Journal of Cellular Physiology

The Regulation of Foxp3 Expression in Regulatory $CD4^+CD25^+$T Cells: Multiple Pathways on the Road

LIANJUN ZHANG[1,2] AND YONG ZHAO[1]*

[1]*Transplantation Biology Research Division, State Key Laboratory of Biomembrane and Membrane Biotechnology, Institute of Zoology, Chinese Academy of Sciences, Beijing, China*

[2]*Graduate School of the Chinese Academy of Sciences, Beijing, China*

Regulatory T cells (Treg cells) have been well documented to have a crucial physiological role in preventing the development of autoimmune diseases and keeping self-tolerance. Foxp3, a recently identified member of the forkhead transcription factors, serves as a master regulator for the development and function of $CD4^+CD25^+$Treg cells. Though it is well defined that Foxp3 expression is sufficient to program $CD4^+CD25^+$Treg cell development, the physiological factors initiating intracellular Foxp3 expression remain poorly understood so far. In the present manuscript, we try to summarize the recent advances regarding the regulatory roles of T-cell receptor (TCR), co-stimulatory molecules, interleukin-2 (IL-2), transforming growth factor-β (TGF-β) and beyond pathways on Foxp3 expression.
J. Cell. Physiol. 211: 590–597, 2007.

One of the hallmarks of the immune system is the self–nonself discrimination directed to fight against the invading pathogens whereas maintaining tolerance to self-antigens in vertebrates (von Boehmer and Kisielow, 1990). Immune tolerance of T cells is achieved through both central and peripheral strategies. The former occurs in the thymus mainly by clonal deletion mediated by negative selection (von Boehmer and Kisielow, 1990), the latter mainly includes the ignorance, anergy, Th1/Th2 derivation, active immunosuppression, and so on. Recently, great progress has been made on the identification and characterization of regulatory T cells (Treg cells) which play important roles in immune tolerance to self and transplant antigens (Jiang and Lechler, 2003; Sakaguchi, 2004, 2005; Waldmann et al., 2004; Schwartz, 2005). In addition to many subpopulations of T cells with regulatory properties including $CD4^+CD45RB^{low}$T cells, $CD4^+CD62L^{low}$T cells, interleukin-10 (IL-10)-secreting Tr1 cells, TGF-β-producing Th3 cells, $CD8^+CD28^-$T cells, $CD4^-CD8^-$T cells and a little subset of NKT cells, $CD4^+CD25^+$Treg cells representing about 5–10% of both the mouse and human peripheral $CD4^+$T cells have been demonstrated to play a crucial role in keeping immune homeostasis and tolerance (Sakaguchi et al., 1996; Sakaguchi, 2000; Yi et al., 2006). Basically, there are two major categories of $CD4^+CD25^+$Treg cells, the naturally occurring $CD4^+CD25^+$Treg cells (nTreg cells) originally from the thymus and the induced $CD4^+CD25^+$Treg cells (iTreg cells) produced in the periphery (Sakaguchi, 2003b; Maggi et al., 2005; Taams and Akbar, 2005; Weber et al., 2006).

Foxp3, a novel member of the forkhead transcription factors, is recently identified to be essential for the development and function of $CD4^+CD25^+$Treg cells (Fontenot et al., 2003, 2005b; Hori et al., 2003; Khattri et al., 2003; O'Garra and Vieira, 2003; Rao and Zhao, 2005; Wan and Flavell, 2005; Ziegler, 2006). A mutation of *Foxp3* results in the deficiency of $CD4^+CD25^+$Treg cells in the thymus and in the periphery, and thus subsequently causes the scurfy and immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) phenotype characterized by severe multi-organ autoimmune diseases in mice and humans, respectively (Bennett et al., 2001; Brunkow et al., 2001). Unlike other Treg cell-associated markers, such as CD25 (the α chain of IL-2R), cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), gluticorticoid-induced TNF-α receptor (GITR) which are also expressed in recently activated T cells, Foxp3 is preferentially expressed in $CD4^+CD8^-CD25^+$ thymocytes and mature $CD4^+CD25^+$T cells but not in $CD4^+CD25^-$T cells in mice. Importantly, Foxp3 has been demonstrated to serve as the master regulator to program $CD4^+CD25^+$Treg cell

Abbreviations: CTLA-4, cytotoxic T lymphocyte associated antigen-4; CsA, cyclosporin A; EAE, experimental autoimmune encephalomyelitis; Foxp3, forkhead box protein 3; GITR, gluticorticoid induced TNF-α receptor; IL-2, interleukin-2; IPEX, immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome; LAT, linker for activation of T cells; MAPK, mitogen-activated protein kinases; NFAT, nuclear factor of activated T cells; PD1, programmed death 1; PI3Ks, phosphoinositide 3-kinases; PLC, phospholipase C; STAT, Signal transducer and activator of transcription; TCR, T-cell receptor; TGF-β, transforming growth factor-beta; TLR, toll-like receptor; Treg cells, regulatory T cells.

Contract grant sponsor: National Natural Science Foundation for Key Programs;
Contract grant number: C30630060.
Contract grant sponsor: Distinguished Young Scholars;
Contract grant number: C03020504.
Contract grant sponsor: National Basic Research Program;
Contract grant number: 973 Program, 2003CB515501.
Contract grant sponsor: Quality Vocational Colleagues of Chinese Academy of Sciences;
Contract grant number: 2003-85.
Contract grant sponsor: Scientific Research Foundation for the Returned Overseas Chinese Scholars of State Education Ministry;
Contract grant number: 2005-546.

*Correspondence to: Prof. Yong Zhao, State Key Laboratory of Biomembrane and Membrane Biotechnology, Institute of Zoology, Chinese Academy of Sciences, Beisihuan Xi Road #25, Haidian, Beijing, China 100080. E-mail: zhaoy@ioz.ac.cn

Received 4 September 2006; Accepted 28 November 2006

DOI: 10.1002/jcp.21001

development in the periphery as in the thymus, because conventional $CD4^+CD25^-$T cells and even the $CD8^+$T cells showed similar regulatory phenotypes and acquired the immunosuppressive activities when they were forced to express Foxp3 (Fontenot et al., 2003; Hori et al., 2003). But how is Foxp3 expression tightly regulated? Is there any difference on the Foxp3 expression pattern between nTreg cells and iTreg cells? The physiological factors and pathways initiating intracellular Foxp3 expression and promoting its immune regulatory characteristics remain poorly understood. Nevertheless, recent studies have revealed that signaling pathways initiated by T-cell receptor (TCR), co-stimulatory molecules, IL-2R, transforming growth factor-β (TGF-β) receptor and beyond are closely associated with Foxp3 expression. However, there is a lack of evidence concerning negative regulation of Foxp3 expression. In this manuscript, we attempted to summarize the current understanding of the physiological regulation of the aforementioned signaling events on Foxp3 expression and mainly focus on its positive regulation.

TCR signaling pathway and Foxp3 expression

TCR undoubtedly has a determinant role in conferring $CD4^+CD25^+$Treg cell specificity and their differentiation (Picca et al., 2006). It is suggested that generation of $CD4^+CD25^+$Treg cells may require higher affinity interaction between the agonist peptides/MHC II and TCR within the thymus in contrast to the process of conventional $CD4^+$T cells production (Pacholczyk et al., 2002; Cabarrocas et al., 2006). Support for this notion has been provided by analyzing Treg cell development in mice expressing a transgenic TCR and its cognate ligand in the thymus. Recent studies show that TCR transgenic $CD4^+$T cells can adopt the regulatory cell phenotype with a higher frequency when they encounter their cognate antigen in the thymus. Based on these observations, engagement of transgenic TCR by a high-affinity self-ligand is expected to initiate signaling cascades that ultimately induce Foxp3 expression and commit thymocytes to Treg cell lineage. In addition, the peripheral conversion into $CD4^+CD25^+$Treg cells from conventional T cells in vitro supported that the TCR signaling was also critically required for Foxp3 expression in mature T cells (Chen et al., 2003). One recent study demonstrated that 10–30% of human mature T cells displayed the dual-specific characteristics, that is, some human T cells expressed two functional TCRs as determined by flow cytometric analysis. Furthermore, they found that human $CD4^+CD25^+$Treg cells expressed two functional TCRs at an increased frequency compared to their $CD25^-$ counterparts. Interestingly, the $CD4^+CD25^+$Treg cells expressing two TCRs displayed more Foxp3 expression than that of $CD4^+CD25^+$Treg cells expressing only one TCR (Tuovinen et al., 2006).

It is reported that Foxp3 expression can be induced at the $CD4^+CD8^+$thymocyte stage but occurs preferentially at the $CD4^+$ stage or during the transition to this stage. They also argue that the adult thymi are superior to neonatal ones to provide the unique niche required for Foxp3 expression. In addition, it is proposed the factors, largely associated with the thymic medulla, are contributing to Foxp3 induction. However, the nature of these signals is largely unknown (Fontenot et al., 2005b).

The TCR downstream signalling pathways are relatively complex and there are many branches to deliver both the positive and negative signals from the cell membrane to the nucleus. An early event following TCR engagement is the Lck-mediated phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs) on the zeta chains of the TCR complex, providing the docking sites for the tyrosine kinase ZAP-70. ZAP-70 then becomes active and catalyzes the phosphorylation of a number of membrane-associated adaptor molecules which then initiate a series of intracellular signal transduction pathways (Fig. 1). Among them there are two major sets of intracellular pathways mediated by phospholipase C-γ (PLC-γ) and the small G proteins, respectively. The linker for activation of T cells (LAT) is one membrane-associated adaptor protein considered to play a crucial role in T-cell development and activation. A single amino acid mutation (Y136F) in LAT leads to the severe autoimmune diseases in mice because of the impairment of the PLC-γ signaling transduction. Zhang and colleagues found that $CD4^+CD25^+Foxp3^+$Treg cells were nearly absent in both thymus and peripheral lymphoid organs of LAT (Y136F) mice, indicating TCR downstream LAT-PLC-γ1 signaling pathways played an important role in regulating Foxp3 expression and $CD4^+CD25^+$Treg cell development (Koonpaew et al., 2006). The cleavage of phatidylinositol (4,5) bisphosphate (PI(4,5)P2) by PLC-γ1 produces two critical secondary messengers: inositol (1,4,5) trisphosphate (IP3) and diacylglycerol (DAG). IP3 accumulation initiates the intracellular calcium mobilization, which then activates the nuclear factor of activated T cells (NFAT) signaling. Whereas DAG triggers the PKC pathway and results in phosphorylation of IκB which in turn leads to NFκB activation and nuclear translocation (Fig. 1). Disruption of the signaling components of the NFkB pathway, including NFkB-inducing kinase (NIK) and TNF receptor-associated factor 6 (Traf6), leads to the impairment of regulatory T-cell generation. In addition, both NIK- and Traf6-deficient mice succumb to multiorgan autoimmune disease by 2 weeks of age (Kajiura et al., 2004; Akiyama et al., 2005; Kim and Rudensky, 2006).

NFAT has been demonstrated to lead to immune responses or anergy in the presence or absence of AP-1, respectively. In

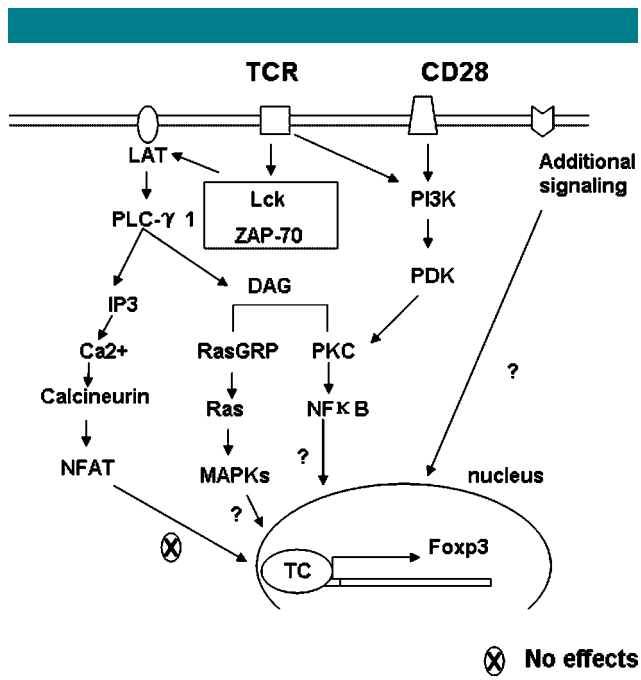

Fig. 1. The schematic diagram shows that in combination with additional signals, the TCR downstream molecules may provide critical signals for Foxp3 expression and $CD4^+CD25^+$Treg cell differentiation in mice. Though the detailed information about the involvement of TCR downstream molecules in Foxp3 expression is poorly understood, LAT-PLC-γ may contribute to this process to some extent. In addition, it was suggested that NFAT might not be responsible for mouse but for human Foxp3 expression. The potential participation of the NFκB, PI3Ks and Ras-MAPK signaling pathways in Foxp3 expression needs further investigation.

addition, NFAT was recently suggested to cooperate with Foxp3 in regulating $CD4^+CD25^+$Treg cell differentiation and their suppressive activities (Rudensky et al., 2006; Wu et al., 2006). In a recent study, the critical transcription factors responsible for the expression of human Foxp3 have been elucidated. Using the reporter construct analysis, Schmidt-Weber et al. described several binding sites for NFAT and AP-1 in the promoter region which were critical positive *cis*-acting elements for human Foxp3 expression and cyclosporin A (CsA) significantly inhibited the activation-induced Foxp3 expression (Mantel et al., 2006). However, several studies indicated that NFAT might may not be responsible for murine Foxp3 expression, as Bopp et al. (2005) reported that mice with a combined NFATc2/c3 deficiency had normal development of Foxp3-expressing $CD4^+CD25^+$Treg cells, but rather rendered conventional $CD4^+$T cells unresponsive to $CD4^+CD25^+$Treg cell-mediated immunosuppression. Consistently, our recent studies showed that after administration of CsA for several consecutive weeks in mice, the percentages of $Foxp^+CD25^+CD4^+$Treg cells in the periphery was significantly decreased, but the expression level of intracellular Foxp3 at a single $CD4^+CD25^+$Treg cell was not markedly affected both in the thymus and the peripheral immune organs (Wang et al., 2006; Wang and Zhao, 2006).

Based on these observations, it should be noted that some differences indeed exist for the Foxp3 expression pattern between the mouse and human $CD4^+CD25^+$Treg cells. Human Foxp3 consists of two spliced forms in comparison with only one in mice. In addition, the Foxp3 mRNA expression may not be confined to the $CD4^+CD25^+$Treg cells in humans, because human $CD4^+CD25^-$T cells express Foxp3 at both the mRNA and protein levels upon antigen-specific or polyclonal stimulation (Walker et al., 2003; Morgan et al., 2005; Roncador et al., 2005), but no detectable Foxp3 expression in activated murine $CD4^+CD25^-$T cells has been observed. The major differences between mouse and human Foxp3 expression pattern are summarized in Table 1.

The Ras-MAP kinase (MAPK) pathway has been recognized to be involved in multiple aspects in T-cell biology, both of the guanine exchange factors Sos and RasGRP1 are critical for Ras activation. But the more recently identified RasGRP1 is commonly used in T-cells and plays an important role for T-cell development (Fig. 1). RasGRP deficiency also leads to the lymphoproliferative disorders resembling the scurfy phenotypes (Ebinu et al., 1998; Dower et al., 2000; Hogquist, 2001). In addition, phosphoinositide 3-kinases (PI3Ks) mediate various signaling events involved in cell proliferation, survival, differentiation and metabolism. In T cells, PI3Ks can be triggered by TCR, co-stimulatory receptors and some cytokine receptors (Reif et al., 1997; Fang and Liu, 2001; Rangachari and Penninger, 2004). However, the direct association of the Ras-MAPK or PI3Ks signaling cascades with Foxp3 expression and the development of $CD4^+CD25^+$Treg cells has not been reported so far. Thus, further investigation for the involvement of LAT-PLC-γ signaling and also other TCR downstream signals in Foxp3 expression is definitely needed. Nevertheless, reported data suggest that TCR engagement is preliminarily required for Foxp3 expression and the differentiation of both nTreg cells and iTreg cells (Fig. 2), although the detailed signaling pathways are not clear yet.

Co-stimulatory signaling pathways and Foxp3 expression

It is widely accepted that T-cell activation requires at least two signals. The first one is specific and initiated by the interaction between TCR and the MHC-peptide complex; the engagement of CD28 by B7 molecules on the antigen presenting cells (APCs) provides the nonspecific co-stimulatory signals essential for T-cell full activation and function (Greenwald et al., 2005). Similarly, Foxp3 expression and the production of $CD4^+CD25^+$Treg cells both in the thymus and in the periphery critically depend on the co-stimulatory signaling. $CD28^{-/-}$ and $B7\text{-}1^{-/-}/B7\text{-}2^{-/-}$ ($CD80^{-/-}/CD86^{-/-}$) mice show significantly reduced numbers of $CD4^+CD25^+$Treg cells in the thymus and the periphery, indicating that CD28:B7 interaction is required for the development and maintenance of $CD4^+CD25^+$Treg cells. In addition, Tai et al. (2005) observed that CD28-mediated Foxp3 expression was independent on IL-2 production. Under physiological circumstances, the conversion of $CD4^+CD25^-$T cells into $CD4^+CD25^+$Treg cells in vivo may also require B7 co-stimulation as mature $CD4^+CD25^-$T cells failed to be converted into Foxp3-expressing $CD4^+CD25^+$Treg cells after adoptively transferred into the secondary syngeneic $B7^{-/-}$ recipient mice (Liang et al., 2005).

$CD4^+CD25^+$Treg cells constitutively express the intracellular and surface CTLA-4 (Takahashi et al., 2000). CTLA-4 deficient mice display the phenotypes critically resembling the Foxp3 mutant ones (Tivol et al., 1995), indicating a close link may exist between CTLA-4 and $CD4^+CD25^+$Treg cells. Recently, CTLA-4 has been suggested to be required for TGF-β induced Foxp3 expression as CTLA-4 deficient T cells cannot be induced to express Foxp3 in the presence of TGF-β, the detailed information about CTLA-4 in Foxp3 expression will be discussed below together with the TGF-β (Zheng et al., 2006). However, some reports showing that $CD4^+CD25^+$Treg cells develop normally in CTLA-4 deficient mice argue against the above descriptions. The controversies need to be clarified. On the other hand, it has been shown that CD80 and CD86, the natural ligands for CD28 and CTLA-4, perform distinct expression pattern and in consequence they may not act equivalently. Zheng et al. demonstrate that CD86 and CD80 differentially modulate the suppressive function of human $CD4^+CD25^+$Treg cells. CD80 has been reported to play a critical role in the TGF-β-induced Foxp3 expression whereas CD86 has had no effect (Zheng et al., 2004, 2006). This is in consistence with the observation that the interaction of CTLA-4 and CD80 is of higher affinity than that of CD86, while CD28 is postulated to interact with CD86 more effectively than CD80.

In addition, other co-stimulatory molecules may also contribute to Foxp3 expression and $CD4^+CD25^+$Treg cell development and function. Blockade of the programmed death 1 (PD1)-PD-L pathway with the anti-PD1 mAb significantly interrupted the vascular endothelium-induced Foxp3 expression and the

TABLE 1. The expression patterns of Foxp3 in humans and mice

	Humans	Mice
Mutant phenotype	IPEX phenotype, multiorgan autoimmune diseases	Scurfy phenotype, multiorgan autoimmune diseases
Alternative splicing	Two spliced forms	No spliced form
Activation induced Foxp3 expression	Foxp3 can be induced in $CD4^+$CD25-T cells upon activation, more like an activation marker	No detectable Foxp3 expression in $CD4^+$CD25-T cells upon antigen stimulation
Transcription factors involved in Foxp3 expression	NFAT, AP-1	Unidentified, but the NFAT may not be responsible for Foxp3 expression

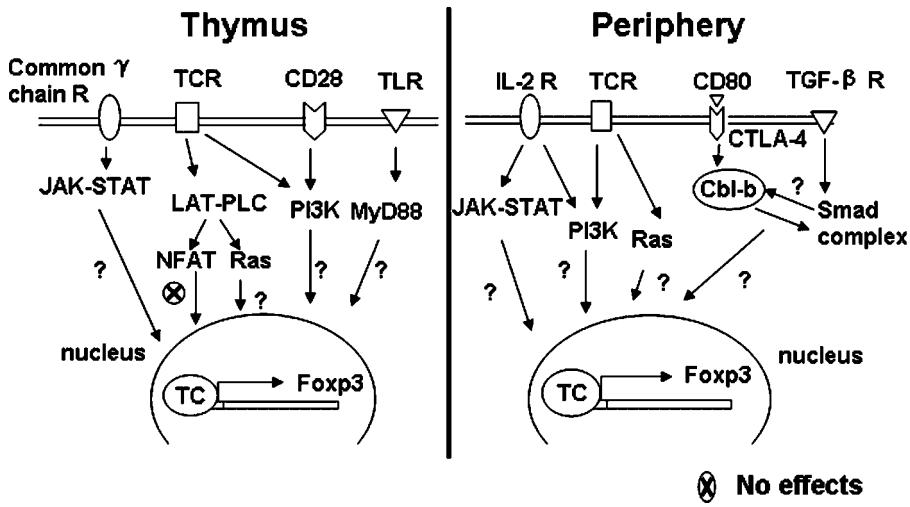

Fig. 2. The schematic diagram shows the potential signaling pathways involved in Foxp3 regulation in both nTreg cells and iTreg cells in mice. An emerging body of data suggested that TCR and co-stimulatory signaling were critical for Foxp3 expression and $CD4^+CD25^+$Treg cell differentiation both in the thymus and in the periphery. The difference may lie in that CD28 is required for Foxp3 expression and the development of nTreg cells in the thymus whereas the peripheral Foxp3 induction and the production of iTreg cells preferentially depend on the engagement CTLA-4 by B7 molecules. Though the precise signaling molecules involved in Foxp3 expression remains obscure, the LAT-PLC signaling and JAK-STAT signaling may partially participate in this process. In addition, Foxp3 expression may need a transcriptional complex formed by several transcription factors and their co-factors. Notably, TGF-β plays a critical role in Foxp3 induction and conversion into $CD4^+CD25^+$Treg cells from $CD4^-CD25^-$ conventional T cells in the periphery. However, it may not be involved in the Foxp3 expression in nTreg cells in the thymus, because TGF-β or Smad3 deficient mice have functional nTreg cells with normal Foxp3 expression. In addition, the TLR signaling have recently been identified to influence the Foxp3 expression in nTreg cells.

conversion into the $CD4^+CD25^+$Treg cells from $CD4^+CD25^-$T cells (Krupnick et al., 2005), indicating that PD1-PD-L interaction seems to be critical for Foxp3 expression and the conversion into $CD4^+CD25^+$Treg cells in mice. OX40-OX40 ligand (OX40-L) interaction may be important for the development and homeostasis of $CD4^+CD25^+$Treg cells, as the significantly reduced number of $CD4^+CD25^+$Treg cells in OX40-deficient mice and the increased $CD4^+CD25^+$Treg cells in constitutively active OX40-L expressing mice were observed (Takeda et al., 2004). Similarly, in a mouse asthma model, pulmonary dendritic cells (DCs) in the bronchial lymph nodes exposed to respiratory allergen could induce Foxp3 expression and the development of Treg cells. Moreover, this process depends on T-cell co-stimulation via the inducible costimulator (ICOS)-ICOS-ligand pathway. So it seems likely that distinct co-molecules may be required for the induction of Foxp3 expression in different experimental systems (Stock et al., 2004). Based on the preliminary data, the different roles of co-stimulatory molecules in $CD4^+CD25^+$Treg cells and effector T cells are summarized in Table 2.

Repertoire analysis for the $CD4^+CD25^+$Treg cells and $CD4^+CD25^-$ effector cells recently discovered that the same TCR could be expressed by both self-reactive T cells and $CD4^+CD25^+$Treg cells (Hsieh et al., 2006). Of note, it is well recognized that the strength of TCR stimulation and co-stimulation are closely related to Foxp3 induction. The suboptimal but not the strongest TCR stimulation leads to the highest percentage of Foxp3 induction in the periphery, similarly, the appropriate strength of co-stimulation is critical for Foxp3 expression. Thus, the signaling intensity and duration might be involved in committing the developing thymocytes with the same antigen specificity into pathogenic autoreactive T cells or protective Treg cells. However, both the TCR and the co-stimulatory signaling pathways are shared by the differentiation of conventional T cells and $CD4^+CD25^+$Treg cells, further research is essential to identify the unique molecules or the critical signals responsible for Foxp3 expression and the programming of $CD4^+CD25^+$Treg cells. Based on the preliminary data, the different roles of co-stimulatory molecules in $CD4^+CD25^+$Treg cells and effector T cells are summarized in Table 2.

TABLE 2. The roles of different co-stimulatory molecules in Treg cells and effector T cells

Comolecules	Ligands	Treg cells	Effector T cells
CD28	CD80,CD86	Indispensable for n Treg cell development and homeostasis	Required for T-cell activation
CTLA-4	CD80,CD86	Important for the suppressive function of Treg cells and also peripheral production of i Treg cells	Deliver the negative signals to T-cell
PD-1	PD-L1,PD-L2	PD-1-PD-L1 interaction was essential for peripheral Foxp3 induction and Treg cell differentiation in some experimental systems	Negative co-stimulatory molecule, interaction with PD-L1 on APCs may switch off auto-reactive T cells and induce peripheral tolerance
ICOS	B7RP-1	Involved in Treg cell differentiation and their suppressive activities	ICOS can inhibit as well as stimulate T-cell responses
OX-40	OX40-L	Associated with Treg cell development, homeostasis and suppression	Regulation of effector or memory T-cell responses
BTLA	HVEM	Uncharacterized	Downregulation of T-cell activation

IL-2 and Foxp3 expression

IL-2 has long been known as a potent T-cell growth factor essential for T-cell proliferation and function. Surprisingly, the IL-2 deficient mice succumb to severe lymphoproliferative disorders several weeks after birth in contrary to our expectations of their immunodeficiency phenotype (Antony et al., 2006). Obviously, significantly lower levels of $CD4^+CD25^+$Treg cells in the periphery have been observed in these IL-2 deficient mice. Thus, one possibility has been proposed that IL-2 is not the optimal T-cell growth factor in vivo but rather an inducer of self-tolerance through the maintenance of $CD4^+CD25^+$Treg homeostasis (Fontenot et al., 2005a; Zhang et al., 2005). In general, it has been demonstrated that IL-2 may not be indispensable for Foxp3 expression and the commitment of nTreg cells in the thymus but is critically required for their peripheral survival and homeostasis (Nishimura et al., 2004; D'Cruz and Klein, 2005; Kretschmer et al., 2005; Fontenot et al., 2005a). The peripheral Foxp3 induction and iTreg cell generation may depend on IL-2 signaling to some extent.

The IL-2R consists of three different chains, CD25, CD122 (the β chain), and CD132 (the common γ chain). Both the IL-2R β and γ chains are required for the optimal signal transduction, but only the trimeric receptor containing the α chain can bind IL-2 with higher affinity. In the absence of cytokine, the IL-2R α chain is associated with several families of inactive protein tyrosine kinases. The Janus kinase (JAK)—signal transducer and activator of transcription (STAT) signaling is the well characterized pathway activated by a variety of growth-regulating signals as well as diverse cytokines to mediate their biological function (Liu et al., 1997; Horvath, 2004). Upon cytokine binding, the receptor-associated JAKs are activated, the activated JAKs then create docking sites for the STAT transcription factors by phosphorylation of specific tyrosine residues on receptor subunits followed by STAT dimerization and translocation into the nucleus to initiate certain gene expression. Among many JAKs–STATs partners, the JAK1/JAK3-STAT5 signaling cascade is specifically initiated by IL-2. JAK3 is a critical kinase related to the signal transduction of the common γ chain cytokines. Compared to the normal Foxp3 expression and development of $CD4^+CD25^+$Treg cells in $JAK3^{+/-}$ mice, it was reported that $JAK3^{-/-}$ mice showed no Foxp3 expression and $CD4^+CD25^+$Treg cells accounted for only 0.2% of the $CD4^+$ T cells (Mayack and Berg, 2006). Furthermore, IL-2 selectively upregulates Foxp3 expression in purified human $CD4^+CD25^+$T cells but not in $CD4^+CD25^-$T cells in vitro via the binding of STAT3 and STAT5 proteins to a highly conserved STAT binding site located in the first intron of the Foxp3 gene (Zorn et al., 2006). Antov et al. (2003) found that transient activation of STAT5 was sufficient to increase $CD4^+CD25^+$Treg cell numbers in IL-2-deficient mice, similar results were observed in human studies showing that the patients homozygous for a missense A630P mutation in STAT5b displayed decreased numbers of $CD4^+CD25^+$Treg cells. Importantly, $CD4^+CD25^+$Treg cells in STAT5b (A630P/A630P) people had very low expression of Foxp3, whereas those in STAT5b (wt/A630P) people displayed normal level of Foxp3 expression (Cohen et al., 2006). Targeted mutation of some other IL-2R downstream signaling components also significantly influenced the development or function of $CD4^+CD25^+$Treg cells, for example, STAT1-deficient mice expressing a transgenic TCR against myelin basic protein spontaneously developed experimental autoimmune encephalomyelitis (EAE) with sharply increased frequency. Experimental studies showed that the development of $CD4^+CD25^+$Treg cells were significantly impaired in the absence of STAT1 which then led to the markedly increased susceptibility to EAE (Nishibori et al., 2004). With regard to the significance of IL-2 in $CD4^+CD25^+$Treg cell biology, the detailed information needs to be collected in the future.

It is worthy to be noted that IL-2, CD25 or CD122 deficient mice had markedly reduced $CD4^+CD25^+$Treg cells. However, Foxp3 is not expressed in thymocytes or peripheral T cells in IL-2R$\gamma^{-/-}$ mice, indicating the critical role of common γ chain in Foxp3 expression and $CD4^+CD25^+$Treg cell commitment (Fontenot et al., 2005a). Thus, we infer from the above descriptions that other cytokines sharing the common γ chain may compensate for IL-2 in nTreg cell commitment to some extent.

TGF-β signaling pathway and Foxp3 expression

The highly evolutionarily conserved TGF-β proteins have been implicated in many fundamental biological processes. In the immune system, TGF-β has been well recognized for its effects on the growth, differentiation and apoptosis of T cells. However, it has recently been reported that TGF-β may facilitate immune tolerance via its involvement in the regulation of Foxp3 expression and $CD4^+CD25^+$Treg cell function, though considerable controversies exist (Chen et al., 2003; Fu et al., 2004; Schramm et al., 2004; Csencsits et al., 2005; Li et al., 2005; Hartwig et al., 2006).

In peripheral lymphoid organs, the frequency of $CD4^+CD25^+$Treg cells and Foxp3 expression was increased by TGF-β1 overexpression and decreased by the impairment of TGF-β-signaling, demonstrating that TGF-β might play an important role in the regulation of Foxp3 expression in the periphery (Schramm et al., 2004). Chen et al. (2003) first demonstrated that in the presence of TGF-β and TCR stimulation in vitro, naive mouse $CD4^+CD25^-$T cells could be converted into $CD4^+CD25^+$Treg cells through induction of the Foxp3 expression. Further studies both in mice and humans suggested that TGF-β was able to induce the expression of Foxp3 and facilitated the subsequent acquisition of the suppressive characteristics in $CD4^+CD25^-$T cells upon antigen-specific or polyclonal activation (Yamagiwa et al., 2001; Fantini et al., 2004; Fu et al., 2004; Kretschmer et al., 2005; Park et al., 2005). Zheng et al. (2006) demonstrated that CTLA-4 engagement of CD80 shortly after T-cell activation was required for TGF-β to induce the expression of Foxp3 and the development of suppressive activities of $CD4^+CD25^-$T cells. Cbl-b, a RING-type E3 ubiquitin ligase, is critical for T-cell anergy and controls the threshold for T-cell activation (Zhang et al., 2002; Krawczyk et al., 2005). It is suggested that Cbl-b could degrade the TCR downstream signaling molecules to promote the T-cell signaling attenuation or termination. Strikingly, Wohlfert et al. (2006) observed the significant relevance of cbl-b, which was tightly regulated by CD28 and CTLA-4 (Liu and Gu, 2002; Li et al., 2004), with the Foxp3 expression and $CD4^+CD25^+$Treg cells. The direct evidence for the involvement of Cbl-b in Foxp3 expression is provided by showing that Cbl-b$^{-/-}$ effector T cells prevented the TGF-β-mediated induction of Foxp3 expression and the convention into Treg cells (Wohlfert et al., 2006). In this regard, we may speculate that engagement of CTLA-4 by B7 molecules delivers specific signals to Cbl-b which then cooperates with TGF-β signaling molecules to initiate the Smad2/3 phosphorylation, subsequently the Smad2/4 or Smad3/4 signaling complex forms and translocates into the nucleus and recruits coactivators that contain histone-acetyl transferase activity, for example, CBP/p300, to activate Foxp3 expression (Zhang et al., 2006) (Fig. 2). On the other hand, Fantini et al. (2004) showed that the TGF-β-induced Foxp3 could directly bind to the inhibitory Smad7 promoter region to turn off its expression. Thus, it forms a feedback regulation of TGF-β signaling that may result in accumulation of Foxp3 expression and then facilitates the

conversion into $CD4^+CD25^+$Treg cells. However, some reports showed that TGF-β or Smad3 deficient mice had functional $CD4^+CD25^+Foxp3^+$Treg cells. Therefore, we may propose that TGF-β may not be responsible for Foxp3 expression in nTreg cells but actually participates in Foxp3 induction in iTreg cells in the periphery (Fig. 2). However, TGF-β alone is not able to induce Foxp3 expression, IL-2 is required for this process. IL-2-deficient $CD4^+CD25^-$T cells fail to express Foxp3 and to be converted into $CD4^+CD25^+$Treg cells in the presence of TGF-β, in addition, neutralization of IL-2 can also abolish the TGF-β mediated induction of immunosuppressive activity. So it is predicted that IL-2 and TGF-β play different but complementary roles in TGF-β-induced Foxp3 expression. Moreover, a recent study described that IL-4R-binding cytokines, such as IL-4 and IL-13, facilitate the generation of Foxp3-expressing Treg cells (Skapenko et al., 2005). Though the in vitro models cannot fully recapitulate the complex situation in vivo, one could recognize that the appropriate multi-cytokine microenvironment might be important for Foxp3 expression as well as the development and function of $CD4^+CD25^+$Treg cells as its role on effector T cells (Wan and Flavell, 2006).

Toll like receptor signaling and Foxp3 expression

Toll like receptors (TLRs) have been well demonstrated to play critical roles in innate immunity as well as the adaptive immunity (Takeda and Akira, 2004; Liew et al., 2005; Liu et al., 2005; Kawai and Akira, 2006). The bi-directional effects of $CD4^+CD25^+$Treg cells and APCs have attracted much attention recently. The interaction with DCs or macrophages may affect the immunosuppressive activities of $CD4^+CD25^+$Treg cells to some extent via TLRs. In addition to the indirect roles via the APC-mediated effects, TLRs have been recently identified to modulate Foxp3 expression and the suppressive activities of $CD4^+CD25^+$Treg cells directly (Sakaguchi, 2003a; Peng et al., 2005; Liu et al., 2006; Sutmuller et al., 2006). The comparative study on TLR expression suggested that $CD4^+CD25^+$Treg cells selectively express TLR-4, TLR-5, TLR-7, and TLR-8 whereas both $CD4^+CD25^+$Treg cells and conventional T-cell express TLR1, TLR2, and TLR6 in mice (Caramalho et al., 2003). It was found that TLR2 was expressed intracellularly in both activated effector T cells and $CD4^+CD25^+$Treg cells but not on resting T cells. The TLR2 ligand-Synthetic bacterial lipoprotein (BLP) could transiently suppress the Foxp3 mRNA expression at the first 8–15 h after T-cell activation (Liu et al., 2006). Recently, Netea et al. (2004) observed the decreased numbers of circulating $CD4^+CD25^+$Treg cells in the blood of $TLR2^{-/-}$ mice whereas the $TLR4^{-/-}$ mice had normal $CD4^+CD25^+$Treg cell development. Consistent with this observation, the deficiency of the critical adaptor molecule MyD88 for TLR2 signalling resulted in significant low numbers of $CD4^+CD25^+$Treg cells compared with wild-type littermates (Sutmuller et al., 2006). But MyD88 is also responsible for TLR4 downstream signalling, thus one possibility might be that the MyD88-independent pathways mediated by TLR4 were involved in Foxp3 expression and $CD4^+CD25^+$Treg cells development to some extent. Another possibility is that TLR2 or TLR4 may differently regulate $CD4^+CD25^+$Treg cells indirectly via APCs or other approaches. Furthermore, engagement of TLR5 with its ligand flagellin did not break the anergic state of human $CD4^+CD25^+$Treg cells, but rather enhanced the expression of Foxp3 and potently increased their immunosuppressive capacity (Crellin et al., 2005). Therefore, It is of great significance to explore the underlying molecular mechanisms for the involvement of different TLRs in Foxp3 expression as well as Treg cell development and function.

Other pathways and Foxp3 expression

Recently, Wang et al. described that the altered encephalitogenic T-cell responses and added severity of EAE in IFN-γknock out mice was closely associated with the decreased frequency or impaired function of $CD4^+CD25^+$Treg cells. Further investigation showed that IFN-γ was capable of Foxp3 induction in vitro in both mouse and human experimental systems. The Foxp3 induction and the subsequent conversion of $CD4^+CD25^-$T cells into $CD4^+CD25^+$Treg cells was abrogated only by anti-IFN-γmAb but not the antibodies targeted to other cytokines including TGF-β (Wang et al., 2006). As STAT1 was the critical mediator for IFN-γ signaling in some circumstances as well as the increased susceptibility to EAE observed in STAT1 deficient mice, thus the STAT1 signaling might be involved in the expression of Foxp3 and the development of $CD4^+CD25^+$Treg cells induced by IFN-γ. Although the IFN-γ facilitated Foxp3 expression in the EAE model, whether IFN-γ plays a similar role in Foxp3 expression in other models or in physiological situations need to be addressed.

$CD4^+CD25^+$Treg cells play critical roles in maternal tolerance to the fetus, infection and tumor progression (Saito et al., 2005). It has been shown that some hormones and the tumor derivatives can induce Foxp3 expression. Tumor-derived Cyclooxygenase (COX)-2 and its product prostaglandin (PG) E2 induce Foxp3 expression at both mRNA and protein levels and enhance Foxp3 promoter activity in human lymphocytes (Baratelli et al., 2005; Sharma et al., 2005). Increased expression of Foxp3 was observed in pregnant mice, moreover, estrogen has been shown to enhance the Foxp3 expression both in vitro and in vivo (Polanczyk et al., 2004). GPR83 is a G protein coupled receptor with differential expression pattern between $CD4^+CD25^+$Treg cells and $CD4^+CD25^-$T cells as determined by the microarray analysis, it has been reported that $CD4^+CD25^+$Treg cells but not $CD4^+CD25^-$T cells preferentially expressed the GPR83. Recently, Hansen et al. demonstrated that in vivo acquisition of immunosuppressive activity in GPR83-transduced $CD4^+$T cells was associated with the induction of Foxp3, indicating that GPR83 might be critically involved in the peripheral generation of $Foxp3^+$Treg cells in vivo (Veldman et al., 2006). Now much more efforts are paid to elucidate how these molecules and signaling pathways are related to Foxp3 expression and thus Treg cell commitment.

Negative regulators for Foxp3 expression

So far, we have limited information available for the negative regulation of Foxp3. However, IL-6, inducible cAMP early repressor (ICER), and the *tax* gene of the human T lymphotropic virus type 1 (HTLV-Tax) are the postulated negative regulators for Foxp3 expression (Yamano et al., 2005; Bettelli et al., 2006; Bodor et al., 2006; Oh et al., 2006). TLR-triggered IL-6 production by activated DCs has been reported to abrogate the immunosuppressive activity without affecting the proliferation of Treg cells (Kubo et al., 2004). As described above, activation of $CD4^+Foxp3^-$ T cells induced Foxp3 expression in the presence of TGF-β. However, Bettelli et al. recently reported that IL-6 could completely inhibit the generation of $Foxp3^+$ Treg cells induced by TGF-β. In combination with IL-6, TGF-β promotes the production of the mostly pathogenic Th17 cells rather than the $Foxp3^+$Treg cells. Taken together, IL-6, an acute phase protein induced during inflammation, significantly suppresses TGF-β-induced Foxp3 expression and Treg cell generation (Bettelli et al., 2006).

It is reported that upon TCR activation, forskolin treatment diminishes Foxp3 expression in the $CD4^+CD25^+$Treg cells, indicating that forskolin-induced ICER may be involved in the downregulation of Foxp3. Likewise, T cells constitutively

expressing ICER are resistant to Foxp3 induction. In addition, the authors propose that ICER, induced upon CTLA-4/B7 engagement, uncouples the coactivator CBP. This impairs recruitment of Smads to the Foxp3 promoter and thus Foxp3 expression is hindered (Bodor et al., 2006).

Several studies have suggested infection with HTLV-1 is associated with the development of a number of inflammatory conditions. Defects in Foxp3 expression and Treg population may be responsible for the occurrence of lymphoproliferation and inflammatory autoimmune diseases in HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) patients (Yamano et al., 2005; Oh et al., 2006) Decreased levels of Foxp3 mRNA and protein expression in HIV-infected patients are observed compared with those in $CD4^+CD25^+$T cells from healthy individuals. Furthermore, the virus-encoded transactivating HTLV-I *tax* gene has been demonstrated to have a direct inhibitory effect on Foxp3 expression, and Foxp3 expression is inversely correlated with HTLV-1 tax proviral DNA load. But it remains to be determined how the *tax* signaling involves the reduced Foxp3 expression in HAM/TSP patients (Yamano et al., 2005; Oh et al., 2006).

Concluding remarks and perspectives

At present, even with the accumulating data showing the signaling molecules involved in positive and negative regulation on Foxp3 expression, we still can not draw a full picture for the dynamic regulation on Foxp3 expression yet. The regulation of Foxp3 expression may be quite complex and need to be coordinated by several signaling pathways. The establishment of a $CD4^+CD25^+$Treg cell line will greatly facilitate our exploring the molecular mechanism underlying its transcriptional and translational regulation. A better and more detailed understanding about Foxp3 specific upstream signaling molecules will significantly help us to deal with autoimmune diseases, inflammatory diseases, graft rejection and tumors via modulation of $CD4^+CD25^+$Treg cells in purpose. Thus, the manipulation of key signaling pathways on Foxp3 expression may hold great clinical therapeutic potential.

Acknowledgments

The authors would like to thank Mr. Aqeel Javeed for his review of our manuscript and Dr. Yanyan Qu for her helpful discussion and valuable suggestion.

Literature Cited

Akiyama T, Maeda S, Yamane S, Ogino K, Kasai M, Kajiura F, Matsumoto M, Inoue J. 2005. Dependence of self-tolerance on TRAF6-directed development of thymic stroma. Science 308:248–251.

Antony PA, Paulos CM, Ahmadzadeh M, Akpinarli A, Palmer DC, Sato N, Kaiser A, Heinrichs C, Klebanoff CA, Tagaya Y, Restifo NP. 2006. Interleukin-2-dependent mechanisms of tolerance and immunity in vivo. J Immunol 176:5255–5266.

Antov A, Yang L, Vig M, Baltimore D, Van Parijs L. 2003. Essential role for STAT5 signaling in $CD25^+CD4^+$ regulatory T cell homeostasis and the maintenance of self-tolerance. J Immunol 171:3435–3441.

Baratelli F, Lin Y, Zhu L, Yang SC, Heuze-Vourc'h N, Zeng G, Reckamp K, Dohadwala M, Sharma S, Dubinett SM. 2005. Prostaglandin E2 induces FOXP3 gene expression and T regulatory cell function in human $CD4^+$ T cells. J Immunol 175:1483–1490.

Bennett CL, Christie J, Ramsdell F, Brunkow ME, Ferguson PJ, Whitesell L, Kelly TE, Saulsbury FT, Chance PF, Ochs HD. 2001. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. Nat Genet 27:20–21.

Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, Weiner HL, Kuchroo VK. 2006. Reciprocal developmental pathways for the generation of pathogenic effector T(H)17 and regulatory T cells. Nature 441:235–238.

Bodor J, Fehervari Z, Diamond B, Sakaguchi S. 2006. Regulatory T cell-mediated suppression: potential role of ICER. J Leukoc Biol 81:161–167.

Bopp T, Palmetshofer A, Serfling E, Heib V, Schmitt S, Richter C, Klein M, Schild H, Schmitt E, Stassen M. 2005. NFATc2 and NFATc3 transcription factors play a crucial role in suppression of $CD4^+$ T lymphocytes by $CD4^+$ $CD25^+$ regulatory T cells. J Exp Med 201:181–187.

Brunkow ME, Jeffery EW, Hjerrild KA, Paeper B, Clark LB, Yasayko SA, Wilkinson JE, Galas D, Ziegler SF, Ramsdell F. 2001. Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse. Nat Genet 27:68–73.

Cabarrocas J, Cassan C, Magnusson F, Piaggio E, Mars L, Derbinski J, Kyewski B, Gross DA, Salomon BL, Khazaie K, Saoudi A, Liblau RS. 2006. $Foxp3^+$ $CD25^+$ regulatory T cells specific for a neo-self-antigen develop at the double-positive thymic stage. Proc Natl Acad Sci USA 103:8453–8458.

Caramalho I, Lopes-Carvalho T, Ostler D, Zelenay S, Haury M, Demengeot J. 2003. Regulatory T cells selectively express toll-like receptors and are activated by lipopolysaccharide. J Exp Med 197:403–411.

Chen W, Jin W, Hardegen N, Lei KJ, Li L, Marinos N, McGrady G, Wahl SM. 2003. 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 198:1875–1886.

Cohen AC, Nadeau KC, Tu W, Hwa V, Dionis K, Bezrodnik L, Teper A, Gaillard M, Heinrich J, Krensky AM, Rosenfeld RG, Lewis DB. 2006. Cutting edge: Decreased accumulation and regulatory function of $CD4^+CD25$high T cells in human STAT5b deficiency. J Immunol 177:2770–2774.

Crellin NK, Garcia RV, Hadisfar O, Allan SE, Steiner TS, Levings MK. 2005. Human $CD4^+$ T cells express TLR5 and its ligand flagellin enhances the suppressive capacity and expression of FOXP3 in $CD4^+CD25^+$ T regulatory cells. J Immunol 175:8051–8059.

Csencsits K, Wood SC, Lu G, Bishop DK. 2005. Transforming growth factor-beta1 gene transfer is associated with the development of regulatory cells. Am J Transplant 5:2378–2384.

D'Cruz LM, Klein L. 2005. Development and function of agonist-induced $CD25^+Foxp3^+$ regulatory T cells in the absence of interleukin 2 signaling. Nat Immunol 6:1152–1159.

Dower NA, Stang SL, Bottorff DA, Ebinu JO, Dickie P, Ostergaard HL, Stone JC. 2000. RasGRP is essential for mouse thymocyte differentiation and TCR signaling. Nat Immunol 1:317–321.

Ebinu JO, Bottorff DA, Chan EY, Stang SL, Dunn RJ, Stone JC. 1998. RasGRP, a Ras guanyl nucleotide-releasing protein with calcium- and diacylglycerol-binding motifs. Science 280:1082–1086.

Fang D, Liu YC. 2001. Proteolysis-independent regulation of PI3K by Cbl-b-mediated ubiquitination in T cells. Nat Immunol 2:870–875.

Fantini MC, Becker C, Monteleone G, Pallone F, Galle PR, Neurath MF. 2004. Cutting edge: TGF-beta induces a regulatory phenotype in $CD4^+CD25($ T cells through Foxp3 induction and down-regulation of Smad7. J Immunol 172:5149–5153.

Fontenot JD, Gavin MA, Rudensky AY. 2003. Foxp3 programs the development and function of $CD4^+CD25^+$ regulatory T cells. Nat Immunol 4:330–336.

Fontenot JD, Rasmussen JP, Gavin MA, Rudensky AY. 2005a. A function for interleukin 2 in Foxp3-expressing regulatory T cells. Nat Immunol 6:1142–1151.

Fontenot JD, Rasmussen JP, Williams LM, Dooley JL, Farr AG, Rudensky AY. 2005b. Regulatory T cell lineage specification by the forkhead transcription factor foxp3. Immunity 22:329–341.

Fu S, Zhang N, Yopp AC, Chen D, Mao M, Chen D, Zhang H, Ding Y, Bromberg JS. 2004. TGF-beta induces Foxp3 + T-regulatory cells from $CD4^+$ CD25—Precursors. Am J Transplant 4:1614–1627.

Greenwald RJ, Freeman GJ, Sharpe AH. 2005. The B7 family revisited. Annu Rev Immunol 23:515–548.

Hartwig UF, Nonn M, Khan S, Meyer RG, Huber C, Herr W. 2006. Depletion of alloreactive T cells via CD69: Implications on antiviral, antileukemic and immunoregulatory T lymphocytes. Bone Marrow Transplant 37:297–305.

Hogquist K. 2001. RasGRP: The missing link for Ras activation in thymocytes. Trends Immunol 22:69.

Hori S, Nomura T, Sakaguchi S. 2003. Control of regulatory T cell development by the transcription factor Foxp3. Science 299:1057–1061.

Horvath CM. 2004. The Jak-STAT pathway stimulated by interleukin 6. Sci STKE 2004:tr9.

Hsieh CS, Zheng Y, Liang Y, Fontenot JD, Rudensky AY. 2006. An intersection between the self-reactive regulatory and nonregulatory T cell receptor repertoires. Nat Immunol 7:401–410.

Jiang S, Lechler RI. 2003. Regulatory T cells in the control of transplantation tolerance and autoimmunity. Am J Transplant 3:516–524.

Kajiura F, Sun S, Nomura T, Izumi K, Ueno T, Bando Y, Kuroda N, Han H, Li Y, Matsushima A, Takahama Y, Sakaguchi S, Mitani T, Matsumoto M. 2004. NF-kappa B-inducing kinase establishes self-tolerance in a thymic stroma-dependent manner. J Immunol 172:2067–2075.

Kawai T, Akira S. 2006. TLR signaling. Cell Death Differ 13:816–825.

Khattri R, Cox T, Yasayko SA, Ramsdell F. 2003. An essential role for Scurfin in $CD4^+CD25^+$ T regulatory cells. Nat Immunol 4:337–342.

Kim JM, Rudensky A. 2006. The role of the transcription factor Foxp3 in the development of regulatory T cells. Immunol Rev 212:86–98.

Koonpaew S, Shen S, Flowers L, Zhang W. 2006. LAT-mediated signaling in $CD4^+CD25^+$ regulatory T cell development. J Exp Med 203:119–129.

Krawczyk CM, Jones RG, Atfield A, Bachmaier K, Arya S, Odermatt B, Ohashi PS, Penninger JM. 2005. Differential control of CD28-regulated in vivo immunity by the E3 ligase Cbl-b. J Immunol 174:1472–1478.

Kretschmer K, Apostolou I, Hawiger D, Khazaie K, Nussenzweig MC, von Boehmer H. 2005. Inducing and expanding regulatory T cell populations by foreign antigen. Nat Immunol 6:1219–1227.

Krupnick AS, Gelman AE, Barchet W, Richardson S, Kreisel FH, Turka LA, Colonna M, Patterson GA, Kreisel D. 2005. Murine vascular endothelium activates and induces the generation of allogeneic $CD4^+25^+Foxp3+$ regulatory T cells. J Immunol 175:6265–6270.

Kubo T, Hatton RD, Oliver J, Liu X, Elson CO, Weaver CT. 2004. Regulatory T cell suppression and anergy are differentially regulated by proinflammatory cytokines produced by TLR-activated DCs. J Immunol 173:7249–7258.

Li D, Gal I, Vermes C, Alegre ML, Chong AS, Chen L, Shao Q, Adarichev V, Xu X, Koreny T, Mikecz K, Finnegan A, Glant TT, Zhang J. 2004. Cutting edge: Cbl-b: One of the key molecules tuning CD28- and CTLA-4-mediated T cell costimulation. J Immunol 173:7135–7139.

Li MO, Wan YY, Sanjabi S, Robertson AK, Flavell RA. 2006. Transforming Growth Factor-beta Regulation of Immune Responses. Annu Rev Immunol 24:99–146.

Liang S, Alard P, Zhao Y, Parnell S, Clark SL, Kosiewicz MM. 2005. Conversion of $CD4^+$ $CD25^($ cells into $CD4^+$ $CD25^+$ regulatory T cells in vivo requires B7 costimulation, but not the thymus. J Exp Med 201:127–137.

Liew FY, Xu D, Brint EK, O'Neill LA. 2005. Negative regulation of toll-like receptor-mediated immune responses. Nat Rev Immunol 5:446–458.

Liu YC, Gu H. 2002. Cbl and Cbl-b in T-cell regulation. Trends Immunol 23:140–143.

Liu KD, Gaffen SL, Goldsmith MA, Greene WC. 1997. Janus kinases in interleukin-2-mediated signaling: JAK1 and JAK3 are differentially regulated by tyrosine phosphorylation. Curr Biol 7:817–826.

Liu GW, Xia XP, Zhao Y. 2005. Research progress in toll-like receptor signal pathways. Chin J Zool 40:117–121.

Liu H, Komai-Koma M, Xu D, Liew FY. 2006. Toll-like receptor 2 signaling modulates the functions of $CD4^+CD25^+$ regulatory T cells. Proc Natl Acad Sci USA 103:7048–7053.

Maggi E, Cosmi L, Liotta F, Romagnani P, Romagnani S, Annunziato F. 2005. Thymic regulatory T cells. Autoimmun Rev 4:579–586.
Mantel PY, Ouaked N, Ruckert B, Karagiannidis C, Welz R, Blaser K, Schmidt-Weber CB. 2006. Molecular mechanisms underlying FOXP3 induction in human T cells. J Immunol 176:3593–3602.
Mayack SR, Berg LJ. 2006. Cutting edge: An alternative pathway of $CD4^+$ T cell differentiation is induced following activation in the absence of gamma-chain-dependent cytokine signals. J Immunol 176:2059–2063.
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. 2005. Expression of FOXP3 mRNA is not confined to $CD4^+CD25^+$ T regulatory cells in humans. Hum Immunol 66:13–20.
Netea MG, Sutmuller R, Hermann C, Van der Graaf CA, Van der Meer JW, van Krieken JH, Hartung T, Adema G, Kullberg BJ. 2004. Toll-like receptor 2 suppresses immunity against Candida albicans through induction of IL-10 and regulatory T cells. J Immunol 172:3712–3718.
Nishibori T, Tanabe Y, Su L, David M. 2004. Impaired development of $CD4^+$ $CD25^+$ regulatory T cells in the absence of STAT1: Increased susceptibility to autoimmune disease. J Exp Med 199:25–34.
Nishimura E, Sakihama T, Setoguchi R, Tanaka K, Sakaguchi S. 2004. Induction of antigen-specific immunologic tolerance by in vivo and in vitro antigen-specific expansion of naturally arising $Foxp3^+CD25^+CD4^+$ regulatory T cells. Int Immunol 16:1189–1201.
O'Garra A, Vieira P. 2003. Twenty-first century Foxp3. Nat Immunol 4:304–306.
Oh U, Grant C, Griffith C, Fugo K, Takenouchi N, Jacobson S. 2006. Reduced Foxp3 protein expression is associated with inflammatory disease during human t lymphotropic virus type 1 Infection. J Infect Dis 193:1557–1566.
Pacholczyk R, Kraj P, Ignatowicz L. 2002. Peptide specificity of thymic selection of $CD4^+CD25^+$ T cells. J Immunol 168:613–620.
Park O, Grishina I, Leung PS, Gershwin ME, Prindiville T. 2005. Analysis of the Foxp3/scurfin gene in Crohn's disease. Ann N Y Acad Sci 1051:218–228.
Peng G, Guo Z, Kiniwa Y, Voo KS, Peng W, Fu T, Wang DY, Li Y, Wang HY, Wang RF. 2005. Toll-like receptor 8-mediated reversal of $CD4^+$ regulatory T cell function. Science 309:1380–1384.
Picca CC, Larkin J3rd, Boesteanu A, Lerman MA, Rankin AL, Caton AJ. 2006. Role of TCR specificity in $CD4^+$ $CD25^+$ regulatory T-cell selection. Immunol Rev 212:74–85.
Polanczyk MJ, Carson BD, Subramanian S, Afentoulis M, Vandenbark AA, Ziegler SF, Offner H. 2004. Cutting edge: Estrogen drives expansion of the $CD4^+CD25^+$ regulatory T cell compartment. J Immunol 173:2227–2230.
Rangachari M, Penninger JM. 2004. Negative regulation of T cell receptor signals. Curr Opin Pharmacol 4:415–422.
Rao E, Zhao Y. 2005. Foxp3: A critical transcription factor for the development of regulatory T cells. Prog Biochem Biophy 32:106–110.
Reif K, Lucas S, Cantrell D. 1997. A negative role for phosphoinositide 3-kinase in T-cell antigen receptor function. Curr Biol 7:285–293.
Roncador G, Garcia JF, Garcia JF, Maestre L, Lucas E, Menarguez J, Ohshima K, Nakamura S, Banham AH, Piris MA. 2005. FOXP3, a selective marker for a subset of adult T-cell leukaemia/lymphoma. Leukemia 19:2247–2253.
Rudensky AY, Gavin M, Zheng Y. 2006. FOXP3 and NFAT: Partners in tolerance. Cell 126:253–256.
Saito S, Sasaki Y, Sakai M. 2005. CD4(+)CD25high regulatory T cells in human pregnancy. J Reprod Immunol 65:111–120.
Sakaguchi S. 2000. Regulatory T cells: Key controllers of immunologic self-tolerance. Cell 101:455–458.
Sakaguchi S. 2003a. Control of immune responses by naturally arising $CD4^+$ regulatory T cells that express toll-like receptors. J Exp Med 197:397–401.
Sakaguchi S. 2003b. The origin of FOXP3-expressing $CD4^+$ regulatory T cells: thymus or periphery. J Clin Invest 112:1310–1312.
Sakaguchi S. 2004. Naturally arising $CD4^+$ regulatory t cells for immunologic self-tolerance and negative control of immune responses. Annu Rev Immunol 22:531–562.
Sakaguchi S. 2005. Naturally arising Foxp3-expressing $CD25^+CD4^+$ regulatory T cells in immunological tolerance to self and non-self. Nat Immunol 6:345–352.
Sakaguchi S, Toda M, Asano M, Itoh M, Morse SS, Sakaguchi N. 1996. T cell-mediated maintenance of natural self-tolerance: Its breakdown as a possible cause of various autoimmune diseases. J Autoimmun 9:211–220.
Schramm C, Huber S, Protschka M, Czochra P, Burg J, Schmitt E, Lohse AW, Galle PR, Blessing M. 2004. TGFbeta regulates the $CD4^+CD25^+$ T-cell pool and the expression of Foxp3 in vivo. Int Immunol 16:1241–1249.
Schwartz RH. 2005. Natural regulatory T cells and self-tolerance. Nat Immunol 6:327–330.
Sharma S, Yang SC, Zhu L, Reckamp K, Gardner B, Baratelli F, Huang M, Batra RK, Dubinett SM. 2005. Tumor cyclooxygenase-2/prostaglandin E2-dependent promotion of FOXP3 expression and $CD4^+$ $CD25^+$ T regulatory cell activities in lung cancer. Cancer Res 65:5211–5220.
Skapenko A, Kalden JR, Lipsky PE, Schulze-Koops H. 2005. The IL-4 receptor alpha-chain-binding cytokines, IL-4 and IL-13, induce forkhead box P3-expressing $CD25^+CD4^+$ regulatory T cells from $CD25^{(}CD4^+$ precursors. J Immunol 175:6107–6116.
Stock P, Akbari O, Berry G, Freeman GJ, Dekruyff RH, Umetsu DT. 2004. Induction of T helper type 1-like regulatory cells that express Foxp3 and protect against airway hyper-reactivity. Nat Immunol 5:1149–1156.
Sutmuller RP, den Brok MH, Kramer M, Bennink EJ, Toonen LW, Kullberg BJ, Joosten LA, Akira S, Netea MG, Adema GJ. 2006. Toll-like receptor 2 controls expansion and function of regulatory T cells. J Clin Invest 116:485–494.
Taams LS, Akbar AN. 2005. Peripheral generation and function of $CD4^+CD25^+$ regulatory T cells. Curr Top Microbiol Immunol 293:115–131.
Tai X, Cowan M, Feigenbaum L, Singer A. 2005. CD28 costimulation of developing thymocytes induces Foxp3 expression and regulatory T cell differentiation independently of interleukin 2. Nat Immunol 6:152–162.
Takahashi T, Tagami T, Yamazaki S, Uede T, Shimizu J, Sakaguchi N, Mak TW, Sakaguchi S. 2000. Immunologic self-tolerance maintained by CD25(+)CD4(+) regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4. J Exp Med 192:303–310.
Takeda K, Akira S. 2004. TLR signaling pathways. Semin Immunol 16:3–9.
Takeda I, Ine S, Killeen N, Ndhlovu LC, Murata K, Satomi S, Sugamura K, Ishii N. 2004. Distinct roles for the OX40-OX40 ligand interaction in regulatory and nonregulatory T cells. J Immunol 172:3580–3589.
Tivol EA, Borriello F, Schweitzer AN, Lynch WP, Bluestone JA, Sharpe AH. 1995. Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. Immunity 3:541–547.
Tuovinen H, Salminen J, Arstila TP. 2006. Most human thymic and peripheral blood $CD4^+CD25^+$ regulatory T cells express two T cell receptors. Blood 108:4063–4067.
Veldman C, Pahl A, Beissert S, Hansen W, Buer J, Dieckmann D, Schuler G, Hertl M. 2006. Inhibition of the transcription factor Foxp3 converts desmoglein 3-specific type 1 regulatory T cells into Th2-like cells. J Immunol 176:3215–3222.
von Boehmer H, Kisielow P. 1990. Self-nonself discrimination by T cells. Science 248:1369–1373.
Waldmann H, Graca L, Cobbold S, Adams E, Tone M, Tone Y. 2004. Regulatory T cells and organ transplantation. Semin Immunol 16:119–126.
Walker MR, Kasprowicz DJ, Gersuk VH, Benard A, Van Landeghen M, Buckner JH, Ziegler SF. 2003. Induction of FoxP3 and acquisition of T regulatory activity by stimulated human $CD4^+CD25^($ T cells. J Clin Invest 112:1437–1443.
Wan YY, Flavell RA. 2005. Identifying Foxp3-expressing suppressor T cells with a bicistronic reporter. Proc Natl Acad Sci USA 102:5126–5131.
Wan YY, Flavell RA. 2006. The roles for cytokines in the generation and maintenance of regulatory T cells. Immunol Rev 212:114–130.
Wang HJ, Zhao Y. 2006. $CD4^+CD25^+$Foxp3+Treg cells, immunosuppressive agents and transplant tolerance. Modern Immunol 26:353–356.
Wang Z, Hong J, Sun W, Xu G, Li N, Chen X, Liu A, Xu L, Sun B, Zhang JZ. 2006. Role of IFN-gamma in induction of Foxp3 and conversion of CD4 CD25 T cells to CD4 Tregs. J Clin Invest 116:2434–2441.
Weber SE, Harbertson J, Godebu E, Mros GA, Padrick RC, Carson BD, Ziegler SF, Bradley LM. 2006. Adaptive islet-specific regulatory CD4 T cells control autoimmune diabetes and mediate the disappearance of pathogenic Th1 cells in vivo. J Immunol 176:4730–4739.
Wohlfert EA, Gorelik L, Mittler R, Flavell RA, Clark RB. 2006. Cutting edge: Deficiency in the E3 ubiquitin ligase Cbl-b results in a multifunctional defect in T cell TGF-beta sensitivity in vitro and in vivo. J Immunol 176:1316–1320.
Wu Y, Borde M, Heissmeyer V, Feuerer M, Lapan AD, Stroud JC, Bates DL, Guo L, Han A, Ziegler SF, Mathis D, Benoist C, Chen L, Rao A. 2006. FOXP3 controls regulatory T cell function through cooperation with NFAT. Cell 126:375–387.
Yamagiwa S, Gray JD, Hashimoto S, Horwitz DA. 2001. A role for TGF-beta in the generation and expansion of $CD4^+CD25^+$ regulatory T cells from human peripheral blood. J Immunol 166:7282–7289.
Yamano Y, Takenouchi N, Li HC, Tomaru U, Yao K, Grant CW, Maric DA, Jacobson S. 2005. Virus-induced dysfunction of $CD4^+CD25^+$ T cells in patients with HTLV-I-associated neuroimmunological disease. J Clin Invest 115:1361–1368.
Yi HF, ZHen Y, Jiang L, Zheng J, Zhao Y. 2006. The phenotypic characterization of naturally occurring regulatory $CD4^+CD25^+$T cells. Cell and Mol Immunol 3:189–195.
Zhang J, Bardos T, Li D, Gal I, Vermes C, Xu J, Mikecz K, Finnegan A, Lipkowitz S, Glant TT. 2002. Cutting edge: Regulation of T cell activation threshold by CD28 costimulation through targeting Cbl-b for ubiquitination. J Immunol 169:2236–2240.
Zhang H, Chua KS, Guimond M, Kapoor V, Brown MV, Fleisher TA, Long LM, Bernstein D, Hill BJ, Douek DC, Berzofsky JA, Carter CS, Read EJ, Helman LJ, Mackall CL. 2005. Lymphopenia and interleukin-2 therapy alter homeostasis of $CD4^+CD25^+$ regulatory T cells. Nat Med 11:1238–1243.
Zhang L, Yi H, Xia XP, Zhao Y. 2006. Transforming growth factor-beta: An important role in $CD4^+CD25^+$ regulatory T cells and immune tolerance. Autoimmunity 39:269–276.
Zheng Y, Manzotti CN, Liu M, Burke F, Mead KI, Sansom DM. 2004. CD86 and CD80 differentially modulate the suppressive function of human regulatory T cells. J Immunol 172:2778–2784.
Zheng SG, Wang JH, Stohl W, Kim KS, Gray JD, Horwitz DA. 2006. TGF-beta requires CTLA-4 early after T cell activation to induce FoxP3 and generate adaptive $CD4^+CD25^+$ regulatory cells. J Immunol 176:3321–3329.
Ziegler SF. 2006. FOXP3: Of mice and men. Annu Rev Immunol 24:209–226.
Zorn E, Nelson EA, Mohseni M, Porcheray F, Kim H, Litsa D, Bellucci R, Raderschall E, Canning C, Soiffer RJ, Frank DA, Ritz J. 2006. IL-2 regulates FOXP3 expression in human $CD4^+CD25^+$ regulatory T cells through a STAT dependent mechanism and induces the expansion of these cells in vivo. Blood 108:1571–1579.