinite heart graft survival in 40% of recipients. This effect was associated with donor-specific T-cell hyporesponsiveness induced via both the direct and indirect pathways.

In addition to these immunosuppressant agents, several other pharmacologic agents with anti-inflammatory properties (e.g. vitamin D, aspirin, and *N*-acetyl cysteine) target DC function [74]. However, the advantage of using the aforementioned classic immunosuppressive agents to 'program' regulatory DCs lies in the fact that this approach provides a relatively safe passage into preclinical, and potentially, clinical trials, as these agents currently constitute the mainstay therapy for graft rejection. Thus, administration of regulatory DCs in the setting of conventional immunosuppression would not require a

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dramatic departure from current clinical strategies, and in addition, those immunosuppressive agents may further promote retention of the regulatory DC phenotype. Based on these studies, it is becoming increasingly evident that regulatory DC administration (or *in vivo* targeting strategies), most likely in combination with other pharmacologic and biologic agents, can play a significant role in the suppression of acute and chronic allograft rejection. The question is whether we are ready to apply DC therapy in the clinic.

Are regulatory DCs ready for the clinic and if so, where do we start?

The goal of preclinical research is to develop diagnostic and therapeutic tools/protocols to improve patient care. The decision as to when to translate small or large animal work to the clinic must be made weighing the evidence suggesting a possible beneficial effect versus the risk and magnitude of side effects, as well as the urgency of need for clinical improvements to enhance patient outcomes. Support for the clinical translation of regulatory DC therapy in transplantation can be found in DC vaccine trials for cancer. The first report of a clinical study utilizing a DC vaccine was published in *Nature Medicine* in 1996 [79]. Subsequently, more than 1000 patients have received DC vaccines in an attempt to promote immunity to tumors. Most of these studies have utilized myeloid DCs generated from monocytes, or alternatively $CD34^+$ cells. Monocytes are readily accessible in peripheral blood, and myeloid DCs are easily generated from monocytes [80]. Although there were pitfalls in design in many of the early trials, testing of DC immunotherapy in cancer has generally proven to be safe, with minimal side effects, and has been found to be effective in some patients (even though most patients had late-stage, advanced cancer) [80]. Some of the early DC vaccine trials utilized immature rather than mature DCs without untoward effects (indeed, this was the impetus for the previously discussed landmark paper of Dhodapkar *et al.* [73], in which effector T-cell function was silenced in two human subjects in an Ag-specific manner by s.c. administration of Ag-pulsed immature DCs, resulting in induction of Tregs). Although investigators have suggested that more preclinical work is needed prior to larger clinical cancer immunotherapy trials, it has been suggested that well performed, phase 1–2 studies with quality control measures and appropriate clinical and immunologic outcomes should proceed [80].

Certainly, there is much to be learned about optimization of human regulatory DC therapy for clinical organ transplantation. Variables such as cell dose, single versus multiple doses (and frequency), and route of administration (although the i.v. route seems the most appropriate for promotion of tolerance) need to be evaluated. Donor hematopoietic cell infusion via i.v. injection has been well tolerated in our experience of BM cell infusion following organ transplantation [81,82] and vaccine trials for cancer therapy [80]. Optimal timing of cell therapy relative to transplantation remains an important issue and warrants further preclinical investigation. In addition, the importance of DC stability/viability and specific migratory patterns *in vivo* postinfusion are key factors that require further analysis. Given the relative permissiveness of small animal models to tolerance induction and possible species differences in DC biology, many of these questions may not be fully answered until phase 1–2 trials are initiated in human transplant recipients. It is the opinion of the authors that we are not far from that position. Cell therapy (platelet transfusion, BM cell infusion, islet cell transplantation, DC vaccines, and others) has a very good safety record [83,84] and in the setting of a patient population with significant need, the potential benefits may justify the risks. Our own work in this regard continues in a preclinical nonhuman primate model. Recently, we have generated regulatory DCs from rhesus monkeys by vitamin D and IL-10-conditioning of monocyte-derived cells. These cells, that express low levels of surface MHC and costimulatory molecules, are poor stimulators of allogeneic T cells *in vitro* and are resistant to maturation induced by a potent pro-inflammatory cytokine cocktail (IL-1β, TNFα, IFNγ, IL-6, PGE2) (Fig. 2). Their potential to induce Ag-specific T-cell hyporesponsiveness suggests a regulatory DC-generating strategy that, coupled with conventional immunosuppressant cover, could improve outcomes in clinical organ transplantation (i.e. reduce dependence on chronic immunosuppressive drug therapy). These cells are currently being evaluated for their ability to regulate alloimmune responses *in vivo* and their ability to affect organ allograft survival.

An obvious patient population to which regulatory DC therapy could apply is live-donor organ allograft recipients with kidney the most prevalent organ transplanted in this manner. Our proposed strategy would require DC generation (from circulating blood monocytes) with modification of these cells to render them stably immature and i.v. administration of the DCs in advance (7–14 days) of transplantation under steady-state conditions.

In conclusion, there are strong indications that regulatory DCs can play a role in clinical organ transplantation. As we contemplate the introduction of this therapy in the clinic, we find encouragement in the safety of human DC vaccines and reassurance in the knowledge that this therapy can be introduced in the context of current immunosuppressive strategies. As discussed by Mirenda *et al.* [8],

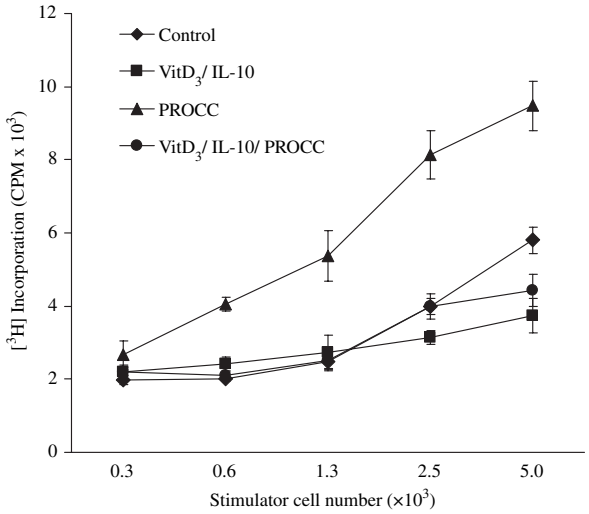

Figure 2 Vitamin D_3/IL-10-treated rhesus monkey DC are weak stimulators of allogeneic $CD2^+$ T cells and are resistant to maturation. Rhesus DC were cultured from $CD14^+$ monocytes for 7 days in complete RPMI media supplemented with human GM-CSF (1000 U/ml) and human IL-4 (1000 U/ml). Control DC received no further treatment; Vitamin D_3/IL-10 DC were generated by adding Vitamin D_3 (20 nM) to cultures on day 1 and 5 and human IL-10 (600 U/ml) on day 5 only. 5 ng/ml TNFα, 20 ng/ml IL-6, 10 ng/ml IL-1β and 1 nM PGE2 were added to DC cultures on day 5 to generate a pro-inflammatory cytokine cocktail (PROCC) and $VitD_3$/IL-10/PROCC DC. DC were co-cultured with allogeneic rhesus $CD2^+$ T cells and proliferation was determined by (^{3}H) thymidine incorporation on day 10. The results are shown as the mean CPM ± 1 SD and are representative of at least 5 experiments.

prospective infusion of mobilized regulatory DCs (i.e. with G-CSF [85–87]) or regulatory DCs propagated from blood pheresis product (Fig. 2) into graft recipients, followed by conventional immunosuppression cover, with the goal of inducing immunoregulation, is applicable in the clinic – albeit in the context live donor transplants. Further exploratory work with regulatory DCs given at the time of transplant and/or subsequently is needed to ascertain the efficacy/applicability of regulatory DCs in the context of deceased donor (heart and lung) transplantation. Well-designed phase 1–2 studies with appropriate safety, as well as immunological monitoring, may not be far off.

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