REVIEW

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Expanding and converting regulatory T cells: a horizon for immunotherapy

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Abstract

The human immune system is a myriad of diverse cellular populations, each contributing to maintaining an effective and optimal immune response against infectious agents. It is important to maintain a "self-check" in the immune system so that responses do not go haywire, leading to the development of autoimmune diseases. Regulatory/suppressor T (Treg) cells are a specialized subpopulation of T cells that suppress the activation, expansion, and function of other T cells, thereby maintaining homeostasis through a fine balance between reactivity to foreign and self antigens. Tregs are characterized by surface expression of interleukin (IL)-2 receptor α chain (CD25) and intracellular expression of forkhead box protein P3 (FoxP3). There are at least two important functional populations of Treg cells, namely natural Treg (nTreg), which are continuously derived from the thymus, and induced Treg (iTreg), which are converted from naive T cells. The development and function of both nTreg and iTreg cells are regulated by several factors, such as antigen T-cell receptor, co-stimulatory receptors (i.e., cytotoxic T lymphocyte-associated antigen, or CTLA-4), and cytokines (IL-2, IL-10, and tumor growth factor-β, or TGF-β). In addition, the TGF-β inhibitor ALK5, retinoid acid, and rapamycin influence the expansion of nTreg cells and the conversion of iTreg cells *in vitro* and *in vivo*. The heightening of Treg expansion may be harnessed to therapeutic methods for the treatment of autoimmune diseases and the induction of transplantation tolerance.

Key words: nTreg, iTreg, FoxP3, expansion, conversion.

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INTRODUCTION

Many autoaggressive diseases result from an imbalance of immune homeostasis. Regulatory/suppressor T (Treg) cells are the most important regulators of such immune homeostasis, maintaining an immune response strong enough to combat foreign aggression (such as infections) but down-regulating its self reactivity to avoid autoimmune diseases. There are at least two different Treg subsets (based on their origin, specificity, and functionality), namely natural Treg (nTreg) and induced Treg (iTreg) cells. Both Treg populations are characterized by high-level expression of surface interleukin-2 receptor α chain (IL-2Rα, CD25) and intracellular expression of a master switch transcription factor called forkhead box protein P3 (FoxP3). While nTreg cells constitutively develop in and are released from the thymus, iTreg cells are converted in peripheral lymphoid tissue by the activation of mature $CD4^+$ T cells via specific antigenic and cytokine stimulation (Bluestone and Abbas 2003). So far, nTreg and iTreg cells are indistinguishable by their cell surface markers (Horwitz et al. 2008). However, these populations differ predominantly in their stability and potency of immuosuppression. In particular, nTreg cells are more stable and more potent than iTreg cells in maintaining self tolerance and preventing autoimmunity (Gupta et al. 2008). It was suggested that FoxP3 expression in thymocytes is dependent on T-cell receptor (TCR)/MHC ligand interactions (Fontenot et al. 2005). However, a recent report showed that some $CD4^-CD8^-$ double-negative (DN) human thymocytes express FoxP3 in the absence of a TCR, suggesting that nTreg cells can develop in the thymus from these DN thymocytes (Tuovinen H et al. 2008).

$CD4^+CD8^+$ double-positive thymocytes may provide trans-acting factors limiting $CD4^-CD8^-$ thymocytes to become nTreg cells (Pennington et al. 2006). Furthermore, $FoxP3^-CD4^+CD8^-$ thymocytes can also be precursors of nTreg cells and are poised to express FoxP3 without further TCR engagement, requiring only stimulation by IL-2 or IL-15 (Lio and Hsieh 2008). Once generated, nTreg cells migrate from the thymus to peripheral tissues, where they prevent the activation of self reactive T cells. Maintaining nTreg cells requires IL-2 signaling (Turka and Walsh 2008) and co-stimulation by CD28 to promote their self-renewal through homeostatic proliferation, hence supporting their survival (Salomon et al. 2000). nTreg cells are always $CD4^+$ and express high levels of CD25 as well as cytotoxic T-lymphocyte antigen 4 (CTLA-4) and the tumor necrosis factor (TNF) superfamily member glucocorticoid--induced TNF receptor family-related protein (GITR). While not all nTreg cells uniformly express CTLA-4 and GITR markers, they all express FoxP3 as the hallmark protein regulating their development and function. Mutations in the FOXP3 gene abrogate the development of nTreg cells or impair their suppressive function, causing hyperactivation of self-reactive T cells, leading to autoimmune diseases such as polyendocrinopathy, inflammatory bowel disease (IBD), immunodysregulation polyendocrinopathy enteropathy X-linked syndrome, type-1 diabetes, multiple sclerosis (MS), and many other ailments (Sakaguchi 2004).

Apart from nTreg cells, additional regulatory populations are iTreg cells that can be defined in immunity also as "adaptive Treg" cells. Such iTreg populations may arise from mature "naïve" $CD4^+$ T cells under certain conditions of antigen stimulation *in vivo* or by culturing $CD4^+$ T cells with different cytokines or chemical/antigenic stimulators *in vitro*. Since iTreg cells do not originate from the thymus, their level of CD25 expression is highly variable (Bluestone and Abbas 2003). While the development of intra-thymic nTreg and extra--thymic iTreg cells may be regulated by different factors, IL-2 and tumor growth factor (TGF)-β play a central role in the maintenance of nTreg cells and in the conversion of iTreg cells. A published report showed that thymus-derived nTreg cells can educate peripheral $CD4^+CD25^-$ cells to develop suppressive activity via co--stimulatory effects of IL-2 and TGF-β. These converted $CD4^+CD25^+$ iTreg cells in turn induce other alloactivated $CD4^+CD25^-$ cells to become potent suppressor cells by mechanisms that surprisingly require both cell contact and TGF-β and IL-10 (Zheng et al. 2004). TGF-β administration to $CD25^-$ cells from the periphery can induce FoxP3 expression in these cells, resulting in the induction of converted $CD4^+CD25^+$ iTreg cells. The iTreg cells are functionally active *in vivo* with therapeutic potential in T cell-mediated chronic intestinal inflammation (Fantini et al. 2006).

As mentioned above, $FoxP3^+$ Treg cells can be derived naturally in the thymus or from naïve T cells in the periphery. Many scientists have attempted to expand nTreg cells and induce the conversion of iTreg cells. Herein we describe various *in vivo* and *in vitro* methods for increasing the numbers of these populations.

IN VITRO EXPANSION OF nTreg CELLS

nTreg cells are present in very low numbers in a normal host and have an anergic phenotype because of which it is difficult to harness their tolerogenic potential to treat autoimmunity and transplant rejection. Several *in vitro* methods to specifically expand nTreg cells have been described with different requirements. Tang et al. (Tang et al. 2004) demonstrated a method for expanding antigen-specific nTreg cells presorted from autoimmune-prone non-obese diabetic (NOD) mice up to 200--fold using a combination of anti-CD3 and anti-CD28 monoclonal antibodies (mAbs) and IL-2. These expanded nTreg cells can function both *in vitro* and *in vivo* to suppress effector T-cell functions, suggesting an approach for cellular immunotherapy against autoimmunity (Tang et al. 2004). Dendritic cells (DCs) can also be used for *in vitro* expansion of nTreg cells. For instance it was shown that DCs isolated from NOD mice could be used to expand islet autoantigen-specific $CD25^+CD4^+$ Treg cells from BDC2.5 mice. These *in vitro* expanded nTreg cells were much more suppressive than their freshly isolated counterparts, as observed by both *in vitro* suppression assay and their ability to suppress autoimmune diabetes *in vivo* (Tarbell et al. 2004). In a similar study, autologous DCs pulsed with an allopeptide from $H2\text{-}K^b$ were used to expand nTreg cells purified from CBA mice. Following adoptive transfer into CBA mice, these *in vitro* expanded nTreg cells preferentially accumulated in the graft draining lymph nodes and within the graft and prevented CBK, but not third-party, skin graft rejection (Golshayan et al. 2007).

It may be important to look at the cytokine signaling pathways involved in the development of Treg cells to better understand the requirements of nTreg expansion. Suppressors of cytokine signaling (SOCS) proteins are a family of SH2 domain-containing intracellular inhibitors of cytokine signal transduction that act by several different mechanisms. In a recent study an increased *in vitro* expansion of $FoxP3^+$ Treg cells from naïve $CD4^+$ T cells was shown when they were cocultured with $SOCS\text{-}3^{-/-}$ DCs compared with wild-type DCs. These $SOCS3^{-/-}$ DCs expressed lower levels of MHC class II, CD40, CD86, and IL-12 than wild-type DCs and showed constitutive activation of STAT3. The expansion of $FoxP3^+$ nTreg cell was blocked by anti--TGF-β Ab and $SOCS3^{-/-}$ DCs produced higher levels of TGF-β than wild-type cells, suggesting the essential role of TGF-β in the expansion of $FoxP3^+$ nTreg cells. The authors also showed that adoptive transfer of $SOCS3^{-/-}$ DCs reduced the severity of experimental autoimmune encephalomyelitis (Matsumura et al. 2007).

In addition to cytokine and molecular requirements,

some researchers have also described methods for *in vitro* Treg expansion using certain chemical molecules. Rapamycin (RAPA) is known to play a role in expanding murine $CD4^+CD25^+FoxP3^+$ Treg cells and preserving their suppressive function. A recent report demonstrated that activation of human $CD4^+$ T cells in the presence of RAPA leads to the growth of $CD4^+CD25^+FoxP3^+$ Tregs and selective depletion of $CD4^+CD25^-$ T effector cells. These RAPA-expanded nTreg cells suppress the proliferation of both syngeneic and allogeneic T cells. A defect in freshly isolated $CD4^+CD25^+$ nTreg cells has been reported in type-1 diabetic patients. Nevertheless, RAPA promotes the expansion of functional $CD4^+CD25^+FoxP3^+$ nTreg cells also in type-1 diabetic patients. Together, the *in vitro* expansion of nTreg cells might be a novel and safe approach for cellular immunotherapy in T cell-mediated diseases (Battaglia et al. 2006).

IN VITRO CONVERSION OF iTreg CELLS

Naïve $CD4^+FoxP3^-$ cells can be converted to functional $CD4^+CD25^+FoxP3^+$ iTreg cells *in vitro* in the presence of TGF-β (Chen et al. 2003); IL-2 promotes the TGF-β-mediated conversion of iTreg cells (Zheng et al. 2007). *In vitro* interferon (IFN)-γ treatment of $CD4^+CD25^-$ T cells also led to conversion to $CD4^+$ iTreg cells, as characterized by increased expression of FoxP3 and enhanced regulatory function, including suppression of experimental autoimmune encephalomyelitis (EAE) in mice (Wang et al. 2006). Looking at the role of DCs in the conversion of iTreg cells, it was shown that naïve $CD4^+CD25^-$ T cells cultured with β-cell peptide-pulsed DCs from NOD mice and TGF-β1 can be converted into $CD4^+CD25^+FoxP3^+$ iTreg cells. These iTreg cells prevented the development of diabetes and protected syngeneic islet grafts from established destructive autoimmunity (Luo et al. 2007). Another study demonstrated that mesenteric lymph node $CD103^+$ DCs promote the conversion of naïve $CD4^+$ T cells into iTreg cells. The conversion into iTreg cells by $CD103^+$ DCs was found to be dependent on TGF-β and the dietary metabolite retinoic acid (Coombes et al. 2007).

Though many methods have been presented for the conversion of peripheral naïve T cells into iTreg cells, a recent report showed the kinetics and dynamics of Treg conversion from $CD4^+FoxP3^-$ T cells in the presence of TGF-β. In particular, up to 90% $FoxP3^+$ iTreg cells can be induced following cultivation of naïve $CD4^+$ T cells in the presence of TGF-β. Conversion takes place only when TGF-β is added within a 2–3 day window of CD3/CD28 mAb stimulation. However, loss of FoxP3 was observed within 4 days of TGF-β withdrawal. Also, adoptive transfer of *in vitro* converted iTregs into wild-type mice resulted in loss of FoxP3 expression among the majority of transferred cells within 2 days; these $FoxP3^-$ cells localized mainly to the blood, spleen, lung, and liver, suggesting that *in vitro* conversion of naïve $CD4^+$ T cells into iTreg cells is a transient state mainly depending on the presence of TGF-β. However, some transferred $FoxP3^+$ iTreg cells persisted and could be detected in the lymph nodes and bone marrow 28 days after transfer (Selvaraj and Geiger 2007).

Conversion of $CD4^+$ cells into iTreg cells has also been reported using copolymer-1 (COP-1), a drug used for the treatment of MS. COP-1 has the capability to induce the conversion of peripheral $CD4^+CD25^-$ to $CD4^+CD25^+$ iTreg cells through the activation of transcription factor FoxP3. The induction of FoxP3 in $CD4^+$ T cells by COP-1 was mediated through its ability to induce the production of IFN-γ and, to a lesser extent, TGF-β. It was shown that *in vitro* treatment and administration with COP-1 significantly increased FoxP3 expression in $CD4^+$ T cells and converted them to $CD4^+CD25^+$ iTreg cells in wild-type B6 mice but not in IFN-$\gamma^{-/-}$ mice (Hong et al. 2005).

IN VIVO EXPANSION OF nTreg CELLS

As opposed to *in vitro* studies, both the *in vivo* expansion of nTreg cells and the *in vivo* conversion of naïve $CD4^+$ cells into iTreg cells are hard to achieve because of the absence of a fully controlled environment as well as a lack of understanding of their *in vivo* requirements. However, limited *in vivo* studies in mice are available for the expansion of nTreg cells and the generation of iTreg cells to increase suppressive activity.

Consistent with the *in vitro* studies, the *in vivo* experiments also revealed that IL-2 was an important growth factor for nTreg cell expansion. *In vivo* IL-2 administration not only expanded a Treg population, but also induced IL-10 production and suppressive activity (Brandenburg et al. 2008). Although daclizumab, a humanized antibody blocking the IL-2 receptor α chain, has prevented transplant rejection, it did not reduce the number of functional Treg cells upon clearance of the antibody (Kreijveld et al. 2007). Apart from IL-2, IFN-γ was found to play an important role in the induction of FoxP3 *in vivo*. In particular, severity of EAE in IFN-$\gamma^{-/-}$ mice directly correlated with the reduced frequency and function of $CD4^+CD25^+FoxP3^+$ Treg cells compared with wild-type mice (Wang et al. 2006).

Major studies that examined the *in vivo* expansion of nTreg cells were actually aimed at the treatment of autoimmune diseases using new drugs that resulted in a prolonged survival mediated via the increased numbers of Treg cells. For example, *in vivo* treatment with NCX-1015, a nitric oxide derivative of prednisolone, protected mice against experimental colitis with an elevated population of Treg cells in the lamina propria, which negatively modulated the intestinal inflammation (Fiorucci et al. 2002). In the transplantation field, induction of transplant tolerance always associates with increased number and suppressive function of Treg

cells. For instance, a brief peritransplant treatment with anti-CD3 immunotoxin and deoxyspergualin of multiple nonhuman primates induced long-term stable transplant tolerance. The frequency of Treg cells was elevated three-fold in graft-tolerant vs. normal monkeys (Asiedu et al. 2005). Since acetylation of several lysines in the forkhead domain of Foxp3 is required for optimal Treg function, a recent study suggests that administration of an histone/protein deacetylases (HDAC) inhibitor to mice increased FoxP3 expression, hence the numbers and suppressive function of Treg cells. Therefore, therapy with HDAC inhibitor decreased IBD and promoted donor-specific allograft tolerance (Tao et al. 2007).

Therapy with low-dose nucleosomal histone $H4_{71–94}$ peptide prevented the development of lupus in mice and resulted in a markedly prolonged lifespan by the generation of $CD4^+CD25^+$ Treg cells. Especially, tolerization with $H4_{71–94}$ peptide presented by plasmacytoid DCs produced increased amounts of TGF-β and diminished IL-6 on stimulation via the Toll-like receptor-9 pathway. Such $H4_{71–94}$ peptide plasmacytoid DC therapy blocked lupus autoimmune disease by expansion of autoantigen-specific nTreg cells and by suppression of pro-inflammatory Th17 cells that otherwise infiltrated the kidneys of untreated lupus mice (Kang et al. 2007). Thus regulation is accomplished by selective expansion of nTreg cells with down-regulation of the pro-inflammatory T-cell response (such as Th17 and Th1 cells).

IN VIVO CONVERSION OF iTreg CELLS

It has long been controversial whether $CD4^+CD25^+$ Treg cells can develop outside the thymus from other $CD4^+$ T-cell lineages under natural conditions, i.e., with the expression of the natural TCR repertoire and with exposure to the natural endogenous antigen load. However, several studies have demonstrated an *in vivo* conversion of $CD4^+CD25^-$ T cells to $CD4^+CD25^+$ iTreg cells. When $CD4^+CD25^-$ T cells from $CD45.1^+$ mice were adoptively transferred into congenic $CD45.2^+$ mice, approximately 5–12% of the transferred cells were converted to $CD4^+CD25^+$ iTreg cells within 6 weeks. These converted iTreg cells became FoxP3 positive, failed to proliferate after stimulation, and suppressed the proliferation of effector T cells in an *in vitro* assay. It was also shown that conversion of $CD4^+CD25^-$ T cells into $CD4^+CD25^+FoxP3^+$ iTreg cells can take place in thymectomized mice, but not in $B7^{-/-}$ mice, suggesting that B7 co-stimulation is essential for the *in vivo* conversion (Liang et al. 2005).

It was also possible to induce the conversion of $FoxP3^+$ iTreg cells *in vivo* by administration of minute antigen doses with suboptimal DC activation. The conversion could be enhanced by addition of TGF-β or by limiting IL-2 production. Subsequently, the iTreg population can be further expanded by delivering an antigen in immunogenic condition (Kretschmer et al. 2005). Couper et al. (Couper et al. 2007) tested two anti-CD25 antibodies, i.e., 7D4 and PC61, for their ability to deplete and repopulate with $CD4^+CD25^+$ iTregs in malaria-infected mice. It was observed that the 7D4/PC61-treated mice underwent an acute malaria infection with a rapid repopulation of the spleen by $CD25^+FoxP3^+$ iTreg cells, with malaria infection driving the conversion of $CD4^+CD25^-FoxP3^-$ cells into $CD4^+CD25^-FoxP3^+$ iTreg cells (Couper et al. 2007).

It was previously demonstrated that cyclosporine (CsA) blocks activation-induced cell death (AICD) of T effector cells, and is therefore detrimental to tolerance induction by the co-stimulation blockade. In contrast, the RAPA-preserved AICD process augmented the co-stimulatory blockade-induced tolerance. In a study looking into the effects of CsA and RAPA on the generation of Treg cells with FoxP3 as the reporter gene system, it was observed that RAPA promoted *de novo* conversion of alloantigen-specific iTreg cells, whereas CsA completely inhibited this process. Thus the differential effects of RAPA and CsA on iTreg cells favor the use of RAPA in shifting the balance from aggressive to protective-type alloimmunity (Gao et al. 2007).

Treg CELLS: BANE OR BOON

It is now evident that both nTreg and iTreg cells play an essential role in controlling patterns of the immune responses, including the prevention of autoimmune diseases as well as the induction of tolerance of allografts. Indeed, elevated levels of Treg cells were observed in patients suffering from malaria, tuberculosis, and other diseases, suggesting that Treg cells control the immune response in infectious diseases (Couper et al. 2007; Scott-Browne et al. 2007). As shown in several experimental models and described in clinical practice, Treg cells are fundamental in preventing the development of autoimmune diseases (Costantino et al. 2008). However, the use of Treg cells for immunotherapy has been limited by the inability to define the antigenic specificities of these cells as well as the difficulties in raising the large number of Treg cells required for effective suppression. The new methods of *ex vivo* expansion or *in vivo* induction may be designed for a new potent immunotherapy to treat patients with autoimmune diseases or recipients undergoing transplantation. Such an immunotherapy may be combined with other procedures such as stem-cell transplantation or gene therapy for genetic diseases. The challenge is to carefully combine the biological studies in animal models followed by clinical trials to optimize the immune responses.

However, the *in vivo* application of nTreg and iTreg cells for therapy requires addressing a number of questions about their potency and stability. As described in our review, there are problems with the stability of Foxp3 expression of iTreg cells. Indeed, the vast majority of adoptively transferred iTreg cells may lose FoxP3 expression, whereas only a small fraction of iTreg cells

may maintain potent suppressor function (Selvaraj and Geiger 2007). Thus iTreg cells have problems with stability and potency when used *in vivo* for immunotherapy. Despite these challenges, an adoptive transfer of iTreg cells protected syngeneic islet grafts from destruction by autoimmune response in NOD mice (Luo et al. 2007). Overall, understanding these optimizing factors should contribute to improved immunotherapy with nTreg and iTreg cells.

REFERENCES

Asiedu CK, Goodwin KJ, Balgansuren G et al (2005) Elevated T regulatory cells in long-term stable transplant tolerance in rhesus macaques induced by anti-CD3 immunotoxin and deoxyspergualin. J Immunol 175:8060–8068

Battaglia M, Stabilini A, Migliavacca B et al (2006) Rapamycin promotes expansion of functional CD4+CD25+FOXP3+ regulatory T cells of both healthy subjects and type 1 diabetic patients. J Immunol 177:8338–8347

Bluestone JA, Abbas AK (2003) Natural versus adaptive regulatory T cells. Nat Rev Immunol 3:253–257

Brandenburg S, Takahashi T, de la RM et al (2008) IL-2 induces in vivo suppression by CD4(+)CD25(+)Foxp3(+) regulatory T cells. Eur J Immunol 38:1643–1653

Chen W, Jin W, Hardegen N et al (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

Coombes JL, Siddiqui KR, rancibia-Carcamo CV et al (2007) A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. J Exp Med 204:1757–1764

Costantino CM, Baecher-Allan CM, Hafler DA (2008) Human regulatory T cells and autoimmunity. Eur J Immunol 38:921–924

Couper KN, Blount DG, de Souza JB et al (2007) Incomplete depletion and rapid regeneration of Foxp3+ regulatory T cells following anti-CD25 treatment in malaria-infected mice. J Immunol 178:4136–4146

Demirkiran A, Hendrikx TK, Baan CC et al (2008) Impact of immunosuppressive drugs on CD4+CD25+FOXP3+ regulatory T cells: does in vitro evidence translate to the clinical setting? Transplantation 85:783–789

Fantini MC, Becker C, Tubbe I et al (2006) Transforming growth factor beta induced FoxP3+ regulatory T cells suppress Th1 mediated experimental colitis. Gut 55:671–680

Fiorucci S, Antonelli E, Distrutti E et al (2002) NCX-1015, a nitric-oxide derivative of prednisolone, enhances regulatory T cells in the lamina propria and protects against 2,4,6-trinitrobenzene sulfonic acid-induced colitis in mice. Proc Natl Acad Sci USA 99:15770–15775

Fontenot JD, Rasmussen JP, Williams LM et al (2005) Regulatory T cell lineage specification by the forkhead transcription factor foxp3. Immunity 22:329–341

Gao W, Lu Y, El EB et al (2007) Contrasting effects of cyclosporine and rapamycin in de novo generation of alloantigen-specific regulatory T cells. Am J Transplant 7:1722–1732

Golshayan D, Jiang S, Tsang J et al (2007) In vitro-expanded donor alloantigen-specific CD4+CD25+ regulatory T cells promote experimental transplantation tolerance. Blood 109:827–835

Gupta S, Shang W, Sun Z (2008) Mechanisms regulating the development and function of natural regulatory T cells. Arch Immunol Ther Exp 56:85–102

Hinz S, Pagerols-Raluy L, Oberg HH et al (2007) Foxp3 expression in pancreatic carcinoma cells as a novel mechanism of immune evasion in cancer. Cancer Res 67:8344–8350

Hong J, Li N, Zhang X et al (2005) Induction of CD4+CD25+ regulatory T cells by copolymer-I through activation of transcription factor Foxp3. Proc Natl Acad Sci USA 102:6449–6454

Horwitz DA, Zheng SG, Wang J et al (2008) Critical role of IL-2 and TGF-beta in generation, function and stabilization of Foxp3+CD4+ Treg. Eur J Immunol 38:912–915

Kang HK, Liu M, Datta SK (2007) Low-dose peptide tolerance therapy of lupus generates plasmacytoid dendritic cells that cause expansion of autoantigen-specific regulatory T cells and contraction of inflammatory Th17 cells. J Immunol 178:7849–7858

Kreijveld E, Koenen HJ, Klasen IS et al (2007) Following anti--CD25 treatment, a functional CD4+CD25+ regulatory T-cell pool is present in renal transplant recipients. Am J Transplant 7:249–255

Kretschmer K, Apostolou I, Hawiger D et al (2005) Inducing and expanding regulatory T cell populations by foreign antigen. Nat Immunol 6:1219–1227

Liang S, Alard P, Zhao Y et al (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

Lio CW, Hsieh CS (2008) A two-step process for thymic regulatory T cell development. Immunity 28:100–111

Liston A, Rudensky AY (2007) Thymic development and peripheral homeostasis of regulatory T cells. Curr Opin Immunol 19:176–185

Luo X, Tarbell KV, Yang H et al (2007) Dendritic cells with TGF-beta1 differentiate naive CD4+CD25– T cells into islet-protective Foxp3+ regulatory T cells. Proc Natl Acad Sci USA 104:2821–2826

Matsumura Y, Kobayashi T, Ichiyama K et al (2007) Selective expansion of foxp3-positive regulatory T cells and immunosuppression by suppressors of cytokine signaling 3-deficient dendritic cells. J Immunol 179:2170–2179

Mucida D, Park Y, Kim G et al (2007) Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. Science 317:256–260

Pennington DJ, Silva-Santos B, Silberzahn T et al (2006) Early events in the thymus affect the balance of effector and regulatory T cells. Nature 444:1073–1077

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

Salomon B, Lenschow DJ, Rhee L et al (2000) B7/CD28 costimulation is essential for the homeostasis of the CD4+CD25+ immunoregulatory T cells that control autoimmune diabetes. Immunity, 12:431–440

Scott-Browne JP, Shafiani S, Tucker-Heard G et al (2007) Expansion and function of Foxp3-expressing T regulatory cells during tuberculosis. J Exp Med 204:2159–2169

Selvaraj RK, Geiger TL (2007) A kinetic and dynamic analysis of Foxp3 induced in T cells by TGF-beta. J Immunol 178:7667–7677

Tang Q, Henriksen KJ, Bi M et al (2004) In vitro-expanded antigen-specific regulatory T cells suppress autoimmune diabetes. J Exp Med 199:1455–1465

Tao R, de Zoeten EF, Ozkaynak E et al (2007) Deacetylase inhibition promotes the generation and function of regulatory T cells. Nat Med 13:1299–1307

Tarbell KV, Yamazaki S, Olson K et al (2004) CD25+ CD4+ T cells, expanded with dendritic cells presenting a single autoantigenic peptide, suppress autoimmune diabetes. J Exp Med 199:1467–1477

Tuovinen H, Kekalainen E, Rossi LH et al (2008) Cutting edge: human CD4–CD8– thymocytes express FOXP3 in the absence of a TCR. J Immunol 180:3651–3654

Turka LA, Walsh PT (2008) IL-2 signaling and CD4+ CD25+ Foxp3+ regulatory T cells. Front Biosci 13:1440–1446

Wan YY, Flavell RA (2007) Regulatory T cells, transforming growth factor-beta, and immune suppression. Proc Am Thorac Soc 4:271–276

Wang Z, Hong J, Sun W et al (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

Wing K, Onishi Y, Prieto-Martin P et al (2008): CTLA-4 control over Foxp3+ regulatory T cell function. Science 322:271–275

Zheng SG, Wang JH, Gray JD et al (2004) 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 172:5213–5221

Zheng SG, Wang J, Wang P et al (2007) 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 178:2018–2027