Tolerogenic dendritic cells: cytokine modulation comes of age

Sergio Rutella, Silvio Danese, and Giuseppe Leone

Dendritic cells (DCs) include a heterogeneous family of professional APCs involved in initiation of immunity and in immunologic tolerance. Specifically, peripheral tolerance can be achieved and maintained by promoting regulatory T-cell (Treg) responses and/or T-cell anergy or deletion. Until recently, immature developmental stages of DC differentiation were believed to induce T-cell anergy or Treg cells, whereas DCs transformed into mature DCs by activation stimuli were thought to represent immunogenic DCs capable of inciting primary T-cell responses. This paradigm has been challenged by the demonstration of T-reg cell expansion by antigen-bearing, fully mature DCs. Similarly, semimature DCs with a distinctive interleukin 10 (IL-10)+IL-12− cytokine production profile might be endowed with tolerogenic functions, supporting the concept that DC maturation per se should no longer be considered as a distinguishing feature of immunogenic as opposed to tolerogenic DCs (TDCs).

Cytokine-modulated TDCs reflect an incomplete or altered status of monocyte differentiation and promote in vitro induction of Treg cells and/or in vivo protection from autoimmune diseases. Several growth factors, including IL-10, transforming growth factor β (TGF-β), granulocyte colony-stimulating factor (G-CSF), hepatocyte growth factor (HGF), and vasoactive intestinal peptide (VIP), modulate DC maturation and favor the differentiation of TDCs. From a therapeutic standpoint, cytokine-modulated TDCs might be beneficial for prevention and/or treatment of posttransplantation graft-versus-host disease (GVHD) and autoimmunity.

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Perspective

Introduction

Dendritic cells (DCs) are highly specialized antigen (Ag)–presenting cells (APCs) that integrate a variety of incoming signals and orchestrate the immune response.1 Bidirectional interactions between DCs and Ag-experienced T cells initiate either an immunogenic or a tolerogenic pathway and are of crucial importance in autoimmune diseases and in transplantation medicine.2 Conventional DC subsets described in humans include myeloid DCs (mDCs) and plasmacytoid DCs (pDCs).3 Myeloid DCs develop from CD11c+HLA-DR+ blood precursors and undergo activation and maturation in response to triggering of toll-like receptors (TLRs), a class of pattern recognition receptors engaged by microbial products.1 DC maturation is paralleled by up-regulation of major histocompatibility complex (MHC) class II and costimulatory CD80/CD86 molecules and by production of interleukin (IL)–12. Plasmacytoid DCs might differentiate either from a common blood DC precursor or from a committed lymphoid progenitor; express CD123, CD4, and CD62L; and secrete type I interferon (IFN) in response to viruses and/or TLR9 ligands.3 In secondary lymphoid organs of mice, different subpopulations of DCs have been identified, namely, CD8a− lymphoid DCs, CD8a+ myeloid DCs, and Langerhans cell–derived DCs.4 In addition, expression of B220 on mouse DCs identifies a functional counterpart of human pDCs.4

DC ability to induce tolerance has been demonstrated initially by experiments on immature DCs residing in peripheral lymphoid tissues.5 Under steady-state conditions, immature DCs capture apoptotic bodies arising from cell turnover and, upon migration to draining lymph nodes, silence T cells to self-Ags.6 Short-lived, migratory DCs might transfer tissue-derived peptides to longer-lived tolerogenic DCs (TDCs) upon reaching the lymph node. Interestingly, self-Ag transport, processing, and presentation for tolerance induction by DCs require partial maturation.7 In the absence of inflammation or TLR triggering, DCs will not produce IL-12, and DCs will be arrested at a semimature stage. Also, DC residence in a tolerizing milieu, for example, in mucosal or immune-privileged sites, affects DC capacity of priming Treg cells. DCs isolated from Peyer patches, lungs, or the anterior chamber of the eye display a mature phenotype, secrete IL-10 but not IL-12, and drive the development of IL-10–producing regulatory T (Treg) cells.7 Even more intriguing, fully mature, immunologically competent DCs can generate “tolerogenic” peptides upon processing of a self-Ag, thyroid peroxidase.8 Accordingly, Ag-loaded, mature DCs can expand CD4+CD25+ Treg cells with retained ability to suppress the proliferation of nonregulatory T cells.9 Thus, a growing body of experimental evidence indicates that DC maturation per se is neither a distinguishing feature of immunogenic as opposed to TDCs nor a control point for initiating immunity (Figure 1).

Given the remarkable functional plasticity of both mDCs and pDCs, it is presently believed that the net effect of Ag dose, DC lineage and maturation status, DC stimulation by pathogen-derived products, and cytokine milieu at sites of inflammation determines whether an immunogenic or a tolerogenic T-cell response will develop.1,5

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TDCs: a definition by function

Although expression of signaling lymphocyte activation molecule (SLAM), programmed cell death ligand-1 (PD-L1), DEC-205 (CD205), and inhibitory receptors of the immunoglobulin-like transcript (ILT) family (ILT3/ILT4) has been associated with DC tolerogenicity, TDCs conceivably correspond to functional subtypes rather than to unique DC lineages in vivo.\textsuperscript{10-13}

Promotion of Treg-cell conversion from naive T cells and/or expansion of pre-existing Treg cells, release of IL-10, and expression of indoleamine 2,3-dioxygenase (IDO) might underlie DC function in inducing peripheral tolerance (Figure 2).\textsuperscript{5,14,15} DCs, either at immature or at semimature stage, can differentiate both naturally occurring CD4\textsuperscript{+}CD25\textsuperscript{+} Treg cells and adaptive, IL-10–producing Tr1 cells either in vitro or in vivo,\textsuperscript{16,17} and overexpression of Jagged-1 on DCs induces Ag-specific, transforming growth factor (TGF)–β–producing Treg cells.\textsuperscript{18} Immature mDCs convert naive T cells into IL-10–producing Tr1 cells in vitro, whereas Ag-bearing TDCs injected subcutaneously in humans induce IL-10–producing, influenza-specific CD8\textsuperscript{+} T cells and mediate a decline of IFN-γ–producing T cells.\textsuperscript{19,20} Additionally, mature DCs expand CD4\textsuperscript{+}CD25\textsuperscript{+} Tregs with retained suppressive activity and are capable of inhibiting diabetes development in NOD mice.\textsuperscript{5,21,22} These studies highlight that mature DCs might be critical for the restimulation and/or expansion of functional Treg cells (Figure 1).

IDO mediates consumption of tryptophan and induces T-cell suppression by activation of GCN2 kinase in T cells, altered APC function, bystander suppression, and/or generation of Treg cells.\textsuperscript{14,23} In mice, IDO expression segregates with B220\textsuperscript{+} pDCs and CD8alpha\textsuperscript{+} splenic DCs and with CD19\textsuperscript{+} pDCs contained within tumor-draining lymph nodes.\textsuperscript{24,25} In humans, CD123\textsuperscript{+}CCR6\textsuperscript{+} DCs might acquire IDO functional activity after activation by IFN-γ or PGE\textsubscript{2} or after CD80/CD86 ligation by cytotoxic T-lymphocyte Ag 4 (CTLA4)/CD28 on Treg cells.\textsuperscript{26,27}

Intriguing evidence has been provided on the differential dynamics of DC–T-cell encounters in intact lymph nodes in the context of immunity and tolerance. During the course of tolerance induction by in vivo targeting of ovalbumin to CD205 on DCs, T cells remain motile and establish transient interactions (lasting 30-90 seconds) with one or multiple Ag-bearing DCs (10-13 per hour).\textsuperscript{28} Conversely, stable and long-lasting DC–T-cell contacts culminate in productive T-cell responses. A functional consequence of the proposed model is that multiple brief signals delivered by a high proportion of DCs would be required to maintain peripheral T-cell tolerance.\textsuperscript{28}

Cytokines as inducers of TDCs

DCs can be licensed to become tolerogenic by a variety of cytokines (Table 1). Conventionally, IL-10 and/or TGF-β have been implicated in the in vitro differentiation of DCs. IL-10 interferes with the granulocyte macrophage (GM)–CSF/IL-13–induced differentiation of human monocytes to DCs but rather promotes the generation of macrophages with enhanced endocytic activity and poor Ag-presenting function.\textsuperscript{29} In mice, IL-10–elicited CD11c\textsuperscript{low}/CD45RB\textsuperscript{high} TDCs acquire plasmacytoid morphology and immature phenotype and promote Tr1 differentiation in vitro and T-cell anergy in vivo.\textsuperscript{30} TGF-β–treated murine APCs induce CD8\textsuperscript{+} Treg cells, suppress de novo experimental autoimmune encephalomyelitis (EAE), and modulate ongoing EAE.\textsuperscript{31} Tumor necrosis factor factor α (TNF-α) differentiates murine semimature IL-12\textsuperscript{high} DCs, expressing high levels of MHC class II and costimulatory molecules.\textsuperscript{32} Such thyroglobulin (TG)–pulsed, TNF-α–matured DCs induce IL-10–secreting CD4\textsuperscript{+}CD25\textsuperscript{+}FoxP3\textsuperscript{+} Treg cells, capable of suppressing TG-specific T cells in a cell contact–
dependent manner. Similarly, GM-CSF induces murine semimature IL-12\textsuperscript{low} DCs in vivo with high expression of MHC class II and costimulatory molecules.

More recently, novel and unrecognized roles have been attributed to individual cytokines in the promotion of DC tolerogenicity (Table 1). Vasoactive intestinal peptide (VIP) is a neuropeptide released by immune cells in response to Ag stimulation and a potent anti-inflammatory agent. Exposure of murine BM-derived DCs to VIP translates into the differentiation of maturation-resistant, IL-10–secreting APCs. DCs modulated with VIP express high levels of MHC but low levels of costimulatory molecules and induce anergic Tr1-like cells. Also, IL-21 polarizes the GM-CSF–driven differentiation of murine BM-derived DCs toward immature DCs that inhibit Ag-specific T-cell proliferation.

In humans, IL-16 and thrombopoietin govern the generation of CD34\textsuperscript{+} cell-derived IL-10\textsuperscript{+}IL-12\textsuperscript{low} TDCs, co-expressing ILTIs, CD80/CD86, and MHC class II, and with impaired ability to present influenza virus to autologous CD4\textsuperscript{+} T cells. INF-\(\gamma\) (IL-28/IL-29)–treated DCs acquire an MHC class II/III and costimulation\textsuperscript{low}IL-12p70\textsuperscript{low} phenotype and induce an IL-2–dependent proliferation of CD4\textsuperscript{+}CD25\textsuperscript{+}FoxP3\textsuperscript{+} Treg cells. Macrophage (M)–CSF and IL-4 can induce monocyte-derived IL-10\textsuperscript{+}IL-12\textsuperscript{neg} TDCs. Granulocyte (G)–CSF indirectly favors the in vitro differentiation of peripheral blood monocytes into TDCs through the release of IL-10 and IFN-\(\gamma\). G-CSF–induced TDCs possess a mature, HLA-DR\textsuperscript{+}CD80/CD86\textsuperscript{+} phenotype and release trace amounts of IL-12p70. Furthermore, G-CSF–driven TDCs activate the in vitro differentiation of IL-10/TGF-\(\beta\)–producing Tr1 cells, capable of suppressing bystander T cells in a cytokine-dependent manner. Similarly, naive CD4\textsuperscript{+} T cells exposed to G-CSF in vivo differentiate into IL-10–producing Tr1 cells after in vitro allo-Ag challenge. Hepatocyte growth factor (HGF) has been implicated in angiogenesis and promotion of tumor cell migration and invasiveness. HGF skew monocyte differentiation toward IL-10–producing, ILT3\textsuperscript{+}CD209\textsuperscript{+} costimulation\textsuperscript{low} TDCs. HGF-differentiated monocytes induce the in vitro development of CD4\textsuperscript{+}CD25\textsuperscript{+}FoxP3\textsuperscript{+} Treg cells that suppress conventional, nonregulatory T cells in a cell contact–dependent manner. HGF evokes a unique gene signature in monocytes, consisting of overexpression of genes potentially implicated in immune tolerance, for example, IDO, C5R1, CCL2, complement component C1q, and IL-10. Mechanistically, DC production of IL-10 and DC expression of ILT3 and IDO cooperate to the in vitro differentiation of Treg cells by HGF-modulated TDCs.

Thymic stromal lymphopoietin (TSLP) is produced by epithelial cells of thymic Hassall’s corpuscles. TSLP-treated thymic DCs express CD80/CD86 and MHC class II but release negligible amounts of IL-12p70 and promote the conversion of CD4\textsuperscript{+}CD25\textsuperscript{+} thymocytes into CD4\textsuperscript{+}CD25\textsuperscript{+}FoxP3\textsuperscript{+} Treg cells. Interestingly, TSLP-activated DCs exert limited effects on the expansion of preformed CD4\textsuperscript{+}CD25\textsuperscript{+} thymocytes, supporting the notion that cytokine-modulated semimature TDCs might be preferentially implicated in the conversion of naive T cells into Tregs.

TDCs in preclinical models of immune-mediated diseases and in organ transplantation

Because DCs play an undisputed role in inciting autoimmune diseases and in instigating transplant rejection, therapeutic harnessing of peripheral tolerance by TDCs represents an attractive avenue for future clinical applications. Animal models of autoimmunity
have provided proof of principle in favor of therapeutic benefits of cytokine-modulated TDCs (Table 1).

In mice, Ag-pulsed, TGF-β2–treated TDCs induce regulatory CD8+ T cells and suppress ongoing EAE.31 TNF-α–differentiated, semimature DCs induce IL-10–secreting, Ag-specific Treg cells and protect from EAE.43 Host-matched TDCs differentiated with IL-10/TGF-β, and GM-CSF reduce serum levels of pro-inflammatory cytokines, induce mixed CD25+ and IL-10+ subpopulations of Tregs with retained graft-versus-leukemia (GVL) responses, and protect from lethal GVHD.44 Semimature DCs induced by GM-CSF expand TG-specific, IL-10+ Treg cells. Additionally, host-matched, VIP-differentiated TDCs possess therapeutic effect in murine CD4+CD25− Foxp3+ Tregs.45 TDCs treated with TSLP, thymic stromal lymphopoietin, and MBP, myelin basic protein.

### Table 1. Cytokines involved in the in vitro or in vivo differentiation of TDCs

<table>
<thead>
<tr>
<th>Cytokine, experimental model</th>
<th>Additional stimuli applied during in vitro differentiation</th>
<th>Sources</th>
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<tr>
<td>TNF-α</td>
<td>Suppression of EAT</td>
<td>Virgini et al32, Menges et al43</td>
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<td></td>
<td>Suppression of EAE</td>
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<tr>
<td>GM-CSF</td>
<td>Suppression of EAT after in vivo provision of GM-CSF</td>
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<tr>
<td>IL-10/TGF-β</td>
<td>Protection from lethal GVHD</td>
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<tr>
<td>G-CSF</td>
<td>Differentiation of human monocyte-derived DCs</td>
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<tr>
<td></td>
<td>Provision of G-CSF in vivo to bone marrow donor mice</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Provision of G-CSF in vivo to mice with EAE, diabetes, and lupus nephritis</td>
<td>NA</td>
</tr>
<tr>
<td>IFN-α</td>
<td>Differentiation of human monocyte-derived DCs</td>
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<td></td>
<td>Differentiation of mouse bone marrow–derived DCs for subsequent use in posttransplantation GVHD</td>
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<td>M-CSF</td>
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<td>IL-10</td>
<td>Differentiation of human monocyte-derived DCs</td>
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<tr>
<td>TSLP</td>
<td>Differentiation of human CD4+CD25+Foxp3+ thymocytes by TSLP-treated CD11c+ thymic DCs</td>
<td>None</td>
</tr>
</tbody>
</table>

EAT indicates experimental autoimmune thyroiditis; EAE, experimental autoimmune encephalomyelitis; NA, not applicable; TSLP, thymic stromal lymphopoietin; and MBP, myelin basic protein.
Permanent acceptance of organ allografts has not been gener-
ally achieved with TDC therapy alone. Blockade of the CD40-
CD154 signaling pathway might be synergistic with DC therapy,
thus promoting the survival of skin allografts. Pharmacologi-
tically treated DCs that are decommissioned from full maturation
might also contribute to the maintenance of transplantation
tolerance through the promotion of Treg-cell differentiation.
Simultaneous targeting of DCs and Treg cells might be a desirable
approach to induce transplantation tolerance, and a self-maintaining regulatory
loop has been recently described in a murine model of cardiac
transplantation, where TDCs induce Treg cells and Treg cells, in
turn, program the generation of TDCs. Importantly, inclusion of
TDCs in future therapeutic regimens might minimize dependence
on nonspecific immunosuppressive drugs currently administered
for transplant rejection.

Conclusions and future perspectives

Theoretically, TDCs for clinical application might be obtained after
in vivo cytokine administration or might be generated ex vivo from
monocyte populations with good manufacturing practice (GMP)–
grade cytokine cocktails. Cytokines possess druglike properties,
such as potency, but also have disadvantages, such as a short
half-life. Considerable effort has been devoted to engineering
cytokines with enhanced half-lives and, for instance, pegylated
G-CSF manifests a remarkable potency in inducing the differen-
tiation of Treg cells that protect against mouse acute GVHD.
Pharmacologic arrest of DC maturation or use of genetically
engineered DCs expressing immunosuppressive molecules might
selectively enhance DC tolerogenicity. Among the various drugs
implicated in the promotion of DC tolerogenicity, 1α,25-
dihydroxyvitamin D3 induces the differentiation of TDCs with
up-regulated expression of ILT3, low IL-10, and enhanced IL-12
secretion. The armamentarium of inhibitory pharmacologic agents
is expected to increase as more compounds are evaluated for the
ability to affect DC functions.

Transfection of DCs with a gene construct encoding a modified
CTLA4 molecule translates into deficient expression of CD80/
CD86 and induction of T-cell anergy and might represent an
attractive means of restoring tolerance in autoimmune diseases.
A human HGF expression vector administered in liposomal formula-
tion decreases IL-12, IFN-γ, and TNF-α expression in tissues, thus
improving mice survival from acute GVHD. It is tempting to
speculate that the beneficial effects of HGF treatment on GVHD
reported in this study might be, at least in part, attributed to the
cytokine-driven promotion of DC tolerogenicity.

An important issue to be considered when designing DC-based
immunotherapy protocols is whether TDCs might inadvertently
receive in vivo maturation signals in an inflammatory microen-
vironment and incite unwanted T-cell responses as fully mature DCs.
To date, several reports have demonstrated a stable phenotype of
cytokine-modulated DC preparations, indicating that TDCs differ-
entiated in the presence of G-CSF, IL-21, VIP, or low-dose
GM-CSF might be resistant to further maturation-inducing
stimuli. This concern is further mitigated by recent findings
that endogenous modulators produced at sites of inflammation, for
example, PGF2 and histamine, might interfere with DC maturation
and promote DC tolerogenic functions.

GVHD represents a privileged clinical setting for human trials
of TDCs/Treg cells because of predictable time of onset, profound
host lymphopenia favoring homeostatic proliferation of infused
and/or in vivo-generated Tregs, broad alloreactive repertoire, and
relative ease of procurement of donor-type monocytes and/or
CD4+CD25+ Treg cells. Theoretically, TDCs might be differenti-
ated from host-type monocytes challenged with donor-derived cells
in the presence of immunoregulatory cytokines and/or drugs. It is
conceivable that TDCs must be administered repeatedly after
disease onset, as TDC half-life approaches 17 to 18 days in animal
models of GVHD. Ongoing clinical trials in haploidentical stem
cell transplantation will determine whether donor cells cultured ex vivo
with IL-10 in the presence of irradiated host cells provide immune
reconstitution with anergic, host-specific Tr1 cells.

It remains to be determined whether TDCs induce undesired in
vivo systemic immunosuppression through the generation of Treg
cells. Whereas activation of Treg cells is Ag-specific, activated
Treg cells might induce Ag-nonspecific suppression. Importantly,
TDC therapy of GVHD is associated with maintenance of GVHD
activity, thus re-assuring of preserved antitumor T-cell responses in
TDC-treated animals. In vitro experimental evidence further
indicates that the tolerizing capacity of semimature TDCs might be
restricted to Ag-specific CD4+ T cells and leave CD8+ T-cell
effector functions unaffected. Collectively, basic findings on DC
functional plasticity provide grounds for optimism in clinical
translation of TDCs to human immune-mediated disorders. Manipu-
lation of DC effector functions by external stimuli warrants further
investigations and will hopefully lead to safe and efficacious
TDC-based therapies in the near future.

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