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TGFβ1 and T_{reg} cells: alliance for tolerance

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Abstract

Transforming growth factor β1 (TGFβ1), an important pleiotropic, immunoregulatory cytokine, uses distinct signaling mechanisms in lymphocytes to affect T-cell homeostasis, regulatory T (T_{reg})-cell and effector-cell function and tumorigenesis. Defects in TGFβ1 expression or its signaling in T cells correlate with the onset of several autoimmune diseases. TGFβ1 prevents abnormal T-cell activation through the modulation of Ca²⁺–calcineurin signaling in a *Caenorhabditis elegans* Sma and *Drosophila* Mad proteins (SMAD)3 and SMAD4-independent manner; however, in T_{reg} cells, its effects are mediated, at least in part, through SMAD signaling. TGFβ1 also acts as a pro-inflammatory cytokine and induces interleukin (IL)-17-producing pathogenic T-helper cells (T_h IL-17 cells) synergistically during an inflammatory response in which IL-6 is produced. Here, we will review TGFβ1 and its signaling in T cells with an emphasis on the regulatory arm of immune tolerance.

Introduction: TGFβ1 in T-cell tolerance

Self-tolerance is achieved primarily by negative selection of immature thymocytes and also through regulatory T (T_{reg})-cell activity in the periphery. Natural T_{reg} (nT_{reg}) cells develop in the thymus, are primarily self-antigen specific and express CD4, CD25 and forkhead box P3 (FOXP3). They home to peripheral tissues to maintain self-tolerance [1]. T_{reg} cells are also generated in the periphery during an active immune response and are called adaptive or induced T_{reg} cells. T_{reg}-cell generation and function is regulated by several important factors. These include cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), glucocorticoid-induced tumour necrosis factor receptor (GITR), CD28 and CD25 on T cells and CD103, B7-1 and B7-2 on antigen-presenting cells (APCs), as well as transcription factor FOXP3 and the cytokines interleukin (IL)-10 and transforming growth factor β1 (TGFβ1) [1–6]. T_{reg} cells are classified into various subsets depending on their surface markers, transcription factors and the cytokines they secrete [7]. The nT_{reg} cells are the major T_{reg} cells found under homeostatic conditions. These cells induce self-tolerance and prevent autoimmunity. They express FOXP3 and are thought to suppress naïve T cells through cell–cell contact and membrane-bound TGFβ1 [8] (Table 1). T_{reg} cells are also induced in the periphery from CD4⁺CD25[−] T cells *de novo*, which are called inducible or adaptive T_{reg} cells. These cells inhibit effector T cells primarily through the production of cytokines, such as IL-10 and TGFβ1. IL-10-producing T_{reg} cells are called T_h1 cells, whereas TGFβ1-secreting T_{reg} cells are called T_h3 cells. A subset

of CD8 T cells (CD8⁺CD122⁺) also suppresses the T-cell response through IL-10 production (reviewed in [7,9,10]). T_r1 cells are localized primarily in mucosal tissues, such as the colon, where they control inflammatory responses [11].

TGFβ1 is an important immunoregulatory cytokine that is involved in the maintenance of self-tolerance and T-cell homeostasis [2]. It is produced by several immune and non-immune cell types and functions in a both autocrine and paracrine manner [2,12,13]; T_{reg} cells are the primary source of TGFβ1 in the control of autoimmune disease. Last year, there were several important studies on the role of TGFβ1 and its signaling in T cells, both as a pro- and anti-inflammatory cytokine. Severe autoimmune diseases are induced in mice by blocking TGFβ signaling in T cells [14–16]. TGFβ signaling in T cells under inflammatory conditions is also important for the development of experimental autoimmune encephalomyelitis (EAE), a mouse model of human multiple sclerosis [17]. IL-6 inhibits *Foxp3* expression and FOXP3⁺ T_{reg} cells are increased in *Il6* knockout (KO) mice, which are resistant to EAE induction [17,18]. Because, in T cells, TGFβ1 induces both FOXP3 and orphan nuclear receptor (RORγt) in the presence of IL-2 and IL-6, respectively, it is suggested that IL-6 counteracts the TGFβ1 effect on T_{reg}-cell generation [19]. Here, we will review recent breakthroughs regarding the function of TGFβ1 in immune tolerance and we will discuss the implications for therapeutic intervention in auto-immune and inflammatory diseases.

TGFβ1 signaling in T cells

TGFβ1 signals primarily through membrane-bound serine/threonine kinase receptors and its signaling intermediates vary depending on cell type and response [19,20]. In T cells, TGFβ1 also signals through a *Caenorhabditis elegans* Sma and *Drosophila* Mad proteins (SMAD)-independent Ca²⁺-calcineurin-nuclear factor of activated T cells (NF-AT) cascade that inhibits naïve T-cell activation [21,22]. Lack of TGFβ ligand-binding might prevent translocation of FK506 binding protein 12 (FKBP12) from the TGFβ1-receptor complex to a ternary complex with the IP₃ receptor and calcineurin, where it prevents Ca²⁺ channels from leaking [23]. Although TGFβ1 inhibits naïve T-cell responses, it also induces *Foxp3* expression in T cells and causes T_h- to T_{reg}-cell conversion and subsequent expansion through SMAD signaling [24]. TGFβ1 also induces *Ctla4* expression in naïve T cells, which, in turn, is responsible for the induction of *Foxp3* in inducible T_{reg} cells (Figure 1) [25]. Conditional deletion in T cells of the gene (*Tgfb2*) for the TGFβ receptor type II (TGFβR2) confirms that TGFβ1 signaling is essential for T-cell homeostasis and T_{reg}-cell suppressor function [14,15].

TGFβ1 induces T_h17-cell differentiation from naïve CD4⁺ cells on αCD3 and lipopolysaccharide (LPS) stimulation in the presence of dendritic cells (DCs). However, T_h17 cells fail to develop from dominant negative (DN)-TGFβRII CD4⁺ T cells under the same culture conditions, suggesting that TGFβ signaling is required for T_h17-cell differentiation [26]. The nuclear orphan receptor RORγt is required for the development of T_h17 cells. TGFβ1 and IL-6 induce RORγt independently. FOXP3, which is induced by TGFβ1, inhibits RORγt-induced IL-17 expression [27]. Thus, a balance of IL-6 and TGFβ1 determines whether a T cell becomes a T_{reg} or a T_h17 cell.

TGFβ1 inhibits both T_h1- and T_h2-cell differentiation by inhibiting T-bet (*Tbx21*) and *Gata3* expression, respectively. TGFβ1 inhibits *Ifng* expression through the inhibition of *Tbx21* and *Stat4*, which are known to induce T_h1-cell differentiation [12,28]. The inhibitory effect of TGFβ1 on *Tbx21* expression is mediated through Src homology region 2 domain-containing protein tyrosine phosphatase-1 (SHP-1) [29], a negative regulator of T-cell receptor (TCR) signaling and T_{reg}-cell generation. TGFβ1 also inhibits T_h2-cell differentiation by inhibiting *Gata3* expression in mouse but not human T cells [30,31]. GATA3 induces T_h2-cell differentiation by inducing *Il5*, *Il13* and *Il4* expression and repressing *Il12r* expression [32]. It

is unclear whether TGF β 1 uses different signaling pathways in inhibiting T_h1- and T_h2-cell differentiation. Our data indicate that TGF β 1 inhibits T_h1-cell differentiation through a Ca²⁺-calcineurin pathway, although it is less clear whether this is the pathway used to inhibit T_h2-cell differentiation [21]. Alternatively, it is possible that TGF β 1 induces T_h3 or T_h17 cells, depending on the cytokine environment, thereby causing an indirect suppression of T_h1- and T_h2-cell differentiation.

Phenotypes of TGF β 1-deficient mice

Tgfb1^{-/-} mice die at weaning age from a multifocal autoimmune disease caused by self-reactive T cells [2,33]. Phenotypes of *Tgfb1*^{-/-} mice on various gene-KO backgrounds are summarized in Table 2. Consistent with recent observations that TGF β 1 and IL-6 are essential for T_h17-cell differentiation, T_h17 cells are absent in *Tgfb1*^{-/-} mice, suggesting that *Tgfb1*^{-/-} T cells cause autoimmune disease without differentiating into T_h17 cells [19,34]. Because *Tgfb1*^{-/-} T cells produce interferon (IFN)- γ but not IL-17, it is possible that IFN- γ produced by T_h1 cells inhibits T_h17-cell development [22,34,35]. *Tgfb1*^{-/-} T cells do differentiate into T_h17 cells *in vitro* in the presence of added TGF β 1 and anti-CD3, suggesting that the lack of T_h17-cell development in *Tgfb1*^{-/-} mice is owing to the presence of abundant IFN- γ , the absence of TGF β 1 or both. In *Tgfb1*^{-/-} *Ifng*^{-/-} mice, inflammation is eliminated only in the liver but not in the heart and lungs [35]. This suggests that pathogenic T_h17 cells might be induced by IL-6 in the absence of IFN- γ and TGF β 1, hence causing disease and early lethality in *Tgfb1*^{-/-} *Ifng*^{-/-} mice. EAE disease is exacerbated in IFN- γ -deficient mice. An interpretation of these data is that the deficiency of T_h1 cells exacerbates autoimmune disease owing to a lack of inhibition of T_h17-cell differentiation that results in T_h17-cell expansion by TGF β 1 and IL-6. Because *Tgfb1*^{-/-} mice produce increased amounts of IFN- γ , it is possible that T_h1 cells cause disease in *Tgfb1*^{-/-} mice, whereas T_h17 cells cause disease in *Tgfb1*^{-/-} *Ifng*^{-/-} mice. We have observed that, in *Tgfb1*^{-/-} mice, CD8 T cells also cause inflammation without CD4 helper T cells, although it is unclear whether the inflammation is caused by T_h17 or CD8 T cells in *Tgfb1*^{-/-} *Ifng*^{-/-} mice [35,36] (Table 2). More recently, Korn *et al.* have shown that IL-21 is required for T_h17-cell generation. Because FOXP3⁺ T_{reg} cells are increased at the expense of effector T cells in *Il6*^{-/-} mice, T_h17 cells could be generated in T_{reg}-cell-deleted IL-6-deficient mice, suggesting that IL-6 inhibits TGF β 1-induced T_{reg}-cell generation, thereby enabling TGF β 1-induced T_h17-cell generation [17,18]. However, IL-6 is more potent than IL-21 in inducing IL-17 and inhibiting FOXP3 production in the presence of TGF β 1.

In contrast to the situation with *Ctla4*^{-/-} mice, treatment of day-2 *Tgfb1*^{-/-} mice with cytotoxic T-lymphocyte-associated protein 4-immunoglobulin fusion protein (CTLA-4-Ig), which binds to B7-1 with high affinity and blocks co-stimulation through both CD28 and CTLA-4 [37], does not reverse the severe autoimmune disease [38] (T. Doetschman *et al.*, unpublished). These data suggest that TGF β 1-deficient T cells do not require co-stimulatory signals through CD28 for their activation and autoimmune response [2]. Recently, we demonstrated, in *Tgfb1*^{-/-} mice, that elimination of self-antigen-reactive TCR-bearing T cells by genetic combination strongly inhibits activation of T cells and eliminates inflammation [33]. We and others have also shown, using TCR-transgenic mice, that T_{reg}-cell generation and maintenance in the periphery is not TGF β 1 dependent [39] (R. Bommireddy *et al.*, unpublished). This suggests that TGF β 1 is important for the prevention of self-reactive T-cell activation, T_{reg}-cell suppressor function or both. This is in contrast to the observations made in TGF β 2-deficient mice, which show a progressive loss of T_{reg} cells in the periphery as they develop inflammation. The loss of T_{reg} cells in these mice is owing to a cell-intrinsic defect in their survival mechanism [14,15], suggesting that TGF β signaling through TGF β 2 is essential for T_{reg}-cell survival in the periphery and that other TGF β ligand(s) might provide survival signals for the maintenance of T_{reg} cells in the periphery of *Tgfb1*^{-/-} mice. Together, these studies indicate that TGF β 1 regulates the activation threshold levels of T cells in response to self-antigen recognition.

TGF β function in T_{reg} cells

Initial studies by Nakamura *et al.* and Piccirillo *et al.* have generated controversy regarding the role of TGF β 1 in T_{reg}-cell function [40,41]. Although neutralizing TGF β antibody blocked T_{reg}-cell function in the first study, soluble TGF β R2 failed to block T_{reg}-cell suppressor function in the other. The mechanism by which T_{reg} cells suppress the T-cell response is now becoming clear with recent observations made in *Tgfb1* and *Tgfb2* conditional-knockout (CKO) mice. Recently, Li *et al.* reported that T-cell-specific deletion of TGF β 1 causes T-cell activation and defective T_{reg}-cell function, suggesting that TGF β 1 is required for suppressor function [42]. Consistently, T-cell-specific ablation of *Tgfb2* also causes T-cell activation and autoimmunity. These TGF β R2-deficient T cells are not suppressed by wild-type T_{reg}-cells, which suggests that TGF β signaling in effector T cells is essential for regulation [1,14,15].

TGF β 1-deficient T_{reg} cells exhibit suppressor function both *in vitro* and in adoptive-transfer models of colitis and transgenic *dn-Tgfb2* T cells are also inhibited by T_{reg} cells [43]. However, *dn-Tgfb2* mice live longer because trans-gene expression is leaky [41]. In mice with *Lck/Cre*-mediated CKO of *Tgfb2* in T cells, those cells with a TGF β R2 deficiency are resistant to T_{reg}-cell suppression and have a similar T-cell phenotype as do *Tgfb1*^{-/-} mice [14,15]. Also, neutralizing antibody to TGF β 1 abolishes suppressor function of TGF β 1-deficient T_{reg} cells *in vivo* in an adoptive-transfer model of colitis, suggesting that paracrine TGF β 1 produced within recipient mice or other cell types in the case of *in vitro* studies might mediate the suppressor function of TGF β 1-deficient T_{reg} cells [39,44]. More recently, Li *et al.* have shown, using *Tgfb1* CKO mice (T-cell-specific deletion of TGF β 1), that FOXP3⁺ T_{reg} cells are increased in *Tgfb1* CKO mice but are defective in their suppressor function. They have also found that TGF β 1-deficient T_{reg} cells fail to inhibit IFN production and T-cell activation *in vivo* and *in vitro* and also fail to protect mice from developing colitis in the adoptive-transfer model of colitis [42]. TGF β 1-independent T_{reg} cells are unlikely to induce dominant tolerance because *Tgfb1*^{-/-} mice die at approximately weaning age from multi-organ autoimmunity [2, 33].

A decrease in functional T_{reg} cells results in autoimmune diseases, such as diabetes and inflammatory bowel disease [45]. Over-expression of TGF β 1 in mouse T cells also increases the proportion of T_h3 cells. These T_h3 cells are protective in adoptive-transfer models of EAE and rescue IL-2-deficient mice from autoimmune disease [46]. This suggests that the TGF β 1 produced by T_h3 cells might induce the conversion of T_h cells to T_{reg} cells in a cell-cell contact-dependent manner (infectious tolerance) [47]. TGF β 1 induces *Foxp3* in T cells, which, in turn, inhibits *Smad7* expression (inhibitory SMAD) in T_{reg} cells in a positive autoregulatory manner. This is in contrast to the induction of *Smad7* in naïve T cells in response to TGF β signaling. Inhibition of *Smad7* by *Foxp3* also results in enhanced expression of SMAD3/4-response genes [24].

Our recent studies suggest that TGF β 1 does not regulate *Foxp3* expression directly because the generation and maintenance of CD4⁺CD25⁺ T_{reg} cells (FOXP3⁺) are actually increased in transgenic TCR-expressing *Tgfb1*^{-/-} mice. CTLA-4 is required for *Foxp3* expression in activated T cells in the presence of TGF β 1, although it is not required for *Foxp3* expression in nT_{reg} cells that develop in the thymus [48]. However, expression of FOXP3 alone in T_{reg} cells is not sufficient for their function because FOXP3⁺ T_{reg} cells in *Tgfb1*^{-/-} mice are unable to prevent the activation of CD4⁺CD25⁻ T cells, which suggests that TGF β 1 is required for their suppressor function (R. Bommireddy *et al.*, unpublished).

Mouse models in which TGF β signaling is disrupted develop cell-autonomous autoimmune disease (Table 3), suggesting an important physiological role for TGF β 1-dependent T_{reg} cells. In nonobese diabetic (NOD) mice, which develop spontaneous autoimmune type 1 diabetes,

CD4⁺CD25^{hi} T_{reg} cells, but not CD4⁺CD25⁺ cells, exhibit regulatory function and the absence of T_{reg}-cell function in CD4⁺CD25⁺ cells is owing to a lack of TGFβ1 production [2,49]. In these mice, the proportion of TGFβ1-producing T_{reg} cells decreases with age and correlates with disease onset and progression [50]. Consistent with this observation, Green *et al.* have shown that TGFβ signaling-deficient T cells are not inhibited by T_{reg} cells *in vivo*, suggesting that TGFβ-dependent T_{reg} cells are major factors in tolerance induction [51]. T_{reg}-cell numbers are reduced in the periphery of mice either lacking TGFβR2 or expressing a *dn-Tgfr2* in T cells [14,15,52]. These data suggest that autocrine TGFβ1 in T_{reg} cells is important for their maintenance, expansion and suppressor function. This is also supported by the finding that *Tgfb1* KO mice, *Tgfr2* CKO in T cells and *dn-Tgfr2* transgenic mice all exhibit similar autoimmune phenotypes, with the delay of onset of autoimmunity in the *dn-Tgfr2* mice being a result of transgene leakiness (Table 3). Together, these *in vivo* and *in vitro* studies suggest that TGFβ1-deficient T_{reg} cells are not effective in a TGFβ-deficient environment [39,44] (Figure 2). Further studies are required to understand the molecular mechanisms that control the expression of TGFβ1 in T_{reg} cells during infection and inflammatory diseases.

T_{reg} cells in tolerance and autoimmune diseases

T_{reg} cells inhibit T_h-cell proliferation in response to the TCR (αCD3) and CD4⁺CD25⁺-depleted CD4⁺ T cells proliferate more vigorously than undepleted CD4⁺ T cells *in vitro* [1]. T_{reg} cells inhibit IL-2 and IFN-γ production by T_h cells (Figure 2) and they also inhibit colitis, diabetes and graft-versus-host disease [11,53,54]. T_{reg}-cell functions have been observed both in CD4⁺CD25⁺ and CD4⁺CD25⁻ T cells. These data indicate that T_{reg} cells, whether they express CD25 or not, act as suppressor cells that exhibit immunoregulatory function. T_{reg}-cell development and function has been studied extensively in NOD mice ([2] and references therein).

Immune regulation is achieved by tightly controlled interactions among cytokines, surface receptors and transcription factors [55]. Deficiency in all or several of these, such as TGFβ1, CTLA-4 or FOXP3, leads to severe auto-immune disease and over-expression of either TGFβ1 or FOXP3 protects mice from autoimmune diseases [1,46,56]. Many autoimmune diseases have been attributed to the loss of T_{reg}-cell function, which involves the production of TGFβ1 and IL-10 [1]. In IL-10-deficient mice, which develop colitis, TGFβ signaling is also deficient, suggesting that IL-10 regulates TGFβ signaling positively in intestinal epithelial cells [57].

FOXP3 in T_{reg}-cell generation

The *Foxp3* gene (Scurfin; forkhead/winged helix transcription factor) is essential for the development and maintenance of T_{reg} cells in the periphery. A defect in the human *FOXP3* gene leads to IPEX (immune deficiency, polyendocrinopathy, enteropathy, X-linked) syndrome, a systemic autoimmune disease, suggesting that FOXP3 is important for T-cell tolerance [58,59]. Recent studies from Rudensky's lab suggest that targeted depletion of FOXP3-expressing T_{reg} cells in mice results in severe autoimmune disease [60]. We propound the excellent reviews by Rudensky and Ziegler on the role of FOXP3 in immune tolerance [5,61]. FOXP3 complexed with NF-AT binds strongly to the *Il2* promoter, thereby repressing its expression; the same complex induces *Il2ra* (CD25) and *Ctla4* expression [62]. TGFβ1 inhibits NF-AT activation in T cells and also induces *Foxp3* *in vitro* [24,63].

Recently, Ono *et al.* have shown that FOXP3 interacts physically with and inhibits the function of transcription factor acute myeloid leukemia-1 (AML-1)/Runt-related transcription factor 1 (Runx1), which is important for T-cell development and IL-2 and IFN-γ expression in CD4⁺ T cells [64]. These data suggest that FOXP3 induces T_{reg}-cell generation through inhibition of IL-2 by interacting with other transcription factors that are involved in IL-2 induction. FOXP3

also induces Toll-like receptor (TLR)10 in T_{reg} cells by binding to the promoter elements of *TLR10* directly and by activating its expression [65]. TLR10 is expressed only in T_{reg} cells but not in non-T_{reg} cells and its expression is enhanced by TCR stimulation in a calcineurin-dependent manner. NF-AT cooperates with FOXP3 in inducing *TLR10*. Recently, it was shown that mere expression of FOXP3 by TGFβ1 in human CD4⁺ T cells does not confer suppressive properties to these T_{reg} cells, suggesting that additional factors are required for FOXP3⁺ T_{reg}-cell suppressor function [66]. All these data suggest that FOXP3 is essential for the development of T_{reg} cells but is not sufficient to confer suppressor function. Although FOXP3 inhibits IL-2 within T_{reg} cells, it must depend on some other factors, such as TGFβ1 or CTLA-4, in order to suppress effector T-cell responses.

CTLA-4 in T_{reg}-cell generation and function

CTLA-4, a negative-regulatory molecule, is expressed constitutively on nT_{reg} cells and is required for their suppressor function [1]. *Ctla4*^{-/-} mice develop a lethal lymphoproliferative disorder, suggesting that CTLA-4 has an important role in preventing self-reactive T-cell activation. Over-expression of *Foxp3* delays the development of auto-immune disease in *Ctla4*^{-/-} mice, suggesting that FOXP3 compensates for the loss of CTLA-4 [58]. We propound a Sansom and Walker review on the role of CTLA-4 in T-cell regulation [67].

Ctla4^{-/-} T_{reg} cells cause suppression of T-cell proliferation in a TGFβ1-dependent manner *in vitro* [3]. Because cross-linking of CTLA-4 on activated T cells induces TGFβ1 production and because TGFβ1 accelerates CTLA-4 expression *in vitro*, it is plausible that CTLA-4-deficient T cells might not exhibit suppressor function owing to a deficiency in TGFβ1 production *in vivo* [68]. Activation of CTLA-4 by cross-linking enhances T_{reg}-cell production of TGFβ1 and its surface accumulation at the site of cell-cell interactions and a loss of membrane-bound TGFβ1 is associated with the onset of diabetes in NOD mice [8,45,68]. Oida *et al.* also showed that blocking TGFβ signaling with a receptor-kinase inhibitor abrogated the suppressive function of T_{reg} cells, suggesting that TGFβ is the primary mediator of T_{reg}-cell suppressor function [8]. These observations suggest that TGFβ1 production is both regulated tightly and important for CD4⁺CD25⁺CTLA-4⁺ T_{reg}-cell function *in vivo* (Figure 2).

The role of signal strength in T-cell activation and tolerance

For productive immune responses, T cells require at least two signals: a primary signal provided by the TCR or CD3 and a secondary co-stimulatory signal provided by CD28 interacting with CD80 and CD86 (B7-1 and B7-2) on APCs. Stimulation of T cells with ionomycin alone leads to anergy in the absence of protein kinase C (PKC) activation. Stimulation of T cells with phorbol 12-myristate 13-acetate (PMA) leads to the activation of nuclear factor-κB (NF-κB) and activator protein-1 (AP-1, a complex of two proteins, FOS and JUN), which prevents anergy induction by ionomycin [69]. FOXP3⁺ T_{reg} cells are anergic to stimulation by αCD3 and αCD28. TGFβ1 induces the conversion of naïve T cells to T_{reg} cells that express *Foxp3* in the presence of αCD3 and αCD28 [24]. These data suggest that activation-signal strength instructs T cells to either become effector T cells or anergic (a state of unresponsiveness); TGFβ1 modulates TCR signal strength through its positive effects on casitas B-lineage lymphoma B (CBL-B) and CTLA-4 production and its negative effects on calcineurin [21, 63,70]. Although CBL-B-deficient T cells express FOXP3, TGFβ1-induced FOXP3 expression is dependent on CBL-B to a large extent [70]. CBL-B-deficient effector T cells are also resistant to suppression by T_{reg} cells and TGFβ, suggesting that TGFβ exerts its suppressor functions through CBL-B [71]. Blockade of co-stimulatory signals through CD4 or CD40L induces peripheral tolerance [72]. CD4⁺CD25⁺ T_{reg} cells can be generated from CD4⁺CD25⁻ T cells *in vivo* after treatment with non-mito-genic αCD3 F(ab')₂ fragments, suggesting that tolerance and the immune response to antigens are regulated by signal strength

[47,49]. Similarly, another study by Ochi *et al.* using oral therapy of α CD3, which induces LAP⁺ [latency-associated peptide (positive)] CD4⁺ T cells, confirms that TGF β 1-dependent T_{reg}-cell generation is important for preventing autoimmune disease [73].

DCs in antigen presentation and T-cell tolerance

DCs have important roles in antigen presentation and effective T-cell responses. If naïve T cells encounter antigen on immature DCs (iDCs), they are tolerized and become T_{reg} cells rather than effector T cells. Co-stimulatory ligands (CD80 and CD86) are up-regulated on DC maturation. These mature DCs are more potent in activating naïve T cells to become effector T cells (Figure 2). Co-stimulatory molecules are also important for the generation of nT_{reg} cells [74]. Elimination of these molecules by gene ablation results in decreased T_{reg} cells. Bluestone *et al.* demonstrated that T_{reg} cells interact directly with APCs, such as DCs, *in vivo* and prevent further arrest of effector T cells on DCs [53]. CTLA-4 down-modulates B7 molecules on DCs *in vitro* and these conditioned DCs (DCs that have encountered T_{reg} cells) induce poor T-cell proliferation [75]. Conditioned DCs might produce the immunosuppressive cytokine TGF β 1, whereas those DCs that encounter naïve T cells might produce the pro-inflammatory cytokine IL-12 and induce a T_H1 response. Consistently, CTLA-4 blockade results in decreased production of TGF β 1 and indoleamine 2,3-dioxygenase (IDO) and an increased T-cell response to viral infection [76]. Naïve T-cell interactions with conditioned DCs in a TGF β 1-rich environment could be tolerized rather than activated. T_{reg}-cell-produced TGF β 1 can act on both DCs and naïve T cells and can down-regulate their responses. TGF β 1 prevents DC maturation by inhibiting co-stimulatory-molecule expression [77].

DCs express IDO on interaction with CTLA-4, which is expressed constitutively on T_{reg} cells [78]. IDO catabolizes tryptophan and leads to immunosuppression by T-cell anergy [79]. T_{reg} cells induce reverse signaling in DCs on their engagement with DCs through CTLA-4 and its ligand CD80 on DCs. Because T_{reg} cells induce TGF β 1 in APCs, it is possible that there is signaling cross-talk in DCs that leads to TGF β 1 production. CTLA-4-Ig treatment causes T-cell anergy, which is dependent on IDO expression, because IDO-deficient DCs are not capable of inducing T-cell tolerance [80]. However, it is unclear whether TGF β 1 production is also impaired in IDO-deficient DCs. CTLA-4-Ig-treated *Tgfb1*^{-/-} mice do not live longer than untreated *Tgfb1*^{-/-} mice and they show no reduction in the severity of inflammation as compared with that in control *Tgfb1*^{-/-} mice, therefore, we speculate that CTLA-4-Ig treatment induces tolerance through TGF β 1 production [38,81] (T. Doetschman, unpublished). More recently, Coombes *et al.* has shown that integrin CD103-expressing DCs are involved in peripheral T_{reg}-cell generation, especially in the intestine, and are responsible for oral tolerance. These DCs capture food antigens and present them to T cells to induce oral tolerance. Blocking retinoic acid (RA) receptors prevented the FOXP3 induction by CD103⁺ DCs, suggesting that this process is dependent on the Vitamin A metabolite RA. CD103⁺ DCs express higher levels of *Tgfb2*, latent TGF β -binding protein 3 (*Ltbp3*) and tissue-plasminogen activator compared with CD103⁻ DCs [82]. Consistently, Mucida *et al.* have also found that RA inhibits TGF β 1-induced ROR γ t and Th17-cell generation in the presence of IL-6 from splenocytes *in vitro*, whereas it increased TGF β 1-induced FOXP3⁺ T_{reg}-cell generation [83]. These data suggest an important role for TGF β in the induction of oral tolerance by DCs in the gut.

Concluding remarks

The recent explosion in publications on T_{reg} cells and the role of TGF β 1 in tolerance induction has led to the conclusion that TGF β 1 is central to self-tolerance. The primary role for TGF β 1 is to prevent T-cell activation by self-Ag presentation. A strong co-stimulatory environment over-rides the suppressor function of TGF β 1 and enables T cells to respond to invading

pathogens. T_{reg} cells have an important role in maintaining lymphocyte homeostasis and perturbations in T_{reg}-cell generation or function lead to autoreactive T-cell expansion. The majority of T_{reg} cells depend on TGFβ1 for their suppressor function, although they do not need to produce it. However, TGFβ signaling is essential for T cells to respond to T_{reg}-cell function. CTLA-4 and FOXP3 are also important for T_{reg}-cell generation, maintenance and function. Although FOXP3 is important for the generation of T_{reg} cells, CTLA-4 is important for the maintenance of the T_{reg}-cell pool in the periphery because it is involved in the induction of *Foxp3* and the production of TGFβ1 [67]. It is noteworthy that, in humans, unlike mouse, TGFβ inhibits T_H17 differentiation [84]. Current studies focusing on the role of TGFβ1 in T-cell subsets using CKO mice will help to clarify the complex roles for TGFβ1 in these processes (Box 1). Present approaches to restoring the T_{reg}-cell pool using antibody therapy or gene-transfer therapy will lead to better treatment options for autoimmune diseases. However, either blocking T_{reg} function or depleting T_{reg} cells will boost the antitumor immune responses.

Outstanding questions

- Unlike TGFβ1, FOXP3 is an intracellular transcription factor expressed only in T_{reg} cells. How does it mediate the suppressor function of T_{reg} cells?
- Because FOXP3-deficient mice develop a similar autoimmune phenotype as *Tgfb1*^{-/-} mice, does FOXP3 induce TGFβ1 expression in T_{reg} cells?
- Because TGFβ1 inhibits autoimmunity but does not inhibit the immune response to invading pathogens, is TGFβ1 expression regulated by the strength of the TCR signals?

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References

1. Miyara M, Sakaguchi S. Natural regulatory T cells: mechanisms of suppression. *Trends Mol. Med* 2007;13:108–116. [PubMed: 17257897]
2. Bommireddy R, Doetschman T. TGFβ, T-cell tolerance and anti-CD3 therapy. *Trends Mol. Med* 2004;10:3–9. [PubMed: 14720578]
3. Tang Q, et al. Distinct roles of CTLA-4 and TGFβ in CD4⁺CD25⁺ regulatory T cell function. *Eur. J. Immunol* 2004;34:2996–3005. [PubMed: 15468055]
4. Shevach EM. Regulatory/suppressor T cells in health and disease. *Arthritis Rheum* 2004;50:2721–2724. [PubMed: 15457438]
5. Rudensky A. Foxp3 and dominant tolerance. *Philos. Trans. R. Soc. Lond. B Biol. Sci* 2005;360:1645–1646. [PubMed: 16147528]
6. Annacker O, et al. Essential role for CD103 in the T cell-mediated regulation of experimental colitis. *J. Exp. Med* 2005;202:1051–1061. [PubMed: 16216886]
7. Beissert S, et al. Regulatory T cells. *J. Invest. Dermatol* 2006;126:15–24. [PubMed: 16417213]
8. Oida T, et al. TGFβ-mediated suppression by CD4⁺CD25⁺ T cells is facilitated by CTLA-4 signaling. *J. Immunol* 2006;177:2331–2339. [PubMed: 16887994]
9. Izcue A, et al. Regulatory T cells suppress systemic and mucosal immune activation to control intestinal inflammation. *Immunol. Rev* 2006;212:256–271. [PubMed: 16903919]
10. Graca L, et al. Dominant transplantation tolerance. *Curr. Opin. Immunol* 2003;15:499–506. [PubMed: 14499256]

11. Uhlig HH, et al. Characterization of Foxp3⁺CD4⁺CD25⁺ and IL-10-secreting CD4⁺CD25⁺ T cells during cure of colitis. *J. Immunol* 2006;177:5852–5860. [PubMed: 17056509]
12. Li MO, et al. Transforming growth factor- β regulation of immune responses. *Annu. Rev. Immunol* 2006;24:99–146. [PubMed: 16551245]
13. Schmidt-Weber CB, Blaser K. Regulation and role of transforming growth factor- β in immune tolerance induction and inflammation. *Curr. Opin. Immunol* 2004;16:709–716. [PubMed: 15511662]
14. Marie JC, et al. Cellular mechanisms of fatal early-onset autoimmunity in mice with the T cell-specific targeting of transforming growth factor- β receptor. *Immunity* 2006;25:441–454. [PubMed: 16973387]
15. Li MO, et al. Transforming growth factor- β controls development, homeostasis, and tolerance of T cells by regulatory T cell-dependent and -independent mechanisms. *Immunity* 2006;25:455–471. [PubMed: 16973386]
16. Rubtsov YP, Rudensky AY. TGF β signalling in control of T-cell-mediated self-reactivity. *Nat. Rev. Immunol* 2007;7:443–453. [PubMed: 17525753]
17. Bettelli E, et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature* 2006;441:235–238. [PubMed: 16648838]
18. Korn T, et al. IL-21 initiates an alternative pathway to induce proinflammatory T(H)17 cells. *Nature* 2007;448:484–487. [PubMed: 17581588]
19. Weaver CT, et al. Th17: an effector CD4 T cell lineage with regulatory T cell ties. *Immunity* 2006;24:677–688. [PubMed: 16782025]
20. Chen W, Wahl SM. TGF β : receptors, signaling pathways and autoimmunity. *Curr. Dir. Autoimmun* 2002;5:62–91. [PubMed: 11826761]
21. Bommireddy R, et al. TGF β 1 inhibits Ca²⁺-calcineurin-mediated activation in thymocytes. *J. Immunol* 2003;170:3645–3652. [PubMed: 12646629]
22. Bommireddy R, et al. TGF β 1 regulates lymphocyte homeostasis by preventing activation and subsequent apoptosis of peripheral lymphocytes. *J. Immunol* 2003;170:4612–4622. [PubMed: 12707339]
23. Shou W, et al. Cardiac defects and altered ryanodine receptor function in mice lacking FKBP12. *Nature* 1998;391:489–492. [PubMed: 9461216]
24. Fantini MC, et al. Cutting edge: TGF β induces a regulatory phenotype in CD4⁺CD25⁺ T cells through Foxp3 induction and down-regulation of Smad7. *J. Immunol* 2004;172:5149–5153. [PubMed: 15100250]
25. Zheng SG, et al. TGF β requires CTLA-4 early after T cell activation to induce FoxP3 and generate adaptive CD4⁺CD25⁺ regulatory cells. *J. Immunol* 2006;176:3321–3329. [PubMed: 16517699]
26. Veldhoen M, et al. Signals mediated by transforming growth factor- β initiate autoimmune encephalomyelitis, but chronic inflammation is needed to sustain disease. *Nat. Immunol* 2006;7:1151–1156. [PubMed: 16998492]
27. Ivanov II, et al. The orphan nuclear receptor ROR γ t directs the differentiation program of proinflammatory IL-17(+) T helper cells. *Cell* 2006;126:1121–1133. [PubMed: 16990136]
28. Lin JT, et al. TGF β 1 uses distinct mechanisms to inhibit IFN- γ expression in CD4⁺ T cells at priming and at recall: differential involvement of Stat4 and T-bet. *J. Immunol* 2005;174:5950–5958. [PubMed: 15879087]
29. Park IK, et al. TGF β 1 inhibits T-bet induction by IFN- γ in murine CD4⁺ T cells through the protein tyrosine phosphatase Src homology region 2 domain-containing phosphatase-1. *J. Immunol* 2005;175:5666–5674. [PubMed: 16237056]
30. Gorelik L, et al. Cutting edge: TGF β inhibits Th type 2 development through inhibition of GATA-3 expression. *J. Immunol* 2000;165:4773–4777. [PubMed: 11045997]
31. Lund R, et al. Identification of novel genes regulated by IL-12, IL-4, or TGF β during the early polarization of CD4⁺ lymphocytes. *J. Immunol* 2003;171:5328–5336. [PubMed: 14607935]
32. Zhou M, Ouyang W. The function role of GATA-3 in Th1 and Th2 differentiation. *Immunol. Res* 2003;28:25–37. [PubMed: 12947222]
33. Bommireddy R, et al. Self-antigen recognition by TGF β 1-deficient T cells causes their activation and systemic inflammation. *Lab. Invest* 2006;86:1008–1019. [PubMed: 16865088]

34. Mangan PR, et al. Transforming growth factor- β induces development of the T(H)17 lineage. *Nature* 2006;441:231–234. [PubMed: 16648837]
35. Gorham JD, et al. Genetic regulation of autoimmune disease: BALB/c background TGF β 1-deficient mice develop necroinflammatory IFN- γ -dependent hepatitis. *J. Immunol* 2001;166:6413–6422. [PubMed: 11342667]
36. Bommireddy R, et al. Elimination of both CD4(+) and CD8(+) T cells but not B cells eliminates inflammation and prolongs the survival of TGF β 1-deficient mice. *Cell. Immunol* 2004;232:96–104. [PubMed: 15922720]
37. Manzotti CN, et al. Integration of CD28 and CTLA-4 function results in differential responses of T cells to CD80 and CD86. *Eur. J. Immunol* 2006;36:1413–1422. [PubMed: 16708397]
38. Mamura M, et al. CD28 disruption exacerbates inflammation in *Tgf- β 1^{-/-}* mice: *in vivo* suppression by CD4⁺CD25⁺ regulatory T cells independent of autocrine TGF β 1. *Blood* 2004;103:4594–4601. [PubMed: 15016653]
39. Fahlen L, et al. T cells that cannot respond to TGF β escape control by CD4(+)CD25(+) regulatory T cells. *J. Exp. Med* 2005;201:737–746. [PubMed: 15753207]
40. Nakamura K, et al. Cell contact-dependent immunosuppression by CD4 (+)CD25(+) regulatory T cells is mediated by cell surface-bound transforming growth factor β . *J. Exp. Med* 2001;194:629–644. [PubMed: 11535631]
41. Piccirillo CA, et al. CD4(+)CD25(+) regulatory T cells can mediate suppressor function in the absence of transforming growth factor β 1 production and responsiveness. *J. Exp. Med* 2002;196:237–246. [PubMed: 12119348]
42. Li MO, et al. T cell-produced transforming growth factor- β 1 controls T cell tolerance and regulates Th1- and Th17-cell differentiation. *Immunity* 2007;26:579–591. [PubMed: 17481928]
43. Kullberg MC, et al. TGF β 1 production by CD4(+)CD25(+) regulatory T cells is not essential for suppression of intestinal inflammation. *Eur. J. Immunol* 2005;35:2886–2895. [PubMed: 16180248]
44. Marie JC, et al. TGF{beta}1 maintains suppressor function and Foxp3 expression in CD4⁺CD25⁺ regulatory T cells. *J. Exp. Med* 2005;201:1061–1067. [PubMed: 15809351]
45. Gregg RK, et al. A sudden decline in active membrane-bound TGF{beta} impairs both T regulatory cell function and protection against autoimmune diabetes. *J. Immunol* 2004;173:7308–7316. [PubMed: 15585854]
46. Carrier Y, et al. Th3 cells in peripheral tolerance. *I. Induction of Foxp3-positive regulatory T cells by Th3 cells derived from TGF β T cell-transgenic mice*. *J. Immunol* 2007;178:179–185. [PubMed: 17182553]
47. Graca L, et al. Dominant tolerance: activation thresholds for peripheral generation of regulatory T cells. *Trends Immunol* 2005;26:130–135. [PubMed: 15745854]
48. Zheng SG, et al. Natural and induced CD4⁺CD25⁺ cells educate CD4⁺CD25⁻ cells to develop suppressive activity: the role of IL-2, TGF β , and IL-10. *J. Immunol* 2004;172:5213–5221. [PubMed: 15100259]
49. Chatenoud L. CD3-specific antibodies restore self-tolerance: mechanisms and clinical applications. *Curr. Opin. Immunol* 2005;17:632–637. [PubMed: 16214320]
50. Pop SM, et al. Single cell analysis shows decreasing FoxP3 and TGF{beta}1 coexpressing CD4⁺CD25⁺ regulatory T cells during autoimmune diabetes. *J. Exp. Med* 2005;201:1333–1346. [PubMed: 15837817]
51. Green EA, et al. CD4⁺CD25⁺ T regulatory cells control anti-islet CD8⁺ T cells through TGF{beta}–TGF{beta} receptor interactions in Type 1 diabetes. *Proc. Natl. Acad. Sci. U. S. A* 2003;100:10878–10883. [PubMed: 12949259]
52. Schramm C, et al. TGF β regulates the CD4⁺CD25⁺ T-cell pool and the expression of Foxp3 *in vivo*. *Int. Immunol* 2004;16:1241–1249. [PubMed: 15249539]
53. Tang Q, et al. Visualizing regulatory T cell control of autoimmune responses in nonobese diabetic mice. *Nat. Immunol* 2006;7:83–92. [PubMed: 16311599]
54. Edinger M, et al. CD4(+)CD25(+) regulatory T cells preserve graft-versus-tumor activity while inhibiting graft-versus-host disease after bone marrow transplantation. *Nat. Med* 2003;9:1144–1150. [PubMed: 12925844]

55. Kane LP, et al. Signal transduction by the TCR for antigen. *Curr. Opin. Immunol* 2000;12:242–249. [PubMed: 10781399]
56. Grewal IS, et al. Expression of transgene encoded TGF β in islets prevents autoimmune diabetes in NOD mice by a local mechanism. *J. Autoimmun* 2002;19:9–22. [PubMed: 12367555]
57. Ruiz PA, et al. IL-10 gene-deficient mice lack TGF β /Smad signaling and fail to inhibit proinflammatory gene expression in intestinal epithelial cells after the colonization with colitogenic *Enterococcus faecalis*. *J. Immunol* 2005;174:2990–2999. [PubMed: 15728512]
58. Khattri R, et al. An essential role for Scurfin in CD4⁺CD25⁺ T regulatory cells. *Nat. Immunol* 2003;4:337–342. [PubMed: 12612581]
59. Gambineri E, et al. Immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance (IPEX), a syndrome of systemic autoimmunity caused by mutations of FOXP3, a critical regulator of T-cell homeostasis. *Curr. Opin. Rheumatol* 2003;15:430–435. [PubMed: 12819471]
60. Williams LM, Rudensky AY. Maintenance of the Foxp3-dependent developmental program in mature regulatory T cells requires continued expression of Foxp3. *Nat. Immunol* 2007;8:277–284. [PubMed: 17220892]
61. Ziegler SF. FOXP3: of mice and men. *Annu. Rev. Immunol* 2006;24:209–226. [PubMed: 16551248]
62. Wu Y, et al. FOXP3 Controls regulatory T cell function through cooperation with NFAT. *Cell* 2006;126:375–387. [PubMed: 16873067]
63. Chen CH, et al. Transforming growth factor β blocks Tec kinase phosphorylation, Ca²⁺ influx, and NFATc translocation causing inhibition of T cell differentiation. *J. Exp. Med* 2003;197:1689–1699. [PubMed: 12810687]
64. Ono M, et al. Foxp3 controls regulatory T-cell function by interacting with AML1/Runx1. *Nature* 2007;446:685–689. [PubMed: 17377532]
65. Bell MP, et al. Forkhead Box P3 regulates TLR10 expression in human T regulatory cells. *J. Immunol* 2007;179:1893–1900. [PubMed: 17641056]
66. Tran DQ, et al. Induction of FOXP3 expression in naive human CD4⁺FOXP3[−] T cells by T cell receptor stimulation is TGF β -dependent but does not confer a regulatory phenotype. *Blood* 2007;110:2983–2990. [PubMed: 17644734]
67. Sansom DM, Walker LS. The role of CD28 and cytotoxic T-lymphocyte antigen-4 (CTLA-4) in regulatory T-cell biology. *Immunol. Rev* 2006;212:131–148. [PubMed: 16903911]
68. Chen W, et al. Engagement of cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) induces transforming growth factor beta (TGF β) production by murine CD4(+) T cells. *J. Exp. Med* 1998;188:1849–1857. [PubMed: 9815262]
69. Macian F, et al. Transcriptional mechanisms underlying lymphocyte tolerance. *Cell* 2002;109:719–731. [PubMed: 12086671]
70. Wohlfert EA, et al. Cutting edge: Deficiency in the E3 ubiquitin ligase Cbl-b results in a multifunctional defect in T cell TGF β sensitivity *in vitro* and *in vivo*. *J. Immunol* 2006;176:1316–1320. [PubMed: 16424156]
71. Wohlfert EA, et al. Resistance to CD4⁺CD25⁺ regulatory T cells and TGF β in Cbl-b^{−/−} mice. *J. Immunol* 2004;173:1059–1065. [PubMed: 15240694]
72. Graca L, et al. Cutting edge: Anti-CD154 therapeutic antibodies induce infectious transplantation tolerance. *J. Immunol* 2000;165:4783–4786. [PubMed: 11045999]
73. Ochi H, et al. Oral CD3-specific antibody suppresses autoimmune encephalomyelitis by inducing CD4(+)CD25(−)LAP(+) T cells. *Nat. Med* 2006;12:627–635. [PubMed: 16715091]
74. Salomon B, et al. B7/CD28 costimulation is essential for the homeostasis of the CD4⁺CD25⁺ immunoregulatory T cells that control autoimmune diabetes. *Immunity* 2000;12:431–440. [PubMed: 10795741]
75. Oderup C, et al. Cytotoxic T lymphocyte antigen-4-dependent down-modulation of costimulatory molecules on dendritic cells in CD4⁺ CD25⁺ regulatory T-cell-mediated suppression. *Immunology* 2006;118:240–249. [PubMed: 16771859]
76. Hryniewicz A, et al. CTLA-4 blockade decreases TGF β , IDO, and viral RNA expression in tissues of SIVmac251-infected macaques. *Blood* 2006;108:3834–3842. [PubMed: 16896154]

77. Lyakh LA, et al. TGF β and vitamin D3 utilize distinct pathways to suppress IL-12 production and modulate rapid differentiation of human monocytes into CD83⁺ dendritic cells. *J. Immunol* 2005;174:2061–2070. [PubMed: 15699136]
78. Grohmann U, et al. CTLA-4-Ig regulates tryptophan catabolism *in vivo*. *Nat. Immunol* 2002;3:1097–1101. [PubMed: 12368911]
79. Munn DH, et al. GCN2 kinase in T cells mediates proliferative arrest and anergy induction in response to indoleamine 2,3-dioxygenase. *Immunity* 2005;22:633–642. [PubMed: 15894280]
80. Mellor AL, et al. Cutting edge: induced indoleamine 2,3 dioxygenase expression in dendritic cell subsets suppresses T cell clonal expansion. *J. Immunol* 2003;171:1652–1655. [PubMed: 12902462]
81. Tivol EA, et al. CTLA4Ig prevents lymphoproliferation and fatal multiorgan tissue destruction in CTLA-4-deficient mice. *J. Immunol* 1997;158:5091–5094. [PubMed: 9164923]
82. Coombes JL, et al. A functionally specialized population of mucosal CD103⁺ DCs induces Foxp3⁺ regulatory T cells via a TGF β - and retinoic acid-dependent mechanism. *J. Exp. Med* 2007;204:1757–1764. [PubMed: 17620361]
83. Mucida D, et al. Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. *Science* 2007;317:256–260. [PubMed: 17569825]
84. Acosta-Rodriguez EV, et al. Interleukins 1 β and 6 but not transforming growth factor- β are essential for the differentiation of interleukin 17-producing human T helper cells. *Nat. Immunol* 2007;8:942–949. [PubMed: 17676045]
85. Shevach EM. From vanilla to 28 flavors: multiple varieties of T regulatory cells. *Immunity* 2006;25:195–201. [PubMed: 16920638]
86. Shull MM, et al. Targeted disruption of the mouse transforming growth factor- β 1 gene results in multifocal inflammatory disease. *Nature* 1992;359:693–699. [PubMed: 1436033]
87. Engle SJ, et al. Transforming growth factor β 1 suppresses nonmetastatic colon cancer at an early stage of tumorigenesis. *Cancer Res* 1999;59:3379–3386. [PubMed: 10416598]
88. Letterio JJ, et al. Autoimmunity associated with TGF β 1-deficiency in mice is dependent on MHC class II antigen expression. *J. Clin. Invest* 1996;98:2109–2119. [PubMed: 8903331]
89. Kobayashi S, et al. Beta 2-microglobulin-deficient background ameliorates lethal phenotype of the TGF β 1 null mouse. *J. Immunol* 1999;163:4013–4019. [PubMed: 10491004]
90. Gorelik L, Flavell RA. Abrogation of TGF β signaling in T cells leads to spontaneous T cell differentiation and autoimmune disease. *Immunity* 2000;12:171–181. [PubMed: 10714683]
91. Lucas PJ, et al. Disruption of T cell homeostasis in mice expressing a T cell-specific dominant negative transforming growth factor β II receptor. *J. Exp. Med* 2000;191:1187–1196. [PubMed: 10748236]
92. Tivol EA, et al. Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. *Immunity* 1995;3:541–547. [PubMed: 7584144]
93. Yang X, et al. Targeted disruption of SMAD3 results in impaired mucosal immunity and diminished T cell responsiveness to TGF β . *EMBO J* 1999;18:1280–1291. [PubMed: 10064594]
94. Kim BG, et al. Smad4 signalling in T cells is required for suppression of gastrointestinal cancer. *Nature* 2006;441:1015–1019. [PubMed: 16791201]
95. Chiang YJ, et al. Cbl-b regulates the CD28 dependence of T-cell activation. *Nature* 2000;403:216–220. [PubMed: 10646609]
96. Lorenz U, et al. Lack of SHPTP1 results in src-family kinase hyperactivation and thymocyte hyperresponsiveness. *Proc. Natl. Acad. Sci. U. S. A* 1996;93:9624–9629. [PubMed: 8790380]

Figure 1.

Cross-talk between TCR and TGF β signaling networks. TCR activation on recognition of MHC–Ag complexes on APCs leads to the phosphorylation of ZAP70, PLC γ and Lck. PLC γ hydrolyses PIP $_2$ to generate DAG and IP $_3$. DAG binds to and activates PKC, whereas IP $_3$ binds to its receptor on the endoplasmic reticulum (ER) and releases Ca $^{2+}$ into cytoplasm. Activated PKC activates NF- κ B by degrading I- κ B through phosphorylation. Increased cytosolic Ca $^{2+}$ leads to the activation of calcineurin (CN), which, in turn, activates NF-AT through dephosphorylation. Active NF- κ B and NF-AT translocate to the nucleus and induce the gene transcription that is required for the T-cell response. Co-stimulatory signals through CD28 activate the JNK/p38 MAPK pathway, which activates AP-1 (FOS/JUN). CD28 co-stimulatory signals inhibit *Cblb*, which is a negative regulator of TCR signals. Signaling through CD28 activates AKT, which, in turn, inhibits SMAD2/3 phosphorylation. Co-stimulatory signaling through CTLA-4, which is a negative regulator of TCR signaling, induces *Cblb*. TGF β -receptor signaling activates SMAD2/3 through phosphorylation. Activated SMAD2/3 binds to SMAD4 and the complex translocates to the nucleus. FOXP3 inhibits SMAD7, which is an inhibitory SMAD that inhibits TGF β signaling. CBL-B also mediates TGF β signaling in *Foxp3* induction. CD25 is the α subunit of the high-affinity receptor for the IL-2 complex and IL-2 is essential for TGF β 1-mediated induction of FOXP3. FKBP12, which is associated with the TGF β receptor, is released on ligand binding. FKBP12 binds to the IP $_3$ receptor and modulates Ca $^{2+}$ release from ER.

Figure 2.

Tolerance induction by T_{reg} cells. Antigen presentation by iDCs to T cells induces tolerance because iDCs do not provide co-stimulation (a). However, Ag presentation by mature DCs activates T cells and the inflammatory environment can trigger the activation of autoreactive T cells (b). (c) T_{reg} cells that express CTLA-4 and TGFβ1 in addition to CD28 interact with DCs and induce TGFβ1 production (conditioned DCs). Co-stimulatory molecules (CD80 and CD86) are down-modulated by TGFβ1 on conditioned DCs. Conditioned DCs induce tolerance in T cells by presenting Ag without co-stimulation and by TGFβ1 secretion.

Table 1Regulatory T-cell subsets^a

T_{reg} subset	Regulatory mechanisms	FOXP3 expression	Function
nT _{regs} (CD4 ⁺ CD25 ⁺)	Cell–cell contact dependent, cytokines (TGFβ1)	Yes	Induces self-tolerance, inhibits allergy and allograft rejection
T _r 1 cells	IL-10	No	Inhibits mucosal inflammation
T _h 3 cells (iT _{reg} cells)	TGFβ1	FOXP3	Inhibits autoimmunity
CD3 ⁺ CD4 ⁺ CD8 [−] (DN) T cells	Fas–FasL	No	Inhibits allograft rejection
CD4 ⁺ CD25 [−] T _{reg} cells (anergic CD4 ⁺ cells)	Cell–cell contact dependent	?	Inhibits allograft rejection
CD8 ⁺ CD122 ⁺	IL-10	No	Inhibits T-cell responses and IFN-γ production
NK-T _{reg} cells	IL-4, IL-10, TGFβ, cytotoxicity	?	Suppression of autoimmunity, elimination of tumors

^aReviewed in [7,85].

Table 2Phenotypes of TGF β 1-deficient mice on various genetic backgrounds

Genotype of KO mice	Survival age	Phenotype	Comments	Refs
<i>Tgfb1</i> ^{-/-}	3–4 wks	Severe multi-organ inflammation	Lymphocytes, macrophages and neutrophils cause the lesions	[86]
<i>Tgfb1</i> ^{-/-} <i>Rag</i> ^{*-/-} (lymphocyte-deficient <i>Tgfb1</i> ^{-/-} mice)	3–6 months	Mild inflammation in the gut and lungs	Macrophages and neutrophils cause the lesions. Inflammation in <i>Tgfb1</i> ^{-/-} mice is caused by lymphocytes primarily	[87]
<i>Tgfb1</i> ^{-/-} <i>Cd4</i> ^{-/-} (CD4 ⁺ T-cell-deficient <i>Tgfb1</i> ^{-/-} mice)	3–4 wks	Severe multi-organ inflammation	CD4 ⁺ T cells are not essential for inflammation in <i>Tgfb1</i> ^{-/-} mice	[36]
<i>Tgfb1</i> ^{-/-} <i>Cd8</i> ^{-/-} (CD8 ⁺ T-cell-deficient <i>Tgfb1</i> ^{-/-} mice)	3–4 wks	Severe multi-organ inflammation	CD8 ⁺ T cells are not essential for inflammation in <i>Tgfb1</i> ^{-/-} mice	[36]
<i>Tgfb1</i> ^{-/-} <i>Igh6</i> ^{-/-} (B-cell-deficient <i>Tgfb1</i> ^{-/-} mice)	4–6 wks	Severe multi-organ inflammation	T cells, macrophages and neutrophils are sufficient for inflammation in <i>Tgfb1</i> ^{-/-} mice	[36]
<i>Tgfb1</i> ^{-/-} <i>nu/nu</i> (Athymic nude <i>Tgfb1</i> ^{-/-} mice; T-cell deficient)	2–3 months	Mild inflammation in gut and lungs	T cells are essential for inflammation in <i>Tgfb1</i> ^{-/-} mice	[36]
<i>Tgfb1</i> ^{-/-} <i>Mhc2</i> ^{-/-} (MHC class II-deficient <i>Tgfb1</i> ^{-/-} mice)	3–4 wks	No CD4 ⁺ T cells, mild inflammation, no autoantibodies	MHC class II antigen presentation exacerbates inflammation in <i>Tgfb1</i> ^{-/-} mice	[88] (Doetschman, unpublished)
<i>Tgfb1</i> ^{-/-} <i>B2m</i> ^{-/-} (MHC class I-deficient <i>Tgfb1</i> ^{-/-} mice)	2–3 months	No CD8 ⁺ T cells, mild inflammation, reduced autoantibodies	MHC class I antigen presentation causes autoimmunity	[89]
<i>Tgfb1</i> ^{-/-} <i>Il6</i> ^{-/-}	1–3 months	Mild inflammation	IL-6 mediates inflammation in <i>Tgfb1</i> ^{-/-} mice	(Doetschman, unpublished)
<i>Tgfb1</i> ^{-/-} <i>Ifnγ</i> ^{-/-}	3–8 wks	Inflammation is eliminated only in the liver	IFN- γ is required for hepatic inflammation in <i>Tgfb1</i> ^{-/-} mice	[35]
<i>Tgfb1</i> ^{-/-} <i>Il4</i> ^{-/-}	3 wks	Inflammation severity is similar to that in <i>Tgfb1</i> ^{-/-} mice	IL-4 is not essential for inflammation in <i>Tgfb1</i> ^{-/-} mice	[35]
<i>Tgfb1</i> ^{-/-} <i>DO11.10</i> (TCR transgenic <i>Tgfb1</i> ^{-/-} mice)	2–3 months	Mild inflammation	Self-antigen recognition by T cells causes inflammation and autoimmunity in <i>Tgfb1</i> ^{-/-} mice	[33]
<i>Tgfb1</i> ^{-/-} <i>Cd28</i> ^{-/-}	2–3 wks	Inflammation in DKO mice is	T-cell co-stimulation is not essential for	[38]

Genotype of KO mice	Survival age	Phenotype	Comments	Refs
		more severe than in <i>Tgfb1</i> ^{-/-} mice	autoimmune disease in <i>Tgfb1</i> ^{-/-} mice	

*Rag** refers to *Rag1* or *Rag2* genes.

Table 3TGF β signaling is essential for the induction of self-tolerance *in vivo*

Knockout or inactivation mice	Phenotype	Comments	Refs
<i>Tgfb1</i>	Multifocal inflammatory autoimmune disease mediated by IFN- γ and IL-6 and T-cell dependent. Mice die within 3 weeks after birth.	TGF β 1 is essential to prevent T-cell activation. TCR down-modulation is an indication of self-antigen recognition <i>in vivo</i> . Disease is self-antigen specific.	[22,86]
<i>Dn- Tgfb2</i> (in T cells)	Mice develop autoimmune inflammatory disease approximately 5 months after birth but are resistant to the induction of EAE.	TGF β signaling in T cells is essential for tolerance induction. Transgenic-receptor expression might be leaky, hence the delay.	[90] [26,91]
<i>Tgfb2</i> (deleted in T cells)	Mice develop multi-organ autoimmune disease similar to <i>Tgfb1</i> KO mice. T-cell activation is not inhibited by wild-type T _{reg} cells. Increased apoptosis of T _{reg} cells and T cells with age and inflammation.	TGF β signaling is essential for prevention of activation of T cells and survival. TGF β signaling is not required for generation of T _{reg} cells but is essential for maintenance of T _{reg} cells in the periphery. T-cell-intrinsic defect of TGF β signaling causes spontaneous activation but no disease.	[14,15]
<i>Ctla4</i>	Massive lymphoproliferative inflammatory autoimmune disease mediated by T _H 2 cells. Mice die approximately 3 weeks after birth.	CTLA-4 is essential for T _{reg} -cell development and function. Over-expression of <i>Foxp3</i> rescues these mice from lethal autoimmune disease.	[58,92]
<i>Foxp3</i>	Massive lymphoproliferative inflammatory autoimmune disease mediated by both T _H 1 and T _H 2 cells. Mice die approximately 3 weeks after birth.	Foxp3 is essential for T _{reg} -cell development and function. Retroviral transfection of CD4 ⁺ T cells programs them to become T _{reg} cells. Up-regulates IL-10.	See [58] and references in [1]
<i>Smad3</i>	Multifocal formation of pyogenic abscesses within the wall of the stomach and the intestine. Involted thymus, smaller spleen and enlarged lymph nodes.	No apparent autoimmune diseases but impaired mucosal immunity. Increased number of activated T cells. Inflammation and cancer in the colon is due to activation of T cells against gut bacteria.	[93]
<i>Smad4</i> (in T cells)	Increased T _H 2 cytokines, gastrointestinal cancer.	Mice live longer (9–12 months).	[94]
<i>Cblb</i>	CD28-independent proliferation of T cells, mice are more susceptible for EAE, effector T cells are resistant to TGF β 1-inhibitory effect.	Mice are normal and live longer.	[95]
<i>Ptpn6</i> (SHP-1) (<i>motheaten</i> mice)	Thymocytes are hyper-responsive to TCR stimulation, increased T _{reg} -cell generation, T cells are resistant to TGF β 1-inhibitory effect.	Mice die early (2–3 wks) after birth. Effector T-cell resistance to T _{reg} -cell suppression is the cause of T-cell activation.	[29,96]