

Tolerogenic Dendritic Cells: Key Regulators of Peripheral Tolerance in Health and Disease

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Key Words

Dendritic cells · Immunotherapy · Maturation · Peripheral tolerance · Regulatory T cells · Tolerance

Abstract

Dendritic cells (DCs) as professional antigen-presenting cells are able to induce immunity and tolerance in different conditions, which leads to strong immune responses against hostile agents or tolerance to self-antigens. Although for subsets of DCs, a regulatory function has been shown, the role of the microenvironment is momentous to generate tolerogenic DCs (tDCs). Different microorganisms and tumor cells escape from immune responses by producing mediators and components that influence DCs to show tolerogenic characteristics. In this review, tDC induction was explained in steady state and disease conditions. Moreover, an overview was presented on the efforts to generate in vitro tDCs, their clinical applications and the problems which remain to be solved in this field.

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Introduction

When Ralph Steinman and Zanvil A. Cohn [1] discovered dendritic cells (DCs) as ‘large stellate cells’ for the first time in 1973, it was hard to imagine that these

cells would precisely determine the outcome of immune responses [2, 3]. Depending on their phenotypic and functional states, DCs may promote immunogenic or tolerogenic responses [4, 5]. Nevertheless, many questions are left answered about the importance of different receptors and their activation. DCs express various types of innate immune receptors called pattern recognition receptors that recognize pathogen structures and trigger DC activation. Although various Toll-like receptors, such as TLR3, TLR4, TLR5, TLR7 and TLR8, induce immune activation, others can silence immune responses through tolerance induction in DCs. For example, TLR2 activation induces IL-10 production, inhibits TLR7/TLR9 signaling and prevents IFN- α and - β production [6, 7].

Despite the immunogenic ability of DCs in raising immune responses, which has been attributed to them for decades as the main and only role in the immune system, they have also been ascribed opposite roles in tolerance induction and silencing of immune responses [8, 9]. DCs play a critical role in the induction of different well-known subsets of T cells, such as Th1, Th2, Th17 and regulatory T cells (T_{reg}s). Previous studies showed that delivering protein antigens such as ovalbumin (OVA) to DCs using DEC-205 antibody chemically coupled to OVA induce T-cell tolerance and α -DEC-205:OVA-injected mice became unresponsive to OVA rechallenge. In the steady state, DCs play a critical role in the induction

of tolerance against self-antigens [8]. Moreover, complete ablation of DCs breaks self-tolerance of CD4⁺ T cells and results in fatal autoimmunity [10].

How can DCs maintain a fine balance between tolerance and immunity? The early answers have relied on the maturation state of DCs. In other words, DCs have to deliver two signals simultaneously to stimulate T cells; first, the major histocompatibility complex (MHC)-peptide complex and, second, CD28 stimulation mediated particularly by B7-1 and B7-2 molecules. DCs without CD28 stimulation signals are not able to stimulate T cells and are called immature DCs. As a result, some terms have been coined among immunologists such as 'mature' and 'immature' states of DCs. Studies have demonstrated that injection of immature DCs can induce antigen-specific T_{reg}s and prevent autoimmune diseases while mature DCs promote immunity against pathogens and tumors [11, 12]. Later, it has been revealed that the production of inflammatory cytokines, e.g. IL-12, is required for mature DCs to induce Th1 cells and lack of IL-12 production induces anergic T cells or T_{reg}s [13, 14].

Tolerogenic DCs (tDCs) or semimature DCs are cells which express costimulatory molecules but are unable to produce proinflammatory cytokines. Therefore, they will not induce Th1/Th17 responses while efficiently inducing T_{reg}s. Although both immature and semimature DCs induce T_{reg}s, the latter have advantages over the others. For example, tDCs will change much less than immature DCs in vivo after being exposed to the inflammatory microenvironment [15]. Nowadays, tDCs have introduced new possibilities to treat inflammatory diseases, including autoimmune diseases, allergic responses and allograft rejections. However, in-depth understanding of their essence and optimization of their generation will be required for their potential application in the treatment of different inflammatory diseases.

DCs: Inducers of Tolerance and Immunity

At first, mature DCs were defined as cells which provide both signals to T cells, including the presentation of antigens and costimulation, and consequently induce immunity, while immature DCs provide only the first one and induce anergic responses. Although it was logically possible and convincing at the time, there were still some discordant studies which showed that phenotypic mature DCs (high expression of costimulatory molecules) could not always induce Th1 responses but they can promote T_{reg}s [16, 17]. Therefore, an improved perspective toward

DC maturation to explain their dual function is necessary. Thus, the three-signal hypothesis emerged as a concept which is still working [18]. According to this theory, the secretion of proinflammatory cytokines (e.g. IL-12) determines outcome [13, 19]. In contrast to those which produce only MHC-peptide and costimulatory molecules, DCs providing three essential signals induce Th1 responses [16]. That is why many studies which have not evaluated the type of cytokine secreted from DCs but only assessed the expression of costimulatory molecules should be meticulously considered [20]. Interestingly, different subsets of T_{reg}s require different levels of costimulatory molecule presentation on DCs. For example, strong B7 costimulation is required to maintain the level of natural T_{reg}s, but no or a reduced level of B7 costimulation is required to induce Foxp3⁺ and T_{reg}s. This issue was well reviewed by Pletinckx et al. [21].

Microenvironment

The microenvironment of DCs (fig. 1) includes cell-cell interaction, microbial flora-DC interaction and apoptotic bodies derived from dying cells interacting with DC.

Different cells influence DCs by three mechanisms: secreting and expressing various molecules, generating microvesicles and modifying cellular junctions. For example, lung stromal cells produce a high amount of TGF- β and thymic stromal lymphopoietin (TSLP) to induce tDCs in the lung, which prevents airway inflammation and excessive immune responses to airway antigens [22].

T_{reg}s induce tDCs in peripheral organs. This interaction includes both contact-dependent and contact-independent mechanisms. Table 1 demonstrates some molecules involved and their receptors expressed on DCs and cytokines secreted from T_{reg}s to induce tDCs. Recent studies showed that in subjects that develop low zone tolerance as a protective mechanism against contact allergies, the cross talk between T_{reg}s and tDCs plays a pivotal role. Exposure to a low dose of allergens induces CD4⁺CD25⁺ regulatory cells that contact CD11c⁺ DCs via gap junctions and induce them to show tolerogenic function. Consequently, allergen-specific CD8⁺ T_{reg} responses are induced by tDCs and inhibit contact hypersensitivity [23, 24]. Other cells, in addition to T_{reg}s, induce tolerance in DCs. In the liver, a large number of anti-inflammatory cytokines are produced from stellate cells, hepatocytes and Kupffer cells to induce tDCs. For example, Kupffer cells produce PGE-2, IL-10 and TGF- β ; consequently, they provide a tolerogenic environment

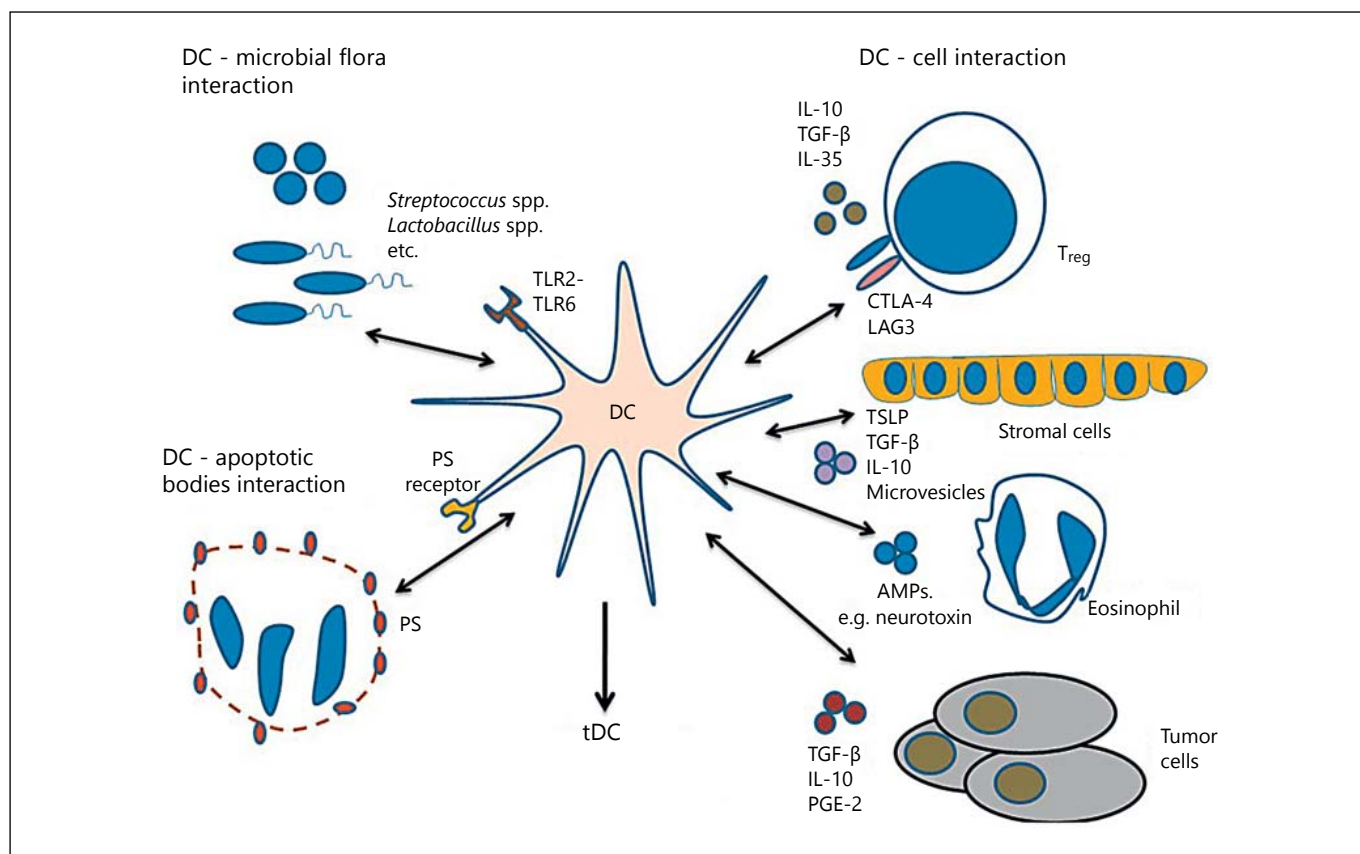


Fig. 1. The microenvironment of DC is classified into three groups including interaction between DCs and surrounding cells, microbial flora residing in the gut and apoptotic bodies. AMPs = Anti-

microbial peptides; LAG3 = lymphocyte activation gene 3; CTLA-4 = cytotoxic T-lymphocyte antigen 4.

Table 1. Different effects of T_{reg}s on DCs: interaction between T_{reg}s and DCs is mediated either through contact-dependent or -independent pathways

Molecules or cytokines from T _{reg} s	Receptors on DCs	Mechanisms of action
TGF-β	TGF-βR	Inhibiting maturation
IL-10	IL-10R	Reducing CD80/86/MHC II, sustaining CTLA-4 expression, inducing B7H4 molecules on antigen-presenting cells
CTLA-4	CD80/86	Reducing proliferation of T cells and IDO induction
LAG3	MHC II	Inhibiting DC maturation
IL-35	IL-35R	Inhibiting IL-12 production

[25, 26]. PGE-2 increases CCL22 secretion from DCs which recruit T_{reg}s by binding to CCR4 on T_{reg}s [27, 28]. It also inhibits IFN-α production from plasmacytoid DCs (pDCs) [29]. Endothelial cells in the liver also produce TSLP, retinoic acid (RA) and vitamin D, which

provide a tolerogenic environment. Presumably, this is the reason why lower-dose treatments are needed for liver allografts to survive in the long term [30]. In addition, an emerging heterogeneous cell population called myeloid-derived suppressor cells (MDSCs) has been

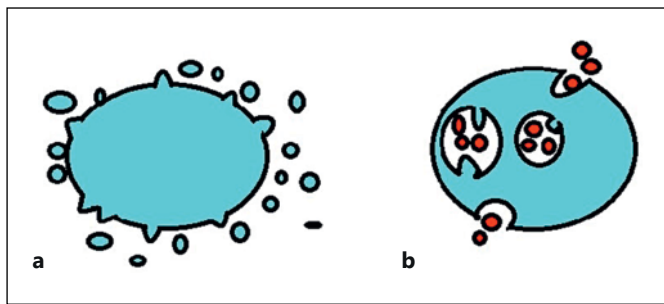


Fig. 2. Formation of exosomes and ectosomes. **a** Ectosomes are microvesicles shed from the cell membrane. **b** In contrast, exosomes are budding inward from endosomes which migrate towards the cell membrane and are released after their accumulation in endosomes.

shown to influence DCs and suppress T cells via the production of anti-inflammatory mediators, e.g. IL-10, nitric oxide, arginase and reactive oxygen species. MDSCs are regulatory cells composed of granulocyte, macrophage and DC precursors, and their tolerogenic properties (e.g. tDCs) opened new possibilities to induce tolerance in vivo. However, MDSCs are mostly not terminally differentiated cells, and their exposure to mediators such as all-trans-RA induce them toward mature inflammatory antigen-presenting cells such as F4/80+ macrophages and CD11c+ DCs. Apparently, tDCs maintain their stability more than MDSCs in different settings [31, 32].

Microvesicles secreted from different cells, another way for the communication between cells, have attracted attention for their tolerogenic potential. Exosomes are microvesicles formed by inverse budding of the membrane into the lumen of an endocytic compartment. Therefore, multivesicular structures will form and release their exosomes upon fusion with the cell membrane. Peche et al. [33] showed that intravenous injection of exosomes derived from immature DCs promoted allograft heart survival by inducing tolerance in CD4+ T cells. They also demonstrated that the tolerogenic response was antigen specific [33, 34]. Ectosomes are another type of microvesicles released by cell membrane budding, a process which is called ectocytosis. The difference between exosome and ectosome formation is depicted in figure 2. Ectosomes control inflammatory responses by inducing tolerance in human DCs. They are released very early after activation of cells, e.g. polymorphonuclear leukocytes. Depending on the origin of the cells or their activation status, they may promote tolerance or immunity. For example, ectosomes

derived from monocytes and platelets have been defined as immunostimulators and upregulators of IL-1 production.

Disruption of E-cadherin junctions happens regularly following mechanical trauma to the skin. Langerhans cells migrate to lymph nodes after trauma and intend to show typical tDC characteristics [35, 36]. Interestingly, Riedl et al. [37] demonstrated that disrupting E-cadherin junctions in Langerhans cells induced phenotypic maturation, but they did not assess cytokine secretion and the type of effects they may have on T cells. The disrupted Langerhans cells express a large number of costimulatory molecules, CD86 and CD80, and present captured and finely processed antigens to T cells without proinflammatory cytokine secretion while generating high amounts of IL-10. Consequently, responses of specific T_{reg} s will be induced in secondary lymphoid tissue [35, 38].

Interaction of the Microbial Flora with DCs

Recently, it has been shown that commensal bacteria which reside in the gut are beneficial for the host. Germ-free mice have an underdeveloped spleen and gut-associated lymphoid tissue but regain their normal state after TLR ligand injection or colonization with appropriate flora [39]. Therefore, it is very important to maintain a fine balance between immunity against microbial invaders and tolerance to the beneficial microbes. Consequently, the interaction between commensals and host cells (intraepithelial cells and DCs) reached, under evolutionary pressure, a level in which the bacterial components induce regulatory signals. For example, when zymosan, an extract of the cell wall of *Saccharomyces cerevisiae* and other fungi, binds to TLR2/TLR6 and dectin-1 as zymosan receptors, it induces regulatory signals which promote tDCs. DCs cultured in the presence of zymosan produce IL-10 and induce Foxp3+ CD4+ T_{reg} s [40]. The network between DCs, intraepithelial cells and the microbial flora is strictly controlled and leads to a fine balance which prevents immunity against beneficial commensal bacteria while inducing highly effective immune responses against the pathogens attacking the gut. Commensal bacteria influence DCs in both direct and indirect modes. For example, bacteria directly influence DCs to prevent NF- κ B activity while indirectly influencing DCs by inducing secretion of anti-inflammatory mediators from intraepithelial cells. Table 2 demonstrates various commensal bacteria inhibiting DC maturation and activation.

Table 2. Commensal bacteria influence intestinal DCs to induce tolerance

Commensal bacteria	Mechanism of actions
<i>Lactobacillus casei</i>	Suppresses degradation of the inhibitor of NF- κ B
<i>Lactobacillus</i> spp.	Inhibits activation of NF- κ B
<i>Bacteroides</i> spp.	Inhibits activation of NF- κ B
<i>Escherichia coli</i>	Inhibits activation of NF- κ B
<i>Bacteroides thetaiotaomicron</i>	Decreases transcription of NF- κ B in the nucleus Increases Rel-A export from the nucleus

Interaction between Apoptotic Bodies and DCs

Apoptotic bodies interact with particular scavenging receptors on DCs to induce a tolerogenic phenotype, which is in contrast to necrotic cells that induce mature immunogenic DCs [41, 42]. At the end of an immune challenge against foreign antigens, activated immune cells die in a finely controlled way, named ‘apoptosis’ or activation-induced cell death. In addition, apoptosis occurs normally in some tissues in a constant way. For example, thousands of intestinal epithelial cells die, which are continuously replaced by new intestinal epithelial cells. Possibly, one of the mechanisms to induce a tolerogenic environment at privileged sites (e.g. the uterus) is the natural cell turnover and the generation of apoptotic bodies, which leads to tolerance induction in DCs. Consistent with this notion, injecting a virus into the eye, an immune-privileged organ, recruits immune cells. Approximately 48 h following the injection, inflammatory cells become apoptotic and tolerance against viral antigens will occur. In contrast, inhibiting apoptosis in recruited inflammatory cells prevents tolerance induction to viral antigens [43]. Therefore, apoptotic bodies carrying particular antigens cause antigen-specific tolerance. One of the inspiring applications of this concept is to prolong allograft survival using donor- and recipient-derived apoptotic cells functioning as both an antigen source and immunosuppressing mediators [44].

The characteristics of apoptotic cells differ from those of viable cells and lead to their recognition and clearance by phagocytic cells. Clearance is mediated by particular receptors, e.g. phosphatidylserine (PS) receptor, Mer tyrosine kinase, complement receptor C1qR, CD36 and $\alpha_v\beta_5$, $\alpha_v\beta_3$ integrins [45]. PS is an anionic phospholipid mostly present in the inner leaflet of the cell membrane in viable cells. However, when cells are prepared for apoptosis, PS molecules flip to the external leaflet. Now-

adays, it is clear that PS induces regulatory signals upon binding to its receptor [46]. Inhibiting PS recognition prevents the clearance of apoptotic bodies. For example, milk fat globule epidermal growth factor 8 (MFG-8) develops autoimmunity, while adding recombinant MFG-8 to culture media restores the normal state in vitro, which may be explained by the clearance capability of phagocytes. MFG-8 is a molecule secreted from CD68+ tingible body macrophages which facilitate apoptotic body clearance by phagocytes via the recognition of PS molecules [47]. It has been suggested that the mechanism to induce tolerance upon α -CD3 antibody injection is also based on the interaction of apoptotic bodies with immature DCs. Anti-CD3 monoclonal antibody eliminates many T cells and induces their apoptosis after injection [48].

Pathogens Have Learned Ways to Induce tDCs

The ceaseless interaction between the immune system and microbial invaders has advanced both sides of the battle. In the case of microbes, they have developed some tolerogenic products inducing tolerance upon the interaction with DCs. Interestingly, regulatory molecules are present in all types of pathogens, including bacteria, viruses and parasites. Microbes can silence immune responses through tolerance induction in DCs. Therefore, many components of various pathogens have been recognized as inducers of tDCs. For example, DCs cultured in the presence of the B subunit of cholera toxin produce IL-10 and induce Foxp3+ T_{reg}s [49]. Cholera toxin also suppresses IL-12 production and maturation of DCs [30]. Table 3 shows some regulatory molecules expressed or secreted by microbes to induce tDCs. Similarly, tumors effectively escape immune responses via the expression and secretion of mediators which directly influence DCs

Fig. 3. Interaction between T_{reg} and tDC. tDCs release mediators such as IL-10, TGF- β , RA, PGE-2 and NO. In addition to these mediators, tDCs express surface inhibitory molecules such as ILT-3 and ILT-4. Collectively, they convert naïve T cells to T_{reg} s. In contrast, T_{reg} s produce cytokines (e.g. TGF- β , IL-35 and IL-10) and inhibitory molecules such as cytotoxic T-lymphocyte antigen (CTLA)-4 and lymphocyte activation gene 3 (LAG3) that influence DCs and inhibit their activation.

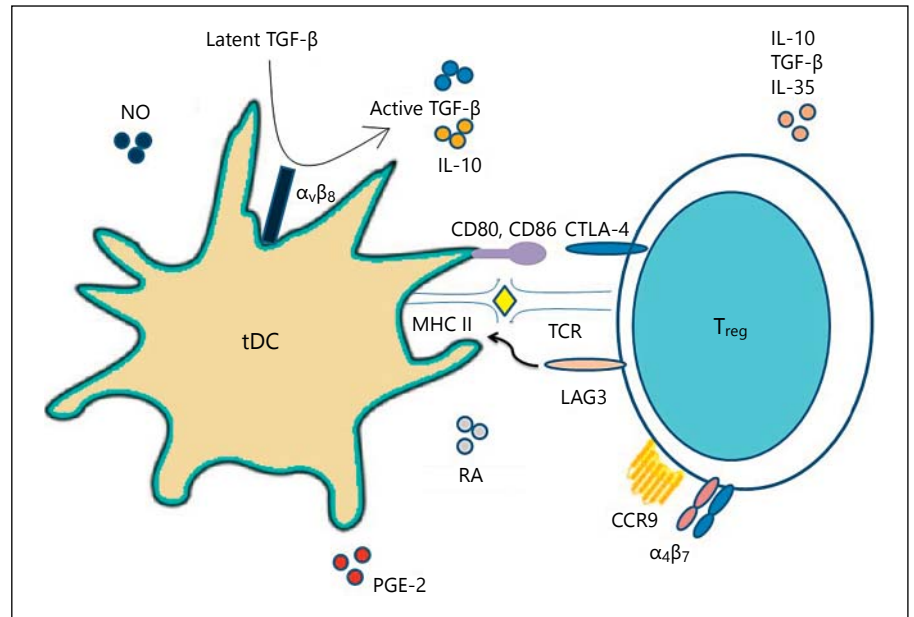


Table 3. Components of the pathogens developed to induce tDCs

Component	Microbial origin	Receptor on DCs
Filamentous hemagglutinin	<i>Bordetella pertussis</i>	TLR4
Lysophosphatidylserine	<i>Schistosoma mansoni</i>	TLR2
LcrV	<i>Yersinia pestis</i>	TLR2/TLR6
Zymosan	<i>Saccharomyces cerevisiae</i>	TLR2/dectin1
Lewis antigen of LPS	<i>Helicobacter pylori</i>	DC-SIGN
Lysophosphatidic acid	<i>Lactobacillus acidophilus</i>	DC-SIGN
Cholera toxin	<i>Vibrio cholerae</i>	Membrane-bound gangliosides
Monosaccharide Gal/GalNAc	<i>Streptococcus, Lactobacillus</i>	MGL1
Polysaccharide A	<i>Bacteroides fragilis</i>	TLR2
Viral IL-10	Epstein-Barr virus	IL-10R
Viral IL-10	Cytomegalovirus	IL-10R

to induce tolerogenic characteristics. TGF- β , IL-10, PGE-2 and VEGF are regularly produced by malignant cells to silence immune responses [50].

T_{reg} Induction by tDCs

In 1978, careful evaluation of mixed lymphocyte reactions by Steinman and Witmer [51] showed that DCs are professional antigen-presenting cells which effectively stimulate T lymphocytes. tDCs have strong capacity to induce and expand T_{reg} s and their own special characteristics like secreting anti-inflammatory mediators or expressing indoleamine 2,3 dioxygenase (IDO), galectins and surface molecules, which help them to

function as inducers and stimulators of T_{reg} s. Some of the mechanisms mediated by these factors to induce T_{reg} s are briefly explained below and demonstrated in figure 3.

T_{reg} s are divided into two main groups: naturally occurring T_{reg} s and peripheral T_{reg} s. The first one are generated in the thymus, possibly induced by tDCs and epithelial cells in the tissue. There are many challenges which remain to be solved in this field. Apparently, TSLP is one of the mediators which influence thymic DCs to show a tolerogenic phenotype and function [52]. It is worth noting that the main effect attributed to TSLP is to drive Th2 responses in the respiratory tract by influencing DCs dur-

Table 4. Mediators and combinations used to generate tDCs in vitro

Groups	Examples
Immunosuppressive drugs	Dexamethasone, rapamycin, mycophenolic acid, cyclosporin A
Anti-inflammatory mediators	TNF- α , GM-CSF (low dose), PGE-2, G-CSF, IL-10, apoptotic bodies
Chemicals	Vitamin D, aspirin, aryl hydrocarbon receptor ligands
Genetic modifications	Knocked in DCs or upregulating SOCS1, IL-10 Knocked out DCs or downregulating Rel-B
Coculture with other cells	Coculture with mesenchymal stem cells

ing allergies. Therefore, there should be other mediators to influence DCs in the thymus to show tolerogenic characteristics and induce natural T_{reg}s.

tDCs play a significant role in the maintenance of peripheral tolerance against self-antigen. Many studies have demonstrated the critical functions of IL-10, TGF- β , IDO and RA to induce T_{reg}s by DCs. For example, IL-10 induces the expression of immunoglobulin-like transcript (ILT)-3 and ILT-4, which mediate suppression in T cells. Mice genetically deficient in $\alpha_v\beta_8$ integrin expression or deficient in the expression of surface TGF- β receptor on T cells clearly show autoimmune manifestations [30]. It has been proven that DCs matured with TLR ligands (e.g. polyinosinic:polycytidylic acid) secrete a less latent form of TGF- β than immature DCs. Mature DCs produce the primary form of TGF- β which needs to be converted by $\alpha_v\beta_8$ integrin to the latent form [53]. One of the main known producers of TGF- β is CD8+ splenic DCs, which effectively induce T_{reg}s [54]. Seemingly, non-DC antigen-presenting cells cannot induce T_{reg}s, even in the presence of exogenous TGF- β . RA induces the expression of SOCS3 (a suppressor of cytokine signaling), which inhibits p38 MAPK and inflammatory cytokines. One of the main and important functions of RA is to recruit T and B lymphocytes to gut-associated lymphoid tissue by inducing expression of CCR9 and $\alpha_4\beta_7$ on the surface of lymphocytes. CCR9 binds to CCL25 (secreted by epithelial cells) and $\alpha_4\beta_7$ binds to MADCAM1 (expressed on endothelial cells). There are still challenges relating to the induction of different subsets of T cells, Th17 and T_{reg}s, by RA producing DCs. Some experiments have shown that interaction between naive T cells and DCs in the presence of high concentrations of RA (10 μ g) induce T_{reg}s while low concentrations lead to Th17 induction [55]. Intriguingly, RA cannot induce Foxp3+ T_{reg}s from naive Foxp3- CD4+ T cells alone and does it only in the presence of TGF- β [56].

In addition to the above-mentioned mediators, another mechanism, particularly in tolerogenic pDCs, is named IDO-dependent T_{reg} induction [57]. Mechanisms of IDO function are not fully determined; however, it partly functions by the destruction and reduction in tryptophan around T cells and production of downstream toxic metabolites (e.g. kynurenine). Reducing tryptophan concentration leads to stopping translation process. Other mechanisms, including mTOR inhibition, proliferation arrest and apoptosis induction, have also been attributed to IDO [58].

Recently, it has been shown that tDCs induce regulatory B cells, a newly defined cell population with a regulatory function [59]. They produce IL-10 and their frequency will be upregulated upon the injection of tDCs to type 1 diabetic patients [59, 60].

Ex vivo Generation of tDCs

After implicating their role in tolerance induction, some laboratories have tried to generate large numbers of tDCs in vitro to exploit their potential to treat diseases. Therefore, many physiological and synthetic mediators have been used to generate tDCs in vitro, which are classified into five groups (table 4) [61].

Cytokines play important roles in the generation of tDCs. DCs, for example, cultured in the presence of G-CSF induce T_{reg}s through IL-10 and TGF- β production [62]. Similar results were obtained by DCs cultured in the presence of IL-10, TGF- β , GM-CSF, hepatocyte growth factor, vasoactive intestinal peptide and PGE-2, inducing functional T_{reg}s. Recent studies showed that DCs that were either differentiated in the presence of IL-10 (DC-10) or incubated as immature DCs with IL-10 induced T cells derived from patients with allergy to produce less IL-5 and IL-13. In other words, tDCs diminished Th2 im-

mune responses [63]. Interestingly, adding blocking antibodies against IL-10 and TGF- β limited suppressive activities of tDCs on effector Th2 cells.

DCs cultured in the presence of vitamin D express high amounts of ILT-3 and ILT-4) [64]. ILT-3 and ILT-4 have been defined as immunoregulatory molecules which promote T_{reg} responses. In addition, vitamin-D3-treated DCs show low expression of CD86, CD80 and CD40 molecules and no secretion of proinflammatory cytokines. Adding to the list, vitamin D3 induces CCL22 production recruiting CCR4+ T_{reg}s. Moreover, an intriguing effect of vitamin D3 may be selective inhibition of the NF- κ B transcription factor in myeloid DC but not in pDCs. Apparently, vitamin D3 is developed during evolution to influence immunogenic DCs (myeloid DCs) instead of pDCs. It should be noted that pDCs help to maintain tolerance in the steady state until being activated by TLR ligands [65].

It took a long time to define the precise mechanisms by which immunosuppressive drugs function. Only a few years ago, it was discovered that DCs are one of the main targets of pharmacological and anti-inflammatory agents. Some studies have clearly demonstrated that anti-inflammatory agents like aspirin can induce tDCs, which effectively induce T_{reg}s. Moreover, immunosuppressive drugs (e.g. rapamycin, micophenolate mofetile and corticosteroids) influence different stages of DCs to induce tDCs. Differentiation, antigen uptake, phenotypic maturation and cytokine secretion are distinct stages influenced by immunosuppressive drugs [66]. The treated cells showed lower expression of costimulatory molecules and IL-12 production. On the other hand, they produced higher levels of IL-10 and induced Foxp3+ T_{reg}s. NF- κ B inhibitors, including deoxyspergualin and the less toxic analogue LF15-0195, are among the drugs effectively inducing tDCs.

The vast understanding about the molecules involved in tolerance induction has offered suitable choices for investigators to downregulate and even completely abrogate some proinflammatory molecules. Many efforts have been made towards knocking out (generation of genetic deficiencies) or silencing (using RNAi technology) proinflammatory molecules and cytokines, e.g. IL-12 and NF- κ B components, to generate DCs with tolerogenic properties [67]. On the other hand, overexpression of inhibitory molecules (e.g. IL-10, TGF- β , CTLA-4 and SOCS1) induces tDCs with a high potential for T_{reg} induction [68].

In a recent study, the clinical applicability of human tDCs incubated with IL-10, TGF- β , dexamethasone, vitamin D3 and rapamycin was studied [69] and IL-10-treated tDCs were found to be the optimal tDCs for functional T_{reg} induction. IL-10-treated tDCs showed superior potential

to suppress T-cell proliferation, to produce anti-inflammatory cytokines and to express inhibitory receptors such as ILT-3 and ILT-4. IL-10-treated DCs also maintained their tolerogenic properties after restimulation with TLR7/TLR8 or TLR2 ligands [69]. Therefore, clinically applicable protocols have been improved to generate tDCs in accordance with current good manufacturing practices.

Considerations Using tDCs for Immunotherapy

Regarding the capability of tDCs to induce tolerance, new possibilities have emerged to cure diseases like autoimmunity, allergies and allograft rejections. An advantage to use these cells is specific therapy. In other words, by modifying only one group of cells *ex vivo*, side effects of immunosuppressive drugs can be prevented. Immunosuppressive drugs influence all host cells upon injection.

Similar to all other medical hypothesis, tDCs should be first tested in the treatment of animal models. Experimental models treated with tDCs have shown significant improvement and have inspired scientists to continue and perform clinical trials using tDC in disease therapy. For example, a recent study [70] showed that CD11c+ DCs induced functional T_{reg}s in the presence of TGF- β and RA. These T_{reg}s were able to prevent graft-versus-host disease upon adoptive transfer into allogenic mice. In addition, the T_{reg}s transferred persisted *in vivo* for a long time. Another study [71] demonstrated that injecting DCs loaded with apoptotic bodies into nonobese diabetic mice prevented the onset of type 1 diabetes. The DCs showed a decreased number of costimulatory molecules, i.e. CD40 and CD80 expression and proinflammatory cytokine secretion. In addition, another study [72] showed a suppressing effect of tDCs on the onset of collagen-induced arthritis. DCs treated with the NF- κ B inhibitor LF 15-0195 were poor allostimulators in mixed lymphocyte reaction and induced specific hyporesponsiveness upon injection to collagen-induced arthritic mice. Treated mice also showed reduced inflammatory cells in their joints [61, 70, 72, 73]. In addition, a phase I clinical trial using tDCs for the first time was done and no safety problems and toxicities were reported in the recipients with type 1 diabetes [60].

Although studies have demonstrated promising results to cure diseases like diabetes, asthma and organ allograft rejections in experimental models, some challenges and considerations remain to be completely solved. These considerations are briefly described below [74, 75].

In spite of great efforts toward using tDCs as a treatment option for diseases, the clinically obtained results

are not satisfactory. Therefore, some experts regretted using this method because of the existence of many variables to prepare large numbers of DCs ex vivo [76]. Nevertheless, others consider tDC therapy a promising tool and try to optimize the procedure. For example, it has been shown that tDCs are not able to migrate towards high endothelial venules in secondary lymphoid tissue after being injected intravenously. It may be necessary to activate high endothelial venules to increase the influx of DCs to local secondary lymphoid tissues [77]. Interestingly, it has been shown that steady-state migratory DCs produce VEGF and stimulate high endothelial venule generation in a systemic manner [78]. In addition, the agents used to generate tDCs have different effects on the migratory potential of DCs. IL-10-treated tDCs, for example, have a low migratory capacity. Therefore, it is necessary to consider the effect of agents on the migratory capacity while selecting inhibitory agents [78].

Immunologists have tried to optimize the procedure of applying tDCs in cell-based therapy. The expected characteristics of tDCs are listed below [75]:

- (1) Sufficient expression of molecules on DCs; for example, the proper amount of MHC II should be presented to increase the amount of antigens and peptides while costimulatory molecules like CD80 or CD86 should be low or deficient. CCR7 expression is also important with regard to migration toward secondary lymphoid tissues.
- (2) Producing a large number of anti-inflammatory cytokines such as IL-10 and TGF- β while secreting low levels of proinflammatory cytokines such as IL-12 and IL-23.
- (3) Maintaining a stable form and function in vivo, even in the presence of proinflammatory conditions.
- (4) Inducing tolerance in the Th2 subset in addition to Th1 and Th17. Some of the cytokines secreted from tDCs induce a Th2 subset. This complexity has challenged the induction of tolerance in conditions such as allergies and asthma.

Other considerations include determining the source of antigens, and optimizing and adapting the route of injection to the different diseases. In the case of rheumatoid arthritis, for example, the best source of antigen and best route to inject tDCs are the synovial fluid and intra-articular injection, respectively. Unfortunately, in the case of diseases like psoriasis, appropriate antigens have not been found to load on ex vivo prepared tDCs and this problem has limited the efficiency of using tDCs for many diseases [79]. Moreover, it has been suggested to inject tDCs directly into skin to treat inflammatory skin diseases by raising the possibility of an interaction between DCs and T cells in dermal secondary lymph nodes as an armamentarium for attacking inflammatory disease [80].

Concluding Remarks

DCs contribute to various clinical conditions. In the past, they were described as cells involved in cancer and infection. Recently, DCs have attracted attention in the treatment of autoimmunity and atherosclerosis. DCs are able to induce both immunity and tolerance. They induce T_{regs} by surface expression of inhibitory molecules or producing anti-inflammatory cytokines. Different agents have been used for more than a decade to generate them in vitro, and new possibilities have emerged to regulate the immune system by tDCs. However, there are still some issues to be considered as follows: optimized generation according to good clinical practice guidelines and maintenance in the tolerogenic form after their injecting into the host. Injection routes and their capacity to migrate to secondary lymph nodes are some other challenges. Admittedly, using tDCs will be an important clinical treatment for T-cell-mediated diseases. However, it is necessary to optimize the procedure to be able to translate the effects of DC observed in experimental models to humans.

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