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Tolerogenic dendritic cell-regulatory T cell interaction and the promotion of transplant tolerance

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Abstract

Evidence has accumulated that targeting of donor alloantigen to quiescent dendritic cells (DC) in situ or adoptive DC therapy is associated with the expansion or induction of regulatory T cells (T reg) in experimental organ transplantation. These Treg can mediate suppression of anti-donor T effector cell responses and promote allograft tolerance. In addition, Treg can exert reciprocal, inhibitory effects on DC that maintain their tolerogenic properties. Several groups have exploited DC to expand alloAg-specific Treg in vitro, followed by adoptive transfer of the Treg to graft recipients, an approach that holds promise for tolerogenic cell therapy in clinical cell and organ transplantation.

Keywords

Dendritic cells; regulatory T cells; transplantation; tolerance

Introduction

Currently, there is considerable interest in the prospect of “tolerogenic cell therapy” for the promotion of clinical bone marrow (BM) or organ transplant tolerance (1,2). Much attention is focussed on two leukocyte populations, namely antigen (Ag)-presenting dendritic cells (DC) and regulatory T cells (Treg). Each exists in various subsets that have the ability to regulate alloreactive T cell function in vitro and in vivo. DC that are either “immature,” “semi-mature,” “maturation-resistant” or “alternatively-activated” (generated in an immunosuppressive milieu, then activated) can regulate innate and adaptive immune responses (1), including CD4⁺ and CD8⁺ T memory cell responses (3,4). These DC also promote experimental transplant tolerance, either alone, or in combination with conventional or experimental immunosuppressive therapy. Properties ascribed to tolerogenic DC, based predominantly on studies of conventional myeloid DC, and including their ability to induce and respond to Treg, are shown in Table 1. In addition to conventional myeloid DC, so-called plasmacytoid DC also display tolerogenic properties, including the ability to induce long-term allograft survival. Like DC, Treg (5) are identified and subdivided on the basis of cell surface marker expression, cytokine production and mechanisms of action (6). The most extensively studied are murine, naturally-occurring, thymus-derived CD4⁺CD25⁺ Treg that constitutively express the transcription factor forkhead box P3 (Foxp3). In addition, two populations of Treg have been described that are induced following Ag recognition. One population is characterized by the

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absence of Foxp3 and the production of the immunosuppressive cytokines, IL-10 and transforming growth factor (TGF) β , and is defined as Type-1 Treg (Tr1). The other population is activated by Ag in the presence of TGF β and induced to express Foxp3 (7).

Early studies on DC tolerogenicity suggested that T cell hypo- or unresponsiveness induced by immature DC was due to inadequate cell surface expression of co-stimulatory molecules, and to the absence (under in vivo steady-state conditions) of endogenous or exogenous danger signals or pro-inflammatory cytokines that otherwise induce DC maturation. However, there is more recent evidence that “immature,” “semi-mature,” “maturation-resistant” or “alternatively-activated” DC can maintain peripheral T cell tolerance by the stimulation and/or induction of Treg. Moreover, when triggered by specific Ag, these Treg can act back on potentially tolerogenic immature DC to block the upregulation of costimulatory molecules and to promote the production of T cell inhibitory factors, such as indoleamine dioxygenase (IDO). Such a “feedback loop” between tolerogenic DC and Treg has been described in experimental organ transplant tolerance (8).

In Situ Targeting of DC to Promote Organ Transplant Tolerance

DC can be targeted in situ in the quiescent steady-state by soluble allopeptides, engineered monoclonal antibodies (mAb) carrying allopeptides, or the infusion of donor-derived apoptotic leukocytes or DC-derived exosomes (nanovesicles <100nm) (9) that are rich in major histocompatibility complex (MHC) Ags. Targeting of allopeptides to DC has limited application in transplantation, given the need to promote unresponsiveness to the complete repertoire of donor alloAgs. Morelli and colleagues (10) have shown that intravenous infusion of early apoptotic donor splenocytes (that enhances the tolerogenic properties of host splenic DC) to quiescent, prospective murine heart allograft recipients, one week before transplant, markedly prolongs graft survival. When these apoptotic cells are combined with a single concomitant injection of anti-CD40L (CD154) mAb, indefinite graft survival (>100 days) is achieved, in the absence of significant transplant vascular pathology. Mechanistic studies have revealed that in situ targeting of recipient DC with donor alloAg in this manner induces specific deletion of specific donor-reactive T cells, and infiltration of long-surviving allografts with CD4⁺Foxp3⁺ T cells (10). While donor early apoptotic cells deliver the complete donor MHC repertoire, and the specificity of recipient APC targeting is high, potential disadvantages of this approach are the unstable nature of early apoptotic cells and the potential presence of contaminating late apoptotic or necrotic cells that could activate host DC. By contrast, donor exosomes are highly stable, although a potential disadvantage to these particles is the presence of heat shock proteins within the exosomes that could activate DC. Nevertheless, the regulatory properties of apoptotic cells and exosomes as novel therapeutic agents to promote transplant tolerance deserve further evaluation.

Use of Stably-Immature DC as “Negative Cellular Vaccines”

Adoptive transfer of tolerogenic DC of donor or recipient origin offers the potential to inhibit immune responses to alloAgs in a donor-specific manner. This has been demonstrated in preclinical rodent models of BM, skin, organ and pancreatic islet cell transplantation, using “immature,” “maturation-resistant” or “alternatively-activated” DC. The latter term refers to DC that have been propagated in the presence of anti-inflammatory or immunosuppressive agents (that suppress DC maturation), then activated with the Toll-like receptor (TLR) 4 ligand bacterial lipopolysaccharide (LPS). Such cells do not make significant amounts of bioactive IL-12, but produce comparatively large amounts of the immunosuppressive cytokine IL-10. Treatment of mice after allogeneic BM transplantation with recipient strain-derived tolerogenic DC (propagated from BM cells in IL-10 and TGF β , then stimulated for 24 hr with LPS) markedly suppresses chronic graft-versus-host disease. This protective effect involves

peripheral generation of alloreactive CD4⁺CD25⁺ Foxp3⁺ Treg from donor-derived CD4⁺CD25⁺ Foxp3⁺ T cells (11). Proof of principle that (autologous) immature DC pulsed with model Ag (keyhole limpet hemocyanin or flu matrix peptide) can induce Ag-specific T cell tolerance, associated with the induction of Treg in healthy human volunteers, has already been demonstrated (12).

In order to minimize the risk of DC maturation (in response to danger signals and other pro-inflammatory factors) following their adoptive transfer to graft recipients, various strategies have been employed to render DC stably immature. These include exposure of immature DC to immunosuppressive cytokines, in particular IL-10 and TGFβ. IL-10-conditioned DC, that induce activation and proliferation of CD4⁺ and CD8⁺ Treg (13), are currently being evaluated for the generation of alloAg-specific Tr1 cells, for the therapy of human BM transplant rejection. Numerous immunosuppressive drugs with distinct mechanisms of action, including corticosteroids, mycophenolate mofetil (MMF) and rapamycin, also promote DC tolerogenicity. Thus, dexamethasone-treated, semi-allogeneic rat DC, infused into prospective renal transplant recipients given a short postoperative course of cyclosporine, accept their allografts indefinitely in the absence of transplant vasculopathy and in association with the generation of indirect pathway Treg (14).

Our own investigations have focussed on the immunosuppressive pro-drug rapamycin, which targets the intracellular mammalian target of rapamycin (mTOR), a serine/threonine kinase that controls many aspects of cellular physiology, including transcription, translation, cell size, cytoskeletal organization and autophagy. DC propagated from murine BM in the presence of clinically-relevant concentrations of rapamycin (RAPA-DC) are phenotypically immature and resistant to costimulatory molecule upregulation following exposure to maturation-inducing stimuli, that include the TLR ligands LPS and CpG, and agonistic anti-CD40 mAb. Significantly, however, expression of the chemokine receptors CCR5 and CCR7 and CD62L is not affected by mTOR inhibition, which allows RAPA-DC to traffic in vivo to secondary lymphoid tissue (15,16). Purified, maturation-resistant RAPA-DC exhibit marked IL-1β-dependent upregulation of cell surface expression of the long (transmembrane) form of the IL-1R family member ST2 (17), shown previously to negatively regulate signal transduction in LPS-stimulated macrophages. ST2 has been identified recently as the receptor for IL-33, a Th2-proliferating cytokine. Compared with control DC, these RAPA-DC have markedly impaired ability to induce allogeneic effector CD4⁺ T cell expansion in vitro, but spare and enrich for alloAg-specific CD4⁺CD25⁺Foxp3⁺ Treg, that exert more powerful suppressive effects on T cell proliferation when compared with freshly-isolated, polyclonal CD4⁺CD25⁺ Treg in vitro (18). Adoptive transfer of donor alloAg-pulsed, host-derived RAPA-DC to quiescent recipients, one week before transplant, followed by a short, post-operative course of minimally effective RAPA leads to indefinite cardiac allograft survival, associated with graft infiltration by CD4⁺Foxp3⁺ Treg (18). Others have shown that RAPA can spare the expansion of Treg, and induce Treg de novo, a property of RAPA that may contribute to its tolerance-sparing/promoting ability in our study and in other experimental animal models, and that has been implicated by observations in organ transplantation (19). Thus, infusion of tolerogenic RAPA-DC (or tolerogenic DC propagated using other pharmacologic agents) combined with RAPA administration to the graft recipient may prove beneficial in therapeutic regimens in clinical organ transplantation aimed at reducing the patients' dependence on immunosuppressive drugs. Modification of the recipient DC phenotype by mTOR inhibition may also have therapeutic potential in hematopoietic cell transplantation (16).

In Vivo Evidence that Tolerogenic DC can Expand or Induce (AlloAg-specific) Treg that Promote/Maintain Allograft Survival

Short-term treatment of mice with immunosuppressive agents that promote DC tolerogenicity (such as $1\alpha,25$ -Dihydroxyvitamin D3 and MMF), can induce tolerance to pancreatic islet allografts, associated with increased frequency of $CD4^+CD25^+$ Treg, that can adoptively transfer transplant tolerance (20). Fujita et al (11) have reported that tolerogenic DC can induce Ag (OVA peptide)-specific generation of $CD4^+CD25^+Foxp3^+$ Treg from $CD4^+CD25^-Foxp3^-$ T cells in vivo. Moreover, in mice deficient in natural Treg, quiescent (but not activated) donor DC bearing alloAg can induce peripheral $CD4^+CD25^+$ Treg de novo, that mediate skin transplant tolerance (21). The expression of a tolerogenic phenotype by host DC in vivo may be maintained by an inhibitory feedback loop between $CD4^+CD25^+$ Treg and tolerogenic DC (8), since $CD4^+CD25^+$ Treg can downregulate costimulatory molecules on APC (22). In humans, studies of heart graft recipients have revealed that rejection-free patients have circulating $CD8^+CD28^-$ alloAg-specific T suppressor cells that upregulate Ig-like transcript (ILT) 3 and 4 on donor APC that render these cells tolerogenic (23). In a heart transplant tolerance model in the mouse, host plasmacytoid DC that acquire donor alloAg and that home to lymph nodes under tolerogenic conditions, induce alloAg-specific $CD4^+CD25^+Foxp3^+$ Treg in vivo and mediate allograft tolerance (24). Collectively, these findings suggest that host DC that acquire donor Ag under non-inflammatory or tolerogenic conditions may maintain tolerance by the induction/expansion of Treg. These Treg may, in turn, actively sustain DC tolerogenicity. Therapeutic strategies that maximise these interactions may be of key significance for the promotion of clinical transplant tolerance.

Use of Immature DC to Select/Expand AlloAg-Specific Treg In Vitro: Implications for Tolerogenic Strategies in the Clinic

Recently, DC have been used in vitro to expand mouse, non-human primate or human alloAg-specific $CD4^+CD25^+$ Treg from the naturally-occurring polyclonal repertoire in the presence of IL-2 (Table 2). Relatively small numbers of these DC-expanded $CD4^+CD25^+$ Treg can suppress T cell proliferative responses to the original stimulating alloAg many more fold than to third party alloAg. Such potent ex vivo DC-expanded Treg suppress graft-versus-host disease in mice across MHC barriers (25), and can synergise with host T cell depletion to promote long-term survival of heart allografts (26).

In our own recent experiments, we have selected mouse alloAg-specific Treg by co-incubation of freshly-isolated $CD4^+CD25^+$ T cells with donor BM-derived immature DC. This procedure rendered a T cell population with a conventional regulatory phenotype: $Foxp3^+$ $GITR^+$, $CTLA4^+$, $CCR7^+$, $CD62L^+$. These cells potently inhibited T effector cell proliferation in vitro at 1:20 and 1:40 Treg:T cell ratios. The suppressive effect was donor-specific, in that no inhibition of effector T cell responses to third party alloAg was observed. Adoptive transfer of these alloAg-specific T cell to vascularized heart allograft recipients, 7 days after transplantation, under cover of a short post-operative course of rapamycin, led to long-term graft survival (MST >150 days) in 80% of the graft recipients (27). This effect was superior to that achieved with polyclonal Treg administration (40% long-term survivors; MST 45 days). The tolerogenic effect was also alloAg-specific, as alloAg-specific Treg selected against third party DC did not prolong graft survival in comparison to host treatment with rapamycin only (MST 30 days). Thus, based on small animal studies, the principle of Treg-based therapy using DC-expanded alloAg-specific Treg has been established.

Evaluation of innovative therapeutic strategies (including cell therapy) that show promise in small animal studies, is often also conducted in outbred, non-human primates, that may provide

a better indication of the potential feasibility, safety and efficacy of the strategy in the clinic. Recently, we have characterized naturally-occurring Treg in the rhesus macaque, and established that in vitro co-culture of purified CD4⁺CD127 (IL-7R)^{-lo} cells for 14 days with immature, maturation-resistant, monocyte-derived rhesus DC in the presence of IL-2 and IL-15, promotes the expansion of alloAg-specific Treg. These Treg suppress proliferative responses of CD4⁺CD25⁻CD127⁺ effector T cells to donor, but not 3rd party alloAgs in 5-day MLR (Fig. 1). These studies confirm the potential of pharmacologically-modified DC to expand alloAg-specific Treg and provide a basis for testing the tolerogenic potential of these alloAg-specific Treg in non-human primate organ transplantation. Eventual clinical application can be envisaged, provided that appropriate clinical grade (GMP) procedures for their isolation, propagation and purification are available.

Conclusion

Both tolerogenic DC and Treg show considerable promise for therapy of allograft rejection and the promotion of donor-specific transplant tolerance. Reciprocal interactions between these regulatory cell populations may be of central importance to the maintenance of tolerance and the success of clinical protocols designed to improve long-term transplant outcomes.

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Figure 1.

Rhesus monkey naturally-occurring Treg expanded in vitro with allogeneic immature DC suppress T cell proliferation and are alloAg-specific. CD4⁺CD127⁻ T cells isolated from normal peripheral blood were co-cultured with immature, maturation-resistant allogeneic monocyte-derived DC (DC:Treg=1:20) for 14 days in the presence of human IL-2 and IL-15. Various numbers of Treg were then co-cultured with autologous CD4⁺CD25⁻CD127⁺ responder T cells (R) in the presence of either donor (allogeneic) or third party irradiated blood mononuclear cell stimulators (S) for 5 days. T cell proliferation was measured by addition of [³H]TdR during the last 18h of culture. Results are means ±1 SD.

Table 1

Properties of tolerogenic DC: inherent and conferred

<ul style="list-style-type: none"> • Ability to acquire and present Ag to Ag-specific T cells (by the direct, indirect or semi-direct pathways) • Low constitutive expression of surface MHC molecules and (low net expression of) costimulatory molecules (e.g. CD80/86 versus PD-L1) • Adequate ability to take up biodegradable micro-particles that release immunosuppressive/immunomodulatory factors • Low IL-12p70; high IL-10/TGFβ/IDO production • Resistance to maturation in response to 'danger signals' (e.g. high-mobility group box 1, TLR ligands) or CD40 ligation • Ability to generate/select/expand (allo)Ag-specific Treg (naturally-occurring or adaptive) 	<ul style="list-style-type: none"> • Ability to respond to Treg by upregulating inhibitory molecules (e.g. IL-10/IDO),- via an inhibitory feedback loop • Expression of death ligands (e.g. FasL [CD95L])/ability to promote apoptosis of effector T cells • Preservation of migratory ability (to secondary lymphoid tissue/T cell areas),- adequate relevant surface chemokine receptors (CCR5; CCR7; CD62L)/responsiveness to cognate ligands • Longevity (in vivo): resistance to innate (NK) or T cell-mediated killing • Adequate transfection efficiency and sustained expression of immunosuppressive transgene products (genetically-modified DC)
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Table 2
Expansion of alloAg-specific Treg by myeloid DC in vitro

Type of DC	Species	Type of Treg	Reference
Immature	human	IL-10-secreting CD4 ⁺ cells	(28)
Immature	human	IL-10/TGFβ-secreting Tr1 cells	(29)
Immature	human *	CD4 ⁺ CD25 ⁺	(30)
Immature	mouse †	CD4 ⁺ CD25 ⁺	(31)
Immature	mouse	CD4 ⁺ CD25 ⁺ Foxp3 ⁺	(18)
'Semi-mature'	cynomolgous macaque (monkey)	CD4 ⁺ CD25 ⁺ CD127 Foxp3 ⁺	(32)
Mature	mouse	CD4 ⁺ CD25 ⁺ Foxp3 ⁺	(25)
Mature	human	CD4 ⁺ CD25 ⁺ Foxp3 ⁺	(33)

* human autologous DC pulsed with allopeptide

† allopeptide-pulsed DC syngeneic with the host