Chapter 14

Foxp3 Expressing Regulatory T-Cells in Allergic Disease

Kayhan T. Nouri-Aria*

Abstract

Allergic diseases such as asthma, rhinitis and eczema are increasing in prevalence worldwide, in particular in industrialised countries affecting up to 20% of the population. Regulatory T-cells (Tregs) have been shown to be critical in T-cell homeostasis and in the maintenance of immune responses, such as prevention of autoimmunity and hampering allergic diseases. The so-called 'natural' $CD4^+CD25^+$ Tregs and/or IL-10-producing Tr1 cells have been shown to be responsible for the protection of immune tolerance and intact immune reactions following exposure to allergens such as aeroallergens or food allergens. In this regard, both cell-cell contact (through membrane bound TGF-β or via suppressive molecules such as CLTA-4) and soluble cytokine-(TGF-β and IL-10) dependent mechanisms have been shown to contribute to the ability of Tregs to operate effectively. The transcription factor Foxp3, a member of the forkhead-winged helix family, appears to be critical in the suppressive abilities of regulatory T-cells. Adoptive transfer of $CD4^+CD25^+$ Tregs from healthy to diseased animals corroborated and provided further evidence of the vital role of these populations in the prevention or cure of certain autoimmune conditions. Clinical improvement seen after allergen immunotherapy for allergic diseases such as rhinitis and asthma has also been associated with the induction of IL-10 and TGF-β producing Tr1 cells as well as Foxp3 expressing $CD4^+CD25^+$ T-cells, resulting in the suppression of Th2 cytokine milieu. Activation and expansion of antigen-specific $CD4^+CD25^+$ Tregs in vivo using adjuvants such as IL-10 or pharmacological agents such as low dose steroids or vitamin D3 could represent novel approaches to induce antigen-specific tolerance in immune-mediated conditions such as allergic asthma, autoimmune disease and the rejection of transplanted organs in man.

Introduction

The pursuit of regulatory T-cells was revived in the 1990s following the observation that athymic nude mice injected with $CD25^+$ -depleted $CD4^+$ cells developed multiorgan autoimmune diseases[1,2] and that these autoimmune incidents could be reversed by the adoptive transfer of $CD4^+CD25^+$ T-cells from healthy mice to the nude mice. This landmark observation provided robust evidence for the putative regulatory role of $CD4^+CD25^+$ T-cells in the control of immune responses and generated considerable interest in all aspects of basic and clinical immunology. The discovery of $CD4^+CD25^+$ Tregs in the peripheral blood and lymphoid tissues in mice was followed by similar findings in man.[3-6] Numerous in vitro studies on $CD4^+CD25^+$ T-cells revealed a critical role for these T populations as the regulator of immune responses in clinical settings including autoimmunity, tumour and microbial infection, transplantation and allergic diseases.

*Kayhan T. Nouri-Aria—Department of Allergy and Clinical Immunology, National Heart and Lung Institute at Imperial College London, Exhibition Road, London SW7 2AZ, England, UK. Email: k.nouri-aria@imperial.ac.uk

Forkhead Transcription Factors: Vital Elements in Biology and Medicine, edited by Kenneth Maiese.

Background

Natural regulatory T-cells (Tregs) constitutively express the high-affinity interleukin-2 (IL-2) receptor α-chain (CD25), a receptor which is crucial for IL-2 signalling and events leading to maintenance, homeostasis and function of Tregs cells in vivo. However, the main source of IL-2 for the survival of $CD4^+CD25^+$ Treg cells seems to be T-cells other than Tregs themselves present in close proximity to $CD4^+CD25^+$ Tregs within lymphoid organs.[7,8] Natural $CD4^+CD25^+$ Tregs are also equipped with a variety of cell surface molecules including cytotoxic T-lymphocyte antigen-4 (CTLA-4, or CD152) and glucocorticoid-induced tumour necrosis factor receptor (GITR), OX40 and programmed death-1 (PD-1) antigen.[9-11] Unlike CD25 which is upregulated on both newly activated and regulatory T-cells, the intensity of CD127 antigen on natural Tregs has been reported to inversely correlate with their suppressive ability, making CD127 unique amongst cell surface markers expressed on Tregs.[12] $CD4^+CD25^+$ Tregs also express high levels of LAG-3 (a CD4-related molecule that binds MHC class II) upon activation (Fig. 1). Tregs may also express CCR4 and CCR6, lymphoid homing receptors CD103 and CD62L and molecules such as perforin and granzyme A.[13] However, none of these surface markers are unique to natural Tregs, but combinations of these molecules would make useful surrogate markes for the functional ability of Tregs.

Thymic selection of $CD4^+CD25^+$ Tregs is regulated by the transcription factor *foxp3*,[13-15] a gene with a pivotal role in the development of functional Tregs. Foxp3 is a member of the forkhead-winged helix family of transcription regulators located on chromosome Xp11.23. Foxp3 full-length protein is encoded by 11 exons and contains a forkhead DNA-binding domain at the C terminus which can bind to the IL-2 promoter and repress IL-2 mRNA transcription.[16] Foxp3 is constitutively expressed at high levels on natural Tregs in both man and mice.[17] Foxp3 can activate or stifle other transcription factors e.g., T-bet (Th1) and STAT6 and GATA3 (Th2) (Table 1), signalling pathways or membrane expression of certain molecules in the periphery. *Scurfy* mice lacking Foxp3 are deficient in $CD4^+CD25^+$ Tregs and develop severe lymphoproliferative and autoimmune disease.[18]

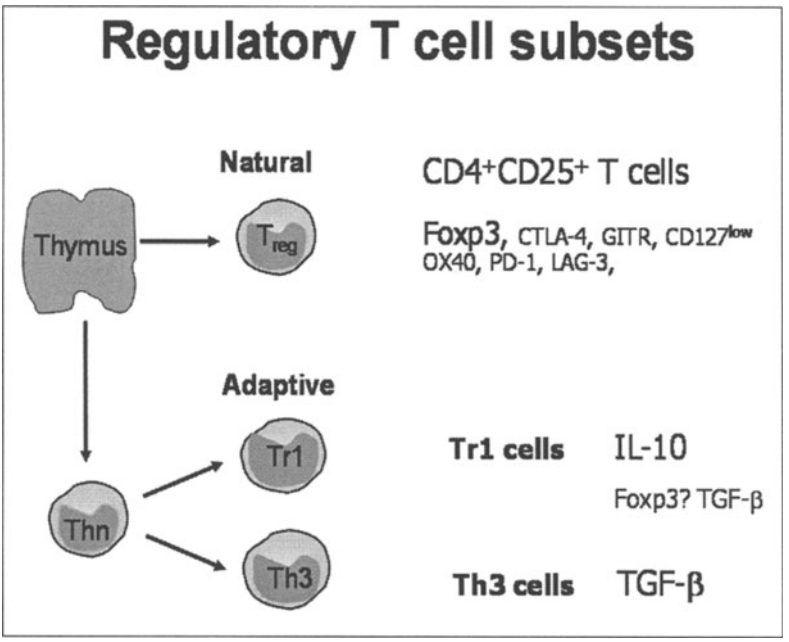

Figure 1. Regulatory T-cells (natural and adaptive-Tr1 and Th3). Cell surface markers, the transcription factor Foxp3 and their cytokines (IL-10 and TGF-β) are depicted. Reproduced with permission from Nouri-Aria KT, Durham Sr. Inflammation & Allergy-Drugs Targets, 2008; 7:237-252.

Table 1. T-cell subsets, their transcription factors and cytokines

Subset	Transcription Factor	Cytokines
Th1 (Autoimmunity)	T-bet, STAT1, STAT4	IFN-γ, IL-2, TNF-α
Th2 (Allergy and helminith infection)	GATA3, STAT6	IL-4, IL-5, IL-9, IL-13
Th17 (Inflammation and autoimmunity)	RORgt (mice), RORC2 (man)	IL-17, IL-25, IL-21, IL-22
Treg (Immunetolerance)	Foxp3 (mice), FOXP3 (man)	IL-10, TGF-β

Mutations in the *foxp3* gene in man result in IPEX syndrome, a condition with the spontaneous development of allergic airways inflammation, hyper IgE and eosinophilia which predominantly affect male offspring.[19] Conversely, the ectopic expression of Foxp3 phenotypically and functionally convert effector T-cells to Tregs with full regulatory function.[20] In mice, Foxp3 expression has been shown to be both necessary and sufficient for Tregs development.[21] Whereas in man there is increasing evidence demonstrating that Foxp3 is also transiently expressed in activated T-cells.[20,21] The observation that Foxp3 mRNA expression in newly activated $CD4^+CD25^+$ cells lacking regulatory function[22] may suggest that in humans Foxp3 alone is not sufficient to command regulatory activity of $CD4^+CD25^+$ cells.

The molecular mechanisms involved in the regulation of Foxp3 expression remain poorly understood. However, it has been established that the transcriptional activity of Foxp3 promoter is dependent on TCR signalling and several AP1- and NFAT-binding sites within the promoter.[23] The binding of STAT5 and STAT3 to conserved binding sites of the Foxp3 locus have also been implicated in the regulation of Foxp3 expression in man.[24] In both peripheral blood and tissues $Foxp3^+CD4^+CD25^+$ Tregs can be induced from $CD4^+CD25^-$ T-cells in the presence of TGF-β and interleukin-2 (IL-2).[25] Demethylation of DNA plays a critical role in the conversion of $CD4^+CD25^-$ T-cells into $Foxp3^+$ Tregs.[26] The mechanism of suppression used by Tregs remains contentious, nonetheless it has been postulated that TGF-β 1 may mediate the immunosuppressive activity of Tregs. In a recent report the in vitro induction of Foxp3 failed to upregulate *EBI3, p35* mRNA, or IL-35 secretion,[27] refuting the earlier suggestion that IL-35 contribute to the suppressive mechanism of human Treg.[28,29]

Regulatory T-Cells in Health and Allergic Conditions

In the last few decades allergic diseases such as asthma, atopic dermatitis and rhinitis have been increasing in prevalence worldwide and in particular in the industrialised countries. The prominent role of natural Tregs in preventing autoimmune diseases, rejection of solid organ transplants and uncontrolled tumour growth collectively suggest $CD4^+CD25^+$ Tregs may also play a critical role in hampering allergic diseases. A low proportion of Tregs and/or a defect in the ability of allergen specific Tregs may be responsible for the increase in the number of individuals with allergy seen in the past 30 years. Thus, better understanding of mechanisms of Tregs controlling Th2 responses, the characteristic feature of allergic conditions, may help in developing more effective therapeutic strategies for treatment of these diseases.

The role of Th2-driven immune responses has been decisively established in the development of allergic diseases. Nevertheless environmental factors and the genetic predisposition of allergic individuals are also believed to contribute, as cofactors, in the severity of these Th2 diseases.[30] Conversely, the lack of responses to allergens in nonatopic healthy individuals and the mechanisms by which such immune tolerance is induced and regulated are poorly understood. This chapter summarises in vitro and in vivo evidence for the functional abilities of regulatory T-cells in controlling effector T-cell responses (cell proliferation and cytokine production) in health and in a number of allergic disorders including asthma, allergic rhinitis and atopic dermatitis as well as in experimental models of allergies. It also discusses the

effects of treatment (glucocorticoids and allergen immunotherapy) on modification of Tregs and the possibility of manipulating these T-cell populations for treatment of allergic conditions.

Health

Despite constant exposure to aeroallergens or food allergens, healthy subjects maintain stable immune tolerance in their gastrointestinal and respiratory systems. The role of $CD4^+CD25^+$ Tregs in controlling immune responses to allergens in man was established[31] by the lack of proliferative responses to allergens and a trend towards cytokine profile of Tr1 cells in healthy volunteers. This was in sharp contrast to T-cell recognition of allergens resulting in elevated Th2 cytokine production (IL-4, IL-5 and IL-13) and heightened proliferative T-cell responses to allergens in atopic individuals.[32] These findings may imply that active immune surveillance by Treg populations operates in healthy nonatopic individual and is possibly absent in atopic subjects. The majority of allergen-specific T-cells in these healthy individuals were of IL-10-secreting Tr1 type. These observations support the notion that an impairment in the ability of Tregs to control exaggerated Th2 responses rather than an imbalance between Th2:Th1 responses, may exist in atopy. It would also provide a plausible explanation as to why no immunological responses are seen in nonatopic healthy volunteers following allergen exposure.

In line with these findings was the lack of proliferative T-cell responses to cows' milk antigen in healthy children. Depletion of $CD4^+CD25^+$ T-cells from PBMCs reversed T-cell recognition of cow's milk antigen, implying that the food tolerance to dietary antigens may be an active process imposed by $CD4^+CD25^+$ Tregs.[33] Contrary to these data, children who had outgrown cows' milk allergy revealed increases in the frequency of circulating $CD4^+CD25^+$ T-cells and decreased in vitro proliferative responses to bovine β-lactoglobulin (a milk protein) when compared with children who remained clinically milk sensitive.[33] A similar scenario of IL-10-associated T-cell-induced anergy has been reported in hyperimmune individuals i.e., bee keepers, who had received multiple bee stings. The intracytoplasmic $IL\text{-}10^+$ cells from these individuals were colocalized to $CD4^+CD25^+$ T-cells with specificity for bee venom antigens.[34]

Experimental Models

The strategic role of $CD25$ in the induction of $CD4^+$ Tregs in a murine model of allergic conjunctivitis was identified following the injection of anti-CD25 in thymectomized mice immunized with ragweed pollen. Subsequent allergen challenge in these mice resulted in severe allergic conjunctivitis as judged by conjunctival eosinophil numbers, ragweed-specific IgE and IgG1 levels, an increased proliferative response and Th2 cytokine production by splenocytes to ragweed allergen, confirming that thymus-derived $CD25^+$ T-cells are involved in the development and the regulation of allergic conjuctivitis. The adoptive transfer of $CD4^+CD25^+Foxp3^+$ T-cells from healthy, naïve mice into ragweed-sensitized mice, suppressed the development of allergic conjuctivitis, reinforcing the perception that Foxp3 expressing Tregs play a pivotal role in the regulation of allergic conditions.[35]

Foxp3 mutant mice, generated by means of knock-in mutagenesis developed an intense multiorgan inflammatory response associated with allergic airway inflammation, hyperimmunoglobulinemia E, eosinophilia and dysregulated Th1 and Th2 cytokine production in the absence of overt Th2 skewing, is consistent with the striking influence of Foxp3 in the development of allergic inflammation.[36]

The influence of IL-10 producing Tr1 cells governing Th2 type responses in experimental model of OVA challenged mice was established by the adoptive transfer of $IL\text{-}10^+CD4^+CD25^+$ cells from naive mice to OVA sensitized mice, resulting in the resolution of inflammatory responses in the bronchial mucosa.[37] This observation is consistent with the low concentration of IL-10 in the bronchoalveolar lavage fluid of adult asthmatic patients compared with healthy controls.[38] Neutralization studies using anti-IL-10 almost abolished the suppressive effect of Tr1 cells in vitro and further strengthened the role of this immunesuppressive cytokine in the control of Th2 responses and in the maintenance of T-cell homeostasis.

Similarly to IL-10, TGF-β is also involved in the control of atopic conditions. Thus in TGF-β knockout mice a higher susceptibility to bronchial hyperreactivity and bronchial inflammation resembling asthmatic reactions in man has been observed.[39] In a mouse model of food allergy, a significant reduction of secreted IgA antibodies, a class of immunoglobulin which is tightly regulated by TGF-β has been demonstrated in the gut. Subsequent recovery from food allergy in these mice was associated with local production of TGF-β.[40]

A failure of immune tolerance rather than a defective in Th1 immunity appears to underlie the immunobiology of Th2-driven allergen-induced airway disease in a mouse model of asthma. The reduction in the bronchial inflammation appears to be the consequence of regulatory processes involving dendritic cell-T-cell interactions. In contrast to OVA-induced murine models of asthma in which mice developed Th2-driven airway disease, the prolonged/three week OVA exposures resulted in the suppression of airway hypersensitivity. The mechanism underlying tolerance by chronic repetitive allergen (OVA) exposure in this rodent model included the recruitment of considerable numbers of Tregs expressing $CD4^+CD25^+Foxp3^+$ plus enhanced TGF-β1 production in the airways, despite tissue eosinophilia and high serum levels of, OVA-specific IgE and IgG_1. The resolution of airway hyperresponsiveness, tissue eosinophilia and Th2 cytokine profile were associated with the accumulation of Foxp3 expressing regulatory T-cells in local draining lymph nodes.[41,42] Understanding of mechanisms involved in airway tolerance to inhaled allergens could potentially help to improve treatment for allergic diseases and asthma.

In another study of murine model, immune tolerance was induced by repeated low-dose aerosolized OVA exposure. In this model, $CD4^+$ T-cells with regulatory effects expressed both cell surface and the soluble form of TGF-β and inhibited the development of an allergic phenotype when these cells were administrated to naive recipient mice challenged with the allergen. Although the blockade of TGF-β particularly interfered with immunosuppression, the severity of suppression was profound when $CD4^+$ T-cells were obtained from the tolerized mice expressing high levels of Foxp3.[43] These findings suggest that the cell surface expression of TGF-β rather than the secreted form of this immunosuppressive cytokine may be responsible for the effective inhibition and blunting the development of allergic responses. The study also suggested antigen-induced tolerance requires cell-cell contact with Tregs expressing Foxp3 and the expression of membrane bound TGF-β as the dominant component in the mechanisms of suppression of allergic responses.[43]

Allergic Rhinitis and Asthma

Allergic rhinitis, a debilitating allergic condition affecting up to 30% of populations in northern Europe and in the USA, is a Th2 predominant disease. The factors driving such Th2 responses have not been fully resolved and pathological mechanisms are unclear. It is however, conceivable that reductions in absolute numbers or a defect in function of the $Foxp3^+CD4^+CD25^+$ in circulation and/or in the nasal mucosa of allergic rhinitics[44] could, in parts, be responsible for the clinical manifestation and the high prevalence of this condition in the western world. In several in vitro studies, $CD4^+CD25^+$ T-cells from aeroallergen sensitive individuals (e.g., grass and birch pollens, cat and house dust mite) were found to be defective resulting in high proliferative responses and IL-5 secretion when $CD4^+CD25^+$ T-cells were cocultured with autologous allergen-stimulated $CD4^+CD25^-$ T-cells.[45-46] The dysregulation of $CD4^+CD25^+$ Tregs in the atopics controlling IL-13 and IL-5 production[32] was most pronounced during the peak of the birch and grass pollen seasons. In contrast, $CD4^+CD25^-$ T-cells from both allergic and nonallergic individuals were efficiently able to suppress T-cell proliferation and Th2 cytokine production to allergens outside of the pollen season with significant levels of Foxp3.

The frequencies of the $Foxp3^+$, $CD4^+CD25^+$ and $Foxp3^+CD4^+CD25^+$ populations were found to be more abundant in the nasal mucosa of healthy controls than in hay fever sufferers, whereas the concentration of IL-2 and IFN-γ secreted by PBMC in hay fever sufferers was significantly greater than in the control group, consistent with a defect in regulatory pathways in this clinical condition. The results further indicate that $CD4^+CD25^+$ Tregs as well as Foxp3 expressing cells may play a crucial

role in immunological imbalance in hay fever, suggesting that Foxp3$^+$CD4$^+$CD25$^+$ T-cells have the potential to act as a new therapeutic target for the treatment of this allergic disorder.[47]

Children with allergic disease have been shown to have fewer CD4$^+$CD25^{hi} T-cells than control subjects. Surprisingly, numbers of CD4$^+$CD25$^+$ and CD4$^+$CD25^{hi} T-lymphocytes were higher in children with persistent allergic rhinitis and/or moderate-severe bronchial asthma than in those with respective milder disease and the frequency of these cells were correlated with total serum immunoglobulin E level.[48] Foxp3 expression of CD4$^+$CD25$^+$ T-cells was elevated in moderate-severe versus mild asthma. Similarly, patients with moderate-severe bronchial asthma had increased expression of IL-10 compared with patients with mild asthma. The suppressive capability of Tregs from patients with more severe asthma appeared to be intact in vitro.[48] On balance, decreased numbers of Tregs in children with allergic airway disease may also represent a defect of the Treg function. The unexpected findings may however represent the recruitment of Foxp3$^+$IL-10$^+$ Tregs from the lymphoid organs to the target organ and/or circulation to combat and dampen of the ongoing allergic inflammation in the lungs of severe asthmatics.

The antigen specificity of Tregs and its influence in the induction and maintenance of tolerance remain contentious. Studies on Tregs from nonallergic healthy subjects demonstrated a profound inhibition of proliferation of effector cells stimulated with influenza antigen as well as birch pollen allergen. This was in sharp contrast with CD4$^+$CD25$^+$ cells from allergic rhinitis patients which were capable of dampening proliferative responses of T-cells to influenza antigen, but not birch pollen. Similarly, the regulation of Th2, but not Th1 cytokine production by CD4$^+$CD25$^+$ cells was impaired in allergic patients, upon stimulation with birch pollen extract. Neutralization of IL-10 led to increased production of IFN-γ and TNF-α in the nonatopic controls, substantiating a dysregulation of allergen-specific Foxp3 expressing CD4$^+$CD25$^+$ T-cells in atopic subjects. The elevated concentration of TNF-α following neutralization of IL-10, however suggests a pro-inflammatory role for this cytokine and that the IL-10 produced by Tregs is possibly involved in promoting tolerance.[49]

Further evidence for the existence of antigen specificity of Foxp3 expressing Tregs was provided by studies of allergen (Der-p 1)- specific and streptokinase (SK) specific-CD4$^+$CD25$^+$Foxp3$^+$ Tregs in the peripheral blood of atopic individuals.[50] CD4$^+$CD25$^+$Foxp3$^+$ T-cells from Der p 1- sensitive atopic individuals when cultured with DCs activated with Der p-1, but not those cultured with either unloaded or SK-loaded DCs, suppressed the proliferative responses of autologous CD4$^+$CD25$^-$ phenotype to Der p 1 or SK respectively.[50] These findings may also imply that the pool of human circulating CD4$^+$CD25^{high}Foxp3$^+$ T-cells consist of Treg populations specialised in the recognition of antigens with different specificities. It has been postulated that natural Tregs with a large repertoire for self-specific T-cell receptors suppress immune responses via contact-dependent mechanisms, whereas the inducible Tregs consist of both self- and nonself-specific cells recognising autoantigens as well as foreign antigens and the latter populations are capable of suppressing a wide range of immune cells via high concentrations of TGF-β 1 (possibly Th3 cells) or IL-10/TGF-β 1 producing Tr1 cells.[51] Although antigen contact is required by Tregs to induce a suppressive mechanism, once they are set in motion, subsequent suppression may not require antigens and can inhibit both in antigen specific and antigen nonspecific fashion.

Mucosal System

The induction of mucosal tolerance through the recruitment of Foxp3 expressing cells has been proposed as an alternative approach for the treatment of respiratory allergy. Long-term efficacy and mechanisms of mucosal tolerance induction were investigated by the means of an experimental model of birch pollen allergy. Two structurally diverse products of Bet v1 allergen i.e., unmodified native three-dimensional major BP allergen Bet v 1 and nonconformational hypoallergenic fragment were applied intranasally before- (prophylactic) or after sensitization (therapeutic) with birch pollen allergen.[52] Both native- and the modified fragment Bet v 1 allergen showed prophylactic and therapeutic effects. The immune tolerance induced with the native Bet v 1 allergen however was associated with the enhanced expression of TGF-β, IL-10 and Foxp3 expressing CD4$^+$ T-cells. These observations provide further evidence on antigen specificity of Tregs and suggest that the native and

conformational structure of antigens rather than modified fragment is an important component in the induction of appropriate immune regulatory effects by Tregs.[52] In this scenario Foxp3 seem to be the dominant molecule for the long-term efficacy of immunosuppression and in the dampening of immunopathology of birch allergy.

Epidemiological studies have indicated that infection with helminth parasites may counteract allergies, possibly by generating Tregs and suppression of the Th2 limb of immunity. To address whether the gastrointestinal nematode Heligmosomoides polygyrus was capable of down-regulating allergic reactions, a rodent model of OVA and house dust mite allergen—Der p 1-induced asthma was used.[53] The administration of the parasite induced suppression of inflammatory cell infiltrates in the lung, but was reversed if mice were treated with anti-CD25. The inhibition of bronchial hyperresponsiveness and airway inflammation was transferable with mesenteric lymph node cells (MLNC) from helminth infected animals to uninfected allergen sensitized mice. MLNC from infected animals showed significant numbers of $CD4^+CD25^+Foxp3^+$ T-cells, high expression of TGF-β and strong interleukin IL-10 responses to parasite antigen. Unexpectedly, MLNC from IL-10-deficient animals also transferred suppression to sensitized hosts, indicating that IL-10 per se may not be the primary suppressor of the allergic response.[52] These data support the contention that helminth infections can elicit a regulatory T-cell population capable of down-regulating allergen- induced lung pathology in vivo. Intervention studies with hookworm in parasite-naïve allergic individuals are currently ongoing in the United Kingdom to test these hypotheses further.[54]

Atopy

Maternal atopic status and the adaptive immune responses to microbial exposure at an early stage in life may decide the outcome of developing allergic disease or atopy during the childhood. Using cord blood mononuclear cells from 50 healthy neonates (31 nonatopic and 19 atopic mothers) and the innate TLR2 agonist peptidoglycan (Ppg) or the adaptive allergen house dust mite Dermatophagoides farinae (Der f 1)[55] as stimuli demonstrated that peptidoglycan was more able to induce high levels of IL-10, IFN-γ, IL-13 and TNF-α cytokine secretion and lymphocyte proliferation than Der f 1. Foxp3 and GITR expression of cord blood mononuclear cells (CBMC) and IL-10 production were also greater in CBMC from neonates without maternal atopy than those with maternal atopy. IL-10 production was highly correlated with the increased expression of Foxp3, GITR and CTLA4, independent of maternal atopy. The increased IL-10 and Foxp3 induction in cord blood mononuclear cells of nonatopic compared to atopic mothers and the induction of IL-10 producing Tregs via TLR2, suggest possible intrinsic defect in the induction of adaptive responses to microbial stimuli which may be associated with atopy.[55]

In a group of children with egg allergy, the ability of $CD4^+CD25^+CD127^{low}$ Treg cells in suppression of IFN-γ production by autologous $CD4^+$ effector T-cells in responses to staphylococcal endotoxin B revealed significantly less Treg cell-associated suppression in the allergic group compared with nonallergic children, although the proportion of circulating $CD4^+CD25^+CD127^{low}$ Treg and Foxp3 expressing cells were similar in both groups.[56]

Maternal atopy is also considered as a strong candidate predicting the development of childhood allergic diseases. Cord blood from offspring of atopic mothers showed fewer Lipid A peptidoglycan-induced $CD4^+CD25^{high}$ Treg cells, lower expression of GITR and Foxp3 and decreased IL-10 and IFN-γ secretion. In contrast IL-17 response to Lipid A was independent of maternal atopy and highly correlated with IL-13 secretion.[57] Similarly, mitogen-induced suppression of T effector cells in cord blood of offspring from atopic mothers was also impaired.[57] These findings imply that impairments in Foxp3 expressing Treg numbers and/or function may be a predisposing factor in the development of atopic diseases in childhood.

Atopic Dermatitis

The high levels of serum IgE in patients with atopic dermatitis was hypothesized to derive from a dysregulation of Tregs controlling IgE synthesis.[58] The frequency of circulating $Foxp3^+CCR4^+CLA^+$ cells was found to be greater in atopic dermatitis with highly elevated

serum IgE compared with low IgE levels with a strong association between Foxp3 expressing cells and the disease severity. $CD25^{hi}$ T-cells appeared to consist of two subsets based on the differential expression of the chemokine receptor CCR6. Although the ratio of $CCR6^+$ and $CCR6^-$ subtypes within the $CD25^{hi}$ subset were similar in atopic dermatitis, the intensity of CCR6 expression was strongly correlated with the suppressive ability of Tregs. $CCR6^-$ populations, in contrast, demonstrated functional characteristics of Th2 effector cells and synthesized large quantities of IL-5 in response to Staphylococcus aureus superantigen derived from the skin colonizing organism, possibly indicating the expression of CD25 on these populations merely represent cell activation.[57] Moreover, the $CCR6^+Foxp3^+CLA^+$ cells had greater suppressive abilities controlling proliferation of effector cells than $CCR6^-$ populations.

Studies on the cutaneous coexpression of Foxp3 and GITR on a panel of different inflammatory skin diseases using dual immunohistochemical staining revealed that Foxp3 and GITR were almost exclusively present on T-cells that express both CD4 and CD25 and were more prevalent in the inflammatory skin conditions than in healthy skin.[58] Similar findings were identified using peripheral blood $CD4^+$ T-cells co-expressing Foxp3 and GITR. In contrast to healthy volunteers whose biopsies showed low numbers of $Foxp3^+GITR^+$ T-cells, cutaneous $Foxp3^+$ T-cells in patients with spongiotic dermatitis, psoriasis and lichen planus showed a frequency of 25-29% and in patients with leishmaniasis this was ~15%.[58] These observations at a glance were suggestive of mechanisms of suppression via molecules of Foxp3 and GITR may be intact in these skin conditions. However, the recruitment of $Foxp3^+GITR^+$ T-cells in the inflamed skin may play a central role in the disease recovery, cessation of immune responses to invasive pathogens and the establishment of immunologic tolerance.

Analysis of Treg cells infiltrated in the skin conditions such as atopic dermatitis and psoriasis showed that $CD25^+$ cells were present in the perivascular and papillar dermis of all lesional specimens and $FoxP3^+$ cells were distributed in the perivascular and interstitial atopic dermatitis dermis. In atopic dermatitis and psoriatic skin, $CD4^+CD25^+FoxP3^+$ T-cells were absent in the lesional region and in the atopy patch test areas of the skin, despite the abundant expression of IL-10 and TGF-β as well as receptors for these cytokines in the dermis.[59] In contrast skin biopsies from healthy volunteers despite having few $Foxp3^+$ T-cells showed an even distribution through the dermis. Double immunostaining demonstrated that $CD25^+FoxP3^+$ cells were distributed in the perivascular, interstitial and periadnexal dermis, in contrast healthy skin specimens featured few $CD25^+$ $FoxP3^+$ cells scattered throughout the dermis. These findings suggest an impaired regulatory T-cell function rather than the absolute numbers of Tregs in the cutaneous lesions may play a key role in the immunopathology of atopic dermatitis.

Modulation of T_{Regs} with Treatment

Glucocorticoids

The administration of glucocorticoids, inhaled or systemic, has been reported to increase Foxp3 and IL-10 expression in the bronchial mucosa in patients with severe asthma. $Foxp3^+$ T-cells were tightly correlated with IL-10 expressing cells but not with the expression of TGF-β1, possibly suggesting the Tr1 nature of the Treg population. The frequency of $CD4^+CD25^+$ T-cells in circulation and the Foxp3 expression by $CD4^+$ T-cells was transient, but significantly greater in patients who received systemic glucocorticoid treatment. In vitro cultures of $CD4^+$ T-cells with corticosteroid induced upregulation of IL-10 and Foxp3 expression on these cells,[60-61] corroborates that glucocorticoids are not only potent immunosuppressor agents with anti-inflammatory effects. They are also capable of inducing the differentiation of $CD4^+$ T-cells towards a Foxp3 expressing Tr1 phenotype with suppressive consequences.

In asthmatic children, inhaled corticosteroid treatment was also associated with an increased proportion of $CD4^+CD25^{hi}$ T-cells in both peripheral blood and bronchial alveolar lavage fluid (BALF) and an improvement in suppression of proliferation and cytokine/chemokine production by $CD4^+CD25^-$ responder T-cells.[62] The role of immunosuppressive drugs, vitamin D3

and dexamethasone, in the induction of IL-10 producing regulatory T-cells from naive $CD4^+$ T-cells have been reported in man and mouse. The newly induced Tregs failed to synthesize IL-5 or IFN-γ, despite retaining strong proliferative capacity. The inhibition nuclear factor (NF)-κ B and activator protein (AP)-1 activities confirmed the influence of these two immunosuppressive agents in the development of Tregs.[60]

The population of $CD4^+CD25^{hi}$ Tregs and Foxp3 mRNA levels in peripheral blood and in the BALF of asthmatic children were found to be lower than in children with chronic cough and healthy control children.[63] Increased percentages of $CD4^+CD25^{hi}$ T-cells in peripheral blood and BALF were identified in asthmatic children after inhaled corticosteroid.[62] In contrast to the asthmatic group, isolated BALF and peripheral blood $CD4^+CD25^{hi}$ T-cells from non-asthmatic subjects suppressed the proliferation and cytokine as well as chemokine production by $CD4^+CD25^-$ responder T-cells. Corticosteroid treatment restored the regulatory activities of $CD4^+CD25^{hi}$ T-cells after inhalation, suggesting that the lung pathology seen in paediatric asthma, as with adult asthma, may stem from impaired regulatory T-cell control of Th2 responses. Pulmonary Tregs may also represent a therapeutic target in paediatric asthma.[63]

Specific Allergen Immunotherapy

The efficacy of allergen specific immunotherapy (SIT) in treatment of selected patients with IgE mediated diseases has been established.[64,65] SIT was initially described about 100 years ago[66] and involves subcutaneous administration of small but increasing doses of allergen using a relatively crude allergen extract.

To date SIT is the only treatment that can alter the natural course of allergic rhinitis, conjunctivitis and allergic reactions to stinging insects.[67] Conventional subcutaneous allergen immunotherapy prevents further allergen sensitisations and the development of asthma in patients with allergic rhinitis.[68] The clinical improvement following allergen immunotherapy is sustained for years after discontinuation, thus SIT is believed to modify the underlying immunological mechanisms of allergic responses.[69] The mechanisms by which allergen immunotherapy reduces allergic symptoms have been studied for decades. Induction of blocking antibody i.e., IgG4, a shift from Th2 to IFN-γ producing Th1 cytokine profile[70,71] and reductions in the numbers of effector cells such as eosinophils, mast cell and basophils in the target organ are amongst the immunological changes observed following successful SIT.

More recently induction of functional $CD4^+CD25^+$ Tregs[72] capable of attenuating allergen-induced proliferation of Th2 cells and their cytokine pattern have been reported with successful SIT. The intracellular IL-10 positive T-cells from patients who had completed a course of allergen immunotherapy was almost exclusively localized to $CD4^+CD25^+$ cells. IL-10-producing $CD4^+CD25^+$ regulatory T-cells have therefore emerged as potential mediators of immune tolerance following grass pollen immunotherapy.[72]

Two subsets of regulatory T-cells, IL-10-producing Type 1 regulatory T-cells and the natural $CD4^+CD25^+Foxp3^+$ Treg cells have been reported to play important roles in the control of allergic inflammation. Successful SIT dampens allergen-specific effector T-cells and activates uncommitted $CD4^+CD25^-$ phenotypes to possibly IL-10 secreting Tr1 populations. Tr1 cells suppress Th2 cells and effector cells of allergic inflammation, such as eosinophils, mast cells, basophils, through IL-10 and possibly TGF-β. Understanding of the mechanisms of $IL\text{-}10^+$ Tr1 cells may be helpful in developing new strategies for treatment of allergic diseases.[73,74]

The effect of house dust mite (HDM) specific immunotherapy on the induction of Tregs expressing markers such as Foxp3, CTLA4, IL-10 and TGF-β was studied using peripheral blood $CD4^+$ T-cells from both HDM sensitive asthmatic- and nonatopic children. This revealed a temporary increase in CTLA-4 at three months after SIT with no significant changes in IL-10 production or in the expression $Foxp3^+$ Tregs. Contrary to these findings were significant increases in TGF-β and Foxp3 expression by $CD4^+CD25^+$ Tregs and the associated clinical improvement following the completion of SIT at one year, suggesting that conventional SIT requires high concentrations of allergens to induce an effective clinical and immunological

tolerance, with TGF-β and Foxp3 as the two sensitive biomarkers for monitoring the response to immunotherapy.[75]

Using house dust mite-sensitive mice, peptide IT increased the number of $CD4^+CD25^+$ Tregs in the peripheral blood and the adoptive transfer of $CD4^+CD25^+$ Tregs precluded the induction of experimental allergic encephalomyelitis.[76] Although these $CD4^+CD25^+$ Tregs showed suppressive capabilities in vitro, their effects in vivo depended on the induction of antigen-specific IL-10 producing Tr-1 cells. As indicated above, Tr1 cells are likely to play a crucial role in the control of allergic disease with major effector mediator cytokines such as IL-10 and TGF-β.

The suppressive mechanisms of Tr1 cells not only involve cytokines IL-10 and TGF-β, but also molecules such as CTLA-4 and PD-1 antigens. IL-10 inhibits CD28 tyrosine phosphorylation and prevents the binding of phosphatidylinositol 3-kinase p85, hence reducing the costimulatory CD28 signalling pathway.[77] Induction of antigen-specific Tr1 cells can thus redirect inappropriate immune responses against allergens using a broad range of suppressor mechanisms.

The autocrine action of IL-10 and TGF-β is important the induction of peripheral T-cell tolerance and plays a crucial role in the mechanisms of allergen-SIT.[78,79] Reactivation of T-cells tolerized by IL-10 and TGF-β can result in the distinct pattern of either Th1 or Th2 cytokine profiles depending on the cytokine milieu in the target organ. Peptide presentation to the anergic T-cells was, however, fully restored in the presence of IL-2 or IL-15 as documented by the secretion of IFN-γ, but no IL-4 could be detected in this system suggesting that the suppression induced by Tregs are reversible.[79] Both IL-10 and TGF-β expressing cells have been reported to increase in the nasal mucosa of grass pollen IT treated patients, with a strong association between TGF-β expressed in the nasal mucosa and the levels of secretory IgA2 produced in the circulation, implying that the immunological changes are not only systemic, but occur in the target organ, i.e., nasal mucosa. Thus, in addition to their cellular inhibitory influence, IL-10 and TGF-β are the critical factors in switching from an inflammatory immunoglobulin E (IgE) to the noninflammatory isotypes IgG4 and IgA respectively, the two classes of Ig with significant values in the outcome of SIT treatment and protection of mucosal surfaces.[78,79]

Another crucial change seen after successful allergen-SIT is a shift in the balance from IgE to IgG, in particular to IgG4 subclass, the latter being under the regulation of IL-10. Two independent studies have reported that the increased IL-10- and TGF-β production by Tregs in vivo may endorse that high and increasing doses of allergens administrated during the course of grass pollen IT are responsible for the proliferation and activation of Tr1 populations. Jutel et al reported the suppression induced by $CD4^+CD25^+Tr1^+$ cells was partially blocked by neutralization of antibodies against secreted forms or membrane-bound IL-10 and TGF-β.[80]

Regulatory T-cells are thought to play an important role in allergic diseases and tolerance induction during specific immunotherapy. In a recent publication, significant numbers of $CD4^+CD25^+$Foxp3 expressing cells were identified in the nasal mucosa of allergic rhinitics who had completed a successful course of SIT. Seasonal increases in $CD4^+Foxp3^+$ and $CD25^+Foxp3^+$ cells in these patients were accompanied by suppression of local allergic inflammation, indicative of the development and differentiation of a regulatory T-cell phenotype post immunotherapy. $CD25^+IL\text{-}10^+$ T-cells were both Foxp3 positive and Foxp3 negative and co-existed in a close microenvironment within the nasal mucosa, providing evidence for the emergence of phenotypically and functionally distinct populations of regulatory cells i.e., "adaptive" Foxp3 expressing Tregs and IL-10 expressing "Tr1" cells following SIT.[81,45] Similar observations have been reported in patients with inflammatory bowel disease, in whom IL-10 producing $Foxp3^+CD4^+CD25^+$ cells were present at increased density in the colon and the presence of IL-10 expressing Tregs were associated with amelioration of colitis.[82] These findings encourage strategies which augment numerically and/or functionally Tregs locally which would be beneficial in the treatment of allergic rhinitis.

Venom Immunotherapy

Venom immunotherapy (VIT) induces long-lasting immune tolerance to hymenoptera venom antigens; however, the underlying mechanisms have yet to be clarified. In a longitudinal

study VIT induced a significant progressive increase in percentage and absolute numbers of $CD25^{bright}Foxp3^{+}$ $CD4^{+}$ regulatory T-cells, with the particular effect on Foxp3 confirmed both at mRNA and protein levels.[83] These changes were unrelated to alterations in the expression of activation markers or imbalances in the naïve/memory T-cell compartments. Interestingly, the increase in the circulating $Foxp3^{+}$Tregs were correlated with a shift from the venom-specific IgE to IgG4, corroborating the findings in grass pollen IT in allergic rhinitis. VIT is also associated with a progressive expansion of circulating regulatory T-cells as defined by high expression of CD25 and/or $CD4^{+}Foxp3^{+}$ T-cells, supporting a role for these cells in the induction of tolerance and unresponsiveness to subsequent venom exposure similar to the phenomenon of natural sting tolerance in bee keepers. Wasp venom- or phospholipase A2-pulsed dendritic cell stimulation of $CD4^{+}CD25^{-}$ T-cells from healthy donors resulted in inhibition of proliferation and Th2 cytokine production by Tregs at 10-fold lower than the optimal concentration. In contrast, IFN-γ production was inhibited at all concentrations, suggesting that the threshold of response is different between allergic and nonallergic individuals.[84]

Less is known about the underlying molecular mechanisms of TGF-β-mediated suppression in allergen immunotherapy. Blocking of CTLA-4 was associated with decreased TGF-β levels within the bronchoalveolar lavage fluid of a murine model of allergic inflammation.[85] TGF-β has been recognized to deviate antibody response from an IgE to an IgA-dominated response in man and in mice post IT.

Based on these observations and the decreased Treg cell populations as possibly one major cause of allergic diseases, the upregulation of Tregs numerically or functionally gives promise of therapeutic potential in the treatment of allergic diseases. Novel strategies should be adopted to improve the clinical efficacy of IT using adjuvants such as IL-10, vitamin D3, or TLR agonist such as CpG. Mycobacterium induced allergen-specific Tregs producing IL-10 and TGF-β protected against airway inflammation in mice.[86-89] The application of adenoviral vectors encoding IL-10 also resulted in a longer suppressive effect, with a very limited half-life of IL-10, hence with less or no side effects. Alternatively immunostimulatory CpG motifs, an agonist of TLR9, may improve clinical efficacy when combined with pollen immunotherapy.

Conclusion

Natural $CD4^{+}CD25^{+}$ Tregs play a critical role in the control of peripheral tolerance to self-antigens and in prevention of allergic diseases including rhinitis, atopic dermatitis and asthma. $CD4^{+}CD25^{+}Foxp3^{+}$ Tregs and IL-10 producing Tr1 cells capable of suppressing Th2 responses to allergens seem to be defective in those who develop allergic sensitization. Significant progress has been made in understanding specific mechanisms resulting in allergic inflammation and IL-4 may be a key factor in preventing the de novo induction of Treg cells and re-induction of allergen tolerance. However the exact mechanism of suppression remains controversial. Better understanding of regulatory mechanisms involved in the development of allergic sensitization and the manipulation of Treg cells holds the promise of effective treatment strategies to prevent and treat allergic diseases. Allergen immunotherapy modifies T-cell responses to allergen and may do so through induction of adaptive Tregs, e.g., IL-10—producing Tr1 cells contributing to the clinical efficacy of the treatment in aeroallergen sensitive individuals. Allergen immunotherapy may enhance the development of allergen-specific Tregs and provide safe, specific and long-term control of allergic diseases and asthma. Co-administration of specific allergen immunotherapy with drugs such as corticosteroids and vitamin D_3, adjuvants, for example IL-10 or CpG, are promising candidates for enhancing and generating antigen specific regulatory responses.

Adoptive transfer of Tregs may represent an effective, donor-specific therapeutic approach, although it can also be cost effective. In vivo induction and/or expansion of Tregs in patients remain an attractive option. This may present a more realistic line to improve allergen specific Tregs and provide significant benefit to allergic rhinitis and asthmatic patients.

Acknowledgement

The author is grateful to Drs. Guy Scadding and Carsten Schmidt-Weber for the reading of the manuscript.

References

1. Sakaguchi S, Sakaguchi N, Asano M et al. Immunologic self-tolerance maintained by activated T-cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol 1995; 155:1151-1164.
2. Asano M, Toda M, Sakaguchi N et al. Autoimmune disease as a consequence of developmental abnormality of a T-cell subpopulation. J Exp Med 1996; 184:387-396.
3. Ng WF, Duggan PJ, Ponchel F et al. Human $CD4^+CD25^+$ cells: a naturally occurring population of regulatory T-cells. Blood 2001; 98:2736-2744.
4. Jonuleit H, Schmitt E, Stassen M et al. Identification and functional characterization of human $CD4^+CD25^+$ T-cells with regulatory properties isolated from peripheral blood. J Exp Med 2001; 193:1285-1294.
5. Dieckmann D, Plottner H, Berchtold S et al. Ex vivo isolation and characterization of $CD4^+CD25^+$ T-cells with regulatory properties from human blood. J Exp Med 2001; 193:1303-1310.
6. Levings MK, Sangregorio R, Roncarolo MG. Human $CD25^+CD4^+$ T regulatory cells suppress naive and memory T-cell proliferation and can be expanded in vitro without loss of function. J Exp Med 2001; 193:1295-1302.
7. Thornton AM, Shevach EM. $CD4^+CD25^+$ immunoregulatory T-cells suppress polyclonal T-cell activation in vitro by inhibiting interleukin 2 production. J Exp Med 1998; 188:287-296.
8. Bayer AL, Yu A, Adeegbe D et al. Essential role for interleukin-2 for $CD4^+CD25^+$ T regulatory cell development during the neonatal period. J Exp Med 2005; 201:769-777.
9. Read S, Malmstrom V, Powrie F. Cytotoxic T-lymphocyte-associated antigen 4 plays an essential role in the function of $CD25^+CD4^+$ regulatory cells that control intestinal inflammation. J Exp Med 2000; 19:295-302.
10. Graca L, Honey K, Adams E et al. Cutting edge: anti-CD154 therapeutic antibodies induce infectious transplantation tolerance. J Immunol 2000; 165:4783-4786.
11. McHugh RS, Whitters MJ, Piccirillo CA et al. $CD4^+CD25^+$ immunoregulatory T-cells: gene expression analysis reveals a functional role for the glucocorticoid-induced TNF receptor. Immunity 2002; 1:311-323.
12. Liu W, Putnam AL, Xu-Yu Z et al. CD127 expression inversely correlates with FoxP3 and suppressive function of human $CD4^+$ Treg cells. J Exp Med 2006; 203:1701-1711.
13. Nouri-Aria KT, Durham SR. Regulatory T-cells and allergic disease. Inflamm Allergy Drug Targets 2008; 7:237-52. Review.
14. Hori S, Nomura T, Sakaguchi S. Control of regulatory T-cell development by the transcription factor Foxp3. Science 2003; 299:1057-1061.
15. Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of $CD4^+CD25^+$ regulatory T-cells. Nat Immunol 2003; 4:330-336.
16. Brandenburg S, Takahashi T, de la Rosa M et al. IL-2 induces in vivo suppression by $CD4^+CD25^+Foxp3^+$ regulatory T-cells. Eur J Immunol 2008; 38:1643-1653.
17. Lin W, Haribhai D, Relland LM et al. Regulatory T-cell development in the absence of functional Foxp3. Nat Immunol 2007; 8:359-368.
18. Khattri R, Cox T, Yasayko SA et al. An essential role for Scurfin in $CD4^+CD25^+$ T regulatory cells. Nat Immunol 2003; 4:337-342.
19. Bennett CL, Christie J, Ramsdell F et al. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. Nat Genet 2001; 27:20-21.
20. Yagi H, Nomura T, Nakamura K et al. Crucial role of FOXP3 in the development and function of human $CD25^+CD4^+$ regulatory T. Int Immunol 2004; 16:1643-1656.
21. Ziegler SF. FOXP3: of mice and men. Annu Rev Immunol 2006; 24:209-226. Review.
22. Schmidt-Weber CB, Blaser K. The role of the FOXP3 transcription factor in the immune regulation of allergic asthma. Curr Allergy Asthma Rep 2005; 5:356-361.
23. Mantel PY, Ouaked N, Ruckert B et al. Molecular mechanisms underlying FOXP3 induction in human T-cells. J Immunol 2006; 176:3593-3602.
24. Zorn E, Nelson EA, Mohseni M et al. IL-2 regulates FOXP3 expression in human $CD4^+CD25^+$ regulatory T-cells through a STAT dependent mechanism and induces the expansion of these cells in vivo. Blood 2006; 108:1571-1579.

25. Fontenot JD, Rasmussen JP, Gavin MA et al. A function for interleukin 2 in Foxp3-expressing regulatory T-cells. Nat Immunol 2005; 6:1142-1151.
26. Janson PC, Winerdal ME, Marits P et al. FOXP3 promoter demethylation reveals the committed Treg population in humans. PLoS ONE 2008; 3:e1612.
27. Allan SE, Song-Zhao GX, Abraham T et al. Inducible reprogramming of human T-cells into Treg cells by a conditionally active form of FOXP3. Eur J Immunol 2008; 38:3282-3289.
28. Bardel E, Larousserie F, Charlot-Rabiega P. Human CD4$^+$CD25$^+$Foxp3$^+$ regulatory T-cells do not constitutively express IL-35. J Immunol 2008; 181:6898-6905.
29. Collison LW, Workman CJ, Kuo TT et al. The inhibitory cytokine IL-35 contributes to regulatory T-cell function. Nature 2007; 450:566-569.
30. Cottrez F, Hurst SD, Coffman RL et al. T regulatory cells 1 inhibit a Th2-specific response in vivo. J Immunol 2000; 165:4848-4853.
31. Akdis M, Verhagen J, Taylor A 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.
32. Ling EM, Smith T, Dao Nguyen X 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.
33. 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.
34. Akdis M, Blaser K, Akdis CA. T regulatory cells in allergy. Chem Immunol Allergy 2006; 91:159-173. Review.
35. Sumi T, Fukushima A, Fukuda K et al. Thymus-derived CD4$^+$ CD25$^+$ T-cells suppress the development of murine allergic conjunctivitis. Int Arch Allergy Immunol 2007; 143:276-81.
36. Lin W, Truong N, Grossman WJ et al. Allergic dysregulation and hyperimmunoglobulinemia E in Foxp3 mutant mice. J Allergy Clin Immunol 2005; 116:1106-15.
37. Van Hove CL, Maes T, Joos GF et al. Prolonged inhaled allergen exposure can induce persistent tolerance. Am J Respir Cell Mol Biol 2007; 36:573-84.
38. Borish L. IL-10: evolving concepts. J Allergy Cli Immunol 1998; 101:293-297.
39. Scherf W, Burdach S, Hansen G. Reduced expression of transforming growth factor beta 1 exacerbates pathology in an experimental asthma model. Eur J Immunol 2005; 35:198-206.
40. Frossard CP, Hauser C, Eigenmann PA. Antigen-specific secretory IgA antibodies in the gut are decreased in a mouse model of food allergy. J Allergy Clin Immunol 2004; 114:377-382.
41. Burchell JT, Wikstrom ME, Stumbles PA et al. Attenuation of allergen-induced Airway Hyperresponsiveness is mediated by airway regulatory T-cells. Am J Physiol Lung Cell Mol Physiol 2008. (Epub ahead of print)
42. Carson WF 4th, Guernsey LA, Singh A et al. Accumulation of regulatory T-cells in local draining lymph nodes of the lung correlates with spontaneous resolution of chronic asthma in a murine model. Int Arch Allergy Immunol 2008; 145:231-43.
43. Ostroukhova M, Seguin-Devaux C, Oriss TB 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.
44. Stock P, DeKruyff RH, Umetsu DT. Inhibition of the allergic response by regulatory T-cells. Curr Opin Allergy Clin Immunol 2006; 6:12-6. Review.
45. 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.
46. Grindebacke H, Wing K, Andersson AC et al. Defective suppression of Th2 cytokines by CD4CD25 regulatory T-cells in birch allergics during birch pollen season. Clin Exp Allergy 2004; 3:1364-1372.
47. Xu G, Mou Z, Jiang H et al. A possible role of CD4$^+$CD25$^+$ T-cells as well as transcription factor Foxp3 in the dysregulation of allergic rhinitis. Laryngoscope 2007; 117:876-80.
48. Lee JH, Yu HH, Wang LC et al. The levels of CD4$^+$CD25$^+$ regulatory T-cells in paediatric patients with allergic rhinitis and bronchial asthma. Clin Exp Immunol 2007; 148:53-63.
49. Thunberg S, Akdis M, Akdis CA et al. Immune regulation by CD4$^+$CD25$^+$ T-cells and interleukin-10 in birch pollen-allergic patients and non-allergic controls. Clin Exp Allergy 2007; 37:1127-1136.
50. Maggi L, Santarlasci V, Liotta F et al. Demonstration of circulating allergen-specific CD4$^+$CD25highFoxp3$^+$ T-regulatory cells in both nonatopic and atopic individuals. J Allergy Clin Immunol 2007; 120:429-436.
51. Nagato T, Kobayashi H, Yanai M et al. Functional analysis of birch pollen allergen Bet v 1-specific regulatory T-cells. J Immunol 2007; 178:1189-1198.
52. Winkler B, Hufnagl K, Spittler A et al. The role of Foxp3$^+$ T-cells in long-term efficacy of prophylactic and therapeutic mucosal tolerance induction in mice. Allergy 2006; 61:173-80.

53. Wilson MS, Taylor MD, Balic A et al. Suppression of allergic airway inflammation by helminth-induced regulatory T-cells. J Exp Med 2005; 202:1199-212.
54. Flohr C, Quinnell RJ, Britton J. Do helminth parasites protect against atopy and allergic disease? Clin Exp Allergy 2009; 39:20-32.
55. Schaub B, Liu J, Höppler S et al. Impairment of T-regulatory cells in cord blood of atopic mothers. J Allergy Clin Immunol 2008; 121:1491-9.
56. Smith M, Tourigny MR, Noakes P et al. Children with egg allergy have evidence of reduced neonatal $CD4^+CD25^+CD127^{lo/-}$ regulatory T-cell function. J Allergy Clin Immunol 2008; 121:1460-6.
57. Schaub B, Campo M, He H et al. Neonatal immune responses to TLR2 stimulation: influence of maternal atopy on Foxp3 and IL-10 expression. Respir Res 2006; 21:7:40-48.
58. Reefer AJ, Satinover SM, Solga MD et al. Analysis of $CD25^{hi}CD4^+$ "regulatory" T-cell subtypes in atopic dermatitis reveals a novel TH2-like population. J Allergy Clin Immunol 2008; 121:415-422.
59. Caproni M, Antiga E, Torchia D et al. FoxP3-expressing T regulatory cells in atopic dermatitis lesions. Allergy Asthma Proc 2007; 28:525-528.
60. de Boer OJ, van der Loos CM, Teeling P et al. Immunohistochemical analysis of regulatory T-cell markers FOXP3 and GITR on $CD4^+CD25^+$ T-cells in normal skin and inflammatory dermatoses. J Histochem Cytochem 2007; 55:891-898.
61. O'Garra A, Barrat FJ, Castro AG et al. Strategies for use of IL-10 or its antagonists in human disease. Immunol Rev 2008; 223:114-131.
62. Xystrakis E, Kusumakar S, Boswell S 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.
63. Hartl D, Koller B, Mehlhorn AT et al. Quantitative and functional impairment of pulmonary $CD4^+CD25^{hi}$ regulatory T-cells in pediatric asthma. J Allergy Clin Immunol 2007; 119:1258-66.
64. Durham SR. Allergen immunotherapy (desensitisation) for allergic diseases. Clin Med 2006; 6:348-351.
65. Nelson HS. Advances in upper airway diseases and allergen immunotherapy. J Allergy Clin Immunol 2007; 119:872-880.
66. Noon L. Prophylactic inoculation against hay fever. Lancet 1911; 1:1572-1573.
67. Frew AJ, Powell RJ, Corrigan CJ et al. Efficacy and safety of specific immunotherapy with SQ allergen extract in treatment-resistant seasonal allergic rhinoconjunctivitis. J Allergy Clin Immunol 2006; 117:319-325.
68. Walker SM, Pajno GB, Lima MT et al. Grass pollen immunotherapy for seasonal rhinitis and asthma: a randomized, controlled trial. J Allergy Clin Immunol 2001; 107:87-93.
69. Akdis CA, Blesken T, Akdis M et al. Role of interleukin 10 in specific immunotherapy. J Clin Invest 1998; 102:98-106.
70. Durham SR, Walker SM, Varga EM et al. Long-Term Clinical Efficacy of Grass-Pollen Immunotherapy. New Engl J Med 1999; 341:468-475.
71. Wachholz PA, Nouri-Aria KT, Wilson DR et al. Grass pollen immunotherapy for hayfever is associated with increases in local nasal but not peripheral Th1:Th2 cytokine ratios. Immunology 2002; 105:56-62.
72. Till SJ, Francis JN, Nouri-Aria K et al. Mechanisms of immunotherapy. J Allergy Clin Immunol 2004; 113:1025-1034. Review.
73. Robinson DS, Larché M, Durham SR. Tregs and allergic disease. J Clin Invest 2004; 114:1389-1397. Review.
74. Verhagen J, Taylor A, Blaser K et al. T regulatory cells in allergen-specific immunotherapy. Int Rev Immunol 2005; 24:533-548. Review.
75. Ajduk J, Marinic I, Aberle N et al. Effect of house dust mite immunotherapy on transforming growth factor beta1-producing T-cells in asthmatic children. Ann Allergy Asthma Immunol 2008; 100:314-22.
76. Mekala DJ, Alli RS, Geiger TL. IL-10-dependent infectious tolerance after the treatment of experimental allergic encephalomyelitis with redirected $CD4^+CD25^+$ T-lymphocytes. Proc Natl Acad Sci USA 2005; 102:11817-11822.
77. Akdis CA, Joss A, Akdis M et al. A molecular basis for T-cell suppression by IL-10: CD28-associated IL-10 receptor inhibits CD28 tyrosine phosphorylation and phosphatidylinositol 3-kinase binding. FASEB J 2000; 14:1666-1668.
78. Nouri-Aria KT, Wachholz PA, Francis JN et al. Grass pollen immunotherapy induces mucosal and peripheral IL-10 responses and blocking IgG activity. J Immunol 2004; 172:3252-3259.
79. Pilette C, Nouri-Aria KT, Jacobson MR et al. Grass pollen immunotherapy induces an allergen-specific IgA2 antibody response associated with mucosal TGF-beta expression. J Immunol 2007; 178:4658-4666.
80. Jutel M, Akdis M, Budak F 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.

81. Radulovic S, Jacobson MR, Durham SR et al. Grass pollen immunotherapy induces Foxp3-expressing $CD4^+$ $CD25^+$ cells in the nasal mucosa. J Allergy Clin Immunol 2008; 121:1267-1272.
82. Mottet C, Uhlig HH, Powrie F. Cutting edge: cure of colitis by $CD4^+CD25^+$ regulatory T-cells. J Immunol 2003; 170:3939-3943.
83. Pereira-Santos MC, Baptista AP, Melo A et al. Expansion of circulating $Foxp3^+CD25^{bright}$ $CD4^+$ T-cells during specific venom immunotherapy. Clin Exp Allergy 2008; 38:291-297.
84. Akdis CA, Akdis M, Blesken T et al. Epitope-specific T-cell tolerance to phospholipase A2 in bee venom immunotherapy and recovery by IL-2 and IL-15 in vitro. J Clin Invest 1996; 98:1676-1683.
85. Taylor A, Verhagen J, Blaser K et al. Mechanisms of immune suppression by interleukin-10 and transforming growth factor-beta: the role of T regulatory cells. Immunology 2006; 117:433-442.
86. Croxford JL, Feldmann M, Chernajovsky Y et al. Different therapeutic outcomes in experimental allergic encephalomyelitis dependent upon the mode of delivery of IL-10: a comparison of the effects of protein, adenoviral or retroviral IL-10 delivery into the central nervous system. J Immunol 2001; 166:4124-4130.
87. Hansen G, Yeung VP, Berry G et al. Vaccination with heat-killed Listeria as adjuvant reverses established allergen-induced airway hyperreactivity and inflammation: role of $CD8^+$ T-cells and IL-18. J Immunol 2000; 164:223-230.
88. Creticos PS, Schroeder JT, Hamilton RG. Immune Tolerance Network Group. Immunotherapy with a ragweed-toll-like receptor 9 agonist vaccine for allergic rhinitis. N Engl J Med 2006; 355:1445-1455.
89. Van Overtvelt L, Lombardi V, Razafindratsita A et al. IL-10-inducing adjuvants enhance sublingual immunotherapy efficacy in a murine asthma model. Int Arch Allergy Immunol 2008; 145:152-62.