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Review

Contextual Regulation of Inflammation: A Duet by Transforming Growth Factor-β and Interleukin-10

Ming O. Li[1,*] and Richard A. Flavell[2,3,*]
[1]Immunology Program, Memorial Sloan-Kettering Cancer Center, New York, NY 10065, USA
[2]Department of Immunobiology
[3]Howard Hughes Medical Institute
Yale University School of Medicine, New Haven, CT 06520, USA
*Correspondence: lim@mskcc.org (M.O.L.), richard.flavell@yale.edu (R.A.F.)
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Transforming growth factor-β (TGF-β) and interleukin-10 (IL-10) are regulatory cytokines with pleiotropic roles in the immune system. The prominent function of TGF-β is to maintain T cell tolerance to self or innocuous environmental antigens via its direct effects on the differentiation and homeostasis of effector and regulatory T cells. A critical route for the regulation of T cells by TGF-β is via activation of a T cell-produced latent form of TGF-β1 by dendritic cell-expressed avβ8 integrin. IL-10 operates primarily as a feedback inhibitor of exuberant T cell responses to microbial antigens. T cells are also the principal producers of IL-10, the expression of which is regulated by IL-27, IL-6, and TGF-β. The collective activity of TGF-β and IL-10 ensures a controlled inflammatory response specifically targeting pathogens without evoking excessive immunopathology to self-tissues.

Introduction

As a consequence of incomplete presentation of self-antigens in the thymus and the highly plastic nature of T cell receptor (TCR) recognition of antigens, autoreactive T cells are present in healthy individuals (Danke et al., 2004). Importantly, these autoreactive T cells are normally prevented from inducing autoimmune diseases. In addition, regulatory mechanisms are in place to prevent collateral tissue damage as a result of exuberant T cell responses to pathogens or innocuous environmental entities such as the commensal flora. How self-inflicted inflammation is controlled in the steady state and during infection is a topic of active research. Previous studies with animal models have established essential functions for the cytokines transforming growth factor-β (TGF-β) and inerleukin-10 (IL-10) in inhibiting T cell-mediated immunopathology (Li et al., 2006b; Moore et al., 2001). In this review, we will discuss recent insights gained in the understanding of the mechanisms by which TGF-β and IL-10 regulate inflammatory T cell responses.

TGF-β Directly Targets Effector T Cells and Treg Cells to Ensure Self-Tolerance

TGF-β belongs to a family of evolutionarily conserved molecules with pleiotropic roles on multiple cell types that affect diverse biological processes, including immune responses (Li et al., 2006b). Three TGF-β members (TGF-β1, TGF-β2, and TGF-β3) are present in mammals; TGF-β1 is the major form expressed in the immune system. Active TGF-β elicits its biological functions through the TGF-β type I (TGF-βRI) and type II (TGF-βRII) receptors. TGF-β engagement of the tetrameric receptor complex activates the kinase activity of the receptors that subsequently phosphorylate downstream targets and activate signaling pathways, which in some cases depend on the transcription-factor Smad proteins (Derynck and Zhang, 2003; Shi and Massague, 2003).

The pivotal functions of TGF-β in immune tolerance were originally revealed in the studies of TGF-β1-deficient mice that develop an early and fatal multi-focal inflammatory disease (Kulkarni et al., 1993; Shull et al., 1992). T cells are essential mediators of the disease because depletion of $CD4^+$ or $CD8^+$ T cells alleviates the immunopathology (Kobayashi et al., 1999; Letterio et al., 1996). The failure to rescue the inflammatory phenotype under germ-free conditions suggests that the T cell-driven inflammation is of autoimmune origin (Boivin et al., 1997). Because TGF-β1 modulates the activities of multiple lineages of leukocytes, it was not clear from these early reports whether TGF-β1 directly controls T cell tolerance. Studies with transgenic mice expressing dominant-negative mutants of TGF-βRII in T cells, and more recently with T cell-specific TGF-βRII-deficient mice, have firmly established T cells as a principal direct TGF-β1 target in vivo (Gorelik and Flavell, 2000; Li et al., 2006a; Lucas et al., 2000; Marie et al., 2006). In the absence of TGF-β signaling, T cells undergo hyperproliferation, activation, and effector T cell differentiation that result in infiltration of leukocytes into multiple vital organs and a neonatal lethal phenotype as severe as that of TGF-β1-deficient mice (Li et al., 2006a; Marie et al., 2006). These observations demonstrate that TGF-β signaling in T cells is indispensable for restraining relentless self-directed T cell responses in peripheral tissues.

Another essential pathway of peripheral T cell tolerance is mediated by $CD4^+$ regulatory T (Treg) cells that express the transcription factor Foxp3 (Sakaguchi, 2004; Zheng and Rudensky, 2007). In the absence of TGF-β signaling in T cells, thymic Treg cell numbers are not reduced (Li et al., 2006a; Marie et al., 2006). However, Treg cells progressively diminish in the peripheral lymphoid organs, notably in the spleens of these mice (Li et al., 2006a; Marie et al., 2006). These findings are in line with an early report using TGFβ1-deficient mice (Marie et al., 2005). Foxp3 controls the genetic program critical for the suppressive

functions of Treg cells. Deficiency of Foxp3 in mice results in T cell-dependent inflammatory phenotype similar in severity to that of T cell-specific TGF-βRII-deficient mice (Brunkow et al., 2001; Fontenot et al., 2003). Therefore, it was an open question whether the loss of T cell tolerance in TGF-βRII-deficient mice was caused by reduced Treg cells or was due to the lack of cell-intrinsic control of effector T cell activation by TGF-β. On the other hand, failed maintenance of TGF-βRII-deficient Treg cells might be caused by environmental changes such as the inflammatory disorder developed in these mice, or it might be a direct consequence of a lack of TGF-β signaling in Treg cells. These questions were addressed in a series of transfer and bone-marrow chimera experiments. Transfer of wild-type Treg cells to neonatal TGF-βRII-deficient mice restored Treg cell numbers to those found in wild-type mice but was unable to correct T cell activation and autoimmune diseases (Li et al., 2006a). Thus, the depletion of Treg cells does not solely account for the loss of T cell tolerance in these mice. These findings are consistent with earlier reports showing that effector T cells expressing a dominant-negative mutant of TGF-βRII cannot be suppressed by Treg cells in colitis, diabetes, or tumor models (Chen et al., 2005; Fahlen et al., 2005; Green et al., 2003; Mempel et al., 2006). The functions of TGF-β signaling in the control of effector T cells and Treg cells have been further investigated in mixed bone-marrow chimera experiments. By creating chimeric mice that harbor both wild-type and TGF-βRII-deficient T cells, T cell-intrinsic effects can be differentiated from T cell-extrinsic environmental causes. Interestingly, the T cell compartments of TGF-βRII-deficient mice exhibit a more activated phenotype and contain lower Treg cell numbers than the wild-type T cell populations in the same chimera (Li et al., 2006a; Marie et al., 2006). These findings have therefore established cell-intrinsic roles for TGF-β signaling in the control of both effector T cells and Treg cells (Figure 1A), which might collectively contribute to the maintenance of T cell tolerance.

TGF-β Regulation of Induced Treg Cell and Th17 Cell Differentiation

In addition to the thymus-produced naturally occurring Treg (nTreg) cells, naive T cells can acquire Foxp3 expression and differentiate into the induced Treg (iTreg) cells in peripheral tissues. TGF-β induces Foxp3 expression and iTreg cell differentiation from $CD4^+CD25^-$ or $CD4^+Foxp3^-$ T cells in vitro (Chen et al., 2003; Fantini et al., 2004; Wan and Flavell, 2005; Zheng et al., 2002). Optimal induction of Foxp3 expression and expansion of TGF-β-induced iTreg cells is dependent on T cell-produced cytokine IL-2 (Davidson et al., 2007; Zheng et al., 2007), which also promotes the differentiation of thymic nTreg cells. Interestingly, overexpression of TGF-β1 in the islets of the pancreas expands Treg cells that protect NOD mice from diabetes (Peng et al., 2004). These observations suggest that when TGF-β is present in excess, it induces the generation and/or maintenance of Treg cells. Endogenous TGF-β might also be involved in the generation of iTreg cells in periphery tissues. Delivery of low-dose agonistic peptide to dendritic cells induces iTreg cell differentiation from antigen-specific naive T cells (Kretschmer et al., 2005). The efficiency of iTreg cell induction is inversely correlated with cell proliferation and is inhibited when T cells are stimulated in the presence of costimulatory signals or with high-dose antigens. Although hyperproliferation is inhibitory for the induction of iTreg cells, converted iTreg cells maintain a stable phenotype and can be expanded upon immunization with antigens in the presence of adjuvant in this model (Kretschmer et al., 2005). Inhibition of TGF-β signaling in T cells via the expression of a dominant-negative mutant of TGF-βRII suppresses iTreg cell generation, which is associated with the enhanced T cell proliferation (Kretschmer et al., 2005). It is an open question whether TGF-β inhibition of T cell proliferation provides the mechanism for its promotion of iTreg cell differentiation in this model. To what extent iTreg cells produced via this pathway contribute to the peripheral Treg cell pool also remains to be determined.

Recent studies have revealed that the gut-associated lymphoid tissue (GALT) might be a particularly important site for the generation of iTreg cells. Transfer of polyclonal $CD4^+Foxp3^-$ T cells to the congenic recipient mice results in the conversion of a subset of these T cells to iTreg cells that accumulate in the GALT (Sun et al., 2007). Administration of oral antigens also induces iTreg cell differentiation from naive $CD4^+Foxp3^-$ transgenic T cells (Coombes et al., 2007). The preferential induction of iTreg cells at the mucosal site has been attributed to the GALT $CD103^+$ dendritic cells that produce high amounts of the vitamin A metabolite retinoid acid (RA). The addition of neutralizing TGF-β antibody or RA receptor inhibitors attenuate the generation of iTreg cells, whereas the presence of RA in T cell culture potentiates TGF-β-induced iTreg cell differentiation (Benson et al., 2007; Coombes et al., 2007; Mucida et al., 2007; Sun et al., 2007). RA produced by GALT dendritic cells is also essential for the induction of the gut-homing receptors on T cells (Iwata et al., 2004). These studies have thus revealed a TGF-β and RA-dependent pathway for the generation and homing of iTreg cells to the GALT (Figure 1A); such a pathway might provide an important mechanism for T cell tolerance to antigens derived from commensal flora or of dietary origin.

TGF-β induction of iTreg cell differentiation is inhibited in the presence of the pro-inflammatory cytokine IL-6 (Bettelli et al., 2006). Instead, TGF-β plus IL-6 leads to the generation of the newly characterized T helper cells, Th17 cells, from naive T cells (Bettelli et al., 2006; Veldhoen et al., 2006a) (reviewed by McGeachy and Cua (2008) in this issue of *Immunity*). Th17 cells produce a distinct subset of cytokines, including IL-17A, IL-17F, and IL-22, that act on a broad range of innate immune and non-hematopoietic cells to regulate host defense and tissue homeostasis (Weaver et al., 2006) [(reviewed in this issue by Ouyang et al. (2008)]. In addition, Th17 cells are the culprits of the adjuvant-induced autoimmune diseases, including experimental allergic encephalomyelitis (EAE) (Cua et al., 2003; Langrish et al., 2005). Although TGF-β and IL-6 are essential in mice for the initial commitment of $CD4^+$ T cells to the Th17 cell lineage, they are not sufficient to induce pathological Th17 cells. Using a transfer model of EAE, a recent study showed that Th17 cells that are expanded in the presence of the pro-inflammatory cytokine IL-23, but not with TGF-β plus IL-6, induce disease in mice (McGeachy et al., 2007). Th17 cells stimulated with TGF-β plus IL-6 produce high amounts of the anti-inflammatory cytokine IL-10 (discussed below) but low amounts of inflammatory chemokines and IL-22. Interestingly, IL-10 produced by Th17 cells that have been expanded with TGF-β plus IL-6 suppresses the immunopathology induced by IL-23-stimulated Th17 cells. These observations

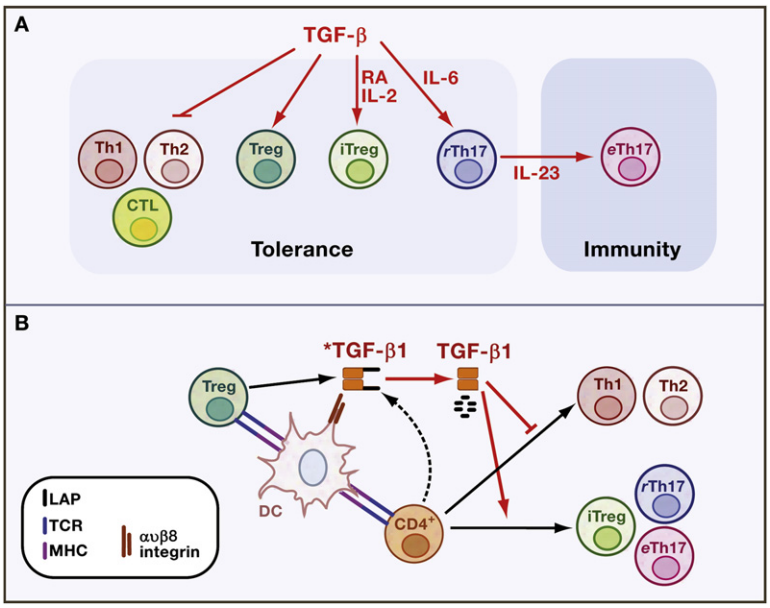

Figure 1. TGF-β Regulation of T Cells

(A) TGF-β controls T cell tolerance via its direct inhibition of T helper 1 (Th1), Th2, and cytotoxic T lymphocyte (CTL) differentiation and the maintenance of regulatory T (Treg) cells. TGF-β promotes the differentiation of induced Treg (iTreg) cells; this differentiation is enhanced by IL-2 and retinoid acid (RA). In the presence of IL-6, TGF-β drives the differentiation of Th17 cells and maintains these cells at a regulatory state (rTh17). IL-23 stimulation of rTh17 cells in the absence of TGF-β induces their differentiation into effector Th17 cells (eTh17) with essential functions in immunity.

(B) A "three-cell" model for TGF-β1-dependent regulation of T cell responses. Upon stimulation by dendritic cells (DCs), Treg cells secrete the latent form of TGF-β1 (*TGF-β1) made of a TGF-β1 dimer associated with the latency-associated protein (LAP). DC-expressed αvβ8 integrin interacts with LAP and induces the degradation of LAP and the release of active TGF-β1. Active TGF-β1 subsequently acts on naive $CD4^+$ T cells via a paracrine mechanism to inhibit their differentiation to Th1 or Th2 cells and to promote their differentiation to iTreg, rTh17, or eTh17 cells. Activated $CD4^+$ T cells produce some amounts of *TGF-β1 that can regulate T cell differentiation through an autocrine route.

suggest that TGF-β plus IL-6 drives the differentiation of Th17 cells to a state with regulatory activities (regulatory Th17, rTh17) and that stimulation and expansion of rTh17 cells by IL-23 in the absence of TGF-β leads to the acquisition of effector Th17 cell activities (effector Th17, eTh17) (Figure 1A). To this end, it is interesting to note that TGF-β inhibits IL-23R expression induced by IL-6 in Th17 cells (Zhou et al., 2007). Therefore, it is conceivable that continued stimulation of Th17 cells by TGF-β might limit their responses to IL-23 and maintain Th17 cells in the nonpathological regulatory state.

The involvement of TGF-β in the differentiation of both Th17 cells and iTreg cells suggests a special kinship between these two lineages of $CD4^+$ T cells. However, the induction of Th17 cells and that of iTreg cells appear to be mutually exclusive. Under the settings that favor Th17 cell differentiation, i.e., TGF-β plus IL-6, iTreg cell generation is inhibited (Bettelli et al., 2006), whereas under the conditions that promote iTreg cell production, i.e., TGF-β plus IL-2 and RA, Th17 cell differentiation is blocked (Laurence et al., 2007; Mucida et al., 2007). The molecular mechanisms underlying the reciprocal differentiation of Th17 and iTreg cells remain to be determined. Th17 cells, presumably in the regulatory state, are present constitutively at high frequencies in the mouse intestinal lamina propria (Ivanov et al., 2006). The GALT is also probably an important site for the generation of iTreg cells. These observations suggest that TGF-β-induced Th17 cells and iTreg cells have important functions in the regulation of mucosal immune responses. To this end, it is interesting to note that TGF-β also promotes B cell class switching to IgA isotypes that are critically involved in the mucosal immune defense (Li et al., 2006b). Therefore, the positive effects of TGF-β on T and B lymphocyte differentiation might have been selected during evolution to fulfill the special needs of mucosal immune homeostasis.

Control of T Cell Responses by T Cell-Produced TGF-β1

TGF-β1 is a widely distributed cytokine that is produced by almost all cell types (Li et al., 2006b). In an early report, liver-specific expression of an active form of TGF-β1 restores the circulating TGF-β1 levels in TGF-β1-deficient mice but fails to rescue the lethal phenotype (Longenecker et al., 2002), suggesting that paracrine and/or autocrine sources of TGF-β1 are essential for the control of T cell tolerance. To address the function of T cell-produced TGF-β1, a recent study used a mouse strain in which the *Tgfb1* gene was inactivated in T cells (Li et al., 2007). In the absence of T cell-produced TGF-β1, mice develop wasting colitis associated with T cell hyperproliferation, activation, and effector T cell differentiation, suggesting that T cell-produced TGF-β1 directly regulates T cells. Previous studies showed that TGF-β signaling in T cells is essential for the maintenance of peripheral Treg cells (Li et al., 2006a; Marie et al., 2006). However, despite decreased numbers of peripheral Treg cells, TGF-βRII-deficient Treg cells undergo enhanced cell proliferation (Li et al., 2006a). Therefore, the TGF-β pathway has a dual function in the suppression of Treg cell proliferation and the maintenance of Treg cells. In the absence of T cell-produced TGF-β1, Treg cells expand in the lymph nodes and spleens, revealing that T cell-produced TGF-β1 is essential for the inhibition of Treg cell proliferation but is dispensable for Treg cell maintenance in these tissues. Interestingly, colonic Treg cells from these mice express lower amounts of Foxp3 than Treg cells from the peripheral lymph nodes (Li et al., 2007). It is possible that the diminished expression of Foxp3 in colonic Treg cells is a consequence of colitis developed in these mice. Alternatively, because TGF-β is involved in the differentiation of iTreg cells in the GALT (discussed in the previous section), T cell-produced TGF-β1 might be required for the de novo generation or maintenance of colonic iTreg cells. These findings suggest that T cell-produced TGF-β1 regulates mucosal T cell tolerance via its inhibition of effector T cell differentiation, and possibly also through the promotion of colonic iTreg cell differentiation (Figure 1B).

Both Treg cells and naive $CD4^+$ T cells produce TGF-β1 upon TCR stimulation in vitro (Li et al., 2007). To further delineate the functions of TGF-β1 produced by the subsets of T cells in vivo, Li et al. used an adoptive-transfer model of colitis. In this model,

transfer of naive $CD4^+$ T cells into lymphopenic hosts results in colitis, which can be prevented by the cotransfer of Treg cells. Early studies showed that Treg cell-mediated protection is abrogated by the treatment of TGF-β neutralization antibody (Powrie et al., 1996) and that naive $CD4^+$ T cells expressing a dominant-negative mutant of TGF-βRII are refractory to Treg cell-induced suppression (Fahlen et al., 2005). Therefore, Treg cell inhibition of colitis is dependent on TGF-β signaling in T cells. However, whether the Treg cell is the source cell type for TGF-β1 is controversial (Fahlen et al., 2005; Kullberg et al., 2005; Nakamura et al., 2004). In a series of transfer experiments, $CD4^+Foxp3^+$ Treg cells isolated from T cell-specific TGF-β1-deficient mice were shown to be incapable of suppressing colitis induced by wild-type naive T cells; this was associated with the failed inhibition of naive T cell differentiation into Th1 cells (Li et al., 2007). The transfer experiments have also shown that, to a lesser extent, TGF-β1 originated from naive T cells could contribute to the protection of colitis. Treg cells express membrane-bound TGF-β1 (Green et al., 2003; Nakamura et al., 2001; Oida et al., 2003), which has been implicated in Treg cell suppression. However, the definitive contribution of surface and/or soluble TGF-β1 produced by Treg cells remains to be determined. In addition, TGF-β1-deficient Treg cells have normal suppressive activity in vitro (Li et al., 2007). Foxp3-deficient mice also develop a more severe phenotype than T cell-specific TGF-β1-deficient mice. Therefore, production of TGF-β1 is likely to be one of many mechanisms that Treg cells utilize to regulate T cell tolerance.

In the EAE model, neutralization of TGF-β1 at the site of immunization inhibits Th17 cell differentiation and disease development (Veldhoen et al., 2006b), suggesting that TGF-β1 regulation of Th17 cell differentiation also occurs via the paracrine and/or autocrine route. T cell production of IL-17 is greatly reduced in T cell-specific TGF-β1-deficient mice (Li et al., 2007). In addition, these mice are highly resistant to EAE, which is associated with the failed differentiation of Th17 cells (Li et al., 2007). These findings demonstrate that T cell-produced TGF-β1 is indispensable for Th17 cell differentiation in vivo (Figure 1B). However, it remains to be determined whether TGF-β1 produced by Treg cells, non-Treg T cells, or both is involved in Th17 cell differentiation. It has been shown that Treg cells potentiate Th17 cell differentiation induced by dendritic cells in vitro (Veldhoen et al., 2006a; Xu et al., 2007). In a transfer model of systemic autoimmune disease, cotransfer of Treg cells inhibits IFN-γ but enhances IL-17 production from effector T cells (Lohr et al., 2006). These observations suggest a pro-inflammatory function for Treg cells in promoting Th17 cell differentiation through the production of TGF-β1. Future studies with Treg cell-specific TGF-β1-deficient mice will help to test this hypothesis.

TGF-β1 Activation by Dendritic Cell-Produced αvβ8 Integrin

TGF-β1 is synthesized and produced as a latent form that needs activation to be functional (Li et al., 2006b). A homodimer of the TGF-β1 propeptide, the latency-associated protein (LAP), is constitutively associated with the mature TGF-β1, which prevents TGF-β1 from binding to its receptors. TGF-β1 activation proceeds via the degradation of LAP or the alteration of LAP conformation; this can be mediated by proteases, including plasmin and matrix metalloproteinases, thrombospondin-1, and integrins αvβ6 or αvβ8 (Annes et al., 2003). Activation of TGF-β1 by αvβ6 or αvβ8 integrin is dependent on their binding to an RGD motif in LAP but proceeds through different mechanisms. αvβ6 integrin induces mechanical traction of LAP and activates TGF-β1 in the absence of its release from the latent complexes (Munger et al., 1999). On the other hand, αvβ8 integrin-induced TGF-β1 activation requires metalloproteinase-mediated degradation of LAP, which results in the release of active TGF-β1 into the extracellular space (Mu et al., 2002). In a recent report, the in vivo function of the integrin-dependent TGF-β1 activation was studied in a mouse model in which the TGF-β1 RGD sequence was substituted with the RGE sequence (Yang et al., 2007). The D-to-E amino acid change abolishes the integrin-dependent activation of TGF-β1 but preserves TGF-β1 processing and secretion. Interestingly, mice homozygous for the *Tgfb1* D-to-E allele develop an autoimmune phenotype that is indistinguishable from that of TGF-β1-deficient mice. These observations demonstrate a pivotal function for the integrin pathway in the activation of TGF-β1 in vivo.

αvβ6 integrin is expressed in epithelial cells. αvβ6-deficient mice are viable and do not suffer from T cell-mediated inflammation. αvβ8 integrin is expressed in multiple cell types, including T cells and dendritic cells. αvβ8 integrin deficiency in mice is embryonic lethal. To investigate the function of αvβ8 integrin in adult mice, researchers have used mouse strains that harbor floxed alleles of *Itgb8* or *Itgav* genes. Ablation of β8 in dendritic cells, but not in T cells, results in colitis in mice; this is associated with T cell activation and differentiation, as well as reduced colonic Treg cells (Travis et al., 2007). In addition, β8-deficient dendritic cells fail to activate TGF-β1 and are unable to induce iTreg cell differentiation in vitro. Similar colitis and T cell phenotypes are present in mice in which the *Itgav* gene is deleted from myeloid cells (Lacy-Hulbert et al., 2007). Intriguingly, inflammation and T cell activation in the dendritic cell-specific αvβ8 integrin-deficient mice are remarkably similar to what is observed in mice with T cell-specific deletion of the *Tgfb1* gene (Li et al., 2007). These findings prompt us to propose a "three-cell" model for TGF-β1-dependent regulation of T cell responses (Figure 1B). In this model, Treg cells, and to a lesser extent naive T cells, produce latent TGF-β1 upon TCR stimulation by dendritic cells. Latent TGF-β1 is activated through mechanisms that are dependent on αvβ8 integrin expressed by dendritic cells, which creates an active TGF-β1 milieu around the dendritic cells. Active TGF-β1 subsequently acts on naive T cells to regulate their activation and differentiation induced by dendritic cells. The involvement of dendritic cells in Treg cell-mediated immune regulation as proposed in this model is consistent with a previous study that showed that Treg cells form long-lasting interactions with dendritic cells in vivo; these interactions subsequently impair the ability of dendritic cells to drive the differentiation of effector T cells (Tang et al., 2006). However, it is important to note that the inflammatory phenotypes developed in T cell-specific TGF-β1-deficient mice are less severe than those of mice with TGF-β1 deficiency in all cells. In addition, dendritic cell-specific β8 integrin-deficient mice suffer from milder inflammation than mice with D-to-E alleles of integrin-activation-defective TGF-β1 mutants. These observations suggest that TGF-β1 produced by cell types other than T cells, and integrins other than dendritic cell-expressed αvβ8, is also important in the regulation of T cells.

Identifying these alternative sources of TGF-β1 or integrins will help us better understand the cellular mechanisms by which TGF-β1 regulates T cells.

IL-10 as a Gatekeeper of Exuberant Immune Reponses to Foreign Antigens

IL-10 is a regulatory cytokine with pleiotropic effects on multiple cell types that express IL-10 receptor 1 (IL-10R1) and IL-10 receptor 2 (IL-10R2) (Moore et al., 2001). IL-10 engagement of its receptor complex initiates diverse signaling, including the activation of the Jak1 and Tyk2 kinases and the Stat3 transcription factor [reviewed in this issue by O'Shea and Murray (2008)], in target cells. An essential function for IL-10 in immune tolerance was first revealed in the study of IL-10-deficient mice that develop lethal colitis in specific-pathogen-free facilities (Kuhn et al., 1993). Colitis in these mice is mediated by T cells that undergo activation and effector T cell differentiation in the absence of IL-10. T cell activation or colitis is inhibited when IL-10-deficient mice are raised under the germ-free conditions (Kuhn et al., 1993; Sellon et al., 1998), demonstrating that IL-10 functions to maintain T cell tolerance to resident enteric bacteria antigens. Similar to pathological microbes, commensal bacteria display the conserved molecular patterns that can be recognized by the innate immune system's pattern-recognition receptors, such as the Toll-like receptors (TLRs). Interestingly, deficiency of the TLR signaling molecule MyD88 blocks the development of colitis in IL-10-deficient mice (Rakoff-Nahoum et al., 2006). These findings suggest that in the steady state, IL-10 inhibits T cell responses induced by TLR-stimulated innate immune cells in recognition of commensal bacteria antigens (Figure 2A).

The adaptive immune system has evolved to combat pathogens efficiently, which is orchestrated by the elaborated effector T cells tailed to the specific pathogens. However, exuberant T cell responses can result in the development of immunopathology and sometimes induce more severe tissue damage than infection itself. Therefore, regulatory mechanisms are in force to control the magnitude of effector T cell responses. The use of various infection models has helped to demonstrate that IL-10 functions to limit the extent of immune responses to foreign pathogens (Moore et al., 2001). This can lead to the dual consequences of either the inhibition of collateral tissue damage, such as that which occurs during *Toxoplasma gondii* or *Trypanosoma cruzi* infection (Gazzinelli et al., 1996; Hunter et al., 1997), or the promotion of a chronic infection state in the case of *Leishmania major* or lymphocytic choriomengitis virus infection (Belkaid et al., 2002; Brooks et al., 2006; Ejrnaes et al., 2006; Kane and Mosser, 2001). The importance of IL-10 in controlling infection is underscored by the finding that some pathogens, such as the Epstern-Barr virus, encode IL-10 homologs in their genome (Moore et al., 1990). The viral IL-10 molecules have regulatory activity similar to that of the host IL-10 and might therefore play an important role in host-pathogen interactions. Taken together, these findings suggest that the IL-10 pathway is a critical regulator of immune responses to microorganisms, including both the innocuous commensal flora and the pathogens.

How IL-10 regulates T cell responses is incompletely understood. IL-10 potently inhibits the functions of antigen-presenting cells such as dendritic cells and macrophages through the repression of inflammatory cytokine production and the inhibition

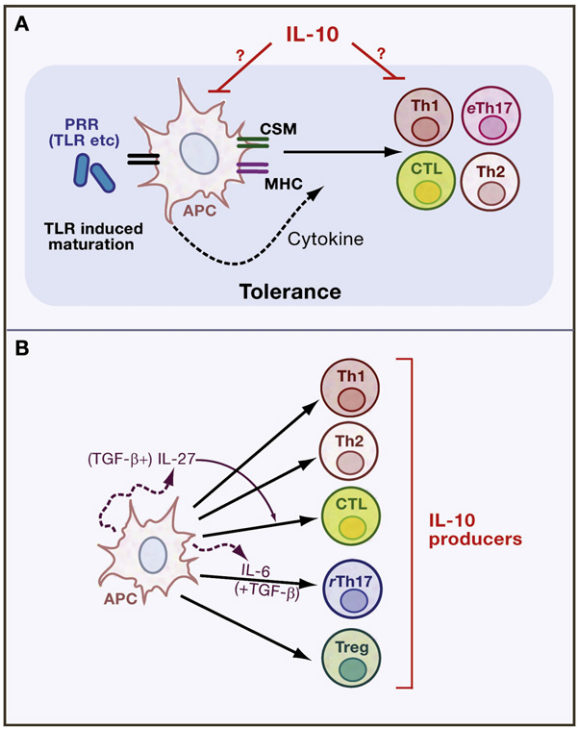

Figure 2. IL-10 Regulation of T Cells

(A) IL-10 controls T cell tolerance at the interface between the innate and the adaptive immune systems. Microbial stimulation of antigen-presenting cells (APCs) via the pattern-recognition receptors (PRRs) such as the Toll-like receptor (TLR) induces APC maturation; this is associated with the induction of major histocompatibility complex (MHC) and costimulatory molecule (CSM) gene expression, and the secretion of innate immune cytokines. IL-10 may directly inhibit the APC maturation process. In addition, IL-10 may act on T cells to inhibit effector T cell differentiation stimulated by the mature APCs.

(B) Upon stimulation by APCs, IL-10 can be produced by multiple T cell types, including Treg, rTh17, Th1, Th2, and CTL cells. IL-10 production in T cells is regulated by the innate immune cytokines IL-27 and IL-6 with the aid of TGF-β.

of MHC class II and costimulatory-molecule expression (Moore et al., 2001). A potential role for IL-10 signaling in macrophages is supported by the study of macrophage and neutrophil-specific Stat3-deficient mice that develop chronic colitis (Takeda et al., 1999). However, because Stat3 is activated by multiple cytokine signaling pathways, the relevance of IL-10 in this model of colitis awaits further investigation. IL-10 might also directly regulate T cells. IL-10 enhances the differentiation of antigen-driven IL-10-producing $CD4^+$ regulatory T (Tr1) cells in vitro (Groux et al., 1997). A recent study showed that during *Listeria monocytogenes* infection, deficiency of IL-10R2 in antigen-specific $CD8^+$ T cells, but not in antigen-presenting cells, leads to the increased expansion of $CD8^+$ T cells (Biswas et al., 2007). Therefore, the IL-10 direct target cells involved in IL-10-mediated regulation of T cell responses remain to be fully characterized and will probably be resolved with the generation of cell-type-specific IL-10 receptor-deficient mice.

Control of T Cell Responses by T Cell-Produced IL-10

IL-10 was originally identified as a molecule that is produced by Th2 cells and inhibits Th1 cell responses (Fiorentino et al., 1989).

Later studies showed that IL-10 is made by Th1, Th2, Th17, and $CD8^+$ T cells, as well as by $CD4^+Foxp3^+$ Treg cells and $CD4^+$ Tr1 cells. In addition, antigen-presenting cells also produce IL-10 in response to pathogen infection. The functions of IL-10 produced by specific cell types have been studied with a strain of conditionally IL-10-deficient mice. Similar to completely IL-10-deficient mice, mice with a T cell-specific inactivation of the *Il10* gene develop colitis and succumb to severe immunopathology upon infection with *T. gondii*; this is associated with enhanced T cell activation and differentiation (Roers et al., 2004). However, abrogation of IL-10 expression in T cells does not affect innate immune responses to lipopolysaccharide, which is controlled by IL-10 produced by macrophages and neutrophils (Siewe et al., 2006). These findings suggest that IL-10 is involved in the feedback control of immune responses and that the functions of effector cells are inhibited by IL-10 produced by the same cell type.

Consistent with the notion that IL-10 can function as a feedback regulator of T cells, recent studies have shown that Th1 cell-produced IL-10 is essential for the inhibition of exaggerated Th1 cell responses during infection. $CD4^+$ T cells producing high amounts of IFN-γ are also the source cells of the IL-10 that inhibits the development of immunopathology upon *T. gondii* infection (Jankovic et al., 2007). These T cells express the Th1 cell lineage transcription factor T-bet, but not the Treg cell marker Foxp3. Therefore, they are by all criteria the classic *T. gondii* antigen-specific Th1 cells. In another infection model using an *L. major* strain (NIH/Sd) that induces nonhealing lesions in infected mice, IL-10 produced by Th1 cells is also found to be responsible for the hindrance of immune responses to *L. major* and as a consequence to promote lesion development (Anderson et al., 2007). Whether similar feedback regulation also operates in Th2, Th17, and $CD8^+$ T cells remains to be determined. It is nevertheless tempting to speculate that IL-10 might have evolved to function as a general inhibitor involved in the autoregulation of effector T cell responses.

In addition to effector T cells, Treg cells produce IL-10. In an adoptive-transfer model of colitis, $CD4^+CD45RB^{low}$ T cell inhibition of $CD4^+CD45RB^{high}$ naive T cell-induced colitis in lymphopenic hosts is reversed by administration of an anti-IL-10 receptor antibody (Asseman et al., 1999). $CD4^+CD45RB^{low}$ T cells from IL-10-deficient mice also fail to protect mice from colitis. These findings are in line with the aforementioned study of T cell-specific IL-10-deficient mice that develop spontaneous colitis. The $CD4^+CD45RB^{low}$ T cell population is enriched for $CD4^+Foxp3^+$ Treg cells. Therefore, it is possible that IL-10 produced by Treg cells is involved in the inhibition of colitis in mice. This conclusion is supported by the observation that mice with the Treg cell-specific deletion of the *Il10* gene develop colitis (Rubtsov et al., 2008, in this issue). In a model of infection with the Friedlin strain of *L. major*, production of IL-10 by Treg cells has also been associated with the failed eradication of the pathogens (Belkaid et al., 2002). Nevertheless, because IL-10 is produced by both Treg cells and effector T cells, the definitive functions of Treg cell-produced IL-10 in this model remain to be established.

Regulation of IL-10 Production in T Cells

The promiscuous expression pattern of IL-10 in T cells prompts the studies on the mechanisms involved in the regulation of IL-10 expression in these cells. To mark IL-10 producers in vivo, several groups have developed IL-10 reporter mouse strains (Calado et al., 2006; Kamanaka et al., 2006; Maynard et al., 2007). IL-10-producing T cells are absent in the thymus but can be detected at varying frequencies in peripheral tissues and are highly abundant in mouse intestines (Kamanaka et al., 2006; Maynard et al., 2007). TCR stimulation of T cells in vivo induces IL-10 expression; the major producers are the Peyer's patch T cells and the intraepithelial lymphocytes in the large and small intestines (Kamanaka et al., 2006). The prominent distribution of IL-10-producing T cells in the intestine is consistent with the critical function of T cell-produced IL-10 in the maintenance of immune tolerance to commensal bacteria antigens in mice. Using dual-reporter mouse strains for IL-10 and Foxp3 has revealed IL-10-producing cells among the $Foxp3^+$ Treg cells in the large intestine and among the $Foxp3^-$ T cells in the small intestine (Kamanaka et al., 2006). So that the lineage relationship of these IL-10-producing T cells could be determined, a series of transfer experiments were performed. Thymic $CD4^+Foxp3^+$ Treg cells give rise to the peripheral $Foxp3^+IL\text{-}10^+$ and $Foxp3^+IL\text{-}10^-$ Treg cells, whereas thymic $CD4^+Foxp3^-$ T cells differentiate into $Foxp3^+IL\text{-}10^+$ or $Foxp3^+IL\text{-}10^-$ Treg cells, as well as the $Foxp3^-IL\text{-}10^+$ T cells (Maynard et al., 2007). Interestingly, all three T cell subsets are present in IL-10-deficient mice but are reduced in mice treated with neutralizing TGF-β antibody (Maynard et al., 2007). These findings have therefore revealed an interrelation between TGF-β and IL-10 in immune tolerance and are also consistent with the known function of TGF-β in iTreg cell differentiation.

TGF-β promotes TCR-stimulated naive T cells to differentiate into iTreg cells but is not sufficient to trigger high IL-10 production in T cells. Recent studies have shown that cytokines IL-27 and IL-6 are important regulators of IL-10-producing T cells (Awasthi et al., 2007; Fitzgerald et al., 2007; McGeachy et al., 2007; Stumhofer et al., 2007) (Figure 2B). IL-27 is a heterodimeric cytokine produced mostly by innate immune cells (Kastelein et al., 2007). Although IL-27 was initially identified as a molecule that promotes Th1 cell differentiation, recent studies have shown that IL-27 limits Th1, Th2, and Th17 cell responses in various infection and autoimmune disease models (Kastelein et al., 2007). The presence of IL-27 in T cell culture inhibits the production of T cell effector cytokines but markedly enhances IL-10 production by both $CD4^+$ and $CD8^+$ T cells (Awasthi et al., 2007; Fitzgerald et al., 2007; Stumhofer et al., 2007). Interestingly, TGF-β further augments IL-27-induced IL-10 expression in $CD4^+$ T cells (Awasthi et al., 2007; Stumhofer et al., 2007), which reveals another connection between TGF-β and IL-10. IL-27 promotion of IL-10 production in $CD4^+$ T cells is observed under neutral, Th1, or Th2 polarizing conditions. The importance of IL-27 in the differentiation of IL-10-producing T cells in vivo has been further corroborated by the observation that $IL\text{-}10^+IFN\text{-}\gamma^+$ Th1 cells are absent in IL-27 receptor-deficient mice infected with *T. gondii* (Stumhofer et al., 2007). In *T. gondii*-infected mice, IL-10 produced by Th1 cells is essential for the inhibition of an excessive Th1 cell response that induces immunopathology in mice (Jankovic et al., 2007). Consistent with an indispensable role for IL-27 in IL-10 production, IL-27 receptor-deficient mice develop lethal $CD4^+$ T cell-mediated inflammation upon *T. gondii* infection (Villarino et al., 2003). IL-6 plus TGF-β-induced Th17

cells produce high amounts of IL-10 whose expression is not further enhanced by IL-27 (Stumhofer et al., 2007). However, similar to IL-27-induced IL-10 expression, Th17 cell production of IL-10 is dependent on the synergistic activity of both IL-6 and TGF-β (Stumhofer et al., 2007). Both IL-6 and IL-27 are type I cytokines with a shared receptor subunit and activate Stat transcription factors in target cells [reviewed in this issue by O'Shea and Murray (2008)]. Interestingly, IL-27 induction of IL-10 expression is dependent on both Stat1 and Stat3, whereas only Stat3 is required for IL-6 promotion of IL-10 production (Stumhofer et al., 2007). In addition, TGF-β-induced IL-10 production in T cells is associated with the binding of the Smad4 transcription factor to the *Il10* gene promoter (Kitani et al., 2003). Nevertheless, the precise epigenetic mechanisms by which Stat, Smad, and other transcription factors regulate IL-10 expression in T cells remain to be determined.

Conclusions

TGF-β1 and IL-10 are regulatory cytokines with pivotal functions in the control of inflammation. TGF-β1 directly targets T cells to ensure immune tolerance to self- and environmental antigens, whereas IL-10 regulates at the interface of innate and adaptive immunity to limit the magnitude of immune responses to microbial antigens. T cells are the essential producers of both TGF-β1 and IL-10 involved in the regulation of T cell responses. Mobilization of T cell-produced TGF-β1 requires the engagement of dendritic cell-expressed αvβ8 integrin that activates the latent form of TGF-β1, whereas T cell production of IL-10 is regulated by innate immune cytokines IL-27 and IL-6. In addition, the TGF-β and IL-10 pathways crosstalk with the participation of TGF-β in the differentiation of IL-10-producing T cells. Additional insights into the molecular and cellular mechanisms by which the dual cytokine pathways regulate peripheral T cell responses will aid the targeting of TGF-β1 and IL-10 for the therapy of various inflammatory disorders.

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