



Published in final edited form as:

*J Clin Immunol.* 2008 November ; 28(6): 647–659. doi:10.1007/s10875-008-9251-y.

## TGF- $\beta$ and Regulatory T Cell in Immunity and Autoimmunity

Yisong Y. Wan and

Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology, The University of North Carolina at Chapel Hill, School of Medicine, CB 7295, 450 West Drive, Chapel Hill, North Carolina, NC 27599-7295, USA wany@email.unc.edu

Richard A. Flavell

Howard Hughes Medical Institute, New Haven, CT 06520, USA

### Abstract

**Introduction**—The immune response is controlled by several inhibitory mechanisms. These mechanisms include regulatory T cells, which exist in multiple classes. Notable among these are Foxp3-expressing regulatory T cells (Treg), NKT cells, and Tr1 cells. Common to these mechanisms are inhibitory cytokines such as interleukin-10 and transforming growth factor-beta (TGF- $\beta$ ). TGF- $\beta$  and Foxp3-expressing Treg cells are critical in maintaining self-tolerance and immune homeostasis.

**Discussions**—The immune suppressive functions of TGF- $\beta$  and Treg cells are widely acknowledged and extensively studied. Nonetheless, recent studies revealed the positive roles for TGF- $\beta$  and Treg cells in shaping the immune system and the inflammatory responses. In this paper, we will discuss the role of these mechanisms in the control of immunity and autoimmunity and the mechanisms that underlie how these molecules control these responses.

### Keywords

TGF- $\beta$ ; Treg; NKT; IL-10

### Introduction

Adaptive and innate immune strategies have evolved in mammals to defend against foreign pathogens to maintain health. With highly antigen-specific surface receptors, T cells are pivotal for adaptive immune responses. Most T cells activate immune responses. Through quasi-random recombinational mechanisms, thymic derived T cells potentially possess infinite numbers of specificities toward foreign as well as self-antigens. Dysregulated self-reactive T cells can lead to autoimmune diseases. Multiple processes therefore are in place to suppress the generation or the function of self-reactive T cells. These T cells can be deleted in the thymus and the periphery. Such elimination processes are however incomplete, resulting in small populations of mostly low-affinity self-reactive T cells in the periphery to potentially initiate an autoimmune response. Fortunately, active immune suppressive mechanisms exist to suppress the function of these autoreactive T cells. Dysregulated immune suppression often results in immune disorders. Autoimmunity and inflammatory diseases can be caused by decreased immune suppression, while cancers are often associated with increased immune suppression. Great progress has been made in understanding the cellular and molecular components of immune suppression. Active immune suppression is mediated mostly through

either cytokines or through specialized cells. The pleiotropic cytokine, transforming growth factor-beta (TGF- $\beta$ ), and the immune-suppressive cell, previously called suppressor cells [1] and now usually termed regulatory T cells (Treg), play critical roles in suppressing the immune response. For years, we have been interested in the immune suppressive functions of TGF- $\beta$  and Treg under normal and immune-pathological conditions. Recently, our and other studies suggest that, in a cell-type and environment-dependent fashion, TGF- $\beta$  and Treg might also positively regulate immune responses. In this paper, we will discuss our views on the functions of TGF- $\beta$  and Treg in the immune regulation.

## TGF- $\beta$ and its Signaling

Consisting of a family of pleiotropic cytokines, TGF- $\beta$  regulates multi-faceted cellular functions including proliferation, differentiation, migration, and survival [2].

Among the three known isoforms of TGF- $\beta$  (TGF- $\beta$ 1, TGF- $\beta$ 2, and TGF- $\beta$ 3), TGF- $\beta$ 1 is predominantly expressed in the immune system. TGF- $\beta$  is synthesized in an inactive form, pre-pro-TGF- $\beta$  precursor. Additional stimuli are required to liberate active TGF- $\beta$ , enabling it to exert its function by binding to its receptor [3–7]. The active form of TGF- $\beta$  can function in either a cell-surface-bound form or a soluble form [8,9].

Activated TGF- $\beta$  binds to its receptor consisting of two subunits, ALK5 and TGF $\beta$ RII, and initiates signaling cascades [10]. Intracellular signal transduction of TGF- $\beta$  is mediated to a great degree via Smad proteins [10,11]. The eight vertebrate Smads identified thus far are grouped into three categories: five receptor-associated Smads (R-Smad1, 2, 3, 5, and 8), one common Smad (Co-Smad4) and two inhibitory Smads (I-Smad6 and 7). Upon TGF- $\beta$  stimulation, activated ALK5 phosphorylates R-Smad-2 and R-Smad-3. Phosphorylated R-Smads associate with Co-Smad4 and translocate into the nucleus to bind to DNA containing a Smad-binding element [12–15]. Smad-independent TGF- $\beta$  signaling pathways have also been reported [16,17]. Through mechanisms yet to be determined, rapid activation of Ras-Erk, TAK-MKK4-JNK, TAK-MKK3/6-p38, Rho-Rac-cdc42 MAPK, and PI3K-Akt pathways occurs when cells are treated with TGF- $\beta$  [18]. MAPKs also coordinate with Smads to modulate TGF- $\beta$  responses [19–21]. Moreover, TGF- $\beta$  receptors activate TRIP-1 and PP2A through direct protein binding to regulate translation initiation [22–24]. Therefore, TGF- $\beta$  exerts its regulation of target cell function via many different signaling pathways. T-cell specific target genes of TGF- $\beta$  are largely unknown; however, the expression of genes important for T-cell differentiation and function, such as GATA3, T-bet, STAT4, interferon- $\gamma$  (IFN- $\gamma$ ), and granzyme-B, are suppressed by TGF- $\beta$  [25–29].

## Regulatory T cells

Immune suppression was proposed in the early 1970s by Gershon et al. [1,30,31]. It was not until the 1990s that Sakaguchi et al. [32] identified a subset of T cells with markedly increased expression of CD25 as suppressor T cells and later referred to as Treg cells. In recent years, substantial progress has been made in identifying different types of Treg cells and in understanding how these cells are generated and function. Based on cell surface markers or cytokine secretion profiles, Tregs can be generally grouped into two categories: naturally occurring Tregs (nTreg) and acquired Treg (aTreg).

### Naturally Occurring Treg

A subset of CD4 T cells that develop in the thymus and constitutively express cell surface IL-2 receptor  $\alpha$  chain (CD25) are termed as nTreg. CD4<sup>+</sup> CD25<sup>+</sup> nTregs comprise approximately 10% of peripheral CD4 T cells in mice and humans. nTregs are critical for maintaining self-tolerance, as disruption of thymic development or peripheral maintenance of these cells

invariably results in the development of autoimmunity. Cell surface molecules, such as cytotoxic T lymphocyte antigen-4 (CTLA-4), glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR), and lymphocyte activation antigen-3 have also been used to differentiate nTreg from conventional T cells [33]. TGF- $\beta$  is expressed in nTreg at high levels as a cell-surface-bound form [9,34]. nTreg cells suppress immune responses in an antigen non-specific fashion in vitro and in vivo [33,35–37]. Foxp3, an X-linked transcription factor belonging to fork-head family, is specifically and highly expressed in nTreg. Thus, Foxp3 is currently used as the most reliable molecular marker for nTreg.

### Acquired Treg

Conventional T cells are able to gain immune-suppressive activities and become aTreg. Tr1 and Th3 cells are two reported types of aTreg. Tr1 cells are often found within the intestinal mucosa and suppress immune reactions toward a variety of cognate antigens [38]. There is no particular surface marker associated with Tr1 cells. However, these cells produce increased levels of interleukin-10 (IL-10) and TGF- $\beta$  [39]. Tr1 cells do not express Foxp3 [40], suggesting that it is a subset of Treg distinct from nTreg. Th3 is another aTreg subset induced primarily from naive CD4 T cells after ingestion of a foreign antigen via the oral route, thereby eliciting oral tolerance [41,42]. While no particular surface marker is associated with these cells, Foxp3 is expressed in Th3 cells [43]. In addition, TGF- $\beta$  is produced at elevated levels by Th3 cells [42]. Whether Th3 cells form a distinct aTreg subset or are activated nTreg remains to be evaluated.

## TGF- $\beta$ Regulated Immune Responses

### Critical Role for TGF- $\beta$ in Immune Suppression

The inhibitory effect of TGF- $\beta$  on the immune system was first described in 1986, where TGF- $\beta$  was found to inhibit the proliferation of human B and T cells [44,45]. However, the definitive evidence to support the critical roles of TGF- $\beta$  in immune suppression were not presented until the analysis of TGF- $\beta$ 1-deficient mice [46,47]. Since then, using genetic-modified mouse models, our and other laboratories have carried out extensive studies to evaluate the immune functions of the components of TGF- $\beta$  signaling networks, including their receptors and intracellular signaling molecules, and furthered the understanding of the suppressive role for TGF- $\beta$  in the immune system. TGF- $\beta$  regulates the adaptive immunity components, such as T cells, as well as the innate immunity components, such as natural killer (NK) cells [48–54]. TGF- $\beta$  suppresses immune responses through at least two ways: inhibiting the function of inflammatory cells and promoting the function of Treg cells.

Multiple types of immune cells can be regulated by TGF- $\beta$  [55]. In the early studies, TGF- $\beta$  was found to suppress T-cell proliferation by inhibiting the production of IL-2, a lymphokine known to potentially activate T cells, NK cells, and other types of cells of the immune system. Addition of exogenous IL-2 partially relieved TGF- $\beta$ -mediated suppression [45]. TGF- $\beta$  suppresses IL-2 production in T cells potentially through direct inhibition of IL-2 promoter activity; a *cis*-acting enhancer DNA element was identified to be critical in suppressing IL-2 production by TGF- $\beta$  [56]. In addition, R-Smad3 is critical in mediating TGF- $\beta$  inhibited IL-2 production, as TGF- $\beta$  failed to suppress IL-2 production in murine T cells that lack this gene [57]. Moreover, the Smad-binding element has been located upstream of the human IL-2 promoter, which is important for Smad-mediated transcriptional suppression of IL-2 [58]. Because addition of exogenous IL-2 did not fully reconstitute T-cell proliferation [45], TGF- $\beta$  inhibits T-cell proliferation also through yet-to-be-defined IL-2-independent mechanisms.

To perform immune function, naive CD4 T cells differentiate into three major types of effector T cells after activation [59]. Based on their cytokine production, CD4 effector T cells, also

named T helper (Th), can be categorized as Th1, Th2, and Th17 cells [60,61]. Th1 cells produce IFN- $\gamma$ , Th2 cells secrete IL-4, IL-13, and IL-5, and Th17 cells express IL-17 and IL-22 [62]. While TGF- $\beta$  partially suppresses T-cell proliferation, we have found that TGF- $\beta$  potently inhibits effector T-cell functions and thus their differentiation into Th1 or Th2 effector cells under tissue culture conditions [63]. Further studies revealed that TGF- $\beta$  regulates effector T-cell function through multiple mechanisms. Distinct sets of transcription factors are preferentially expressed in and are important for Th-cell differentiation. These include T-bet and Stat4 for Th1 differentiation and Gata-3 and Stat6 for Th2 differentiation [59]. While the detailed mechanism remains unknown, our and other studies showed that T-bet and Gata-3 expression is inhibited by TGF- $\beta$  [26,27,64,65] possibly, in the latter case, through a mechanism via blocking Itk kinase activity and calcium influx [25]. Interestingly, effector cytokine production by fully differentiated Th2 cells is unaffected by TGF- $\beta$ , while Th1 cells remain susceptible to TGF- $\beta$  suppression [66]. Therefore, TGF- $\beta$  exerts most of its inhibitory effects on the establishment of effector cell functions. Our recent study demonstrated that Th1-polarizing condition promotes CD122 (IL-2 receptor  $\beta$  chain) expression through T-bet [67]; thereby enhancing the clonal expansion and survival of Th1 cells [67]. Addition of TGF- $\beta$  suppressed CD122 upregulation under Th1-skewing conditions. Therefore, TGF- $\beta$  also limits Th1 effector cell numbers through inhibiting the upregulation of CD122. It was also noted that TGF- $\beta$  inhibited T-cell differentiation independent of T-cell proliferation [68]. Thus, TGF- $\beta$  potentially regulates T-cell proliferation and effector functions through discrete mechanisms with the greatest effects on suppressing their differentiation. While TGF- $\beta$  inhibits the production of pro-inflammatory cytokines, it promotes T-cell production of IL-10, an anti-inflammatory cytokine, likely through direct activation of the IL-10 promoter via Co-Smad4 [69].

Besides regulating CD4 T cells, TGF- $\beta$  controls CD8 T-cell proliferation and effector functions. The expression of effector molecules by CD8 T cells, such as IFN- $\gamma$  and perforin, is inhibited by TGF- $\beta$  [70–73]. Recent studies showed that TGF- $\beta$  is important for Treg-induced inhibition of the exocytosis of granules and cytolytic function of CD8 T cells [74].

TGF- $\beta$  is critical in immune suppression under physiological conditions because TGF- $\beta$ 1<sup>-/-</sup> mice develop a multifocal inflammatory disease associated with increased inflammatory cytokine production [46,47,75]. This phenotype is predominantly mediated through T cells, as depletion of CD4<sup>+</sup> T cells or crossing TGF- $\beta$ 1<sup>-/-</sup> mice onto an MHC class II null background prevented this inflammation [76]. However, from these studies, it was not clear whether T cells are direct targets of TGF- $\beta$  since TGF- $\beta$ 1 acts on multiple cell types. Indeed, TGF- $\beta$  plays an important role in suppressing innate immunity. By expressing a transgene encoding a dominant negative form of TGFRII under the control of CD11c promoter (CD11cTGFDR), we blocked TGF- $\beta$  signaling in NK cells and dendritic cells (DC) [51]. Blockade of TGF- $\beta$  signaling in NK cells caused the accumulation of a large pool of NK cells secreting copious amounts of IFN- $\gamma$ . Increased IFN- $\gamma$  induced Th1 differentiation of CD4 T cells in these mice and resulted in their resistance to *Leishmania* infection. However, blockade of TGF- $\beta$  signaling in DC from these mice did not affect DC homeostasis or interleukin 12 production, suggesting that TGF- $\beta$  differentially affects NK and DC cells. In addition, TGF $\beta$ RII deletion facilitated generation of a highly pathogenic T-cell subset exhibiting hallmarks of NK cells. These TGF $\beta$ RII-deficient NK-like T cells highly elevated IFN- $\gamma$  expression [77]. Further studies are warranted to elucidate TGF- $\beta$  function in the generation and function of innate components and the underlying mechanisms.

To further investigate the intrinsic function of TGF- $\beta$  in T cells, several groups including ours have used transgenic approaches to block TGF- $\beta$  signaling in T cells by expressing dominant negative TGF- $\beta$  receptors [50,52]. In this effort, we generated mice expressing a dominant-negative form of TGF $\beta$ RII from the CD4 promoter (CD4-dnT $\beta$ RII), whose CD4 and CD8 T

cells are refractory to TGF- $\beta$  signaling. These mice developed an autoimmune inflammatory phenotype associated with uncontrolled CD4<sup>+</sup> T-cell differentiation into Th1 effector cells [50]. Without TGF- $\beta$  signaling, both CD4 and CD8 T cells from CD4-dnT $\beta$ RII mice displayed increased effector functions, which led to drastically increased immune rejection of B16 melanoma and EL4 lymphoma in vivo [78]. Nonetheless, CD4-dnT $\beta$ RII mice displayed much less immune pathology than TGF- $\beta$ 1<sup>-/-</sup> mice. This is possibly due to insufficient expression of the transgenes or incomplete inhibition of TGF- $\beta$  signaling. Subsequent studies demonstrated that deletion of TGF- $\beta$ RII in the bone marrow cells results in an immune pathology similar to that found in TGF- $\beta$ 1<sup>-/-</sup> mice [79]. However, the contribution of T cells to such a phenotype remained undetermined. Recently, more definitive studies from our laboratory have uncovered the essential role of TGF- $\beta$  signaling in controlling the development, homeostasis, and tolerance of T cells through both Treg-dependent and Treg-independent mechanisms [67]. Mice with T-cell specific TGF- $\beta$ RII deletion (4cre-RII/RII) developed a progressive wasting disease and succumbed to death by 5 weeks of age. In these mice, a great number of leukocytes infiltrated into multiple non-lymphoid organs, autoantibody levels were elevated, and peripheral T cells displayed activated phenotypes. In addition, deficiency of TGF- $\beta$ RII caused mice to develop fatal autoimmune diseases similar to the TGF- $\beta$ 1<sup>-/-</sup> mice. This phenotype can be attributed to hyperactivation and exaggerated Th1 effector functions of immune cells, especially T cells [67,79]. These findings are in accordance with the results from Rudensky's laboratory, where a different strain of T-cell specific TGF- $\beta$ RII knockout mice were used [77].

T-bet encoded by the *Tbx21* gene is a transcription factor that is critical for IFN- $\gamma$  production and Th1 differentiation of CD4<sup>+</sup> T cells [80]. We attempted to alleviate/rescue the Th1-type immune disorder observed in 4cre-RII/RII mice by creating 4cre-RII/RII mice deficient in the *Tbx21* gene. Much to our surprise, CD4<sup>+</sup> T cells from 4cre-RII/RII-Tbx21<sup>-/-</sup> mice remained activated but with much less IFN- $\gamma$  production. Therefore, TGF- $\beta$  suppresses T-cell activation through a T-bet-independent mechanism, while T-bet remains essential for IFN- $\gamma$  expression. In addition, CD4<sup>+</sup> T-cell numbers were found to be decreased in these mice, likely due to decreased expression of CD122 (IL-2R $\beta$ ), a receptor that is important for both IL-2 and IL-15 signaling. Further analysis revealed that Th1-skewing conditions preferentially upregulated CD122 on CD4<sup>+</sup> T cells in vitro in a T-bet dependent manner [67]. More interestingly, addition of TGF- $\beta$  inhibited the upregulation of CD122 on CD4<sup>+</sup> T cells, suggesting that physiologically TGF- $\beta$  limits CD4<sup>+</sup> effector T-cell numbers through controlling IL-2- and IL-15-driven T-cell expansion. As TGF- $\beta$  potentially inhibits T-bet expression in Th1 cells [26], it remains to be addressed whether TGF- $\beta$  inhibits CD122 expression through T-bet-dependent and/or T-bet-independent mechanisms.

Activation of T cells in 4cre-RII/RII mice might be due to decreased Treg numbers in the periphery [67]. However, using a transfer model, we and others found that spontaneous activation of T cells lacking TGF- $\beta$ RII is refractory to Treg suppression [67,81]. It thus suggests that wild-type Treg are not able to suppress T cells that cannot respond to TGF- $\beta$ . This conclusion is consistent with previous reports [9,82,83]. It does not however establish a Treg-independent role of TGF- $\beta$  in controlling T-cell activation, since Treg-mediated suppression might be through a TGF- $\beta$ -dependent mechanism. Compelling evidence to support that TGF- $\beta$  controls T-cell activation through Treg-independent fashion came from the analysis of 4cre-RII/RII mice with the OTII T cell receptor (TCR) transgene on a *Rag1*<sup>-/-</sup> background, where endogenous Foxp3<sup>+</sup> Treg cells failed to develop. Substantial portions of T cells from these mice displayed an activated phenotype, while only a small percentage of these cells produced effector cytokines, which could be due to lack of stimulation from cognate antigens. Treg-independent TGF- $\beta$ -dependent regulation of immune functions is an area that is poorly understood, and future studies are needed to pursue the mechanisms involved.

Leukocytes and stromal cells are both able to generate TGF- $\beta$ ; which source of TGF- $\beta$  is important and whether TGF- $\beta$  regulates T-cell function as an autocrine, a paracrine, or an endocrine cytokine are interesting questions. We generated T-cell specific TGF- $\beta$ 1 knockout mice and addressed contribution of T-cell-made TGF- $\beta$ 1. These mice developed lethal immunopathology in multiple organs, associating with enhanced T-cell proliferation, activation, and differentiation. In a transfer model, TGF- $\beta$ 1 produced by Treg cells was shown to be required for Th1-cell differentiation and the onset of inflammatory bowel disease, while TGF- $\beta$ 1 generated by conventional T cells also contributed to the inhibitory effects [84]. These findings demonstrated that, to regulate T cells, TGF- $\beta$ 1 functions through an autocrine or a paracrine but may not be an endocrine mechanism. Whether such a mechanism could be applied to other leukocytes or non-lymphoid cells and what mechanisms are involved in achieving localized effects of TGF- $\beta$  are important questions to be addressed in the future.

Another mechanism by which TGF- $\beta$  inhibits immune responses is through promoting the generation of Treg cells by inducing Foxp3 expression. Early studies demonstrated that TGF- $\beta$  was necessary and sufficient for human CD8<sup>+</sup> T cells to acquire suppressive activities [85]. In addition, regulatory activity was induced in human naive (CD45RA<sup>+</sup> RO<sup>-</sup>) CD4 T cells by TGF- $\beta$  after stimulation [86]. TGF- $\beta$  was subsequently demonstrated to induce the expression of Foxp3 in CD4<sup>+</sup> CD25<sup>-</sup> human T cells [87] and in activated murine CD4<sup>+</sup> and CD8<sup>+</sup> T cells as well. In the presence of TGF- $\beta$ 1, *Staphylococcus* endotoxin-B-activated CD8 T cells inhibited the proliferation and effector functions of CD4<sup>+</sup> and CD8<sup>+</sup> T cells. This was accompanied by elevated levels of IL-10 and TGF- $\beta$ 1 [88]. TGF- $\beta$  was later demonstrated to convert mouse CD4<sup>+</sup> CD25<sup>-</sup> into CD4<sup>+</sup> CD25<sup>+</sup> T cells with elevated Foxp3 expression [87, 89].

Studies demonstrated that TGF- $\beta$  is able to convert CD4<sup>+</sup> CD25<sup>-</sup> non-Treg into CD4<sup>+</sup> CD25<sup>+</sup> Treg cells, and this was accompanied with increased Foxp3 expression [87,89]. However, a substantial portion of Foxp3<sup>+</sup> Treg cells are negative for CD25 [90,91]. In these studies, it was not distinguished whether such conversion is due to preferential expansion/survival of the existing Foxp3<sup>+</sup> CD25<sup>-</sup> population or due to de novo Foxp3 expression in the Foxp3<sup>-</sup> CD25<sup>-</sup> population. Evidence for TGF- $\beta$  promoting the conversion of Foxp3<sup>-</sup> cells into Foxp3<sup>+</sup> cells came from our study using Foxp3-monomeric red fluorescent protein (mRFP) knockin mice, where Foxp3-expressing cells are marked by mRFP expression [91]. TGF- $\beta$  induced de novo Foxp3 expression in Foxp3<sup>-</sup> CD4 T cells. Furthermore, only Foxp3<sup>+</sup> CD4<sup>+</sup> cells but not Foxp3<sup>-</sup> CD4<sup>+</sup> counterparts possessed regulatory activities [91].

Although TGF- $\beta$  promotes Treg generation in vitro, it has been controversial whether TGF- $\beta$  is involved in the generation or maintenance of Foxp3-expressing Treg under physiological conditions. Transient expression of TGF- $\beta$  by a transgene specifically expressed in islets promotes the generation of CD4<sup>+</sup> CD25<sup>+</sup> Treg in situ with high Foxp3 expression in diabetes-predisposed non-obese diabetic (NOD) mice [92]. This observation correlated with the suppression of diabetes. In addition, induced Treg cells suppressed the onset of diabetes after adoptive transfer of these cells into NOD mice [92]. These findings demonstrated that TGF- $\beta$  is sufficient to promote the generation of Treg under physiological conditions. Conflicting results have been presented with regards to whether TGF- $\beta$  is essential for the development and maintenance of nTreg. In one study, the CD4<sup>+</sup> CD25<sup>+</sup> Treg population was shown to be decreased in adult mice transgenic for a dominant negative form of TGF- $\beta$  receptor II [93] under the control of the CD2 promoter (hCD2- $\Delta$ kT $\beta$ RII). After being transferred into mice subjected to dextran sodium sulfate-induced colitis, hCD2- $\Delta$ kT $\beta$ RII transgenic CD4<sup>+</sup> CD25<sup>+</sup> cells proliferated poorly compared with wild-type CD4<sup>+</sup> CD25<sup>+</sup> nTreg cells, thus suggesting that TGF- $\beta$  signaling was required for the maintenance and expansion of CD4<sup>+</sup> CD25<sup>+</sup> nTreg in vivo [94]. However, in another transgenic model where a similar form of TGF $\beta$ DNR (dnT $\beta$ RII) is expressed under the control of the CD4 promoter [50], CD4<sup>+</sup> CD25<sup>+</sup>

nTreg cells in CD4-dnT $\beta$ RII transgenic mice developed normally. A slightly increased number of nTreg were found in the periphery of these mice compared to their wild-type counterparts ([83] and our unpublished observation). Peripheral but not thymic nTreg were found to be reduced in 8- to 10-day-old TGF- $\beta$ 1<sup>-/-</sup> mice [81], suggesting an essential function for endogenous TGF- $\beta$ 1 in the maintenance of the peripheral population of Treg. These results contrast that of an earlier study where no defect of Treg development or maintenance was observed in TGF- $\beta$ 1<sup>-/-</sup> mice [95]. A more recent definitive study from our laboratory demonstrated that Foxp3-expressing nTreg cells that lack TGF $\beta$ RII developed normally in the thymus but were poorly maintained in the periphery [67]. Interestingly, in the same study, TGF $\beta$ RII-deficient Treg proliferated faster than the wild-type counterparts in the periphery despite that lower numbers of TGF $\beta$ RII-deficient Treg cells were found, suggesting that TGF- $\beta$  signaling is required to promote the survival of peripheral Treg [67]. The reasons for these discrepancies are unknown but may be related to the different experimental systems and mouse genetic backgrounds used. In addition, because CD25 is also expressed by activated T cells, CD4<sup>+</sup> CD25<sup>+</sup> Treg identified in these studies may have been contaminated by activated T cells to varying degrees in the earlier studies. With the development of enhanced green fluorescent protein (EGFP)-Foxp3 and Foxp3-mRFP knockin mice and Foxp3 intracellular staining, these potential complications can be circumvented by identifying Treg based on Foxp3 expression [67,90,91]. It is coming to a consensus that TGF- $\beta$  is required for the maintenance of Treg in the periphery. We investigated whether T-cell-made TGF- $\beta$  is required for Treg maintenance by studying mRFP-expressing Treg cells from mice lacking TGF- $\beta$ 1. We found that T-cell-produced TGF- $\beta$ 1 is dispensable for the development and maintenance of Treg cells [84], despite the fact that TGF- $\beta$ 1-deficient Treg cells lost their suppressive activities in a transfer model. Thus, self-made TGF- $\beta$ 1 appears to be required for Treg function, while it remains an open question as what source of TGF- $\beta$  is required for Treg maintenance.

### TGF- $\beta$ Promotes Certain Immune Responses

As discussed above, TGF- $\beta$  is prevalently viewed as an immune suppressive cytokine. However, as a pleiotropic cytokine, TGF- $\beta$  has been found to exhibit immune-promoting properties, which has recently attracted much attention.

In early studies, TGF- $\beta$  was found to enhance the proliferation of mouse CD8<sup>+</sup> cells under certain conditions [96] and to increase TNF- $\alpha$  production by both CD4<sup>+</sup> and CD8<sup>+</sup> cells [97]. TGF- $\beta$  accelerates T-cell death in some studies [98,99], while an anti-apoptotic role for TGF- $\beta$  has also been documented [100,101].

Interestingly, TGF- $\beta$  was recently identified to be important for the induction of IL-17-producing cells under inflammatory conditions [102–105]. Th-17 cell is a new class of effector T cells that is gaining increasing attention. Th-17 cells produce IL-17 and IL-22 and are critical for the induction of experimental autoimmune encephalomyelitis (EAE) in mice. Under culture conditions, TGF- $\beta$  in combination with IL-6 promotes Th-17 differentiation. In addition, TGF- $\beta$  is important for the generation of Th-17 cells and the induction of EAE in mice [103,106]. By deleting the TGF- $\beta$ 1 gene specifically in T cells, we found that T-cell-made TGF- $\beta$ 1 is required for the induction of pathogenic Th-17 cells in the intestines and in the spinal cord. myelin oligodendrocyte glycoprotein-induced EAE is drastically ameliorated when T cells cannot generate TGF- $\beta$ 1 [84]. Considering that TGF- $\beta$ 1 can be generated by conventional and Treg cells, it would be important to discern whether either type of cells is required for the generation of Th-17 cells and the induction of EAE in mice.

The lack of TGF- $\beta$  signaling is often associated with increased proliferation and effector function of immune cells. However, two recent independent studies demonstrated that TGF- $\beta$  might promote proliferation and survival of immune cells under certain conditions. The numbers of canonical CD1d-dependent natural killer (NK1.1<sup>+</sup>) T cells and CD8<sup>+</sup> T cells were

decreased upon TGF- $\beta$ RII deletion, suggesting a positive role for TGF- $\beta$  signaling in their development [67]. In the absence of TGF- $\beta$  signaling, OT-II CD4 T cells, which only responded to OVA peptide from chicken ovalbumin, were prone to apoptosis in the periphery and failed to differentiate into effector cells. This phenotype correlates with reduced expression of CD122 on these cells. It suggests that TGF- $\beta$  signaling is required for the survival of some cells. However, it remains to be addressed as to what is the relationship among TGF- $\beta$  promoted T-cell survival, CD122 expression, and Th1 differentiation.

Collectively, these studies highlight the multi-faceted effects of TGF- $\beta$  on various immune functions and emphasize the importance of cellular and environmental contexts in directing the discrete roles of TGF- $\beta$ . What triggers and mediates TGF- $\beta$  to perform such a broad spectrum of functions needs to be elucidated in the years to come.

## Treg-Controlled Immune Responses

Early studies showing that thymectomy of neonatal rodents within 3 days after birth led to lethal autoimmunity suggested that a set of thymus-derived cells are important to maintain self-tolerance in the periphery [107,108]. Upon identification of CD4<sup>+</sup> CD25<sup>+</sup> as markers for Treg cells, Sakaguchi's group used anti-CD25 antibody to deplete these cells, and such manipulation led to lethal autoimmunity due to the loss of peripheral tolerance in adult mice [33]. In 2003, an X-linked forkhead family transcription factor named Foxp3 was identified to be expressed specifically in nTreg among the lymphocyte populations [35,109,110]. Spontaneous monogenetic mutation in the *Foxp3* gene results in systemic autoimmunity in *Scurfy* mice and *IPEX* (X-linked neonatal diabetes mellitus, enteropathy, and endocrinopathy syndrome) patients [111–115]. The disease manifested in *Scurfy* mice was attributed to Treg deficiency. However, T-cell extrinsic elements were reported to contribute to the *Scurfy* phenotype [116], while counter-evidence were also presented [117]. In addition, targeted deletion of *Foxp3* gene in mice led to a phenotype reminiscent of *Scurfy* mice with undetectable CD4<sup>+</sup> CD25<sup>+</sup> T cells [118–120]. Moreover, ectopically overexpressed *Foxp3* gene endowed immune suppressive activities to conventional CD4 and CD8 T cells [112,113,120]. Therefore, Foxp3 controls the development and function of nTreg cells, a type of suppressor T cells that are essential in maintaining self-tolerance and immune homeostasis.

## Mechanisms of Treg-Mediated Immune Suppression

The essential role of Treg in immune suppression is indisputable. Yet, the mechanisms by which Treg cells carry out their function remain ill-defined. Nevertheless, it is generally agreed that Treg suppress immune responses through multiple mechanisms including cell contact dependent and independent mechanisms. Several surface molecules preferentially expressed by Treg cells are proposed to be important for their function. For example, CD25, a high-affinity IL-2 binding receptor, is highly expressed by the nTreg cells [121]. In addition, the peripheral maintenance of nTreg is dependent on IL-2 signaling [122–124], which is also important for the proliferation and survival of activated effector T cells. It is therefore hypothesized that one mechanism of nTreg suppression of conventional T-cell activation is through competition for IL-2 consumption [125,126]. Under culturing conditions, such competition may result in effector T cell death due to deprivation of growth factors, such as IL-2 [127]. However, studies showing that CD25-deficient nTreg cells possess intact suppressive activity question the validity of this hypothesis [123]. CTLA-4, another surface molecule preferentially expressed by nTreg, is important for inhibiting immune activation by competing for costimulatory ligands on T cells [128] and inhibiting the function of antigen-presenting cells [129,130]. Thus, it is suggested that CTLA-4 is important for nTreg-mediated immune suppression. Genetic evidence however argues against the critical roles for CTLA-4 in nTreg function, as the function of CTLA-4<sup>-/-</sup> nTreg functioned normally in vitro and in vivo [131,132]. However, antibody-mediated blockage of CTLA-4 abrogated nTreg function



[131,132], while it remains unknown whether this is due to a non-specific effect or due to a simultaneous block of CTLA-4 and its potential homolog with redundant functions by the antibodies.

Recent studies have unraveled important roles of cytokines in nTreg function. TGF- $\beta$  appears to be critical in mediating nTreg function, as T cells from CD4-dnT $\beta$ RII mice that are unresponsive to TGF- $\beta$  are refractory to nTreg-mediated suppression in vitro and in vivo [9, 82,83]. Despite the fact that TGF- $\beta$  mRNA is not elevated in nTreg cells, it is suggested that the membrane bound form of TGF- $\beta$  is increased in nTreg cells and is important for their function [9,34]. IL-10 is another immune suppressive cytokine preferentially expressed in Treg [133,134] and is important in mediating the functions of these cells [134,135]. Besides suppress effector T cell functions directly, recent study suggests that nTreg dampens immune responses through regulating innate component, such as DC. nTreg can potentially induce tolerogenic DC through CTLA-4 engagement induced tryptophan catabolism [136]. In addition, it appears that nTreg destabilize the interaction between antigenic DC with conventional T cell to prevent the activation of T cells [137]. It is clear now that multiple mechanisms are involved in Treg-mediated immune suppression. Cell-contact-dependent and cell-contact-independent mechanisms critical for controlling Treg function remain to be identified in the future.

### Foxp3 Controls Treg Function

As a single molecule that controls the development and function of Treg, Foxp3 has been under close scrutiny since its discovery. We and others have developed knockin mouse models to track Foxp3-expressing cells with fluorescent proteins [90,91]. Foxp3 can be expressed in thymic-derived Treg cells; its expression can also be induced in conventional T cells in vitro by TGF- $\beta$  regardless of their proliferation status [91]. In addition, Foxp3 expression can be induced in vivo in conventional T cells under suboptimal stimulation [138]. More importantly, in these studies, Foxp3 expression has always been associated with the immune suppressive function. Thus, it is generally thought that Foxp3 serves as an on-and-off switch to positively regulate Treg function in a binary fashion. However, a transient increase of Foxp3 expression in human CD4 T cells did not result in suppressive function. In addition, in many cases of human IPEX patients, functional Foxp3 protein is made but at much reduced levels [139]. Moreover, emerging evidence associates decreased Foxp3 expression in Treg cells with various human autoimmune disorders, such as graft-versus-host disease [140], autoimmune myasthenia gravis [141], and multiple sclerosis [142]. We have also found that intra-islet Treg cells expressed lower levels of Foxp3 than Treg cells from other peripheral lymphoid organs in diabetic NOD mice, while the frequencies of Foxp3-expressing Treg cells among the different compartments were comparable. Thus, Foxp3 may control Treg function in a dose-dependent, non-binary fashion, and decreased Foxp3 expression could lead to impaired Treg function and be causal for immune disorders.

Studying of a mouse model where attenuated Foxp3 expression is serendipitously achieved, we found that reduced Foxp3 expression led to the development of a lethal aggressive lymphoproliferative autoimmune syndrome reminiscent of *Scurfy* mice [143] and T-cell-specific *Foxp3* knockout mice [120]. Compared with WT Treg cells, the surface expression of 'signature genes' for Treg cells, such as CD25, CTLA-4, and GITR [32,144,145], were decreased in these mice [146]. Thus, Foxp3 programs the gene expression of Treg in a tunable and dose-dependent fashion [146].

Further investigation revealed interesting phenotypic changes in the functions of Treg cells, which express decreased Foxp3. Foxp3 is required for the development and maintenance of Treg cells [120]. However, decreased Foxp3 expression did not lead to defective thymic development of Treg cells. This result agrees with the results from a separate study, where GFP complementary DNA was inserted into endogenous *Foxp3*-coding region to mark T cells with

Foxp3 promoter activity but fail to produce functional Foxp3 protein [147]. Substantial numbers of GFP-expressing thymocytes were also detected in these mice [147]. By adoptive transfer assays, we found that attenuated Foxp3 expression did not result in intrinsic defects in the homeostatic expansion/maintenance of Treg cells in the periphery. Extra-thymic generation of Foxp3-expressing T cells can be promoted in vitro by TGF- $\beta$  [89,91,106]. TGF- $\beta$  induced de novo Foxp3 expression normally in T cells with attenuated Foxp3 expression. Therefore, decreased Foxp3 expression did not affect the development, homeostatic expansion/maintenance, or TGF- $\beta$  driven de novo generation of Foxp3-expressing T cells [146]. These findings present a possibility that defective and even ablated Foxp3 expression might not result in the total elimination of the “Treg lineage.” Indeed, Foxp3-null mice generated “Treg lineage” cells in the thymus and can be maintained in the periphery [147]. Thus, in *Scurfy* mice and *IPEX* human patients, Foxp3-expressing T cell subsets are likely to be generated in the thymus and maintained in the periphery. Further studies are warranted to identify and characterize these “Treg lineage” cells lacking functional Foxp3.

In vitro, hypoproliferative (‘anergic’) and immune-suppressive activities are two defining properties for Foxp3-expressing Treg cells that are thought to go hand-in-hand [33]. Intriguingly, upon TCR stimulation in vitro, while Foxp3 low-expressing T cells remained anergic; their immune-suppressive activities were greatly impaired. Thus, anergy and immune suppression are two separable properties of Treg cells that are affected differentially by the expression levels of Foxp3 [146]. In addition, in a T-cell transfer model, the immune-suppressive activities of Foxp3 low-expressing T cells were abolished in vivo, although these cells infiltrated efficiently into lymphoid and non-lymphoid organs. Thus, the suppressive function of Treg requires high level Foxp3 [146]. Interestingly, agreeing with these findings, Foxp3-deficient “Treg lineage” cells remain anergic but without immune suppressive function [147]. Therefore, unlike what has been recognized, Foxp3 may not be the “grand-master” of Treg development. It is emerging that Foxp3 appears to be required for stabilizing “regulatory function” of already differentiated “Treg lineage” cells in vivo or TGF- $\beta$  induced Treg cells in vitro [147–149]. The molecular master-switch controlling the commitment of “Treg lineage” remains to be discovered. In addition, despite the fact that multiple molecules, such as CTLA-4 and CD25, have been suggested to contribute to the suppressive activities of Treg, it remains unsolved whether any of them may be sufficient to render Treg-suppressive function. It would be interesting to investigate whether any of these genes are able to reconstitute the Treg function in the aforementioned mouse models. Genomic-scaled DNA binding profiling studies have been performed to identify Foxp3-specific target genes that might be crucial for Treg function. Over 700 potential targets including promoters, intragenic regulatory elements, and small modulatory RNA/small interfering RNA have been identified [150]. Future efforts are need to sieve through these candidates to pinpoint the critical Foxp3 target genes in controlling the immune suppressive function of Treg cells.

### Positive Roles for Treg in Inflammation

Treg contribute to inflammation by inducing Th-17 indirectly through TGF- $\beta$ . TGF- $\beta$  appears to be critical for the induction of Th-17 cells according to our and other studies [105]. High levels of TGF- $\beta$ , probably existing as a membrane bound form, are found on Treg cells. Therefore, Treg cells have been identified to be an important inducer for Th-17 cells in vitro. Using TGF- $\beta$ 1 knockout mice, future studies need to be performed to address whether Treg is the critical source of TGF- $\beta$  in Th-17 cell induction and thus the onset of EAE.

Foxp3-expressing cells can convert into effector cells. Analysis of Foxp3 low-expressing cells revealed that they developed effector functions producing large amounts of type 2 effector cytokine, such as IL-4, while the changes of IL-2, IFN- $\gamma$ , or IL-17 expression were modest [146]. In another study, “Treg lineage” cells without functional Foxp3 were able to express

Th1, Th2, and Th17 effector cytokines [147]. These findings present an interesting possibility that Foxp3-expressing cells might not always suppress but rather, in some cases, can foster the immune response, e.g. under inflammatory conditions. In fact, TNF- $\alpha$ , a pro-inflammatory cytokine, has been shown to repress the expression of Foxp3 in human Treg cells [151]. It is reasonable to believe that in a highly inflammatory microenvironment, residential Treg decrease their Foxp3 expression, subsequently lose their suppressive function, and might even be converted into effector cells to contribute to the immune responses. Upon resolution of infection, inflammatory cytokines would subside and Foxp3 levels and suppressive activity would be restored.

## Conclusions

The importance of active immune suppression is widely acknowledged. Studies on TGF- $\beta$  and Treg have shed light on how immune suppression functions. Advances in these areas have been and are being translated into clinical benefits. Further investigations are warranted to elucidate the mechanism through which TGF- $\beta$  and Treg control immune responses. In addition, we are starting to realize that under certain conditions, TGF- $\beta$  and Treg can both serve as promoting factors to direct immune responses. To facilitate designing immune therapies against inflammatory diseases and cancers, more studies are needed to reveal alternative functions of TGF- $\beta$  and Treg.

## Acknowledgments

We thank F. Manzo for secretarial assistance. Y.Y.W. is a Cancer Research Institute (CRI) fellow. R.A.F. is an investigator of the Howard Hughes Medical Institute. Work from this laboratory quoted in this review is supported by grants from the NIH and American Diabetes Association (ADA).

## References

1. Gershon RK. A disquisition on suppressor T cells. *Transplant Rev* 1975;26:170–85. [PubMed: 1101469]
2. Blobel GC, Schiemann WP, Lodish HF. Role of transforming growth factor beta in human disease. *N Engl J Med* 2000;342:1350–8. [PubMed: 10793168]
3. Annes JP, Chen Y, Munger JS, Rifkin DB. Integrin alphaVbeta6-mediated activation of latent TGF-beta requires the latent TGF-beta binding protein-1. *J Cell Biol* 2004;165:723–34. [PubMed: 15184403]
4. Annes JP, Munger JS, Rifkin DB. Making sense of latent TGFbeta activation. *J Cell Sci* 2003;116:217–24. [PubMed: 12482908]
5. Crawford SE, Stellmach V, Murphy-Ullrich JE, Ribeiro SM, Lawler J, Hynes RO, Boivin GP, Bouck N. Thrombospondin-1 is a major activator of TGF-beta1 in vivo. *Cell* 1998;93:1159–70. [PubMed: 9657149]
6. Munger JS, Huang X, Kawakatsu H, Griffiths MJ, Dalton SL, Wu J, Pittet JF, Kaminski N, Garat C, Matthay MA, et al. The integrin alpha v beta 6 binds and activates latent TGF beta 1: a mechanism for regulating pulmonary inflammation and fibrosis. *Cell* 1999;96:319–28. [PubMed: 10025398]
7. Yehualaesht T, O'Connor R, Green-Johnson J, Mai S, Silverstein R, Murphy-Ullrich JE, Khalil N. Activation of rat alveolar macrophage-derived latent transforming growth factor beta-1 by plasmin requires interaction with thrombospondin-1 and its cell surface receptor, CD36. *Am J Pathol* 1999;155:841–51. [PubMed: 10487979]
8. Letterio JJ, Roberts AB. Regulation of immune responses by TGF-beta. *Annu Rev Immunol* 1998;16:137–61. [PubMed: 9597127]
9. Green EA, Gorelik L, McGregor CM, Tran EH, Flavell RA. 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;100:10878–83. [PubMed: 12949259]
10. Massague J. TGF-beta signal transduction. *Annu Rev Biochem* 1998;67:753–91. [PubMed: 9759503]

11. Huse M, Muir TW, Xu L, Chen YG, Kuriyan J, Massague J. The TGF beta receptor activation process: an inhibitor- to substrate-binding switch. *Mol Cell* 2001;8:671–82. [PubMed: 11583628]
12. Inman GJ, Nicolas FJ, Hill CS. Nucleocytoplasmic shuttling of Smads 2, 3, and 4 permits sensing of TGF-beta receptor activity. *Mol Cell* 2002;10:283–94. [PubMed: 12191474]
13. Johnson K, Kirkpatrick H, Comer A, Hoffmann FM, Laughon A. Interaction of Smad complexes with tripartite DNA-binding sites. *J Biol Chem* 1999;274:20709–16. [PubMed: 10400705]
14. Shi Y, Wang YF, Jayaraman L, Yang H, Massague J, Pavletich NP. Crystal structure of a Smad MH1 domain bound to DNA: insights on DNA binding in TGF-beta signaling. *Cell* 1998;94:585–94. [PubMed: 9741623]
15. Zawel L, Dai JL, Buckhaults P, Zhou S, Kinzler KW, Vogelstein B, Kern SE. Human Smad3 and Smad4 are sequence-specific transcription activators. *Mol Cell* 1998;1:611–7. [PubMed: 9660945]
16. Engel ME, McDonnell MA, Law BK, Moses HL. Interdependent SMAD and JNK signaling in transforming growth factor-beta-mediated transcription. *J Biol Chem* 1999;274:37413–20. [PubMed: 10601313]
17. Yu L, Hebert MC, Zhang YE. TGF-beta receptor-activated p38 MAP kinase mediates Smad-independent TGF-beta responses. *EMBO J* 2002;21:3749–59. [PubMed: 12110587]
18. Derynck R, Zhang YE. Smad-dependent and Smad-independent pathways in TGF-beta family signalling. *Nature* 2003;425:577–84. [PubMed: 14534577]
19. Blanchette F, Rivard N, Rudd P, Grondin F, Attisano L, Dubois CM. Cross-talk between the p42/p44 MAP kinase and Smad pathways in transforming growth factor beta 1-induced furin gene transactivation. *J Biol Chem* 2001;276:33986–94. [PubMed: 11448947]
20. Funaba M, Zimmerman CM, Mathews LS. Modulation of Smad2-mediated signaling by extracellular signal-regulated kinase. *J Biol Chem* 2002;277:41361–8. [PubMed: 12193595]
21. Kretzschmar M, Doody J, Timokhina I, Massague J. A mechanism of repression of TGFbeta/ Smad signaling by oncogenic Ras. *Genes Dev* 1999;13:804–16. [PubMed: 10197981]
22. Choy L, Derynck R. The type II transforming growth factor (TGF)-beta receptor-interacting protein TRIP-1 acts as a modulator of the TGF-beta response. *J Biol Chem* 1998;273:31455–62. [PubMed: 9813058]
23. Griswold-Prenner I, Kamibayashi C, Maruoka EM, Mumby MC, Derynck R. Physical and functional interactions between type I transforming growth factor beta receptors and Balph, a WD-40 repeat subunit of phosphatase 2A. *Mol Cell Biol* 1998;18:6595–604. [PubMed: 9774674]
24. McGonigle S, Beall MJ, Pearce EJ. Eukaryotic initiation factor 2 alpha subunit associates with TGF beta receptors and 14–3–3 epsilon and acts as a modulator of the TGF beta response. *Biochemistry* 2002;41:579–87. [PubMed: 11781097]
25. Chen CH, Seguin-Devaux C, Burke NA, Oriss TB, Watkins SC, Clipstone N, Ray A. Transforming growth factor beta blocks Tec kinase phosphorylation, Ca<sup>2+</sup> influx, and NFATc translocation causing inhibition of T cell differentiation. *J Exp Med* 2003;197:1689–19. [PubMed: 12810687]
26. Gorelik L, Constant S, Flavell RA. Mechanism of transforming growth factor beta-induced inhibition of T helper type 1 differentiation. *J Exp Med* 2002;195:1499–505. [PubMed: 12045248]
27. Gorelik L, Fields PE, Flavell RA. Cutting edge: TGF-beta inhibits Th type 2 development through inhibition of GATA-3 expression. *J Immunol* 2000;165:4773–7. [PubMed: 11045997]
28. Lin JT, Martin SL, Xia L, Gorham JD. TGF-beta1 uses distinct mechanisms to inhibit IFN-gamma expression in CD4+ T cells at priming and at recall: differential involvement of Stat4 and T-bet. *J Immunol* 2005;174:5950–8. [PubMed: 15879087]
29. Thomas DA, Massague J. TGF-beta directly targets cytotoxic T cell functions during tumor evasion of immune surveillance. *Cancer Cell* 2005;8:369–80. [PubMed: 16286245]
30. Gershon RK, Kondo K. Infectious immunological tolerance. *Immunology* 1971;21:903–14. [PubMed: 4943147]
31. Gershon RK, Kondo K. Cell interactions in the induction of tolerance: the role of thymic lymphocytes. *Immunology* 1970;18:723–37. [PubMed: 4911896]
32. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J Immunol* 1995;155:1151–64. [PubMed: 7636184]

33. Sakaguchi S. Naturally arising CD4+ regulatory t cells for immunologic self-tolerance and negative control of immune responses. *Annu Rev Immunol* 2004;22:531–62. [PubMed: 15032588]
34. Nakamura K, Kitani A, Strober W. Cell contact-dependent immunosuppression by CD4(+)CD25(+) regulatory T cells is mediated by cell surface-bound transforming growth factor beta. *J Exp Med* 2001;194:629–44. [PubMed: 11535631]
35. Sakaguchi S. Naturally arising Foxp3-expressing CD25+CD4+ regulatory T cells in immunological tolerance to self and non-self. *Nat Immunol* 2005;6:345–52. [PubMed: 15785760]
36. Shevach EM. Regulatory T cells in autoimmunity\*. *Annu Rev Immunol* 2000;18:423–49. [PubMed: 10837065]
37. Shevach EM. CD4+CD25+ suppressor T cells: more questions than answers. *Nat Rev Immunol* 2002;2:389–400. [PubMed: 12093005]
38. Groux H, O'Garra A, Bigler M, Rouleau M, Antonenko S, de Vries JE, Roncarolo MG. A CD4+T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature* 1997;389:737–42. [PubMed: 9338786]
39. Roncarolo MG, Bacchetta R, Bordignon C, Narula S, Levings MK. Type 1 T regulatory cells. *Immunol Rev* 2001;182:68–79. [PubMed: 11722624]
40. Vieira PL, Christensen JR, Minaee S, O'Neill EJ, Barrat FJ, Boonstra A, Barthlott T, Stockinger B, Wraith DC, O'Garra A. IL-10-secreting regulatory T cells do not express Foxp3 but have comparable regulatory function to naturally occurring CD4+CD25+ regulatory T cells. *J Immunol* 2004;172:5986–93. [PubMed: 15128781]
41. Faria AM, Weiner HL. Oral tolerance. *Immunol Rev* 2005;206:232–59. [PubMed: 16048553]
42. Weiner HL. Induction and mechanism of action of transforming growth factor-beta-secreting Th3 regulatory cells. *Immunol Rev* 2001;182:207–14. [PubMed: 11722636]
43. Stassen M, Fondel S, Bopp T, Richter C, Muller C, Kubach J, Becker C, Knop J, Enk AH, Schmitt S, et al. Human CD25+ regulatory T cells: two subsets defined by the integrins alpha 4 beta 7 or alpha 4 beta 1 confer distinct suppressive properties upon CD4+ T helper cells. *Eur J Immunol* 2004;34:1303–11. [PubMed: 15114663]
44. Kehl JH, Roberts AB, Wakefield LM, Jakowlew S, Sporn MB, Fauci AS. Transforming growth factor beta is an important immunomodulatory protein for human B lymphocytes. *J Immunol* 1986;137:3855–60. [PubMed: 2878044]
45. Kehl JH, Wakefield LM, Roberts AB, Jakowlew S, Alvarez-Mon M, Derynck R, Sporn MB, Fauci AS. Production of transforming growth factor beta by human T lymphocytes and its potential role in the regulation of T cell growth. *J Exp Med* 1986;163:1037–50. [PubMed: 2871125]
46. Kulkarni AB, Huh CG, Becker D, Geiser A, Lyght M, Flanders KC, Roberts AB, Sporn MB, Ward JM, Karlsson S. Transforming growth factor beta 1 null mutation in mice causes excessive inflammatory response and early death. *Proc Natl Acad Sci USA* 1993;90:770–4. [PubMed: 8421714]
47. Shull MM, Ormsby I, Kier AB, Pawlowski S, Diebold RJ, Yin M, Allen R, Sidman C, Proetzel G, Calvin D, et al. Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. *Nature* 1992;359:693–9. [PubMed: 1436033]
48. Cazac BB, Roes J. TGF-beta receptor controls B cell responsiveness and induction of IgA in vivo. *Immunity* 2000;13:443–51. [PubMed: 11070163]
49. Datto MB, Frederick JP, Pan L, Borton AJ, Zhuang Y, Wang XF. Targeted disruption of Smad3 reveals an essential role in transforming growth factor beta-mediated signal transduction. *Mol Cell Biol* 1999;19:2495–504. [PubMed: 10082515]
50. Gorelik L, Flavell RA. Abrogation of TGFbeta signaling in T cells leads to spontaneous T cell differentiation and autoimmune disease. *Immunity* 2000;12:171–81. [PubMed: 10714683]
51. Laouar Y, Sutterwala FS, Gorelik L, Flavell RA. Transforming growth factor-beta controls T helper type 1 cell development through regulation of natural killer cell interferon-gamma. *Nat Immunol* 2005;6:600–7. [PubMed: 15852008]
52. Lucas PJ, Kim SJ, Melby SJ, Gress RE. Disruption of T cell homeostasis in mice expressing a T cell-specific dominant negative transforming growth factor beta II receptor. *J Exp Med* 2000;191:1187–96. [PubMed: 10748236]

53. Nakao A, Miike S, Hatano M, Okumura K, Tokuhisa T, Ra C, Iwamoto I. Blockade of transforming growth factor beta/Smad signaling in T cells by overexpression of Smad7 enhances antigen-induced airway inflammation and airway reactivity. *J Exp Med* 2000;192:151–8. [PubMed: 10899902]
54. Yang X, Letterio JJ, Lechleider RJ, Chen L, Hayman R, Gu H, Roberts AB, Deng C. Targeted disruption of SMAD3 results in impaired mucosal immunity and diminished T cell responsiveness to TGF-beta. *EMBO J* 1999;18:1280–91. [PubMed: 10064594]
55. Li MO, Wan YY, Sanjabi S, Robertson AK, Flavell RA. Transforming growth factor-beta regulation of immune responses. *Annu Rev Immunol* 2006;24:99–146. [PubMed: 16551245]
56. Brabletz T, Pfeuffer I, Schorr E, Siebelt F, Wirth T, Serfling E. Transforming growth factor beta and cyclosporin A inhibit the inducible activity of the interleukin-2 gene in T cells through a noncanonical octamer-binding site. *Mol Cell Biol* 1993;13:1155–62. [PubMed: 8423782]
57. McKarns SC, Schwartz RH, Kaminski NE. Smad3 is essential for TGF-beta 1 to suppress IL-2 production and TCR-induced proliferation, but not IL-2-induced proliferation. *J Immunol* 2004;172:4275–84. [PubMed: 15034041]
58. Tzachanis D, Freeman GJ, Hirano N, van Puijenbroek AA, Delfs MW, Berezovskaya A, Nadler LM, Boussiotis VA. Tob is a negative regulator of activation that is expressed in anergic and quiescent T cells. *Nat Immunol* 2001;2:1174–82. [PubMed: 11694881]
59. Murphy KM, Reiner SL. The lineage decisions of helper T cells. *Nat Rev Immunol* 2002;2:933–44. [PubMed: 12461566]
60. Mosmann TR, Coffman RL. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu Rev Immunol* 1989;7:145–73. [PubMed: 2523712]
61. Park H, Li Z, Yang XO, Chang SH, Nurieva R, Wang YH, Wang Y, Hood L, Zhu Z, Tian Q, et al. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat Immunol* 2005;6:1133–41. [PubMed: 16200068]
62. Liang SC, Tan XY, Luxenberg DP, Karim R, Dunussi-Joannopoulos K, Collins M, Fouser LA. Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. *J Exp Med* 2006;203:2271–9. [PubMed: 16982811]
63. Gorelik L, Flavell RA. Transforming growth factor-beta in T-cell biology. *Nat Rev Immunol* 2002;2:46–53. [PubMed: 11905837]
64. Gorham JD, Guler ML, Fenoglio D, Gubler U, Murphy KM. Low dose TGF-beta attenuates IL-12 responsiveness in murine Th cells. *J Immunol* 1998;161:1664–70. [PubMed: 9712029]
65. Heath VL, Murphy EE, Crain C, Tomlinson MG, O'Garra A. TGF-beta1 down-regulates Th2 development and results in decreased IL-4-induced STAT6 activation and GATA-3 expression. *Eur J Immunol* 2000;30:2639–49. [PubMed: 11009098]
66. Ludviksson BR, Seegers D, Resnick AS, Strober W. The effect of TGF-beta1 on immune responses of naive versus memory CD4+ Th1/Th2 T cells. *Eur J Immunol* 2000;30:2101–11. [PubMed: 10940900]
67. Li MO, Sanjabi S, Flavell RA. Transforming growth factor-beta controls development, homeostasis, and tolerance of T cells by regulatory T cell-dependent and -independent mechanisms. *Immunity* 2006;25:455–71. [PubMed: 16973386]
68. Sad S, Mosmann TR. Single IL-2-secreting precursor CD4 T cell can develop into either Th1 or Th2 cytokine secretion phenotype. *J Immunol* 1994;153:3514–22. [PubMed: 7930573]
69. Kitani A, Fuss I, Nakamura K, Kumaki F, Usui T, Strober W. Transforming growth factor (TGF)-beta1-producing regulatory T cells induce Smad-mediated interleukin 10 secretion that facilitates coordinated immunoregulatory activity and amelioration of TGF-beta1-mediated fibrosis. *J Exp Med* 2003;198:1179–88. [PubMed: 14557415]
70. Ahmadzadeh M, Rosenberg SA. TGF-beta1 attenuates the acquisition and expression of effector function by tumor antigen-specific human memory CD8 T cells. *J Immunol* 2005;174:5215–23. [PubMed: 15843517]
71. Bonig H, Banning U, Hannen M, Kim YM, Verheyen J, Mauz-Korholz C, Korholz D. Transforming growth factor-beta1 suppresses interleukin-15-mediated interferon-gamma production in human T lymphocytes. *Scand J Immunol* 1999;50:612–8. [PubMed: 10607309]

72. Ranges GE, Figari IS, Espevik T, Palladino MA Jr. Inhibition of cytotoxic T cell development by transforming growth factor beta and reversal by recombinant tumor necrosis factor alpha. *J Exp Med* 1987;166:991–8. [PubMed: 3498791]
73. Smyth MJ, Strobl SL, Young HA, Ortaldo JR, Ochoa AC. Regulation of lymphokine-activated killer activity and pore-forming protein gene expression in human peripheral blood CD8+ T lymphocytes. Inhibition by transforming growth factor-beta. *J Immunol* 1991;146:3289–97. [PubMed: 1827481]
74. Mempel TR, Pittet MJ, Khazaie K, Weninger W, Weissleder R, von Boehmer H, von Andrian UH. Regulatory T cells reversibly suppress cytotoxic T cell function independent of effector differentiation. *Immunity* 2006;25:129–41. [PubMed: 16860762]
75. Rudner LA, Lin JT, Park IK, Cates JM, Dyer DA, Franz DM, French MA, Duncan EM, White HD, Gorham JD. Necroinflammatory liver disease in BALB/c background, TGF-beta 1-deficient mice requires CD4+ T cells. *J Immunol* 2003;170:4785–92. [PubMed: 12707360]
76. Letterio JJ, Geiser AG, Kulkarni AB, Dang H, Kong L, Nakabayashi T, Mackall CL, Gress RE, Roberts AB. Autoimmunity associated with TGF-beta1 -deficiency in mice is dependent on MHC class II antigen expression. *J Clin Invest* 1996;98:2109–19. [PubMed: 8903331]
77. Marie JC, Liggitt D, Rudensky AY. Cellular mechanisms of fatal early-onset autoimmunity in mice with the T cell-specific targeting of transforming growth factor-beta receptor. *Immunity* 2006;25:441–54. [PubMed: 16973387]
78. Gorelik L, Flavell RA. Immune-mediated eradication of tumors through the blockade of transforming growth factor-beta signaling in T cells. *Nat Med* 2001;7:1118–22. [PubMed: 11590434]
79. Leveen P, Larsson J, Ehinger M, Cilio CM, Sundler M, Sjostrand LJ, Holmdahl R, Karlsson S. Induced disruption of the transforming growth factor beta type II receptor gene in mice causes a lethal inflammatory disorder that is transplantable. *Blood* 2002;100:560–8. [PubMed: 12091349]
80. Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH. A novel transcription factor, T-bet, directs Th1 lineage commitment. *Cell* 2000;100:655–69. [PubMed: 10761931]
81. Marie JC, Letterio JJ, Gavin M, Rudensky AY. TGF-beta1 maintains suppressor function and Foxp3 expression in CD4+ CD25+regulatory T cells. *J Exp Med* 2005;201:1061–7. [PubMed: 15809351]
82. Chen ML, Pittet MJ, Gorelik L, Flavell RA, Weissleder R, von Boehmer H, Khazaie K. Regulatory T cells suppress tumor-specific CD8 T cell cytotoxicity through TGF-beta signals in vivo. *Proc Natl Acad Sci U S A* 2005;102:419–24. [PubMed: 15623559]
83. Fahlen L, Read S, Gorelik L, Hurst SD, Coffman RL, Flavell RA, Powrie F. T cells that cannot respond to TGF-beta escape control by CD4(+)/CD25(+) regulatory T cells. *J Exp Med* 2005;201:737–46. [PubMed: 15753207]
84. Li MO, Wan YY, Flavell RA. T cell-produced transforming growth factor-beta1 controls t cell tolerance and regulates Th1- and Th17-cell differentiation. *Immunity* 2007;26:579–91. [PubMed: 17481928]
85. Gray JD, Hirokawa M, Horwitz DA. The role of transforming growth factor beta in the generation of suppression: an interaction between CD8+T and NK cells. *J Exp Med* 1994;180:1937–42. [PubMed: 7964469]
86. Yamagiwa S, Gray JD, Hashimoto S, Horwitz DA. A role for TGF-beta in the generation and expansion of CD4+CD25+regulatory T cells from human peripheral blood. *J Immunol* 2001;166:7282–9. [PubMed: 11390478]
87. Fantini MC, Becker C, Monteleone G, Pallone F, Galle PR, Neurath MF. Cutting edge: TGF-beta induces a regulatory phenotype in CD4+CD25- T cells through Foxp3 induction and down-regulation of Smad7. *J Immunol* 2004;172:5149–53. [PubMed: 15100250]
88. Rich S, Seelig M, Lee HM, Lin J. Transforming growth factor beta 1 costimulated growth and regulatory function of staphylococcal enterotoxin B-responsive CD8+T cells. *J Immunol* 1995;155:609–18. [PubMed: 7608539]
89. Chen W, Jin W, Hardegen N, Lei KJ, Li L, Marinos N, McGrady G, Wahl SM. Conversion of peripheral CD4+CD25- naive T cells to CD4+CD25+regulatory T cells by TGF-beta induction of transcription factor Foxp3. *J Exp Med* 2003;198:1875–86. [PubMed: 14676299]
90. Fontenot JD, Rasmussen JP, Williams LM, Dooley JL, Farr AG, Rudensky AY. Regulatory T cell lineage specification by the forkhead transcription factor foxp3. *Immunity* 2005;22:329–41. [PubMed: 15780990]

91. Wan YY, Flavell RA. Identifying Foxp3-expressing suppressor T cells with a bicistronic reporter. *Proc Natl Acad Sci U S A* 2005;102:5126–31. [PubMed: 15795373]
92. Peng Y, Laouar Y, Li MO, Green EA, Flavell RA. TGF-beta regulates in vivo expansion of Foxp3-expressing CD4+CD25+ regulatory T cells responsible for protection against diabetes. *Proc Natl Acad Sci U S A* 2004;101:4572–7. [PubMed: 15070759]
93. Schramm C, Protschka M, Kohler HH, Podlech J, Reddehase MJ, Schirmacher P, Galle PR, Lohse AW, Blessing M. Impairment of TGF-beta signaling in T cells increases susceptibility to experimental autoimmune hepatitis in mice. *Am J Physiol Gastrointest Liver Physiol* 2003;284:G525–535. [PubMed: 12466145]
94. Huber S, Schramm C, Lehr HA, Mann A, Schmitt S, Becker C, Protschka M, Galle PR, Neurath MF, Blessing M. Cutting edge: TGF-beta signaling is required for the in vivo expansion and immunosuppressive capacity of regulatory CD4+CD25+ T cells. *J Immunol* 2004;173:6526–31. [PubMed: 15557141]
95. Mamura M, Lee W, Sullivan TJ, Felici A, Sowers AL, Allison JP, Letterio JJ. CD28 disruption exacerbates inflammation in Tgf-beta1-/- mice: in vivo suppression by CD4+CD25+regulatory T cells independent of autocrine TGF-beta1. *Blood* 2004;103:4594–601. [PubMed: 15016653]
96. Lee HM, Rich S. Differential activation of CD8+ T cells by transforming growth factor-beta 1. *J Immunol* 1993;151:668–77. [PubMed: 8335902]
97. Gray JD, Liu T, Huynh N, Horwitz DA. Transforming growth factor beta enhances the expression of CD154 (CD40L) and production of tumor necrosis factor alpha by human T lymphocytes. *Immunol Lett* 2001;78:83–8. [PubMed: 11672591]
98. Chung EJ, Choi SH, Shim YH, Bang YJ, Hur KC, Kim CW. Transforming growth factor-beta induces apoptosis in activated murine T cells through the activation of caspase 1-like protease. *Cell Immunol* 2000;204:46–54. [PubMed: 11006017]
99. Sillett HK, Cruickshank SM, Southgate J, Trejdosiewicz LK. Transforming growth factor-beta promotes 'death by neglect' in post-activated human T cells. *Immunology* 2001;102:310–6. [PubMed: 11298829]
100. Chen W, Jin W, Tian H, Sicurello P, Frank M, Orenstein JM, Wahl SM. Requirement for transforming growth factor beta1 in controlling T cell apoptosis. *J Exp Med* 2001;194:439–53. [PubMed: 11514601]
101. Genestier L, Kasibhatla S, Brunner T, Green DR. Transforming growth factor beta1 inhibits Fas ligand expression and subsequent activation-induced cell death in T cells via downregulation of c-Myc. *J Exp Med* 1999;189:231–9. [PubMed: 9892606]
102. Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B. TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. *Immunity* 2006;24:179–89. [PubMed: 16473830]
103. Mangan PR, Harrington LE, O'Quinn DB, Helms WS, Bullard DC, Elson CO, Hatton RD, Wahl SM, Schoeb TR, Weaver CT. Transforming growth factor-beta induces development of the T (H) 17 lineage. *Nature* 2006;441:231–4. [PubMed: 16648837]
104. Veldhoen M, Hocking RJ, Flavell RA, Stockinger B. Signals mediated by transforming growth factor-beta initiate autoimmune encephalomyelitis, but chronic inflammation is needed to sustain disease. *Nat Immunol* 2006;7:1151–6. [PubMed: 16998492]
105. Weaver CT, Harrington LE, Mangan PR, Gavrieli M, Murphy KM. Th17: an effector CD4 T cell lineage with regulatory T cell ties. *Immunity* 2006;24:677–88. [PubMed: 16782025]
106. Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, Weiner HL, Kuchroo VK. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature* 2006;441:235–8. [PubMed: 16648838]
107. Sakaguchi S, Takahashi T, Nishizuka Y. Study on cellular events in post-thymectomy autoimmune oophoritis in mice. II. Requirement of Lyt-1 cells in normal female mice for the prevention of oophoritis. *J Exp Med* 1982;156:1577–86. [PubMed: 6983558]
108. Sakaguchi S, Takahashi T, Nishizuka Y. Study on cellular events in postthymectomy autoimmune oophoritis in mice. I. Requirement of Lyt-1 effector cells for oocytes damage after adoptive transfer. *J Exp Med* 1982;156:1565–76. [PubMed: 6983557]



109. Fontenot JD, Dooley JL, Farr AG, Rudensky AY. Developmental regulation of Foxp3 expression during ontogeny. *J Exp Med* 2005;202:901–6. [PubMed: 16203863]
110. Schwartz RH. Natural regulatory T cells and self-tolerance. *Nat Immunol* 2005;6:327–30. [PubMed: 15785757]
111. Bennett CL, Christie J, Ramsdell F, Brunkow ME, Ferguson PJ, Whitesell L, Kelly TE, Saulsbury FT, Chance PF, Ochs HD. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat Genet* 2001;27:20–1. [PubMed: 11137993]
112. Brunkow ME, Jeffery EW, Hjerrild KA, Paepfer B, Clark LB, Yasayko SA, Wilkinson JE, Galas D, Ziegler SF, Ramsdell F. Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse. *Nat Genet* 2001;27:68–73. [PubMed: 11138001]
113. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 2003;299:1057–61. [PubMed: 12522256]
114. Khattri R, Cox T, Yasayko SA, Ramsdell F. An essential role for Scurfin in CD4+CD25+T regulatory cells. *Nat Immunol* 2003;4:337–42. [PubMed: 12612581]
115. Wildin RS, Ramsdell F, Peake J, Faravelli F, Casanova JL, Buist N, Levy-Lahad E, Mazzella M, Goulet O, Perroni L, et al. X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. *Nat Genet* 2001;27:18–20. [PubMed: 11137992]
116. Chang X, Gao JX, Jiang Q, Wen J, Seifers N, Su L, Godfrey VL, Zuo T, Zheng P, Liu Y. The Scurfy mutation of FoxP3 in the thymus stroma leads to defective thymopoiesis. *J Exp Med* 2005;202:1141–51. [PubMed: 16230479]
117. Liston A, Farr AG, Chen Z, Benoist C, Mathis D, Manley NR, Rudensky AY. Lack of Foxp3 function and expression in the thymic epithelium. *J Exp Med* 2007;204:475–80. [PubMed: 17353370]
118. Kim JM, Rasmussen JP, Rudensky AY. Regulatory T cells prevent catastrophic autoimmunity throughout the lifespan of mice. *Nat Immunol* 2007;8:191–7. [PubMed: 17136045]
119. Williams LM, Rudensky AY. Maintenance of the Foxp3-dependent developmental program in mature regulatory T cells requires continued expression of Foxp3. *Nat Immunol* 2007;8:277–84. [PubMed: 17220892]
120. Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+regulatory T cells. *Nat Immunol* 2003;4:330–6. [PubMed: 12612578]
121. Thornton AM, Shevach EM. CD4+CD25+ immunoregulatory T cells suppress polyclonal T cell activation in vitro by inhibiting interleukin 2 production. *J Exp Med* 1998;188:287–96. [PubMed: 9670041]
122. D’Cruz LM, Klein L. Development and function of agonist-induced CD25+Foxp3+ regulatory T cells in the absence of interleukin 2 signaling. *Nat Immunol* 2005;6:1152–9. [PubMed: 16227983]
123. Fontenot JD, Rasmussen JP, Gavin MA, Rudensky AY. A function for interleukin 2 in Foxp3-expressing regulatory T cells. *Nat Immunol* 2005;6:1142–51. [PubMed: 16227984]
124. Furtado GC, de Lafaille MA Curotto, Kutchukhidze N, Lafaille JJ. Interleukin 2 signaling is required for CD4(+) regulatory T cell function. *J Exp Med* 2002;196:851–7. [PubMed: 12235217]
125. Barthlott T, Moncrieffe H, Veldhoen M, Atkins CJ, Christensen J, O’Garra A, Stockinger B. CD25+CD4+ T cells compete with naive CD4+ T cells for IL-2 and exploit it for the induction of IL-10 production. *Int Immunol* 2005;17:279–88. [PubMed: 15684039]
126. de la Rosa M, Rutz S, Dorninger H, Scheffold A. Interleukin-2 is essential for CD4+CD25+ regulatory T cell function. *Eur J Immunol* 2004;34:2480–8. [PubMed: 15307180]
127. Pandiyan P, Zheng L, Ishihara S, Reed J, Lenardo MJ. CD4+ CD25+Foxp3+ regulatory T cells induce cytokine deprivation-mediated apoptosis of effector CD4+ T cells. *Nat Immunol* 2007;8:1353–62. [PubMed: 17982458]
128. Egen JG, Allison JP. Cytotoxic T lymphocyte antigen-4 accumulation in the immunological synapse is regulated by TCR signal strength. *Immunity* 2002;16:23–35. [PubMed: 11825563]
129. Slavik JM, Hutchcroft JE, Bierer BE. CD28/CTLA-4 and CD80/CD86 families: signaling and function. *Immunol Res* 1999;19:1–24. [PubMed: 10374692]
130. Greenwald RJ, Freeman GJ, Sharpe AH. The B7 family revisited. *Annu Rev Immunol* 2005;23:515–48. [PubMed: 15771580]

131. Boden E, Tang Q, Bour-Jordan H, Bluestone JA. The role of CD28 and CTLA4 in the function and homeostasis of CD4+ CD25+ regulatory T cells. *Novartis Found Symp* 2003;252:55–63. (discussion 63–6, 106–14). [PubMed: 14609212]
132. Read S, Greenwald R, Izcue A, Robinson N, Mandelbrot D, Francisco L, Sharpe AH, Powrie F. Blockade of CTLA-4 on CD4+CD25+ regulatory T cells abrogates their function in vivo. *J Immunol* 2006;177:4376–83. [PubMed: 16982872]
133. Uhlig HH, Coombes J, Mottet C, Izcue A, Thompson C, Fanger A, Tannappel A, Fontenot JD, Ramsdell F, Powrie F. Characterization of Foxp3+CD4+CD25+ and IL-10-secreting CD4+CD25+ T cells during cure of colitis. *J Immunol* 2006;177:5852–60. [PubMed: 17056509]
134. Kamanaka M, Kim ST, Wan YY, Sutterwala FS, Lara-Tejero M, Galan JE, Harhaj E, Flavell RA. Expression of interleukin-10 in intestinal lymphocytes detected by an interleukin-10 reporter knockin tiger mouse. *Immunity* 2006;25:941–52. [PubMed: 17137799]
135. Asseman C, Mauze S, Leach MW, Coffman RL, Powrie F. An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. *J Exp Med* 1999;190:995–1004. [PubMed: 10510089]
136. Fallarino F, Grohmann U, Hwang KW, Orabona C, Vacca C, Bianchi R, Belladonna ML, Fioretti MC, Alegre ML, Puccetti P. Modulation of tryptophan catabolism by regulatory T cells. *Nat Immunol* 2003;4:1206–12. [PubMed: 14578884]
137. Tadokoro CE, Shakhar G, Shen S, Ding Y, Lino AC, Maraver A, Lafaille JJ, Dustin ML. Regulatory T cells inhibit stable contacts between CD4+T cells and dendritic cells in vivo. *J Exp Med* 2006;203:505–11. [PubMed: 16533880]
138. Kretschmer K, Apostolou I, Hawiger D, Khazaie K, Nussenzweig MC, von Boehmer H. Inducing and expanding regulatory T cell populations by foreign antigen. *Nat Immunol* 2005;6:1219–27. [PubMed: 16244650]
139. Torgerson TR, Ochs HD. Immune dysregulation, polyendocrinopathy, enteropathy, X-linked: forkhead box protein 3 mutations and lack of regulatory T cells. *J Allergy Clin Immunol* 2007;120:744–50. (quiz 751–2). [PubMed: 17931557]
140. Miura Y, Thoburn CJ, Bright EC, Phelps ML, Shin T, Matsui EC, Matsui WH, Arai S, Fuchs EJ, Vogelsang GB, et al. Association of Foxp3 regulatory gene expression with graft-versus-host disease. *Blood* 2004;104:2187–93. [PubMed: 15172973]
141. Balandina A, Lecart S, Dartevelle P, Saoudi A, Berrih-Aknin S. Functional defect of regulatory CD4 (+)CD25+ T cells in the thymus of patients with autoimmune myasthenia gravis. *Blood* 2005;105:735–41. [PubMed: 15454488]
142. Huan J, Culbertson N, Spencer L, Bartholomew R, Burrows GG, Chou YK, Bourdette D, Ziegler SF, Offner H, Vandenbark AA. Decreased FOXP3 levels in multiple sclerosis patients. *J Neurosci Res* 2005;81:45–52. [PubMed: 15952173]
143. Lyon MF, Peters J, Glenister PH, Ball S, Wright E. The scurfy mouse mutant has previously unrecognized hematological abnormalities and resembles Wiskott–Aldrich syndrome. *Proc Natl Acad Sci USA* 1990;87:2433–7. [PubMed: 2320565]
144. Ono M, Shimizu J, Miyachi Y, Sakaguchi S. Control of autoimmune myocarditis and multiorgan inflammation by glucocorticoid-induced TNF receptor family-related protein (high), Foxp3-expressing CD25+ and CD25– regulatory T cells. *J Immunol* 2006;176:4748–56. [PubMed: 16585568]
145. Takahashi T, Tagami T, Yamazaki S, Uede T, Shimizu J, Sakaguchi N, Mak TW, Sakaguchi S. Immunologic self-tolerance maintained by CD25(+)CD4(+) regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4. *J Exp Med* 2000;192:303–10. [PubMed: 10899917]
146. Wan YY, Flavell RA. Regulatory T-cell functions are subverted and converted owing to attenuated Foxp3 expression. *Nature* 2007;445:766–70. [PubMed: 17220876]
147. Gavin MA, Rasmussen JP, Fontenot JD, Vasta V, Manganiello VC, Beavo JA, Rudensky AY. Foxp3-dependent programme of regulatory T-cell differentiation. *Nature* 2007;445:771–5. [PubMed: 17220874]

148. Zheng Y, Josefowicz SZ, Kas A, Chu TT, Gavin MA, Rudensky AY. Genome-wide analysis of Foxp3 target genes in developing and mature regulatory T cells. *Nature* 2007;445:936–40. [PubMed: 17237761]
149. Hill JA, Feuerer M, Tash K, Haxhinasto S, Perez J, Melamed R, Mathis D, Benoist C. Foxp3 transcription-factor-dependent and -independent regulation of the regulatory T cell transcriptional signature. *Immunity* 2007;27:786–800. [PubMed: 18024188]
150. Zheng Y, Rudensky AY. Foxp3 in control of the regulatory T cell lineage. *Nat Immunol* 2007;8:457–62. [PubMed: 17440451]
151. Valencia X, Stephen G, Goldbach-Mansky R, Wilson M, Shevach EM, Lipsky PE. TNF downmodulates the function of human CD4+CD25hi T-regulatory cells. *Blood* 2006;108:253–61. [PubMed: 16537805]