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Tolerogenic Dendritic Cells: All Present and Correct?

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Abstract

Although well-recognized for their sentinel role and, when activated, their immunostimulatory function, bone marrow-derived dendritic cells (DC) possess inherent tolerogenic ability. Under quiescent conditions, these cells maintain central and peripheral self tolerance. When appropriately-conditioned, *in vitro* or *in vivo*, they inhibit innate and adaptive immunity to foreign Ags, including memory T cell responses. This suppressive function is mediated by various mechanisms, including the expansion and induction of Ag-specific regulatory T cells. Extensive experience in rodent models and recent work in non-human primates, indicate the potential of pharmacologically-modified, tolerogenic DC (tolDC) to regulate alloimmunity *in vivo*, and to promote lasting, alloAg-specific T cell unresponsiveness and transplant survival. While there are many questions yet to be addressed concerning the functional biology of tolDC in humans, these cells offer considerable potential as natural, safe, and Ag-specific regulators for long-term control of the outcome of organ and hematopoietic cell transplantation. This minireview surveys recent findings that enhance understanding of the functional biology and therapeutic application of tolDC, with special reference to transplantation.

Keywords

tolerance; dendritic cells; T cells; transplantation

Introduction

Dendritic cells (DC) are professional antigen (Ag)-presenting cells (APC) that comprise distinct subsets with functional plasticity. For extensive background information on DC tolerogenicity and its relation to transplant tolerance, the reader is referred to a recent (2007) comprehensive review (1). Topics that will be covered in this mini-review are summarized in Table 1.

Central tolerance depends on intrathymic DC

In the thymus, central tolerance is accomplished via negative selection of self- or foreign Ag-reactive thymocytes, - a highly efficient process that is mediated by Ag-presenting cells (APC) and by the induction of regulatory T cells (Treg). Recently, using mouse bone marrow (BM) chimeras, in which the hematopoietic cell-derived compartment was rendered deficient in Ag presentation or T cell activation, Proietto et al (2) showed that thymic DC were the major hematopoietic cells underlying both of these processes *in vivo*. The (minor) thymic DC subset implicated in polyclonal Treg generation was comparatively mature, efficient at presentation of Ags on major histocompatibility complex (MHC) class II, and

expressed high levels of the chemokines CCL17 and CCL22 that promote interaction with CD4⁺ thymocytes expressing the receptor, CCR4. Such DC, or their precursors migrating into the thymus from the blood via thymic-homing receptors, may induce both negative selection of T cells specific for Ag expressed peripherally, and Treg development. These findings are consistent with reports of the ability of alloAg-pulsed host DC, injected intrathymically, to promote donor-specific tolerance to organ allografts. Notably, the ability to induce Treg can be conferred on DC by thymic stromal lymphopoietin (TSLP), that is produced by thymic stromal cells. A recent study reveals that, TSLP-conditioned BM-derived DC with this capacity can protect non-obese diabetic (NOD) mice from type-1 (autoimmune) diabetes (3).

DC control peripheral tolerance in the steady-state

Under steady-state conditions, DC are also thought to play an important role in the induction of peripheral T cell tolerance by various mechanisms. These include the production of suppressive factors (IL-10; transforming growth factor [TGF]β; indoleamine dioxygenase [IDO]), induction of Treg, and clonal deletion of autoreactive T cells. Recent data have linked TGFβ signaling with IDO in regulating the spontaneous tolerogenicity of DC in mice (4). Other regulatory molecules, -programmed death-1 (PD-1) and cytotoxic T lymphocyte Ag (CTLA)4 appear to be crucial for peripheral CD8⁺ T cell tolerance induction by resting DC, whereas engagement of P-selectin glycoprotein ligand-1 by P-selectin on DC induces IDO, IL-10 and TGFβ genes and enhances their capacity to generate CD4⁺CD25⁺ forkhead box (Fox)p3⁺ Treg. Studies performed on human tolerogenic (tol) DC and CD4⁺ T cells indicate a crucial role for the inducible costimulator (ICOS) in T cells in the induction of peripheral tolerance, mediated mainly by an IL-10-independent mechanism (5).

To test whether maintenance of self tolerance is dependent on DC, efforts have been made to profoundly deplete DC in mice. This can be achieved, transiently, using DC subset-specific mAbs, or by conditional (CD11c⁺) DC depletion, as in CD11c-diphtheria toxin receptor transgenic mice, without the development of autoimmunity. Only when mice are constitutively depleted of all DC subsets, - conventional DC, plasmacytoid DC and Langerhans cells, does progressively severe, ultimately fatal spontaneous autoimmune disease ensue (6), likely reflecting the priming of T cells by a very small residual population of DC, or by other hematopoietic APCs.

DC can subvert CD4⁺ and CD8⁺ memory T cell responses

Overcoming the resistance of memory T cells to immunomodulation continues to present a serious challenge in transplantation. High frequencies of Ag-experienced memory T cells correlate with enhanced rejection risk and are a major hurdle to transplant induction in humans. Generally, it is thought that, unlike naive T cells, memory T cells are not susceptible to inactivation. Recently, human 'alternatively-activated', monocyte-derived DC (moDC) (conditioned by exposure to vitamin (Vit)D3 and dexamethasone, then lipopolysaccharide [LPS]-stimulated), were shown to induce (IL-10-independent) anergy in allogeneic memory T cells (7), that suppressed autologous primary CD4 and CD8 T cell responses. The induction of anergy in these memory T cells was prevented by exogenous IL-12p70, but not by depleting CD4⁺CD25^{hi} T cells. Moreover, the tolDC did not expand Foxp3⁺ T cells preferentially from memory T cells. Similar earlier studies had shown that co-cultures of dexamethasone-conditioned human moDC and alloreactive CD4⁺ memory T cells rendered the T cells hyporesponsive to restimulation with mature DC.

Memory CD8⁺ T cells are a potent barrier to transplant tolerance induction. In a recent study, Kenna et al (8) tested the outcome of presentation of genetically-targeted, tolerogenic cognate Ag, by steady-state DC to fully differentiated, long-lived CD8⁺ memory T cells in

vivo. Adoptively-transferred CD8⁺ memory T cells underwent initial expansion, followed by partial deletion, leaving residual T cells unresponsive to further antigenic stimulation. These findings suggest that CD8⁺ memory T cells may be more susceptible to inactivation of effector function than previously realized. Targeting of effector and memory T cells by transfer of Ag-encoding hematopoietic cells that can differentiate into resting (tol) DC, may provide an effective approach to therapy of established immune-mediated disease.

Plasmacytoid DC and tolerance

Plasmacytoid DC (pDC) are 'unconventional' DC, characterized by their ability, following activation, to mediate innate anti-viral immunity via production of type-1 interferon (IFN). They can also direct adaptive T cell responses (e.g. therapeutic immunity to tumor Ags) (9) and contribute to peripheral T cell tolerance. Thus, both immature and activated/mature pDC can induce Treg. Indeed, a crucial role has been described for alloAg-presenting host pDC that induce Ag-specific Treg in graft-draining lymphoid tissue and mediate tolerance to vascularized organ grafts. pDC also appear to function as tolerogenic liver DC. Thus, it has been shown recently that pDC induce efficient CD4⁺ and CD8⁺ T cell tolerance to orally administered Ags that reach the liver via the blood (10). Moreover, pDC in the liver microenvironment (where they are highly-enriched) promote in vivo tolerance by inducing anergy or deletion of Ag-specific T cells, that constantly traffick there from the blood (10). These findings have implications for understanding the inherent immune privilege of liver allografts (see below) and their capacity to transfer oral tolerance and prevent systemic Ag-specific T cell immunity. Recently, expression of the gut-specific homing chemokine receptor CCR9 on immature pDC from secondary lymphoid tissue has been reported to define tolerogenic pDC, that potently induce Treg function and suppress Ag-specific responses in vitro and in vivo (11). The latter include acute graft-versus-host disease (GVHD) induced by allogeneic CD4⁺ T cells in irradiated BM transplant recipients.

Tolerogenic DC function following allogeneic hematopoietic stem cell transplantation

Applications of allogeneic hematopoietic stem cell (HSC) transplantation have been limited due to the morbidity and mortality associated with GVHD, caused by donor T cells recognizing and reacting to MHC and minor histocompatibility (miH) Ag differences between donor and recipient. Potent 'regulatory DC', generated in vitro from mouse BM with immunosuppressive cytokines (IL-10 and TGFβ), have been described that exhibit greater capacity to protect mice from acute and chronic GVHD than 'classic' tolDC. This effect is achieved via the generation of Ag-specific, anergic CD4⁺ T cells and the peripheral induction of CD4⁺Foxp3⁺ Treg. More recently, the likely, naturally-occurring counterparts of these regulatory DC (CD49b⁺CD200R3⁺) have been identified by Sato and colleagues in mice (12). These DC protect against cutaneous chronic GVHD in a MHC-compatible/miH Ag-incompatible model via similar mechanisms, including enhanced production of donor-derived CD4⁺CD25⁺Foxp3⁺ Treg. The generation of human counterparts of these regulatory DC is a promising approach to immunotherapy that may allow Ag-specific therapeutic intervention for prevention of chronic GVHD.

Hepatic DC and inherent liver tolerogenicity

The hepatic microenvironment appears to program hematopoietic progenitor cell differentiation into tolDC (13). Moreover, liver DC have been implicated in the inherent tolerogenicity of hepatic allografts. Within the liver microenvironment, constant exposure to microbial products from the gut may confer refractory behavior on intrahepatic DC in response to Toll-like receptor (TLR) ligation (e.g. TLR4 ligation by bacterial endotoxin [=

lipopolysaccharide; LPS]), a phenomenon referred to as 'endotoxin tolerance', as demonstrated in mouse models. Moreover, compared with their counterparts in secondary lymphoid tissues, murine liver DC are poor instigators of allogeneic effector T cell responses and can induce alloreactive T cell hyporesponsiveness. Recent observations of normal human liver DC have confirmed these findings (14). Thus, unlike blood DC, human liver DC produce substantial amounts of IL-10 upon TLR4 ligation, and generate more suppressive CD4⁺CD25⁺Foxp3⁺ Treg and IL-4-producing Th2 cells via an IL-10-dependent mechanism. Of significance, hepatic TLR4 modulates the magnitude of systemic CD8⁺ effector T cell responses, as has been demonstrated using a mouse orthotopic liver transplant model. Thus, due to both downregulated innate immune responsiveness and the ability to regulate T cell function, liver DC may promote hepatic tolerogenicity. Indeed, unlike murine MHC-mismatched wild-type livers, that are accepted without immunosuppressive therapy and induce donor-specific tolerance, liver allografts from *fms-like tyrosine kinase 3* ligand-deficient mice, that lack interstitial DC, are rejected acutely (15). Interestingly, the tolerogenic potential of donor DC may not be restricted to liver allografts. Thus, costimulation blockade with anti-CD154 mAb or CTLA4Ig induces long-term survival of wild-type (WT) hearts, but not those from fractalkine receptor (CX3CR1)^{-/-} (DC-deficient) donors (16). Compared to WT donors, DC depletion in transgenic donors before heart transplant exacerbated chronic rejection under costimulation blockade.

Selective in situ targeting/delivery of auto- or alloAg to DC promotes T cell tolerance

Defects in central and peripheral tolerance are believed to underlie T cell-mediated destruction of pancreatic islet β cells in autoimmune diabetes, that is a persistent problem following islet cell transplantation for this disease. Addressing the concept that targeting of Ag to tolerogenic DC in vivo may be useful for therapy of immune-mediated disease (1), Mukhopadhyaya et al (17) delivered a mimotope peptide recognized by diabetogenic CD8⁺ T cells to DC in autoimmune NOD mice via the endocytic receptor CD205 expressed on these cells. Incidentally, this receptor has also been employed recently to target CD40 small interfering RNA-containing immunoliposomes to DC in vivo, resulting in CD40 gene silencing and immune modulation (18). Following initial islet Ag-specific T cell proliferation, the autoreactive T cells were deleted and tolerance ensued. These data support DC targeting of self Ags for therapy of chronic T-cell-mediated autoimmune disease.

In situ delivery of inhibitory donor apoptotic cells to quiescent host DC is an innovative approach to therapy of transplant rejection. In a recent report, Morelli and colleagues (19) describe the therapeutic impact of targeting recipient DC with early apoptotic (but not live) donor splenocytes on the development of chronic allograft vasculopathy (CAV). This pathologic process underlies late graft failure, and is thought to be mediated via indirect allo-recognition through T cells and alloAbs. In a fully MHC-mismatched mouse heart graft model, CAV was reduced significantly by prospective infusion of early apoptotic cells, that targeted to conventional DC in host secondary lymphoid tissue. The effect was associated with reduced indirect CD4⁺ T cell alloresponses and alloAb production, and mediated by deletion, enhanced Foxp3 expression, IL-10 secretion and reduced IFN γ release by indirect pathway CD4⁺ T cells. This was accompanied by reduced cross-priming of anti-donor CD8⁺ T cells. Whether these salutary effects of a well-tolerated procedure can be translated to a large animal model is currently under investigation.

Uterine DC, alloimmunity, and the role of HLA-G

Recent insights have been gained into the role of DC within the maternal decidua in the success of implantation and acceptance of the semi-allogeneic fetus. It has been argued that

expression of HLA-G at the human fetal-maternal interface and in transplanted tissue makes this a key molecule in regulation of alloimmune responses. HLA-G-immunoglobulin-like transcript (ILT) receptor interaction promotes the development of tolDC. Thus, ligation of transgenic ILT4 on murine DC by HLA-G reduces peptide presentation by MHC class II and prolongs skin allograft survival. Recent evidence suggests that SHP-2 protein tyrosine phosphatase and the IL-6 signal transducer and activator of transcription 3 (STAT3) signaling pathway play crucial roles in the modulation of DC differentiation by ILT4 and HLA-G (20).

Use of tolerogenic DC to promote allograft survival

It is now well-recognized that in many rodent models, immature, 'alternatively-activated', or genetically- or pharmacologically-modified, maturation-resistant DC of either donor or host origin, can promote indefinite organ allograft survival (1). A recent approach is the use of RNA interference to generate (RelB-silenced) tolDC that prevent organ allograft rejection. Alternatively, DC can be used to expand or induce Ag-specific Treg that, following their adoptive transfer, suppress alloreactivity and promote long-term graft survival or inhibit established, islet-directed autoimmunity (21). A recent finding suggests that VitD3- and dexamethasone-treated human DC (IL-10^{hi}, IL-12^{lo}) might be the most suitable tools to treat chronic inflammatory disease (22). Not only is there evidence that quiescent endogenous DC have intrinsic ability to induce and maintain a tolerant state, but quiescent DC (treated with a VitD3 analogue to prevent maturation) and bearing histocompatibility Ag, induce skin allograft tolerance, T cell unresponsiveness and the appearance of Treg both in secondary lymphoid tissue and the tolerated graft (23). Waldmann and colleagues have proposed that dominant or 'infectious' transplant tolerance may result from continuous conversion of naive to regulatory T cells as the result of exposure to donor graft Ags processed by such quiescent host DC (23).

Many reports show how diverse anti-inflammatory and immunosuppressive drugs confer tolerogenic properties on DC. Inhibition of the serine/threonine protein kinase mammalian target of rapamycin (mTOR) not only suppresses cytokine-driven T cell proliferation, but also prevents conventional DC maturation and renders DC resistant to activation induced either by TLR or CD40 ligation, or proinflammatory cytokines (24). These rapamycin-conditioned DC (R-DC) upregulate expression of the IL-1R family member ST2, that negatively regulates TLR signaling. Moreover, they enrich for alloAg-specific Treg and can promote indefinite heart allograft survival when adoptively-transferred to prospective graft recipients given a short, post-operative course of minimally effective rapamycin. Compatible with their tolerogenic phenotype, R-DC of host origin prolong allogeneic BM graft survival and reduce the severity of GVHD following HST transplantation (25). Significantly, CCR5, CCR7 and CD62L expression on DC is not affected by mTOR inhibition, permitting adoptively transferred DC-R to migrate in vivo to secondary lymphoid tissue, where immune regulation is required (25).

In mice, the infusion of mesenchymal stem cells (that have been shown to inhibit DC function) and rapamycin promotes heart allograft tolerance, associated with high frequencies of tolDC and CD4⁺CD25⁺Foxp3⁺ Treg in host spleens. In other reports, an inhibitory feedback loop between Treg and DC has been described in experimental heart transplant tolerance. DC in these tolerant heart recipients showed enhanced expression of IDO (26),- that can be induced by Treg and that promotes allogeneic T cell apoptosis. Adoptive transfer of the tolDC from primary graft recipients augmented Treg and prolonged graft survival in naive, secondary heart graft recipients, and synergized with Treg to induce tolerance. Another immunosuppressive enzyme that has been implicated in the in vivo

immunosuppressive properties of tolDC in experimental organ transplantation is heme oxygenase-1.

Extrapolation from rodent to human studies

Much of what we know about DC development and function is based largely on studies of cells isolated from rodent models or propagated in vitro, either from murine BM or human blood-borne precursors in response to appropriate hematopoietic growth factors. Phenotypic differences between rodent and human DC can make direct extrapolation of function difficult, and much remains to be uncovered about human DC development and function, especially in vivo. Issues confronting human DC biology in relation to the monitoring, outcome and therapy of organ and HSC transplantation (Table 2) need to be more fully addressed. Although there is proof-of-principle that human autologous immature moDC pulsed with nominal Ag can inhibit Ag-specific effector T cell responses, and induce Ag-specific Treg in healthy individuals, there are, as yet, no reports of DC therapy in clinical HSC or organ transplantation.

Non-human primates (NHP) are important pre-clinical models for assessment of promising strategies for transplant tolerance. The recent characterization of NHP tolDC that can inhibit T cell proliferation, generate alloAg-specific Treg, and modulate alloimmune reactivity in vivo, are encouraging pointers to their potential efficacy in NHP cell and organ transplantation. Although not predicted by rodent studies, the potential risk that such 'negative vaccination' could lead to processing and presentation of alloAg by endogenous DC, possibly leading to increased alloimmunity needs to be carefully monitored. Alternative models for testing therapeutic strategies with tolDC that could help define the most appropriate tolDC subset or DC-specific in vivo targeting approaches are humanized mice, such as NOD-SCID- $\gamma c^{-/-}$ or Rag2 $^{-/-}$ $\gamma c^{-/-}$ animals, engrafted with human CD34 $^{+}$ HSC. Despite model limitations, humanized mice could help establish the longevity, functional stability, migratory patterns, optimal numbers and frequency and route of tolDC administration, that may help guide clinical testing. Support for clinical evaluation of tolDC therapy in humans comes from numerous DC vaccine trials in cancer, that have generally proved safe, with minimal side effects, and with efficacy in some patients. Indeed, initial testing of tolDC in human autoimmune disease is already underway.

Conclusions

Considerable progress has been made in understanding how DC mediate tolerance under steady-state or anti-inflammatory conditions, and how these cells can be manipulated to suppress alloimmunity and promote transplant tolerance in small animal models. Despite many unanswered questions and the hurdles to be overcome, tolDC offer considerable potential as natural regulators of immunity that provide the opportunity for safe, Ag-specific and comparatively inexpensive long-term control of allograft rejection.

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Table 1

Tolerogenic DC

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- Maintain central and peripheral tolerance to self Ag in the quiescent steady-state
 - Mediate oral and liver tolerance
 - Use various mechanisms to control T cell survival, proliferation and function
 - Can be targeted in situ with auto- or alloAgs to suppress adverse immune responses
 - Can inhibit memory T cell responses
 - Reciprocal DC-Treg interactions underlie experimentally-induced transplant tolerance
 - TolDC can be generated in vitro for therapy of graft rejection, GVHD and autoimmune disorders
 - Human DC can induce Ag-specific T cell tolerance in vivo
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Table 2**Challenges facing DC-based therapy for tolerance induction in transplantation**

<ul style="list-style-type: none">• Choice of type (sub-set) and source of DC (bone marrow versus blood)• Form of (donor) alloAg (cell-free lysate, MHC peptides; early apoptotic cells; exosomes)• Stable DC immaturity/tolerogenicity/specificity, ensuring minimal risk of host sensitization• Optimal route, timing, dose and frequency of administration (in non-human primates; humans)• Optimal immunosuppressive agents for combination therapy• Outcomes that can be monitored adequately in the laboratory and clinic• Overcoming heterologous immunity/memory/late graft failure• Regulatory issues: safety (GMP compliance); standardization/quality control• Commercialization of therapeutic product
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