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Therapeutic potential of TGF-β-induced CD4+Foxp3+ regulatory T cells in autoimmune diseases

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Abstract

Foxp3+ T regulatory cell (Treg) subsets play a crucial role in the maintenance of immune homeostasis against self-antigen. The lack or dysfunction of these cells is responsible for the pathogenesis and development of many autoimmune diseases. Therefore, manipulation of these cells may provide a novel therapeutic approach to treat autoimmune diseases. In this review, we provide current opinions concerning the classification, developmental and functional characterizations of Treg subsets. A particular emphasis will be focused on the therapeutic role of TGF-β-induced $CD4^+Foxp3^+$ cells (iTregs) in established autoimmune disease. Moreover, the similarity and disparity of iTregs and naturally occurring, thymus-derived $CD4^+CD25^+Foxp3^+$ regulatory T cells (nTregs) have also be discussed. While the proinflammatory cytokine IL-6 can convert nTregs to IL-17-producing cells, iTregs induced by TGF-β are resistant to the effects of this cytokine. Understanding this difference may play a key role in determining how Tregs can be used in the treatment of established autoimmune diseases.

Keywords

Autoimmune diseases; Immunoregulation; Regulatory T cells; TGF-β; Foxp3; Th17 cells

Introduction

One of the key features of the immune system is its ability to discriminate self antigen from non-self antigens, providing effective immune responses against microbial antigens but

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preventing deleterious autoimmune responses [1]. Although several mechanisms, such as clonal deletion (physiological deletion of self-reactive lymphocytes) and anergy (functional inactivation of self-reactive lymphocytes), have all been suggested as mechanisms responsible for the immune unresponsiveness to self antigens, there is also a substantial evidence that T cell–mediated active suppression of self-reactive T cells by suppressor or regulatory T (Treg) cells is another essential mechanism of self-tolerance in the periphery [1,2].

Gershon and Kondo first suggested thymocytes contained a population of suppressor T cells in the early 1970s [3]. Other data supporting the existence of a suppressor T cell population in normal thymocytes came from experiments in which neonatal thymectomy (NTx) of normal mice between day 2 and 4 after birth resulted in the development of autoimmune thyroiditis, gastritis, orchitis, prostatitis, and sialoadenitis [4]. Nonetheless, a resurgent interest in Treg cells was restored in 1995 when Sakaguchi's group found a $CD4^+$ subset that constitutively expresses CD25 (IL-2 receptor α-chain) in the thymus and displays the suppressive activity [5]. These thymus-derived, naturally-occurring $CD4^+CD25^+$ (nTreg) cells can migrate to periphery 3 days after birth and constitute 5–10% of peripheral $CD4^+$ T cells in normal naïve mice. Removal of $CD4^+CD25^+$ T cells elicited many autoimmune diseases that are similar with that in NTx mice. Reconstitution with normal $CD4^+CD25^+$ T cells within a limited period after NTx prevented the development of autoimmunity [5].

The discovery of Foxp3, a major X-linked forkhead/winged helix transcription factor associated with nTreg development and function, provides an important molecular insight into this essential cell lineage [6–8]. Foxp3 is mainly expressed on endogenous $CD4^+CD25^+$ Treg cells but not on conventionally activated $CD4^+$ cells in mouse [1]. A mutation of Foxp3 gene leads to total lack of the $CD4^+Foxp3^+$ Treg cells and development of Scurfy mice that display the most severe form of multi-organ inflammation [9]. The development of $Foxp3^{gfp}$ "knock-in" mice that express fluorescent proteins under control of the Foxp3 promoter provides an important tool to investigate phenotype and functional characteristics of purified and viable Treg cells since Foxp3 staining cannot be used to isolate living Tregs [9].

$CD4^+CD25^+$ cells also exist in humans although only the $CD4^+CD25^{bright}$ cell population display the immune suppressive effect [10]. Recent evidence suggests that although Foxp3 expression in murine lymphocytes appears to be a definitive marker for Treg cells, this may not be the case for human Tregs. New data demonstrate that FOXP3 (the analog of murine Foxp3) may be upregulated in rapidly proliferating human T cells and might be viewed as an activation marker for human T cells [11–13]. Nonetheless, mutations of the human FOXP3 gene cause a disease called IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome), which is characterized by autoimmune disease in multiple endocrine organs [14–16].

Classification of Treg cells

It is now well recognized that the Treg cell family consists of multiple heterogeneous subsets that include $CD4^+$, $CD8^+$, $CD4^-CD8^-$ T cells, NK T cells and γδ T cells [17,18].

Among these Treg subsets, $CD4^+$ Treg cells are better understood than other Treg cell subsets since they are key players in the Treg cell network and display many important functional characteristics. $CD4^+$ Treg subsets can be further classified into two main populations, thymus-derived, natural $CD4^+CD25^+Foxp3^+$ cells (nTregs) as described above and those that can be induced from $CD25^-$ precursors in peripheral lymphoid organs. Induced Treg cells can be developed by the administration of self antigen, peptide or anti-CD3 antibody *in vivo* [19–22] or induced *ex vivo* with IL-10 (IL-10-producing type 1 regulatory T cells, Tr1 cells) [23] or with IL-2 and TGF-β (transforming growth factor β) (iTreg) *in vitro* [24–27]. Induced iTreg cells developed *in vivo* and Tr1 cells may not express Foxp3 and their role in the autoimmunity has been reviewed elsewhere [28, 29]. Conversely, iTregs induced *ex vivo* by IL-2 and TGF-β express Foxp3 and share some similar phenotypic and functional characteristics with nTregs although they may also exhibit substantial differences in the development and functionality. In this review, we will compare and contrast nTreg and iTreg cells and focus on the potential therapeutic role of iTreg cells in autoimmune diseases.

Similarities and disparities between nTreg and iTreg cells

Most $CD4^+$ nTreg and iTreg cells express CD25, α chain of IL-2 receptor. iTreg cells can be induced from $CD4^+CD25^-$ precursors cells extrathymically [24]. When Foxp3 was identified as a specific hallmark for nTreg cells, studies immediately confirmed that the combination of IL-2 and TGF-β was able to induce $CD25^-Foxp3^-$ precursors to express Foxp3, and with this expression came suppressive activity [25–27]. Both Treg subsets express CTLA-4 (cytotoxic T lymphocyte antigen 4), GITR (gluccorticoid-induced tumor necrosis factor receptor), CCR4 (chemokine receptor) and CD62L (L-Selectin), and most are previously activated cells (i.e. $CD45RB^{low}$ in the mouse and CD45RO in the human) [30].

Both Treg cells subsets produce little IL-2 and IFN-γ, but they may produce immunosuppressive cytokines such as active TGF-β and IL-10 [24,31]. Both of them also express membrane-bound TGF-β [32,33]. They display a weak proliferative capacity when stimulated with anti-CD3 but the addition of exogenous IL-2 can restore their proliferation [25–27]. Both Treg cell subsets suppress T cell immune response *in vitro* similarly. They suppress CD4+ effector cell activation, proliferation and cytokine production, as well as CD8+ effector cell activation, proliferation and cytotoxicity activity *in vitro*.

Whereas the adoptive transfer of nTreg cells can prevent the induction of autoimmune gastritis, colitis, EAE, diabetes, lupus and rescue the multiorgan autoimmune disease in Scurfy mice [7, 34–37], adoptive transfer of iTreg cells similarly suppressed lupus, diabetes, EAE and colitis [38–40]. Although the mechanism of action of Treg cells is still poorly understood, it is generally believed that cell-cell contact is required for the *in vitro* suppressive activity of both Treg cell subsets, and cytokines such as TGF-β and/or IL-10 are possibly required for the *in vivo* suppressive activity of nTreg cells [35, 41, 42]. We recently demonstrated that TGF-β and/or IL-10 are also required for the inhibitor effect of iTreg cells on mouse lupus-like syndromes (Zhou XH and Zheng SG, unpublished data). Both Treg cell subsets can also possess the ability to induce conventional $CD4^+CD25^-$ cells to become a

new generation of iTreg cells *in vitro* and *in vivo* [26, 43–45]. Nonetheless, there are substantial differences in the development and functionality between nTreg and iTreg cells.

A distinction is often made between nTregs that develop in thymus and iTregs that develop extrathymically [46]. Although endogenous $CD4^+CD25^+Foxp3^+$ cells in the periphery were viewed as nTregs [1], we believe these cells may be a mixture of nTregs and iTregs since infection, tumor, food and allergens can enlarge this cell population [30]. There are no specific phenotypic hallmarks that can distinguish iTregs from nTregs. By definition, $CD4^+CD25^+Foxp3^+$ cells found in the thymus can be viewed as nTregs.

IL-2 and TGF-β play an essential role in the differentiation and development of iTreg cells. Other TGF-β superfamily members such as Activin A and BMP also have independent or synergistic role with TGF-β in iTreg induction [47–50], but they are redundant for the development of nTreg cells since $CD4^+CD25^+$ cells exist in the thymus of TGF-β/TGF-β receptor deficient mice, or IL-2 and IL-2 receptor deficient mice [51, 52]. However, these cytokines are essential for the maintenance of Foxp3 expression and for the survival of both subsets *in vitro* and *in vivo* [47, 52].

Additionally, whereas the CD28/B7 signal pathway is essential for the development of nTreg cells, this signal pathway is not needed for the development of iTregs although this signal pathway promotes the expansion of iTreg cells [53, 54]. In addition, although the lack of a functional CTLA-4/B7 signal pathway markedly interferes with the development of iTregs, CTLA-4 deficient mice have a normal frequency and function of nTreg cells, notwithstanding other reports that CTLA-4 is important for the suppressive activity of nTreg cells [35, 55]. Another member of the CD28 and CTLA-4 receptor family, inducible T cell co-stimulator (ICOS), also favors Treg development [56]. By contrast, Ox40, a member of the tumor necrosis factor (TNF)–TNF receptor (TNFR) superfamily, negatively regulates the development and function of both nTregs and iTregs [57, 58].

nTreg and iTreg cells also differ in their plasticity and stability in an inflammatory milieu. Recent studies revealed that the combination of IL-6 and TGF-β can induce mouse T cells to produce IL-17 [59, 60]. IL-23 produced by immunogenic DCs promotes the survival and expansion of these cells and enables these T cells to become into pro-inflammatory Th17 cells [61]. Although these cells seem to play an important role in immune defense against certain bacterial infections, expansion of these cells is also closely related to the pathogenesis and development of many autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, psoriasis and possibly SLE. The TGF-β/TGF-β receptor signaling pathway is essential for the development of Th17 cells [62], probably via an upregulation of RORγt expression, a transcription factor needed for IL-17 [63]. Interestingly endogenous TGF-β produced by $Foxp3^+CD4^+CD25^+$ nTreg cells enables IL-6 to convert some of these cells to Th17 thus losing their previous phenotypic characteristics and functional activities [33, 64]. nTreg cells can also be converted into Th2 cells even in a Th1-polarizing environment if Foxp3 expression is attenuated [65]. We also observed that nTregs can be converted into Th1 cells when they were strongly stimulated with anti-CD3 in the presence of IL-6 [62]. More recently, Tsuji M et al reported that the environment of the Peyer's patches (PPs) down-regulates Foxp3 expression by nTregs and induce them to become

CD4+ follicular B helper T(T_{FH}) which is required B cell function in the germinal centers [66].

Unlike nTreg cells, we recently found that iTregs were completely resistant to the IL-6-driven Th1 and Th17 cell conversion. This resistance cannot be explained by the lack of TGF-β production by iTregs. In fact, we observed that both nTregs and iTregs expressed and produced similar levels of membrane-bound TGF-β and active TGF-β. In addition, we also observed that iTregs are unable to convert into Th1/Th17 cells after exogenous TGF-β was added to the cultures [33]. Recently, we conducted an experiment *in vivo* to better establish the stability of both Tregs in live animals. When nTregs were adoptively transferred into mice with evident CIA, >50% of these cells in draining lymph nodes lost Foxp3 expression in one week after cell transfer. The majority of $Foxp3^{-}$ cells began to express IL-17 and IFN-γ but not IL-4. Conversely, iTregs cells mostly maintained Foxp3 expression and did not convert into Th1/Th2/Th17 cells [67]. The differences of stability can contribute to their different functionality in autoimmune diseases.

Although both nTregs and iTregs are known to express Foxp3, recent studies have revealed that the Foxp3 gene locus and enhancer in nTregs and iTregs are structurally distinct. Floess et al reported that while complete demethylation of CpG motifs within the *foxp3* locus in nTregs was observed, only partial demethylation of CpG motifs was found in iTregs. They also demonstrated that DNA methylation affects Treg stability and suppressive capacity *in vitro* [68]. More information came from LaI et al, who identified a unique and evolutionarily conserved CpG-rich island of the Foxp3 nonintronic upstream enhancer and discovered that a specific site within it was unmethylated in nTregs but not in iTregs [69]. It is still arguable, however, that methylation of the Foxp3 locus or enhancer affects the stability and functionality of iTregs. We and others have found that iTregs expressed stable Foxp3 if these cells were restimulated with IL-2 and/or TGF-β [47]. Recently, we observed that addition of all-trans retinoic acid (atRA) to TGF-β not only promoted Foxp3 expression, but also sustained Foxp3 expression and survival although atRA did not change the Foxp3 locus methylation [70]. In addition, these groups found that the addition of the DNMT (DNA methyltransferase) inhibitor 5-aza-2'-deoxycytidine (AzaC) can induce demethylation of the Foxp3 locus and enhancer, suggesting that epigenetic regulation can overcome the problem of stability and functionality in iTreg cells. It is noted iTreg cells can be induced *in vivo* and these iTreg cells displayed both stable Foxp3 expression and demethylated TSDR (Treg-specific demethylated region) [71]. It is unknown why iTregs generated *in vitro* and *in vivo* have a different methylation status in TSDR.

Therapeutic role of iTreg cells in autoimmune diseases

Adoptive transfer of nTregs or iTregs can rescue Scurfy mice and prevent many autoimmune diseases [7, 34–37], suggesting that manipulation of these cells may have the potential to treat or cure autoimmune diseases.

To be of use as therapeutic agents for inflammatory and autoimmune diseases, Treg cells must be able to inhibit ongoing T cell responses and reverse established pathology. Although one group showed the therapeutic ability of $CD4^{+}CD25^{+}$ nTreg cells to reverse

established colitis [72], most studies revealed that the therapeutic effects of nTregs on established autoimmune diseases is fairly unsatisfactory. While adoptive transfer of nTregs can markedly prevent the development of CIA, injection of these cells to established CIA was almost unable to suppress the disease progression [73]. A study from Daikh's lab observed that the effect of adoptive transfer of *in vitro* expanded CD4+CD25+CD62L+ cells from (NZBxNZW) F1 (BWF1) lupus-prone mice into BWF1 mice that had developed proteinuria is modest [37]. Additionally, nTregs also did not suppress Th17 cell-mediated autoimmune gastritis (AIG) [75].

There are several reasons may explain for this unsatisfactory effect of iTregs on established autoimmune diseases: Firstly, the low frequency and poor growth make sufficient numbers for adoptive immunotherapy a significant challenge. Although expansion *in vitro* can overcome this problem, it has been observed that repeated stimulation leads to the diminished Foxp3 expression and the suppressive activity [76]. Secondly, it is very likely that when antigen-specific effector T cells are induced, nTregs can not counteract the effector T cell response and function. For example, T cells from lupus mice and other autoimmune diseases may be resistant to treatment with nTregs although the functional properties of nTregs from these diseases are normal [77]. Additionally, when inflammation is established, a large amount of proinflammatory cytokines, such as IL-6, IL-1 and TNF-α is released. It has been reported that increased levels of IL-6 released by activated DCs abolished the suppressive ability of nTregs [78]. TNF-α possibly has a similar ability to affect the suppressive activity of nTregs [79]. Lastly, it is possible that there is a dynamic alteration of nTregs in an inflammatory milieu. It has been recently reported by Strober's lab and our group that nTregs can be converted into IL-17-producing cells (Th17) when stimulated with IL-6 [33, 64], we have observed that these nTregs lost Foxp3 expression and suppressive activity when they had been stimulated with IL-6 [33]. As Th17 cells have been demonstrated to be involved in the pathogenesis of many autoimmune diseases, it, therefore, raises a concern that not only does nTreg therapy lack the therapeutic effect on the established autoimmune diseases, but may even worsen the autoimmune diseases since high levels of IL-6 and other pre-inflammatory cytokines have been identified in many autoimmune diseases [80].

Unlike nTregs, several studies have revealed that manipulation of iTregs may have a therapeutic potential on established autoimmune diseases. Adoptively transferred iTreg to EAE mice were active both prophylactically and after priming, suggesting iTreg are highly potent suppressors of autoimmune encephalomyelitis [40]. Similarly, iTregs not only controlled acute onset of T1D elicited by Th1 effectors in SCID recipients and protected NOD mice from spontaneous disease when administered during the preinsulitis phase, but also suppressed the disease progression during the established insulitis phase [39]. Aricha R et al also reported that administration of *ex vivo*-generated iTreg cells after the induction of experimental autoimmune myasthenia gravis can significantly ameliorate disease manifestations [81]. We have also demonstrated that a single injection of iTregs not only prevented other parental T cells from inducing lymphoid hyperplasia, B cell activation, and an immune complex glomerulonephritis, but also decreased the titer of anti-DNA, suppressed proteinuria, and doubled survival when cells are adoptively transferred to

animals that had already developed anti-dsDNA Abs [38]. Others have confirmed this effect [82]. More interestingly, while both nTregs and iTregs can suppress AIG initiated by Th1 and Th2 cells, only Ag-specific iTregs but not nTregs suppressed Th17-mediated AIG [75]. Recently, we found that adoptive transfer of iTregs to established CIA resulted in superior therapeutic effects when compared to nTreg cells [67]. Taken together, these data indicate that iTregs may have an advantageous therapeutic effect on the established autoimmune diseases.

iTregs in mouse and human

While iTregs in mouse have been well studied, it is unclear if similar protocols can generate iTregs in humans. Unlike mouse CD4+ cells, stimulation with polyclonal mitogens without exogenous TGF-β induced transient FOXP3 expression, and endogenous TGF-β produced by $CD4^+$ cells or contaminated in culture medium may account for the FOXP3 induction [83]. The addition of TGF-β to the cultures markedly increased FOXP3 expression after one week of culture, however, these cells were not anergic, produced high levels of IL-2, and experienced vigorous proliferation when restimulated with anti-CD3 antibody [83]. These cells were unable to suppress T cell proliferation *in vitro* and therefore one questions whether IL-2 and TGF-β has ability to induce iTreg cells in humans [83]. Furthermore, it must be remembered that unlike mouse cells, FOXP3 expression is not a specific hallmark for human Treg cells since it is also transiently expressed on activated proliferating human T cells [11–13].

As nTregs and iTregs differ in their principal antigen specificities and strength of T-cell receptor (TCR) stimulation needed for their generation [30], we believe that both TCR stimulation strength and other co-stimulatory factors may affect the differentiation of TGF-β-induced iTregs. In fact, we have previously reported that human CD4+ cells can be induced to become Treg with IL-2 and TGF-β. CD4+ cells stimulated with staphylococcal enterotoxin B and these cytokines become potent suppressors of T cell-dependent IgG production [24]. Additionally, naive $CD4^+$ cells stimulated with alloantigens and IL-2 and TGF-β also become Treg that suppress the development of allospecific CTL activity [84].

It is likely that the FOXP3 locus remains methylated in human TGF-β-induced $CD4^+$ cells affects their suppressive activity. LaI et at observed that addition of AzaC to TGF-β restored the ability of the latter to induce CD4+ iTregs in humans and they believed that AzaC induces demethylation of Foxp3 gene enhancer, then endows these human CD4+ with suppressive capacity [69]. However, our group recently found that addition of atRA to TGF-β also enabled human $CD4^+$ cells to become suppressor cells (Lu L et al. Manuscript submitted). The addition of atRA did not significantly change methylation of FOXP3 locus but markedly promoted $CD4^+$ cells to become mature CD4+ cells that expressed high levels of CD45RO and lost CD127 expression although they also increased FOXP3 expression. We therefore believe that after 1 wk these human $CD4^+$ cells are only partially differentiated suppressor cells. We observed that upon repeated stimulation, the cells became anergic, and some then expressed membrane-associated TGF-β, and T cell proliferation was strongly suppressed (Wang J and Zheng SG. Unpublished data). Thus, we believe that polyclonal stimulation of human CD4+ cells with IL-2 and TGF-β induces both mouse and human

CD4+CD25− cells to become $Foxp3^+CD4^+CD25^+$ suppressor cells, but the human cells need more time or other cofactors to function in a manner similar to mouse suppressor cells.

Conclusion

We have summarized the similarities and differences of both nTregs and iTregs and highlighted the differences of stability and functionality of Treg cell subsets in the inflammatory environment. While nTregs can be converted into Th1/Th17 cells when stimulated with IL-6 and the therapeutic effects on established autoimmune diseases are unsatisfactory, iTregs are stable and seem to be effective in the treatment of established autoimmune diseases. Although there are different requirements for the induction of iTregs in humans compared to mouse, it is likely that we can expect the future development of human iTregs using TCR stimulation with IL-2, TGF-β and other additives such as AzaC or atRA. These encouraging findings underscore the need to explore the therapeutic potential of iTregs in human immune-mediated diseases. In this approach, iTreg cells are induced from naïve $CD4^+$ precursor cells in a patient, enriched, expanded *ex vivo*, and reinfused. This approach is advantageous because the iTreg product can be analyzed phenotypically and functionally prior to infusion, providing another level of safety. Furthermore, cell dosage can be tightly controlled [85].

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References

1. 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]
2. Shevach EM. CD4+ CD25+ suppressor T cells: more questions than answers. Nat Rev Immunol. 2002; 2:389–400. [PubMed: 12093005]
3. Gershon RK, Kondo K. Cell interactions in the induction of tolerance: the role of thymic lymphocytes. Immunology. 1970; 18:723–37. [PubMed: 4911896]
4. Kojima A, Prehn RT. Genetic susceptibility to post-thymectomy autoimmune diseases in mice. Immunogenetics. 1981; 14:15–27. [PubMed: 7035348]
5. 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]
6. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. Science. 2003; 299:1057–61. [PubMed: 12522256]
7. 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]
8. 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]
9. 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]
10. Baecher-Allan C, Brown JA, Freeman GJ, Hafler DA. CD4+CD25high regulatory cells in human peripheral blood. J Immunol. 2001; 167:1245–53. [PubMed: 11466340]

11. Morgan ME, van Bilsen JH, Bakker AM, Heemskerk B, Schilham MW, Hartgers FC, Elferink BG, van der Zanden L, de Vries RR, Huizinga TW, Ottenhoff TH, Toes RE. Expression of FOXP3 mRNA is not confined to CD4+CD25+ T regulatory cells in humans. Hum Immunol. 2005; 66:13–20. [PubMed: 15620457]
12. Allan SE, Crome SQ, Crellin NK, Passerini L, Steiner TS, Bacchetta R, Roncarolo MG, Levings MK. Activation-induced FOXP3 in human T effector cells does not suppress proliferation or cytokine production. Int Immunol. 2007; 19:345–54. [PubMed: 17329235]
13. Yu JN, Ma JL, Wang JH, Tao XJ, Li XP, Zou HJ, Zheng SG. FOXP3, is still a specific marker for human regulatory T cells? Chin J Allergy Clin Immunol. 2008; 2:5–10.
14. Chatila TA, Blaeser F, Ho N, Lederman HM, Voulgaropoulos C, Helms C, Bowcock AM. JM2, encoding a fork head-related protein, is mutated in X-linked autoimmunity-allergic disregulation syndrome. J Clin Invest. 2000; 106:R75–81. [PubMed: 11120765]
15. Wildin RS, Ramsdell F, Peake J, Faravelli F, Casanova JL, Buist N, Levy-Lahad E, Mazzella M, Goulet O, Perroni L, Bricarelli FD, Byrne G, McEuen M, Proll S, Appleby M, Brunkow ME. X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. Nat Genet. 2001; 27:18–20. [PubMed: 11137992]
16. 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]
17. Tang Q, Bluestone JA. The Foxp3+ regulatory T cell: a jack of all trades, master of regulation. Nat Immunol. 2008; 9:239–44. [PubMed: 18285775]
18. Horwitz DA, Zheng SG, Gray JD, Wang JH, Ohtsuka K, Yamagiwa S. Regulatory T cells generated ex vivo as an approach for the therapy of autoimmune disease. Semin Immunol. 2004; 16:135–43. [PubMed: 15036237]
19. Hahn BH, Singh RP, La Cava A, Ebling FM. Tolerogenic treatment of lupus mice with consensus peptide induces Foxp3-expressing, apoptosis-resistant, TGFbeta-secreting CD8+ T cell suppressors. J Immunol. 2005; 175:7728–37. [PubMed: 16301683]
20. Ochi H, Abraham M, Ishikawa H, Frenkel D, Yang K, Basso AS, Wu H, Chen ML, Gandhi R, Miller A, Maron R, Weiner HL. Oral CD3-specific antibody suppresses autoimmune encephalomyelitis by inducing CD4+ CD25− LAP+ T cells. Nat Med. 2006; 12:627–35. [PubMed: 16715091]
21. Kang HK, Michaels MA, Berner BR, Datta SK. Very low-dose tolerance with nucleosomal peptides controls lupus and induces potent regulatory T cell subsets. J Immunol. 2005; 174:3247–55. [PubMed: 15749855]
22. Brand DD, Myers LK, Whittington KB, Latham KA, Stuart JM, Kang AH, Rosloniec EF. Detection of early changes in autoimmune T cell phenotype and function following intravenous administration of type II collagen in a TCR-transgenic model. J Immunol. 2002; 168:490–8. [PubMed: 11751997]
23. 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]
24. Zheng SG, Gray JD, Ohtsuka K, Yamagiwa S, Horwitz DA. Generation ex vivo of TGF-beta-producing regulatory T cells from CD4+CD25− precursors. J Immunol. 2002; 169:4183–9. [PubMed: 12370347]
25. 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]
26. Zheng SG, Wang JH, Gray JD, Soucier H, Horwitz DA. Natural and induced CD4+CD25+ cells educate CD4+CD25− cells to develop suppressive activity: the role of IL-2, TGF-beta, and IL-10. J Immunol. 2004; 172:5213–21. [PubMed: 15100259]

27. 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]
28. Chatenoud L, Bluestone JA. CD3-specific antibodies: a portal to the treatment of autoimmunity. Nat Rev Immunol. 2007; 7:622–32. [PubMed: 17641665]
29. Battaglia M, Gregori S, Bacchetta R, Roncarolo MG. Tr1 cells: from discovery to their clinical application. Semin Immunol. 2006; 18:120–7. [PubMed: 16464609]
30. Horwitz DA, Zheng SG, Gray JD. Natural and TGF-beta-induced Foxp3(+)CD4(+) CD25(+) regulatory T cells are not mirror images of each other. Trends Immunol. 2008; 29:429–35. [PubMed: 18676178]
31. 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]
32. 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]
33. Zheng SG, Wang J, Horwitz DA. Cutting edge: Foxp3+CD4+CD25+ regulatory T cells induced by IL-2 and TGF-beta are resistant to Th17 conversion by IL-6. J Immunol. 2008; 180:7112–6. [PubMed: 18490709]
34. DiPaolo RJ, Glass DD, Bijwaard KE, Shevach EM. CD4+CD25+ T cells prevent the development of organ-specific autoimmune disease by inhibiting the differentiation of autoreactive effector T cells. J Immunol. 2005; 175:7135–42. [PubMed: 16301616]
35. Read S, Malmström V, Powrie F. Cytotoxic T lymphocyte-associated antigen 4 plays an essential role in the function of CD25(+)CD4(+) regulatory cells that control intestinal inflammation. J Exp Med. 2000; 192:295–302. [PubMed: 10899916]
36. Kohm AP, Carpentier PA, Anger HA, Miller SD. Cutting edge: CD4+CD25+ regulatory T cells suppress antigen-specific autoreactive immune responses and central nervous system inflammation during active experimental autoimmune encephalomyelitis. J Immunol. 2002; 169:4712–6. [PubMed: 12391178]
37. Scalapino KJ, Tang Q, Bluestone JA, Bonyhadi ML, Daikh DI. Suppression of disease in New Zealand Black/New Zealand White lupus-prone mice by adoptive transfer of ex vivo expanded regulatory T cells. J Immunol. 2006; 177:1451–9. [PubMed: 16849451]
38. Zheng SG, Wang JH, Koss MN, Quismorio F Jr, Gray JD, Horwitz DA. CD4+ and CD8+ regulatory T cells generated ex vivo with IL-2 and TGF-beta suppress a stimulatory graft-versus-host disease with a lupus-like syndrome. J Immunol. 2004; 172:1531–9. [PubMed: 14734731]
39. Weber SE, Harbertson J, Godebu E, Mros GA, Padrick RC, Carson BD, Ziegler SF, Bradley LM. Adaptive islet-specific regulatory CD4 T cells control autoimmune diabetes and mediate the disappearance of pathogenic Th1 cells in vivo. J Immunol. 2006; 176:4730–9. [PubMed: 16585566]
40. Selvaraj RK, Geiger TL. Mitigation of experimental allergic encephalomyelitis by TGF-beta induced Foxp3+ regulatory T lymphocytes through the induction of anergy and infectious tolerance. J Immunol. 2008; 180:2830–8. [PubMed: 18292504]
41. Fahlén 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]
42. Hara M, Kingsley CI, Niimi M, Read S, Turvey SE, Bushell AR, Morris PJ, Powrie F, Wood KJ. IL-10 is required for regulatory T cells to mediate tolerance to alloantigens in vivo. J Immunol. 2001; 166:3789–96. [PubMed: 11238621]
43. Zheng SG, Meng L, Wang JH, Watanabe M, Barr ML, Cramer DV, Gray JD, Horwitz DA. Transfer of regulatory T cells generated ex vivo modifies graft rejection through induction of tolerogenic CD4+CD25+ cells in the recipient. Int Immunol. 2006; 18:279–89. [PubMed: 16415106]

44. Jonuleit H, Schmitt E, Kakirman H, Stassen M, Knop J, Enk AH. Infectious tolerance: human CD25(+) regulatory T cells convey suppressor activity to conventional CD4(+) T helper cells. J Exp Med. 2002; 196:255–60. [PubMed: 12119350]
45. Qiao M, Thornton AM, Shevach EM. CD4+ CD25+ [corrected] regulatory T cells render naive CD4+ CD25− T cells anergic and suppressive. Immunology. 2007 Apr; 120(4):447–55. Epub 2007 Jan 17 Erratum in: Immunology 2007, 121, 146. [PubMed: 17244157]
46. La Cava A. Tregs are regulated by cytokines: implications for autoimmunity. Autoimmun Rev. 2008; 8:83–7. [PubMed: 18771756]
47. Zheng SG, Wang J, Wang P, Gray JD, Horwitz DA. IL-2 is essential for TGF-beta to convert naive CD4+CD25− cells to CD25+Foxp3+ regulatory T cells and for expansion of these cells. J Immunol. 2007; 178:2018–27. [PubMed: 17277105]
48. Davidson TS, DiPaolo RJ, Andersson J, Shevach EM. Cutting Edge: IL-2 is essential for TGF-beta-mediated induction of Foxp3+ T regulatory cells. J Immunol. 2007; 178:4022–6. [PubMed: 17371955]
49. Huber S, Stahl FR, Schrader J, Lüth S, Presser K, Carambia A, Flavell RA, Werner S, Blessing M, Herkel J, Schramm C. Activin a promotes the TGF-beta-induced conversion of CD4+CD25− T cells into Foxp3+ induced regulatory T cells. J Immunol. 2009; 182:4633–40. [PubMed: 19342638]
50. Lu L, Ma J, Wang X, Wang J, Zhang F, Yu J, He G, Xu B, Brand DD, Horwitz DA, Shi W, Zheng SG. Synergistic effect of TGF-beta superfamily members on the induction of Foxp3(+) Treg. Eur J Immunol. 2009 Nov 26.
51. Piccirillo CA, Letterio JJ, Thornton AM, McHugh RS, Mamura M, Mizuhara H, Shevach EM. CD4(+)CD25(+) regulatory T cells can mediate suppressor function in the absence of transforming growth factor beta1 production and responsiveness. J Exp Med. 2002; 196:237–46. [PubMed: 12119348]
52. Fontenot JD, Rasmussen JP, Gavin MA, Rudensky AY. A function for interleukin 2 in Foxp3-expressing regulatory T cells. Nat Immunol. 2005; 6:1142–1151. [PubMed: 16227984]
53. Salomon B, Lenschow DJ, Rhee L, Ashourian N, Singh B, Sharpe A, Bluestone JA. B7/CD28 costimulation is essential for the homeostasis of the CD4+CD25+ immunoregulatory T cells that control autoimmune diabetes. Immunity. 2000; 12:431–40. [PubMed: 10795741]
54. You S, Leforban B, Garcia C, Bach JF, Bluestone JA, Chatenoud L. Adaptive TGF-beta-dependent regulatory T cells control autoimmune diabetes and are a privileged target of anti-CD3 antibody treatment. Proc Natl Acad Sci U S A. 2007; 104:6335–40. [PubMed: 17389382]
55. Zheng SG, Wang JH, Stohl W, Kim KS, Gray JD, Horwitz DA. TGF-beta requires CTLA-4 early after T cell activation to induce FoxP3 and generate adaptive CD4+CD25+ regulatory cells. J Immunol. 2006; 176:3321–9. [PubMed: 16517699]
56. Herman AE, Freeman GJ, Mathis D, Benoist C. CD4+CD25+ T regulatory cells dependent on ICOS promote regulation of effector cells in the prediabetic lesion. J Exp Med. 2004; 199:1479–89. [PubMed: 15184501]
57. So T, Croft M. Cutting edge: OX40 inhibits TGF-beta- and antigen-driven conversion of naive CD4 T cells into CD25+Foxp3+ T cells. J Immunol. 2007; 179:1427–30. [PubMed: 17641007]
58. Vu MD, Xiao X, Gao W, Degauque N, Chen M, Kroemer A, Killeen N, Ishii N, Chang Li X. OX40 costimulation turns off Foxp3+ Tregs. Blood. 2007; 110:2501–10. [PubMed: 17575071]
59. Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B. TGF-beta 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]
60. 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]
61. Annunziato F, Cosmi L, Liotta F, Maggi E, Romagnani S. Type 17 T helper cells-origins, features and possible roles in rheumatic disease. Nat Rev Rheumatol. 2009; 5:325–31. [PubMed: 19434074]

62. Lu L, Wang J, Zhang Fm, Chai Y, Brad DD, Wang XH, Horwitz DA, Shi W, Zheng SG. Role of Smad and non-Smad signals in the development of Th17 and regulatory T cells. J Immunol. 2010 in press.
63. Manel N, Unutmaz D, Littman DR. The differentiation of human T(H)-17 cells requires transforming growth factor-beta and induction of the nuclear receptor RORgammat. Nat Immunol. 2008; 9:641–9. [PubMed: 18454151]
64. Xu L, Kitani A, Fuss I, Strober W. Cutting edge: regulatory T cells induce CD4+CD25−Foxp3− T cells or are self-induced to become Th17 cells in the absence of exogenous TGF-beta. J Immunol. 2007; 178:6725–9. [PubMed: 17513718]
65. Wan YY, Flavell RA. Regulatory T-cell functions are subverted and converted owing to attenuated Foxp3 expression. Nature. 2007; 445:766–70. [PubMed: 17220876]
66. Tsuji M, Komatsu N, Kawamoto S, Suzuki K, Kanagawa O, Honjo T, Hori S, Fagarasan S. Preferential generation of follicular B helper T cells from Foxp3+ T cells in gut Peyer's patches. Science. 2009; 323:1488–92. [PubMed: 19286559]
67. Kong N, Zhou XH, Fan HM, Liu ZM, Brand DD, Zou HJ, Zheng SG. TGF-β-Induced $CD4^+Foxp3^+$ Cells but not natural $CD4^+CD25^+$ cells suppress established collagen-induced arthritis. Arthritis & Rheumatism. 2009; 60:S4.
68. Floess S, Freyer J, Siewert C, Baron U, Olek S, Polansky J, Schlawe K, Chang HD, Bopp T, Schmitt E, Klein-Hessling S, Serfling E, Hamann A, Huehn J. Epigenetic control of the foxp3 locus in regulatory T cells. PLoS Biol. 2007; 5:e38. [PubMed: 17298177]
69. Lal G, Zhang N, van der Touw W, Ding Y, Ju W, Bottinger EP, Reid SP, Levy DE, Bromberg JS. Epigenetic regulation of Foxp3 expression in regulatory T cells by DNA methylation. J Immunol. 2009; 182:259–73. [PubMed: 19109157]
70. Zhou XH, Kong N, Wang J, Zou HJ, Fan HM, Brand DD, Liu ZM, Zheng SG. All-trans retinoid acid restores the stability and functionality of nTregs in the Inflammatory milieu. Arthritis & Rheumatism. 2009; 60:S736.
71. Polansky JK, Kretschmer K, Freyer J, Floess S, Garbe A, Baron U, Olek S, Hamann A, von Boehmer H, Huehn J. DNA methylation controls Foxp3 gene expression. Eur J Immunol. 2008; 38:1654–63. [PubMed: 18493985]
72. Mottet C, Uhlig HH, Powrie F. Cutting edge: cure of colitis by CD4+CD25+ regulatory T cells. J Immunol. 2003; 170:3939–43. [PubMed: 12682220]
73. Morgan ME, Flierman R, van Duivenvoorde LM, Witteveen HJ, van Ewijk W, van Laar JM, de Vries RR, Toes RE. Effective treatment of collagen-induced arthritis by adoptive transfer of CD25+ regulatory T cells. Arthritis Rheum. 2005; 52:2212–2221. [PubMed: 15986351]
74. Bardos T, Czipri M, Vermes C, Finnegan A, Mikecz K, Zhang J. $CD4^+CD25^+$ immunoregulatory T cells may not be involved in controlling autoimmune arthritis. Arthritis Res Ther. 2003; 5:R106–13. [PubMed: 12718754]
75. Huter EN, Stummvoll GH, DiPaolo RJ, Glass DD, Shevach EM. Cutting edge: antigen-specific TGF beta-induced regulatory T cells suppress Th17-mediated autoimmune disease. J Immunol. 2008; 181:8209–13. [PubMed: 19050237]
76. Chai JG, Tsang JY, Lechler R, Simpson E, Dyson J, Scott D. CD4+CD25+ T cells as immunoregulatory T cells in vitro. Eur J Immunol. 2002; 32:2365–75. [PubMed: 12209650]
77. Monk CR, Spachidou M, Rovis F, Leung E, Botto M, Lechler RI, Garden OA. MRL/Mp CD4+,CD25− T cells show reduced sensitivity to suppression by CD4+,CD25+ regulatory T cells in vitro: a novel defect of T cell regulation in systemic lupus erythematosus. Arthritis Rheum. 2005; 52:1180–4. [PubMed: 15818683]
78. Pasare C, Medzhitov R. Toll pathway-dependent blockade of CD4+CD25+ T cell-mediated suppression by dendritic cells. Science. 2003; 299:1033–6. [PubMed: 12532024]
79. Valencia X, Stephens 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]
80. Linker-Israeli M, Deans RJ, Wallace DJ, Prehn J, Ozeri-Chen T, Klinenberg JR. Elevated levels of endogenous IL-6 in systemic lupus erythematosus. A putative role in pathogenesis. J Immunol. 1991; 147:117–23. [PubMed: 2051017]

81. Aricha R, Feferman T, Fuchs S, Souroujon MC. Ex vivo generated regulatory T cells modulate experimental autoimmune myasthenia gravis. J Immunol. 2008; 180:2132–9. [PubMed: 18250419]

82. Su H, Ye DQ, Wang BL, Fang XH, Chen J, Wang Q, Li WX, Zhang N. Transforming growth factor-beta1-induced CD4+CD25+ regulatory T cells in vitro reverse and prevent a murine lupus-like syndrome of chronic graft-versus-host disease. Br J Dermatol. 2008; 158:1197–209. [PubMed: 18410422]

83. Tran DQ, Ramsey H, Shevach EM. Induction of FOXP3 expression in naive human CD4+FOXP3 T cells by T-cell receptor stimulation is transforming growth factor-beta dependent but does not confer a regulatory phenotype. Blood. 2007; 110:2983–90. [PubMed: 17644734]

84. 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]

85. Riley JL, June CH, Blazar BR. Human T regulatory cell therapy: take a billion or so and call me in the morning. Immunity. 2009; 30:656–65. [PubMed: 19464988]