The interactions of dendritic cells with antigen-specific, regulatory T cells that suppress autoimmunity

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Abstract

Dendritic cells (DCs) are important for several aspects of the development and function of CD4+ CD25+ regulatory T cells (Tregs), which are critical for maintaining peripheral tolerance and preventing autoimmunity. In cultures from human thymus, dendritic cells (DCs) conditioned with thymic stromal lymphopoietin (TSLP) mediate the production of Tregs from CD4+ CD25− thymocytes. In cultures from mouse lymphoid organs, CD86-rich DCs induce the proliferation and improved suppressive function of antigen-specific Tregs. DC-expanded, antigen-specific Tregs show greatly enhanced efficacy relative to polyclonal populations in blocking experimental autoimmunity. In several animal models including NOD diabetes, Tregs directed to one autoantigen are able to block autoimmunity induced by multiple antigens from the target organ. Distinct states of DC differentiation or maturation are likely to be important for the emerging roles of DCs in the biology of Tregs, particularly the control of autoimmunity in an antigen-dependent manner.

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1. CD4+ CD25+ regulatory cells as a critical element for peripheral T cell tolerance

To avoid autoimmune disease, self-reactive T cells that escape thymic deletion are normally kept in check by numerous peripheral tolerance mechanisms. These include mechanisms intrinsic to the cell such as anergy, deletion and unresponsiveness due to TCR downregulation. In addition, extrinsic regulatory cells can suppress the response of other T cells. A number of types of regulatory cells exist. One set is often termed “induced” with the initial example being the IL-10 producing, CD4+ Tr1 cell [1,2]. In addition, multiple injections of the glycolipid, α-galactosyl ceramide, stimulates NKT cells to down-modulate T cell responses by inducing IL-10 producing regulatory DCs [3], while tumor induced IL-13-producing NKT cells stimulate a CD11b+Gr1+ cell to produce TGFβ, which in turn induces regulatory T cells [4,5].

Another major set of extrinsic Treg develops in the thymus and expresses both CD4, the accessory molecule for recognition of MHC class II products, and CD25, the high affinity receptor for interleukin-2 (IL-2) [6,7]. Over the past 10 years, these “natural” CD4+ CD25+ suppressor T cells have been found to be important regulators of many T cell responses, particularly autoimmunity [8–10]. For example, depletion of CD25+ cells in NOD mice leads to the acceleration of autoimmune diabetes and induction of autoimmunity in other organs [11]. Recent data indicates that similar CD4+ CD25+ regulatory T cells can develop in the periphery from CD4+ CD25− T cells [12]. In this review, unless otherwise indicated, regulatory T cells (Tregs) refer to “natural” CD4+ CD25+ T cells.

The transcription factor, Foxp3, is critical for the differentiation of Tregs [13,14], and the field is now changing considerably because of the availability of effective antibodies to identify Foxp3+ T cells. The identification of humans and mice that are deficient in Foxp3 was a critical finding in the understanding of natural Tregs. In a disease called IPEX (an X-linked syndrome characterized by immune dysfunction polyendocrinopathy and enteropathy), in which the Foxp3 gene is altered, children can die at 1–2 years of life from severe insulin dependent or
type I diabetes mellitus (T1D) and other autoimmune diseases [15,16]. In some pedigrees the documented mutation would likely lead to deficiencies in the Foxp3 protein. Likewise, scurfy mice have a similar syndrome to IPEX and have a frameshift mutation in the Foxp3 gene which results in a protein lacking the DNA-binding forkhead domain [16,17]. Recent data suggest that Foxp3 is almost exclusively expressed in this CD4+CD25+ lineage of cells [13,18]. Therefore, it does appear that the development of Tregs via Foxp3 literally keeps us alive by preventing autoimmunity like T1D and inflammatory bowel disease.

In this review we will summarize some examples of the efficacy of antigen-specific, CD4+ CD25+ Tregs, including the newly recognized roles of dendritic cells (DCs) in influencing this critical limb of the immune system.

2. Selection of Tregs in the thymus

The thymus is a major site for the development of CD4+ CD25+ T cells. Developing T cells first undergo positive and negative selection based on the avidity of the T cell receptor (TCR) to self antigens expressed in the thymus. T cells must have at least a low avidity for self peptide/MHC complexes to be positively selected, and then those T cells with a high avidity for self are deleted via negative selection. It is currently thought that some positively selected CD4+ T cells with a substantial avidity for self avoid negative selection but develop into CD25+ regulatory T cells [19]. NOD mice, which develop autoimmune diabetes, are known to have defects in negative selection and thymic structure [20–24], but it is not clear how or if these defects affect selection of Tregs.

Self-specific TCRs are enriched on Tregs when compared to other CD4+ T cells. For example, in mice expressing HA as a surrogate autoantigen, many high affinity HA-specific T cells survived deletion, and were selected as Tregs [25]. However, thymocytes with a lower affinity HA-specific TCR were not selected as Tregs in these HA-expressing mice. Similarly, when DO.11 TCR transgenic mice specific for an OVA peptide were crossed to mice expressing OVA systemically, DO.11 T cells expressing OVA "self" specific TCR (identified with a mAb to the clonotypic receptor) were selected as Tregs, whereas those T cells expressing other potentially foreign-specific TCRs were selected as CD4+CD25− non-regulatory T cells [26]. Therefore, the antigen specificity of the Treg repertoire is likely to be influenced by the repertoire of self antigens that is presented in the thymus to newly developed, single CD4+ thymocytes.

Recent studies with human thymus have shown that CD4+ but CD25− thymocytes undergo extensive proliferation and differentiation into suppressive CD4+CD25+ T cells when cocultured with DCs that have been conditioned by thymic stromal lymphopoietin (TSLP), a cytokine produced in the Hassall’s corpuscles [27]. These TSLP conditioned DCs are primarily, if not exclusively located in the thymic medulla, and express the high levels of CD80 and CD86 that are known to be necessary for selection of Tregs [28]. Therefore, medullary DCs, or more specifically a subset of TSLP-conditioned more mature DCs, are likely to be important for the development of Tregs in the thymus (Fig. 1A). These recent findings also illustrate another theme of this review, which is the differentiation or maturation state of the DC significantly impacts the outcome of the DC-Treg interaction.

Fig. 1. DCs display multiple interactions with Tregs. Three separate major interactions of DCs with Tregs can be identified. First, in the thymus, a population of DCs induced by the cytokine TSLP encourage selection, expansion and differentiation of functional CD4+CD25+ Tregs from a CD4+CD25− population. Second, in the periphery, DCs expressing intermediate to high levels of CD80 or CD86 (B7) induce antigen-specific proliferation and expansion of functionally active Tregs, with IL-2 being an important growth factor. Third, Tregs may suppress effector T cell responses in part by down-regulating the ability of less mature DCs to effectively present antigen.
3. Proliferation and antigen specificity requirements of Tregs in vitro

Initial studies indicated that, in contrast to CD4+ CD25− T cells, Tregs did not proliferate in response to TCR stimulation in culture, but the Tregs could be induced to divide in the presence of anti-CD3 and exogenous IL-2 [29–31]. The observed anergic state of Tregs in vitro can in part be explained by the type of antigen presenting cell (APC) used in most studies. Generally, unfractionated spleen cells are used as a source of APCs, and we have found that this population is relatively weak at inducing the expansion of antigen-specific Tregs even with exogenous IL-2 [32–34], possibly as we will consider below, because DCs in a fresh spleen cell suspension are largely in an immature functional state [35,36].

In contrast, Tregs proliferate more extensively in vitro if presented with antigen or anti-CD3 on mature DCs, especially in the presence of exogenous IL-2 [32–34,37] (Fig. 1B). While DC-stimulated Tregs can proliferate in the absence of exogenous IL-2, it is likely that sufficient IL-2 is derived from Foxp3-negative cells which are present in small numbers in Tregs selected on the basis of CD25 expression. This proliferation does not come at the expense of function, since the resulting DC-expanded Tregs show suppressive activity that is equal to or greater than the unexpanded cells. Therefore, it is possible to use mature DCs loaded with specific antigen and IL-2 to expand antigen-specific Tregs ex vivo, and the larger numbers of cells so obtained make experimental studies more feasible.

The maturation state of the DC is again critical in these experiments. On the one hand, a mature DC is used to expand functional Tregs, and in addition, the capacity of mature DC to expand CD4+ CD25− effector cells is not suppressed by Tregs. Pašare and Medzhitov have presented evidence that TLR stimulation of DCs leads to IL-6 production, which protects the CD4+ CD25− T cell from suppression by Tregs [38]. However, when one wishes to assay the suppressive function of Tregs, the APCs that are used to present antigen to CD4+ CD25− cells contain immature DCs [39], implying that Tregs are able to suppress immature DC function (Fig. 1C).

In most publications, polyclonal Tregs were shown to suppress responses of polyclonal CD4+ CD25− T cells when both were stimulated with anti-CD3. In other experiments, CD4+ CD25− cells from a TCR transgenic mouse of one specificity were cultured with Tregs isolated from a TCR transgenic of a second specificity, and no suppression was observed if only the cognate peptide for the responder cells was added [29,40]. If the peptide for which the Tregs were specific was added (or if anti-CD3 was added), the response of CD4+ CD25− T cells to their distinct peptide was suppressed [29,40]. This showed that Tregs needed to be triggered through their TCR to suppress, although once triggered, the antigen specific Tregs suppress T cell responses to other antigens. The selection and expansion of antigen specific Tregs is important because as will be described below, antigen-specific Tregs are much more effective than polyclonal populations in blocking autoimmunity in mice.

4. Proliferation and antigen specificity requirements of Tregs in vivo

Many studies now provide evidence that Tregs also proliferate in response to IL-2 in vivo. A clear example is that mice lose Tregs when treated with neutralizing polyclonal anti-IL-2 antibody, while NOD mice treated with anti-IL-2 exhibit accelerated diabetes [11]. Increased autoimmunity takes place in mice genetically deficient for either IL-2 or the IL-2 receptor [41–43]. These mice lack Tregs, and when transferred with Tregs, autoimmunity in vivo is blocked [44–46]. Thus, IL-2 is essential for maintenance of Tregs in vivo.

The in vivo proliferative activity of Tregs was followed in mice by injecting syngenic CD62LhTregs into mice expressing a different thyl allele. A subset of the injected cells was quiescent and long-lived, whereas a second subset marked by high CD44 expression divided and expanded early, acquired an activated phenotype and then diminished in numbers. These two subsets were also observed when BrdU incorporation was used to assess Treg turnover [47]. In addition, when HA-specific Tregs were injected into mice expressing HA under the rat insulin promoter, the cells only divided in the pancreatic draining lymph node [47], which is known to contain DCs that are presenting antigens derived from islet β cells [48,49]. Similarly, OVA-specific Tregs respond in vivo only in the draining pancreatic lymph node when OVA is expressed as a self-antigen in β cells [50]. Immunization with antigen in IFA leads to proliferation of TCR transgenic, antigen-specific Tregs in skin draining lymph nodes [50,51]. In summary, antigen-specific Treg are not anergic and can proliferate in vivo, with the proliferation taking place in lymphoid organs draining the site of antigen deposition.

The observed expansion of Tregs in vivo is likely to be initiated by antigen presenting DCs. When Tregs (either freshly isolated or expanded first in vitro with DCs) are transferred into mice, the Tregs proliferate when challenged with DCs bearing the corresponding antigens [32,33]. In addition, the observed clustering of Tregs with DCs in inflamed intestine in a colitis model could be important for either the proliferation or suppressor function of Tregs, or both [52]. A recent approach to understanding the role of DCs in expanding Tregs in vivo involves the adoptive transfer of TCR transgenic CD4+ T cells along with the targeting of the corresponding antigen to DCs in situ using antibodies to the DEC-205 endocytic receptor that is expressed at high levels on DCs [53–55]. When antigen is delivered in this way to the DCs within intact lymphoid tissues, the total population of TCR transgenic T cells expands, but the fraction of CD4+ CD25+ TCR transgenic T cells seems to preferentially increase in numbers [56]. More intriguingly, when CD4+ CD25− T cells from RAG knockout mice are studied, the targeting of antigen in low doses to DCs leads to a preferential increase in CD4+ CD25+ regulatory T cells, suggesting that there is some way for DCs in vivo to induce the differentiation of Tregs from CD4+ CD25− T cells [57]. These observations suggest two themes. One is that Tregs are undergoing constant renewal in vivo either in a homeostatic manner in response to IL-2 or in response to
endogenous self antigens presented on DCs. The other is that DC-dependent pathways exist to expand antigen specific Tregs in vivo, which may be critical to the control of autoimmune disease.

5. Enhanced efficacy of disease-specific Tregs in blocking a spontaneous model of autoimmunity, diabetes in NOD mice

NOD mice develop spontaneous autoimmune diabetes that resembles human T1D in many respects [58,59]. For example, in both human and NOD mice, T and B cells that react with multiple islet beta cell proteins are pathogenic [60]. Insulitis in NOD mice develops between 5 and 9 weeks of age whereas overt diabetes, defined by the inability to normalize blood glucose due to the destruction of insulin-producing β cells, does not occur until 12–25 weeks. In NOD mice, polyclonal Tregs inhibit diabetes development [61–64]. However, large numbers of polyclonal Tregs are required to see an effect on disease, presumably because the frequency of islet-specific Tregs is small. In different systems, a minimum of (2–5) × 10^6 polyclonal Tregs, and in some cases multiple injections, have been needed to block diabetes development [62–64]. For example, NOD mice treated with TNF early in life exhibit accelerated diabetes, but several injections of NOD Tregs, if started early, substantially delay diabetes [64]. Other studies with polyclonal cells have demonstrated the importance of CD62L expression on the active NOD Tregs [62].

Recently, using islet specific, BDC2.5 TCR transgenic T cells, we have shown that islet-specific Tregs are much more potent than polyclonal Tregs at suppressing T1D in NOD mice [33]. BDC2.5 is a diabetogenic NOD CD4+ TCR transgenic line that is specific for an autoantigen expressed in the secretory granules of pancreatic β cells [65]. Although the specific recognized protein is not known, T cells from BDC2.5 mice respond to purified islets as well as a series of mimotope peptides [66]. Tregs sorted from BDC2.5 mice can be expanded 5–10-fold using NOD bone-marrow derived DCs pulsed with a BDC2.5 mimotope peptide. These expanded Tregs maintained clonotype expression and showed enhanced in vitro suppression [33]. Tregs expanded from BDC2.5 mice with these CD86-high DCs were potent inhibitors of autoimmune diabetes, including diabetes mediated by T cells containing clones for many different β cell autoantigens. This was tested by transfer of 10^7 spleen cells from diabetic NOD mice into NOD.scid mice, which results in diabetes 2–4 weeks after transfer. Mice that received small numbers (5000–50,000) of DC-expanded BDC2.5 Tregs along with the diabetogenic cells showed a marked delay in the development of diabetes or they did not develop disease at all. In contrast, polyclonal (antigen non-selected) Treg populations from NOD mice had no detectable disease-inhibiting activity at 20-fold higher doses [33]. In Fig. 2, an example of this experimental result is given. Here, 10^4 DC-expanded BDC2.5 Tregs completely prevented transfer of diabetes to NOD.scid mice, but 10^5 Tregs from NOD mice expanded with DCs and anti-CD3 gave no protection. The inability of 10-fold more polyclonal Tregs to mediate the same suppression observed with antigen-specific Tregs suggests a relevant physiologic and potentially therapeutic role for DCs in expanding CD4+ CD25+ suppression in an antigen and disease-specific manner.

DC-expanded BDC2.5 Tregs also blocked diabetes when given up to 2 weeks after the transfer of diabetic spleen cells to NOD.scid mice. At this later time point, the lymphoid organs had been repopulated, so the mice were no longer lymphopenic, and pathogenic T cells had already infiltrated the pancreas [33]. The ability of BDC2.5 Tregs to block at this pathogenic stage indicates that homeostatic expansion of the Tregs (as occurs during lymphopenia) is not necessary for diabetes suppression and that the Tregs can still suppress diabetes after insulitis has begun [33].

In a separate system, BDC2.5 Tregs were also expanded with anti-CD3 instead of DCs along with high doses of IL-2, and these expanded islet-specific Tregs were more potent at blocking ongoing diabetes relative to polyclonal NOD Tregs expanded with anti-CD3 and high doses of IL-2 [67]. Interestingly, when compared in a similar NOD.scid transfer model, the number of these anti-CD3 expanded Tregs necessary to block diabetes is much higher than the number needed using DC-expanded Tregs [67], which may be due to the lower levels of BDC2.5 TCR on Tregs expanded with anti-CD3 as compared to those expanded with DCs [33]. Nevertheless, it is important from the perspectives of both pathogenesis and therapy that Tregs expanded with either anti-CD3 or with DCs plus specific antigen are able to prevent diabetes when given late in the pathogenic process, after Treg inflammation has likely progressed for some time.

Tregs specific for pancreatic islet β cell antigens may receive their essential TCR signal from APCs that are found in the pancreatic draining lymph node and/or in the pancreatic islets themselves. In a mouse model described by Green and colleagues, diabetes develops synchronously at 10 weeks when
CD80 and TNF are expressed as transgenes in the islets. In these mice, Tregs accumulate in the pancreatic lymph node and islets, and very low numbers of Tregs isolated from the pancreatic lymph node, but not other lymph nodes, prevent diabetes development when transferred into these TNF/CD80-expressing mice [68]. Similarly, in another system, CD4+ CD25+ CD69− T cells isolated directly from the pancreas of BDC2.5 mice had potent diabetes-suppressive capacity and expressed high levels of Foxp3 and IL-10 compared to Tregs isolated from other tissues[69]. Together, these studies provide substantial evidence that islet-antigen specificity is important for the diabetes-inhibitory capacity of Tregs in the NOD model and that the number and/or potency of Tregs are enriched in the pancreatic lymph nodes and the diseased islets.

6. Antigen-specific Tregs in other autoimmune diseases

Experimental autoimmune encephalomyelitis (EAE), which is used as a mouse model for multiple sclerosis, involves immunization with CFA and different myelin antigens, including myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG) or proteolipid protein (PLP) or whole CNS homogenate. Unlike spontaneous autoimmune models, such as diabetes in NOD mice or lupus in NZBW, mice, the induction of EAE requires exogenous administration of both antigen and ligands for Tolllike receptors. EAE can also be induced by TCR transgenic T cells specific for myelin antigens as long as the transgenic T cells are comprised primarily of effector autoreactive T cells and depleted of autoreactive Tregs. In one such EAE system, disease develops in mice expressing a MBP-specific TCR transgene when on a RAG−/− background. EAE is blocked if wildtype TCR transgenic mice are studied, or if one transfers Treg populations derived from wildtype TCR transgenic mice, which also contain endogenously rearranged TCRs. When Tregs from wildtype mice were separated based on expression of the TCR, clonotype-positive Tregs (specific for MBP) showed better suppression of disease than clonotype-negative Tregs in the RAG−/− [70]. In another system, regulatory T cells induced by PLP (likely of both the Treg and Trl variety) suppressed EAE induced by PLP or CNS homogenate containing many antigens, but not EAE induced by MOG or MBP [71]. Again, Tregs of one specificity could block autoimmunity induced by multiple antigens, but when disease was induced by only one protein, Tregs specific for another protein were not effective. The authors conclude that the second result is due to an inability of the Tregs to be fully activated by their cognate antigen.

In another autoimmune model, thyroiditis develops in the PVG rat strain after irradiation and thyomectomy at 3 weeks, and is mediated by CD4+ T cells. Regulatory T cells taken from rats in which the thyroid was ablated in utero were not able to prevent thyroiditis, but still could prevent diabetes [72]. This suggests that exposure to specific self antigen from the thyroid is necessary for either thymic selection or peripheral maintenance of Tregs specific for that antigen.

Antigen dependence of Tregs was also found in elegant studies of autoimmune ovarian disease (AOD). The disease develops in female mice that have been thymectomized at day 3, which is before the time when Tregs develop in the thymus [73]. In this model, Tregs were isolated from non-thymectomized female mice (which had been exposed to ovarian antigens), and these were better at suppressing AOD than Tregs from male mice. This result indicates that exposure to ovarian autoantigen is essential for the development of disease-preventing Tregs. The enhanced ability of Tregs from females to block AOD was seen only when these transferred Tregs were given to recipients that lacked ovarian antigens early in life (mice were ovarioectomized at birth, and then 3 weeks later, the mice were given ovary grafts to function as targets for AOD) [74]. An earlier study showed if spleen cells from males were given to mice with ovarian antigens, these cells gained the capacity to suppress as well as those from female mice [75]. Similarly, tolerance to ovarian antigens in non-thymectomized female mice was observed only with continuous exposure to these antigens [76]. In contrast, Tregs from both male and female mice had an equal ability to block dacryoadenitis, which is autoimmune infiltration of the lacrimal glands that also develops in the day 3 thymectomized mice [74]. These results indicate that antigen exposure in the periphery is able to generate effective antigen-specific tolerance by Tregs.

Together, as summarized in Table 1, the data from mouse models for diabetes and other autoimmune diseases show the importance of antigen specificity for the function of Tregs in blocking organ-specific autoimmunity, and also the role of antigen in maintaining these cells in the periphery. In many of these models, Tregs of one specificity can block autoimmunity mediated by T cells specific for many autoantigens (see Table 1). This could be because Tregs block the function of APCs presenting both the antigen for which the Treg is specific as well as other autoantigens (Fig. 1C). Additional experiments will be required to determine if suppression of autoimmunity is more effective using a collection of Tregs that recognize a spectrum of autoantigens in the diseased organ.

7. The effect of DCs in different states of maturation for intrinsic peripheral tolerance

The importance of DCs for eliciting T cell immunity by efficiently immunizing naïve T cells has been recognized for some
time. More recently, DCs also have been found to be important for tolerance induction [77]. The decision between T cell immunity and tolerance is decided in part by the activation state of the DC. In the first experiments, antigen was selectively delivered to DCs within intact lymphoid tissues by incorporating the antigen into a monoclonal antibody to an endocytosis receptor on DCs, DEC-205 or CD205. This delivery greatly increases the efficiency with antigens are captured and presented by DCs, and focuses the presentation in DCs, or in this case, the DEC-high, CD8α high subset. If antigens were presented by DCs to T cells in the steady state, tolerance resulted [53–55]. This tolerance was preceded by early proliferation, followed by either deletion or anergy of the antigen-specific T cells. In contrast, if the antigen was presented in the context of inflammatory signals such as anti-CD40, which activate or mature the DC, the result was immunity [53,54,78]. In a parallel set of experiments, uptake and processing of dying cells was shown to be efficient in the CD8α DC subset [79]. This led to tolerance of T cells specific for antigens in the apoptotic cells, or immunity if the DCs were induced to mature [80].

These results were extended significantly by Probst et al. in an analysis of tolerance to a surrogate self antigen expressed selectively in DCs using an estrogen responsive CD11c promoter. When naïve mice (rather than adoptively transferred TCR transgenic T cells) were given tamoxifen to induce expression of the antigen, CD8α T cell tolerance developed [81]. Importantly, this required ligation of PD-1 and to a lesser extent CTLA-4 on the T cells [82]. Thus, peripheral tolerance induced by DCs requires “costimulation” and is not simply a result of presentation of antigen or signal one in the absence of signal two, and the interaction with a steady-state or immature DC can lead to intrinsic tolerance of T cells.

8. The effect of DCs in different states of maturation on CD4+ CD25+ Tregs

While immature DCs within lymphoid tissues are able to induce an intrinsic form of tolerance in the steady state, DCs in different states of differentiation or maturation also impact on the biology of Tregs. We had already mentioned that in the human thymus, it seems necessary to mature DCs with TSLP in order for the DCs to mediate expansion and differentiation of CD4+ CD25+ Tregs from CD4+ CD25— thymocytes. Likewise, with peripheral Tregs, we have found that mature DCs are much more active than immature cells in expanding Tregs in the presence of IL-2. These mature DCs developed in bone marrow cultures either “spontaneously” or in larger numbers with the addition of LPS, and were enriched by selection for high CD86 expression [32,33].

These observations are attributed to the fact that the proliferative response of Tregs is more dependent on CD80/86 expression than CD4+ CD25— T cells. The antigen-dependent proliferation induced by bone marrow DCs from mice deficient for both CD80 and CD86 was blocked to a greater extent when CD4+ CD25+ cells were the responders than when CD4+ CD25— cells were tested [32]. Likewise in vivo, CD80/86 knockout mice are still capable of mounting an autoimmune effector response, but these mice have a deficiency in Tregs and this results in development of rapid autoimmunity on the susceptible NOD background [61]. Signaling through CD28 is again required for selection of Tregs in the thymus [61], and has been replicated in recent experiments on the capacity of TSLP conditioned DCs to expand and differentiate human CD4+ CD25+ Tregs from CD4+ CD25— thymocytes [27]. Lastly, CD80/86–CD28 interactions are important for both survival and proliferation of Tregs in the periphery [83].

We suspect that this pathway, i.e., the expansion of peripheral Tregs by mature CD86-rich DCs, functions to block autoimmunity during most immune responses to microbial antigens. During infection, DCs are presenting a mixture of antigens derived from the microbe as well as self tissues and the environment. The Treg repertoire is likely to be dominated by cells specific for these “harmless” self antigens, and these Tregs undergo expansion at the same time as effectors are developing to microbial antigens. The expansion of Tregs may contribute to the regulation of autoimmunity and chronic inflammation. As suggested above and summarized in Fig. 1C, Tregs may be able to suppress DCs that are presenting other antigens, including foreign antigens. Indeed, when antigen-pulsed mature DCs were used to immunize mice, a greater Th1 response was observed when Tregs were depleted [84]. Tregs are also induced during Leishmania major infection and are important for down-modulation of the Leishmania-specific immune response [85]. These Tregs could be induced either by proliferation or by differentiation from CD25— cells. Similar induction of antigen-specific Tregs have been observed in other infectious models and may be due to cross-reactivity between self and microbial antigens (reviewed in [86]).

In addition to the expansion of Tregs by mature DCs during infection in vivo, there may be DCs in a distinct or an intermediate maturation state that can be used to preferentially expand Tregs in the steady state. These DCs may have intermediate levels of CD80 and CD86 but lack other functions of mature DCs, such as the production of immune enhancing cytokines, e.g., IL-12 and type I interferons. Another marker, CD103, was recently shown to identify DCs that are important for maintaining a proper balance of Tregs to effector cells and avoiding autoimmune colitis; CD103-deficient mice were unable to mediate suppression of colitis by wildtype regulatory T cells, suggesting that CD103+ DCs in the recipient were necessary for suppression [87]. The concept of an alternatively activated DC, one that displays some but not all of the features of a classical LPS-matured APC, playing a role in peripheral tolerance has been described previously [88,89]. Many of these studies have been carried out on DCs that are then injected into mice. The studies from von Boehmer’s lab indicate that there are DCs within lymphoid tissues that are able to expand CD4+ CD25+ Tregs from CD4+ CD25— precursors (using TCR transgenic T cells and antigen selectively targeted repeatedly and in small amounts to DCs) [57]. It will be important to determine the in vivo populations that correspond functionally to the “regulatory” DCs in these different systems.

Another potentially important type of DCs for Treg biology are DCs that are stimulated to produce TGFβ. These DCs
may preferentially encourage either the differentiation and/or expansion of Tregs but not effector T cells. Tumor-bearing mice contain an increase in an immature DC subset that is CD11c+CD11b+ and CD80/86 low or negative, secretes TGFβ, and promotes Treg proliferation in vivo [90]. This suggests that tumors provide a stimulus that matures the DCs along an alternative pathway, which in turn encourages Treg-induction. Once the nature of this stimulus that induces TGFβ producing DCs is understood, it may be possible to mimic that stimulus to induce an increase of antigen specific Tregs in the context of autoimmunity.

9. Defects in Tregs and DCs in autoimmune disease

There is evidence for defects in Tregs in many autoimmune diseases. In both multiple sclerosis and psoriasis patients, although there seem to be normal numbers of Tregs in peripheral blood, these cells display less suppressive activity than Tregs from controls [91]. In Myasthenia Gravis, there are also normal numbers of Tregs in the thymus, but these cells have lower expression of Foxp3 and lower suppressive activity [92]. Also, patients with rheumatoid arthritis have Tregs with compromised function that can be restored with anti-TNF treatment [93]. In mice, depletion of Tregs results in autoimmunity [94]. Tregs isolated from NOD mice at later stages of diabetes pathogenesis are less able to regulate than those isolated from younger mice, and this is associated with a decrease in Foxp3+ TGFβ+ cells among the CD4+ CD25+ CD62L+ population in the pancreas and pancreatic lymph nodes of older NOD females [63,95].

Alterations in DC function have also been noted in NOD mice. DCs isolated from NOD mice have a less-mature phenotype than control strains such a C57BL/6 [96–98]. After LPS treatment, a smaller percentage of NOD bone marrow derived DCs are high for CD80 or CD86 [33]. The correlation of immature DCs with autoimmunity seems paradoxical with a role for DCs in steady-state tolerance. However, less mature DCs would have lower levels of costimulation that are important for extrinsic tolerance, i.e., Treg homeostasis. Consistent with this finding is the fact that many immune-stimulatory treatments, including CFA, BCG, polyIC, TNF and LPS actually prevent diabetes development if given at the right stage in the disease development (around the time of initiation of insulitis), and this prevention may be mediated by a regulatory T cell population [99–103].

Similar to the decrease in autoimmunity after immune stimulation in mice, autoimmune disease incidence in humans is increased in populations with a lower incidence of infection, sometimes called the “hygiene hypothesis” [104]. Specifically, type 1 diabetes incidence is lower in individuals who attended day care in the first year of life (where, presumably the infants were exposed to more infections) [105]. Therefore, it is possible that NOD mice, and genetically predisposed individuals in certain “hygienic” environments, cannot maintain sufficient numbers of Tregs to prevent autoimmunity because the level of co-stimulatory molecules that the DCs express is insufficient.

10. Future applications: using DCs to induce antigen-specific Tregs

Now that it is evident that antigen-specific Tregs are more effective in blocking autoimmunity and that DCs are able to expand Tregs in an antigen-specific manner, it seems timely to extend these findings and try to design therapies that should be able to specifically dampen autoreactivity but not general immunity. A critical next step in our view, both to understand Treg biology and to design human therapies, is to expand antigen-specific Tregs from the polyclonal repertoire. Masteller and colleagues recently used complexes of an MHC class II molecule and a diabetogenic peptide to preferentially expand β cell reactive Tregs from the polyclonal repertoire of NOD mice [106]. In the experiment they described, these Tregs were able to prevent diabetes in half of the CD28−/− mice when given a few weeks before diabetes develops. The future will see more studies that test if DCs can be used to expand antigen-specific Tregs from polyclonal populations and then determine if autoimmunity can be prevented and even treated. A parallel example involves the use of Tregs to suppress transplantation immunity, both graft versus host and host versus graft disease. In this light, we have observed that DCs and be used to expand alloreactive Tregs in vitro, and that these alloreactive Tregs have greatly enhanced capacity to specifically suppress the mixed leukocyte reaction in vitro and graft versus host disease in vivo [34]. Currently, little is known about the function of antigen-specific human Tregs compared to polyclonal populations, or about the ability to expand functional Tregs from autoimmune individuals.

One limitation of designing immunotherapies targeted at one peptide is picking a relevant peptide. Even in the case of human autoimmunity where different susceptible HLA alleles have been identified, the set of peptides potentially necessary for tolerance induction could be large. An advantage of using DCs as APCs for Tregs is that they are efficient in the presentation of antigens from cellular sources [79,80,107]. In mice, injection of apoptotic cells leads to tolerance mediated by DCs that have engulfed the apoptotic cells. Ex vivo DCs could be used to process islets or β cells. In this way one could expand disease-specific Tregs that are appropriate for an individual’s HLA molecules and to multiple β cell antigens.

Another possibility is to target specific autoantigens selectively to DCs in vivo, or particularly DC subsets and maturation states. Targeting antigens to DCs via a DEC-205 antibody in the absence of immune stimulatory signals leads to immune tolerance via anergy or deletion [53–55] while in other systems it is possible to expand Tregs [37]. It also may be possible to enrich tolerance over immunity via the specific DC subset or developmental state that is targeted, e.g., a TGFβ producing DC [90].

11. Summary

DCs and Tregs are known to interact in several important ways (Fig. 1). In the thymus, TSLP-induced DCs enhance the development of Tregs from CD4+CD25− thymocytes. In vitro studies of peripheral T cells show that mature DCs induce
antigen-specific proliferation of functionally active Tregs. Likewise, Tregs respond to DCs and specific antigen within peripheral lymphoid organs in vivo. These antigen-specific Tregs can block ongoing autoimmunity in many systems, including NOD diabetes, EAE and AOD (Table 1), and in the case of spontaneous autoimmune diabetes in NOD mice, antigen-specific Tregs are much more potent than polyclonal populations in suppressing disease. Defects in both DCs and Tregs have been described in human autoimmunity, illustrating the importance of these cell types for maintaining tolerance. In addition to the previously-described role of immature DCs in steady-state intrinsic tolerance, mature DCs expressing costimulatory molecules CD80/86 are likely important for induction of extrinsic tolerance, i.e., self-specific Tregs, during an inflammatory response. Separately, DCs in an intermediate maturation state may be important for the selective induction and/or expansion of Tregs in vivo relative to effector T cells. Once these processes are better understood, the knowledge can be used to design therapies to induce tolerance in autoimmune patients.

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