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Natural and Induced Tolerogenic Dendritic Cells

Courtney A. Iberg and Daniel Hawiger

Dendritic cells (DCs) are highly susceptible to extrinsic signals that modify the functions of these crucial APCs. Maturation of DCs induced by diverse proinflammatory conditions promotes immune responses, but certain signals also induce tolerogenic functions in DCs. These “induced tolerogenic DCs” help to moderate immune responses such as those to commensals present at specific anatomical locations. However, also under steady-state conditions, some DCs are characterized by inherent tolerogenic properties. The immunomodulatory mechanisms constitutively present in such “natural tolerogenic DCs” help to promote tolerance to peripheral Ags. By extending tolerance initially established in the thymus, these functions of DCs help to regulate autoimmune and other immune responses. In this review we will discuss the mechanisms and functions of natural and induced tolerogenic DCs and offer further insight into how their possible manipulations may ultimately lead to more precise treatments for various immune-mediated conditions and diseases. The Journal of Immunology, 2020, 204: 733–744.

Dendritic cells (DCs) are APCs critical for the initiation of T cell immune responses and for the governing of immune tolerance. Together with other types of cells in the thymus, DCs have important roles in mediating central tolerance, combining thymic deletion of self-reactive T cells and a production of thymically derived regulatory T (tTreg) cells in a cumulative process aimed at preventing overt anti-self responses (1). However, because of differing efficiencies of antigenic presentation in the thymus and the periphery, and cross-reactivity of TCRs, the mature peripheral T cell repertoire still contains T cells that may be reactive to self (2, 3). These self-reactive peripheral T cells can then be primed in the periphery, even by low-affinity peptides that are below their original thresholds for negative selection in the thymus (2, 4), ultimately increasing the risk of autoimmune responses against self-antigens (2, 4–7).

The priming of self-reactive peripheral T cells is controlled by tTreg cells (8). However, the functions of tTreg cells may be overwhelmed by specific proinflammatory autoimmune activation; also, in some individuals, the development of tTreg cells may be compromised (2, 4, 9, 10). Various animal models of autoimmune diseases initiated in healthy animals after immunization with specific self-antigens either in the presence of adjuvants or in the context of an introduced infectious agent have demonstrated that specific priming of preexisting self-reactive T cells mediates an autoimmune process (2, 4–7). Therefore, pathways of thymic tolerance need to be extended by the specific mechanisms operating in the peripheral immune system. Particularly, autoimmune responses can be ameliorated or even completely prevented by the Ag-specific, peripherally formed Treg (pTreg) cells that are induced extrathymically by DCs (7, 11).

Dendritic cells in peripheral tolerance

The roles of DCs in peripheral tolerance have been established by multiple lines of independent experimental evidence. The disturbance of tolerance and immune homeostasis caused by the absence of DCs and their subsets was observed in various experimental systems that relied on a specific in vivo killing of DCs expressing diphtheria toxin receptor or diphtheria toxoid A subunit or by other means, such as a chemical depletion of DCs (12–16). These results are in agreement with other early studies that identified the roles of DCs in the induction of peripheral tolerance by employing methods of specific delivery of defined Ags to DCs in vivo, tracking the uptake of proteins to DCs, and the transgenic expression of ectopic Ags as cytosolic proteins in DCs (17–20). The combination of specific Ag-targeting methods with various genetic models of DCs has allowed for further advances in our understanding of the importance of DCs in governing peripheral tolerance, as we also recently discussed in Ref. 11.

The specific functions of DCs depend, in part, on the developmentally determined diversity of DC subsets reviewed...
extensively by Murphy and colleagues (21) and Merad et al. (22). Both human and murine DCs consist of two main populations, conventional DC (cDC) and plasmacytoid DC (pDC), both of which develop from progenitors in the bone marrow and then differentiate into various subsets present throughout multiple tissues (23, 24). The cDC population can be further divided into the DC1 and DC2 subsets, as defined by the transcription factors required for their development. The DC1 subset, which requires the transcription factors Irf8, Id2, and Batf3 for development, is distinguished by the expression of XCR1 and further characterized by the expression of additional cell surface molecules, including B and T lymphocyte associated (BTLA), CD8ε, and DEC-205. In contrast, the development of DC2s is governed by the transcription factors Irf4 and Notch2, and these DCs are distinguished by cell-surface expression of CD172a (SIRPα) as well as DCIR2 and CD11b (11, 23, 24). Although not a main focus of this review, pDCs, characterized by the expression of cell surface molecules (including B220, bone marrow stromal Ag 2 [BST2], and Siglec-H), are primarily involved in antiviral responses but also have some roles in tolerance (11, 25). Importantly, CD141+ (BDCA-3+) XCR1+ BTLA+ human DC1s and CD1c+ CD172a+ CD11b+ human DC2s share many developmental, phenotypical, and functional similarities with their murine counterparts (21, 26).

In addition to their roles in tolerance, DCs have crucial functions in the initiation of immune responses. The efficient priming of immune responses by specific DC subsets requires additional signals from the proinflammatory environment that can be sensed through specific pattern recognition receptors (PRRs) (27–29). These signals lead to the DC acquisition of enhanced properties to induce immune responses in a process referred to as maturation. Overall, the model of such proinflammatory DC maturation postulates increased proinflammatory cytokine production and increased cell surface expression of costimulatory and MHC molecules and chemokine ligands or receptors in response to microbial and other proinflammatory stimulation (28, 30). In addition to this maturation process resulting in increased immune responses, specific extrinsic signals were also proposed to induce tolerogenic differentiation of DCs. The experiments using bone marrow–derived DCs (BMDCs), monocyte-derived DCs (moDCs), and DCs obtained ex vivo showed that some PRR agonists, as well as various other physiological and pharmaceutical agents, can allow for the induction of DCs with tolerogenic functions. Further, the experiments in vivo revealed that, in response to certain signals in specific anatomical sites (including the intestines and airways), some DCs help to maintain immune homeostasis toward commensal organisms and other Ags, even under partially proinflammatory conditions (28, 31–33). We propose to refer to such DCs that acquire tolerogenic properties either in vitro or in vivo as “induced tolerogenic DCs” (itDCs), as partially based on the terminology first introduced by Maldonado and von Andrian (34).

However, even in the absence of specific extrinsic signals, generally referred to as steady-state conditions, many DCs that are present in the spleen and other lymphoid organs do not necessarily remain as “immature” immunological bystanders but instead have important roles in initiating and maintaining tolerance to available peripheral Ags (11). These DCs inherently promote in T cells various mechanisms of tolerance, including T cell anergy, T cell deletion, and a conversion of pTreg cells (11). We therefore propose to refer to such DCs as “natural tolerogenic DCs” (ntDCs) (Fig. 1).

Establishing peripheral tolerance by natural tolerogenic functions of DCs

The physiological steady state can be defined by the undisturbed expression of cytokines and other molecules contributing to the baseline conditions that, together with stromal cells of the secondary lymphoid tissues, provide a framework for the interactions between DCs and T cells (35, 36). Initially, it was postulated that, in the steady state, DCs remain immature akin to BMDCs or moDCs that are characterized by lower expression of MHC and costimulatory molecules when cultured in the absence of maturation signals (35). However, the available experimental evidence has clearly shown that, even in the steady state, DCs can constitutively initiate active mechanisms of tolerance in T cells, such as the conversion of pTreg cells (7, 11). These general contradictions were recognized early by Lutz and Schuler, who proposed that the division between “immature DCs” and “mature DCs” (as defined by DC phenotypes) did not necessarily correspond with “tolerogenic DCs” and “immunogenic DCs,” respectively. Instead, divisive tolerogenic and immunogenic maturation processes were proposed (31), further supported by the identification of transcriptional determinants of certain tolerogenic and immunogenic maturation states in DCs as well as by the emerging concept of “homeostatic maturation” of DCs under steady-state conditions (37–40). For example, a decrease in E-cadherin–mediated cell–to–cell contact results in specific increases in expression of MHC class II (MHCII) and costimulatory molecules (39). The concept of DC maturation in the steady state was additionally defined by other observations of multiple specific gene expression changes comparable, in scope, to those observed under TLR agonist–mediated maturation (30). This process of maturation under homeostatic conditions has also been proposed to result in functions of DCs necessary to induce active mechanisms of tolerance (38). Although the specific mechanisms governing DC functions in vivo under steady-state conditions following their initial development from the bone marrow precursors are still being uncovered, it is clear that these processes are continuous, and some possibly cell autonomous, resulting in the stable numbers and phenotypes of DCs expressing crucial molecules involved in tolerance (Fig. 2 and as discussed below).

In the steady state, DCs can induce multiple mechanisms of tolerance in T cells including anergy, but a de novo conversion of pTreg cells bestows a dominant and long-lasting tolerance to peripheral Ags (7, 11, 41–44). Although in the steady state Ags initially acquired by all cDCs can induce tolerance, DC1s are more prone to induce tolerogenic effects as compared with DC2s (7, 11, 42). The specialization among DCs can be attributed to different localization of DCs within a local architecture of immune organs, differences in the efficiencies of processing and presentation of Ags to T cells, and the specific immunomodulatory mechanisms in DC1s and DC2s (11, 45–48). The immunomodulatory pathways are of particular importance in the mediation of a tolerogenic partnership of DCs and T cells (49). Importantly, the engagement of immunomodulatory axes (including programmed death
ligand-1 (PD-L1)/programmed death-1 (PD-1), CD80/CD86/Ctla-4, and B7h/ICOS) can promote Foxp3 expression, pTreg cell induction, and tolerance (7, 11, 49). Specifically, the PD-L1/PD-1 axis promotes immune tolerance via PD-L1’s competition with costimulatory CD28 for binding with B7-1, as well as by the recruitment of SHP-2 by PD-1, which negatively impacts TCR signaling (50). Moreover, Ctla-4 (CD152) expressed on the T cell surface competes with the costimulatory molecule CD28 for binding with CD80/CD86 (B7-1 and B7-2) present on the surface of the DC. Although Ctla-4 can remove CD80/CD86 from the DC surface, it also directly negatively regulates CD4+ and CD8+ T cell activation by dampening TCR signaling, hindering IL-2 production, and preventing cell cycle progression (51–53). Finally, B7h (ICOS-L or B7RP-1) is constitutively expressed on DCs for binding with CD80/CD86 (B7-1 and B7-2) present on the surface of the DC, although Ctla-4 can remove CD80/CD86 from the DC surface, it also directly negatively regulates CD4+ and CD8+ T cell activation by dampening TCR signaling, hindering IL-2 production, and preventing cell cycle progression (51–53). Finally, B7h (ICOS-L or B7RP-1) is constitutively expressed on DCs, and expression of its receptor, ICOS, is induced upon T cell activation (49). Related to other members of the CD28 Ig superfamily, ICOS (together with its binding partner B7h) has proven important in the development of Treg cells, and a deficiency in ICOS signaling has rendered mice more susceptible to various autoimmune pathologies, although ICOS functions are required for the progression of some autoimmune processes (54–58).

Among various cytokines governing immune responses, the presence of TGF-β is often correlated with maintaining immune homeostasis. The absence of TGF-β leads to spontaneous lymphoproliferation and inflammatory disease, and TGF-β also influences the expression of IL-10, an important protolerogenic cytokine (34, 49, 59, 60). By acting directly on T cells, TGF-β promotes differentiation of both Treg and some effector T cells (43, 61–65). Metabolites, such as retinoic acid (RA), enhance tolerogenic properties of cytokines including TGF-β, further promoting the induction of Foxp3 expression and the amelioration of autoimmune disease (66–70).

In addition to the immunomodulatory pathways described above, which can directly contribute to inducing Foxp3 expression in pTreg cells, recent results identified the roles of BTLA and CD5 in promoting pTreg cell homeostasis via the modulation of the sensitivity of the developing pTreg cells to effector-differentiating cytokines (7, 42, 71, 72). Among the DC populations, BTLA is expressed specifically in DC1s (72). During the interactions between the BTLAhi DC1s and T cells in the steady state, BTLA signals through herpesvirus entry mediator (HVEM) in naive CD4+ T cells to activate MEK and, subsequently, the transcription factor ETS1 to increase expression of Cd5 (42, 72). High CD5 expression then allows for a conversion of these T cells to Foxp3+ pTreg cells by interfering with mammalian target of rapamycin (mTOR) activation in response to effector-differentiating cytokines, such as IL-4, IL-6, and IFN-γ (7, 42, 71).

BTLAhi DC1s reside in lymphoid organs, including the spleen, and these resident lymphoid tissue DCs are ideally positioned to capture systemic self-antigens, including those derived from apoptotic cells (20, 73, 74). Although the specific roles of BTLA in governing tolerance among CD8+ T cells remain unclear, DC1s can maintain tolerance to
self-antigens by presenting endogenous Ags to both CD4+ and CD8+ T cells (73, 75). DC1s also have important roles in eliciting Th1 responses as well as in cross-priming CD8+ cytotoxic T cells (23). Such versatile functions of some DC1s may reflect complex mechanisms mediated by BTLA and HVEM (76–79), yet it is also interesting to speculate that at least some of these proimmune functions may be performed by DC1s characterized by low expression of BTLA.

Nevertheless, it is clear that apoptotic materials are an abundant source of tissue self-antigens crucial for the maintenance of immune tolerance (20, 80, 81). The relationship between the uptake, processing, and presentation of apoptotic materials and the functional characteristics of DCs is complex. The phagocytic scavenger receptor CD36 has long been recognized for its role in facilitating the uptake of apoptotic materials via recognition of phosphatidylserine found on the outer leaflets of the membranes of apoptotic cells (74, 82). Consistent with the notion that DC1s are primarily responsible for the constitutive uptake of apoptotic materials, CD36 is expressed highly on DC1s compared with DC2s and pDCs (83, 84). In addition to being a source of Ags, an exposure to apoptotic materials may also enhance the tolerogenic properties of some DCs. An engagement of CD36 can inhibit maturation of moDCs induced by proimmunogenic stimuli and can induce tolerogenic BMDCs that resist LPS stimulation and induce Foxp3+ pTreg cell development (85, 86), also discussed later in the text.

In contrast, necrotic-type materials derived from injured cells can be recognized by Clec9a (DNGR-1), a C-type lectin expressed by some DC1s, resulting in inflammation and maturation of DCs (87, 88). However, under steady-state conditions, the resident lymphoid tissue BTLAhi DC1s (as well as some migratory DCs) exhibit inherent tolerogenic properties (11). The ability of DC1s to uptake, process, and present Ags to T cells in the steady state has been demonstrated by using multiple methods, including a direct targeting of Ags to such DCs by using chimeric Abs specific for DEC-205 and other surface receptors (11, 17, 89). Importantly, the combination of genetic models including a DC-specific deletion of Irf4 (resulting in an increased DC1/DC2 ratio) or a deletion of Batf3 (resulting in decreased numbers of DC1s) and targeted Ag delivery to DC-specific molecules has helped to further clarify the specific functions of DC subsets (11, 17, 42, 49).

In addition to the tolerance promoted by resident lymphoid tissue DCs, tolerance to self-antigens is also promoted by migratory DCs that transport Ags from the nonlymphoid tissue to the lymph nodes (LNs). Such migratory DC1s, especially those found in skin and parenchymal organs, undergo homeostatic maturation in the steady state and have tolerogenic functions (40, 90–92). However, the tolerogenic functions of many migratory DCs are also induced at certain anatomical locations (such as the intestines) upon exposure to specific extrinsic stimuli (as discussed below).

**Inducing tolerogenic DCs for the maintenance of homeostasis**

In contrast to resident lymphoid tissue DCs and DCs migrating from the parenchymal organs in the steady state, many DCs that are exposed to various environmental stimuli present in the intestines, airways, and skin are constantly at risk for undergoing immunogenic maturation. However, as discussed earlier in the text, these DCs do not induce detrimental
immune responses and instead induce tolerogenic functions. The specific tolerogenic mechanisms employed by iTDCs remain an area of active investigation, but it is clear that, despite the presence of proinflammatory mediators, these DCs are typically characterized by an elevated production of various anti-inflammatory cytokines and other regulatory molecules (11, 93–99).

Among such specific anatomical sites, the skin represents a crucial barrier that is in constant contact with foreign Ags and commensal microbes and that requires intricately regulated immune responses orchestrated by DCs (93, 100). The lungs are another crucial anatomical site that is continually exposed to commensals and pathogens, therefore also requiring active immunoregulation. Correspondingly, DCs obtained from patients with chronic obstructive pulmonary disease were shown to produce IL-10, leading to the induction of Tr1 regulatory cells (95, 101, 102). The intestines are yet another key organ that remains in constant contact with a large number of commensal bacteria and that can also be exposed to potentially pathogenic microbes. Oral administration of Ags is well established to lead to the induction of Foxp3+ Treg cells, thereby helping to maintain homeostasis (103).

It has recently been suggested that the specific anatomical organization of gut-draining LNs may play a role in the balance of tolerance and immunity, as proximal and distal gut-draining LNs supported primarily tolerogenic and immunogenic responses, respectively (47). In addition to such anatomic specialization, other studies have demonstrated that the presence of commensal microbes from the human gut, dietary metabolites, and some other biologically active molecules results in the formation of iTDCs that can differentiate Foxp3+ Treg cells and regulatory Tr1 cells and decrease the numbers of effector T cells (33, 104–107). Accordingly, the pTreg cell–inducing functions of Irf8/Batf3-dependent CD103+CD11b+ DC1s help maintain a local immune homeostasis within the gut-associated lymphoid tissues as well as at other mucosal surfaces and also some immune-privileged sites, such as the eye (12, 13, 32, 108–110). Although DC2s are generally less efficient at inducing pTreg cells, some iTDCs in the intestines still promote both Treg cell–independent and Treg cell–dependent tolerance (111, 112).

Recent work has identified that stimulation through certain PRRs and the presence of specific cytokines and metabolites can actively divert DCs toward tolerogenic functions [(34, 47, 93, 101, 113) and (Fig. 3)]. In contrast to their proimmunogenic roles, some PRRs may contribute to a tolerogenic sensitization (29, 33, 114–117). However, the impact of individual PRRs on the induction of tolerogenic DCs is likely to be context dependent. For example, BMDCs differentiated in the presence of splenic stroma were found to produce high amounts of IL-10 and dampen naive CD4+ T cell responses in culture (36). Paradoxically, an additional stimulation with TLR-2, -3, -4, and -9 agonists of DCs cocultured with splenic stromal cells further enhances their tolerogenic state, resulting in heightened production and secretion of CXCR3 chemokine IFN-γ–inducible protein 10 (IP-10) and a corresponding decrease in Th1 proliferation (118). Nevertheless, certain TLRs such as TLR-2 appear to be more tolerogenic than other TLRs. In a murine disease model of arthritis, the microbial commensals’ stimulation of TLR-2 on APCs promotes Treg cell suppressive functions and dampens IFN-γ production, whereas stimulation of TLR-4 promotes Th17- and IL-17–driven pathology (119). Various TLRs may also physically associate as heterodimers to promote contrasting responses depending on the specific composition of each heterodimer. Studies using the Yersinia pestis virulence factor LcrV showed that recognition of LcrV by a TLR-2/TLR-6 heterodimer could lead to iTDC induction, complete with IL-10 production and Tr1 induction, whereas recognition of LcrV by a TLR-2/TLR-1 heterodimer resulted in IL-12 production and induction of effector Th1 cells (120).

A particular agonist may also lead to divergent immune responses by stimulating different PRRs. Early reports suggested that zymosan, a glucan derived from yeast cell walls, promotes IL-10 production and tolerance by concomitant engagement of TLR-2 and dectin-1 (121). However, later reports from ex vivo and in vivo experimental systems suggested that divergent responses may arise from zymosan’s stimulation of these PRRs. It was found that TLR-2 ligation by zymosan increases the expression of Raldh2 by DCs, leading to the production of RA and the eventual promotion of Treg cells via the suppression of effector differentiation. In contrast, ligation of dectin-1 increases Th1 and Th17 differentiation and exacerbates autoimmunity (116). In some instances, the concomitant signaling of specific combinations of other PRRs (such as TLR-2 and TLR-4 or TLR-3, -4, or -5 and DC-SIGN) may result in tolerogenic profiles, including increased IL-10 production and lower costimulatory molecule expression by DCs (122, 123).

In addition to TLRs, signaling via G-protein–coupled receptors (such as GPR109a and GPR81) extends DC-mediated tolerance in the gut. The G-protein–coupled receptor GPR109a is a receptor for commensal bacteria–produced butyrate and niacin that induce production of IL-10 and Aldh1a1 in DCs (124). Similarly, the G-protein–coupled receptor GPR81 is expressed in intestinal DCs and macrophages and has been shown to be activated by lactate, a product of microbial fermentation that is present in abundance in the colon (125, 126). The genetic deletion of GPR81 results in a decrease in tolerance protecting from colitis, as evidenced by an increase in proinflammatory cytokine production, a decrease in regulatory factors such as IL-10, and a decrease in IDO1 expression; correspondingly, pharmacological activation of GPR81 has been shown to lessen murine colitis severity (126). Also, other molecules, such as DC-SIGN, play a specific role in itDC induction, as well as in the induction of tolerogenic functions in macrophages, by promoting specific mechanisms of tolerance (such as the production of IL-10) in response to various microorganisms (117, 127, 128). However, the binding of DC-SIGN (possibly in concert with the binding of TLRs) to various ligands, including cell wall components and modified oligosaccharides derived from bacterial LPS, results in divergent tolerogenic or immunogenic DC–mediated immune responses and may also lead to Th1 responses (122, 129–131).

Certain cytokines and metabolites have crucial roles in inducing and governing the functions of iTDCs as well as in shaping the responses of DCs to PRR ligands. For example, the
addition of IL-10 to human DC cultures may lead to decreased expression of MHCII (HLA-DR) and costimulatory molecules, resulting in T cell anergy (132, 133). In the small intestine, RA is locally found at high concentrations due to the metabolism of dietary vitamin A (109, 134). This localized presence of RA then promotes the expression of Raldh2 and production of additional RA by CD103+ DCs in the lamina propria, ultimately resulting in the increased induction of IL-10–producing Foxp3+ Treg cells (as well as in the inhibition of TGF-β–mediated Th17 cell induction and in the imprinting of gut-homing receptors on T cells) (66, 108, 109, 135–137). Consistent with its protolerogenic roles, IL-10 can then downregulate DC expression of MHCII and costimulatory molecules and reverse the effects of proinflammatory cytokines, such as IL-6 and TNF-α (60). Further, IL-10 treatment of human DCs upregulates TLR-2 expression in response to LPS administration, consistent with the protolerogenic functions observed following TLR-2 activation (as discussed above). Such treated DCs also decrease expression of IL-12-related cytokines, possibly further indicating collaborative roles of IL-10 and TLR-2 in the dampening of the immune response (138).

In addition to IL-10, the IL-12 cytokine family member IL-35, predominantly produced by Treg cells, was proposed to dampen T cell responses (139). Similarly, IL-37 decreases the production of proinflammatory cytokines induced by LPS stimulation, and the forced expression of IL-37b in murine skin DCs promotes tolerogenic DC induction, thereby affecting contact hypersensitivity challenge (140, 141). Among other cytokines, IL-27 was initially considered to be proinflammatory (142). However, other studies indicated the role of IL-27 in suppressing the differentiation of effector T cells in autoimmune models, such as experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS) (143–145). Still, it remained unknown how IL-27 signaling directly affected DCs and subsequent T cell responses. A more recent report highlighted the role of IL-27 and the immunoregulatory molecule CD39 expressed by DCs. Specifically, CD39, whose expression is induced by LPS, reduces NLRP3 inflammasome activation in DCs, thereby reducing subsequent Th1 and Th17 effector T cell differentiation (146).

Other extensively studied physiological factors that function in iTDC promotion are ligands for the aryl hydrocarbon receptor (AHR), which is important in the regulation of the balance between the formation of regulatory and effector T cells (147). The endogenous ligands of AHR, such as 2-(1'H-indole-3'-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE), also act directly on DCs by skewing them toward a tolerogenic profile characterized by decreased production of inflammatory cytokines like IL-6 and IL-12 and increased production of suppressive TGF-β and IL-10 (104, 148). In DCs, AHR can induce expression of IDO1, an immunosuppressive enzyme that catabolizes tryptophan into kynurenine (Kyn) and other metabolites (148). Further, the upregulation of IDO1 and Socs2 expression and increased RA production by DCs contribute to a decrease in the proinflammatory cytokine milieu and the subsequent induction of Foxp3+ Treg cells (149). Together, these findings indicate a crucial role of ITE and AHR signaling in the induction of iTDCs that could have therapeutic roles in multiple types of autoimmune responses (150).

Vitamin D3 represents another key physiological factor that may induce tolerogenic DCs (151–153). The active form of vitamin D3 induces immunoregulatory properties upon binding to the vitamin D receptor (VDR), which is selectively expressed by various cell types, including intestinal and skin epithelial cells, osteoblasts, CD4+ and CD8+ T cells, and also multiple APCs, including macrophages, monocytes, and DCs (151, 154). VDR agonists increase IL-10 production.
but decrease expression of IL-12 and CD80/CD86 and CD40 by DCs (155). Additionally, itDCs induced by VDR agonists, such as calcitriol and paricalcitol, are poor inducers of Ag-specific effector T cells but are potent inducers of Treg cells (151–153, 156). It was further demonstrated that DCs may also synthesize the active form of vitamin D3, therefore providing a local source of this crucial immunomodulant, altering immune cell trafficking, and further increasing the DC secretion of the chemokine CCL22, which attracts Treg cells (157–161). Overall, VDR agonists are promising therapeutics for autoimmune diseases, transplantation tolerance, and allergies (151–153, 156).

Other physiological factors may also play a role in itDC promotion. Vasoactive intestinal peptide decreases production of proinflammatory TNF-α and IL-6 and increases production of IL-10 in human DCs despite a pre-exposure to LPS, thereby mediating T cell anergy (162). Also, an addition of seminal plasma to differentiating moDCs yielded high regulatory cytokine and low proinflammatory cytokine production profiles in such moDCs (163).

In addition to these physiological factors, 14-dehydroergosterol (14-DHE), an ergosterol analogue–based compound derived from fermented wheat bran, induces tolerogenic properties in DCs, although the exact mechanisms are unclear (164, 165). Similarly, multiple pharmacological agents, including chemically modified TLR ligands, JAK inhibitors, corticosteroids, cisplatin, antibiotics, probiotics (including bacterial species of the Lactobacillus, Bifidobacterium, and Streptococcus genera), and dietary supplements (such as zinc) have been shown to promote tolerogenic profiles in BMDCs and moDCs. These profiles are characterized by changes at both the transcriptional and translational levels, ultimately leading to decreased production of the inflammatory cytokines TNF-α and IL-6, decreased expression of costimulatory molecules, increased expression of coinhibitory molecules like PD-L1, and the production of the anti-inflammatory cytokines IL-10 and TGF-β (166–169).

Among the intrinsic signaling pathways involved in sensing specific extrinsic factors that induce tolerogenic properties in DCs, Wnt/β-catenin is a major molecular pathway involved in the promotion of itDCs and the increased production of anti-inflammatory cytokines (such as TGF-β, RA, and IL-10) and, conversely, in blocking the production of NF-κB–induced proinflammatory cytokines (32, 33, 67, 170). Deficiencies in Wnt receptors LRPS/6 and β-catenin–signaling mechanisms enhance proinflammatory cytokine production and Th1/Th17 effector responses and increase disease severity in mouse models including colitis and MS (67, 171–174). Noncanonical Wnt signaling mediated by Wnt5a is also involved in the promotion of tolerance by modifying DC maturation and IL-10 production in response to TLR agonists (175). In addition to Wnt/β-catenin, mTOR emerges as an important regulator of DC functions that may also affect tolerogenic mechanisms (176, 177).

Harnessing the tolerogenic functions of DCs for therapeutic applications

The functions of DCs as inducers of tolerance represent important therapeutic opportunities. Modulation of such DC-induced tolerance can help block different forms of autoimmune and also impact other types of immune responses relevant for transplantations as well as tumor immunology (11). Both ntDCs and itDCs are relevant for such therapeutic manipulations, and the targeted delivery of Ags to DEC-205+ and other DCs has proven to be a powerful way to reinforce tolerance to self-antigens implicated in the autoimmune process (89). For example, the spontaneous induction of peripheral tolerance in response to Ags derived from organs insulated from the immune system (such as the CNS), is likely less efficient (7, 11). This results in an increased potential for autoimmune diseases such as in animal models of MS, which can be readily provoked after an immunization of healthy animals with CNS Ags (7, 178, 179). However, such autoimmune responses can be blocked by tolerance induced by targeted delivery of various tissue-specific Ags to DCs, allowing for efficient Ag presentation to self-reactive T cells (11, 17, 18, 89).

In these initial experiments, DCs were targeted in vivo with anti–DEC-205 chimeric Ab to deliver a potentially encephalitogenic peptide Ag derived from myelin oligodendrocyte glycoprotein, which was genetically fused to the Ab molecule, to prevent subsequently induced EAE (18). These early results were then extended to other EAE models and various encephalitogenic Ags that were delivered through DC-specific molecules, as recently reviewed in Refs. 11 and 89. The targeting of Ags to DCs has also been successful in mediating tolerance in multiple different models of autoimmunity, including diabetes, colitis, and arthritis, as well as in a model of graft-versus-host disease (180–183). Overall, this specific delivery of Ags results in tolerogenic mechanisms that prevent autoimmunity.

In addition to the delivery of Ags specifically targeted to DCs, other studies found that certain formulations of Ags (such as nanoparticles or specifically modified cellular material) could lead to their in vivo acquisition by DCs and to the amelioration of autoimmune processes including EAE and diabetes (184–187). Despite generally lacking cell target specificity, these methods showed promise in the treatment of ongoing autoimmune processes, particularly when additionally coupled with agents known to induce the formation of itDCs under proinflammatory conditions (149). Some of those methods also incorporated DC-specific Abs to enhance the specificity of the delivery system (188), although most of such Ab-coupled immunogenic particles have been tested for new vaccine approaches (189). In addition to controlling autoimmunity, DC-mediated tolerance holds promise in mitigating transplant rejection (as recently reviewed by Thomson et al. in Ref. 190). Importantly, DCs can cooperate with other types of immune cells, such as NKT cells, to prevent graft rejections (191).

In human systems, the most available options thus far have been to induce itDCs ex vivo, analogous to the induction of itDCs from murine BMDCs, and to subsequently treat them with agents that can potentially further promote such itDC differentiation in vivo. Either murine BMDCs or moDCs derived from humans afflicted with an autoimmune disease were treated in vitro with the pharmacologic agents PEGylated–TLR-7 ligand, dexamethasone plus monophosphoryl lipid A, tocitakinib, or prednisolone and were then used to delay disease onset or ameliorate disease severity in diabetes, rheumatoid arthritis, EAE, and myasthenia gravis, respectively (166, 167, 169, 192–194). Also, treatment with IL-10 leads to the formation of itDCs that likely possess clinical relevance (195).
Further, the treatment of BMDCs with the anti-tumor drug cisplatin in conjunction with various TLR agonists results in increased IL-10 production by the BMDCs as well as the prevention of Th1 and Th17 responses (196).

In contrast to the generally beneficial functions of tolerogenic DCs in the prevention of autoimmunities as discussed above, the tumor microenvironment can skew DCs toward tolerogenic functions, thereby diminishing tumor rejection (177, 197–202). In an effort to induce anti-tumor immunity, multiple DC-based immunotherapies against cancer have been gaining importance, as recently reviewed by Sancho and colleagues (203). Even more breakthroughs that will determine the contributions of various DCs to tumor-immune evasion and allow for the harnessing of such DCs for anti-tumor therapies are expected.

Conclusions
The recent years have seen a growing understanding of DC functions in both the initiation and the regulation of immune responses. Overall, whereas functions of iDCs contribute to the maintenance of homeostasis under potentially proinflammatory conditions, nDCs help to establish tolerance under steady-state conditions. Importantly, the new insights are providing us with a framework for exploiting the DC-mediated mechanisms of tolerance for more effective immunotherapies, which will, hopefully, be burdened by fewer side effects.

Disclosures
The authors have no financial conflicts of interest.

References


151. Reuter, S., H. Martin, H. Beckert, M. Bros, E. Montemanni, C. Belz, A. Heinz, S. H. Im, H. Reth, F. Arntz, and S. H. Im. 2013. Recognition of TollR7 and TLR17 by the aryl hydrocarbon receptor.

152. Reuter, S., H. Martin, H. Beckert, M. Bros, E. Montemanni, C. Belz, A. Heinz, S. H. Im, H. Reth, F. Arntz, and S. H. Im. 2013. TollR7 and TLR17 recognition by the aryl hydrocarbon receptor.


155. Reuter, S., H. Martin, H. Beckert, M. Bros, E. Montemanni, C. Belz, A. Heinz, S. H. Im, H. Reth, F. Arntz, and S. H. Im. 2013. Recognition of TollR7 and TLR17 by the aryl hydrocarbon receptor.

156. Reuter, S., H. Martin, H. Beckert, M. Bros, E. Montemanni, C. Belz, A. Heinz, S. H. Im, H. Reth, F. Arntz, and S. H. Im. 2013. TollR7 and TLR17 recognition by the aryl hydrocarbon receptor.


158. Reuter, S., H. Martin, H. Beckert, M. Bros, E. Montemanni, C. Belz, A. Heinz, S. H. Im, H. Reth, F. Arntz, and S. H. Im. 2013. TollR7 and TLR17 recognition by the aryl hydrocarbon receptor.

159. Reuter, S., H. Martin, H. Beckert, M. Bros, E. Montemanni, C. Belz, A. Heinz, S. H. Im, H. Reth, F. Arntz, and S. H. Im. 2013. TollR7 and TLR17 recognition by the aryl hydrocarbon receptor.


162. Reuter, S., H. Martin, H. Beckert, M. Bros, E. Montemanni, C. Belz, A. Heinz, S. H. Im, H. Reth, F. Arntz, and S. H. Im. 2013. TollR7 and TLR17 recognition by the aryl hydrocarbon receptor.

163. Reuter, S., H. Martin, H. Beckert, M. Bros, E. Montemanni, C. Belz, A. Heinz, S. H. Im, H. Reth, F. Arntz, and S. H. Im. 2013. TollR7 and TLR17 recognition by the aryl hydrocarbon receptor.

164. Reuter, S., H. Martin, H. Beckert, M. Bros, E. Montemanni, C. Belz, A. Heinz, S. H. Im, H. Reth, F. Arntz, and S. H. Im. 2013. TollR7 and TLR17 recognition by the aryl hydrocarbon receptor.

165. Reuter, S., H. Martin, H. Beckert, M. Bros, E. Montemanni, C. Belz, A. Heinz, S. H. Im, H. Reth, F. Arntz, and S. H. Im. 2013. TollR7 and TLR17 recognition by the aryl hydrocarbon receptor.
the abundant production of IL-10, thereby promoting Th2- and Tr1-biased T-cell immunity. *Oncotarget* 7: 33765–33782.


