

REVIEW

Regulatory T cells and asthma

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Experimental
Allergy

Abstract

Airway inflammation in asthma is characterized by activation of T helper type-2 (Th2) T cells, IgE production and eosinophilia. In many cases, this process is related to an inappropriate T cell response to environmental allergens, and other T cell-dependent pathways may also be involved (such as Th17). Regulatory T cells (Tregs) are T cells that suppress potentially harmful immune responses. Two major subsets of Treg are CD25^{hi}, Foxp3⁺Tregs and IL-10-producing Tregs. There is evidence that the numbers or function of both subsets may be deficient in patients with atopic allergic disease. Recent work has extended these findings into the airway in asthma where Foxp3 expression was reduced and CD25^{hi} Treg-suppressive function was deficient. In animal models of allergic airways disease, Tregs can suppress established airway inflammation and airway hyperresponsiveness, and protocols to enhance the development, recruitment and function of Tregs have been described. Together with studies of patients and *in vitro* studies of human T cells, these investigations are defining potential interventions to enhance Treg function in the airway in asthma. Existing therapies including corticosteroids and allergen immunotherapy act on Tregs, in part to increase IL-10 production, while vitamin D3 and long-acting β -agonists enhance IL-10 Treg function. Other possibilities may be enhancement of Treg function via histamine or prostanoid receptors, or by blocking pro-inflammatory pathways that prevent suppression by Tregs (activation of Toll-like receptors, or production of cytokines such as IL-6 and TNF- α). As Tregs can also suppress the potentially beneficial immune response important for controlling infections and cancer, a therapeutic intervention should target allergen- or site-specific regulation.

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Immune mechanisms in asthma

A majority of patients with asthma have an atopic, allergic background. The prevailing consensus is that the immunological basis of atopic sensitization and allergic disease results from inappropriate T helper type-2 T cell responses to common environmental proteins termed allergens. Th2-associated cytokines such as IL-4 and IL-13 are important in driving IgE production to allergens, IL-5 contributes to the eosinophilic inflammation that characterizes allergic disease and IL-9 and IL-13 are thought to be important for bronchial hyperresponsiveness [1]. Th2 cytokines and local mucosal IgE production are also features of non-atopic or intrinsic asthma, although what drives this process is unknown [2].

Recent advances in both immunology and clinical phenotyping of asthma have raised the possibility that other mechanisms may drive pathology in some patients with asthma, or co-exist with Th2-type inflammation in many patients. For example, both cluster analysis of clinical, pathological and physiological parameters [3]

and biopsy studies [4] of patients with severe asthma suggested eosinophilic and non-eosinophilic subsets. Th17 T cells are a more recently described subset driven by IL-23, which produce IL-17 and are associated with neutrophilic inflammation [5]. This subset can also be derived with TGF- β and IL-6, as discussed below. IL-17 expression by both T cells and eosinophils is increased in the asthmatic airway [6], and in animal models, IL-17 was required during initial allergic sensitization but reduced Th2-dependent inflammation in established disease [7], whereas transfer of Th17 cells together with Th2 cells in naïve mice augmented airway inflammation [8]. Neutrophilic inflammation in asthma has been associated with development of fixed airflow obstruction [9] although whether this results from Th17-type T cell activation or is restricted to asthma is uncertain. Recently, a variety of cytokines derived from epithelium, fibroblasts and other airway structural cells have been shown to have an important potential for interaction with Th2 T cells, mast cells and eosinophils: these include IL-25 [10], IL-33 [11] and TSLP [12].

It may be that different phenotypes in asthma result from different patterns of genetic predisposition to atopy, Th2 responses, Th17 responses, epithelial cell activation and neutrophilic inflammation. Much more research is required to link genotypes and phenotypes, and in particular to define how these determine disease progression and response to treatment [13].

Why do asthmatics develop Th2-driven airway inflammation?

It is clear that whole populations are exposed to ubiquitous allergens such as pollens and dust mites, so why does everyone not develop allergic sensitization, and why are all atopics not asthmatic? The answers to these questions probably lie in a mixture of genetic susceptibility to both atopy and asthma (possibly through changes in airway structural cells such as epithelium), timing and dose of allergen exposure and co-exposure to infectious agents. At the level of immunological sensitization, it has become clear that Th2-cell responses to common allergens can be detected in non-atopic healthy individuals under certain conditions [14]. Along with other inappropriate T cell reactivity, such as that to self-antigens, there is increasing evidence that at least one mechanism for preventing activation of potentially harmful T cells is active suppression by regulatory or suppressive T cells [15]. One possible explanation for development of allergic airway inflammation in asthma is that such suppression is either defective or overwhelmed.

Regulatory T cells

There is now evidence for a variety of suppressive or regulatory T cell (Treg) subsets that either develop as a normal part of the immune system (termed naturally occurring Tregs) or in response to particular antigenic exposure (adaptive Tregs) [16]. The best characterized are CD4⁺ T cells that constitutively express high levels of surface CD25 (the IL-2 receptor α chain: CD25^{hi} T cells) [17]. Unlike CD4⁺ Th cells, these CD25^{hi} cells do not proliferate or produce cytokines when stimulated with antigen in the laboratory, but actively suppress proliferation and cytokine production from other T cells (termed effectors). They owe their suppressive phenotype in large part to the expression of high levels of the transcription factor Foxp3, because removal or reduction of Foxp3 abrogates their ability to suppress and adding Foxp3 to CD25 negative effector T cells renders these suppressive [18–21]. Patients with defects in *foxp3* develop a syndrome termed immunodysregulation, polyendocrinopathy, enteropathy and X-linked (IPEX), which includes development of autoimmunity and atopic allergic disease [22]. How Foxp3 renders the cell suppressive is unclear: although it prevents the cell from developing into an

IL-17-producing effector by inhibiting expression of transcription factor ROR γ T [23], it is itself inhibited by expression of the Th1-associated transcription factor T-bet and the Th2-associated factor GATA3 in developing CD4⁺ T cells [24, 25]. Foxp3 directly associates with the transcription factor AML1/Runx1, which in turn is required for activation of the *IL-2* and *IFN- γ* genes [26], and also increases expression of the micro-RNA miRNA 155, which, via suppression of cytokine synthesis (SOCS)-1, can down-regulate STAT5 activation and hence IL-2 production [27]. How CD25^{hi} Tregs suppress is also uncertain. A variety of suppressive pathways are described including cytokines such as IL-10 and TGF- β , cell surface molecules such as CTLA4, PD-1 and BTLA [15, 17] and recently a suppressive cytokine termed IL-35 was described (although whether this is important for human Tregs is less clear) [28, 29]. These cells can suppress effector T cells both directly and via effects on antigen-presenting cells such as dendritic cells. CD25^{hi} Tregs formed aggregates around dendritic cells, which depended on expression of the adhesion molecule LFA-1, and subsequently, via a CTLA-4-dependent pathway, down-regulated DC expression of the co-stimulation molecules CD80 and CD86 [30]. Thus, these cells compete with naïve T cells for access to DCs and reduce their ability to activate effector T cell responses. CD25^{hi} Tregs can also directly suppress effector cell functions of mast cells [31] and neutrophils [32]. It is likely that Tregs can suppress by a variety of mechanisms in a context-dependent manner. For example, it was recently shown, using a Foxp3-specific conditional knockout of the IL-10 allele, that IL-10 production by Tregs was required to control immune responses at environmental interfaces such as the colon or the lung, but not to prevent development of autoimmune disease [33]. Expression of ICOS by human Foxp3⁺ Tregs was shown to distinguish two subsets: ICOS⁺ cells inhibited dendritic cell function via IL-10, and T cells via TGF- β , whereas ICOS⁻ Foxp3⁺ Tregs used TGF- β only [34].

In addition to CD25^{hi} Tregs, there is a subset of IL-10-producing Tregs [35]. These cells were derived *in vitro* after stimulation of naïve T cells in the presence of the immunosuppressive drugs dexamethasone and vitamin D3 [36], in animal models after peptide treatment [37], and in patients after immune reconstitution [38]. These cells do not express high levels of Foxp3 [35]. Other Treg subtypes including TGF- β -producing cells are described [39]. The inter-relation between these different regulatory subsets is uncertain.

CD25^{hi} T cells were first characterized in animal models of autoimmune disease but have been associated with a variety of animal models and human disease [17]. Although a failure of suppression may result in immunological diseases such as diabetes [40] or rheumatoid arthritis (RA) [41], or transplant rejection [42], over-exuberant suppression has been associated with progression and

metastasis of some cancers [43] and with progression of some infectious diseases [44]. Thus, the equilibrium between suppression and activation of immune responses to maintain health is a fine balance and therapeutic intervention in this pathway would ideally be antigen or site specific.

Regulatory T cells in allergy and asthma

Both CD25^{hi} and IL-10-producing Tregs have been implicated in preventing Th2 responses to allergens. When peripheral blood CD4⁺CD25⁺ T cells were separated from the CD4⁺ T cell population from non-atopic non-allergic individuals, the remaining CD4⁺CD25⁻ T cells showed brisk proliferative and cytokine responses to allergen stimulation *in vitro* [14]. CD4⁺CD25⁺ T cells could inhibit proliferation of and cytokine production by these allergen-responsive T cells. Furthermore, the suppressive effects of CD4⁺CD25⁺ T cells on allergen-stimulated CD4⁺CD25⁻ T cells were reduced when cells from atopic allergic patients were studied and still further reduced when patients had active allergic rhinitis (AR) during the pollen season [14, 45].

After allergen stimulation *in vitro*, IL-10-producing, IL-4-producing and IFN- γ -producing T cells could be isolated from peripheral blood of atopic allergic donors and healthy controls [46]. IL-10-producing T cells had the capacity to suppress allergen-driven Th2 cytokine production from IL-4-producing effector cells, through mechanisms including IL-10, TGF- β , CTLA4 and PD-1. The frequency of these IL-10-producing Tregs was reduced and that of IL-4-producing cells increased in blood from atopic allergic donors. The frequency of these allergen-specific IL-10-producing Tregs was 0.1–0.007% of the total CD4⁺ T cells. Whether they are the allergen-specific subset of the CD4⁺CD25⁺ population or represent a separate cell type is unclear.

These findings suggested that CD4⁺CD25⁺ regulatory and IL-10-producing T cells have the potential to suppress pathogenic Th2 responses and that this suppression may be defective or overridden in patients with allergic disease.

A similar reduction of suppression by peripheral blood CD4⁺CD25⁺ T cell from house dust mite (HDM)-allergic asthmatic children compared with non-atopic controls is reported [47]. Hartl et al. [48] addressed the issue of whether CD4⁺CD25⁺ Tregs are present in the airways and whether their function is reduced in asthma. In bronchoalveolar lavage fluid (BALF) from children with asthma, the percentage of BAL T cells that were CD4⁺CD25⁺ and levels of Foxp3 mRNA were lower than in BAL from healthy controls or children with chronic cough. The percentage of both CD4⁺CD25⁺ T cells and Foxp3 mRNA in BAL correlated positively with FEV₁ for children with asthma. They also demonstrated reduced suppression of effector T cell proliferation and cytokine production by BAL CD4⁺CD25⁺ T cells from asthmatic children compared with those from BAL of healthy con-

trols. After 4 weeks of treatment of asthmatics with inhaled corticosteroids, the percentages of both CD4⁺CD25⁺ T cells and Foxp3 were increased in BAL.

Part of the explanation for this reduction in the frequency of CD4⁺CD25⁺ Tregs in the lung may relate to the observed reduction in the response of these cells in peripheral blood from asthmatics to the chemokines CCL1 and XCL1 [49, 50]. Potential interactions of Tregs in asthma are shown in Fig. 1.

Regulatory T cells in animal models of allergic airway disease

The development of animal models of allergic airways disease has allowed a specific intervention to determine potential mechanisms in allergic asthma [51]. Transfer of antigen-specific Tregs from a DO11.10 T cell-receptor transgenic mouse could prevent development of airway hyperresponsiveness (AHR) and Th2-mediated airway inflammation, and suppress established AHR and airway inflammation in an ovalbumin-inhaled challenge model [52]. This suppression was dependent on induction of IL-10 from recipient T cells but not on IL-10 production by the transferred CD4⁺CD25⁺ Tregs. In a study of two strains of mouse, one (A/J) susceptible to development of AHR and airway inflammation in response to inhaled HDM extract, and one relatively resistant (C3H), depletion of CD4⁺CD25⁺ T cells was shown to increase AHR and inflammation in the 'resistant' C3H mice [53]. This effect was associated with an increase in the numbers and activation status of pulmonary myeloid DCs, suggesting that the reported *in vitro* effects of CD4⁺CD25⁺ Tregs on DC/T cell interaction [30] are also relevant *in vivo*. In a long-term challenge model, CD4⁺CD25⁺ T cells could prevent development of airway mucus hyper-secretion, smooth muscle hypertrophy and collagen synthesis but not reverse established remodelling [54]. Animal models have also allowed identification of novel mechanisms of suppression by Tregs, including IL-35, which comprises a heterodimer, the Epstein-Barr virus induced gene 3 (EBI3) and the IL-12 p35, is preferentially expressed by Foxp3-positive Tregs and suppressed T cell proliferation [28]. However, as with all animal models, it is important to confirm such findings in human cells, and IL-35 was not detected in human CD4⁺CD25⁺Foxp3⁺ Tregs [29]. Further investigation will be required to determine the role of IL-35 in suppression by human Tregs.

Why is the balance between regulatory and effector T cells skewed towards activation in asthma?

Inherent defects in regulatory T cells

There are several possible explanations for the finding of a reduced suppressive activity of Tregs in allergy and asthma. One would be that atopic and/asthmatic

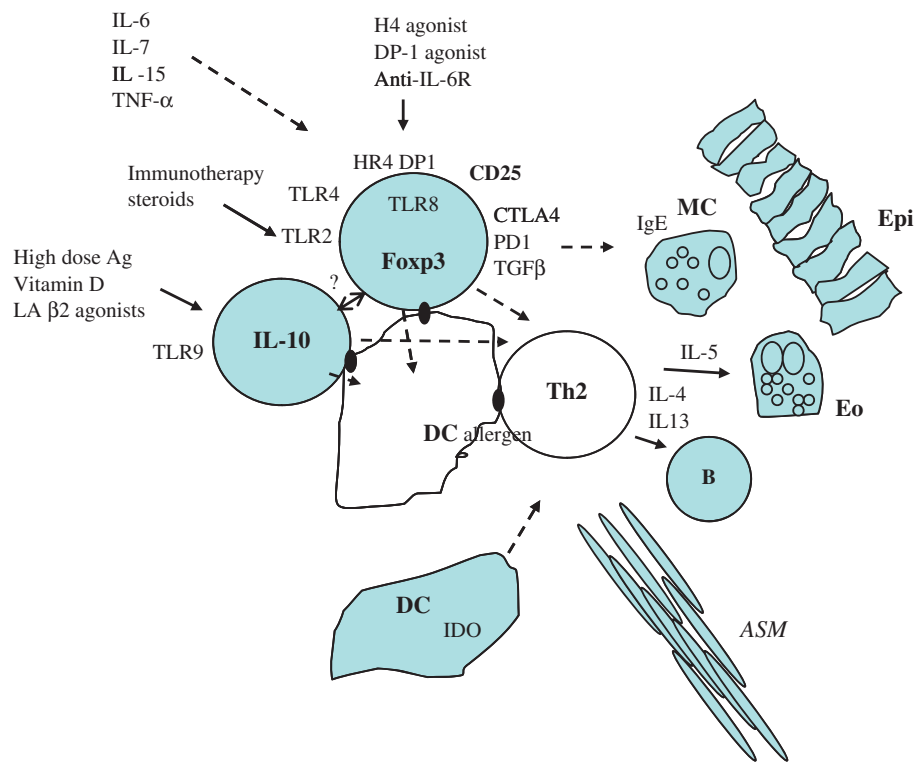


Fig. 1. Possible interactions between regulatory T cells (Treg) and the asthmatic airway. Th2 cells are activated by allergen presented by dendritic cells (DC) and act on eosinophils via IL-5, and B cells (switching to IgE production), epithelium (Epi) and airway smooth muscle (ASM) via IL-4 and IL-13. Allergen-specific IgE activates mast cells (MC). CD25^{hi} Foxp3⁺ Tregs can use a variety of regulatory pathways (including TGF- β , IL-10, CTLA-4 and PD-1; interrupted arrows), to suppress Th2 cells directly or via DCs, and can also directly suppress MC function. IL-10-producing Tregs also act via DCs or directly on T cells. The suppressive function of CD25^{hi} Tregs is reversed by IL-6, IL-7, IL-15 and TNF- α (interrupted arrows). High-dose antigen, vitamin D3 and long-acting β 2-agonists can all augment IL-10 production from IL-10-producing Tregs, while allergen immunotherapy and corticosteroids appear to enhance regulation by both subsets of Tregs. Agonists for histamine receptor 4 and β -prostanoid 1 receptor, and an antagonist for IL-6, augment Treg function (possibly via DCs). CD25^{hi} Tregs can induce IL-10-producing Tregs under some circumstances. Some DCs are also suppressive via the actions of indoleamine 2,3 dioxxygenase. The interactions of Tregs with Epi and ASM are uncertain. See the text for further details.

individuals have an inherent defect that reduces their suppressive potential. The reduced expression and function of CTLA-4 by CD4⁺CD25⁺ Tregs from patients with RA was related to the reduced ability to suppress effector T cell cytokine production [55]. However, to date, no such generalized defect in Treg function has been described in allergic or asthmatic patients: indeed, suppression of anti-CD3-stimulated CD4⁺CD25⁺ T cells did not differ between Tregs from allergic and control subjects [14], suggesting that the observed reduction in Treg-suppressive function is allergen specific. If a defect in Treg function predisposes to atopy and/or asthma, one might also expect an association with increased prevalence of autoimmune disease. Although some studies have detected such a link, others show no relation or even an inverse association [56, 57].

The hygiene hypothesis

An important hypothesis to explain the increased prevalence of asthma and allergy in Western populations is termed the hygiene hypothesis, which suggests that lack

of exposure to infectious agents and environmental microbes during childhood may predispose to allergy [58]. At the immunological level, this hypothesis has been extended to suggest a failure of development of protective Treg responses to allergens [59]. However, this may not be the only immunological explanation for the hygiene hypothesis as an absence of immune deviation from Th2 towards Th1 responses to allergens may also play a role [60]. These mechanisms may be differentially activated by different pathogens or environmental microbes. For example, helminth infections are associated with IL-10 responses and protect against development of atopic allergic disease [61], and helminth infection could prevent development of airway inflammation by inducing Tregs in an animal model of allergen challenge [62]. A heat-killed suspension of *Mycobacterium vaccae* also protected against airway inflammation in a mouse model and this protection was via IL-10-producing T cells and TGF- β [63]. In contrast, the protective effect of growing up in a farming environment may be via Toll-like receptor (TLR)-mediated immune deviation towards Th1 responses to bacteria present in cowsheds [64].

Further understanding of how Treg responses develop (or fail to) will be important. The allergen dose may be important. High-level exposure to bee venom was shown to induce protective IL-10-producing T cell responses in bee keepers [65], and exposure to high concentrations of cat allergen appeared to be protective against development of asthma, possibly because of development of 'modified Th2' responses characterized by the production of IL-10 [66]. For bee keepers, the protective IL-10 T cell response was short lived, waning over weeks during the winter months, when they were not working with bees. Repeated low-dose inhaled allergen can lead to immune tolerance and reduced airway Th2 inflammation upon subsequent inhaled allergen challenge in animal models. In a mouse model, such a tolerance was associated with development of Foxp3⁺ Tregs with surface TGF- β , which activated the Notch-/HES-1 signalling pathway in target cells [67, 68]. High-dose-inhaled tolerance induced IL-10 production from dendritic cells and IL-10-producing Tregs [69]. In a rat model, repeated airway challenge prevented AHR and inflammation by induction of CD4⁺CD25⁺Foxp3⁺ Tregs, which acted to prevent maturation of airway dendritic cells [70]. The relationship between breastfeeding and allergy is controversial and recent studies have not confirmed a protective effect [71, 72]. In a mouse model, breastfeeding was shown to transfer allergens from mother to neonate and protect from airway inflammation and AHR upon subsequent inhaled challenge of the offspring [73]. This effect was mediated by Tregs and was dependent on TGF- β . Studies on breastfeeding in humans should take into account allergen exposure of the mother. Certainly, avoidance of peanuts during pregnancy and lactation may be ill advised for avoidance of peanut allergy [74].

Infection and inflammation affect the balance between regulation and activation of T cell responses

An important factor in determining the outcome of allergen exposure both during development and in adult life may be co-incident infection and exposure to microbial products, as discussed above. These agents both activate innate immunity and modulate adaptive immune responses through their interaction with pathogen-associated molecular patterns receptors including TLRs. These receptors have both direct and indirect effects on Treg function. Activation of TLR4 by bacterial lipopolysaccharide (LPS) blocked the suppressive effect of CD4⁺CD25⁺ T cells, in part via induction of IL-6 [75]. However, LPS was reported to enhance the proliferation and regulatory activity of CD4⁺CD25⁺ Tregs [76]. In contrast, TLR2 ligation enhanced the proliferation of both Tregs and effector T cells and reduced Foxp3 expression by Tregs [77], while TLR8 ligation reversed suppression by CD25⁺ Tregs [78]. Recently, TLR9 was shown to be highly expressed by IL-10-producing Tregs

(but not by CD4⁺CD25⁺ Tregs, or Th1 and Th2 effector T cells), and CpG oligonucleotides (ligands for TLR9) reduced IL-10 production and suppression by these cells [79]. A variety of pro-inflammatory cytokines can overcome suppression by Tregs. For example, IL-7 and IL-15, at concentrations detectable in synovial fluid from children with juvenile RA, prevented suppression by CD4⁺CD25⁺ Tregs from the joint tissue [80]. TNF- α may also play a role in overcoming Treg function in inflamed joints in RA [41, 81]. There is little information on the effect of cytokines present in sites of allergic inflammation on Treg function in asthma, although TNF is reported to overcome suppression by peripheral blood CD4⁺CD25⁺ Tregs from asthmatic individuals [47]. TNF receptor antagonists are effective in the treatment of RA and, in at least some studies, asthma [82]; it may be that part of their effect is to restore Treg function at sites of inflammation.

Can regulatory T cell function be enhanced in allergy and asthma?

Inhaled corticosteroids are the mainstay of anti-inflammatory treatment for asthma, often in combination with long-acting β 2-agonists [83]. Pre-incubation with fluticasone propionate increased the subsequent suppressive actions of CD4⁺CD25⁺ Tregs in allergen-stimulated cultures, and this enhancement of suppression was related to increased IL-10 production by these cells [84]. Corticosteroids also increased Foxp3 expression by CD25⁺ T cells [85], and corticosteroid treatment of children with asthma enhanced the suppressive ability of CD25⁺ T cells isolated from BALF [48]. Corticosteroids, together with vitamin D3, induce the IL-10 Treg phenotype *in vitro*, and long-acting β -agonists can enhance IL-10 production from these cells [86]. A small percentage of asthmatics fail to respond to corticosteroid treatment. CD4⁺ T cells from these steroid-resistant asthmatics do not show steroid induction of IL-10 upon stimulation *in vitro*. This *in vitro* steroid resistance was reversed by treatment of patients with vitamin D3 [87]. Relative vitamin D deficiency is not uncommon in Western populations [88], and it will be of considerable interest to determine whether vitamin D3 or analogues can enhance regulation in the context of allergic asthma.

Allergen-specific immunotherapy has long been used for AR and asthma and is associated with the modulation of Th2 responses [89]. It is suggested that both CD25⁺ and IL-10-producing Treg function may be enhanced by immunotherapy: CD25⁺ T cells produced increased IL-10 in allergen-stimulated peripheral blood cells from immunotherapy-treated patients, and the numbers of CD25⁺Foxp3⁺ T cells and IL-10⁺ cells recruited to the nasal mucosa during the pollen season were increased in patients who had been treated with immunotherapy [90–93]. Further understanding of the mechanisms

of immunotherapy, and which of these mechanisms is responsible for the long-term effects of this treatment on clinical symptoms and Th2 inflammation will be important if we are to improve immunotherapy for asthma. In a mouse model of sublingual immunotherapy, vitamin D3 and dexamethasone increased the efficacy of immunotherapy with an increase in local Foxp3⁺ T cells [94].

In murine systems, a number of factors that can either enhance development or recruitment of Tregs have been defined. TGF- β acts on naïve CD4⁺ T cells to enhance Foxp3 expression and could induce CD25⁺Foxp3⁺ Tregs that suppressed airway inflammation in an HDM model [95]. Of note, TGF- β also induces development of Th17 T cells from naïve precursors, although there is an additional requirement for IL-6 [96]. Blockade of the IL-6 receptor led to expansion of CD4⁺CD25⁺ Tregs in the airway and suppression of airway inflammation in a mouse model [97]. The histamine receptor-4 agonist, 4-methylhistamine, given intra-tracheally before allergen challenge reduced airway inflammation and AHR upon subsequent allergen challenge, in a mouse model, and acted *in vitro* to increase migration of human CD4⁺CD25⁺Foxp3⁺ T cells [98]. Similarly, activation of the μ -prostanoid 1 receptor suppressed AHR and inflammation in a mouse model by modulating dendritic cell function and increasing Foxp3⁺ Treg numbers in the airway [99]. Osteopontin has immunoregulatory effects on Th1 inflammation and was also shown to suppress established airway inflammation via an effect on dendritic cells [100]. Blocking the IL-2 receptor β chain also reduced AHR and inflammation to airway challenge in a mouse model, possibly tilting the balance towards Treg activity [101]. Whether these findings can be translated into therapies that increase Treg function in human asthma remains to be seen. An important consideration will be the safety of such potential treatments. Tregs also suppress immunity with anti-tumour and anti-infective capacities. It may be that non-allergen-specific approaches can be combined with allergen-specific therapy such as immunotherapy to enhance allergen-specific T cell tolerance. Of note using two mouse strains devoid of naturally occurring Tregs, Curotto de Lafaille et al. [102] showed that a strain that could develop adaptive Foxp3⁺ Tregs could suppress IL-4 production and induce mucosal tolerance in an airway challenge model, whereas IL-5 and eosinophilia was controlled by IFN- γ -dependent mechanisms even in the absence of Foxp3. Thus, Tregs are not the only suppressive pathway to consider in asthma.

An important factor in the equilibrium between regulation and inflammation in the asthmatic airway is the mechanism of recruitment of effector and Tregs. CD4⁺CD25⁺ Tregs have been characterized as expressing a range of chemokine receptors broadly similar to Th2 effector T cells, namely CCR4, 5, 6 and 8 [103]. Although no differences in chemokine receptor expression or re-

sponsiveness were found between atopic and non-atopic donors [103], it has been reported that CD4⁺CD25⁺ Tregs from asthmatics had reduced responsiveness to CCL1, the ligand for CCR8, and a relative deficiency of surface XCL1 expression [49, 50]. Furthermore, it was suggested that XCL1 could enhance the *in vitro* suppressive function of CD25⁺ Tregs from asthmatic individuals. If Tregs could be selectively recruited to and retained in the asthmatic airway this might have therapeutic potential.

Other T cell subtypes that can regulate airway inflammation

In addition to CD4⁺CD25⁺ and IL-10-producing Tregs, other T cell subtypes have been described to have the potential to suppress airway inflammation in asthma. A human IL-10-producing NK T cell subset is described, which, at least *in vitro*, could suppress allergen responses from effector T cells [104]. CD8 memory T cells were shown to be long lived in many tissues, including the lung, and could be activated by antigen presentation or by cytokines (IL-12 and IL-18) alone to produce IFN- γ and to stimulate IL-12 production by dendritic cells. Using a transgenic T cell receptor model, Leggat et al. [105] showed that non-allergen-specific CD8 T cells could suppress allergen sensitization in a mouse model. Thus, CD8 memory T cells may bridge the innate and adaptive immune response, and under some circumstances (such as infections), modulate development of allergic sensitization. There may also be cross talk between Th1 and chronically stimulated Th2 cells in the airway in individuals exposed to multiple allergens; further work is needed in this area. Other suppressive pathways may also be important in asthma. A subset of human dendritic cells that express indoleamine 2,3 dioxygenase (IDO) and are suppressive was described [105, 106], and recently the HDM protein Der p 1 was shown to reduce IDO activity in DCs from HDM-allergic individuals [107]. In addition, dexamethasone suppression of allergic airway inflammation in a mouse model was shown to be in part via activation of the IDO pathway [108].

Conclusion: regulatory T cells and asthma

Most of the current information relating to the role or the potential for Tregs in asthma relates more to allergy in general. With the increasing emphasis on different subtypes of asthma [2–4] with differing mixes of inflammation, repair and remodelling, there is a need for more information on the potential and contribution of Tregs to these different aspects both in clinical 'endotypes' [109] and in animal models. Current data from patients with asthma confirm that the relative 'defect' of peripheral blood CD4⁺CD25⁺ Treg function seen in allergy is also seen in allergic asthmatics [47], and extend this finding to

airway T cells [48]. Furthermore, possible defects in chemokine receptor expression by Tregs in asthmatics may in part explain reduced Treg numbers in the airway [49, 50]. However, these studies have focused on T cell function *in vitro* and are paralleled by studies showing the ability of Tregs to reduce airway inflammation *in vivo* in animal models, and there are few studies that address other aspects of asthma such as airway remodelling, fibroblast activation, smooth muscle changes and epithelial repair. It is of note that TGF- β , which is an important effector cytokine for Tregs, is also implicated in fibroblast activation and initiation of airway remodelling [110]. This may also be the case for other mediators such as actin and osteopontin. Thus, at least some aspects of airway remodelling in asthma may directly result from the regulatory response to allergic airway inflammation. In a mouse model of repeated airway challenge, CD4⁺CD25⁺ Tregs could reverse inflammation but not established airway remodelling [54], and it will be important to explore further the ability of Tregs of different subtypes to modulate (suppress or even increase) different aspects of asthma pathophysiology. With the available tools of animal models and potential for sampling the human airway, it should be possible to define the biology of Tregs in asthma with greater precision and move towards specific immune intervention. Perhaps the most immediately attractive areas are in the enhancement of IL-10 production via corticosteroids and/or vitamin D, either alone or combined with allergen-specific immunotherapy.

Acknowledgements

The author thanks Dr Victor Iraola for critically reviewing the manuscript.

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