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Dendritic cells as inducers of peripheral tolerance

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Abstract

Mechanisms of tolerance initiated in the thymus are indispensable for establishing immune homeostasis, but they may not be sufficient to prevent tissue-specific autoimmune diseases. In the periphery dendritic cells (DCs) play a critical tolerogenic role, extending the maintenance of immune homeostasis and blocking autoimmune responses. Here we review these essential roles of DCs in orchestrating mechanisms of peripheral T cell tolerance that were determined by methods of targeted delivery of defined antigens to DCs *in vivo* in combination with various genetic modifications of DCs. Further, we discuss how the functions of DCs empowered by specific delivery of T cell antigens could also be harnessed for tolerance induction in clinical settings.

Peripherally induced tolerance

The fundamental ability of the immune system to respond against foreign pathogens and tumors while preventing an autoimmune response against self-antigens underlie the potential challenges first recognized by Paul Ehrlich who, over a hundred years ago, used the term “horror autotoxicus” to define an immune attack against an organism’s healthy tissues [1]. Positive and negative selection in the thymus, combined with a production of thymically derived **regulatory T (tReg) cells**, all shape the T cell receptor (TCR) repertoires and responsiveness of T cells to prevent overt anti-self responses [2, 3]. However, it is clear that the mature repertoire still contains T cells with a degree of reactivity to self [4]. This self-reactivity is explained by several factors, such as insufficient thymic deletion in case of some tissue restricted antigens (TRAs), which are presented to T cells in the thymus less efficiently (in comparison to their presentation by specialized antigen presenting cells (APCs) in the peripheral immune system) [5]. More broadly, it is clear that T cell receptors (TCR) are cross-reactive to some degree, that is, they recognize multiple, sometimes even unrelated, peptides (“molecular mimics”) presented by major histocompatibility complex (MHC) molecules [6, 7]. Although the specific “fit,” or “affinity,” between cross-reactive peptides and specific TCRs may differ significantly, such cross-reactivity increases the risk of some peripheral T cells remaining reactive against self-antigens [8–11]. A risk of

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autoimmunity is further increased because, especially during infections, some self-reactive peripheral T cells can be primed even by low-affinity peptides that are below their original thresholds for negative selection [5, 10–12]. Additionally, a degree of self-reactivity correlates with increased TCR signaling during thymic selection and increased expression of CD5; these CD5^{hi} cells can be self-reactive but nevertheless survive thymic selection and therefore may also present greater risks of autoimmune responses [3, 13, 14].

Therefore, additional mechanisms of tolerance are necessary to prevent autoimmune activation of peripheral self-reactive T cells. Functions of Treg cells are indispensable to maintain immune homeostasis, and the absence of Treg cells leads to overt auto-aggressive activation of the immune system [15]. However, thymically-produced tTreg cells may be overwhelmed by specific pro-inflammatory autoimmune activation; also, in some individuals, the development of self-antigen specific tTreg cells may be compromised [5, 10, 11, 16]. Similarly, in various animal models of autoimmune diseases, the autoimmune process can be 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, ultimately leading to the priming of the pre-existing self-reactive T cells [11, 17]. Overall, self-reactive T cells continue to persist in the peripheral immune system, and, for multiple reasons, thymically-imposed mechanisms of tolerance may fail to prevent a specific immune priming of such self-reactive T cells, ultimately leading to the autoimmune process [5, 10, 11, 17, 18].

Crucially, specific mechanisms of tolerance originating in the peripheral immune system can further prevent activation of self-reactive T cells that escaped thymic deletion or failed to be inhibited by the functions of tTreg cells [19]. In a process analogous to its functions in medullary thymic epithelial cells (mTEC), the Autoimmune Regulator (AIRE) mediates expression of TRAs in peripheral non-hematopoietic **stromal cells** and induces deletion of self-reactive T cells [20]. However, antigens derived from apoptotic cells represent a critical, and arguably more abundant source of tissue self-antigens, and their presentation to both CD4⁺ and CD8⁺ T cells relies on the functions of DCs [21–25]. Although **cross-presented antigens** acquired from various tissues may lead to deletion of CD8⁺ T cells, the tolerance spontaneously induced by DCs in this way may be particularly important for the maintenance of immune homeostasis to self- and oral antigens within the intestine [25–29]. In contrast, the spontaneous induction of mechanisms of peripheral tolerance including CD4⁺ T cell deletion, **anergy** and conversion of peripheral (p)Treg cells in response to antigens from organs that are more insulated from the immune system (such as the central nervous system (CNS)), may be less efficient [17]. Therefore, spontaneously induced peripheral tolerance induced by DCs may not prevent autoimmune responses against the CNS and other organs whose antigens are not sufficiently available for the specific induction of mechanisms of peripheral tolerance. However, such tolerogenic functions of DCs can be unmasked and enabled by a targeted delivery of various tissue-specific antigens, allowing for their efficient presentation to self-reactive T cells [30–32].

Establishing the roles of DCs as key inducers of peripheral tolerance

Initially, DCs were considered to be unlikely candidates for tolerance induction. Since their early discovery by Ralph Steinman, DCs have been recognized as instrumental for orchestrating the priming of effector T cells [33]. In keeping with such originally-identified functions of DCs, the recent advances in deciphering molecular pathways governing the development and homeostasis of DCs have further delineated such roles of DCs and their subsets in the priming of effector T cells against various infectious agents and tumors as well as in the activation of autoimmune T cells as recently reviewed by Durai and Murphy in [34].

However, efficient priming of T cells by DCs leading to immune responses requires additional signals from the pro-inflammatory environment that can be sensed by DCs through specific pattern recognition receptors (PRRs) [35–38]. The acquisition of such additionally enhanced properties to prime T cells is also often referred to as a “maturation” of DCs. In contrast, a lack of T cell priming in the absence of pro-inflammatory stimuli has initially led to a characterization of such DCs as potentially tolerogenic immature bystanders under steady state conditions [23, 37]. In early experiments, it was observed that, in the absence of additional differentiation signals, human DCs had a reduced capacity to stimulate T cell responses *in vitro*. Additionally, a transfer of autologous DCs, as well as *in vivo* expansion of endogenous DCs, could enhance oral tolerance and also prevent autoimmunity in various models of diabetes and **experimental autoimmune encephalomyelitis (EAE)**, an animal model of multiple sclerosis (MS) [39–42]. Similarly, pulsing DCs with influenza proteins led to decreased specific immune responses upon infusion of such DCs into patients [43]. In some other *in vivo* animal studies, the binding of xenogeneic and aggregated immunoglobulins to DCs resulted in inhibition of the corresponding anti-globulin immune responses [44, 45]. However, the specific outcome of T cell stimulation elicited by DCs under non-inflammatory conditions had been difficult to measure. To overcome these limitations, an experimental system was developed to deliver defined peptide antigens directly to DCs *in vivo* for their subsequent presentation to CD4⁺ T cells [30]. Such antigens were included as fusion proteins with the constant regions of a recombinant **chimeric antibody** specific for the lectin receptor DEC205 (CD205, LY75) present on some conventional DCs (cDCs) [30] and (Box 1). The use of fusion proteins helps to avoid unintentional stoichiometric differences in the amounts of antigenic molecules present in a DC-targeting reagent. Further, the original constant regions were replaced with the engineered murine IgG1 constant regions that also had mutations minimizing their non-specific binding to Fc receptors [30]. In DCs, DEC205 mediates an efficient endocytic pathway allowing for robust processing and presentation of antigens from proteins binding to this DC-surface lectin receptor [23]. Although DEC205 can be also expressed in other immune cells such as germinal center B cells, the expression of DEC205 in B cells is considerably increased *in vivo* only after an immunization using adjuvants or *in vitro* after activation with LPS, anti-CD40, and IL-4 [23, 46, 47]. Therefore, in the steady state, delivery of antigens through the DEC205 receptor leads to specific antigenic presentation by DCs, and these early experiments helped to unequivocally establish that in the steady state the outcome of the initial antigenic stimulation of CD4⁺ T cells by DCs results in an abortive

T cell activation, thereby leading to tolerance [30]. Furthermore, by using MHCI-restricted and cross-presented antigens, subsequent experiments extended the initially established tolerogenic functions of the DEC205⁺ DCs to the induction of tolerance among CD8⁺ T cells [48]. The antigen-specific CD4⁺ T cells tolerance induced by DEC205⁺ DCs *in vivo* was further leveraged to mediate an effective protection from autoimmunity in an animal model of MS [32]. In contrast, an activation of DCs through ligation of their surface CD40 prevented a tolerogenic outcome of T cell activation and resulted in the priming of antigen-specific effector T cells [30]. The approach originally developed to deliver antigens through DEC205 was subsequently applied to other receptor molecules expressed on mouse and some human DCs including CD11c, DC-SIGN, Langerin (CD207), Trem14, DNGR-1/CLEC9A, and DCIR2, which were targeted with antigens to help provide essential *in vivo* functional characterization of such DCs [30, 49–56] and (Box 1). Besides cDCs, plasmacytoid DCs (pDCs) were also found to have functions in peripheral and oral tolerance as recently reviewed by Swiecki and Colonna in [57]. These tolerogenic functions of mouse and human pDCs were also confirmed using antibody-based targeting of various antigens through surface molecules including blood dendritic cell Ag 2 (BDCA2), **Siglec-H** and DC immunoreceptor (DCIR). These studies have shown that, despite the lower ability of pDCs to activate naïve T cells, antigens presented by pDCs could nevertheless lead to various forms of tolerance in responding T cells [58–60]. It should be noted that, in addition to the functions in peripheral tolerance, pDCs were also proposed to bring peripherally acquired antigens back to the thymus to enhance central deletion of T cells [61]. Overall, the methods of delivery of defined antigens to various DCs by antibodies have become widely used for studies of various T cell responses induced by mouse and human DCs as well as a development of novel vaccines as reviewed by Figdor and colleagues in [62]. In some such studies, the chemical conjugates between whole antigenic proteins and DC-specific antibodies were used, although the specificity and efficacy of a targeted delivery using such conjugates may be less stringent in the absence of the specific design and modifications introduced into the recombinant chimeric antibodies, as discussed above.

Box 1

Targeting of antigens to various types of DCs

The targeting of known antigens (Ags) to elicit cognate CD4⁺ T cell responses may be employed to induce tolerance and reduce autoimmune disease. Multiple recombinant chimeric antibodies with variable regions specific for various cell surface receptor molecules expressed on DCs have been produced based on the original design (Box 1). Examples of such chimeric recombinant antibodies that target antigens to either all CD11c⁺ cDCs or specific DCs within the DC1 or DC2 lineages include anti-CD11c-Ag, which targets antigens to all CD11c⁺ DCs [55]; anti-DEC205-Ag, which targets antigens to DCs within the DC1 lineage [30]; anti-Langerin-Ag, which targets antigens to migratory DCs [52]; and anti-DCIR2-Ag, which targets antigens to some DCs within the DC2 lineage [49]. Additionally, anti-Siglec-H-Ag [60] targets tolerogenic antigens to Siglec-H⁺ pDCs. In addition to targeting of DCs with recombinant chimeric antibodies antigens may also be delivered to DCs using single-chain fragment variable (scFv) containing antigenic peptides as well as antibodies which are produced via chemical

conjugation of the antigen protein to the heavy chain of the original monoclonal antibodies such as anti-DNGR-1/CLEC9A or anti-DEC205 [56, 106, 116]. Further, other methods of non-targeted antigen delivery have been used, such as oral administration of proteins that may be processed and presented by cDCs in the intestines [27]. Additionally, tolerogenic microparticles are predominantly taken up by monocytes and macrophages and possibly such antigens might then be also further acquired by DCs [109].

Figure I. Delivery of defined antigens to DCs *in vivo* using recombinant chimeric antibodies
T cell antigens are included as fusion proteins added to the carboxy termini of the heavy chains of a recombinant chimeric antibody. The murine IgG1 constant regions were additionally engineered to minimize their non-specific binding to Fc receptors and were then combined with the variable regions specific for surface endocytic receptor DEC205 expressed on murine DCs as described in [30].

In addition to the extensive results obtained from experiments that used antigen targeting, the general functions of DCs in peripheral tolerance were also confirmed by other independent experimental methods that relied on transgenic expression of ectopic antigens as cytosolic proteins in DCs or by tracking the uptake of such proteins to DCs [22, 63]. Moreover, consistent with the tolerogenic functions of such DCs, an ablation of DCs induced by a treatment with diphtheria toxin (DT) in mice expressing the DT receptor

(DTR) in DEC205⁺ DCs or all CD11c⁺ DCs was shown to lead to spontaneous autoimmune T cell activation and increased formation of effector T cells [64, 65]. However, a similar ablation of DCs did not always cause a disturbed homeostasis of peripheral T cells [66], possibly reflecting differences in deletion of specific DC populations in addition to complex physiologic conditions associated with the uptake of self-antigens and a subsequent activation of self-reactive T cells, as discussed above. Nevertheless, in other experimental systems including specific DTR expression in either chemokine receptor (XCR1)⁺ DCs or in the entire DC lineage controlled by the transcription factor Zbtb46, as well as in experiments that relied on a chemical depletion of some DCs, the absence of DCs or their subsets disturbed tolerance and immune homeostasis [27, 28, 67].

Exploring tolerogenic mechanisms of peripheral DCs in vivo

Results of the initial experiments that examined the fate of antigen-specific T cells responding to defined antigens presented by DCs in the steady state revealed that responding T cells underwent a partial deletion combined with an ensuing induction of T cell unresponsiveness in the surviving cells (anergy) [30, 32]. Subsequent studies found a substantial portion of the **Foxp3**^{neg} T cells that responded to DCs also converted into Foxp3-expressing peripheral regulatory T cells (pTreg cells) and documented the expansion of some pre-existing tTreg cells [68, 69]. Other studies found a similar extrathymic conversion by specialized CD103⁺ DCs presenting intestinal and food antigens within gut associated lymphoid tissues (GALT) of Foxp3⁺ Treg cells contributing to maintenance of local immune homeostasis [27, 28, 70–73]. Recent results of experiments in an EAE model using *Hopx*^{-/-} mice that have specific deficiencies in functions and survival of pTreg cells (but not tTreg cells) helped to further establish the essential roles of pTreg cells in the maintenance of DC-induced tolerance and prevention of organ-specific autoimmunity [54, 74]. Overall, these results suggested that specialized tolerogenic DCs in steady state are not immature bystanders but rather inducers of active processes in T cells to promote mechanisms of peripheral tolerance including a conversion of pTreg cells. Consistent with such actively induced mechanisms, various immune mediators and environmental signals including materials from dead cells and some microbial products may enhance tolerogenic properties of DCs as reviewed by Manicassamy and Pulendran in [75], Sancho and Reis e Sousa in [76] and Mellman in [37]. The specific tolerogenic mechanisms of such tolerogenic types of DCs and the roles of pTreg cells have become an area of intense investigation [37, 54, 68, 70, 71, 73, 77, 78].

Combining the methods of antigen delivery to DCs with diverse genetic mouse models lacking specific subtypes of DCs facilitated the understanding of specific tolerogenic functions of DCs [27, 52, 55] and (Figure 1). The two main lineages of CD11c⁺ cDCs (termed “DC1” and “DC2”) are characterized by the expression of specific surface markers and require different transcription factors for their development and proper localization and functions in lymphoid tissues [34, 79, 80]. In murine lymphoid organs, XCR1^{hi}CD172a^{lo} DC1 can additionally express DEC205, CD8a, Langerin, and DNCR-1/CLEC9A, whereas some XCR1^{lo}CD172a^{hi} DC2 may also express DCIR2 [34, 79, 80]. Many transcription factors govern DC1 and DC2 development, including Batf3 and IRF8 which are required for the development of the DC1 subset and IRF4, which is required for normal numbers of DC2

[34, 79, 80]. The results of experiments using *Batf3*^{-/-} mice confirmed that DC1 contained DCs that can induce pTreg cells and organ-specific tolerance [55, 81]. However, *Batf3*^{-/-} mice show no spontaneous autoimmune inflammation, possibly in part due to the heterogeneity of DCs within the DC1 lineage and an incomplete absence of such DCs in certain genetic backgrounds of experimental mice [82, 83]. These results are also consistent with the observations from mice with a decreased DC1 population following a deletion of IRF8 in cDCs, which results in a decreased induction of pTreg cells in intestines in response to orally administered antigen (although other forms of tolerance appear to remain intact) [27]. In contrast, DC2 are generally less efficient at induction of pTreg cells and tolerance, although some DC2 could also induce pTreg cells in the intestines and expand the existing tTreg cells as well as promote other forms of Treg cell-independent tolerance [84–86]. However, a delivery of antigens to DCs via anti-DCIR2 chimeric antibodies failed to efficiently induce pTreg cells in the peripheral lymphoid organs [52]. Also, in the intestines, a deletion of DCIR2⁺ DCs using a DTR expression system failed to exacerbate sensitivity to inflammation, whereas a deletion of CD103⁺CD11b⁻ DC1 exacerbated such inflammation (although in this case a Treg-independent mechanism was proposed) [87].

The heterogeneous T cell responses following their activation by DCs in the steady state also became evident in other recent experiments, which utilized a delivery of T cell antigens to all cDCs targeted through the CD11c integrin with an anti-CD11c chimeric antibody. Although an antigenic presentation by all CD11c⁺ DCs initially induced anergy in the responding T cells, such tolerance could not be maintained because the anergic T cells failed to convert into pTreg cells [55]. Strikingly, in the *Irf4*^{-/-} mice characterized by the decreased numbers of DC2 in lymphoid organs and a correspondingly increased proportion of the lymphoid resident DC1 population, the same antigens delivered to all CD11c⁺ DCs resulted in a robust induction of pTreg cells [55]. Therefore, efficient induction of pTreg cells and long-lasting immune tolerance relies on compartmentalization of the presentation of antigens by specialized DCs. This idea is also in agreement with the results from other earlier experiments that found an induction of pTreg cells in response to antigens delivered to DC1 through DNGR-1/CLEC9A expressed on CD8⁺ DCs or Langerin expressed on chemokine receptor CCR7-dependent migratory CD103⁺ DCs [52, 56]. However, a precise phenotypic and functional delineation of the tolerogenic DCs that induce pTreg cell conversion in response to systemic antigens is complicated by the diverse expression of DEC205, Langerin, and DNGR-1/CLEC9A on migratory and resident subpopulations of DC1 [34, 79, 80, 83]. Also, DC1 have potent functions in antiviral and anti-tumor immune responses particularly under pro-inflammatory conditions [34, 38]. Therefore, a more precise definition of tolerogenic DCs that can efficiently direct pTreg cell differentiation and tolerance requires a consideration of additional functional and phenotypic properties of such DCs that induce specific tolerogenic responses in T cells, as discussed below.

Recent studies suggest that mechanisms of T cell anergy and pTreg cell conversion may be intimately linked and that induction of an anergic state can precede a subsequent conversion of initially-tolerized T cells into pTreg cells [55, 88]. However, not all anergic T cells can become pTreg cells, and the specific mechanisms governing the fates of T cells responding to tolerogenic DCs remain unclear. The differentiation of some anergic T cells into pTreg cells could be facilitated by functions of CD73, neuropilin 1 (Nrp1), and folate receptor 4

(FR4) expressed on such T cells [88]. Also, differences in the efficiencies of processing and presentation of antigens by the DC1 and DC2 populations could potentially contribute to a preferential induction of pTreg cells by tolerogenic DCs [49]. DCs that induce Treg cells are also characterized by the production of multiple immunomodulatory metabolites, cytokines, and enzymes, including **retinoic acid (RA)**, **transforming growth factor β (TGF β)**, IL-10, CD39, and others [70, 89–94]. RA together with TGF β can promote induction of pTreg cells, and production of RA and TGF β in intestinal DCs can be regulated by beta-catenin [90, 95]. Additionally, these DCs require expression of TGF β -activating integrin $\alpha(v)\beta 8$ for efficient induction of pTreg cells and prevention of colitis [96]. Furthermore, specific immunomodulatory molecules such as CTLA-4, ICOS, and PD-1 have important functions in Treg cell induction and regulation of tolerance by DCs [63, 97–102]. In addition to its roles in regulating T cell motility during their interactions with DCs, PD-1 can directly induce expression of Foxp3 in T cells [63, 97–100, 102]. In contrast to the mechanisms that directly induce Foxp3 expression in T cells, a recently-described pathway depends on thymic-independent functions of CD5 to modulate a sensitivity of peripheral CD5^{hi} T cells to effector-differentiating cytokines thereby instructing such potentially auto-reactive T cells to convert into pTreg cells to avoid autoimmune activation [103]. This mechanism is consistent with the previously proposed precursors for pTreg cells that are present among peripheral T cells [104, 105]. However, expression of CD5 in T cells represents a spectrum rather than discrete thresholds, and some CD5^{lo} T cells can be auto-reactive consistent with the ability of low-affinity peptides to prime self-reactive peripheral T cells [5, 10–12]. To promote a conversion of such broad-affinity T cells into pTreg cells during responses to systemic antigens, expression of CD5 in these T cells can be additionally increased through an engagement of a **BTLA** receptor HVEM by the tolerogenic BTLA^{hi} DEC205⁺ DCs of DC1 lineage present in lymphoid organs [55]. Overall, the induction of pTreg cells and tolerance by DCs constitutes a complex and dynamic process that may be co-orchestrated by specific immunomodulatory molecules expressed by tolerogenic DCs that help steer the initially-tolerized T cells toward their conversion into pTreg cells (Figure 2). Future research will undoubtedly clarify and provide more details regarding the various functional relationships among these multiple pathways.

Exploiting functions of DCs for immunotherapies against autoimmunity

Ever since the first experiments that showed the potential of DCs loaded with specific antigens in modulating immune responses, the utilization of DC functions has been proposed for vaccine purposes but also for the immunotherapy protocols to both enhance immune tolerance and to abrogate tolerogenic responses that might impede anti-tumor immunity [23, 37]. Particularly, the delivery of defined antigens to DCs *in vivo* opened new possibilities of leveraging these methods as potentially therapeutically-relevant applications. In the first of such experiments, DCs were targeted *in vivo* with anti-DEC205 chimeric antibody to deliver a potentially-encephalitogenic antigen, myelin oligodendrocyte glycoprotein (MOG), to prevent subsequently-induced EAE [32]. Further, these early results were thereafter extended to other EAE models using multiple encephalitogenic antigens delivered through diverse molecules expressed on both conventional and plasmacytoid DCs [52, 54, 55, 60, 106–108]. Such DC-induced protection from EAE was mediated by the crucial functions of

the *de novo* induced pTreg cells [54]. These results confirmed the paradigm that to avoid induction of autoimmunity, tolerance needs to be established before the immune system is challenged with specific antigens under the pro-inflammatory conditions. At present, it remains less clear how to enhance tolerogenic functions of DCs by targeting with antigens during the on-going autoimmune process. However, the results of experiments that show a successful amelioration of the relapsing-remitting EAE after a delivery of antigen-coupled-microparticles that may induce apoptosis in inflammatory monocytes, raise interesting questions of whether such tolerogenic antigens might also be presented by tolerogenic DCs [109]. In general, the ability of tolerogenic DCs to convert self-reactive T cells into pTreg cells may be of particular interest in autoimmune disorders that are characterized by a decreased thymic production of self-antigen specific tTreg cells and a correspondingly increased development of self-reactive T cells [16]. Since the increased amounts of peripheral self-reactive T cells in the absence of sufficient numbers of corresponding tTreg cells pose an especially high risk for autoimmunity, the tolerogenic functions of DCs to convert such self-reactive T cells into pTreg cells may have a crucial role in maintaining immune homeostasis under such conditions. In addition to EAE, the targeting of antigens to DCs has been shown to induce tolerance to prevent autoimmunity in multiple different models. In the transgenic mouse model for type 1 diabetes, in which hemagglutinin (HA) is expressed under the insulin promoter (resulting in about 40% of these mice becoming diabetic in the presence of HA-specific T cells), the targeting of HA to DEC205⁺ DCs prevented disease in nearly all such mice [110]. Crucially, similar protection was also achieved in the spontaneous **NOD model**, where the delivery of natural insulin antigens to DEC205⁺ DCs also induced conversion of pancreatic β -cell-reactive TCR tg BDC2.5 CD4⁺ T cells into long-lived Foxp3⁺ pTreg cells [111]. Also in the NOD model, a delivery to either DEC205⁺ or DCIR2⁺ DCs of peptides specific for diabetogenic CD4⁺ and CD8⁺ T cells led to the deletion of such autoreactive T cells and other forms of Treg cell-independent tolerance, protecting from diabetes [85, 112]. In other models of autoimmunity, targeted delivery of specific antigens to DEC205⁺ DCs led to similar protection from disease. In a VILLIN-HA transgenic mouse model of inflammatory bowel disease (IBD) where HA is expressed in the intestinal epithelium, a delivery of HA to DEC205⁺ DCs prevented IBD induced by HA-specific T cells [113]. Finally, targeted delivery of **proteoglycan (PG)** to DEC205⁺ DCs prevented the subsequent induction of a PG-induced arthritis [114]. In addition to the models of autoimmunity, targeting DEC205⁺ DCs with the immunodominant domain of human type XVII collagen prolonged the survival of skin grafts in a skin transplantation model [115].

Concluding remarks

The long and intense research into the functions of DCs has resulted in an appreciation of their versatile functions in both priming and regulation of immune responses. Some of the earliest studies, including those demonstrating that DCs were incapable of initiating T cell priming in the absence of pro-inflammatory stimuli, paved the way for later studies, which demonstrated that the delivery of specific antigens could induce T cell tolerance and reduce autoimmune disease. Recently, studies have become even more challenging and innovative, combining targeted antigen delivery with genetic models to determine the active roles of

specific immunomodulatory pathways and different DC subsets in maintaining immune tolerance. Throughout these and other studies, DCs have proven time and again to be major players in the delicate balance between tolerance and immunity, though there are still many questions to be answered (see Outstanding Questions). Undoubtedly, future research will allow us to even more fully understand the central roles of DCs in the regulation of immune responses and to better harness the power of DCs to activate and modulate specific T cell responses as new frontiers for the treatment of diseases ranging from autoimmunity to infection to cancer.

Outstanding questions

How do various immunomodulatory mechanisms mediated by DCs and their subsets work in concert to promote specific tolerogenic T cell responses and maintenance of long-lasting tolerance?

What are the specific mechanisms in T cells and DCs that underlie the related processes of T cell anergy and pTreg cell differentiation that are both induced by tolerogenic DCs?

How can specific tolerogenic mechanisms mediated by DCs in the steady state also be leveraged to tame the on-going autoimmune process under pro-inflammatory conditions?

How can a specific regulation of an inadvertent induction of antigen-specific tolerance by DCs impeding anti-tumor immune responses be exploited for novel anti-cancer therapies?

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Glossary

Anergy

a phenomenon in which an immune cell is rendered unresponsive.

BTLA (“B and T Lymphocyte-Associated”)

a molecule expressed by B and T cells as well as by some dendritic cells; the DEC205⁺ DC1 dendritic cells expressing BTLA are considered to be tolerogenic and promote regulatory T cell differentiation.

Chimeric Antibody

an antibody whose structure includes, a variable (V) region specific for a cell surface molecule, a species-specific constant (C) region engineered to minimize non-specific binding *in vivo*, and a genetically fused antigenic peptide of choice.

Cross-Presented Antigens

refer to the unique abilities of the DC1 population to process peptides obtained from the extracellular proteins for presentation by MHCI complexes; fundamental for the anti-tumor and anti-viral immunity mediated by DCs as well as induction of tolerance by deletion of autoreactive CD8⁺ T cells.

EAE (“Experimental Autoimmune Encephalomyelitis”)

a common murine model of multiple sclerosis (MS) provoked by immunization of animals with neuronal antigens; disease severity is measured based on a standardized scale.

Foxp3 (“Forkhead Box P3,” “Scurfin”)

a transcription factor that is a master regulator of regulatory T cell development.

NOD Model (“Non-Obese Diabetic Model”)

a murine model of type I diabetes

Proteoglycan

a macromolecule composed of a protein bound to glycosaminoglycans; is found in abundance in connective tissues; may be an inducer of arthritis.

Regulatory T Cell (“Treg Cell”)

a subset of CD4⁺ T cells known to dampen the immune response; hallmarks of Treg cells include expression of the transcription factor Foxp3 and the cell surface molecule CD25.

Retinoic Acid (“RA”)

a vitamin A metabolite; with transforming growth factor β , is involved in regulatory T cell differentiation.

Siglec-H

a murine siglec-like molecule that contains two immunoglobulin domains; is expressed on some macrophages and on plasmacytoid dendritic cell precursors of the spleen, lymph nodes, bone marrow, and blood.

Stromal Cells (“Stroma”)

Specialized connective and epithelial tissue cells; involved in the shaping of the immune response, in part due to their involvement in thymic selection, regulatory T cell differentiation, and removal of some auto-specific T cells in the periphery.

Transforming Growth Factor β (“TGF β ”)

secreted polypeptide cytokine; involved in various mechanisms, including regulatory T cell differentiation, cellular proliferation, and apoptosis.

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Trends

Peripherally-induced T cell tolerance is required to extend the maintenance of immune homeostasis and to block autoimmune responses.

Dendritic cells (DCs) are key inducers of peripheral tolerance. The tolerogenic functions of DCs can be directed and enhanced by the *in vivo* targeted delivery of defined T cell antigens.

The crucial pathways and molecular mechanisms regulating peripheral tolerance are being uncovered by approaches combining methods of *in vivo* targeted delivery of T cell antigens to DCs and various genetic modifications of DCs.

The tolerogenic functions of DCs and their specific mechanisms of tolerance, including the induction of *de novo* converted peripheral regulatory T cells (pTreg cells), can become a foundation of new immunotherapies

Figure 1. Experimental identification of tolerogenic functions of various DCs using methods of antigen delivery and genetic mouse models lacking specific types of DCs

(A) Targeting of antigens to DC1 through either DEC205, DNGR-1/CLEC9A or Langerin results in induction of pTreg cells [52, 56, 68]. (B) Targeting of antigens to all cDCs through CD11c results in inefficient induction of pTreg cells because a majority of such DCs are DC2 and lack an ability to efficiently promote a conversion of pTreg cells [55]. (C) BATF3-dependent DC1 are required for pTreg cell induction and in *Batf3*^{-/-} mice characterized by a decreased DC1:DC2 ratio, pTreg cells are converted inefficiently despite antigen targeting through DEC205 [55]. (D) Conversion of pTreg cells depends on the DC1:DC2 ratio. The induction of pTreg cells following an antigen targeting through CD11c to all cDCs is restored in *Irf4*^{-/-} mice characterized by the increased proportion of DC1 [55]. (E) The Langerin⁺ migratory DCs within DC1 promote pTreg cell induction, and following an ablation of Langerin⁺ DCs pTreg cells are converted only inefficiently despite antigen targeting through Langerin [52]. (F) IRF8-dependent DC1 are required for pTreg cell induction in the intestines in response to oral antigens, and such pTreg cell induction is compromised in mice with the genetic *Irf8* deficiency in DCs that results in a decreased DC1:DC2 ratio [27].

Figure 2. Multiple immunomodulatory mechanisms that contribute to specific DC-induced T cell tolerance and conversion of pTreg cells

Programmed death ligand (PDL)1-PD1, B7H-ICOS, CD80/86-CTLA4/CD28, IL-2, IL-10, retinoic acid (RA), transforming growth factor (TGF) β , BTLA-HVEM, and CD5 have multiple direct or indirect roles in induction of T cell tolerance and promotion of pTreg cell differentiation including a transition of anergic T cells into pTreg cells by tolerogenic DCs [15, 55, 63, 70, 89–93, 97–103]. Such tolerogenic pathways may be possibly initiated by various specialized DCs within DC1 lineage.