

Review

TGF- β and ‘Adaptive’ Foxp3⁺ Regulatory T cells

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In naïve T cells transforming growth factor-beta (TGF- β) induces Foxp3, a transcription factor essential for programming and developing T regulatory cells (Treg cells). This finding reveals a physiological factor which can turn on the Foxp3 gene and establishes an experimental approach to induce antigen-specific Treg cells as a potential therapy for human diseases. While this role for TGF- β is well confirmed, several critical questions remain largely unanswered and await further investigation. In this regard, it is imperative to understand the molecular pathways by which TGF- β signaling initiates and regulates Foxp3 expression. It is also important to elucidate which factors and/or cytokines influence the TGF- β -mediated conversion of naïve T cells and how to create an immunologically regulatory milieu to facilitate Treg cell generation *in vivo*. In this short article, we will highlight the key findings and recent progress in the field, discuss the molecular mechanisms underlying the TGF- β -mediated induction of Foxp3, and attempt to outline the challenges ahead.

Keywords: TGF- β , smads, Foxp3⁺ adaptive Treg, Th17, dendritic cells, immune tolerance, human T cell, mucosal system, transcription factors

Introduction

Nearly all cell types in rodents and humans can produce and respond to TGF- β . First discovered as a growth factor for non-immune cells, TGF- β has gradually been recognized as a critical cytokine in regulating immune responses (Sporn and Roberts, 1989; Strober et al., 1997; Weiner, 1997; Letterio and Roberts, 1998; Sporn, 1999; Chen and Wahl, 2002; Rubtsov and Rudensky, 2007; Li and Flavell, 2008). The earliest noticed role of TGF- β in the immune system was its inhibitory effect on the growth and activation of immune cells. In this regard, TGF- β potently counteracts macrophage activation (Tsunawaki et al., 1988; Wahl et al., 1990), prevents the maturation of dendritic cells (DCs) (Steinman et al., 2003; Liu, 2004), inhibits B cell antibody production, with the notable exception of IgA (Kehrl et al., 1986a; Coffman et al., 1989), and most relevantly to this review, suppresses T cell activation and proliferation (Kehrl et al., 1986b). In fact, TGF- β has long been known to be a powerful inhibitor of both Th1 and Th2 differentiation (Fiorentino et al., 1989; Sad and Mosmann, 1994). However, we have more recently discovered that TGF- β , in the context of T cell receptor (TCR) stimulation, induces Foxp3 expression in naïve CD4⁺CD25[−]Foxp3[−] T cells and converts them into Foxp3⁺ regulatory T cells (termed ‘adaptive’ or ‘induced’ Treg cells) (Chen et al., 2003). This TGF- β -mediated conversion of Foxp3⁺ Treg cells occurs not only in murine (Chen et al., 2003;

Fantini et al., 2004; Fu et al., 2004; Wan and Flavell, 2005; Bettelli et al., 2006; Davidson et al., 2007; Luo et al., 2007; Zheng et al., 2007), but also in human CD4⁺ T cells (Fantini et al., 2004; Amarnath et al., 2007; Tran et al., 2007). Importantly, the TGF- β -dependent induction of peripheral (Kretschmer et al., 2005; Luo et al., 2005) and thymic ‘natural’ (Liu et al., 2008) Foxp3⁺ Treg cells has also been shown to occur in mice *in vivo*. While this finding has been well received and confirmed by many laboratories in the field, a number of critical questions remain unanswered. Here we highlight the key findings and recent progress in the field and outline the imminent questions and issues that need to be addressed concerning TGF- β and Treg cells.

TGF- β induction of Foxp3⁺ Treg cells

CD4⁺CD25⁺Foxp3⁺ Treg cells are instrumental in the maintenance of immunological tolerance to self (Sakaguchi, 2000; Shevach, 2002; Bluestone and Abbas, 2003; Powrie and Maloy, 2003; Fontenot and Rudensky, 2005; Schwartz, 2005; von Boehmer, 2005; Waldmann et al., 2006; Hill et al., 2007a). The majority of Foxp3⁺ Treg cells are generated and developed in the thymus; these are termed ‘natural’ Tregs. Whether Foxp3⁺ Treg cells could be generated from peripheral CD4⁺ T cells remained unclear until 2003. We have long had an interest in understanding TGF- β regulation of T cell immunity and tolerance (Chen and Wahl, 1999, 2002, 2003; Chen, 2006); even before Foxp3 was identified as the ‘master gene’ for the development of natural Treg cells

(Fontenot et al., 2003; Hori et al., 2003; Khattry et al., 2003), we had shown that TGF- β was able to induce CD4⁺CD25⁺CTLA-4⁺ anergic/suppressor T cells from naïve CD4⁺ T cells in mice [Chen W, et al., J. Leuk. Biol. (suppl.), 2001, p102, Abstract 362]. Following the discovery of Foxp3 as the critical gene in programming CD4⁺CD25⁺ Treg cells, we demonstrated that TGF- β , concomitant with TCR stimulation, induced Foxp3 expression in naïve peripheral CD4⁺CD25⁺ Foxp3[−] T cells and converted them into Foxp3⁺ Treg cells (Chen et al., 2003). Importantly, the TGF- β -converted 'adaptive' or 'induced' Foxp3⁺ Treg cells were phenotypically and functionally indistinguishable from the natural Foxp3⁺ Treg cells generated in the thymus; adaptive Treg cells potentially inhibit TCR-driven T cell proliferation *in vitro* and when adaptively transferred *in vivo*. At the time, this finding was unexpected. The dominant view for Foxp3⁺ Treg cells was that they were generated only in the thymus, and once a T cell left the thymus and entered the periphery it would not be able to develop into a Treg cell. Following our initial demonstration that TGF- β could induce Foxp3 in naïve CD4⁺ T cell populations, other groups have confirmed this in both mice and humans (Fantini et al., 2004; Fu et al., 2004). Subsequently, Foxp3 transgenic mice, in which only Foxp3⁺ T cells express GFP or RFP, were utilized to demonstrate that TGF- β indeed converted CD4⁺Foxp3[−] T cells into Foxp3⁺ regulatory T cells (Wan and Flavell, 2005; Bettelli et al., 2006). Importantly, we and others have verified that TGF- β signaling in CD4⁺ T cells is absolutely required for the induction of Foxp3 expression, as CD4⁺ T cells deficient in TGF- β signaling cannot be converted to Foxp3⁺ Treg cells *in vitro* or *in vivo* (Apostolou et al., 2008; Liu et al., 2008). IL-2 has been found to be critical in facilitating TGF- β -mediated induction of Foxp3⁺ expression in CD4⁺ naïve T cells; although it alone is unable to induce Foxp3 (Davidson et al., 2007; Zheng et al., 2007). TGF- β -mediated induction of Foxp3⁺ Treg cells has also been observed *in vivo* in a variety of experimental settings. Waldmann and colleagues demonstrated that *in vivo* neutralization of TGF- β by specific antibodies dramatically reduced the increase in Foxp3⁺ Treg cells induced by anti-CD4 antibody in models of transplantation (Cobbold et al., 2004). Moreover, systemic increases in TGF- β were shown to substantially increase Foxp3⁺ Treg cell numbers in mice (Luo et al., 2005; Perruche et al., 2008), and following adaptive transfer of naïve CD4⁺Foxp3[−] T cells, the conversion to Foxp3⁺ Treg cells was shown to be dependent on TGF- β *in vivo* (Apostolou et al., 2008; Belkaid and Oldenhove, 2008). Thus, only a few years after its initial discovery, TGF- β -mediated induction of Foxp3⁺ Treg cells has become an accepted paradigm and a potentially very exciting research area. However, it is important to determine why TGF- β , a ubiquitous and non-specific cytokine in the immune system, could induce Foxp3⁺ transcription in such a specific manner; at present no other cytokines or soluble factors have been able to replace TGF- β in the induction of Foxp3 in naïve CD4⁺ T cells.

The TGF- β -mediated induction of Foxp3 in naïve CD4⁺ T cells theoretically supports the notion of plasticity in CD4⁺ T cells; it also provides a simple experimental approach to generate unlimited numbers of antigen (Ag)-specific Foxp3⁺ Treg cells required for use as potential therapies for autoimmune diseases, chronic

inflammation, allergy and allograft rejection. This point is particularly important considering the difficulty in expanding natural Foxp3⁺ Treg cells *in vitro* and the fact that a heterogeneous pool of natural Treg cells with different Ag specificities could be limited in their clinical use owing to potential non-specific suppression of other immune responses. In line with this, Ag-specific Foxp3⁺ Treg cells induced *in vitro* with TGF- β potentially inhibit autoimmune diseases in mice, whereas the same number of TCR-activated natural Treg cells were ineffective in suppressing the same disease (Huter et al., 2008). Furthermore, the experimental system of TGF- β -mediated induction of Foxp3 in CD4⁺ T cells facilitated the identification of the initiating factors (e.g. TGF- β plus IL-6) responsible for promoting Th17 cell differentiation (Bettelli et al., 2006; Ivanov et al., 2006). Despite the envisaged therapeutic application of TGF- β -induced Foxp3⁺ regulatory cells, some unresolved issues exist regarding their ability to suppress immune functions. Although the majority of studies demonstrated that TGF- β -induced Foxp3⁺ Treg cells show potent immunosuppressive activity *in vitro* and *in vivo* (Chen et al., 2003; Fantini et al., 2004; Wan and Flavell, 2005; Bettelli et al., 2006; Davidson et al., 2007; You et al., 2007; Apostolou et al., 2008), there remains some controversy (Hill et al., 2007b). The reasons for the discrepancy in suppressive function are unknown, but might be associated with the individual experimental systems. Microarray analysis of Treg cells, both natural and induced, showed that the TGF- β -induced Foxp3⁺ cells exhibited only some elements of a 'Treg signature' despite full expression of Foxp3 (Hill et al., 2007b). Moreover, it has been shown that the Foxp3 locus is differentially methylated in natural and induced regulatory T cells (Floess et al., 2007). Such data raise interesting questions concerning the difference between natural and induced Foxp3⁺ T cells. However, caution should be taken when interpreting these data and making conclusions regarding the differences in gene-expression profiles or the differential methylation patterns between natural and induced Foxp3⁺ Treg cells, as one cannot assume that the differentiation status and/or the environmental influences are the same between natural Tregs isolated from mice and induced Tregs harvested from culture dishes following stimulation with TCR plus TGF- β for a few days. Nevertheless, TGF- β indeed plays an essential role in converting naïve T cells into Foxp3⁺ Treg cells. The challenges ahead, however, include understanding the underlying mechanisms and developing a system *in vivo* to induce Ag-specific Treg cells for future clinical applications.

The role of antigen-presenting cells in the conversion of adaptive Foxp3⁺ Treg cells

Antigen-presenting cells (APCs), and in particular DCs, play an important role in the TGF- β -mediated Foxp3⁺ Treg cell conversion in the peripheral immune system. Studies have demonstrated that DCs can induce Foxp3⁺ Treg cells in the presence of exogenous TGF- β and by the secretion of endogenous TGF- β . However, which DCs can do this most potently remains unclear. Is there a specific DC subset responsible for the induction of Foxp3? Or

does the activation status of the DCs determine their ability to induce Foxp3? Immature DCs (iDCs), such as those isolated from the spleen of naïve mice, induced Foxp3⁺ Treg cells from CD4⁺Foxp3[−] T cells in the presence of TGF-β (Luo et al., 2007). However, mature DCs (mDCs) expanded natural Foxp3⁺ Treg cells without the addition of exogenous IL-2 (Tarbell et al., 2004). Most strikingly, two groups independently identified that DCs in the lamina propria (Lp) of the gut, could specifically convert naïve CD4⁺ T cells into Foxp3⁺ Treg cells (Coombes et al., 2007; Sun et al., 2007). Lp DCs expressing CD103 were shown to convert naïve CD4⁺ T cells into Foxp3⁺ Treg cells through the production of endogenous TGF-β, although both CD103⁺ and CD103[−] DCs showed a similar ability to convert Treg cells in the presence of exogenous TGF-β (Belkaid and Oldenhove, 2008). In addition, CD103⁺ DCs also produced retinoic acid (RA) which further amplified the TGF-β induction of Treg cells.

In addition to DCs, recent reports have demonstrated that macrophages may also play a role in converting Foxp3⁺ Treg cells specifically within the mucosal immune system (Denning et al., 2007; Savage et al., 2008); the difference in the ability of DCs and macrophages in this conversion process is still to be elucidated.

In considering that both DCs and macrophages induce Foxp3⁺ Treg cells, a critical question is what triggers these APCs to produce the TGF-β that leads to the generation of Foxp3⁺ Treg cells. Both macrophages and DCs possess the capacity to engulf and digest apoptotic cells; we recently demonstrated that iDCs, upon uptake and digestion of apoptotic T cells, produce TGF-β, which can then convert naïve CD4⁺ T cells to Foxp3⁺ Treg cells (Perruche et al., 2008). Thus both iDCs and macrophages (Fadok et al., 1998; Chen et al., 2001; Gandhi et al., 2007) can produce TGF-β upon phagocytosis of apoptotic cells, suggesting a link between T cell apoptosis and T cell immunoregulation.

When and where does TGF-β-mediated Foxp3 induction occur *in vivo*?

Despite compelling evidence that TGF-β-mediated induction of Foxp3 expression occurs *in vitro* and in several experimental systems *in vivo*, an important question remains outstanding; to what extent do 'adaptive' Foxp3⁺ Treg cells contribute to the endogenous pool of Foxp3⁺ Treg cells in un-manipulated mice? Furthermore, in what situations does the *de novo* conversion of naïve CD4⁺ T cells to Foxp3⁺ Treg cells occur, and in which organs and/or tissues does this peripheral conversion to Foxp3⁺ cells take place? In naïve mice in the steady state, the conversion to Foxp3⁺ Treg cells is likely trivial in the peripheral lymph nodes and spleen. In the absence of significant challenges from foreign Ags or abnormally high endogenous Ags, the Foxp3⁺ Treg cells in the naïve mouse are homeostatically maintained by yet unidentified mechanisms. It is hard to envision that there is any need to generate any additional Foxp3⁺ Treg cells in the quiet lymph nodes and spleen of a healthy animal. However,

this may not be the case in certain lymphoid tissues, the most salient example being the mucosal system of the gut. In the gut, particularly in the gut-associated lymphoid tissues, T cells are constantly faced with large amounts of Ags derived from food, pathogens or commensal bacteria. The dynamic changes in the environmental Ags makes it unlikely, in fact unimaginable, that in the gut only natural Foxp3⁺ Treg cells (which most likely respond to self Ags) exist without the generation of induced Foxp3⁺ Treg cells in response to Ags in an environment rich in TGF-β. Indeed, studies have clearly demonstrated that in the Lp Foxp3⁺ Treg cells are found in significantly higher numbers than in either peripheral lymph nodes or the spleen (Belkaid and Oldenhove, 2008). In fact, the gut as a site for the induction of regulatory T cells was described well over a decade ago (Weiner, 1997). Weiner and colleagues defined populations of regulatory T cells generated following the induction of oral tolerance to fed protein Ag (Chen et al., 1994; Friedman and Weiner, 1994). More recently, Foxp3⁺ T cells have been shown to be induced following oral exposure to protein Ag via a TGF-β-dependent mechanism (Mucida et al., 2007). Thus, in the gut, it seems likely that the Foxp3⁺ Treg cells contain a substantial portion of induced Foxp3⁺ Treg cells converted from CD4⁺Foxp3[−] T cells in the mucosa. As discussed, CD103⁺ DCs may play a particular role in this generation of induced Foxp3⁺ Treg cells in the gut (Belkaid and Oldenhove, 2008). However, the exact proportion of the Foxp3⁺ regulatory T cell population that are induced in the mucosal system is undefined.

In addition to the gut, it would be reasonable to envisage that other mucosal systems, the respiratory and genitourinary systems, might also harbor such conversion of Foxp3⁺ Treg cells, but evidence for this is lacking. Thus, in the steady-state, the *de novo* conversion of Foxp3⁺ Treg cells may be minimal in the regular lymph nodes and spleen, but may occur constantly in mucosal lymphoid tissues. Furthermore, it can be anticipated that the *de novo* TGF-β-dependent conversion of naïve CD4⁺ T cells to Foxp3⁺ Treg cells can occur in all lymph nodes and the spleen when the immune system is challenged by exogenous and/or endogenous Ags, as long as the appropriate cytokine milieu exists in the microenvironment, such as sufficient levels of TGF-β and the absence of inhibiting pro-inflammatory cytokines (discussed below). However, evidence to support this notion awaits further experiments.

Factors influencing the TGF-β-mediated conversion of naïve T cells to Foxp3⁺ Treg cells

Whether the TGF-β-mediated Foxp3⁺ Treg cell conversion occurs *in vitro*, and probably also *in vivo*, is not simply dependent upon the signals received through TCR and TGF-β receptors; other factors and/or cytokines in the microenvironment also influence this conversion. IL-2 is critical in assisting TGF-β-mediated induction of Foxp3 in naïve CD4⁺ T cells *in vitro* (Davidson et al., 2007; Zheng et al., 2007). This could also be true *in vivo*; conditional knockout mice lacking both the IL-2 and TGF-β receptors have no CD4⁺CD25⁺ Foxp3⁺ Treg cells in the periphery (Liu et al.,

2008). RA has been shown to enhance TGF- β -mediated induction of Foxp3 (Coombes et al., 2007; Mucida et al., 2007; Sun et al., 2007), but inhibit TGF- β -mediated induction of IL-10 in naïve CD4⁺ T cells (Maynard et al., 2009). However, the mechanism by which RA potentiates TGF- β -mediated induction of Foxp3 remains largely unknown; RA effects have been shown to be direct, enhancing Smad3-dependent signaling, and indirect, by relieving inhibition from a CD4⁺ CD44^{hi} subset (Hill et al., 2008; Xiao et al., 2008).

On the other hand, it has been shown that pro-inflammatory cytokines such as IL-6 antagonize TGF- β -mediated induction of Foxp3 in CD4⁺ T cells, consequently switching the differentiation program towards a Th17 effector CD4⁺ T cell lineage (Bettelli et al., 2006; Zhou et al., 2007). Intriguingly, IL-4, the Th2 signature cytokine, abrogated almost all TGF- β -mediated induction of Foxp3 in CD4⁺ T cells and promoted the differentiation of a unique subset of T cells producing IL-9 and IL-10 (Dardalhon et al., 2008; Veldhoen et al., 2008). Despite these observations, the underlying molecular mechanisms by which these factors/cytokines regulate TGF- β -mediated induction of Foxp3 expression are undefined. Nevertheless, these findings have highlighted the dynamic and complex nature of T cell differentiation *in vitro*, and particularly, *in vivo*. This can help in better understanding when Foxp3⁺ Treg cells are generated and when Th17 effector cells are differentiated in physiological, as well as pathological, settings.

'Adaptive' Foxp3⁺ Treg cells in humans

The demonstration that TGF- β -mediated induction of Foxp3 in naïve CD4⁺ T cells could occur in mice, inevitably raised the question of whether this could also occur in human T cells. In the pre-Foxp3 era it had been reported that TGF- β was an important factor in the expansion of CD4⁺CD25⁺ regulatory cells from human peripheral blood (Yamagiwa et al., 2001). Following the identification of Foxp3, initial studies suggested that human CD4⁺CD25⁺ T cells could be induced to express Foxp3 in the absence of exogenous TGF- β (Walker et al., 2003). However, subsequent analysis revealed that TGF- β is indeed required for the induction of Foxp3 in human T cells stimulated with TCR and CD28 antibodies (Amarnath et al., 2007; Tran et al., 2007). Most notably, inclusion of neutralizing anti-TGF- β antibodies abrogated Foxp3 induction in culture; the TGF- β could be produced from the stimulated T cells or by the activation of latent TGF- β in the serum of the culture medium. In addition, exogenous TGF- β has been shown to further up-regulate Foxp3⁺ T cell induction (Fantini et al., 2004; Amarnath et al., 2007; Tran et al., 2007). Thus, a TGF- β signal is required for the induction of Foxp3 in CD4⁺Foxp3⁺ T cells in both mice and humans. However, an unresolved question in humans is whether all Foxp3⁺ T cells induced *in vitro* are immunosuppressive, as observed in cultures of mouse CD4⁺ T cells. It has been reported that a subset of Foxp3⁺ T cells expressing low levels of Foxp3 (Foxp3^{low}) lack typical immunosuppressive function, failing to inhibit TCR-stimulated T cell proliferation (Gavin et al., 2006; Tran et al., 2007). Thus, it has been

argued that Foxp3 in human T cells may be only an activation marker and not a specific 'lineage' marker for the regulatory phenotype. The jury is still out on this issue, but its resolution is important, as it will directly affect future experimental designs for the induction of Ag-specific regulatory T cells which have potential therapeutic applications in human diseases.

Molecular mechanisms of TGF- β -mediated induction of Foxp3

Despite the general consensus that TGF- β in the context of TCR stimulation induces Foxp3 expression in naïve CD4⁺ T cells, the underlying molecular mechanisms are ill defined. At present, all that is certain is that both TCR and TGF- β signaling are essential; either one alone fails to turn on Foxp3. Several questions need to be resolved; first and foremost is the transcription of the Foxp3 gene attributed to the direct effect of the TGF- β signaling pathway or due to TCR signaling elements which are indirectly affected by TGF- β treatment? If the former, is this effect mediated by Smad-dependent or -independent pathways (Derynck and Zhang, 2003)? Studies have shown, at least *in vitro*, that Smad3 (Tone et al., 2008) and/or Smad4 (our unpublished data) are required for the TGF- β -mediated induction of Foxp3 in naïve CD4⁺ T cells; CD4⁺ T cells lacking either gene showed a marked defect in Foxp3 induction. Interpretation of this piece of *in vitro* data has been complicated by the fact that neither Smad3 knockout mice nor T-cell-specific Smad4 knockout mice showed significant changes in thymic or peripheral CD4⁺CD25⁺Foxp3⁺ Treg cell number in naïve mice (Maruyama and Chen, manuscript in preparation). It remains unknown if there are changes in Foxp3⁺ cells when these transgenic mice are challenged in models of infection, inflammation, tumor or transplantation. Despite the noted defect in TGF- β -mediated induction of Foxp3 in Smad3 and Smad4 null T cells, it is unlikely that Smad2/3 and/or Smad4 could turn on the Foxp3 gene by directly binding to and functioning at the Foxp3 promoter, as it has been shown that Smad3 binds to the enhancer rather than the promoter of Foxp3 gene (Tone et al., 2008). As it is generally believed that the binding of Smad proteins to their target genes is weak, it is hard to conceive that phosphorylated Smad2/3 (P-Smad2/3) could initiate Foxp3 expression in the absence of other cofactors or partners. In addition, TGF- β -induced P-Smad2/3 in cells occurs almost immediately following TGF- β treatment (a few minutes to a few hours depending on cell types). If the P-Smad2/3 could directly turn on the Foxp3 gene, Foxp3 mRNA should be detected within a few minutes or, maximally, hours after TGF- β treatment. Yet, the expression of Foxp3 mRNA cannot be detected until about 12–24 h following TGF- β treatment of CD4⁺ naïve T cells. Thus, the lag in time between the activation of P-Smad2/3 and expression of Foxp3 mRNA is too long to support this possibility. Therefore, it is likely that P-Smad2/3 and/or Smad4, act with a, or several, yet unidentified intermediate co-partners, to turn on the expression of the Foxp3 gene.

It is also possible that TGF- β may induce Foxp3 expression through a Smad-independent pathway (Derynck and Zhang, 2003). This possibility could suggest that the TCR signal, altered by the TGF- β signal, directs Foxp3 transcription in CD4⁺

T cells. In this scenario, the TGF- β signal serves as a co-factor, likely an inhibitory force, and the TCR signal as the direct player. Experimental evidence favoring this possibility includes the demonstration that some T cell transcription factors, such as NFAT and AP-1, directly bind to the Foxp3 promoter (Wu et al., 2006). Gata-3, a critical transcription factor in Th2 cell differentiation, can also bind to the Foxp3 promoter (Mantel et al., 2007). Moreover, ROR- γ t, a master transcription factor of Th17 cell differentiation, physically interacts with Foxp3, potentially highlighting a level of mutual regulation between Foxp3⁺ and Th17 cells (Zhou et al., 2008). Although this possibility remains open and needs rigorous investigation, it is difficult to envisage that TGF- β signaling simply serves as an inhibitory factor to indirectly influence TCR signaling during the induction of Foxp3. If the TGF- β signal was only an inhibitory force, in theory it would be possible that any factor(s) suppressing TCR signaling could induce Foxp3, in the absence of TGF- β . Yet, no other factors or cytokines have been shown to replace TGF- β in the generation of Foxp3⁺ CD4⁺ T cells. Studies have suggested that some factors or molecules could up-regulate Foxp3 in CD4⁺ T cells *in vitro* and *in vivo* in the absence of exogenous TGF- β ; however, such data should be interpreted with caution before the role of TGF- β signaling is rigorously excluded.

Taken together, we can propose the following model to explain how TGF- β induces Foxp3 in CD4⁺ T cells (Figure 1). TGF- β , via Smad-dependent and -independent pathways, activates/inhibits transcription factors associated with TGF- β signaling that also possess the ability to interact with TCR signaling pathways. Such transcription factors may act directly by binding to the Foxp3 promoter to switch on the gene or indirectly by regulating additional transcription factors that can turn the Foxp3 gene on or off.

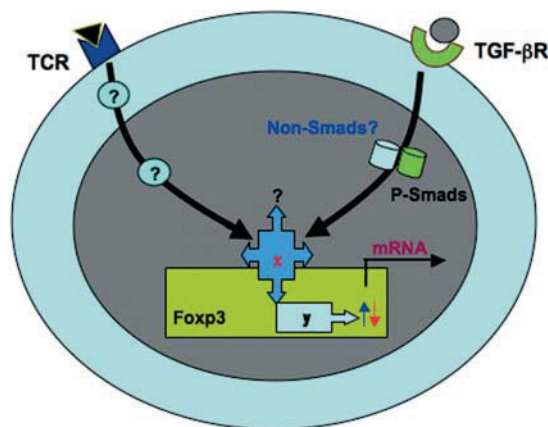


Figure 1 A proposed model for TGF- β induction of Foxp3 in CD4⁺ naive T cells. TGF- β , via Smad-dependent and/or -independent pathways, activates/inhibits transcription factors (x) that also possess the ability to interact with TCR signaling pathways. Such transcription factors may act directly by binding to the Foxp3 promoter to switch on the gene or indirectly by regulating additional transcription factors (y) that can turn the Foxp3 gene on or off. TCR, T cell receptor; TGF- β R, TGF- β receptors. The question marks indicate unknown players. The blue and red arrows indicate on and off regulation, respectively.

Concluding remarks

Compelling evidence has now demonstrated that TGF- β , concomitant with TCR stimulation, induces Foxp3 expression in naive CD4⁺ T cells, converting them to Treg cells. This TGF- β -mediated conversion has been shown to occur both *in vitro* and *in vivo* in mice, and may also happen in humans. While this phenomenon has been well confirmed, challenges ahead include identifying the underlying molecular mechanisms by which TGF- β turns on the Foxp3 gene. Ideally this would lead to the establishment of experimental procedures to induce Ag-specific Treg cells for therapeutic applications in human immunopathological diseases.

Acknowledgements

We thank Drs T. Maruyama and P. Zhang, MIU, NIDCR, NIH for critically reading the manuscript. We apologize to those whose work was not cited here due to space limitations. The authors carried out this work as employees of the National Institutes of Health.

Funding

The research in authors' laboratory was supported by the Intramural Research Program of the National Institute of Dental and Craniofacial Research of the National Institutes of Health.

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