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## Role of regulatory T cells in human diseases

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The discovery of regulatory T lymphocytes (Treg) that are actively involved in maintaining immune tolerance has led to new insights into mechanisms of tolerance breakdown in human diseases, including those resulting from allergic, autoimmune, or infectious causes. Congenital deficiency of CD4<sup>+</sup>CD25<sup>+</sup> Treg cells caused by loss-of-function mutations in the gene encoding Foxp3 triggers a syndrome of lymphoproliferation and myeloproliferation, autoimmunity, and allergic dysregulation, whereas deficient allergen-specific Treg cell responses have been associated with a number of allergic and autoimmune disorders. Tolerization to allergens and autoantigens is associated with augmentation of Treg cell numbers and suppressive function, suggesting the manipulation of Treg cell activity as a potential strategy for future therapeutic interventions in allergic and autoimmune diseases. (*J Allergy Clin Immunol* 2005;116:949-59.)

**Key words:** *Regulatory T cells, Foxp3, dysregulation polyendocrinopathy enteropathy-X-linked, X-linked autoimmunity-allergic dysregulation, allergy, autoimmunity, lymphoproliferation, immunotherapy, IgE*

Regulatory T (Treg) cells are subsets of T cells involved in the maintenance of peripheral self-tolerance by actively suppressing the activation and expansion of autoreactive T cells. Several types of Treg cells have been characterized, most prominently natural and inducible CD4<sup>+</sup>CD25<sup>+</sup> Treg cells. The latter have been propelled to the forefront of immunologic investigation by virtue of the identification in human subjects and in mice of a syndrome of lymphoproliferation, autoimmunity, and allergic dysregulation resulting from the absence of CD4<sup>+</sup>CD25<sup>+</sup> Treg

### Abbreviations used

CTLA-4:	Cytotoxic T lymphocyte-associated antigen 4
IL-2R:	IL-2 receptor
IPEX:	Immune dysregulation polyendocrinopathy enteropathy-X-linked
SOCS-1:	Suppressor of cytokine signaling 1
Stat:	Signal transducer and activator of transcription
TCR:	T-cell receptor
TLR:	Toll-like receptor
Treg cell:	Regulatory T cell
XLAAD:	X-linked autoimmunity-allergic dysregulation

cells because of deleterious mutations in the transcriptional regulator Foxp3. This review focuses on the role of CD4<sup>+</sup>CD25<sup>+</sup> Treg cells in human disease, with a particular emphasis on insights gained from studying human subjects and rodent models with failure of CD4<sup>+</sup>CD25<sup>+</sup> Treg cell development and function caused by Foxp3 deficiency. The consequences of CD4<sup>+</sup>CD25<sup>+</sup> Treg cell deficiency or dysfunction in the development of human allergic and autoimmune diseases are examined.

### NATURAL CD4<sup>+</sup>CD25<sup>+</sup> Treg CELLS

Compelling evidence indicates a key role for Treg cells in the maintenance of self-tolerance. These cells mediate dominant suppression of autoreactive T cells normally present in the periphery, as well as downregulating immune responses to foreign antigens.<sup>1,2</sup> Among the several subpopulations of Treg cells identified to date, the naturally arising CD4<sup>+</sup>CD25<sup>+</sup> Treg cells have emerged as being particularly critical for the maintenance of immunologic tolerance.<sup>3</sup> CD4<sup>+</sup>CD25<sup>+</sup> Treg cells arise in the thymus, represent 5% to 10% of CD4<sup>+</sup> T cells in the periphery, and constitutively express the IL-2 receptor (IL-2R)  $\alpha$  chain (CD25), cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), and glucocorticoid-induced TNF receptor family-related gene (GITR).<sup>4-7</sup> As detailed in this review, CD4<sup>+</sup>CD25<sup>+</sup> Treg cells also express a transcriptional regulator, Foxp3, that acts as a master switch gene for their development and function.<sup>8</sup> Consistent

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with their function in maintaining self-tolerance, depletion of CD4<sup>+</sup>CD25<sup>+</sup> Treg cells precipitates autoimmunity against multiple tissues.<sup>9</sup>

CD4<sup>+</sup>CD25<sup>+</sup> Treg cells are anergic and do not produce IL-2.<sup>10</sup> When stimulated, they suppress the proliferation and cytokine production of conventional CD4<sup>+</sup>CD25<sup>-</sup> T cells, as well as that of CD8<sup>+</sup> T cells and established T<sub>H</sub>1 and T<sub>H</sub>2 cells.<sup>11-14</sup> CD4<sup>+</sup>CD25<sup>+</sup> Treg cells produce TGF- $\beta$  and IL-10, 2 cytokines endowed with immunosuppressive functions that play critical functions in Treg cell biology (discussed later in this review). Suppression by CD4<sup>+</sup>CD25<sup>+</sup> Treg cells can proceed by means of several mechanisms that might be differentially used depending on the microenvironment and the immunopathology being suppressed.<sup>15</sup> *In vitro*, the dominant immunosuppressive mechanism used by CD4<sup>+</sup>CD25<sup>+</sup> Treg cells appears to be cell contact and CTLA-4 dependent but might proceed independently of IL-10 and TGF- $\beta$ .<sup>15</sup> *In vitro*-activated CD4<sup>+</sup>CD25<sup>+</sup> Treg cells express granzyme A and display perforin-dependent cytotoxicity against autologous target cells, suggesting a function for direct killing in immunosuppression.<sup>16</sup> In contrast, suppression in several *in vivo* systems could be demonstrated not only to be CTLA-4 dependent but also IL-10 dependent, TGF- $\beta$  dependent, or both.<sup>15</sup> TGF- $\beta$  might act by directly engaging TGF- $\beta$  receptor type II on target effector cells and might also be important for peripheral homeostasis of CD4<sup>+</sup>CD25<sup>+</sup> Treg cells, indicating a pleiotropic function of this cytokine in supporting immunosuppression by Treg cells.<sup>17-19</sup>

### CD4<sup>+</sup>CD25<sup>+</sup> Treg CELL DEVELOPMENT IN THE THYMUS AND PERIPHERY

Natural CD4<sup>+</sup>CD25<sup>+</sup> Treg cells develop in the thymus. Neonatal thymectomy is associated with the development of autoimmunity secondary to CD4<sup>+</sup>CD25<sup>+</sup> Treg cell deficiency.<sup>20</sup> Mechanisms regulating CD4<sup>+</sup>CD25<sup>+</sup> Treg cell development in the thymus remain unclear. One set of studies support a mechanism whereby CD4<sup>+</sup>CD25<sup>+</sup> Treg cell development involves high-affinity interaction of their T-cell receptor (TCR) with peptide/MHC ligands, especially those presented by thymic epithelial cells, at levels of avidity approaching those associated with clonal deletion.<sup>21-24</sup> An alternative model suggests that the development of CD4<sup>+</sup>CD25<sup>+</sup> Treg cells is induced by mechanisms other than recognition of self-agonist peptides but that CD4<sup>+</sup>CD25<sup>+</sup> Treg cells are more resistant to agonist-induced clonal deletion, leading to the selective survival of CD4<sup>+</sup>CD25<sup>+</sup> Treg cells at high-avidity TCR peptide-MHC interactions.<sup>25</sup>

In the periphery the majority of natural Treg cells constitutively express high levels of CD25 (CD25<sup>high</sup>), but a significant minority express low levels of CD25 (CD25<sup>low</sup>).<sup>26,27</sup> Both populations are immunosuppressive, and both express the transcription factor Foxp3. It is thought that the CD25<sup>low</sup> population represents a population of natural Treg cells undergoing homeostatic proliferation, tissue infiltration, or both.<sup>26</sup> The maintenance of

CD4<sup>+</sup>CD25<sup>+</sup> Treg cells in the periphery is critically dependent on the action of 2 cytokines, IL-2 and TGF- $\beta$ . Although CD4<sup>+</sup>CD25<sup>+</sup> Treg cells do not produce IL-2, they are dependent on this cytokine for development in the thymus and for homeostasis and activation of suppressor function in the periphery (see discussion later in this review).<sup>28</sup> In studies on TGF- $\beta$ -deficient mice, TGF- $\beta$  was found to be necessary to maintain Foxp3 expression, regulatory function, and pool size of peripheral CD4<sup>+</sup>CD25<sup>+</sup> Treg cells but was not required for development of CD4<sup>+</sup>CD25<sup>+</sup> Treg cells in the thymus.<sup>19</sup>

### NATURAL VERSUS ADAPTIVE (OR INDUCIBLE) CD4<sup>+</sup>CD25<sup>+</sup> Treg CELLS

In addition to natural CD4<sup>+</sup>CD25<sup>+</sup> Treg cells, another species of CD4<sup>+</sup>CD25<sup>+</sup> Treg cells can be derived *in vitro* through treatment of peripheral CD4<sup>+</sup>CD25<sup>-</sup> T cells with anti-TCR and anti-CD28 antibodies in the presence of TGF- $\beta$ .<sup>27,29-31</sup> These so-called adaptive CD4<sup>+</sup>CD25<sup>+</sup> Treg cells express Foxp3 and exhibit attributes typical of the natural CD4<sup>+</sup>CD25<sup>+</sup> Treg cells, such as suppression of *in vitro*-induced antigen- and mitogen-driven T-cell proliferation and downregulation of allergic lung inflammation induced by ovalbumin in transgenic mice expressing an ovalbumin peptide-specific TCR.<sup>29</sup> Whereas the production of murine adaptive CD4<sup>+</sup>CD25<sup>+</sup> Treg cells *in vitro* by cross-linking of the TCR and CD28 stimulation is absolutely dependent on TGF- $\beta$ , it has been reported that human adaptive CD4<sup>+</sup>CD25<sup>+</sup> Treg cells can be induced *in vitro* in the absence of TGF- $\beta$ , but this observation was not reproduced in independent studies.<sup>32,33</sup>

The relationship between natural and adaptive CD4<sup>+</sup>CD25<sup>+</sup> Treg cell populations is of significant interest. Do adaptive Treg cells derive from committed natural (thymically educated and committed) Treg cells that cycle between CD4<sup>+</sup>CD25<sup>+</sup> and CD4<sup>+</sup>CD25<sup>-</sup> states in the periphery, or could they be derived *de novo* in an antigen-specific manner from non-Treg, naive T-cell population? At least 2 different *in vivo* experimental approaches indicate that adaptive Treg cells could be derived *de novo* from naive CD4<sup>+</sup> T cells in the periphery. Using thymectomized TCR-transgenic mice on a RAG knockout background, Apostolou and Von Boehmer<sup>34</sup> demonstrated that CD4<sup>+</sup>CD25<sup>+</sup> Foxp3<sup>+</sup> Treg cells could be derived *de novo* from peripheral CD4<sup>+</sup> naive T cells through continuous infusion of a submitogenic concentration of an agonistic peptide. The second approach used RAG-deficient mice repopulated with monoclonal T- and B-cell populations that are otherwise devoid of natural CD4<sup>+</sup>CD25<sup>+</sup> Treg cells. Whereas intraperitoneal immunization of these mice is associated with the induction of hyper-IgE and lung inflammation, oral antigen administration is associated with tolerance induction mediated by oral antigen-induced CD4<sup>+</sup>CD25<sup>+</sup> Foxp3<sup>+</sup> Treg cells.<sup>35</sup> These results from both *in vitro* and *in vivo* experimental systems reveal a surprising plasticity of the CD4<sup>+</sup>CD25<sup>-</sup> populations to develop *de novo* into CD4<sup>+</sup>CD25<sup>+</sup> Treg cells, with

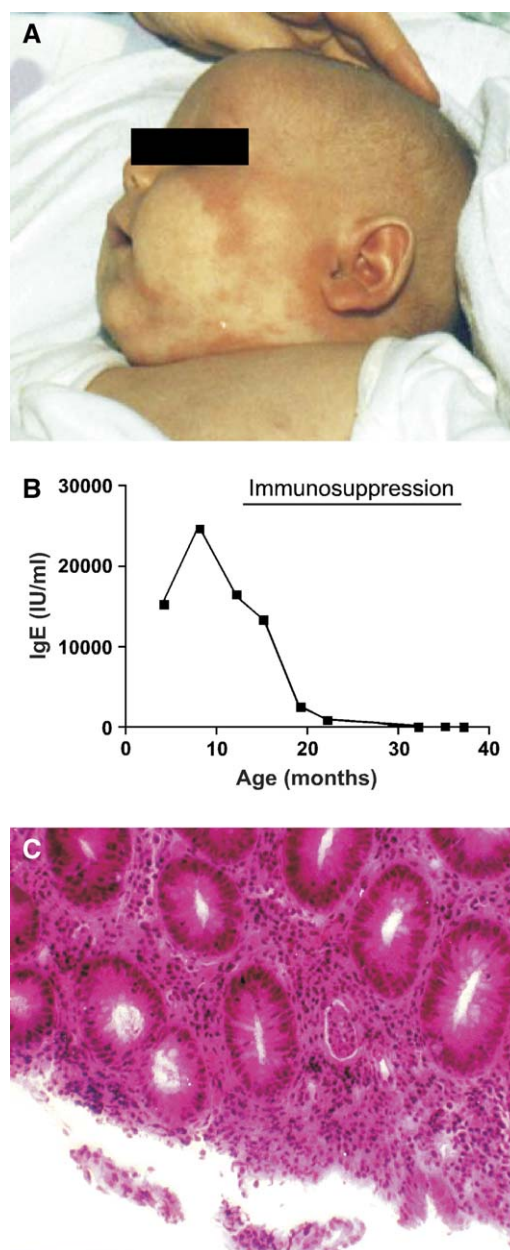
obvious implications for the treatment of human diseases with *ex vivo*-derived Treg populations or with immunotherapy, as is already used in allergic diseases.

A more general question relates to the temporal requirement for Foxp3 expression to maintain natural CD4<sup>+</sup>CD25<sup>+</sup> Treg cell phenotype in the periphery. Transgenic expression of Foxp3 in the thymus fails to rescue the lethal phenotype of Foxp3-deficient mice, which is consistent with a requirement for Foxp3 expression in the periphery.<sup>36</sup> However, whether Foxp3 function is continuously required to maintain CD4<sup>+</sup>CD25<sup>+</sup> Treg cells in the periphery throughout the lifespan of the organism remains an open question.

### Foxp3, A MASTER SWITCH GENE FOR CD4<sup>+</sup>CD25<sup>+</sup> Treg CELLS

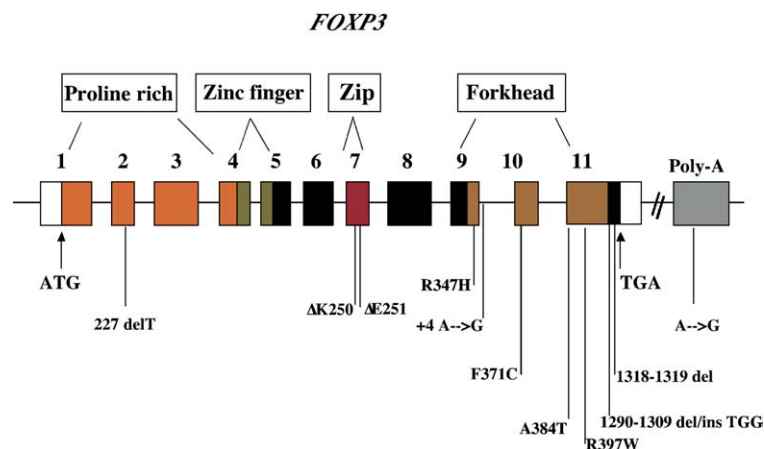
The identification of the forkhead-type factor Foxp3 as a master switch gene for CD4<sup>+</sup>CD25<sup>+</sup> Treg cells has provided a pivotal breakthrough in our understanding of CD4<sup>+</sup>CD25<sup>+</sup> Treg cell development and function. Foxp3 was originally identified as the gene product affected in a lethal X-linked recessive lymphoproliferative disease in mice and human subjects.<sup>37</sup> Loss-of-function mutations in the gene encoding Foxp3 underlie the lymphoproliferative disease of the scurfy mouse. Male mice with Foxp3 deficiency die at about the third week of age because of unrelenting infiltrative lymphoproliferative disease that involves several organs.<sup>37-40</sup> Foxp3-deficient mice also experience allergic dysregulation with striking hyper-IgE levels and eosinophilia in the absence of overt T<sub>H</sub>2 skewing.<sup>40</sup> The striking hyper-IgE level is a distinguishing feature of CD4<sup>+</sup>CD25<sup>+</sup> Treg cell deficiency in that it is shared by other mouse models lacking in CD4<sup>+</sup>CD25<sup>+</sup> Treg cells.<sup>41</sup> Importantly, the scurfy phenotype can be reproduced by targeted mutagenesis of *Foxp3* and is rescued by a *Foxp3* transgene, which is consistent with the causative role for Foxp3 in disease pathogenesis.<sup>40,42</sup>

*FOXP3* mutations also underlie a homologous autoimmune lymphoproliferative disorder in human subjects, termed immune dysregulation polyendocrinopathy enteropathy-X-linked (IPEX) syndrome and X-linked autoimmunity-allergic dysregulation syndrome (XLAAD).<sup>43-47</sup> Male subjects with this syndrome present with neonatal autoimmune type 1 diabetes with islet cell destruction by infiltrating T cells. A more general predilection to autoimmunity is manifest, including polyendocrinopathy, autoimmune hemolytic anemia, and autoimmune enteropathy. Another prominent feature of IPEX/XLAAD is severe allergic inflammation with eczema and food allergy. The IgE levels can be extremely increased and accompanied by intense peripheral eosinophilia and (unlike the case of the mouse models discussed below) evidence of overt T<sub>H</sub>2 skewing.<sup>43,48</sup> The latter might reflect the evolution of the disease under the influence of ongoing immunosuppressive therapy. Many patients have persistent secretory diarrhea, which might be contributed to by both food allergy-induced eosinophilic gastroenteropathy and



**FIG 1.** Disease manifestations of Foxp3 deficiency in human subjects. **A**, Eczema-like skin lesions on the face of a child with IPEX. **B**, IgE levels in a child with IPEX followed over the first 3 years of life. The child was started on immunosuppressive therapy on the diagnosis of his disease at the age of 2 months. **C**, Enteritis-like picture in IPEX. Biopsy specimen of the sigmoid colon in a child with IPEX showing a colitis-like picture with infiltration by a mixed cellular infiltrate (hematoxylin and eosin staining).

autoimmune inflammatory bowel disease-like enteropathy (Fig 1). Despite aggressive therapy, the outcome of IPEX/XLAAD is poor, with most patients dying in childhood. Bone marrow transplantation has been attempted in some patients and is potentially curative.<sup>46,49,50</sup> In both mice and human subjects, female carriers are asymptomatic, which is consistent with X-linked recessive inheritance.



**FIG 2.** *FOXP3*: gene organization and mutations in IPEX. The gene encoding Foxp3 is composed of 11 coding exons (numbered 1 through 11) and 2 upstream noncoding exons (not shown). The positions of the translational start and stop codons (ATG and TGA) are indicated. The predicted functional domains include a proline-rich N-terminal region, a zinc finger, leucine zipper (*Zip*), and forkhead domains. Unfilled portions of exons 1 and 11 represent untranslated regions. Mutations identified in *FOXP3* have been summarized elsewhere.<sup>46,47</sup>

Foxp3 is a member of the forkhead family of transcription factors defined by the presence of a winged helix (forkhead) DNA-binding domain. In addition to a C-terminal forkhead homology domain, Foxp3 also contains a single C2H2 zinc finger motif and a leucine zipper domain, both located midway through the protein. The latter has been implicated in studies on other Foxp subfamily members (Foxp1, Foxp2, and Foxp4) in the formation of Foxp homodimers and heterodimers.<sup>51,52</sup> A proline-rich N-terminal domain mediates transcriptional suppression (Chatila TA, unpublished observations, August 2005). Single amino acid deletions in the leucine zipper domain and missense and nonsense mutations and deletions in the forkhead homology domain have been associated with the development of IPEX in human subjects, which is consistent with a functionally critical role of the respective domain in Foxp3 function (Fig 2).<sup>43</sup> A 2-bp insertion in exon 8 of Foxp3 leading to a translational frame shift and a premature stop codon underlines the genetic defect in the scurfy mouse.<sup>42</sup>

Foxp3 is a transcriptional regulator that has been described to suppress transcription from nuclear factor of activated T cells and nuclear factor  $\kappa$ B response elements.<sup>53,54</sup> Foxp3-mediated transcriptional suppression of key T-cell cytokine genes, including those encoding IL-2, IL-4, and IFN- $\gamma$ , underlies the inability of CD4<sup>+</sup>CD25<sup>+</sup> Treg cells to secrete these cytokines. The role of the respective domains in transcriptional regulation by Foxp3 remains elusive, as is the mechanism by which it interacts with and suppresses transcriptional activation.

### Foxp3 DEFICIENCY: DISEASE MANIFESTATIONS AND PATHOGENESIS

The immunopathology of Foxp3 deficiency results from unchecked T-cell activation.<sup>55,56</sup> That this is due to

failure of Foxp3-directed CD4<sup>+</sup>CD25<sup>+</sup> Treg cell development is supported by the observation that expression of Foxp3 in peripheral CD4<sup>+</sup>CD25<sup>-</sup> cells transforms these cells into Treg cells endowed with suppressive properties.<sup>8,39</sup> Also, adoptive transfer of CD4<sup>+</sup>CD25<sup>+</sup> Treg cells rescues scurfy mice from disease, and Foxp3-transduced CD4<sup>+</sup>CD25<sup>-</sup> T cells suppressed wasting and colitis induced by the transfer of CD4<sup>+</sup>CD25<sup>-</sup> T cells into RAG-deficient mice, at least in the short term.<sup>39,57</sup> These results established an essential function for Foxp3 in CD4<sup>+</sup>CD25<sup>+</sup> Treg cell development.

Although in both mice and human subjects loss-of-function in the gene encoding Foxp3 mutations unleash a lethal syndrome of autoimmunity and lymphoproliferation, the precise mechanisms by which the disease evolves remain poorly understood. Affected mice, both the original scurfy mice and the more recently derived strains with targeted loss-of-function *Foxp3* mutations-deletions, are phenotypically indistinguishable from healthy littermate control animals during the first week to 10 days of life; thereafter, the disease evolves aggressively, and death can ensue within a few days to weeks.<sup>37-40</sup> Disease progression is associated with lymphoid and myeloid hyperplasia and a concomitant intense mixed inflammatory infiltrate involving several organs, including the liver, lung, pancreas, stomach, skin, and gut.<sup>37-40</sup> The overwhelming majority of CD4<sup>+</sup> and CD8<sup>+</sup> T cells are activated, and both T<sub>H</sub>1 and T<sub>H</sub>2 cytokines are abundantly expressed. T<sub>H</sub>1 and T<sub>H</sub>2 cytokines are also found at high levels in the circulation. This massive dysregulated production of cytokines suggests a role for cytokine-mediated tissue injury in disease pathogenesis. Support for this concept comes from animal models associated with cytokine dysregulation, such as one with deficiency of the negative cytokine receptor regulator suppressor of cytokine signaling 1 (SOCS-1) and another with deficiency of the immunoregulatory cytokine TGF- $\beta$ .<sup>58,59</sup> Inactivation of signal



transducer and activator of transcription 6 (Stat6; SOCS-1 deficiency) and IFN- $\gamma$ /Stat1 (SOCS-1 and TGF- $\beta$  deficiency) rescues autoimmune liver injury associated with these models.

In Foxp3-deficient mice the spectrum of organ involvement and disease manifestations vary depending on the strain background (Wen Lin and Talal Chatila, manuscript in preparation). Affected mice on the BALB/c background experience a more aggressive disease and have a shorter lifespan compared with those on the C57BL/6 background. Unlike human disease, the murine form is not associated with type 1 diabetes in the absence of a diabetes-prone genetic background. These differences point to disease-modifying genes that act to alter disease outcome and presage phenotypic variability in disease manifestations in human subjects.

### **Treg CELL DEFICIENCY, LYMPHOPROLIFERATION, AND AUTOIMMUNITY CAUSED BY DEFECTS ALONG THE IL-2/IL-2R PATHWAY**

A substantial proportion (up to 40%) of patients with an IPEX-like phenotype lack detectable mutations in the *FOXP3* gene.<sup>47</sup> An IPEX-like phenotype has also been described in female subjects, which is suggestive of autosomal inheritance.<sup>60</sup> These observations have led to the search for mutations in other genes that are associated with Treg cell deficiency. Of particular interest are candidate genes along the IL-2/IL-2R axis. Treg cells express all 3 components of the IL-2R, including the  $\alpha$ ,  $\beta$ , and  $\gamma$  chains (CD25, CD122, and CD132, respectively).<sup>61</sup> Treg cells do not secrete IL-2 but are dependent on the provision of this cytokine by paracrine sources to expand in the periphery and activate their immunosuppressive function.<sup>28</sup> Whereas deficiency of IL-2R $\gamma$  chain (CD132) is associated with X-linked severe combined immunodeficiency,<sup>62</sup> genetic defects in the other components of the IL-2/IL-2R axis, all of which are autosomal, are predictive of abnormalities in the development and function of CD4<sup>+</sup>CD25<sup>+</sup> Treg cells. The number of CD4<sup>+</sup>CD25<sup>+</sup> Treg cells is profoundly reduced in mice lacking in IL-2, IL-2R $\alpha$ , IL-2R $\beta$ , or both isoforms of the downstream Stat5 (Stat5a and Stat5b). Mice deficient in IL-2, IL-2R $\alpha$ , IL-2R $\beta$ , or both isoforms of Stat5 (Stat5a and Stat5b) experience a fatal lymphoproliferative and autoimmune disease.<sup>63-67</sup> Importantly, reconstitution with CD4<sup>+</sup>CD25<sup>+</sup> Treg cells rescues the lethal autoimmunity and lymphoproliferative disease of IL-2, IL-2R $\alpha$ , and IL-2R $\beta$  chain-deficient mice, which is consistent with a cardinal role for Treg cell deficiency in the phenotypic manifestation of these mutant mice.<sup>61,68</sup>

Mutations in component genes of the IL-2/IL-2R pathway have been recognized in human subjects. Sharfe et al<sup>69</sup> reported the case of a child with IL-2R $\alpha$  chain deficiency caused by a 4-bp deletion in the coding sequence, resulting in a translational shift and leading to a complete deficiency of CD25.<sup>70</sup> The affected infant

presented with cytomegalovirus-induced pneumonitis with subsequent evolution of a frank autoimmune lymphoproliferative disease that was cured by bone marrow transplantation. A second case of IL-2R $\alpha$  chain deficiency caused by compound heterozygote mutations in the IL2-R $\alpha$  chain gene has recently been documented in a child with an IPEX-like clinical picture but normal Foxp3 (James Verbsky, Medical College of Wisconsin, personal communication, August 2005). Thus mutations in components of the IL-2/IL-2R axis might give rise to an overlapping clinical picture with that of IPEX.

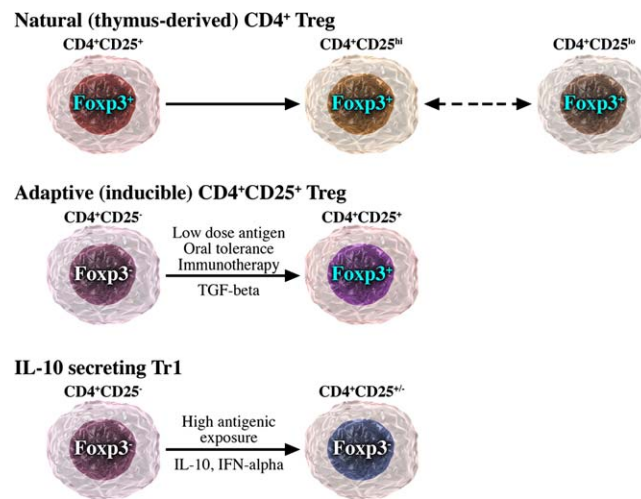
### **OTHER CANDIDATE GENE DEFECTS LEADING TO CD4<sup>+</sup>CD25<sup>+</sup> Treg CELL DYSFUNCTION**

In addition to components of the IL-2/IL-2R pathway, putative defects in other candidate genes might also give rise to an autosomal IPEX-like phenotype. CTLA-4 is constitutively expressed on CD4<sup>+</sup>CD25<sup>+</sup> Treg cells and mediates negative regulatory function in T-cell activation.<sup>71</sup> Its deficiency in mice gives rise to a syndrome of autoimmunity and lymphoproliferation that strongly overlaps with that of Foxp3-deficient mice.<sup>72,73</sup> Human CTLA-4 deficiency has not been described to date, although polymorphisms in the CTLA-4 locus have been associated with human autoimmune endocrinopathies.<sup>74</sup>

Involvement of granzyme A and the perforin pathway in CD4<sup>+</sup>CD25<sup>+</sup> Treg cell-mediated cytotoxicity and immunosuppression has been discussed earlier.<sup>16</sup> A number of congenital defects affect granzyme/perforin-dependent cytotoxicity, including perforin deficiency or the exocytosis of the cytotoxic granules of cytotoxic T cells and natural killer cells (including mutations in *SAP*, *Munc13-4*, *Rab27a*, *CHS-1*, *RGGT*, and others).<sup>75</sup> All these mutations give rise to syndromes of hemophagocytic lymphohistiocytosis, characterized by uncontrolled expansion of virus-specific CD8<sup>+</sup> T cells in the wake of specific viral infections. Defective cytolytic function of CD4<sup>+</sup>CD25<sup>+</sup> Treg cells has been postulated to be instrumental in the evolution of these disorders.<sup>16</sup>

### **CD4<sup>+</sup>CD25<sup>+</sup> Treg CELLS VERSUS OTHER Treg POPULATIONS**

In addition to natural and adaptive CD4<sup>+</sup>CD25<sup>+</sup> Treg cells, there are other Treg populations that play an important role in maintaining peripheral tolerance (Fig 3). The IL-10-secreting regulatory T cells (IL-10 Treg cells) encompass a heterogeneous group of Treg cells that are induced in the periphery in response to antigenic stimulation and are characterized by the secretion of large amounts of IL-10.<sup>76</sup> A subgroup of IL-10 Treg cells are the Treg type 1 cells or T<sub>R</sub>1 cells. These cells were originally identified in patients with severe combined immunodeficiency who have successfully undergone HLA-mismatched bone marrow transplantation.<sup>77</sup> T<sub>R</sub>1 cells



**FIG 3.** Natural and induced Treg cell populations. *Upper panel*, Natural (thymic) CD4<sup>+</sup>CD25<sup>+</sup> T cells develop in the thymus in a Foxp3-dependent manner and are exported to the periphery, where they constitutively express Foxp3. Although a majority of natural Treg cells express high levels of CD25 (CD25<sup>hi</sup>), a minority express lower levels (CD25<sup>lo</sup>). Both populations are immunosuppressive, and both express Foxp3. It is thought natural Treg cells might cycle between the 2 phenotypes. *Middle panel*, CD4<sup>+</sup>CD25<sup>-</sup> Foxp3<sup>-</sup> cells can be induced to differentiate into CD4<sup>+</sup>CD25<sup>+</sup> Foxp3<sup>+</sup> adaptive or inducible Treg cells under special conditions, including low antigen exposure, oral tolerance, and immunotherapy. Induction *in vitro* can be achieved by means of stimulation in the presence of TGF- $\beta$ . *Lower panel*, IL-10-secreting T<sub>H</sub>1 cells and related Treg cells are Foxp3<sup>-</sup> and are induced *in vivo* on high antigenic exposure, especially during chronic exposure, and can be induced *in vitro* by means of stimulation in the presence of IL-10.

secrete IL-10, IL-5, and IFN- $\gamma$  but little IL-2 and exert their suppressive function through an IL-10-dependent mechanism.<sup>78,79</sup> T<sub>R</sub>1 cells might play a critical role in limiting pathologies associated with a high antigenic load, such as infections and severe inflammation.<sup>76</sup> Other IL-10 Treg cells can be induced *in vitro* by antigenic or mitogenic stimulation in the presence of corticosteroids and vitamin D3 and secrete IL-10 but not other T<sub>H</sub>1 or T<sub>H</sub>2 cytokines.<sup>80,81</sup> These, too, are effective in suppressing experimental models of autoimmune disease, such as experimental allergic encephalomyelitis. Yet another Treg population, T<sub>H</sub>3, has been described that is preferentially generated after oral antigen administration and that secretes large amounts of TGF- $\beta$ .<sup>82,83</sup>

Currently, the relationship between the different Treg populations, including natural and adaptive CD4<sup>+</sup>CD25<sup>+</sup> Treg cells, IL-10-secreting Treg cells, and TGF- $\beta$ -secreting T<sub>H</sub>3 cells, remains unclear. Unlike CD4<sup>+</sup>CD25<sup>+</sup> Treg cells, IL-10-secreting Treg cells derived *in vitro* in the presence of dexamethasone and vitamin D3 express little or no Foxp3.<sup>81</sup> Nevertheless, it should be noted that corticosteroids have been described to induce Foxp3,<sup>84</sup> and there has yet to be a formal demonstration that IL-10-secreting Treg cells can be derived from lymphocytes of Foxp3-deficient animals. It has been suggested that the IL-10-secreting Treg cells and CD4<sup>+</sup>CD25<sup>+</sup> Treg cells represent 2 separate and specialized populations: the first endowed with an anti-inflammatory capacity and infiltrates injured tissues to control inflammation and tissue destruction through the release of IL-10 and TGF- $\beta$ , whereas the latter has a central homeostatic function to regulate

T-cell proliferation through direct cell-cell contact mechanisms.<sup>85</sup> There have been very few studies that have compared these different populations head to head in the same disease models, and the results seem to indicate significant redundancy.<sup>86</sup> The availability of mice whose Foxp3 expression is coupled with reporter fluorescent proteins<sup>26,27</sup> and the application of genomic profiling approaches to the study of Treg cells offers to clarify the relationship between the different Treg populations.<sup>26</sup>

In addition to its expression in CD4<sup>+</sup> T cells, Foxp3 is also expressed in a small population of CD8<sup>+</sup> T cells. Foxp3<sup>+</sup> CD8<sup>+</sup> Treg cells have been described in both human subjects and rodents and appear to share many of the phenotypic features with their CD4<sup>+</sup>CD25<sup>+</sup> counterparts, including constitutive expression of CD25, CTLA-4, and TGF- $\beta$ .<sup>87-89</sup> They have been implicated in the maintenance of transplant tolerance in rodent models of allograft rejection.<sup>89,90</sup>

## ROLE OF Treg CELLS IN HUMAN ALLERGIC DISEASES

The allergic dysregulation associated with Foxp3 mutations in human subjects and in rodents pointed to the function of Treg cells in regulating the allergic response. Such a role in human allergic diseases has been corroborated by several other lines of evidence. Human peripheral blood from nonatopic donors inhibits the proliferation and T<sub>H</sub>2 cytokine production by CD4<sup>+</sup>CD25<sup>-</sup> T cells on their stimulation *in vitro* with allergen. More recently,

Treg cells from atopic subjects were found to be less effective in suppressing allergen-stimulated proliferation of autologous CD4<sup>+</sup>CD25<sup>+</sup> T cells than those of nonatopic individuals, suggesting defective allergen-specific Treg cell activity in atopic subjects.

A prominent feature of IPEX syndrome is the frequent occurrence of food allergy, which is indicative of a role for Treg cells in tolerance to food antigens. Insight into the role of Treg cells in establishing tolerance to foods was provided by studies on children with allergy to cow's milk. It was found that those children who outgrew their cow's milk allergy had a higher frequency of  $\beta$ -lactoglobulin-specific CD4<sup>+</sup>CD25<sup>+</sup> Treg cells and decreased proliferation of PBMCs to  $\beta$ -lactoglobulin compared with children who had clinically active allergy.<sup>91</sup> Depletion of Treg cells from PBMCs of cow's milk-tolerant children significantly boosted their proliferation in response to  $\beta$ -lactoglobulin to levels exceeding those of similarly depleted PBMCs of children with active allergy. These results suggested that mucosal induction of tolerance against dietary antigens is associated with the development of antigen-specific CD4<sup>+</sup>CD25<sup>+</sup> Treg cells.

A role for CD4<sup>+</sup>CD25<sup>+</sup> Treg cells in atopic dermatitis has also been suggested by the ubiquitous manifestation of atopic dermatitis in children with IPEX and the occurrence of atopic dermatitis-like lesions in mice with loss-of-function *Foxp3* mutations. Ou et al<sup>92</sup> studied the effects of the superantigen staphylococcal enterotoxin B on CD4<sup>+</sup>CD25<sup>+</sup> Treg cell function in subjects with atopic dermatitis. CD4<sup>+</sup>CD25<sup>+</sup> Treg cells of patients with atopic dermatitis were found to have normal immunosuppressive function. However, after staphylococcal enterotoxin B stimulation, CD4<sup>+</sup>CD25<sup>+</sup> Treg cells were no longer anergic, and they lost their immunosuppressive activity. This suggested that superantigens might augment T-cell activation in patients with atopic dermatitis in part by promoting the breakdown of CD4<sup>+</sup>CD25<sup>+</sup> Treg cell immunosuppressive function.

## EFFECT OF THERAPEUTIC INTERVENTIONS ON Treg CELLS IN ALLERGIC DISEASES

Immunotherapy is associated with hyporesponsiveness of T cells to allergens, decreased allergen-induced production of T<sub>H</sub>2 cytokines, and in some cases a shift toward a T<sub>H</sub>1 profile. These alterations have been linked to the induction by immunotherapy of Treg cells that suppress the ongoing T<sub>H</sub>2-driven allergic response. In studies on allergic subjects and immunotherapy to house dust mite, birch pollen, and grass pollen allergens, the suppression of allergen-responsive T cells after immunotherapy appears to be IL-10 and TGF- $\beta$  dependent and is associated with expansion of IL-10-producing, allergen-specific CD4<sup>+</sup>CD25<sup>+</sup> Treg cells.<sup>93,94</sup> Interestingly, suppression of allergen responsiveness in healthy donors also involves a similar population of IL-10-secreting CD4<sup>+</sup>CD25<sup>+</sup> cells that constitutively express CTLA-4 and CD25 and mediate suppression in an IL-10-, TGF- $\beta$ -, and CTLA-4-

dependent manner.<sup>95</sup> Although the phenotype of these allergen-specific Treg populations cited above is indicative of CD4<sup>+</sup>CD25<sup>+</sup> Treg cells, their capacity to express Foxp3 was not determined.

Corticosteroids are potent anti-inflammatory and immunosuppressive agents that are particularly useful in the treatment of allergic, inflammatory, autoimmune, and graft-versus-host diseases. In addition to their direct inhibitory effects on immune cell activation and cytokine production, corticosteroids have been noted to promote Treg cell development and function. A prominent effect of corticosteroids is the induction of IL-10 production by T cells and macrophages. *In vitro* mitogenic stimulation of human and murine T cells in the presence of corticosteroids and vitamin D3 promotes their differentiation into IL-10-producing T<sub>R</sub>1-type cells that are effective in suppressing experimental allergic encephalomyelitis.<sup>80</sup> Corticosteroids might separately promote the function of CD4<sup>+</sup>CD25<sup>+</sup> Treg cells, as assayed in suppressing allergen-stimulated *in vitro* T-cell cultures, in part by increasing IL-10 production.<sup>96</sup> More recently, *in vitro* treatment with corticosteroids was found to upregulate Foxp3 expression in CD4<sup>+</sup> T lymphocytes of healthy donors.<sup>84</sup> Significantly, inhaled or systemic corticosteroid treatment was associated with increased Foxp3 expression in freshly isolated CD4<sup>+</sup> T cells of asthmatic subjects, and the frequency of CD4<sup>+</sup>CD25<sup>+</sup> T cells was also increased after systemic corticosteroid treatment.<sup>84</sup> Foxp3 expression in CD4<sup>+</sup> T cells induced by corticosteroids tightly correlated with that of IL-10, suggesting a causal link. Collectively, these results suggest that a component of the immunomodulatory actions of corticosteroids involves upregulation of Treg cell activity.

Induction of airway hyperresponsiveness and allergic airway inflammation in murine asthma models could be ameliorated by prior treatment with *Mycobacterium vaccae*.<sup>97-99</sup> This effect was mediated by CD4<sup>+</sup>CD45Rb<sup>low</sup> T cells, which are enriched in CD4<sup>+</sup>CD25<sup>+</sup> Treg cells, and proceeded in an IL-10- and TGF- $\beta$ -dependent manner. Furthermore, the transfer of *M vaccae*-primed and antigen-sensitized Treg cells could protect recipient allergic mice from airway inflammation. In clinical trials treatment with *M vaccae* was found to be effective in ameliorating moderate-to-severe atopic dermatitis in children, suggesting the potential to modulate Treg cell function in allergic disease by treatment with microbial products.<sup>100</sup> Its success in human subjects with asthma has, however, been more equivocal,<sup>101,102</sup> suggesting additional factors, such as concurrent allergen exposure (to prime antigen-specific T<sub>R</sub> cells), might be important for optimal outcome of this therapy.<sup>103</sup>

## CD4<sup>+</sup>CD25<sup>+</sup> Treg CELLS AND ENVIRONMENTAL MODIFIERS: AT THE NEXUS OF THE HYGIENE HYPOTHESIS?

A likely environmental agent involved in disease pathogenesis in Foxp3 deficiency is the microbial flora,

which interfaces with the host immune system through Toll-like receptors (TLRs) expressed in various cell types, including macrophages, dendritic cells, selective T- and B-cell subsets, and epithelial cells.<sup>104</sup> CD4<sup>+</sup>CD25<sup>+</sup> Treg cells selectively express TLR-4, TLR-5, TLR-7, and TLR-8, and LPS induces their proliferation and suppressor activity.<sup>105</sup> Hence Treg cells might play an important role in preventing excess activation of the immune system by the commensal flora. There appears to be a delicate balancing act mediated by Toll receptor-signals in Treg cells versus antigen-presenting cells. On one hand, LPS administration augments CD4<sup>+</sup>CD25<sup>+</sup> Treg cell suppressor activity.<sup>105</sup> On the other hand, LPS-treated antigen-presenting cells have been shown to inhibit the suppressive activity of Treg cells by stimulating the secretion of IL-6, which directly inhibits the suppressor effects of Treg cells.<sup>106</sup> This balancing act might break down in Foxp3 deficiency, leading to excessive activation of cells of the innate immune system by the commensal flora and subsequently leading to immunopathology. It is tempting to speculate that decreased stimulation by the microbial flora resulting in hypofunction of the CD4<sup>+</sup>CD25<sup>+</sup> Treg cells might be one of the causes of increased allergic responses, as stipulated by the hygiene hypothesis.

## ROLE OF Treg CELLS IN HUMAN AUTOIMMUNE DISEASE

Several human autoimmune diseases have been noted to associate with alterations in CD4<sup>+</sup>CD25<sup>+</sup> Treg cell activity.<sup>107</sup> Some, such as multiple sclerosis and the polyglandular syndrome type II, were associated with normal numbers but defective activity of peripheral blood CD4<sup>+</sup>CD25<sup>+</sup> Treg cells.<sup>108,109</sup> A similar dissociation between the numbers and functional activity of CD4<sup>+</sup>CD25<sup>+</sup> Treg cells was found in the thymuses of patients with myasthenia gravis compared with those of healthy subjects, which correlated with decreased expression of Foxp3.<sup>110</sup> A more complex picture emerged in studies of patients with rheumatoid arthritis and idiopathic juvenile arthritis. Peripheral blood CD4<sup>+</sup>CD25<sup>+</sup> Treg cells of patients with rheumatoid arthritis were found to be effective in suppressing proliferation of autologous CD4<sup>+</sup>CD25<sup>+</sup> Treg cells but not the secretion by these T cells of inflammatory cytokines, including TNF- $\alpha$  and IFN- $\gamma$ .<sup>111</sup> The defect in suppressing inflammatory cytokine secretion was reversed with anti-TNF- $\alpha$  therapy. Yet in other studies that directly examined the frequency and function of CD4<sup>+</sup>CD25<sup>+</sup> Treg cells in the affected joints of patients with rheumatoid arthritis and other rheumatic conditions, it could be demonstrated that the population of CD4<sup>+</sup>CD25<sup>+</sup> Treg cells was expanded in the target joints compared with in the peripheral blood and that patients with prognostically favorable disease exhibited an increased frequency of CD4<sup>+</sup>CD25<sup>+</sup> Treg cells in their joints.<sup>112,113</sup> These results would argue for a reactive function of CD4<sup>+</sup>CD25<sup>+</sup> Treg cells in limiting joined inflammation in arthritis.

The capacity of immunotherapy with autoantigens to modulate the immune response by upregulating Treg cell activity was documented in a study on patients with rheumatoid arthritis.<sup>114</sup> Oral immunotherapy with an autoantigenic peptide that stimulates autoreactive T cells was associated with suppression of peptide-induced T-cell proliferation, a change in the cytokine profile from a T<sub>H</sub>1 to a T<sub>H</sub>2 response, and an expansion of the Foxp3<sup>+</sup> CD4<sup>+</sup>CD25<sup>+</sup> Treg population in circulation. These results highlight the potential for using tolerance-inducing regimens that promote Treg cell function in autoimmune diseases.

## ROLE OF Treg CELLS IN HUMAN INFECTIOUS DISEASE

The function of Treg cells in infectious diseases can reflect either positively or negatively on disease outcome, depending on whether the disease is acute and self-limiting or chronic and persistent, and is associated with tissue damage caused by a robust immune response.<sup>115,116</sup> There are several examples of infections in which a favorable outcome hinges on carefully balancing the effector and regulatory components of the immune response. For example, the number of CD4<sup>+</sup>CD25<sup>+</sup> Treg cells in the circulation inversely correlated with liver damage in human hepatitis C virus infection, indicating a favorable function of CD4<sup>+</sup>CD25<sup>+</sup> Treg cells in dampening the damaging immune response.<sup>117</sup> In HIV infection CD4<sup>+</sup>CD25<sup>+</sup> Treg cells inversely correlate with disease progression. In murine colitis models the capacity of effector T cells transferred into an immunodeficient host and triggered by the commensal flora to cause intense intestinal inflammation is held in check by cotransfer of Treg populations.<sup>118</sup> By the same token, loss of CD4<sup>+</sup>CD25<sup>+</sup> Treg cells in Foxp3 deficiency in rodents and in human subjects results in intense gastrointestinal inflammation, with a pathologic and immunologic profile that closely matches that seen in mice with severe combined immunodeficiency syndrome reconstituted with effector CD4<sup>+</sup> T cells (Martin G. Martin and Talal A. Chatila, unpublished observations, August 2005). This delicate balance between regulatory and effector arms of the immune response could be tipped in favor of the pathogens, as is especially demonstrable in murine models of malaria or viral infections.<sup>14,119</sup> Perhaps most intriguing is the direct targeting of Treg cells by pathogens and their products. The capacity of the staphylococcal superantigen B to modulate Treg cell function in atopic dermatitis has been previously discussed. CD4<sup>+</sup>CD25<sup>+</sup> Treg cells are themselves the target of viral infection with human T cell lymphotropic virus type 1 and HIV. The former can act through the human T cell lymphotropic virus type 1 transactivator Tax protein to directly suppress Foxp3 expression and inhibit Treg cell function.<sup>120</sup> Depletion of CD4<sup>+</sup>CD25<sup>+</sup> Treg cells because of their infection with HIV might hasten disease progression.<sup>121</sup>



## CONCLUSIONS

It is now clear that Treg cells play a central role in maintaining peripheral tolerance to self-antigens and in regulating the immune response to non-self-antigens. Genetic defects in the development and function of Treg cells, most notably Foxp3 deficiency, have been associated with the development of syndromes of autoimmunity, lymphoproliferation, and allergic dysregulation. These have provided fascinating experiments of nature that have helped probe the function of Treg cells in human diseases. There is a pressing need to further elucidate molecular mechanisms governing the development and function of CD4<sup>+</sup>CD25<sup>+</sup> Treg cells, as well as to identify parallel pathways governing the development and function of other Treg populations, including T<sub>R</sub>1 and T<sub>H</sub>3 cells. A similar need exists in identifying the relationship between the different Treg populations and the development of reliable and easy-to-use markers to track their numbers and activities in human diseases. The manipulation of Treg populations for therapeutic purposes promises to be a burgeoning field of investigation, with the potential for a wide range of clinical applications.

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