nature immunology

REVIEW

A well adapted regulatory contrivance: regulatory T cell development and the forkhead family transcription factor Foxp3

Jason D Fontenot[1] & Alexander Y Rudensky[1,2]

The random generation of antigen receptors in developing lymphocytes results in a considerable risk of autoimmunity. Regulatory T cells (T_{reg} cells) act in a dominant, trans-acting way to actively suppress immune activation and maintain immune tolerance. Here, we discuss the principal advances in our understanding of the molecular mechanisms of T_{reg} cell development and function with particular emphasis on the forkhead transcription factor Foxp3. Accumulating evidence suggests that T_{reg} cells represent a dedicated T cell lineage and that Foxp3 functions as the T_{reg} cell lineage specification factor. The aggressive early-onset lymphoproliferative syndrome resulting from Foxp3 deficiency identifies T_{reg} cells as vital mediators of immunological tolerance to self and Foxp3 as the mediator of the genetic mechanism of dominant tolerance.

The adaptive immune system of higher vertebrates provides a more efficient and specific immune defense against infectious microorganisms than that afforded by the innate immune system. The hallmark of the adaptive immune system is the random generation of antigen receptors in developing lymphocyte clones through a process of somatic cell gene rearrangement mediated by the recombination-activating gene recombinase. The essentially unlimited specificities of this anticipatory recognition system provides an efficient counterbalance to the short reproduction cycles and high mutation rates of infectious microorganisms. However, the diversity of antigen recognition afforded by the system also poses the threat of autoimmunity because of the generation of self-reactive receptors. Moreover, the emergence of major histocompatibility complex (MHC)–restricted T cell recognition exacerbates this threat, because to develop, T cells must express antigen receptors that interact with self peptide–MHC complexes.

The potential for "horror autotoxicus," or autoimmunity, was recognized by Paul Ehrlich more than 100 years ago when he noted that a "well adapted regulatory contrivance" must exist to counter this problem[1]. Expanded and developed in the framework of the clonal selection hypothesis, the idea of immunological 'tolerance' proposed a requirement for the elimination of autoreactive lymphocyte clones during development[2,3]. A vast body of experimentation over the past half-century has elucidated both cellular and molecular mechanisms leading to deletion or functional inactivation of autoreactive T and B cells in the primary lymphoid organs, thymus and bone marrow, respectively, or in the periphery. Collectively, these mechanisms act in a cell-intrinsic way and are therefore dubbed 'recessive tolerance', as elimination of an individual autoreactive lymphocyte clone does not affect other self-reactive clones. Over the past decade a population of so-called 'regulatory T cells' (T_{reg} cells) has been linked to the prevention of autoimmunity. T_{reg} cells act in a dominant, trans-acting way to actively suppress immune activation, thereby functioning as critical mediators of self-tolerance and immune homeostasis. Accumulating experimental evidence suggests that the immunosuppressive potential of these cells can be harnessed therapeutically to treat autoimmunity and facilitate transplantation tolerance or can be targeted to potentiate tumor immunotherapy. In this review we discuss the principal advances in our understanding of the molecular mechanisms of T_{reg} cell development and function. We focus on the identification of the forkhead transcription factor Foxp3 as the unique molecular marker of T_{reg} cells and its function as the T_{reg} cell lineage specification factor.

Dominant tolerance and T_{reg} cells

The idea of a dominant form of immunological tolerance involving a specialized population of 'suppressor' T cells acting both to terminate conventional immune responses and to prevent autoimmune pathology was proposed over 30 years ago[4]. Early studies envisaged suppressor cell cascades involving multiple suppressor factors, anti-idiotypic T cell networks and 'suppressor-inducer' and 'contra-suppressor' cells[5]. However, the mechanisms responsible for these suppressive phenomena were never characterized at the molecular or biochemical level. Moreover, key findings of those studies were found to be demonstrably inaccurate and the field of suppressor T cell biology was largely discredited[6]. The present renaissance of the study of dominant tolerance can be traced mainly to the observation that mice thymectomized early in life develop organ-specific autoimmunity and that cells derived from adult spleen can prevent this disease[7]. Using this model or models of lymphopenia-induced autoimmunity, several groups made critical

[1]Department of Immunology, and [2]Howard Hughes Medical Institute, University of Washington School of Medicine, Seattle, Washington 98195, USA. Correspondence should be addressed to J.D.F. (fontenot@u.washington.edu) or A.Y.R. (aruden@u.washington.edu).

Published online 22 March 2005; doi:10.1038/ni1179

observations demonstrating that specific $CD4^+$ T cell subsets, fractionated based on expression of various cell surface markers, including CD5 and CD45RB, are capable of preventing autoimmune disease[8,9]. Efforts to better define the subset of cells mediating suppression of autoimmunity culminated in the identification of $CD4^+$ T cells constitutively expressing the interleukin 2 receptor-α (IL-2Rα) chain (CD25) as being highly 'enriched' in suppressor activity[10]. These 'naturally arising' $CD4^+CD25^+$ T_{reg} cells became the best candidates for the T cell population mediating dominant tolerance to self. To emphasize their origin and importance, T_{reg} cell production has been called the third function of the thymus[11].

An overview of functional properties of T_{reg} cells

The identification of a cell surface marker (CD25) allowing the enrichment of T_{reg} cells has greatly facilitated the more rigorous study of T cell–mediated dominant tolerance. Another important milestone in the field was the development of an *in vitro* T cell suppression assay[12–14]. After T cell receptor (TCR) crosslinking *in vitro*, T_{reg} cells are unable to proliferate or produce IL-2 but are able to inhibit proliferative responses and cytokine production by effector T cells. However, this *in vitro* anergy belies a more complex activity *in vivo*. The aforementioned adoptive transfer experiments suggest that T_{reg} cells are capable of self-renewal, as transfer of relatively small numbers of T_{reg} cells afforded a long-lasting protection against autoimmunity. In addition, T_{reg} cells are capable of robust MHC class II–dependent proliferation in lymphopenic conditions, after specific TCR stimulation or after transfer into mice genetically deficient in T_{reg} cells[15–21]. Additionally, an *in vitro* protocol has been developed for the expansion of T_{reg} cell populations after TCR and CD28 engagement in the presence of very high concentrations of IL-2, which could allow for isolation and cloning of antigen-specific T_{reg} cells[22]. Thus, despite their apparent *in vitro* anergy, T_{reg} cell populations are capable of robust expansion *in vivo* and their early description as anergic cells is misleading.

The important issue of the mechanism(s) of suppressive action by T_{reg} cells remains unresolved (reviewed by von Boehmer[23] in this issue). Although *in vitro* suppression is contact dependent and is insensitive to transforming growth factor-β (TGF-β) or IL-10 blockade, both IL-10 and TGF-β have been linked to suppression mediated by T_{reg} cells in several *in vivo* experimental models[24–26]. Reverse signaling through crosslinking of B7 (CD80 and CD86) on the cell surface of antigen-presenting cells or activated T cells, mediated by cytotoxic T lymphocyte–associated antigen 4 (CTLA-4) expressed by T_{reg} cells, has been proposed as yet another effector mechanism of suppression[27]. However, like IL-10 and TGF-β, CTLA-4 does not seem to be a nonredundant mechanism of suppression, as T_{reg} cells isolated from mice with the targeted deletions of genes encoding each of these molecules are suppressive *in vitro*[28,29]. The interplay of these mechanisms in T_{reg} cell function *in vivo* remains to be further defined. In addition, after activation, human T_{reg} cells may directly kill activated CD4 and CD8 T cells in a perforin- or granzyme-dependent way[30]. Finally, it has been proposed that T_{reg} cells may suppress immune activation by 'soaking up' T cell growth factors such as IL-2 (refs. 17,31). It is likely the predominant effector mechanism of T_{reg} cell–mediated suppression may vary depending on the specific tissue and inflammation type being studied. T_{reg} cell–specific gene targeting may help to elucidate the suppressive mechanisms operating *in vivo*.

Other important, unresolved issues are whether T_{reg} cells show constitutive suppressive activity *in vivo* and whether this activity requires maintenance by 'tonic' T_{reg} cell TCR and cytokine signals or whether the induction of suppressive mechanisms requires stronger TCR and/or cytokine receptor stimuli. Evidence supporting the idea of involvement of TCR signals in inducing the suppressive function of T_{reg} cells *in vivo* has begun to emerge from the analysis of T_{reg} cells derived from TCR-transgenic mice in the presence or absence of cognate ligand. Indirect evidence for the involvement of specific TCR stimulation required for T_{reg} cell–mediated suppression has come from the finding of much more efficient protection against organ-specific autoimmunity mediated by T_{reg} cells found in the organ-draining lymph nodes than by T_{reg} cells found in nondraining lymph nodes or spleen[32].

As for the involvement of cytokines in affecting suppressive properties of T_{reg} cells, early reports indicated that T_{reg} cell–mediated *in vitro* suppression could be overridden by provision of large amounts of IL-2 (ref. 24). However, more recent studies have suggested that T_{reg} cells are capable of suppressing IL-2 mRNA induction in responder cells even in the presence of large amounts of IL-2 (ref. 33). Moreover, two groups have reported that T_{reg} cell suppressive activity *in vitro* is dependent on IL-2, as it is abrogated in the presence of IL-2-neutralizing antibodies[33,34]. Those data and results suggesting that the maintenance of CD25 expression on T_{reg} cells depends on IL-2 (ref. 35) suggest the possibility of a relatively simple regulatory network whereby the maintenance and suppressive activity of T_{reg} cells is conditional on IL-2 production by nonregulatory T cells. Furthermore, increased amounts of IL-2 resulting from immune activation may fuel the expansion of the T_{reg} cell population (discussed below).

Thymic development of T_{reg} cells

Initial observations of autoimmunity that is preventable after transfer of peripheral $CD4^+$ T cells in neonatally thymectomized mice provided evidence for the thymic origin of T_{reg} cells[9]. Indeed, subsequent studies have demonstrated the presence of a subset of $CD25^+$ cells in the CD4 single-positive thymocyte compartment in mice and humans[36–38]. These $CD25^+$ CD4 single-positive thymocytes are capable of suppression in adoptive transfer models and in *in vitro* suppression assays[37]. In addition to its suppressive capacity and CD25 expression, this thymocyte subset displays markers characteristic of peripheral T_{reg} cells, including increased expression of CTLA-4, glucocorticoid-inducible tumor necrosis factor receptor (GITR) and OX40 and resistance to deletion[38,39].

The fact that thymectomy in mice on day 3 after birth, but not adult thymectomy, results in autoimmunity suggests that T_{reg} cells are produced relatively late during neonatal development. Early reports indicated that there are very few $CD25^+$ T cells in the periphery before day 3 of life[40]. However, subsequent examination of mice thymectomized at day 3 has shown reduced but considerable numbers of $CD25^+CD4^+$ thymocytes and peripheral T cells[41]. These cells seem to be fully functional, as they mediate protection against autoimmunity when transferred together with $CD45RB^{hi}CD4^+$ T cells into lymphopenic hosts[41]. Thus, the autoimmunity developing in neonatally thymectomized mice may be due to a quantitative imbalance between regulatory and nonregulatory T cells. However, it is possible that the T_{reg} cells in neonatal mice are qualitatively different. This last possibility is suggested by the observation that almost no neonatal $CD25^+CD4^+$ T cells express the adhesion molecule CD103 (α_E integrin), whereas in adult mice, 8–15% of the $CD25^+CD4^+$ T_{reg} population is $CD103^+$ (ref. 41). That study also noted reduced Foxp3 mRNA in neonatal versus adult thymocytes. One interpretation of those results is that the neonatal thymus is less efficient than the adult thymus in supporting T_{reg} cell development (discussed below).

T_{reg} cell development: the function of the TCR

TCR signaling is important in T cell lineage commitment during thymocyte development. This is best demonstrated by the differentiation of thymocytes bearing MHC class I–restricted TCRs into $CD8^+$ T cells

and the differentiation of thymocytes bearing MHC class II–restricted TCRs into $CD4^+$ T cells[42]. An essential function for TCR signals in the development of T_{reg} cells was suggested by the finding that TCR-transgenic mice on a recombination-activating gene–deficient background (which lack endogenous TCR rearrangements) do not develop T_{reg} cells, whereas most TCR-transgenic mice expressing functional recombination-activating genes contain varying numbers of T_{reg} cells[43,44]. A vivid demonstration of how essential TCR specificity is in T_{reg} cell biology was provided by the development of spontaneous autoimmune experimental allergic encephalomyelitis in mice transgenic for myelin basic protein–specific TCRs and lacking recombination-activating gene expression. While in the presence of functional recombination-activating genes, these TCR-transgenic mice are protected from experimental allergic encephalomyelitis[45,46]. This particular TCR is not subject to deletion by its cognate ligand in the thymus. However, the experiments suggest that small numbers of T cells expressing endogenously rearranged TCRα or TCRβ chains are capable of preventing autoimmunity in this TCR-transgenic mouse model. The idea of a specific function for TCR-ligand interactions in the development of T_{reg} cells is supported by the observed increase in the proportion of T_{reg} cells that develop in TCR-transgenic mice when the mice are bred onto a strain expressing a transgene encoding the cognate ligand for the particular TCR[18,47–49]. Increased expression of many attenuators of TCR signaling and cell survival factors, including CD5, PD-1, CTLA-4, OX40 and GITR, at both the mRNA and protein level in T_{reg} cells is in agreement with the suggested increased affinity of T_{reg} cell TCRs for self peptide–MHC complexes[16,39,50]. Consistent with involvement of TCR signaling in T_{reg} cell development, genetic deficiencies in components of the transcription factor NF-κB activation pathway 'downstream' of TCR signaling, including IκB kinase-2, Bcl-10 and PKC-θ, result in reduced relative numbers of T_{reg} cells[51,52]. An investigation of T_{reg} cell TCR specificities using retrovirus-mediated transfer of T_{reg} cell TCRs into nonregulatory monoclonal $CD25^-CD4^+$ T cells showed an increased avidity of T_{reg} cell TCRs for self peptide–MHC class II complexes reflected in rapid peptide–MHC class II–dependent population expansion of the transduced cells in lymphopenic hosts[53]. In aggregate, these results suggest an essential function for TCR-ligand interactions in the development of T_{reg} cells and indicate that naturally arising T_{reg} cell lineage commitment is induced in response to self-reactive TCRs with an avidity range for self peptide–MHC somewhere between that required for positive and negative selection. This model of T_{reg} cell development may also explain the preponderance of T_{reg} cells in the $CD4^+$ T cell lineage, as $CD4^+$ T cell lineage commitment is thought to be favored by TCR signals of increased strength or duration[54,55].

An alternative model of T_{reg} cell lineage commitment suggests that T_{reg} cell fate is not determined by the strength of TCR-ligand interactions but may result from an unknown invariant signal (perhaps Notch-Notch ligand interactions) or selection on an unconventional TCR ligand, different from that required for nonregulatory T cell selection[56]. This model is based on the observation that although the proportion of T_{reg} cells in the thymus of mice expressing a particular TCR transgene increases after encounter with the cognate ligand, the absolute number of T_{reg} cells does not increase or increases only modestly. Consistent with published observations[38], it was noted that T_{reg} cells are more resistant to TCR-induced apoptosis[56]. Thus, the enrichment in T_{reg} cells observed in TCR–cognate ligand transgenic systems is hypothesized to result from the preferential elimination of nonregulatory $CD4^+$ T cells rather than from increased production of T_{reg} cells. To accommodate the finding of self-reactive TCRs displayed by T_{reg} cells, this model argues that TCR-ligand interactions serve as survival or expansion signals after T_{reg} cell lineage commitment, allowing developing T_{reg} cells to compete for 'space' with the bulk of 'nonregulatory' thymocytes. In contrast to an induced mechanism of dominant tolerance envisioned by TCR affinity models of T_{reg} cell lineage commitment, this model suggests that the mechanism of dominant tolerance is 'hard-wired'.

The best explanation for the available data is probably a combination of the two models. Specifically, we propose that a TCR signal within a certain avidity range is required but not sufficient for T_{reg} cell lineage commitment, and that one (or more) additional unidentified and likely limiting signal(s) is (are) needed (discussed below; **Fig. 1**). Not all anergic T cells that escape negative selection have regulatory properties[57]. Furthermore, the developmental delay in the appearance of a fully competent T_{reg} cells in neonatal mice may be due to diminished availability of such a factor. It is possible that thymic epithelial cells may be particularly suited to support T_{reg} cell lineage commitment.

The idea of a specific function for thymic epithelial cells in T_{reg} development was suggested by allogeneic thymic graft experiments[58,59]. Subsequently, a T_{reg} cell compartment of a size comparable to that in wild-type mice was found in mice expressing MHC class

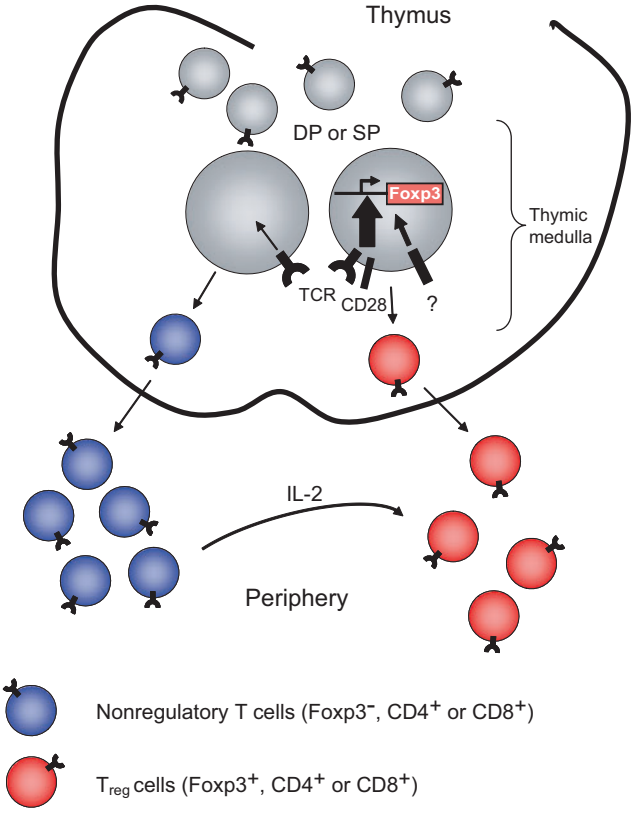

Figure 1 T_{reg} cell lineage specification by Foxp3. In this inductive model of T_{reg} cell lineage commitment, high-avidity TCR–self peptide–MHC interactions augmented by CD28 signaling and an additional, unidentified, perhaps limiting, signal converge to induce Foxp3 expression and thus T_{reg} cell lineage commitment. The developmental delay in the appearance of a fully competent T_{reg} cells in neonatal mice may be due to diminished availability of this unidentified signal. In this model, Foxp3 expression defines the T_{reg} cell lineage, irrespective of CD25 expression or MHC restriction. Because TCR signals of an increased strength or duration favor the development of MHC class II–restricted $CD4^+$ T cells, Foxp3-expressing T_{reg} cells are highly enriched in but are not restricted to this population. Production of IL-2 by peripheral T cells expands the T_{reg} cell population but is not required for T_{reg} cell development. DP, double-positive; SP, single-positive; ±, with or without.

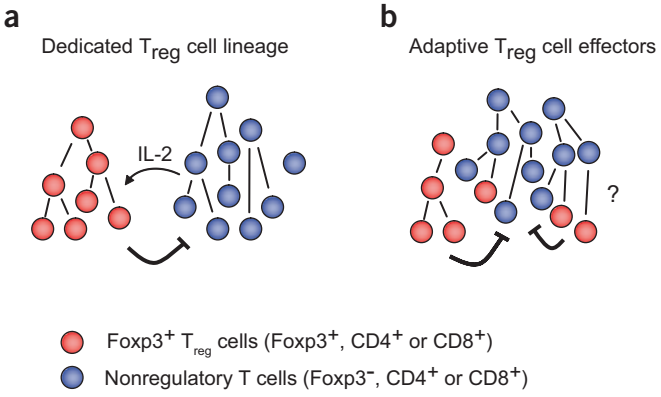

Figure 2 Models of dominant tolerance. (**a**) Dedicated T_{reg} cell lineage. In this model, T_{reg} cell lineage commitment through induction of Foxp3 expression occurs preferentially in thymocytes and recent thymic emigrants, whereas induction of Foxp3-expression in mature peripheral nonregulatory T cells in both humans and mice in physiological conditions is a rare and relatively inefficient process. Activation of effector T cells results in production of IL-2, which expands the T_{reg} cell population during immune activation. T_{reg} cells function mainly to control T cell self-reactivity and autoimmune inflammation. As a consequence of this function, T_{reg} cells cause downmodulation of inflammation associated with pathogen-specific immune responses while not specifically developing to limit these responses. (**b**) Adaptive T_{reg} cell effectors. In this model, T_{reg} cells are generated both in the thymus and *de novo* from effector nonregulatory T cells as a consequence of immune activation. The signals inducing Foxp3 expression in peripheral T cells *in vivo* have yet to be clearly defined. Here, Foxp3 induction in effector nonregulatory cells acts as a form of negative feedback to directly regulate conventional antigen-specific immune responses. One prediction of this model is the generation of pathogen-specific Foxp3-expressing T_{reg} cells during the course of an ongoing infection. In this model, Foxp3 functions not as a T_{reg} cell lineage specification factor but as a transcriptional regulator of an immunosuppressive effector program.

II molecules only on thymic cortical epithelial cells[60]. Furthermore, expression of a cognate TCR ligand on thymic stromal cells favored the development of CD25$^+$ T_{reg} cells expressing a corresponding TCR transgene, whereas ligand expression on bone marrow–derived cells resulted in the development of anergic CD25$^-$CD4$^+$ T cells with regulatory properties[48,49].

What peptide ligands are recognized by TCRs displayed by developing T_{reg} cell precursors in the thymus? The very high diversity of TCRs of T_{reg} cells in the periphery and in the thymus suggests that these cells are capable of interacting with any peptide–MHC class II ligand in a certain avidity range. Some T_{reg} cell TCRs seem to recognize ubiquitously expressed MHC class II–bound peptides present in high copy numbers[53] (C.S. Hsieh and A.Y.R., unpublished data). The presence of T_{reg} cells in mice with MHC class II–bound peptide repertoires mostly limited to a single predominant peptide supports this idea[60,61]. It is also likely that a subset of T_{reg} cells can preferentially react with tissue-specific peptides present in low copy numbers in the thymus. Based on the avidity model of T_{reg} cell lineage commitment, we would predict a higher affinity of T_{reg} cells for these low-abundance tissue-specific peptides. It is reasonable to further suggest that tissue-specific peptides are displayed for T_{reg} cell recognition by medullary epithelial cells in an autoimmune regulator protein–dependent way[62]. However, the possibility of potential 'holes' in the T_{reg} cell TCR repertoire contributing to autoimmunity found in autoimmune regulator protein–deficient mice remains purely speculative at present.

T_{reg} cell development: costimulation and cytokines

Considering the essential involvement of TCR signals in T_{reg} cell lineage development, it is not surprising that costimulatory signals mediated by CD28 engagement of CD80 or CD86 molecules are important in shaping the repertoire and size of the T_{reg} cell compartment. T_{reg} cell numbers are very much diminished in mice lacking CD28 or CD80 and CD86 (ref. 63). It seems that this T_{reg} cell deficiency is due to a combination of a direct effect on T_{reg} cell thymocyte development and diminished peripheral IL-2 (discussed below) produced by nonregulatory T cells in the absence of CD28-CD80 or CD28-CD86 interactions[64]. Nevertheless, T_{reg} cells generated in the absence of CD28 are functional[63]. This indicates that T_{reg} cell lineage commitment can occur in the absence of CD28. Although CTLA-4 can influence thymocyte development and has been suggested as a potential effector molecule of T_{reg} cells, functional T_{reg} cells have been reported in CTLA-4–deficient mice[29]. Thus, CTLA-4 is also not required for T_{reg} cell lineage development.

It is possible that T_{reg} cell lineage commitment in the thymus can be facilitated by cytokine signals. The presence of high concentrations of TGF-β induces regulatory function in TCR-stimulated CD25$^-$CD4$^+$ T cells *in vitro*[65–68]. However, *in vivo*, TGF-β seems to be dispensable for thymic T_{reg} cell development but involved in the maintenance of peripheral T_{reg} cell numbers and functionality[69,70] (Marie and A.Y.R., unpublished data).

Signals mediated by IL-2–IL-2R have been linked to the development, maintenance, survival and expansion of T_{reg} cell populations[71]. Consistent with critical involvement of IL-2 signals in T_{reg} cell biology, IL-2- and IL-2R-deficient mice suffer from lymphoproliferative autoimmune disorders. However, the protection afforded by adoptive transfer of peripheral CD4$^+$ T cells isolated from IL-2-deficient mice but not from CD25-deficient mice in a TCR-transgenic model of experimental allergic encephalomyelitis suggests that T_{reg} cells are present even in the absence of IL-2 (ref. 72). A subsequent report[35] by that same group supports this conclusion. Thus, it seems likely IL-2 signal is also dispensable for T_{reg} cell lineage commitment in the thymus. However, definitive proof of this possibility has not yet been attained. Thus, although CD28 and IL-2 have a considerable effect on the size of the developing T_{reg} cell subset, the nature of the putative nonredundant signal involved in T_{reg} cell lineage commitment in the thymus together with the TCR remains to be identified.

T_{reg} cells and Foxp3

Although CD25 expression has been useful in defining the T_{reg} cell population in nonimmune mice and humans, accurate discrimination between T_{reg} cells and recently activated nonregulatory T cells, which upregulate CD25, during immune activation associated with autoimmune pathology or infection is almost impossible. Increased expression of CD25, as well as GITR, CTLA-4 and lymphocyte activation gene 3, on activated nonregulatory T cells suggests that expression of these molecules does not functionally define the T_{reg} cell population and raises the possibility that not all T_{reg} cells express these molecules. Therefore, a principal challenge is to identify a unique functional molecular marker of T_{reg} cells. The identification of such a molecule should help to resolve a fundamental issue regarding the nature of dominant tolerance. Two general models for T cell–mediated immunosuppression have been considered: that T_{reg} cells represent a dedicated functional lineage (**Fig. 2a**), or that T_{reg} cells represent a 'plastic' phenotype(**Fig. 2b**). The first model suggests the existence of a factor responsible for specifying a T_{reg} cell lineage, which therefore serves as the mediator of the genetic mechanism of dominant tolerance. The second argues that T cell–mediated immunosuppression is not the

purview of a dedicated T_{reg} cell lineage but is the consequence of a dynamic balance between cells expressing different amounts of cytokine receptors and different cytokine production profiles[73].

The identification of mutations in the gene encoding Foxp3 as the cause of the fatal human autoimmune disorder 'immune dysregulation, polyendocrinopathy, enteropathy, X-linked' (IPEX) and the analogous disease in a spontaneous mutant mouse, scurfy, was a breakthrough in the field and led to subsequent studies that argue for the idea of T_{reg} cells as a dedicated functional lineage[74–77] (reviewed by Sakaguchi[78] in this issue). At a very young age, human patients with this autoimmune syndrome present with massive lymphoproliferation, early-onset insulin dependent diabetes mellitus, thyroiditis, eczema, severe enteropathy and food allergies preventing normal food intake, and additional autoimmune pathologies such as autoimmune hemolytic anemia and thrombocytopenia, as well as severe infections[79]. Similar sequelae are found in scurfy mutant and Foxp3-deficient mice, including severe dermatitis, aggressive lymphoproliferation resulting in gross enlargement of secondary lymphoid organs, lymphocytic infiltration of multiple organs, hypergammaglobulinemia and autoimmune hemolytic anemia[21,80]. Affected males succumb to the IPEX syndrome between 3 and 4 weeks of age. Analysis of the scurfy mutant before the identification of the causative mutation demonstrated that the disease is mediated by T cells, with $CD4^+$ T cells being the primary effectors of the disease[81–83]. In fact, polyclonal activation of $CD4^+$ T cells and, to a lesser extent, $CD8^+$ T cells is found in Foxp3-deficient mice as early as 7 days of age (ref. 21 and J.D.F. and A.Y.R., unpublished observations). In addition to showing substantially increased production of a broad spectrum of proinflammatory cytokines, the *in vitro* responses of T cells isolated from scurfy mice show a decreased activation threshold and lesser dependence on costimulation[84,85]. Furthermore, transgenic overexpression of Foxp3 results in reduced numbers of peripheral T cells, and the remaining T cells show impaired responses to TCR ligation[86]. Those studies along with phenotypic similarities to TGF-β– and CTLA4-deficient mice led to the proposal that Foxp3 may mediate a general mechanism of negative regulation of T cell activation.

Foxp3 belongs to a large family of functionally diverse transcription factors based on its winged helix–forkhead DNA-binding domain (forkhead box (Fox)). These proteins have been classified into subfamilies (indicated by the letter after 'Fox') based on phylogenetic analysis of homology in the forkhead domain only, and each has been assigned a unique number (at the end of the name)[87]. In addition to the C-terminal forkhead domain, Foxp3 also contains a Cys_2His_2 zinc finger domain and a coiled-coil–leucine zipper motif. Homology among full-length human, mouse and rat Foxp3 is very high, suggesting a highly conserved function. At present there is very little understanding of the function of Foxp3 at the molecular level. Foxp3 binds DNA, localizes to the nucleus and can act as a transcriptional repressor[88]. Identification of consensus forkhead binding domains adjacent to NFAT transcription factor binding sites in the promoters of several cytokine genes, including those encoding IL-2, IL-4 and tumor necrosis factor, led to the proposal of a model of Foxp3-mediated transcriptional inhibition or repression in which Foxp3 antagonizes NFAT function by competition for DNA binding sites[88]. Based on those and other studies, it has also been proposed that Foxp3 is induced in a variety of cell types as a general mechanism of negative immune regulation by repressing production of inflammatory cytokines. However, so far there has been no characterization of Foxp3 target genes or the transcriptional program specified by Foxp3.

The devastating lymphoproliferative autoimmune disease resulting from Foxp3 deficiency affects mutant males but not heterozygous female carriers for both humans and mice. Those observations and the fact that random X-chromosome inactivation is maintained in T cells from heterozygous female carriers suggest that control of the pathology in heterozygous females is mediated by a cell-extrinsic mechanism consistent with the idea of dominant tolerance. That consideration and some resemblance between the range of target organs affected by the disease in Foxp3 mutant mice and that in mice depleted of $CD25^+CD4^+$ T cells prompted examination of the function of Foxp3 in T_{reg} cell biology. Analysis of Foxp3 expression in T cells at both the mRNA and protein level has shown high expression in $CD25^+CD4^+$ T_{reg} cells and low expression of Foxp3 in naive and, notably, recently activated $CD25^-CD4^+$ T cells[21,89,90]. Those results suggest that Foxp3 is a specific molecular marker of T_{reg} cells and, unlike CTLA-4, GITR and lymphocyte activation gene 3, allows for discrimination between T_{reg} cells and activated nonregulatory T cells. Analysis of the origin of $CD4^+CD25^+$ T_{reg} cells in chimeric mice containing a 1:1 mixture of allelically marked bone marrow stem cells derived from Foxp3-deficient and wild-type mice showed that Foxp3-deficient bone marrow cannot give rise to $CD4^+CD25^+$ T_{reg} cells, thus demonstrating that $CD4^+CD25^+$ T_{reg} cell development is critically dependent on Foxp3 expression[21]. In agreement with that finding, transgene-driven Foxp3 overexpression in mice results in an increase in the $CD4^+CD25^+$ T_{reg} cell subset and acquisition of suppressive properties by $CD4^+CD25^-$ and $CD8^+$ T cells, although these cells are not as efficient at inhibiting $CD25^-CD4^+$ T cell *in vitro* responses as are $CD4^+CD25^+$ T_{reg} cells[89]. The essential involvement of Foxp3 in programming T_{reg} cell function was further demonstrated by the acquisition of regulatory properties by $CD4^+CD25^-$ T cells after retroviral transduction with Foxp3 (refs. 21,90). However, in those experiments, only some of the Foxp3-expressing cells acquired the characteristics of $CD4^+CD25^+$ T_{reg} cells, including high expression of CD25 and suppressor activity. One possible interpretation of those results is that the acquisition of regulatory properties after expression of Foxp3 in peripheral nonregulatory T cells is conditional on TCR specificity or the expression of additional cofactors either pre-existing or acquired in the process of activation and differentiation.

Foxp3 as the T_{reg} cell lineage specification factor

Viewed as a whole, the available data demonstrate essential involvement of Foxp3 in the development and function of $CD4^+CD25^+$ T_{reg} cells. However there are several issues that remain unresolved. The results from transgenic and retroviral overexpression studies raise the issue of the relationship among Foxp3, CD25 and the T_{reg} cell population. Although $CD4^+CD25^+$ T_{reg} cells have high expression of Foxp3, low expression of Foxp3 has been detected in $CD4^+CD25^-$ and $CD8^+$ T cells[21,72,87]. Do all T cells have low expression of Foxp3, or do subsets of cells with high expression of Foxp3 exists in these populations? Along the same lines, the issue of a cell-intrinsic role for Foxp3 in nonregulatory T cell function has not been conclusively resolved. Does low Foxp3 expression modulate T cell activation in nonregulatory T cells? Analysis of mice expressing a 'knock-in' allele encoding a green fluorescent protein–Foxp3 fusion protein suggests that Foxp3 expression is highly restricted to T_{reg} cells and that the low expression of Foxp3 detected in $CD4^+CD25^-$ and $CD8^+$ T cells is due to the presence of $Foxp3^+$ T_{reg} cells in these populations[91].

Another principal issue in the field is the frequency of the conversion of mature, nonregulatory T cells to T_{reg} cells in the periphery as well as the *in vivo* conditions necessary for such conversion. Does a subset of nonregulatory T cells upregulate Foxp3 and acquire T_{reg} cell activity during the course of conventional immune responses as part of a negative feedback loop? Do experimental manipulations that generate increased numbers of T_{reg} cells expand a preexisting T_{reg} cell population or convert them from nonregulatory T cells? If nonregulatory T cells can acquire T_{reg} cell phenotype, is this a transient phenotype

or a terminal differentiation into T_{reg} cells, and does such conversion occur during the course of a conventional immune response? Unlike mouse $CD25^-CD4^+$ T cells, human $CD25^-CD4^+$ T cells are reported to upregulate Foxp3 after *in vitro* activation[92]. However, because Foxp3 expression was analyzed in bulk T cell populations in those studies, it is unclear if the observed increase in Foxp3 expression was due to expansion of a small population of $Foxp3^+$ $CD25^-$ T_{reg} cells or conversion of nonregulatory T cells. The same caveat holds true for reports of TGF-β–mediated upregulation of Foxp3 by nonregulatory T cells[66–69]. In contrast to the aforementioned report suggesting that human $CD25^-CD4^+$ T cells upregulate Foxp3 after *in vitro* activation[92], published results analyzing mouse T cells *in vitro*[21,90], our own single-cell analysis of mouse T cells *in vitro* and *in vivo*[91] and another report analyzing activation of human $CD25^-CD4^+$ T cells[93] have shown no upregulation of Foxp3 after activation of nonregulatory T cells. Although more work is required to reconcile the data in human T cells, one possibility is that the function of Foxp3 is different in mice and humans.

From a general perspective, peripheral upregulation of Foxp3 after activation of human $CD25^-CD4^+$ T cells would support a model of dominant tolerance in which T_{reg} cells are generated *de novo* from nonregulatory T cells as a consequence of immune activation and thereby act as negative feedback regulators of conventional immune responses (**Fig. 2b**). We believe, however, that the salient conservation of Foxp3 at the protein and nucleic acid level, in both coding and noncoding sequence, and the notable similarity in autoimmune syndrome found in both mice and humans deficient in Foxp3 challenge the idea of such a scenario.

In conclusion, based on published data and analysis of green fluorescent protein–Foxp3 'knock-in' reporter mice[93], we propose a model in which Foxp3 acts as the T_{reg} cell lineage specification factor. Thus, we argue that expression of Foxp3, irrespective of CD25 expression or MHC restriction, defines the naturally occurring T_{reg} cell lineage. In this model, induction of Foxp3 expression in developing thymocytes commits these cells to the T_{reg} cell lineage (**Fig. 1**). We also suggest that induction of Foxp3 expression in peripheral nonregulatory T cells in both humans and mice in physiological conditions is a relatively rare process (**Fig. 2a**). The issue of whether Foxp3 is required only at the commitment stage of T_{reg} cell differentiation or is also needed for the maintenance of T_{reg} cell function remains to be addressed. The devastating lymphoproliferative autoimmune syndrome found in both mice and humans deficient in Foxp3, and thus T_{reg} cells, demonstrates that T_{reg} cell–mediated dominant tolerance is a vital mechanism of immune homeostasis. We argue that the early onset of this syndrome indicates that this mechanism evolved specifically to control T cell self-reactivity and autoimmune inflammation. Nevertheless, we further speculate that as a consequence of shared characteristics of immune inflammation associated with autoimmunity and microbial exposure, in particular chronic infection, T_{reg} cells cause downmodulation of pathogen-specific immune responses while not specifically developing to limit these responses. The discovery of a dedicated genetic mechanism of dominant tolerance mediated by Foxp3 is manifest to the insight put forward by Paul Ehrlich a century ago.

ACKNOWLEDGMENTS

We thank all the members of the Rudensky laboratory for discussions. Supported by the Cancer Research Institute (J.D.F.), Howard Hughes Medical Institute and National Institutes of Health.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

Published online at http://www.nature.com/natureimmunology/

1. Ehrlich, P. *Collected Studies on Immunity* (J. Wiley & Sons, New York, 1910).
2. Burnet, F.M. A modification of Jerne's theory of antibody production using the concept of clonal selection. *Australian J. Sci.* **20**, 67–69 (1957).
3. Talmage, D.W. Allergy and immunology. *Annu. Rev. Med.* **8**, 239–256 (1957).
4. Gershon, R.K. & Kondo, K. Infectious immunological tolerance. *Immunology* **21**, 903–914 (1971).
5. Dorf, M.E. & Benacerraf, B. Suppressor cells and immunoregulation. *Annu. Rev. Immunol.* **2**, 127–157 (1984).
6. Moller, G. Do suppressor T cells exist? *Scand. J. Immunol.* **27**, 247–250 (1988).
7. Nishizuka, Y. & Sakakura, T. Thymus and reproduction: sex-linked dysgenesia of the gonad after neonatal thymectomy in mice. *Science* **166**, 753–755 (1969).
8. Sakaguchi, S. Naturally arising $CD4^+$ regulatory T cells for immunologic self-tolerance and negative control of immune responses. *Annu. Rev. Immunol.* **22**, 531–562 (2004).
9. Shevach, E.M. Regulatory T cells in autoimmmunity*. *Annu. Rev. Immunol.* **18**, 423–449 (2000).
10. Sakaguchi, S., Sakaguchi, N., Asano, M., Itoh, M. & Toda, M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor α-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J. Immunol.* **155**, 1151–1164 (1995).
11. Seddon, B. & Mason, D. The third function of the thymus. *Immunol. Today* **21**, 95–99 (2000).
12. Thornton, A.M. & Shevach, E.M. $CD4^+CD25^+$ immunoregulatory T cells suppress polyclonal T cell activation *in vitro* by inhibiting interleukin 2 production. *J. Exp. Med.* **188**, 287–296 (1998).
13. Takahashi, T. *et al.* Immunologic self-tolerance maintained by $CD25^+CD4^+$ naturally anergic and suppressive T cells: induction of autoimmune disease by breaking their anergic/suppressive state. *Int. Immunol.* **10**, 1969–1980 (1998).
14. Read, S. *et al.* $CD38^+$ $CD45RB^{low}$ $CD4^+$ T cells: a population of T cells with immune regulatory activities *in vitro*. *Eur. J. Immunol.* **28**, 3435–3447 (1998).
15. Fisson, S. *et al.* Continuous activation of autoreactive $CD4^+$ $CD25^+$ regulatory T cells in the steady state. *J. Exp. Med.* **198**, 737–746 (2003).
16. Gavin, M.A., Clarke, S.R., Negrou, E., Gallegos, A. & Rudensky, A. Homeostasis and anergy of $CD4^+CD25^+$ suppressor T cells *in vivo*. *Nat. Immunol.* **3**, 33–41 (2002).
17. Klein, L., Khazaie, K. & von Boehmer, H. *In vivo* dynamics of antigen-specific regulatory T cells not predicted from behavior *in vitro*. *Proc. Natl. Acad. Sci. USA* **100**, 8886–8891 (2003).
18. Walker, L.S., Chodos, A., Eggena, M., Dooms, H. & Abbas, A.K. Antigen-dependent proliferation of $CD4^+$ $CD25^+$ regulatory T cells *in vivo*. *J. Exp. Med.* **198**, 249–258 (2003).
19. Annacker, O. *et al.* $CD25^+$ $CD4^+$ T cells regulate the expansion of peripheral CD4 T cells through the production of IL-10. *J. Immunol.* **166**, 3008–3018 (2001).
20. Malek, T.R., Yu, A., Vincek, V., Scibelli, P. & Kong, L. CD4 regulatory T cells prevent lethal autoimmunity in IL-2Rβ-deficient mice. Implications for the nonredundant function of IL-2. *Immunity* **17**, 167–178 (2002).
21. Fontenot, J.D., Gavin, M.A. & Rudensky, A.Y. Foxp3 programs the development and function of $CD4^+CD25^+$ regulatory T cells. *Nat. Immunol.* **4**, 330–336 (2003).
22. Tang, Q. *et al.* *In vitro*-expanded antigen-specific regulatory T cells suppress autoimmune diabetes. *J. Exp. Med.* **199**, 1455–1465 (2004).
23. von Boehmer, H. Mechanisms of suppression by suppressor T cells. *Nat. Immunol.* **6**, 338–344 (2005).
24. Shevach, E.M. $CD4^+$ $CD25^+$ suppressor T cells: more questions than answers. *Nat. Rev. Immunol.* **2**, 389–400 (2002).
25. Green, E.A., Gorelik, L., McGregor, C.M., Tran, E.H. & Flavell, R.A. $CD4^+CD25^+$ T regulatory cells control anti-islet $CD8^+$ T cells through TGF-β-TGF-β receptor interactions in type 1 diabetes. *Proc. Natl. Acad. Sci. USA* **100**, 10878–10883 (2003).
26. Asseman, C., Mauze, S., Leach, M.W., Coffman, R.L. & Powrie, F. An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. *J. Exp. Med.* **190**, 995–1004 (1999).
27. Paust, S., Lu, L., McCarty, N. & Cantor, H. Engagement of B7 on effector T cells by regulatory T cells prevents autoimmune disease. *Proc. Natl. Acad. Sci. USA* **101**, 10398–10403 (2004).
28. Piccirillo, C.A. *et al.* $CD4^+CD25^+$ regulatory T cells can mediate suppressor function in the absence of transforming growth factor β1 production and responsiveness. *J. Exp. Med.* **196**, 237–246 (2002).
29. Tang, Q. *et al.* Distinct roles of CTLA-4 and TGF-β in $CD4^+CD25^+$ regulatory T cell function. *Eur. J. Immunol.* **34**, 2996–3005 (2004).
30. Grossman, W.J. *et al.* Human T regulatory cells can use the perforin pathway to cause autologous target cell death. *Immunity* **21**, 589–601 (2004).
31. Barthlott, T., Kassiotis, G. & Stockinger, B. T cell regulation as a side effect of homeostasis and competition. *J. Exp. Med.* **197**, 451–460 (2003).
32. Green, E.A., Choi, Y. & Flavell, R.A. Pancreatic lymph node-derived $CD4^+CD25^+$ Treg cells: highly potent regulators of diabetes that require TRANCE-RANK signals. *Immunity* **16**, 183–191 (2002).
33. Thornton, A.M., Donovan, E.E., Piccirillo, C.A. & Shevach, E.M. Cutting edge: IL-2 is critically required for the *in vitro* activation of $CD4^+CD25^+$ T cell suppressor function. *J. Immunol.* **172**, 6519–6523 (2004).
34. de la Rosa, M., Rutz, S., Dorninger, H. & Scheffold, A. Interleukin-2 is essential for $CD4^+CD25^+$ regulatory T cell function. *Eur. J. Immunol.* **34**, 2480–2488 (2004).
35. Curotto de Lafaille, M.A., Lino, A.C., Kutchukhidze, N. & Lafaille, J.J. $CD25^-$ T cells generate $CD25^+Foxp3^+$ regulatory T cells by peripheral expansion. *J. Immunol.* **173**, 7259–7268 (2004).
36. Stephens, L.A. & Mason, D. CD25 is a marker for $CD4^+$ thymocytes that prevent autoimmune diabetes in rats, but peripheral T cells with this function are found in both $CD25^+$ and CD25- subpopulations. *J. Immunol.* **165**, 3105–3110 (2000).

37. Itoh, M. *et al.* Thymus and autoimmunity: production of CD25+CD4+ naturally anergic and suppressive T cells as a key function of the thymus in maintaining immunologic self-tolerance. *J. Immunol.* **162**, 5317–5326 (1999).
38. Papiernik, M., de Moraes, M.L., Pontoux, C., Vasseur, F. & Penit, C. Regulatory CD4 T cells: expression of IL-2Rα chain, resistance to clonal deletion and IL-2 dependency. *Int. Immunol.* **10**, 371–378 (1998).
39. Shimizu, J., Yamazaki, S., Takahashi, T., Ishida, Y. & Sakaguchi, S. Stimulation of CD25+CD4+ regulatory T cells through GITR breaks immunological self-tolerance. *Nat. Immunol.* **3**, 135–142 (2002).
40. Asano, M., Toda, M., Sakaguchi, N. & Sakaguchi, S. Autoimmune disease as a consequence of developmental abnormality of a T cell subpopulation. *J. Exp. Med.* **184**, 387–396 (1996).
41. Dujardin, H.C. *et al.* Regulatory potential and control of Foxp3 expression in newborn CD4+ T cells. *Proc. Natl. Acad. Sci. USA* **101**, 14473–14478 (2004).
42. Goldrath, A.W. & Bevan, M.J. Selecting and maintaining a diverse T-cell repertoire. *Nature* **402**, 255–262 (1999).
43. Fehervari, Z. & Sakaguchi, S. Development and function of CD25+CD4+ regulatory T cells. *Curr. Opin. Immunol.* **16**, 203–208 (2004).
44. Hsieh, C.S. & Rudensky, A.Y. The role of TCR specificity in naturally arising CD25+CD4+ regulatory T cell biology. *Curr. Top. Microbiol. Immunol.* (in the press).
45. Lafaille, J.J., Nagashima, K., Katsuki, M. & Tonegawa, S. High incidence of spontaneous autoimmune encephalomyelitis in immunodeficient anti-myelin basic protein T cell receptor transgenic mice. *Cell* **78**, 399–408 (1994).
46. Olivares-Villagomez, D., Wang, Y. & Lafaille, J.J. Regulatory CD4+ T cells expressing endogenous T cell receptor chains protect myelin basic protein-specific transgenic mice from spontaneous autoimmune encephalomyelitis. *J. Exp. Med.* **188**, 1883–1894 (1998).
47. Kawahata, K. *et al.* Generation of CD4+CD25+ regulatory T cells from autoreactive T cells simultaneously with their negative selection in the thymus and from nonautoreactive T cells by endogenous TCR expression. *J. Immunol.* **168**, 4399–4405 (2002).
48. Jordan, M.S. *et al.* Thymic selection of CD4+CD25+ regulatory T cells induced by an agonist self-peptide. *Nat. Immunol.* **2**, 301–306 (2001).
49. Apostolou, I., Sarukhan, A., Klein, L. & von Boehmer, H. Origin of regulatory T cells with known specificity for antigen. *Nat. Immunol.* **3**, 756–763 (2002).
50. McHugh, R.S. *et al.* CD4+CD25+ immunoregulatory T cells: gene expression analysis reveals a functional role for the glucocorticoid-induced TNF receptor. *Immunity* **16**, 311–323 (2002).
51. Schmidt-Supprian, M. *et al.* Differential dependence of CD4+CD25+ regulatory and natural killer-like T cells on signals leading to NF-κB activation. *Proc. Natl. Acad. Sci. USA* **101**, 4566–4571 (2004).
52. Schmidt-Supprian, M. *et al.* Mature T cells depend on signaling through the IKK complex. *Immunity* **19**, 377–389 (2003).
53. Hsieh, C.S. *et al.* Recognition of the peripheral self by naturally arising CD25+ CD4+ T cell receptors. *Immunity* **21**, 267–277 (2004).
54. Hernandez-Hoyos, G., Sohn, S.J., Rothenberg, E.V. & Alberola-Ila, J. Lck activity controls CD4/CD8 T cell lineage commitment. *Immunity* **12**, 313–322 (2000).
55. Brugnera, E. *et al.* Coreceptor reversal in the thymus: signaled CD4+8+ thymocytes initially terminate CD8 transcription even when differentiating into CD8+ T cells. *Immunity* **13**, 59–71 (2000).
56. van Santen, H.M., Benoist, C. & Mathis, D. Number of T reg cells that differentiate does not increase upon encounter of agonist ligand on thymic epithelial cells. *J. Exp. Med.* **200**, 1221–1230 (2004).
57. Viret, C. & Janeway, C.A., Jr. Self-specific MHC class II-restricted CD4–CD8- T cells that escape deletion and lack regulatory activity. *J. Immunol.* **170**, 201–209 (2003).
58. Modigliani, Y. *et al.* Lymphocytes selected in allogeneic thymic epithelium mediate dominant tolerance toward tissue grafts of the thymic epithelium haplotype. *Proc. Natl. Acad. Sci. USA* **92**, 7555–7559 (1995).
59. Modigliani, Y. *et al.* Establishment of tissue-specific tolerance is driven by regulatory T cells selected by thymic epithelium. *Eur. J. Immunol.* **26**, 1807–1815 (1996).
60. Bensinger, S.J., Bandeira, A., Jordan, M.S., Caton, A.J. & Laufer, T.M. Major histocompatibility complex class II-positive cortical epithelium mediates the selection of CD4+25+ immunoregulatory T cells. *J. Exp. Med.* **194**, 427–438 (2001).
61. Pacholczyk, R., Kraj, P. & Ignatowicz, L. Peptide specificity of thymic selection of CD4+CD25+ T cells. *J. Immunol.* **168**, 613–620 (2002).
62. Kyewski, B. & Derbinski, J. Self-representation in the thymus: an extended view. *Nat. Rev. Immunol.* **4**, 688–698 (2004).
63. Salomon, B. *et al.* B7/CD28 costimulation is essential for the homeostasis of the CD4+CD25+ immunoregulatory T cells that control autoimmune diabetes. *Immunity* **12**, 431–440 (2000).
64. Tai, X., Cowan, M., Feigenbaum, L. & Singer, A. CD28 costimulation of developing thymocytes induces Foxp3 expression and regulatory T cell differentiation independently of interleukin 2. *Nat. Immunol.* **6**, 152–162 (2005).
65. Fantini, M.C. *et al.* 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 (2004).
66. Zheng, S.G., Wang, J.H., Gray, J.D., Soucier, H. & Horwitz, D.A. Natural and induced CD4+CD25+ cells educate CD4+CD25- cells to develop suppressive activity: the role of IL-2, TGF-β, and IL-10. *J. Immunol.* **172**, 5213–5221 (2004).
67. Chen, W. *et al.* Conversion of peripheral CD4+CD25− naive T cells to CD4+CD25+ regulatory T cells by TGF-β induction of transcription factor Foxp3. *J. Exp. Med.* **198**, 1875–1886 (2003).
68. Park, H.B., Paik, D.J., Jang, E., Hong, S. & Youn, J. Acquisition of anergic and suppressive activities in transforming growth factor-β-costimulated CD4+CD25− T cells. *Int. Immunol.* **16**, 1203–1213 (2004).
69. Schramm, C. *et al.* TGFβ regulates the CD4+CD25+ T-cell pool and the expression of Foxp3 *in vivo*. *Int. Immunol.* **16**, 1241–1249 (2004).
70. Huber, S. *et al.* Cutting edge: TGF-beta signaling is required for the *in vivo* expansion and immunosuppressive capacity of regulatory CD4+CD25+ T cells. *J. Immunol.* **173**, 6526–6531 (2004).
71. Malek, T.R. & Bayer, A.L. Tolerance, not immunity, crucially depends on IL-2. *Nat. Rev. Immunol.* **4**, 665–674 (2004).
72. Furtado, G.C., Curotto de Lafaille, M.A., Kutchukhidze, N. & Lafaille, J.J. Interleukin 2 signaling is required for CD4+ regulatory T cell function. *J. Exp. Med.* **196**, 851–857 (2002).
73. Stockinger, B., Barthlott, T. & Kassiotis, G. T cell regulation: a special job or everyone's responsibility? *Nat. Immunol.* **2**, 757–758 (2001).
74. Brunkow, M.E. *et al.* Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse. *Nat. Genet.* **27**, 68–73 (2001).
75. Chatila, T.A. *et al.* JM2, encoding a fork head-related protein, is mutated in X-linked autoimmunity-allergic disregulation syndrome. *J. Clin. Invest.* **106**, R75–R81 (2000).
76. Wildin, R.S. *et al.* X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. *Nat. Genet.* **27**, 18–20 (2001).
77. Bennett, C.L. *et al.* The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat. Genet.* **27**, 20–21 (2001).
78. Sakaguchi, N. Naturally arising Foxp3-expressing CD25+CD4+ regulatory T cells in immunologic tolerance to self and non-self. *Nat. Immunol.* **6**, 345–352 (2005).
79. Gambineri, E., Torgerson, T.R. & Ochs, H.D. Immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance (IPEX), a syndrome of systemic autoimmunity caused by mutations of FOXP3, a critical regulator of T-cell homeostasis. *Curr. Opin. Rheumatol.* **15**, 430–435 (2003).
80. Godfrey, V.L., Wilkinson, J.E. & Russell, L.B. X-linked lymphoreticular disease in the scurfy (sf) mutant mouse. *Am. J. Pathol.* **138**, 1379–1387 (1991).
81. Godfrey, V.L., Rouse, B.T. & Wilkinson, J.E. Transplantation of T cell-mediated, lymphoreticular disease from the scurfy (sf) mouse. *Am. J. Pathol.* **145**, 281–286 (1994).
82. Godfrey, V.L., Wilkinson, J.E., Rinchik, E.M. & Russell, L.B. Fatal lymphoreticular disease in the scurfy (sf) mouse requires T cells that mature in a sf thymic environment: potential model for thymic education. *Proc. Natl. Acad. Sci. USA* **88**, 5528–5532 (1991).
83. Blair, P.J. *et al.* CD4+CD8− T cells are the effector cells in disease pathogenesis in the scurfy (sf) mouse. *J. Immunol.* **153**, 3764–3774 (1994).
84. Clark, L.B. *et al.* Cellular and molecular characterization of the scurfy mouse mutant. *J. Immunol.* **162**, 2546–2554 (1999).
85. Kanangat, S. *et al.* Disease in the scurfy (sf) mouse is associated with overexpression of cytokine genes. *Eur. J. Immunol.* **26**, 161–165 (1996).
86. Khattri, R. *et al.* The amount of scurfin protein determines peripheral T cell number and responsiveness. *J. Immunol.* **167**, 6312–6320 (2001).
87. Kaestner, K.H., Knochel, W. & Martinez, D.E. Unified nomenclature for the winged helix/forkhead transcription factors. *Genes Dev.* **14**, 142–146 (2000).
88. Schubert, L.A., Jeffery, E., Zhang, Y., Ramsdell, F. & Ziegler, S.F. Scurfin (FOXP3) acts as a repressor of transcription and regulates T cell activation. *J. Biol. Chem.* **276**, 37672–37679 (2001).
89. Khattri, R., Cox, T., Yasayko, S.A. & Ramsdell, F. An essential role for Scurfin in CD4+CD25+ T regulatory cells. *Nat. Immunol.* **4**, 337–342 (2003).
90. Hori, S., Nomura, T. & Sakaguchi, S. Control of regulatory T cell development by the transcription factor Foxp3. *Science* **299**, 1057–1061 (2003).
91. Fontenot, J.D. *et al.* Regulatory T cell lineage specification by the forkhead transcription factor Foxp3. *Immunity* (in the press).
92. Walker, M.R. *et al.* Induction of FoxP3 and acquisition of T regulatory activity by stimulated human CD4+CD25- T cells. *J. Clin. Invest.* **112**, 1437–1443 (2003).
93. Yagi, H. *et al.* Crucial role of FOXP3 in the development and function of human CD25+CD4+ regulatory T cells. *Int. Immunol.* **16**, 1643–1656 (2004).