

Regulatory T cells as potential immunotherapy in allergy

Kajsa Wing^a and Shimon Sakaguchi^{a,b}

Purpose of review

CD4⁺ regulatory T cells are fundamental for the induction and maintenance of immunological tolerance to self and foreign-antigens, including allergens. Here we discuss recent advances in the field of regulatory T cells and how this knowledge can be exploited to treat and prevent allergy.

Recent findings

Current research suggest that naturally occurring CD4⁺CD25⁺ regulatory T cells together with inducible IL-10-producing CD4⁺ regulatory T cells actively control allergic responses and that their function or numbers may contribute to the development or progression of allergy. Indeed, successful treatment of allergy by allergen-specific immunotherapy may depend on the induction of IL-10 secreting CD4⁺ T cells. Work has begun to reveal the impact of various pharmaceutical treatments on naturally occurring CD25⁺ regulatory T cells. In addition, recent findings point to an important role for toll-like receptors in the tuning of regulatory T cell function and homeostasis. This may link the hygiene hypothesis to regulatory T cells and open up new possibilities for early intervention in allergic disease.

Summary

The identification of a role for regulatory T cells in allergic disease has provided a host of new therapeutic possibilities, with the potential prospect of safe and long-term alleviation of allergic diseases.

Keywords

Foxp3, suppression, tolerance

Introduction

Allergic diseases, such as rhinitis, asthma and atopic dermatitis, which follow immune responses to otherwise innocuous antigens, are rarely life-threatening, but have become common chronic diseases in many societies, particularly in the developed part of the world. Indeed, in some western countries every third child needs medical attention by a physician because of allergic disease. Medicine is thus faced with a challenge, and even though a great deal has been learned about the pathogenic mechanisms of allergy, less is known about the underlying cause of disease or how this negative trend can be reversed.

CD4⁺ helper T cells play a key role in allergic disease by driving the anomalous Th2 mediated allergic immune responses in susceptible individuals. Signature cytokines of allergic diseases are IL-4 and IL-13, which instigate IgE production, mucus secretion and airway hyper-responsiveness, and IL-5 that promotes eosinophil infiltration and inflammation [1•]. Additionally, although atopic sensitization is associated with Th2 responses, other factors, including Th1 cytokines may sometimes have adverse effects on clinical manifestation of disease, which is to be considered when creating potential protocols for tolerance induction [2]. Current treatments of allergy and asthma are mainly based on pharmacological interventions with antihistamines or glucocorticoids, which in most cases effectively control the disease but without providing long-term relief or permanent cure. With recent advances in our understanding of various subsets of regulatory T cells (Tregs) including inducible IL-10-producing Tregs and thymically derived naturally occurring CD4⁺CD25⁺ Tregs, such therapeutic goals may now be within reach. CD4⁺Tregs have a unique role in the induction and maintenance of tolerance since they regulate both adaptive and innate immune responses and both Th2 as well as Th1 reactions which makes them an ideal target of immunotherapy. This review will highlight recent progress in the field of Tregs and allergic disease research and discuss the potential role of Tregs in current and future therapy of allergy.

CD4+CD25+Foxp3+ regulatory T cells

The majority of CD4⁺CD25⁺ Tregs (CD25⁺Tregs) emerge from the thymus and constitute 5–10% of peripheral CD4⁺ T cells in healthy mice and humans (Fig. 1). The suppressive mechanism of CD25⁺Tregs is unclear at present but is believed to be mainly cell-contact dependant *in vitro* although suppressive

Curr Opin Allergy Clin Immunol 6:482–488. © 2006 Lippincott Williams & Wilkins.

^aDepartment of Experimental Pathology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto and ^bCore Research for Evolutional Science and Technology (CREST) program, Science and Technology Agency of Japan, Kawaguchi, Japan

Correspondence to Kajsa Wing, Department of Experimental Pathology, Institute of Frontier Medical Sciences Shogoin 53, Kawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan

Tel: +81 75 751 3851; fax: +81 75 751 3820;
e-mail: kajsa@frontier.kyoto-u.ac.jp

Sponsorship: The authors are funded by grants from CREST (Core Research for Evolutional Science and Technology) and AstraZeneca.

Current Opinion in Allergy and Clinical Immunology 2006, 6:482–488

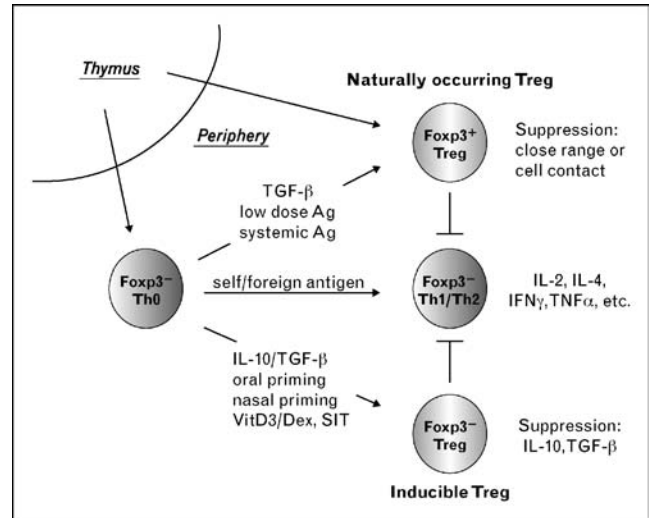
Abbreviations

IPEX	immune dysregulation polyendocrinopathy enteropathy-X-linked syndrome
SIT	specific allergen immunotherapy
TLR	toll-like receptor
Treg	regulatory T cell

© 2006 Lippincott Williams & Wilkins
1528-4050

Figure 1 Relationship of thymically and extrathymically generated regulatory T cells

T cells emerge from the thymus as either conventional naïve T cells (Th0) or fully functional *Foxp3*⁺ regulatory T cells (Tregs). Th0 cells undergo further differentiation and become Th1 or Th2 cells in the periphery after exposure to antigen. Stimulation of Th0 cells under specialized conditions of antigen priming can result in effector cells with a suppressive phenotype that either may or may not express *Foxp3*. Induced *Foxp3*⁺Tregs are virtually indistinguishable from thymically derived *Foxp3*⁺Tregs and similar to thymic Tregs they may suppress effector cells by either short-range or cell-contact dependent mechanisms even though the involvement of suppressive cytokines cannot be excluded. *Foxp3*[−] Tregs are recognized by their secretion of IL-10 or TGF- β and have for example been suggested to be generated in the healthy response to allergens in humans or after SIT therapy in allergic patients. Ag, Antigen; VitD3, vitamin D3; Dex, dexamethasone.



cytokines like IL-10 and TGF- β have been reported to play a role, particularly *in vivo* [3]. CD25⁺Tregs are best recognized by expression of the transcriptional regulator *Foxp3* (*FOXP3* in humans), which appears to serve as a master switch gene for Treg development and function. *Foxp3* expression closely correlates with CD4⁺CD25⁺ T cells in mice and to that of CD4⁺CD25^{high} cells in humans although *Foxp3*⁺CD25[−]/CD25^{low} cells with suppressive activity also exist. Other commonly used CD25⁺Treg markers are CTLA-4 (CD152) and GITR (glucocorticoid induced TNF family-related gene/protein), but neither these nor any other so far described surface markers are exclusively expressed by CD25⁺ Tregs, making it difficult to differentiate Tregs from other T cells, especially after activation [4]. This fact poses a particular problem for CD25⁺Treg research in humans and human disease and has led to inconclusive reports of CD25⁺Treg quantification in disease settings [5]. Moreover, although protocols that aim to purify or expand human CD25⁺Tregs for systemic therapy in patients have already been developed, the success and safety of these in the clinic may very well depend on highly selective and easy to use markers for human CD25⁺Treg [6–8]. Therefore much effort has been devoted to find surface markers that distinguish CD25⁺Tregs from activated nonregulatory T cells. One such marker is CD27, which in combination with CD25 expression was found to better define Treg in inflamed synovium of juvenile idiopathic arthritis patients than CD25 used alone [9]. CD27 is a member of the tumor necrosis factor receptor family and it is expressed by naïve and some memory T cells, but the expression is downregulated in terminally differentiated T cells. Why CD27 expression remains on activated CD25⁺Treg but not on conventional T cells is not known

and the molecule itself has so far not been implicated in the function of Tregs [10]. Overall, CD27 expression appears to be useful as a Treg marker only in a highly inflammatory environment but even so it should be noted that its expression is not exclusively confined to *FOXP3* expressing cells [11]. Recently, two separate groups reported CD127 (IL-7 receptor) expression to be inversely correlated to *FOXP3* expression in freshly isolated human adult and cord blood T cells [12*,13*]. If CD127 was co-stained with CD25 the expression level of *FOXP3* in the resulting CD127^{low}CD25⁺ population reached an average of 87%, although individual variation could be seen [12*]. The main advantage of using CD127 in combination with CD25 is that both *FOXP3*⁺CD25^{high} and *FOXP3*⁺CD25^{low} cells, which have so far been difficult to study, are included in the CD127^{low} cell population (see below).

The discovery of the importance of the *Foxp3* gene in Treg biology and later the possibility to detect *Foxp3* protein on a single cell level has vastly improved our knowledge of naturally occurring Tregs [14–16]. With *Foxp3* as a basis, elegant murine studies have begun to elucidate basic requirements of CD25⁺Treg development and homeostasis, such as their more or less absolute dependency on IL-2 in the periphery, the important role of CD28 during thymic development and the positive impact of TGF- β on *Foxp3* expression [17**,18**,19]. In humans, flow cytometric staining of *FOXP3* protein has shown that *FOXP3*⁺ T cells constitute 5–10% of CD4⁺ T cells in peripheral blood [20]. This is a far greater proportion of CD4⁺ T cells than the early estimations of approximately 2–3%, which were based on CD4⁺CD25^{high} Treg numbers and, in fact, more comparable to levels of CD25⁺Treg reported in

mice [21,22]. Many of these $Foxp3^+$ T cells express CD25 with lower intensity than traditional $CD25^{high}$ -Tregs, making them difficult to purify for functional studies. This fact raises several questions. For example, do functional and immunophenotypic studies based on human $CD25^{high}$ Tregs show the whole truth if large amounts of potential $FOXP3^+$ Treg are missing from Treg isolates? On the other hand, how do we even know that $FOXP3^+CD25^{dim}$ T cells are regulatory cells without reliable surface markers that enable functional assessment of them? In this respect, newly discovered markers such as CD127 might be useful. Finally, if these $FOXP3^+CD25^{dim}$ cells are not regulatory what are they? Recently, it was found that conventional in-vitro stimulation transiently induces $FOXP3$ expression in non-regulatory human $CD4^+$ and $CD8^+$ T cells, but without accompaniment of suppressive ability [23**]. Such transient expression has not been reported in mice and may, together with the production of a second splice variant of *Foxp3*, very well represent unique features of human T cells [24,25]. At the same time, a growing number of reports show that suppressive cells with similar phenotype and function as naturally occurring $CD25^+$ Tregs can be experimentally generated from peripheral $CD4^+CD25^-$ T cells in both mice and humans after antigen stimulation, an event especially evident in the presence of high levels of TGF- β (Fig. 1) [26–31]. Thus, one possibility is that *Foxp3* can be induced in more or less all T cells but certain circumstances are required for a regulatory phenotype to be induced and sustained. The significance of this extrathymic development remains to be elucidated but it points to a previously unappreciated plasticity in the $CD25^+$ Treg population that may be important for the induction of tolerance to self antigens and perhaps even more so to foreign antigen such as allergens.

The role of $CD25^+$ Tregs in allergic disease

The spontaneous development of allergic airway inflammation, hyper-IgE and eosinophilia in addition to various autoimmune syndromes in *Foxp3* mutant mice, and the subsequent rescue of these animals if transferred with wild type $CD25^+$ Tregs, provide compelling evidence for the important role of $CD25^+$ Treg in allergic disease [16,32*]. Humans suffering from immune dysregulation polyendocrinopathy enteropathy-X-linked syndrome (IPEX) are similarly affected and a majority of these patients also have some form of mutation in the *FOXP3* gene [25]. In contrast to models of *Foxp3* mutant mice, in which *Foxp3* message and $CD25^+$ Tregs are completely lacking, IPEX patients may sometimes but not necessarily always be deficient in $CD25^+$ Tregs [15,16,23**,32*,33**]. Rather it appears as if various *FOXP3* mutations in these patients can lead to heterogeneous immunological abnormalities that affect both the function of $CD25^+$ Treg and conventional

T cells [33**]. For example, while *Foxp3*-deficient mice display an upregulated production of both Th1 and Th2 cytokines, some IPEX patients instead produce less IL-2 and IFN- γ than normal donors. Since $CD25^+$ Tregs are highly dependent on the growth factors produced by effector cells this finding suggests that even if $CD25^+$ Tregs exist in IPEX patients they may not be optimally stimulated and therefore not properly functional *in vivo* [32*,33**]. Interestingly, one patient carrying a *FOXP3* mutation that did not result in autoimmunity still had elevated IgE levels and eczema, which illustrates that allergic disease, compared to autoimmune disease, is more easily triggered by immune dysregulation [33**].

Several studies have examined the role of $CD25^+$ Tregs in common allergic diseases. For example, children who had outgrown their cow's milk sensitivity had significantly higher levels of $CD4^+CD25^+$ T cells in their peripheral blood and were less responsive to stimulation with β -lactoglobulin *in vitro* than those who maintained a clinically active allergy [34]. $CD25^+$ Tregs have also been shown to suppress allergic responses to inhaled antigens such as grass or birch pollen [35–37]. Broadly speaking these studies together show that while healthy controls suppress proliferation and Th1/Th2 cytokine production, allergic individuals defectively inhibit Th2 but not Th1 responses to allergen and this is even more pronounced during the pollen season. Thus, $CD25^+$ Tregs in allergic patients may not be dysfunctional *per se* but their function may rather be affected by various genetic and environmental factors resulting in the dysregulation of Th2 responses to allergens.

The role of $CD25^+$ Tregs and adaptive Tregs in atopic dermatitis was recently investigated. One study found that the total number of $CD4^+CD25^+$ T cells but not *FOXP3* mRNA, increased in peripheral blood of atopic dermatitis patients compared with that of healthy controls and asthma patients, indicating that the increase in $CD25^+$ T cells represents activated effector T cells and not Tregs [38]. $FOXP3^+$ cells have also been studied in the skin of atopic dermatitis patients and while one study found plenty of $CD4^+CD25^+$ T cells but no expression of *FOXP3* protein, another study detected an accumulation of $FOXP3^+$ cells, which is usually not seen in healthy skin [39,40]. This is similar to previously published reports in other inflammatory conditions such as rheumatoid arthritis, for which $FOXP3^+$ cells increase in the afflicted joints [5]. Finally, various studies of allergic asthma in the DO11.10 transgenic mouse model have shown that $CD25^+$ Tregs can reduce the recruitment of Th2 cells and eosinophils as well as the secretion of IL-4, although the impact of $CD25^+$ Tregs on airway hyperreactivity appears to be more modest [41,42,43**,44**].

Adaptive Tregs in the allergic response

Recent findings suggest that allergen-specific IL-10 producing T cells with regulatory capacity may be adaptively induced as a component of the normal response to allergens in healthy individuals (Fig. 1) [45]. Moreover, IL-10 producing T cells have been shown to be induced following specific allergen immunotherapy in humans and in mouse models of tolerance induction after respiratory exposure to allergen [46–50]. The relationships between naturally occurring Foxp3^+ Tregs, the inducible Foxp3^+ Tregs aforementioned and the inducible IL-10 producing T cells is currently unclear. Adoptive transfer of ovalbumin-specific CD25^+ Treg to ovalbumin-sensitized recipients was shown to reduce clinical symptoms of asthma through an IL-10 dependant mechanism [44^{••}]. Interestingly, suppression was still evident when ovalbumin-specific CD25^+ Tregs from IL-10 deficient mice were transferred. The authors concluded that CD25^+ Tregs suppressed Th2 mediated responses via the induction of IL-10 production in conventional T cells [44^{••}]. Recent reports propose that inducible Tregs may be differently polarized, perhaps depending on the conditions of induction. That is, Tregs induced in the respiratory tract have been shown to express *IL-10*, *Foxp3* and *GATA3* or alternatively *TGF- β* and *Foxp3* [49,51]. In contrast, administration of ovalbumin together with heat-killed *Listeria monocytogenes* induced Tregs that expressed both *Foxp3* and *T-bet* and secreted IFN- γ as well as IL-10 [50]. It should be mentioned, however, that these induced Tregs were analyzed as bulk populations and not at a single cell level, making it difficult to definitely predict the expression pattern. In summary, tolerance to allergens seems to involve several types of Treg, both thymically derived and peripherally induced ones. Much work remains, however, to fully decipher the relationships between them and their respective roles in the response to allergens.

Regulatory T cells and intervention in allergic disease

During the course of allergic disease, three main points of intervention can be envisaged. The first point is to prevent a dysregulated Th2 response to allergens and this most probably has to be done very early in life. There is overwhelming consensus that the immune system is critically matured and tuned by interaction with commensal bacteria as well as other types of parasites and infections, and conceivably this also applies to the maintenance of Tregs [52,53]. Indeed, recent studies suggest that toll-like receptor (TLR) signalling is important for the function and homeostasis of CD25^+ Treg. MyD88-deficient mice have a lower frequency of Tregs than normal animals, and several TLRs, including TLR 2, 5 and 8, are expressed by CD25^+ Treg, which suggests that bacterial products can modulate Treg directly in addition to indirect effects via antigen presenting cells

[54^{••},55^{••},56[•]]. Interestingly, epidemiological studies show that Th2 cell stimulating helminths may counteract the development of allergies, possibly by induction of Treg [57]. This hypothesis was verified in an experimental model in which mice infected with the gastrointestinal nematode *Heligmosomoides polygyrus* were found to significantly suppress experimentally induced airway allergy to ovalbumin and the house dust mite allergen Der p 1, compared to uninfected controls [58^{••}]. Suppression was accompanied by increased levels of $\text{CD4}^+\text{CD25}^+\text{Foxp3}^+$ T cells, IL-10 and TGF- β . While depletion of CD25^+ T cells reversed suppression, however, IL-10 did not appear to play a role in this model. The antigen specificity of the resulting Treg population in helminth infected mice is not known but one reasonable speculation might be that the parasites cause a general and nonspecific expansion of naturally occurring CD25^+ Tregs, which alters the balance between Tregs and effector cells in favor of tolerance.

The second point of intervention is to block the already established Th2 response to allergens. This can be done either by specific allergen immunotherapy (SIT) or by glucocorticoid treatment. SIT has been around as an effective clinical treatment of IgE mediated diseases for almost a century but it is only recently that the mechanisms behind it have begun to be revealed. One important event might be the induction of allergen specific CD4^+ Tregs that produce IL-10 and perhaps also TGF- β [46,47,59,60]. These Tregs are primarily found among CD25^+ T cells but it is currently unclear how these cells are related to thymically derived FOXP3^+ Tregs. What can be said is that peripheral blood of SIT patients do not display an increase in either CD25^+ , $\text{CD25}^{\text{high}}$ cells or in the level of *FOXP3* expression, which suggests that SIT-induced Tregs might be CD25^+ effector cells that have deviated towards an IL-10 producing phenotype (H. Grindebacke, unpublished data) [46]. One possibility is that naturally occurring CD25^+ Treg somehow facilitate the induction of these IL-10 producing T cells, a phenomenon which has been reported to occur in mice in response to inhaled allergen [44^{••}]. Currently, attempts are being made to rid SIT of severe side effects like anaphylaxis and also shorten the long treatment period. One strategy is to use purer preparations of allergens such as specific peptides to avoid binding of allergen to IgE. So far, there are limited reports on the Treg response after peptide immunotherapy. One study found that the beneficial effect of peptide therapy was not directly dependent on CD25^+ Treg [61]. In another study by the same group, however, whole CD4^+ T cells were found to suppress cell division of CD4^- cells after cat peptide antigen treatment, indicating the induction of a Treg population [62]. Another strategy involves the use of adjuvants in combination with allergens which in mice can enhance induction of

Treg [50,63]. In humans, allergen conjugated to immunostimulatory CpG, which binds to TLR 9, seem to induce long lasting effects in spite of only a short treatment period [64,65]. Animal experiments show an immune deviation towards Th1, but the role of Tregs remains to be assessed [66]. In conclusion, the role of CD25⁺Tregs in SIT remains unclear and detailed studies of the interactions between CD25⁺FOXP3⁺Tregs, conventional CD4⁺ T cells and also antigen presenting cells are warranted.

Glucocorticoids are known to deliver short-term relief from clinical symptoms by downregulation of Th2 responses and the combination of dexamethasone and vitamin D3 was found to induce IL-10 production but not *FOXP3* expression in CD4⁺ T cells in mice and humans [1*,67,68*]. In contrast, another study [69] did detect enhanced *FOXP3* expression in CD4⁺ T cells, both *in vitro* and in asthmatic patients after treatment with steroids. Despite these effects, corticosteroids do not seem to alter the underlying disease mechanisms. A recent study [70*] in mice showed that corticosteroids interfere with production of adaptive Tregs after respiratory exposure with allergen, by abolishing the development of IL-10 producing dendritic cells, which are important for induction of IL-10 producing Tregs. This highlights the fact that corticosteroids have multiple effects on the immune system and that it is the net effect of all these that determines the outcome.

The third way to control the allergic response is to prevent the action of effector molecules such as cytokines, IgE or histamine. The implications on Tregs in this stage of the allergic pathway might be to lower the activation status of effector cells and thus allow for Treg suppression to take place. Recently, IL-6 was shown to play a role in allergic airway inflammation by both enhancing Th2 responses and hindering the expansion of CD25⁺Tregs [43**]. This suggests that anti-IL-6 therapy may be a potential way to not only control clinical manifestations but also to tip the balance in favor of permanent tolerance. Another interesting effector molecule is histamine. It seems that histamine can have several different effects depending on which type of histamine receptor (HR1, HR2) the target cells express. HR1 expressed by Th1 cells enhances their function while HR2 expressed by Th2 cells inhibits the production of Th2 cytokines [71*]. The expression pattern of histamine receptor on CD25⁺Tregs has yet to be reported but may indicate how Treg functionality is affected during allergic inflammation.

Conclusion

Induction or expansion of Tregs is an attractive therapeutic solution for allergic diseases, one that may already be here. In order to take advantage of the full potential of

Treg therapy, however, much remains to be learnt about how these cells develop, how they function and importantly what the respective roles of naturally occurring CD25⁺Tregs versus inducible Tregs are in allergic disease.

Acknowledgements

We thank our colleagues for allowing us to describe prepublication material and acknowledge the helpful discussions with Anna Rudin, Zoltan Fehervari and Masahiro Ono.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 501–504).

- 1 Hawrylowicz CM, O'Garra A. Potential role of interleukin-10-secreting regulatory T cells in allergy and asthma. *Nat Rev Immunol* 2005; 5: 271–283.
- This is an extensive review which focuses on the role of IL-10 and IL-10 producing Treg in allergic disease.
- 2 Heaton T, Rowe J, Turner S, Aalberse RC, *et al.* An immunoepidemiological approach to asthma: identification of in-vitro T-cell response patterns associated with different wheezing phenotypes in children. *Lancet* 2005; 365: 142–149.
- 3 Wing K, Fehervari Z, Sakaguchi S. Emerging possibilities in the development and function of regulatory T cells. *Int Immunol* 2006; 18:991–1000.
- 4 Sakaguchi S. Naturally arising Foxp3-expressing CD25⁺CD4⁺ regulatory T cells in immunological tolerance to self and nonself. *Nat Immunol* 2005; 6: 345–352.
- 5 Dejaco C, Duftner C, Grubeck-Loebenstein B, Schirmer M. Imbalance of regulatory T cells in human autoimmune diseases. *Immunology* 2006; 117: 289–300.
- 6 Earle KE, Tang Q, Zhou X, Liu W, *et al.* In vitro expanded human CD4⁺CD25⁺ regulatory T cells suppress effector T cell proliferation. *Clin Immunol* 2005; 115:3–9.
- 7 Hoffmann P, Boeld TJ, Eder R, Albrecht J, *et al.* Isolation of CD4⁺CD25⁺ regulatory T cells for clinical trials. *Biol Blood Marrow Transplant* 2006; 12: 267–274.
- 8 Hoffmann P, Eder R, Kunz-Schughart LA, Andreesen R, *et al.* Large-scale in vitro expansion of polyclonal human CD4⁺CD25^{high} regulatory T cells. *Blood* 2004; 104:895–903.
- 9 Ruprecht CR, Gattorno M, Ferlito F, Gregorio A, *et al.* Coexpression of CD25 and CD27 identifies FoxP3⁺ regulatory T cells in inflamed synovia. *J Exp Med* 2005; 201:1793–1803.
- The basic finding of this paper may be useful when studying CD25⁺Treg from sites of strong inflammation.
- 10 Koenen HJ, Fasse E, Joosten I. CD27/CFSE-based ex vivo selection of highly suppressive alloantigen-specific human regulatory T cells. *J Immunol* 2005; 174:7573–7583.
- 11 Oh U, Grant C, Griffith C, Fugo K, *et al.* Reduced Foxp3 protein expression is associated with inflammatory disease during human T lymphotropic virus type 1 infection. *J Infect Dis* 2006; 193:1557–1566.
- 12 Liu W, Putnam AL, Xu-Yu Z, Szot GL, *et al.* CD127 expression inversely correlates with FoxP3 and suppressive function of human CD4⁺ T reg cells. *J Exp Med* 2006; 203:1701–1711.
- This, and the paper by Seddiki *et al.* [13*] describe the novel Treg marker CD127 which may be useful for functional studies of freshly isolated human FOXP3⁺ Treg.
- 13 Seddiki N, Santner-Nanan B, Martinson J, Zaunders J, *et al.* Expression of interleukin (IL)-2 and IL-7 receptors discriminates between human regulatory and activated T cells. *J Exp Med* 2006; 203:1693–1700.
- This, and the paper by Liu *et al.* [12*], describe the novel Treg marker CD127, which may be useful for functional studies of freshly isolated human FOXP3⁺ Treg.
- 14 Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 2003; 299:1057–1061.
- 15 Khattri R, Cox T, Yasayko SA, Ramsdell F. An essential role for Scurfin in CD4⁺CD25⁺ T regulatory cells. *Nat Immunol* 2003; 4:337–342.

- 16 Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4⁺CD25⁺ regulatory T cells. *Nat Immunol* 2003; 4:330–336.
- 17 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* 2005; 6:152–162.
- This and the paper by Fontenot *et al.* [18*] systematically investigate the basic requirements for thymic development and peripheral maintenance of naturally occurring Treg.
- 18 Fontenot JD, Rasmussen JP, Gavin MA, Rudensky AY. A function for interleukin 2 in Foxp3-expressing regulatory T cells. *Nat Immunol* 2005; 6:1142–1151.
- This and the paper by Tai *et al.* [17*] systematically investigate the basic requirements for thymic development and peripheral maintenance of naturally occurring Treg.
- 19 Marie JC, Letterio JJ, Gavin MA, Rudensky AY. TGF-beta1 maintains suppressor function and Foxp3 expression in CD4⁺CD25⁺ regulatory T cells. *J Exp Med* 2005; 201:1061–1067.
- 20 Roncador G, Brown PJ, Maestre L, Hue S, *et al.* Analysis of FOXP3 protein expression in human CD4⁺CD25⁺ regulatory T cells at the single-cell level. *Eur J Immunol* 2005; 35:1681–1691.
- 21 Baecher-Allan C, Brown JA, Freeman GJ, Hafler DA. CD4⁺CD25⁺high regulatory cells in human peripheral blood. *J Immunol* 2001; 167:1245–1253.
- 22 Wing K, Ekmark A, Karlsson H, Rudin A, *et al.* Characterization of human CD25⁺ CD4⁺ T cells in thymus, cord and adult blood. *Immunology* 2002; 106:190–199.
- 23 Gavin MA, Torgerson TR, Houston E, DeRoos P, *et al.* Single-cell analysis of normal and FOXP3-mutant human T cells: FOXP3 expression without regulatory T cell development. *Proc Natl Acad Sci USA* 2006; 103:6659–6664.
- This study showed that FOXP3 expression can be transiently induced in human conventional T cells and that FOXP3 expressed at low levels not always correspond to anergic regulatory T cells. This observation may have wide implications on the interpretation of FOXP3 expression in humans.
- 24 Allan SE, Passerini L, Bacchetta R, Crellin N, *et al.* The role of 2 FOXP3 isoforms in the generation of human CD4⁺ Tregs. *J Clin Invest* 2005; 115:3276–3284.
- 25 Ziegler SF. FOXP3: of mice and men. *Annu Rev Immunol* 2006; 24:209–226.
- 26 Apostolou I, von Boehmer H. In vivo instruction of suppressor commitment in naive T cells. *J Exp Med* 2004; 199:1401–1408.
- 27 Knoechel B, Lohr J, Kahn E, Bluestone JA, *et al.* Sequential development of interleukin 2-dependent effector and regulatory T cells in response to endogenous systemic antigen. *J Exp Med* 2005; 202:1375–1386.
- 28 Wan YY, Flavell RA. Identifying Foxp3-expressing suppressor T cells with a bicistronic reporter. *Proc Natl Acad Sci USA* 2005; 102:5126–5131.
- 29 Chen W, Jin W, Hardegen N, Lei KJ, *et al.* Conversion of peripheral CD4⁺CD25⁺ naive T cells to CD4⁺CD25⁺ regulatory T cells by TGF-beta induction of transcription factor Foxp3. *J Exp Med* 2003; 198:1875–1886.
- 30 Walker MR, Carson BD, Nepom GT, Ziegler SF, *et al.* De novo generation of antigen-specific CD4⁺CD25⁺ regulatory T cells from human CD4⁺CD25⁺ cells. *Proc Natl Acad Sci USA* 2005; 102:4103–4108.
- 31 Walker MR, Kaspric DJ, Gersuk VH, Benard A, *et al.* Induction of FoxP3 and acquisition of T regulatory activity by stimulated human CD4⁺CD25⁺ T cells. *J Clin Invest* 2003; 112:1437–1443.
- 32 Lin W, Truong N, Grossman WJ, Haribhai D, *et al.* Allergic dysregulation and hyperimmunoglobulinemia E in Foxp3 mutant mice. *J Allergy Clin Immunol* 2005; 116:1106–1115.
- This study specifically evaluated the allergic inflammation in mice carrying a loss-of-function mutation in the *Foxp3* gene that recapitulates a known disease related mutation of an IPEX patient.
- 33 Bacchetta R, Passerini L, Gambineri E, Dai M, *et al.* Defective regulatory and effector T cell functions in patients with FOXP3 mutations. *J Clin Invest* 2006; 116:1713–1722.
- This paper presents a detailed evaluation of CD25⁺Treg function in four patients with FOXP3 mutations. It indicates a higher degree of complexity in human IPEX patients compared with *Foxp3* deficient or mutant mice.
- 34 Karlsson MR, Rugtveit J, Brandtzaeg P. Allergen-responsive CD4⁺CD25⁺ regulatory T cells in children who have outgrown cow's milk allergy. *J Exp Med* 2004; 199:1679–1688.
- 35 Ling EM, Smith T, Nguyen XD, Pridgeon C, *et al.* Relation of CD4⁺CD25⁺ regulatory T-cell suppression of allergen-driven T-cell activation to atopic status and expression of allergic disease. *Lancet* 2004; 363:608–615.
- 36 Grindebacke H, Wing K, Andersson AC, Suri-Payer E, *et al.* Defective suppression of Th2 cytokines by CD4⁺CD25⁺ regulatory T cells in birch allergies during birch pollen season. *Clin Exp Allergy* 2004; 34:1364–1372.
- 37 Bellinghausen I, Klostermann B, Knop J, Saloga J. Human CD4⁺CD25⁺ T cells derived from the majority of atopic donors are able to suppress TH1 and TH2 cytokine production. *J Allergy Clin Immunol* 2003; 111:862–868.
- 38 Ou LS, Goleva E, Hall C, Leung DY. T regulatory cells in atopic dermatitis and subversion of their activity by superantigens. *J Allergy Clin Immunol* 2004; 113:756–763.
- 39 Verhagen J, Akdis M, Traidl-Hoffmann C, Schmid-Grendelmeier P, *et al.* Absence of T-regulatory cell expression and function in atopic dermatitis skin. *J Allergy Clin Immunol* 2006; 117:176–183.
- 40 Caproni M, Torchia D, Antiga E, Volpi W, *et al.* The effects of tacrolimus ointment on regulatory T lymphocytes in atopic dermatitis. *J Clin Immunol* 2006.
- 41 Jaffar Z, Sivakuru T, Roberts K. CD4⁺CD25⁺ T cells regulate airway eosinophilic inflammation by modulating the Th2 cell phenotype. *J Immunol* 2004; 172:3842–3849.
- 42 Hadeiba H, Locksley RM. Lung CD25⁺ CD4⁺ regulatory T cells suppress type 2 immune responses but not bronchial hyperreactivity. *J Immunol* 2003; 170:5502–5510.
- 43 Doganci A, Eigenbrod T, Krug N, De Sanctis GT, *et al.* The IL-6R alpha chain controls lung CD4⁺CD25⁺ Treg development and function during allergic airway inflammation in vivo. *J Clin Invest* 2005; 115:313–325.
- This study described IL-6 as a novel factor in allergic disease that can drive Th2 cell proliferation and prevent CD25⁺Treg expansion. This suggested that anti-IL-6 treatment could be used to tip the balance in favor of tolerance.
- 44 Kearley J, Barker JE, Robinson DS, Lloyd CM. Resolution of airway inflammation and hyperreactivity after in vivo transfer of CD4⁺CD25⁺ regulatory T cells is interleukin 10 dependent. *J Exp Med* 2005; 202:1539–1547.
- This study showed that CD25⁺Treg can suppress Th2 responses to inhaled allergen by inducing IL-10 producing Treg, but that IL-10 production from CD25⁺Treg themselves are not needed for the tolerance induction.
- 45 Akdis M, Verhagen J, Taylor A, Karamloo F, *et al.* Immune responses in healthy and allergic individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. *J Exp Med* 2004; 199:1567–1575.
- 46 Francis JN, Till SJ, Durham SR. Induction of IL-10+CD4⁺CD25⁺ T cells by grass pollen immunotherapy. *J Allergy Clin Immunol* 2003; 111:1255–1261.
- 47 Jutel M, Akdis M, Budak F, Aebischer-Casaulta C, *et al.* IL-10 and TGF-beta cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. *Eur J Immunol* 2003; 33:1205–1214.
- 48 Gambineri E, Torgerson TR, Ochs HD. 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* 2003; 15:430–435.
- 49 Akbari O, Freeman GJ, Meyer EH, Greenfield EA, *et al.* Antigen-specific regulatory T cells develop via the ICOS-ICOS-ligand pathway and inhibit allergen-induced airway hyperreactivity. *Nat Med* 2002; 8:1024–1032.
- 50 Stock P, Akbari O, Berry G, Freeman GJ, *et al.* Induction of T helper type 1-like regulatory cells that express Foxp3 and protect against airway hyper-reactivity. *Nat Immunol* 2004; 5:1149–1156.
- 51 Ostroukhova M, Seguin-Devaux C, Oriss TB, Dixon-McCarthy B, *et al.* Tolerance induced by inhaled antigen involves CD4⁺ T cells expressing membrane-bound TGF-beta and FOXP3. *J Clin Invest* 2004; 114:28–38.
- 52 Bach JF. The effect of infections on susceptibility to autoimmune and allergic diseases. *N Engl J Med* 2002; 347:911–920.
- 53 Dunne DW, Cooke A. A worm's eye view of the immune system: consequences for evolution of human autoimmune disease. *Nat Rev Immunol* 2005; 5:420–426.
- 54 Peng G, Guo Z, Kiniwa Y, Voo KS, *et al.* Toll-like receptor 8-mediated reversal of CD4⁺ regulatory T cell function. *Science* 2005; 309:1380–1384.
- This and other papers describe the expression and role of various TLRs on CD25⁺Treg. These findings may have implications for the homeostasis and control of CD25⁺Treg. Potentially, this might lead to novel ways of intervening in the induction of allergic disease.
- 55 Suttmoller RP, den Brok MH, Kramer M, Bennink EJ, *et al.* Toll-like receptor 2 controls expansion and function of regulatory T cells. *J Clin Invest* 2006; 116:485–494.
- This and other papers describe the expression and role of various TLRs on CD25⁺Treg. These findings may have implications for the homeostasis and control of CD25⁺Treg. Potentially, this might lead to novel ways of intervening in the induction of allergic disease.

- 56 Crellin NK, Garcia RV, Hadisfar O, Allan SE, *et al.* Human CD4⁺ T cells express TLR5 and its ligand flagellin enhances the suppressive capacity and expression of FOXP3 in CD4⁺CD25⁺ T regulatory cells. *J Immunol* 2005; 175:8051–8059.
- This and other papers describe the expression and role of various TLRs on CD25⁺Treg. These findings may have implications for the homeostasis and control of CD25⁺Treg. Potentially, this might lead to novel ways of intervening in the induction of allergic disease.
- 57 Wilson MS, Maizels RM. Regulation of allergy and autoimmunity in helminth infection. *Clin Rev Allergy Immunol* 2004; 26:35–50.
- 58 Wilson MS, Taylor MD, Balic A, Finney CA, *et al.* Suppression of allergic airway inflammation by helminth-induced regulatory T cells. *J Exp Med* 2005; 202:1199–1212.
- This was the first study to show experimentally that helminth-infection counteracts allergic disease by enhancing Treg responses *in vivo*.
- 59 Gardner LM, Thien FC, Douglass JA, Rolland JM, *et al.* Induction of T 'regulatory' cells by standardized house dust mite immunotherapy: an increase in CD4⁺ CD25⁺ interleukin-10⁺ T cells expressing peripheral tissue trafficking markers. *Clin Exp Allergy* 2004; 34:1209–1219.
- 60 Akdis CA, Blesken T, Akdis M, Wuthrich B, *et al.* Role of interleukin 10 in specific immunotherapy. *J Clin Invest* 1998; 102:98–106.
- 61 Smith TR, Alexander C, Kay AB, Larche M, *et al.* Cat allergen peptide immunotherapy reduces CD4⁺ T cell responses to cat allergen but does not alter suppression by CD4⁺ CD25⁺ T cells: a double-blind placebo-controlled study. *Allergy* 2004; 59:1097–1101.
- 62 Verhoef A, Alexander C, Kay AB, Larche M. T cell epitope immunotherapy induces a CD4⁺ T cell population with regulatory activity. *PLoS Med* 2005; 2:e78.
- 63 Zuany-Amorim C, Sawicka E, Manlius C, Le Moine A, *et al.* Suppression of airway eosinophilia by killed *Mycobacterium vaccae*-induced allergen-specific regulatory T-cells. *Nat Med* 2002; 8:625–629.
- 64 Simons FE, Shikishima Y, Van Nest G, Eiden JJ, *et al.* Selective immune redirection in humans with ragweed allergy by injecting Amb a 1 linked to immunostimulatory DNA. *J Allergy Clin Immunol* 2004; 113:1144–1151.
- 65 Tulic MK, Fiset PO, Christodoulouopoulos P, Vaillancourt P, *et al.* Amb a 1-immunostimulatory oligodeoxynucleotide conjugate immunotherapy decreases the nasal inflammatory response. *J Allergy Clin Immunol* 2004; 113:235–241.
- 66 Tighe H, Takabayashi K, Schwartz D, Van Nest G, *et al.* Conjugation of immunostimulatory DNA to the short ragweed allergen amb a 1 enhances its immunogenicity and reduces its allergenicity. *J Allergy Clin Immunol* 2000; 106:124–134.
- 67 Barrat FJ, Cua DJ, Boonstra A, Richards DF, *et al.* In vitro generation of interleukin 10-producing regulatory CD4⁺ T cells is induced by immunosuppressive drugs and inhibited by T helper type 1 (Th1)- and Th2-inducing cytokines. *J Exp Med* 2002; 195:603–616.
- 68 Xystrakis E, Kusumakar S, Boswell S, Peek E, *et al.* Reversing the defective induction of IL-10-secreting regulatory T cells in glucocorticoid-resistant asthma patients. *J Clin Invest* 2006; 116:146–155.
- This study suggested that vitamin D3 can restore IL-10 production and enhance responsiveness to dexamethasone in glucocorticoid resistant asthma patients, and may thus have clinical implications.
- 69 Karagiannidis C, Akdis M, Holopainen P, Woolley NJ, *et al.* Glucocorticoids upregulate FOXP3 expression and regulatory T cells in asthma. *J Allergy Clin Immunol* 2004; 114:1425–1433.
- 70 Stock P, Akbari O, DeKruyff RH, Umetsu DT. Respiratory tolerance is inhibited by the administration of corticosteroids. *J Immunol* 2005; 175:7380–7387.
- This paper points to the adverse effects glucocorticoids may have on tolerance induction *in vivo*.
- 71 Akdis CA, Simons FE. Histamine receptors are hot in immunopharmacology. *Eur J Pharmacol* 2006; 533:69–76.
- This recent review gives a comprehensive background to the pleiotropic effects of histamine in inflammation and allergic disease.