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Review

Induced Regulatory T Cells: Their Development, Stability, and Applications

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Regulatory T (Treg) cells, as central mediators of immune suppression, play crucial roles in many facets of immune systems. The transcription factor Foxp3 has been characterized as a master regulator of Tregs, and is induced during their thymic development. Foxp3$^+$ Tregs can also be generated from naïve T cells after stimulation in the presence of TGF-β and IL-2; the resulting cells are called induced Tregs (iTregs) when generated *in vitro*, or peripheral Tregs (pTregs) when generated *in vivo*. Compared to tTregs, iTregs have been shown to be unstable, and attempts to generate stable iTregs have been made for clinical applications. We review here the current knowledge on the development of pTregs, iTregs, and their roles and applications.

Trends

iTregs could be a crucial source of regulatory T cells for immune therapy, and clinical applications of iTregs are now ongoing.

Reprogramming of effector or memory T cells into iTregs is especially important for overcoming the issue of antigen-specificity, but this requires methods to efficiently and stably generate those cells.

Epigenetics plays a key role in Treg development and stability, and methods to affect these marks and enhance the stability of iTregs are under active investigation.

pTregs can be induced by some bacterial species, and the microbiota is emerging as an important player in Treg differentiation.

Development of Regulatory T cells

Treg cells suppress excess immunity against a diverse range of antigens, including self-antigens, commensal bacteria-derived antigens, and environmental allergens [1]. Treg cells developed in the thymus are called thymus-derived Treg (tTreg) cells. Treg cells are specified by an expression of the transcription factor Foxp3 (Forkhead box P3), which plays crucial roles in the differentiation, maintenance, and function of Treg cells [2–5]. Deficiency of Foxp3 in both humans and mice results in the lack of Treg cells, and leads to the development of severe systemic inflammatory diseases manifested by autoimmunity, colitis, and allergies [6–8]. In tTreg cells, Foxp3 has been shown to be induced by strong T cell receptor (TCR) signals after the recognition of self-antigen–MHC complexes present on thymic antigen-presenting cells (APCs) with relatively high avidity. Therefore, tTreg cells express a TCR repertoire with a bias for self, and are particularly important in the prevention of autoimmunity [9,10].

The second route for Treg generation is the differentiation from naïve CD4$^+$ T cells at the periphery upon reception of antigen stimulation with an appropriate combination of cytokines, including IL-2 and transforming growth factor (TGF)-β. Foxp3$^+$ Treg cells produced in this way are called induced Treg (iTreg) cells when generated *in vitro* or peripherally induced Treg (pTreg) cells when generated *in vivo*. Although pTreg cells compose only a small percentage of Treg cells as a whole, this cell subset is highly enriched in particular organs, including the gut and maternal placenta. Accordingly, pTreg cells are thought to be particularly important in the establishment of tolerance against commensal bacteria, foods, allergens, and the fetus in a pregnant mother [11–19]. It has been revealed that both of the Treg cell subsets cooperatively maintain immune homeostasis [20]. In this review article, we describe the molecular mechanisms that govern iTreg and pTreg cell differentiation and maintenance, and the clinical applications of iTregs in organ transplantation and the treatment of immunological diseases.

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Induction of Tregs by TGF-β

Signals that induce Foxp3 have been well characterized (Figure 1). The importance of TGF-β for iTreg development has been well established since the discovery that TGF-β could induce Foxp3 in naïve T cells [11]. TGF-β1-deficient mice exhibited normal tTreg development in the thymus, but peripheral Tregs were significantly reduced in number [21].

A major signaling pathway induced by TGF-β is the phosphorylation and activation of Smad transcription factors. In T cells, Smad2 and Smad3 are activated by TGF-β, and subsequently form a heterotrimer with Smad4. By using single- and double-knockout mice, we have demonstrated that Smad2 and Smad3 are redundantly essential for Foxp3 induction [22,23], but these two are not essential for the induction of RORγt, a master regulator of the Th17 program [24]. Like *Tgfb1* knockout mice, T cell-specific *Smad2*- and *Smad3*-deficient mice possess normal tTreg cells in the thymus, but total Treg cell number is decreased in the periphery, which may be due to severe inflammation in *Smad2/3*-double deficient mice [22]. The importance of Smad2 has often been ignored [25,26], but gene disruption studies have clearly demonstrated the redundancy between Smad2 and Smad3 [22,27,28].

Three intronic enhancers, designated 'conserved noncoding sequences' (CNSs) 1, 2, and 3, in addition to a promoter, have been identified at the *Foxp3* gene locus, and were revealed to play important roles in Treg cell differentiation [29,30]. Importantly, CNS1 contains two consecutive Smad-binding sites, and chromatin immunoprecipitation (ChIP) assays confirmed the recruitment of Smad3 into CNS1 [29]. The importance of CNS1 for iTreg/pTreg generation has been demonstrated by generating mice lacking a CNS1 region. These mice exhibit spontaneous allergic Th2-type inflammation in the intestine and lung, and defects in pTreg but not in tTreg

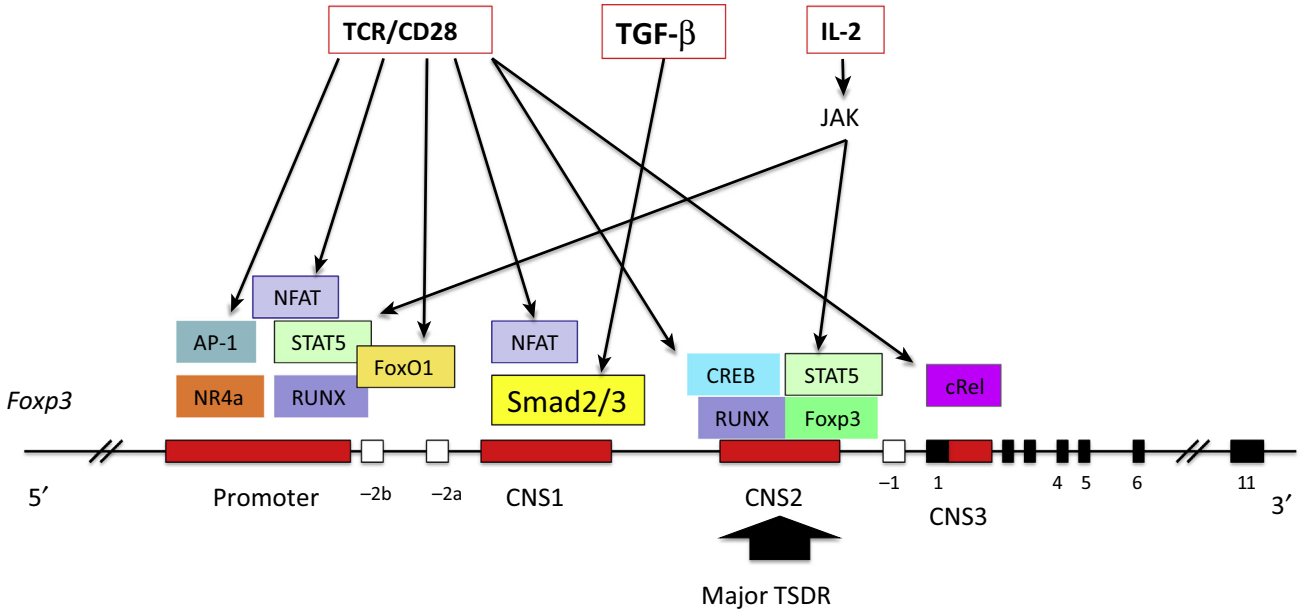

Figure 1. Signals and Transcription Factors Involved in Foxp3 Induction and Stable Expression in Induced Tregs. Activation of the transcription factors Smad2 and Smad3 is essential for Foxp3 induction in induced Tregs (iTregs), and Smads are recruited to the conserved non-coding sequence 1 (CNS1) region. The CNS2 region serves as an enhancer for *Foxp3* transcription and is bound by transcription factors such as Foxp3, STAT5, and cAMP response element-binding protein (CREB). In tTregs, CpG islands of this region are hypomethylated, and constitute a major Treg-specific demethylated region (TSDR). This region is heavily methylated in freshly generated iTregs, therefore these important transcription factors cannot be recruited and Foxp3 expression is unstable. Abbreviations: AP-1, activator protein-1; JAK, Janus kinase; NFAT, nuclear factor of activated T cells; RUNX, Runt-related transcription factor; STAT, signal transducer and activator of transcription; TCR, T cell receptor; TGF, transforming growth factor;.

differentiation [18,30]. Although Smad2 has been shown to have much weaker or lower DNA binding activity than Smad3, it has not been clarified how Smad2 can activate the Foxp3 promoter through CNS1.

Overall, it is clear that TGF-β is a major player in the differentiation of both pTregs and iTregs, but recent evidence has shown that additional factors are required for the efficient and stable differentiation of these cells, which will be discussed below.

pTreg Development in the Intestine

pTregs are mostly present in the intestine, primarily because of the abundant expression of TGF-β there. It should be emphasized that retinoic acid in the gut also promotes pTreg generation. Mucosal dendritic cells (DCs), especially $CD103^+$DCs, induce $Foxp3^+$ Tregs via the production of TGF-β as well as retinoic acid [31,32]. Retinoic acid (RA) induces the binding of retinoic acid receptor (RAR) and retinoid X receptor (RXR) to CNS1 and to a subordinate site in the promoter [26]. This leads to increased histone acetylation in the region of the Smad3 binding sites of CNS1, and increased binding of phosphorylated Smad3 [26].

Recently, commensal bacteria, especially *Clostridia* strains, have been shown to promote the generation of pTregs in the gut [33,34]. Smith *et al.* have suggested that metabolite(s) generated by resident bacterial species regulate Tregs in the gut. Indeed, short-chain fatty acids (SCFAs), bacterial fermentation products of dietary fibers produced by a range of bacteria, restored colonic Treg numbers in mice devoid of gut microbiota and increased Treg numbers in bacteria-colonized mice. Colonic Tregs have been shown to express GPR43, a receptor for SCFAs, and mice fed SCFAs were protected against experimentally induced colitis in a GPR43-dependent manner [35]. In addition, the SCFAs butyrate and propionate, which are produced by commensal microorganisms, facilitate the generation of pTregs [36,37]. Butyrate inhibits histone deacetylase (HDAC) activity and enhances histone H3 acetylation in the promoter and CNS regions of the *Foxp3* locus, which must be another mechanism of enhanced pTreg generation [36,37]. Even though it has been clarified that SCFAs facilitate pTreg development, SCFAs alone cannot induce iTreg cells, and TGF-β is still essential for iTreg cell development, even in the presence of SCFAs.

Although it is not clear where pTreg cells are developed, $CD103^+$ lamina propria dendritic cells (LPDCs) in the intestine are now widely believed to induce pTreg and iTreg cells by providing antigens, RA, and TGF-β [32,38–40]. Moreover, DC-specific disruption of TGF-β receptor II also leads abnormal Treg cell phenotypes [41]. Thus, TGF-β signaling inside LPDCs is also necessary for proper pTreg development. Recently, we proposed that the induction of TGF-β in LPDCs is dependent on the Toll-like receptor-2 (TLR2)-mediated ERK–AP-1 pathway as well as on the autoinduction of TGF-β. Interestingly, Smad3 directly activated the *Tgfb1* promoter, while Smad2 inhibited *Tgfb1* promoter activation [42]. This might lead to confusion of the dependence of pTreg development on Smad2 and Smad3.

Interestingly, most Tregs in the gut coexpress Foxp3 and RORγt, a master regulator of Th17 [43,44]. Although $Foxp3^+ROR\gamma t^+$ T cells display signatures of both Tregs and Th17 cells, gene expression profiles and epigenetic modifications of $Foxp3^+ROR\gamma t^+$ T cells are more similar to those of conventional $Foxp3^+$ Tregs than those of $Foxp3^-ROR\gamma t^+$ Th17 cells. $Foxp3^+ROR\gamma t^+$ T cells in the gut are stable Tregs and have been shown to play essential roles in regulating inappropriate Th2 responses [43]. It should be emphasized that, in addition to RORγt, part of Tregs express T-bet, Gata3, or IRF4, proteins which play important roles in suppressing Th1/Th2 responses [45–48]. It has not been clarified whether pTregs in the gut express these Th1/2 master genes at steady-state levels or under inflammatory conditions.

Differences Between tTregs and iTregs

Several groups have unveiled the developmental and functional differences between tTreg and iTreg cells by using microarray analyses [49,50]. Among the genes differentially expressed, *Ikzf2* (Helios) and *Nrp1* (neuropilin-1) have often been used as markers of tTreg cells as opposed to iTreg and pTreg cells. Nrp1 has been shown to be dispensable for the suppression of autoimmunity and maintenance of immune homeostasis, but necessary for Treg cells when limiting antitumor immune responses or curing established inflammatory colitis [51]. Nrp1 ligation reduced Akt phosphorylation, which increased nuclear localization of Foxo3a, thereby promoting Treg cell stability [51].

Unlike tTregs, iTregs have been shown to be unstable. This is a significant obstacle to the use of *ex vivo*-expanded iTregs for adoptive immune therapy [52]. It has been shown that Foxp3 expression in tTregs is stabilized by demethylation of the CpG islands in the CNS2 region of the *Foxp3* locus [53,54], because demethylation of CNS2 leads to the recruitment of various transcription factors including Stat5, NFAT, Runx1/Cbfβ, CREB, and Foxp3 itself [55,56]. Unstable expression of Foxp3 in iTregs is believed to be associated with strong demethylation of CNS2 (Figure 1) [57,58]. This idea is supported by the fact that sustained IL-2 signals sent by an IL-2–anti-IL-2 antibody complex-induced CNS2 demethylation in iTregs and stabilized Foxp3 expression [59]. Gut Foxp3$^+$RORγt$^+$ pTregs were stable even after transfer to lymphopenic mice, and their CNS2 region was significantly demethylated [44]. It has been shown that demethylation of the CNS2 increases in the subset of *in vitro*-generated iTregs that remain Foxp3$^+$ T cells after adoptive transfer to lymphopenic mice [60]. Thus, there must be a mechanism for converting unstable iTregs to stable iTregs *in vivo*, and finding such mechanism(s) is a key to generating stable iTregs suitable for clinical applications. In particular, the examination of epigenetic modifications is a promising avenue for increasing iTreg stability.

Related to this issue, although the TGF-β signal seems to be essential for pTreg development, pTregs appear to have additional mechanisms for obtaining stability. As mentioned, most pTregs in the gut express RORγt [43,44], and CNS2 of the *Foxp3* locus has been shown to be demethylated [61]. The precise mechanisms underlying such differences are not fully understood at present, but environmental factors of the gut, such as the presence of microbiota and their metabolites, as well as cytokines from various immune cells, may be involved.

Mechanisms of Specific DNA Demethylation in Tregs

Indeed, in addition to the Treg cell transcriptional program, the establishment of a 'Treg cell-specific CpG hypomethylation pattern' appeared to be crucial for Treg development [57]. The CpG methylation patterns between tTreg cells and other CD4$^+$ T cell subsets were surprisingly similar globally, but the Treg-specific demethylated regions (TSDRs) were distributed in genes that were known to be important for Treg cell differentiation and function. Major TSDRs were observed in the CNS2 enhancer of *Foxp3*, *Ctla4*, *Il2ra* (which encodes CD25), *Ikzf4* (which encodes Eos), and *Tnfrs18* (which encodes GITR).

Demethylation of CNS2 of the *Foxp3* locus may be maintained by the stable binding of Foxp3 and the Cbf-β–Runx1 complex or CREB/ATF to the demethylated CNS2 [30,62]. Although TSDR demethylation is probably achieved through an active mechanism that includes recently discovered intermediate steps involving active DNA demethylation pathways, 5-hydroxymethylcytosine (5hmC), and enzymes of the ten-eleven-translocation (Tet) family [63], it was reported that *Tet1* and *Tet2* deletion led to *Foxp3* hypermethylation, impaired Treg cell differentiation and function, and autoimmune disease [64]. Using *Tet2/Tet3* double-deficient mice, Yue *et al.* demonstrated that TET2/3 proteins mediate demethylation of CNS1 and CNS2 in the *Foxp3* locus as well as other TSDRs in tTregs, and the stability of Foxp3 expression in *Tet2/Tet3* double-deficient Tregs is markedly compromised [60]. Vitamin C has been shown to potentiate

TET activity [65,66], and consequently facilitates the demethylation of the *Foxp3* CNS2 region and increases the stability of Foxp3 expression in TGF-β-induced iTreg cells [67]. Hydrogen sulfide (H_2S) has also been shown to be required for tTreg cell differentiation and function, as evidenced by the fact that H_2S deficiency leads to systemic autoimmune disease [64]. H_2S promotes TET1 and TET2 expression, which are recruited to the *Foxp3* locus by TGF-β and IL-2 signaling to maintain *Foxp3* demethylation and Treg cell-associated immune homeostasis. Conversely, methylation of TSDRs is probably dependent on DNA (cytosine-5)-methyltransferase 1 (Dnmt1) because Dnmt1 deficiency in T cells resulted in enhanced Foxp3 induction in thymic and peripheral Foxp3-negative T cells upon TCR engagement [68]. Methyl-binding domain protein-2 (Mbd-2) also has been shown to be necessary for the maintenance of TSDR demethylation because $Mbd2^{-/-}$ Tregs cells exhibited a strong increase in methylation and a marked impairment in the binding of Tet2 at the TSDR site [69].

The targeting of the pathways outlined here should allow the generation of stable iTregs that may be used to achieve long-term tolerance in clinical settings.

Towards the Generation of Antigen-Specific iTregs

Another important aspect of Treg therapies is the use of antigen-specific Tregs. This specificity would allow the establishment of focused tolerance instead of broad immunosuppression that can have severe side-effects. Antigen-specific iTregs can be expanded *in vitro* by co-culturing naïve T cells with dendritic cells in the presence of known antigens and TGF-β, making this procedure applicable for antigen-specific immunotherapy (Figure 2, upper panel). In the autoimmune gastritis model in transgenic mice expressing H^+/K^+ ATPase-specific TCR, antigen-specific TGF-β-induced iTregs were able to prevent gastric pathology when coadministered with antigen-specific transgenic effector T cells [70]. Using similar models, notably Th17-mediated autoimmune gastritis, Hunter *et al.* demonstrated that antigen-specific iTregs have a stronger suppressive capacity than polyclonal tTregs or iTregs [71]. Takasato *et al.* have compared antigen-nonspecific polyclonally expanded iTregs and *ex vivo*-expanded tTregs as well as

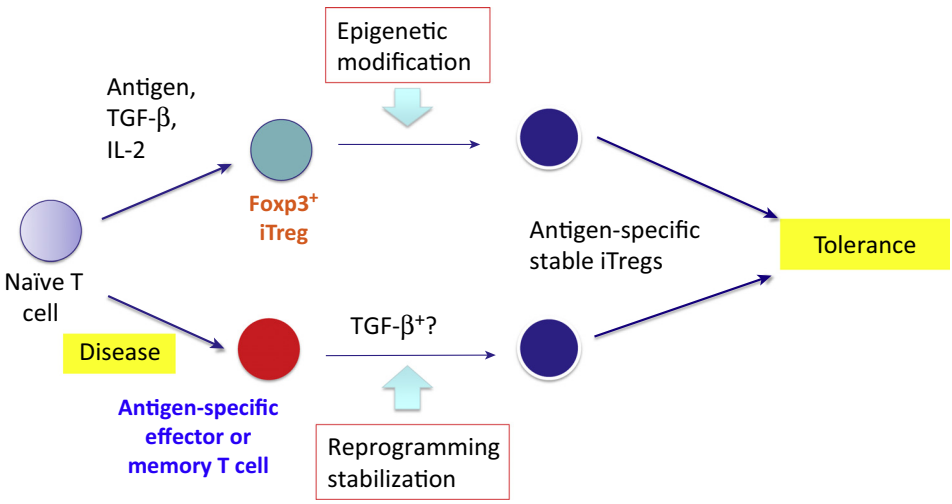

Figure 2. Two Methods to Generate Antigen-Specific Stable iTregs. Naïve T cells become $Foxp3^+$ iTregs in the presence of TGF-β and IL-2 *in vitro*. Such iTregs are usually unstable and easily lose Foxp3 expression; however, by introducing epigenetic modifications, portions of iTregs acquire stable Foxp3 expression (upper model). On the other hand, iTreg may be able to be reprogrammed from pathogenic effector or memory T cells which are generated in autoimmune or allergic diseases (lower line). TGF-β and additional unknown factors may be necessary for this stable reprogramming. These antigen-specific stable iTregs can be used to induce tolerance to a specific antigen in a clinical setting such as transplantation or autoimmunity. iTreg, induced regulatory T cell; TGF, transforming growth factor.

antigen-specific iTregs generated *in vitro* using a heart transplantation model [72]. In this report, they generated antigen-specific iTregs with an MHC peptide identified as a rejection antigen. In the case of organ transplantation, antigen-specific iTregs can be generated via both direct and indirect pathways using dendritic cells from F1 mice, even if a specific allo-antigen peptide is not known [73]. These studies demonstrated that *ex vivo*-generated antigen-specific iTregs induced more potent lifelong immunological tolerance for allogeneic cardiac transplants than did other types of Tregs.

However, for many autoimmune diseases, the target antigens are unknown or the antigens vary among patients. To overcome these obstacles, it would be ideal if iTregs could be generated from effector T cells which accumulate in the inflamed regions or from peripheral memory T cells (Figure 2, lower panel). Unfortunately, it has been shown that effector T cells are resistant to the actions of TGF-β, thus Foxp3 is poorly induced in effector T cells. Notwithstanding, only a few reports have shown conversion of effector or memory T cells to Tregs. Zhang *et al.* showed that Foxp3$^+$ iTregs cannot be induced from effector memory T cells but are inducible from central memory T cells [74]. It has also been reported that antigen-specific memory Th2 cells were redifferentiated into Foxp3$^+$ iTregs by TGF-β when stimulated in the presence of all-*trans* retinoic acid and rapamycin [75]. The 'converted' iTregs from memory Th2 cells suppressed the proliferation and cytokine production of Th2 memory cells *in vitro* and significantly suppressed Th2 memory cell-mediated airway hyper-reactivity, eosinophilia, and allergen-specific IgE production *in vivo*. The mechanism underlying the ability of Foxp3 to be induced in these memory T cells remains to be clarified. Interestingly, it has been shown that human Th1 cells can be converted to Foxp3$^+$ Treg cells by stimulation with programmed death ligand-1 (PDL1) [76]. Such converted Tregs suppressed human-into-mouse xenogeneic graft-versus-host disease (GVHD). Because SHP1/2 pharmacologic inhibition prevented Treg conversion by PDL1, suppression of tyrosine kinases may be involved in the conversion from Th1 to Tregs, although the precise mechanism remains to be clarified. In these experiments, the possibility of contamination by undifferentiated T cells, that can more easily generate iTregs, should also be excluded. Genetic and epigenetic reprogramming of memory T cells into stable and long-lived iTregs will undoubtedly be a challenging but crucial goal to be able to use iTregs in the clinic.

The second problem to be solved is the instability of iTregs *in vivo*. Apparently, in some cases such as cardiac transplantation models [72], iTregs generated *in vitro* are sufficient for therapeutic activity. By contrast, in GVHD models, iTregs are shown to be ineffective because of the instability of iTregs compared with tTregs [52]. This study showed that the majority of adoptively transferred iTregs, but not Foxp3$^+$ tTregs, quickly reverted to Foxp3$^-$ effector T cells. However, recent reports indicated that the concurrent administration of rapamycin with IL-2/anti-IL-2 Ab complexes to the transplant recipients significantly improved Foxp3 stability in iTregs, which then prevented GVHD as efficiently as in tTregs [77]. Why the stability of iTregs varies among disease models is not known. In the case of cardiac transplantation, we showed that tolerogenic DCs were induced by iTreg transfer [72], thus tolerance may be promoted continuously by an 'infectious tolerance' mechanism. The conditions required to induce such self-sustaining tolerance by iTregs remain to be clarified.

The third problem is the need for an efficient method for generating human iTregs. In contrast to mouse naïve T cells, Foxp3 can be easily induced by TCR simulation of human T cells. Such Foxp3$^+$ T cells are not suppressive and easily lose Foxp3 expression. Even though TGF-β induced higher levels of FoxP3 in naïve human T cells *in vitro*, the induced FoxP3-positive cells were neither anergic nor suppressive, and they produced proinflammatory cytokines upon stimulation [78]. The human TGF-β-induced FoxP3$^+$ T cells obviously differed from murine TGF-β-induced FoxP3$^+$ T cells. This may be because most of the human T cells derived from PBMC are not naïve, and contaminating pre-differentiated effector T cells can generate 'pseudo'

Table 1. Factors that Upregulate or Stabilize Foxp3 in iTregs.

Factor	Mechanism	Refs
Retinoic acid	Binding to *Foxp3* enhancer CNS1	[26,31]
Progesterone	Suppression of mTOR	[81]
Vitamin D3	Binding to *Foxp3* enhancer CNS1	[82]
Short-chain fatty acids	Activation of GPR43	[35]
Butyrate	Inhibition of HDAC	[36,37]
Vitamin C	Activation of TET enzymes	[60,67]
Hydrogen sulfide	Induction of TET1 and TET2	[64]
Rapamycin	Inhibition of mTOR	[77,83]
JAK1 inhibitor	Suppression of Th17	[84]
AhR ligands	Suppression of Th17?	[80,85]
C3aR/C5aR antagonist	AKT/Foxo1/3 or cAMP/CREB	[86,87]

Foxp3$^+$ Tregs. Further study will be necessary to establish methods for generating stable and functionally suppressive iTregs.

Induction and Stabilization of iTregs by Reagents and Drugs

The identification of optimal methods for iTreg cell preparation is important. Various reagents have been proposed to upregulate and stabilize Foxp3 expression. Potential avenues based on the mechanism that we have outlined here are currently under investigation and are outlined in Table 1. Although the underlying mechanisms of some of these reagents are not fully understood, a combination of such drugs may facilitate iTreg therapy. In addition to *ex vivo* expansion of iTregs, methods of direct induction of iTregs *in vivo* are also useful. For this purpose, CD4-targeted, biodegradable nanoparticles (NPs) loaded with TGF-β and IL-2 have been used for the expansion of Tregs *in vivo* [79]. Dietary AhR ligands attenuated the delayed-type hypersensitivity response by suppressing Th17 while promoting Treg differentiation [80]. In addition, drugs that can directly promote conversion of naïve or effector T cells into iTregs cells *in vivo* will be very useful. As mentioned, one report suggested that tumor cells overexpressing PDL1 converted Th1 cells into FOXP3$^+$ Treg cells *in vivo* in a human-into-mouse xenogeneic GVHD model [76]. However, so far no such drugs that promote a direct conversion from effector T cells into Tregs *in vivo* have been reported.

Concluding Remarks and Future Directions

Treg cells possess the potential to influence a wide range of immunological diseases ranging from autoimmune conditions to allergies to cancer. The ultimate goal of adoptive transfer of iTreg cells is to control inflammation with minimum adverse effects – such as generalized immunosuppression and infection – through the administration of antigen-specific Treg cells. Given the low frequency of Treg cells in human peripheral blood, a feasible approach is to generate stable antigen-specific Treg cells *in vitro* from non-Treg cells. However, it is first necessary to uncover the mechanisms responsible for Treg-specific epigenetic conversion, especially in human, to be able to generate functional and stable iTregs for clinical application. As shown in this review, mechanisms of epigenetic modifications in tTregs have been uncovered, and it has been shown that such mechanisms can be used to stabilize iTregs, such as the use of vitamin C for TET activation. Understanding of molecular basis for the development and generation of iTregs and pTregs in addition to tTregs may promote the development of novel methods for the application of iTregs to immune therapy.

Outstanding Questions

CNS2 is demethylated in pTregs, and CNS2 methylation is gradually decreased in iTregs after adoptive transfer into lymphopenic mice. The mechanism of demethylation of CNS2 of pTregs and iTregs *in vivo* remained to be clarified. If the factor (s) that induce demethylation *in vivo* can be identified, this would be helpful to establish methods to generate stable iTregs.

Vitamin C induces demethylation of CNS2 of *Foxp3* and TSDR of *CD25*, but not of other TSDRs, including *Ctla4*, *Ikzf4*, and *Tnfrs18*. Because vitamin C enhances the enzyme activity of TET proteins, it is not clear how such gene specific demethylation can occur. The answer to this question may help in developing methods to induce gene-specific DNA demethylation.

Some bacterial species induce pTreg in the gut, but other mucosal surfaces need to be examined as well. Are there any ways to induce pTregs to endogenous self-antigens or exogenous allergens? This would represent another way of inducing tolerance in autoimmune or allergic diseases.

Little is known about human pTregs and iTregs. Are the properties of human pTregs and iTregs similar to those of their murine counterparts? Methods to induce stable human iTregs should also to be established.

How can we reprogram effector or memory T cells to iTregs? Why are effector T cells resistant to the action of TGF-β? If central memory T cells respond to TGF-β, what is the mechanism? Solving these problems is essential to generate antigen-specific iTregs from effector or memory T cells.

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