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Dendritic Cells and $CD4^+CD25^+$ T Regulatory Cells: Crosstalk Between Two Professionals in Immunity versus Tolerance

WanJun Chen

Mucosal Immunology Unit, Oral Infection and Immunity Branch, National Institute of Dental and Craniofacial Research, National Institutes of Heath, 30, Convent Dr. Bethesda, MD 20892

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1. ABSTRACT

Dendritic cells (DCs) are professional antigen presenting cells (APCs). $CD4^+CD25^+$ T regulatory cells (T regs) are recognized as professional regulatory cells. DCs not only initiate T cell immunity by uptake, processing and presentation of specific antigens, but also induce immune tolerance by deletion of T cells and/or induction of regulatory T cells. $CD4^+CD25^+$ T regs maintain immune tolerance by suppressing the function of $CD4^+$ and $CD8^+$ T cells, B cells, macrophages, DCs and NK cells. It would be inconceivable that the delicate balance between immunity and tolerance could be kept impeccable without the crosstalk between DCs and $CD4^+CD25^+$ T regs. This review focuses on the recent development in our understanding of DCs and $CD4^+CD25^+$ T regs in immune tolerance, with transforming growth factor-beta (TGF-β) serving as a potential link between these two professionals.

2. DCs

DCs are a sparsely distributed, migratory population of bone-marrow-derived leukocytes that are specialized in the recognition, uptake, transport, processing and presentation of antigens to T cells (1-3). Firstly discovered as the most important APCs in initiating innate and adaptive immunity to infections and other non-self antigens, DCs have been recently recognized as also a crucial player (determinant) in induction of T cell tolerance. To reconcile the two paradoxical functions of DCs, a number of mechanisms have been introduced. In this regard, it has been demonstrated that DCs, at an "immature" stage of development in the 'steady state' (absence of inflammation), act as sentinels in peripheral tissues, continuously sampling antigenic environment and inducing tolerance by the deletion of naïve peripheral T cells and the induction of regulatory T cells (2, 3). When encountered with infection, inflammation and other

"danger" signals, "immature" DCs migrate to the draining lymph nodes and become "mature" DCs marked by up-regulation of several surface molecules such as CD11c, CD80, CD86, MHC class I and II, CD40 and by acquisition of a unique marker CD83 to initiate T cell immune responses (2, 3).

Immature DCs can reside in the non-lymphoid and lymphoid tissues. DCs in the thymus, Langerhans cells in skin, mucosal DCs in Peyer's patch (PP) in intestine (4) and in lungs and DCs in spleen are examples of immature DCs. Thymic DCs reside almost exclusively in the medulla. One of the most prominent functions of thymic DCs is to delete self-reactive specific T cells (3). In periphery, immature DCs may function as immunoregulatory cells. Immature DCs are able to delete self-antigen or harmless-antigen specific T cells when targeted with the respective antigens under the steady state. In addition, immature DCs possess the ability to capture and digest apoptotic cells and present the antigen of the apoptotic cells to specific T cells, which may result in T cell tolerance. It is however unclear whether immature DCs may also produce immunosuppressive cytokines TGF-β and IL-10 upon the phagocytosis of apoptotic cells as macrophages do (5, 6). Finally, immature DCs may induce IL-10 producing T regulatory (Tr1) cells (7) and $CD4^+CD25^+$ T regs (8), which may actively suppress immune responses.

When encountered with microbial antigens and inflammation, immature DCs will change their phenotype and function to become "mature DCs". During maturation, DCs dampen their endocytic receptor expression and lose their endocytosis capacity(2). Many inflammatory and non-inflammatory antigens induce immature DCs to mature. A number of microbial products may activate DCs through the Toll-like receptors (TLRs) (2). Some proinflammatory stimuli can also drive DCs to mature through pathways distinct from TLRs. These include several TNF family members like TNFα itself, FasL, and CD40L, Fc receptors for immune complexes, IFN-α produced by plasmacytoid DCs (9), certain types of necrotic cells and certain innate cell types such as NK cells, γδT cells, and NKT cells (2). The original hallmark of DC function involves the induction of T cell proliferation and stimulation of T cell-dependent antibody production (2). DCs also control the differentiation of Th1 and Th2 cells. Recent evidence has also demonstrated that mature DCs can expand $CD4^+CD25^+$ Tregs *in vitro* and *in vivo* to control T cell tolerance (8, 10).

3. $CD4^+CD25^+$ T REGS

$CD4^+CD25^+$ T regs constitute 5-10% of $CD4^+$ T cells in peripheral lymphoid tissues and in the thymus in normal mice (11). Phenotypically, $CD4^+CD25^+$ T regs express surface $CD25^+$, $CD62L^+$, $CD45RB^{-/low}$, $GITR^{+hi}$, and $LAG\text{-}3^{+hi}$ and $LAP/TGF\text{-}\beta^+$ and intracellular $CTLA\text{-}4^+$. However, these markers are not inherently specific for $CD4^+CD25^+$ T regs, because $CD4^+CD25^-$ T cells, once stimulated by TCR engagement, also express these molecules. The most specific marker so far for $CD4^+CD25^+$ T regs is Foxp3, because only T regs express this gene (12-14).

Functionally, $CD4^+CD25^+$ T regs, in contrast to $CD4^+CD25^-$ "responder" T cells, show unresponsiveness (anergy) to TCR stimulation in the presence of normal splenic APCs in cultures. $CD4^+CD25^+$ T regs produce no IL-2 (15). Significantly, when co-cultured with normal $CD4^+$ responder T cells or $CD8^+$ T cells, $CD4^+CD25^+$ T regs suppress TCR mediated T cell proliferation of the responder T cells by inhibiting their IL-2 production (16). $CD4^+CD25^+$ T regs can also regulate the function of DCs and other APCs (discussed later). Intriguingly, the immunosuppression mediated by $CD4^+CD25^+$ T regs is antigen non-specific and even occurs among different species. For example, $CD4^+CD25^+$ T regs from mice may suppress rat $CD4^+$ T cell proliferation (17). TCR stimulation is required for $CD4^+CD25^+$ T regs to carry out their immunosuppression. It has been shown that high levels of exogenous IL-2 in cultures not only reverse the anergic state, but also abrogate the suppressive activity of $CD4^+CD25^+$ T regs (18). Amazingly, $CD4^+CD25^+$ T regs preserve and even enhance their potent immunosuppressive ability *in vitro*, once exogenous IL-2 is removed from the cultures. Importantly, cell-contact is required for $CD4^+CD25^+$ T regs to suppress their target cells *in vitro* (18).

In vivo, however, $CD4^+CD25^+$ T regs could vigorously proliferate to their specific antigen(19, 20). Another feature of $CD4^+CD25^+$ T regs *in vivo* is that they always express high levels of CD25. In spite of their own proliferation, $CD4^+CD25^+$ T regs potently inhibit normal $CD4^+$ and $CD8^+$ T cell expansion *in vivo*. Consistently, depletion of $CD4^+CD25^+$ T regs in normal mice either by anti-CD25 antibodies or by thymectomy at day 3 of neonatal life resulted in several types of autoimmune-like inflammation in multiple organs (21). On the other hand, adoptive transfer of $CD4^+CD25^+$ T regs prevents and suppresses diseases in animal models of autoimmune diseases, inflammation and transplantation (22).

$CD4^+CD25^+$ T regs in human possess most of the basic features of their counterpart in mice (23, 24). Human $CD4^+CD25^+$ (particularly $CD25^{+hi}$) T regs also specifically express Foxp3 and are immunosuppressive to normal $CD4^+$ responder T cells when co-cultured(25). The indispensable role of $CD4^+CD25^+$ T regs in maintaining immune tolerance in human has also been gradually recognized. $CD4^+CD25^{+hi}$ T regs play a critical role in transplantation (26). In patients with multiple sclerosis (MS) (27), diabetes (28), SLE (29) or IBD (30, 31), decrease in numbers and/or defect in suppressive activity of $CD4^+CD25^+$ T regs have been observed, although the definite association between the change of T regs and the pathogenesis of the diseases remains to be established. $CD4^+CD25^+$ T regs are also involved in tumor pathogenesis through their regulatory effects on anti-tumor immunity. Many human cancers have some degree of alteration (normally increase) in the number of $CD4^+CD25^+$ T regs in peripheral blood as well as within the solid tumor (32, 33). Although the intratumor $CD4^+CD25^+$ T regs secrete high levels of TGF-β, the underlying mechanisms responsible for the immunosuppression of $CD4^+CD25^+$ T regs remain to be elucidated (33). Recent evidence has started to reveal that

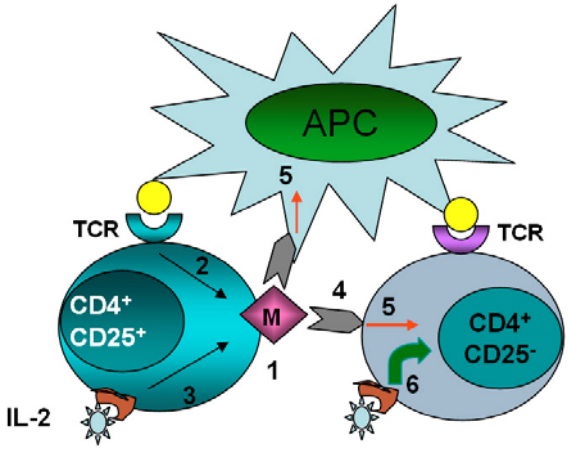

Figure 1. The requirements for candidate molecule (M) mediating immunosuppression of $CD4^+CD25^+$ T regs. 1) M must be on the surface of T regs; 2) After TCR stimulation, M should be enhanced, induced, prevented from degradation or activated to its active form; 3) In the presence of high levels of exogenous IL-2 in cultures, M still expresses or even increases on T regs. Once IL-2 is removed, M should still persist on T regs; 4) The target cell should possess constitutive or inducible receptor for M (M-R); 5) M binds to M-R to deliver a negative (inhibitory) signal to responder cells, which is antigen-nonspecific; 6) High levels of IL-2 could reverse or abrogate suppression of T reg.

$CD4^+CD25^+$ T regs also play a significant role in the pathogenesis of HIV infection. It has been reported that patients with HIV infection have increased or decreased the number of $CD4^+CD25^+$ T regs, which may influence immune responses (34, 35). It is however unclear how $CD4^+CD25^+$ T regs associated with HIV infection are generated and regulated and it is premature to state whether $CD4^+CD25^+$ T regs play a "good" or "bad" or even "ugly" role in HIV infection. Although $CD4^+CD25^+$ T regs have been unanimously recognized as one of the major players in maintaining normal immune tolerance and in influencing pathogenesis of various diseases, two mysteries of T regs however still remain largely unresolved, i.e. mechanism(s) of suppression and pathway(s) of development.

3.1. Mechanism(s) of suppression by $CD4^+CD25^+$ T regs

Despite intensive efforts during the past decade, the molecular mechanisms by which $CD4^+CD25^+$ T regs carry out their immunosuppressive activity still remain a mystery. Based on the unique features of $CD4^+CD25^+$ T regs *in vitro*, several molecules have been indicated in mediating the suppression, including CD25, IL-2, CTLA-4, IL-10, GITR, LAG-3, Granzyme B, Foxp3 and TGF-β. It is surprising however that none of the aforementioned molecules on the list has been accepted by all immunologists yet. It is possible that several molecules/factors are responsible for the suppression, but the idea that one molecule plays a direct role is valid. It would be of interest to analyze each of the proposed molecules on the list to examine which one of them fits the known features of $CD4^+CD25^+$ T regs *in vitro*.

For this purpose, it is essential to outline the minimal requirements for any candidate molecule to be the direct player in mediating suppression of T regs. If we examine the *in vitro* features of $CD4^+CD25^+$ T regs carefully, it would not be difficult to summarize that the ideal molecule must meet at least the following pre-conditions. For easy reading, I mark this potential molecule as M (Figure 1). 1) M has to be on the surface of T regs; 2) After TCR stimulation, M should be enhanced, induced, prevented from degradation or activated to its bioactive form on the cell surface of T regs; 3) When high levels (>100 u/ml) of IL-2 (plus TCR stimulation) are present in cultures, M should be expressed or even increased on T regs. Importantly, once IL-2 is removed, M should still exist on T regs; 4) The target cells (e.g. $CD4^+CD25^-$ responder T cells) should possess constitutive or inducible receptor(s) for molecule M (M-R); 5) Most importantly, the signals delivered from M on T regs to its receptor (M-R) on target cells must be negative, inhibitory and antigen non-specific; 6) Finally, in a co-culture system, high levels of IL-2 could reverse (abrogate) the suppressive signals delivered from M to M-R.

Based on aforementioned pre-conditions, most of the molecules on the list except TGF-β are unlikely the one that directly mediates the immunosuppression by $CD4^+CD25^+$ T regs, because they may meet some of the requirements, but fail to fulfill all preconditions. Although T regs express CD25, there is no evidence that CD25 on T regs could bind surface IL-2 (if any) on target cells to deliver an inhibitory signal. IL-2 is required for the homeostasis and survival of $CD4^+CD25^+$ T regs, but it is unlikely that IL-2 binds CD25 or its other receptors on responder T cells to deliver an inhibitory signal to suppress T cell proliferation. The opposite effect is true. Although Granzyme B mediated cell death might be responsible for some suppressive effects induced by T regs to their target cells (36, 37), it is unlikely the major factor, because ample evidence has clearly shown that most of the suppressed cells still remain alive *in vitro and in vivo* (19, 38). Although LAG-3 is expressed predominantly on $CD4^+CD25^+$ T regs (39), there is no evidence that LAG-3 on T regs and its receptor (ligand?) on responder cells could deliver a suppressive signal. Foxp3 is the most specific marker and the master gene for the development of $CD4^+CD25^+$ T regs, but the role of Foxp3 as a mediator for the suppression of T regs seems unlikely. Foxp3 is a transcription factor in the nucleus (12) and can not be expressed on the surface of T regs. The best scenario (if any) would be that Foxp3 interacts with other suppressive molecules that in turn mediate the suppression by T regs.

Although $CD4^+CD25^+$ T regs may produce higher levels of IL-10, the cell-contact requirement precludes the soluble factor as a main mechanism, and indeed, anti-IL-10 or IL-10 receptor antibodies fail to block immunosuppression *in vitro*. There is no evidence that $CD4^+CD25^+$ T regs have cell membrane-bound IL-10 and that exogenous IL-2 could abrogate IL-10 mediated immunosuppression. Even though IL-10 is not essential for suppression *in vitro*, the involvement of IL-10 in immunosuppression *in vivo* merits careful consideration

(50). It has been established that certain forms of immunity such as colitis can be suppressed by $CD4^+CD25^+$ T cells and require their secretion of IL-10 (31), whereas others such as autoimmune gastritis can be suppressed independently of IL-10 (18). The challenging question is where and how IL-10 is responsible for suppression *in vivo*.

GITR was once thought the most promising candidate in responsible for suppression induced by T regs (17, 40), because anti-GITR antibody, by initiating a signal to $CD4^+CD25^+$ T regs, led to proliferation of T regs and abrogated their suppression to $CD4^+$ responder T cells in the co-culture systems. Recent evidence however has demonstrated that GITR acts mainly on responder T cells (e.g. $CD4^+CD25^-$ T cells) through co-stimulatory effect to produce more IL-2, which then in turn antagonizes the suppressive activity of $CD4^+CD25^+$ T regs (41, 42).

Although accumulated evidence has indicated a role for CTLA-4 in immunosuppression induced by T regs (43-45), the question is whether CTLA-4 is the molecule directly responsible for the suppression or it contributes to the suppression indirectly. The emerging evidence favors the later possibility. Although $CD4^+CD25^+$ T regs express CTLA-4 and CTLA-4 fulfills most of the aforementioned requirements, one fact may exclude CTLA-4 as the direct mediator for the suppression. CTLA-4 binds CD80 and CD86. Although activated $CD4^+$ responder T cells may express CD80 and CD86 to which CTLA-4 on T regs may bind as an 'outside-in' signaling model (46, 47), $CD4^+CD25^+$ T regs in the CTLA-4-/- mice exhibit same suppressive capacity as those in the wild type mice. Thus, it is likely that CTLA-4 is associated with the suppressive mechanisms of T regs by an indirect manner.

Is TGF-β the best candidate in mediating immunosuppression by $CD4^+CD25^+$ T regs? Since both $CD4^+CD25^+$ T regs and TGF-β are indispensable for immune tolerance, it is not surprising that the two are linked. The role for TGF-β in immunosuppression by $CD4^+CD25^+$ T regs was however originally disregarded based on the fact that T regs needed cell-contact, but TGF-β was normally regarded as a soluble cytokine. The discovery that $CD4^+CD25^+$ T regs express cell membrane-bound latent TGF-β (LAP-TGF-β), active TGF-β has re-invigorated investigation in this field (45, 48, 49). When TGF-β is placed into the aforementioned pre-conditions, it is intriguing to note that TGF-β fulfills all the requirements. First, TGF-β is expressed on the cell surface of $CD4^+CD25^+$ T regs. In addition, T regs also express higher levels of TGF-β receptor II (TβRII), a binding receptor for TGF-β, which may explain the anergy of T regs to TCR stimulation, because the binding between autocrine or paracrine membrane-bound TGF-β and TβRII on T regs may inhibit TCR induced proliferation (45). Second, when $CD4^+CD25^+$ T regs are activated via TCR stimulation and in the presence of IL-2, the expression of cell membrane-bound TGF-β on T regs persists and even increases (45, 48-50). Importantly, once exogenous IL-2 is removed, T regs still express high levels of cell membrane-bound TGF-β (45, 49, 50). Third, naïve $CD4^+CD25^-$ T cells express very low levels (if any) of TβRII, but it increases significantly after TCR stimulation (45). In a co-culture system, cell membrane-bound TGF-β on T regs could bind TβRII on $CD4^+CD25^-$ responder T cells to deliver a negative signal, which can be demonstrated by upregulation of P-Smad2/3 in responder T cells (45, 50). Fourth, TGF-β suppresses its target cells in an antigen non-specific manner and even among species (51). Finally, high levels of exogenous IL-2 are able to abrogate TGF-β mediated immunosuppression of T cell activation (45, 52, 53). In addition, a critical role for TGF-β in responsible for suppression by $CD4^+CD25^+$ T regs *in vivo* has been validated. Powrie and her colleagues have found that TGF-β is absolutely required for $CD4^+CD25^+$ T reg suppression of IBD induced by infusion of $CD4^+CD45RB^{hi}$ T cells in mice (43, 54), because co-administration of anti-TGF-β antibody could completely abrogate the suppression. Moreover, TβRII dominant-negative transgenic T responder cells ($CD8^+$ and $CD4^+$) are resistant to suppression by wild type $CD4^+CD25^+$ Tregs *in vivo* (54-56).

Although TGF-β fits well aforementioned preconditions and growing evidence has gradually appreciated it as a critical mediator in suppression induced by $CD4^+CD25^+$ T regs, two pieces of unresolved puzzles still prevent TGF-β from getting universal recognition. First, it remains to be elucidated why some of anti-TGF-β antibodies cannot reverse suppression by T regs in co-cultures. Besides the different systems used in individual laboratory, it is likely that the unique structure and/or conformation of cell membrane-bound TGF-β might prevent the binding of anti-TGF-β antibodies to inactivate its activity, whereas the same antibody could effectively neutralize the activity of soluble TGF-β. The other mystery is why $CD4^+CD25^+$ T cells in TGF-β1-/- mice still possess suppressive function in cultures. Several possibilities need to be considered to resolve this issue. First, TGF-β1 is the dominant (in normal situation), but not the only isoform of TGF-β in regulating immune responses. In the absence of TGF-β1, TGF-β2 and/or 3 may compensate for the TGF-β signal transduction, because all TGF-β1, 2 and 3 bind the same receptors and execute the same signals *in vitro*, although they may not substitute each other *in vivo*. Additionally, the ability of $CD4^+CD25^+$ TGF-β1-/- T cells to effect suppression in cultures must be viewed with caution since maternal transfer of TGF-β and its passive binding to these cells cannot be excluded. It is unclear at present that the cell membrane-bound TGF-β on normal $CD4^+CD25^+$ T regs is solely produced by the T reg or it can be also passively bound onto T regs (45). Finally, it should be noted that in addition to T regs, many other cells including macrophages/monocytes, DCs, epithelial cells, myeloid suppressor cells and tumor cells also produce a large amounts of TGF-β. The TGF-β produced from non-T reg cells may explain the unexpected TGF-β-dependent immunosuppression by TGF-β1-/- $CD4^+CD25^+$ T cells *in vivo* (54).

3.2. Generation of $CD4^+CD25^+$ T reg

It is a general belief at present that "natural" $CD4^+CD25^+$ professional T regs are generated and

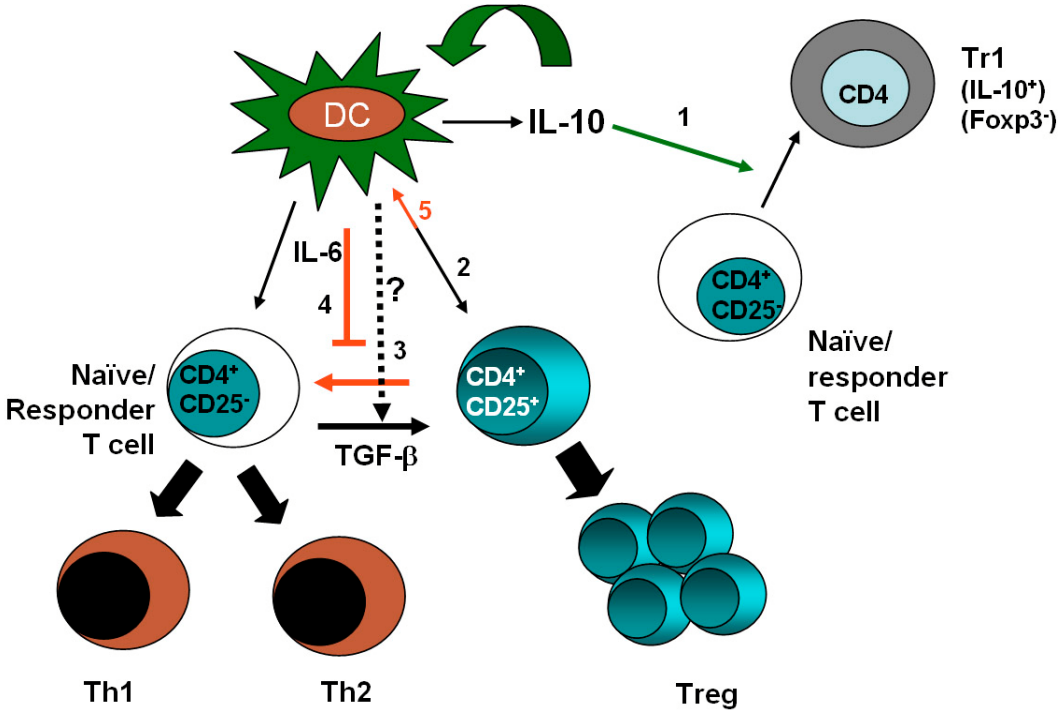

Figure 2. Interaction between DCs and T regs. 1) DCs induce IL-10 producing Tr1. IL-10 downregulates function of DCs; 2) DCs (particularly mature DCs) expend $CD4^+CD25^+$ T regs in cultures and *in vivo*; 3) Can DCs (particular immature DCs) induce/convert $CD4^+CD25^+$ T regs from $CD4^+CD25^-$ naïve T cells? 4) Mature DCs abrogate $CD4^+CD25^+$ T cell suppression of $CD4^+$ T responder cells through IL-6 and unknown factors; 5) $CD4^+CD25^+$ T regs suppress maturation and function of DCs.

developed from the thymus. Considering the importance of the thymus as an incubator (place) to generate $CD4^+CD25^+$ T regs, the detailed pathways and mechanisms by which these T regs are developed remain largely undefined. The current theory suggests that $CD4^+CD25^+$ T regs are derived from a defined lineage and survive from negative selection during a very fine "window" with strong affinity to TCR engagement. One significant question is however why $CD4^+CD25^+$ T regs express $CD25^+$, $CD45RB^{-low}$, intracellular CTLA-4^+ and $GITR^{+hi}$, all of which have been recognized as T cell activation-associated markers, whereas $CD4^+CD25^-$ T cells, which also encounter antigen and survive from negative selection in the thymus, do not express any of those activation-associated markers. Importantly, are peripheral $CD4^+CD25^+$ T regs exclusively generated in the thymus or converted from $CD4^+CD25^-$ T cells? Recent studies in mice and human have revealed that TGF-β, together with TCR stimulation, could convert peripheral $CD4^+CD25^-$ naïve T cells to $CD4^+CD25^+$ T regs through induction of T reg specific gene Foxp3 (26, 52, 57, 58). Strikingly, these TGF-β converted T regs are phenotypically and functionally indistinguishable from the natural "professional" $CD4^+CD25^+$ T regs (52). Thus, $CD4^+CD25^+$ T regs are primarily developed in thymus, but could also be converted from $CD4^+CD25^-$ naïve T cells under certain conditions. The challenging questions are cellular and molecular mechanisms for the development of Tregs in the thymus and the molecular events involved in TGF-β induction of Foxp3 expression.

4. ROLE OF DCS IN THE INDUCTION/EXPANSION OF $CD4^+CD25^+$ T REGS

One of the proposed mechanisms is that inhibitory DCs induce immune tolerance by inducuction and/or expansion of T regs. It has been shown that DCs may induce Tr1 through IL-10 (7, 59, 60) (Figure 2). If a naïve T cell encounters its antigen on immature DCs, it may differentiate into Tr1 rather than a T-effector cell (61). This can be obtained by repetitive exposure of naïve peripheral blood $CD4^+$ T cells to allogeneic immature DCs. In another report, *in vitro* culture of bone marrow cells in the presence of IL-10 induced differentiation of a distinct subset of DCs with a specific expression of CD45RB (60). These $CD11c^{low}$ $CD45RB^{high}$ DCs display plasmacytoid morphology and an immature-like phenotype and secrete high levels of IL-10 after activation. OVA peptide-pulsed $CD11c^{low}$ $CD45RB^{high}$ DCs specifically induce tolerance through the differentiation of Tr1 cells *in vitro* and *in vivo*. Interestingly, antigen-exposed DCs in which RelB function is inhibited lack cell surface CD40, prevent priming of immunity, and suppress previously primed immune responses (62). These $RelB^-CD40^-$ DCs induce Tr1 cells and transfer antigen-specific "infectious" tolerance to primed recipients in an IL-10-dependent fashion. Thus, immature DCs can drive the differentiation of Tr1 cells. Since $CD4^+CD25^+$ Tr1 cells produce IL-10, it would be conceivable that T regs contribute to this process, at least *in vivo*, but the definite correlation remains to be established.

Recent studies have indicated a role of immature DCs in enhancing $CD4^+CD25^+$ T regs, however, whether it is "induction" or "expansion" remains to be determined. By manipulating DCs with different biological reagents and immunologic means *in vitro* and *in vivo*, several groups have shown that DCs are capable of enhancing $CD4^+CD25^+$ T regs. When NOD bone marrow-derived DCs were specifically treated with a mixture of antisense oligonucleotides to down-regulate CD40, CD80, and CD86 primary transcripts *ex vivo*, the engineered DCs promoted an increased prevalence of $CD4^+CD25^+$ T cells in NOD recipients at all ages. Diabetes-free recipients exhibited significantly greater numbers of $CD4^+CD25^+$ T regs compared to untreated NOD mice (63). Human plasmacytoid DCs activated by CpG oligodeoxynucleotides also induced generation of $CD4^+CD25^+$ T regs (64). Coupling of ovalbumin (OVA) to anti-DEC-205 mAb (alpha DEC) induced proliferation of OVA-specific T cells *in vivo* (65). Expansion was however short-lived, caused by DCs and rendered T cells anergic thereafter. Phenotypic analysis revealed that induction of $CD4^+CD25^+CTLA\text{-}4^+$ T cells could suppress proliferation and IL-2 production of effector $CD4^+$ T cells. Vitamin D receptor ligand-treated DCs not only induce Tr1, but also enhance $CD4^+CD25^+$ suppressor T cells (66). It has been reported that mice treated with granulocyte macrophage-colony stimulation factor (GM-CSF) that could induce DCs with a semimature phenotype, showed an increased $CD4^+CD25^+$ T cells producing high levels of IL-10 and suppressing experimental autoimmune thyroiditis (67). Similarly, treatment with granulocyte colony-stimulating factor (G-CSF) protected NOD mice from developing spontaneous diabetes. G-CSF triggered marked recruitment of DCs, particularly immature $CD11c^{(low)}$ $B220^{(+)}$ plasmacytoid DCs, and increased accumulation of functional $CD4^+CD25^+$ T regs that produce TGF-β1 (68). Although thymic DCs almost exclusively reside in the medulla where they play a critical role in negative selection rather than induction of $CD4^+CD25^+$ T regs (19, 69), some reports correlated migration of peripheral DCs to the thymus with generation of $CD4^+CD25^+$ thymic T regs (70). Most recently, it has been shown that Hassall's corpuscles, groups of epithelial cells within thymic medulla, express thymic stromal lymphopietin (TSLP) (71). TSLP-treated human thymic DCs are able to induce proliferation and differentiation of $CD4^+CD8^-CD25^-$ thymic T cells into $CD4^+CD25^+Foxp3^+$ regulatory T cells *in vitro*. Moreover, one study has demonstrated that $CD8\alpha^{(+)}$ DCs can induce a unique population of T helper type 1-like regulatory cells that express the transcription factors Foxp3 and T-bet and produce both IL-10 and IFN-γ (72). Since peripheral $CD4^+CD25^-$ could be converted into $CD4^+CD25^+$ $Foxp3^+$ T regs, which is at least in great part dependent on TGF-β (52, 57, 58), it is of interest to study whether DCs, particularly immature DCs, may play a part in this process. Immature DCs produce TGF-β(73), but what are the stimuli inducing DCs to produce TGF-β? Resolving these issues will help validate the role of DCs in the generation of $CD4^+CD25^+$ T regs (Figure 2).

DCs can overcome anergy and induce proliferation of professional $CD4^+CD25^+$ T regs *in vitro* and *in vivo* (8, 74) (Figure 2). It has been known that $CD4^+CD25^+$ T regs are anergic, unable to proliferate in response to TCR stimulation by normal splenic APCs (16, 18), unless high levels of exogenous IL-2 or anti-CD28 antibodies are present in the cultures. Recent evidence has shown that $CD4^+CD25^+$ T regs can proliferate in the absence of exogenous cytokines in cultures and *in vivo* when stimulated by antigen-loaded mature DCs (8, 74). With high numbers of DCs in cultures, $CD4^+CD25^+$ T regs and $CD4^+CD25^-$ populations initially proliferate to a comparable extent. However, the expansion of $CD4^+$ $CD25^+$ T regs stops by day 5, in the absence or presence of exogenous IL-2, whereas $CD4^+CD25^-$ T cells continue to grow. $CD4^+$ $CD25^+$ T cell growth requires DC-T cells contact and is partially dependent on the production of small amounts of IL-2 by T regs and B7 costimulation by DCs. Interestingly, expanded $CD4^+$ $CD25^+$ T regs still preserve their surface CD25 and other featured molecules and possess even stronger suppressive activity. Intriguingly, mature DCs can expand $CD4^+$ $CD25^+$ T regs in the absence of antigen but in the presence of exogenous IL-2 (8, 74). Importantly, antigen-preloaded DCs can induce proliferation of adoptively transferred $CD4^+$ $CD25^+$ T regs *in vivo*. These exciting studies have opened a new avenue to grow $CD4^+$ $CD25^+$ T regs for potential clinical application. However, human $CD4^+$ $CD25^+$ T regs respond poorly to mature monocyte-derived DCs in the mixed leukocyte reaction (24, 75). The challenging question ahead is to understand the molecular mechanisms by which DCs abrogate the anergic state of $CD4^+$ $CD25^+$ T regs.

5. DC REGULATION OF SUPPRESSIVE ACTIVITY OF $CD4^+CD25^+$ T REGS

In addition to overcoming anergy of T regs, mature DCs may reverse the immunosuppression induced by $CD4^+CD25^+$ T regs (Figure 2). Stimulation of toll-like receptors (TLRs) such as TLR4 or TLR9 in DCs by LPS or CpG reversed the immunosuppression by $CD4^+CD25^+$ T regs, restoring responder T cell proliferation to near normal levels (74, 76). The DC-mediated inhibition of suppressor activity was independent of co-stimulation (74, 76), but was dependent in part on interleukin-6 (IL-6), which was induced by TLRs upon recognition of microbial products. These findings have provided an explanation for DCs (particularly mature DCs) to initiate immune responses rather than tolerance to foreign antigens. Several interesting questions however remain to be answered. For example, if IL-6 affects T regs, what are the signaling pathways to change the behavior of T regs and abrogate their suppressive activity? If IL-6 signals on $CD4^+CD25^-$ responder T cells, how does IL-6 influence the responder T cells to resist to suppression by T regs? What is (are) other molecule(s) that may interact with IL-6 to block the suppression by T regs (74, 76)?

6. $CD4^+CD25^+$ T CELL REGULATION OF DC FUNCTION

$CD4^+CD25^+$ T regs can regulate phenotype and function of DCs. Co-culture of mouse $CD4^+CD25^+$ T regs with mouse bone marrow-derived DCs inhibits maturation

of DCs, characterized by preventing up-regulation of several DC markers including CD11c, CD80, CD86, CD40 and MHC class II, which results in downregulation of DC-mediated T cell function (77). Although $CD4^+CD25^+$ T regs down-regulate surface expression of CD80 and CD86 molecules on DCs, this suppression is at the steady-state level for CD80 mRNA but not for CD86 mRNA *in vitro* (78). Function of mature DCs is under the control of the naturally occurring $CD4^+CD25^+$ T regs, *in vivo*. Depletion of $CD4^+CD25^+$ T regs, *in vivo,* enhanced development of MHC class I and II restricted IFN-γ producing cells and induced higher cytotoxic activity of $CD8^+$ T cells (10). By contrast, priming of T helper (Th)2 cells was downregulated at the same conditions. Development of colitis is dependent on accumulation of activated $CD134L^+$ DCs in mesenteric lymph nodes, which is inhibited by $CD4^+CD25^+$ T regs (31). In a murine model with melanoma, depletion of $CD4^+CD25^+$ T regs elicits long-lasting protective tumor immunity induced by DCs preloaded with stressed tumor cells (79). In humans, it has been shown that co-culture of $CD4^+CD25^+$ T regs with monocyte-derived DCs renders DC inefficient as APCs despite prestimulation with CD40 ligand. In contrast to co-cultured with $CD4^+CD25^-$ T cells, DCs cultured with T regs showed prevention of maturation (80). Although DCs co-cultured with T regs increased IL-10 production, the inhibitory effects of DCs can partially be reverted by neutralizing antibodies to TGF-β, but not to IL-10. The potent $CD4^+CD25^+$ T reg cells suppress the proliferation in a DC-driven allo-mixed lymphocyte reaction (MLR) culture by more than 90% (81). In the peripheral blood of patients with squamous cell carcinoma of the head and neck, the relative levels of HLA-DR expression on myeloid and total DCs positively correlated with the ratios of Th1 and Th2, and the proportion of total circulating DCs was inversely correlated with that of $CD4^+CD25^+$ T regs (82). In addition to DCs, $CD4^+CD25^+$ T regs can exert direct suppressive effects on monocytes/macrophages, thereby affecting subsequent innate/adaptive immune responses (83). Although the underlying cellular and molecular events that are associated with $CD4^+CD25^+$ T regulatory cell regulation of DCs are still largely unknown, it seems that cell-contact and immunoregulatory cytokines are involved. As another mechanism of immunosuppression, CTLA-4 on $CD4^+CD25^+$ T regs upregulate indoleamine 2,3-dioxygenase (IDO) expression that initiate tryptophan catabolism in DCs (84, 85).

7. TGF-β: A MEDIATOR OF CROSSTALK BETWEEN DCS AND $CD4^+CD25^+$ TREGS?

Since TGF-β is one of the most critical immunoregulatory cytokines in regulation of immune responses (51, 86), a role of TGF-β in mediating the cross-talk between DCs and $CD4^+CD25^+$ T regs is inevitably considered. As discussed, $CD4^+CD25^+$ T regs express cell membrane-bound as well as soluble TGF-β (45, 50) that can be responsible for the suppressive activity of T regs. It would be reasonable to envision that $CD4^+CD25^+$ T regs regulate DC function at least in part through TGF-β. TGF-β has been shown to stimulate epithelial Langerhans cell (LC) differentiation from hematopoietic progenitor cells or from a monocytic differentiation pathway (73). It is of interest to note that the effect of TGF-β is a dose dependent in serum-free culture medium. Low concentration of TGF-β (0.5 ng/ml) promotes proliferation and differentiation of $CD34^+$ progenitor to LC-like DCs, but higher concentrations suppresses LC differentiation and proliferation in the same cultures (73). This phenomena is consistent with the differential role of TGF-β in regulation of T cell proliferation (51-53). Importantly, DCs generated from human hematopoietic progenitor cells in the presence of TGF-β lack mature DC features. For example, TGF-β induced DCs fail to express CD83 ($CD83^-$) and have low levels of CD86 ($CD86^{dim}$) (73). Similar evidence was obtained in TGF-β1 mediated differentiation of DCs in cultures of murine bone marrow cells, resulting in DCs expressing $MHCII^{low}$ and $CD86^{dim}$. Effect of TGF-β on differentiation of LC cells was also confirmed *in vivo*. TGF-β1-/- mice selectively lack epidermal LC (87). In contrast to a potential enhancement of differentiation of immature DCs, TGF-β may suppress maturation and function of mature DCs (73). Interestingly, it has been recently shown that splenic stromal cell-derived TGF-β induces mature DCs to differentiate into a type of regulatory DC (88). This type of regulatory DCs strongly inhibited proliferative responses of naïve $CD4^+$ T cells to antigen stimulation by mature DCs.

On the other hand, DCs also produce TGF-β. Immunohistology studies have revealed expression of TGF-β protein in LCs (89). *In vitro*-generated DCs also abundantly synthesize TGF-β1 (73). TGF-β1 mRNA can be detected in LC-type and germinal center-type DCs generated in cultures of $CD34^+$ cord blood. Although TGF-β produced by DCs may play a role in the development of $CD4^+CD25^+$ T regs, a direct connection is still missing. An unresolved but fascinating question is that under which conditions DCs produce TGF-β. Since both immature and mature DCs can produce TGF-β, it would be more informative to analyze TGF-β quantitatively rather than qualitatively and compare it with other inflammatory cytokines and factors to determine the role of DC in inhibition or promotion of T cell responses.

8. SUMMARY

$CD4^+CD25^+$ T regs and DCs represent the two most important populations of cells in regulation of immunity and tolerance. It takes these two types of professionals to complete the task. The question is how they interact. By reinforcing our investigations on underlying molecular events and mechanisms of interaction between $CD4^+CD25^+$ T regs and DCs, and extending our studies to autoimmune diseases, chronic inflammation, transplantation, cancers, bacterial and HIV infections, it may become possible to manipulate host offense and defense.

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Send correspondence to: Dr WJ. Chen, Mucosal Immunology Unit, Oral Infection and Immunity Branch, National Institute of Dental and Craniofacial Research, N ational Institutes of Heath, 30, Convent Dr. Bethesda, MD 20892, Tel: 301-435-7168, Fax: 301-402-1064, E-mail: wchen@mail.nih.gov

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