

Role of regulatory T cells in allergy and asthma

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Th2 cells play a critical role in the pathogenesis of allergy and asthma. However, the immunological mechanisms that downmodulate and protect against the development of these disorders are poorly understood. A spectrum of CD4⁺ T cells, including Th3 cells, T_R cells, CD4⁺CD25⁺ cells and NKT cells play a critical role in regulating these diseases. A better understanding of the role of regulatory cells in allergic diseases may lead to the identification of novel therapeutic targets.

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Abbreviations

AHR	airway hyperreactivity
DC	dendritic cell
EAE	experimental autoimmune encephalomyelitis
IFN	interferon
IL	interleukin
NKT	natural killer T
OVA	ovalbumin
RT-PCR	reverse transcriptase polymerase chain reaction
TGF	transforming growth factor
Th	T helper
T_R	regulatory T
TSLP	thymic stromal lymphopoietin

Introduction

Since 1986, the T helper (Th) 1/Th2 paradigm has dominated our understanding of the pathophysiology of asthma and allergic disease. It has become apparent in recent years, however, that other immunological principles are important in explaining the regulation of asthma and allergy. New paradigms encompassing a more diverse set of cell types must be developed to explain the immunological events that regulate these disorders. The modern view of regulatory T cells began with the observation by Sakaguchi and colleagues [1,2] that depletion of CD25⁺ cells induced multiorgan autoimmunity. The following article will attempt to review the spectrum of CD4⁺ T cells that critically regulate the development of asthma and allergy.

The Th1/Th2 paradigm

Th2 cells, which produce IL-4 (enhancing IgE synthesis), IL-5 (increasing eosinophil growth and differentiation), IL-9 (enhancing mast cell differentiation) and IL-13 (increasing mucus production and inducing airway hyper-reactivity, a cardinal feature of asthma), control and amplify the allergic inflammatory response [3,4]. As Th1 cells crossregulate Th2 cells in some systems, it was assumed that Th1 cells downmodulated the effects of Th2 cells and thus had a beneficial role in allergic disease and asthma. However, it became clear that Th1 cells were themselves pro-inflammatory, and that the development of a Th1-associated inflammatory response could exacerbate asthma and allergic disease [5]. IFN-γ is often present at sites of allergic inflammation and is thought to contribute to the disease. Furthermore, although the transfer of allergen-specific Th1 cells into the lungs of mice did not directly induce airway hyper-reactivity (AHR), it did result in enhanced, rather than reduced, inflammation in the airways. Finally, it became clear that the increase in prevalence of atopic diseases over the past 20 years has been accompanied by an increase in the prevalence of autoimmune, Th1-driven, diseases [6]. This suggested that the environmental changes that have resulted in increased asthma prevalence cannot be solely due to a reduction in Th1 regulatory elements. These observations therefore suggest that forms of immunity other than pure Th1 responses must be effective in regulating the development of asthma and allergic diseases.

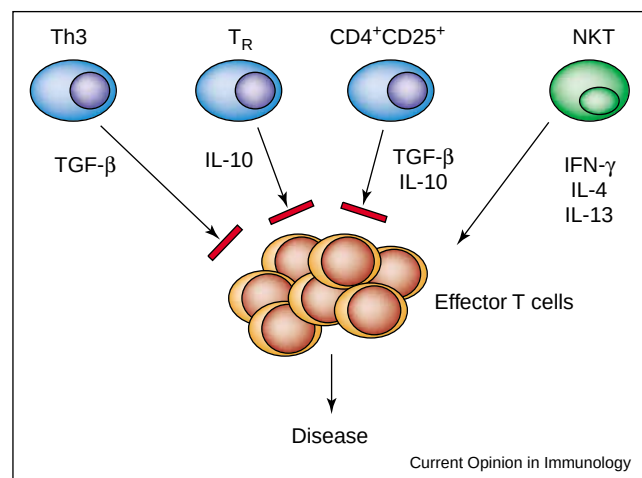
Regulatory T cells

The term ‘regulatory T cell’ refers to cells that actively control or suppress the function of other cells, generally in an inhibitory fashion. Regulatory T cells appear to control the development of autoimmune disease and transplant rejection, and may also play a critical role in controlling asthma and allergy. The specific mechanisms by which these regulatory cells function, and the specific characteristics of these cells, are still being investigated. However, at least four major types of CD4⁺ regulatory T cells have been described: Th3 cells, T_R cells, CD4⁺CD25⁺ cells and natural killer T (NKT) cells (Figure 1).

Th3 cells

Th3 cells were described by Weiner and co-workers [7] as CD4⁺ T cells induced by oral antigen in the mesenteric lymph nodes, which produce TGF-β and varying amounts of IL-4 and IL-10. These T cells suppress the development of experimental autoimmune encephalomyelitis (EAE), suggesting that T cells producing

Figure 1



Subsets of regulatory cells in allergy and asthma. At least four major types of $CD4^+$ regulatory T cells are thought to play a critical role in controlling the expression of asthma and allergy. Although Th3, T_R and $CD4^+CD25^+$ cells can inhibit effector T cells, NKT cells may enhance their differentiation and development.

TGF- β or IL-10 have regulatory or suppressive properties that inhibit the effects of pathogenic autoreactive T cells.

T_R1 and T_R cells

Roncarolo and co-workers [8] showed that chronic activation of both human and murine $CD4^+$ T cells in the presence of IL-10 gave rise to T_R1 cells, a subset of $CD4^+$ T cells. T_R1 cells have a low proliferative capacity and produce high levels of IL-10, low levels of IL-2 and no IL-4. These antigen-specific T cells suppress the proliferation of $CD4^+$ T cells in response to antigen, and prevent the induction of experimental colitis in SCID (severe combined immunodeficiency) mice. Taken together, these observations indicate that T_R1 cells can regulate antigen-specific immune responses and inhibit the function of pathological autoantigen-specific T cells and Th2 cells *in vivo* [9,10]. Other studies showed that pulmonary dendritic cells (DCs) present in the bronchial lymph nodes of mice exposed to respiratory allergen secrete IL-10 and induce the development of T Line 108 cells in a process that required T-cell co-stimulation via the inducible co-stimulator (ICOS)–ICOS ligand pathway [11]. The T_R cells produced IL-10, had potent inhibitory activity and blocked the development of asthma in a murine model.

$CD4^+CD25^+$ cells

$CD4^+CD25^+$ cells were described by Sakaguchi *et al.* [12] as a minor fraction (approximately 10%) of $CD4^+$ T cells that plays critical roles in the prevention of organ-specific autoimmunity and allograft rejection, and in the maintenance of self-tolerance. These cells constitutively

express the IL-2 receptor α chain, they are similar to cells identified as $CD45RB^{lo}$, and they are produced by the thymus as a functionally mature T-cell subpopulation [13,14]. The study of these regulatory cells may be facilitated in the future now that new markers have been identified, which include the glucocorticoid-induced TNF receptor (GITR) [15] and *Foxp3*, a transcription factor that appears to be specific for $CD25^+$ regulatory T cells [16]. The depletion of $CD25^+$ cells results in the development of autoimmune diseases such as thyroiditis, gastritis, type 1 diabetes and inflammatory bowel disease. Furthermore, the depletion of $CD25^+$ cells enhances some forms of tumor immunity, indicating that these cells function to suppress a wide range of immune responses [17,18].

$CD25^+$ cells suppress immune responses through direct cell–cell contact in a process that is dependent on signaling via cytotoxic T lymphocyte antigen 4 (CTLA-4), as well as the secretion of TGF- β . The antigen specificity of $CD25^+$ T cells is not well understood, but studies in TCR transgenic mice suggest that these cells undergo thymic selection and require a TCR with high affinity for self-peptides in a process that is distinct from positive selection and deletion [19]. Importantly, the suppressive effects of $CD25^+$ cells can be blocked by the presence of IL-6, often produced by DCs that have been activated through Toll-like receptor (TLR) pathways [20]. Alternatively, $CD25^+$ T cells may regulate the development of other T cells through competition for ‘space’, independent of specificity or effector function [21,22,23]. Thus, the transfer of almost any naïve $CD4^+$ T cell into a lymphopenic environment may result in a limitation of autoimmune pathology.

What is the relationship between T_R1 cells and $CD25^+$ cells? An analysis of naturally occurring human $CD4^+CD25^+$ T cells at the clonal level revealed that these cells are distinct from IL-10 producing T_R1 cells, and that $CD4^+CD25^+$ T cells do not represent the anergic precursors of IL-10-producing T_R1 cells [24]. Unlike T_R1 cell clones, suppressive $CD4^+CD25^+$ T-cell clones express significantly higher levels of CD25 in comparison to nonsuppressive controls, they do not proliferate in response to cytokines and, most importantly, they do not produce IL-10. Suppressive $CD4^+CD25^+$ T-cell clones, however, produce TGF- β [25], and this molecule, which appears to be bound to the cell membrane, has a small, but significant role in the suppression of proliferation [26]. Furthermore, the characterization of $CD4^+CD25^+$ T cells at the clonal level demonstrates that human $CD4^+CD25^+$ T cells are not a homogenous population of suppressor cells. This conclusion is supported by two recent reports that suggest that one of the functions of $CD4^+CD25^+$ T cells may be to induce the differentiation of IL-10- and TGF- β -producing T_R1 cells [25,27].

NKT cells

NKT cells comprise a population of T cells that express cell surface markers characteristic of both NK cells and conventional T cells, and they have been shown to exert strong regulatory activity in autoimmune diseases [28^{••}], including diabetes mellitus [29], EAE [30], oxazolone induced colitis [31] and in tumor immunity [32]. NKT cells are either CD4⁺ or CD4⁻CD8⁻ double negative, and depend on IL-15 for survival [33[•]]. NKT cells express a limited TCR repertoire and express an invariant V α 14–J α 281 TCR in mice and V α 24–J α 15 TCR in humans [34]. Through this TCR, NKT cells recognize glycolipid antigens presented by the nonpolymorphic MHC class I-like protein CD1d. Most importantly, when activated, NKT cells rapidly produce large quantities of IL-4 and IFN- γ . This rapid production of cytokines is thought to allow NKT cells to exert strong regulatory activity, and recent studies suggest that NKT cells exert potent activity in controlling the development of asthma, as discussed below.

Regulatory T cells in asthma and allergic disease

Regulatory T cells have been studied primarily in the context of tolerance to self-antigens. However, tolerance is also important in the peripheral immune system, in terms of immune responses to antigens encountered in the respiratory and intestinal tracts. These sites are normally continuously exposed to a wide variety of environmental, nonreplicating, nonpathogenic antigens that induce tolerance or hyporesponsiveness rather than active immunity. For example, exposure to cats or dogs in the first year of life reduces the subsequent risk of allergic sensitization to multiple allergens [35,36]. Other studies have shown that children growing up on farms, particularly those with direct contact with animals, were less likely to develop allergies than children growing up in urban environments [37,38], possibly because of exposure to endotoxin [39]. In any case, tolerance to environmental allergens encountered at mucosal surfaces, such as the lungs or intestinal tract, could be mediated by the development of regulatory T cells, or alternatively by clonal deletion or anergy of allergen-specific cells. Moreover, as patients that specifically lack CD25⁺ regulatory T cells (a syndrome caused by mutations in Foxp3, known as X-linked autoimmunity-allergic dysregulation syndrome [XLAAD] or immune dysregulation polyendocrinopathy, X-linked syndrome [IPEX]) develop severe eczema, elevated IgE levels, eosinophilia and food allergy, it is very likely that some form of regulatory T cells control the development of allergic disease and asthma [40].

In support of this theory, it was shown that T cells engineered to secrete TGF- β , in contrast to IFN- γ -secreting Th1 cells, could very effectively reduce airway inflammation and AHR [41]. Furthermore, the blockade

of TGF- β signaling in mature T cells enhanced airway inflammation and AHR, suggesting that the regulation of T cells via TGF- β reduces inflammatory responses in the lungs [42]. Inflammation in asthma can be inhibited not only by TGF- β secreting cells, but also by IL-10 secreting cells. Thus, T cells engineered to secrete IL-10 greatly reduce the development of AHR and airway inflammation [43,44].

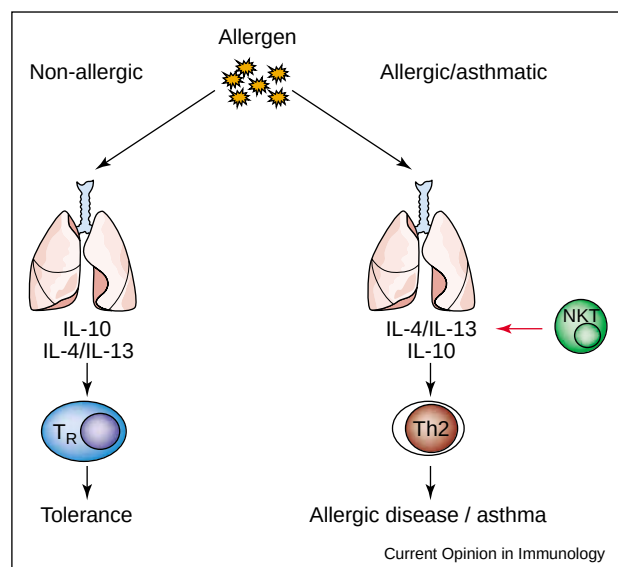
These observations indicate that cells other than Th1 cells are very effective in downmodulating pulmonary inflammation in asthma, and that responses involving TGF- β or IL-10 are very effective in downregulating asthma. These studies support the idea that immune tolerance, which is associated with the production of TGF- β and IL-10, might be important in regulating asthma.

Immune tolerance in asthma and allergy

Immune tolerance can be established by respiratory or oral exposure to the allergen [45–47]. Previous studies provided evidence that CD8⁺ T cells expressing the $\gamma\delta$ TCR were involved in the respiratory tolerance process [48,49], although this finding is now controversial [50,51]. Intranasal (i.n.) delivery of a single immunodominant peptide from an allergen can induce tolerance to the whole protein, in a process called linked suppression [52,53], but this process appears to induce the functional inactivation of antigen-specific CD4⁺ T cells, a form of anergy, rather than induction of regulatory T cells. By contrast, respiratory administration of whole allergens on three consecutive days appears to induce the development of T-cell hyporesponsiveness, associated with the development of T_R cells. The production of ovalbumin (OVA)-specific IgE in these mice was reduced, although the production of OVA-specific IgG₁ and IgG_{2a} was increased, indicating that immune deviation or split tolerance was induced [45]. Most importantly, however, the induction of such respiratory tolerance or immune deviation to OVA greatly inhibited the subsequent development of AHR and airway inflammation [54].

The protective response induced by i.n. exposure to OVA involved the initial activation and rapid proliferation of OVA-specific T cells. DCs were also involved in this tolerance process, as DCs isolated from the lymph nodes of OVA-exposed mice expressed high levels of B7-1, B7-2, CD40 and ICOS ligand [55]. These DCs also had a low capacity to endocytose fluorescein isothiocyanate (FITC)-conjugated dextran, indicating that these DCs behaved as mature DCs rather than immature DCs, which were previously thought to be involved in the development of tolerance [56–60]. Moreover, the mature DCs from tolerized mice transiently produced IL-10, as detected by RT-PCR and by intracellular cytokine staining. The mature DCs induced OVA-specific T cells to proliferate and to produce IL-4 and IL-10, but not IFN- γ

Figure 2



The effects of respiratory allergens in non-allergic versus asthmatic individuals. Exposure to respiratory allergens results in the development of T_R cells and subsequent tolerance in non-allergic individuals. Th2 cells may develop either as an aberration of T_R-cell development, because of inadequate production of IL-10, or as a consequence of enhanced production of IL-4 and IL-13 by NKT cells in particular, which leads to the development of allergy and asthma.

or TGF- β . After repeated stimulation of OVA-specific T cells with the IL-10 producing DCs, IL-4 synthesis in T cells decreased, although IL-10 synthesis persisted (Figure 2).

Because both T_R cells and Th2 cells develop through an intermediate stage where they produce both IL-4 and IL-10 (although in different quantities), because both cell types require ICOS-ICOS-ligand co-stimulation to develop, and because both cell types are associated with mucosal responses, it has been suggested that these two forms of immunity are related. However, Th2 cells, but not T_R cells, produce IL-13 and induce AHR. We believe that, under normal circumstances, exposure to respiratory allergen results in the development of T_R cells and subsequent tolerance. Th2 cells could develop as an aberration of T_R-cell development, possibly because of the inadequate production of IL-10 or enhanced IL-4 and IL-13 production, which would then lead to the development of allergy and asthma. Recent studies suggest that epithelial cells might play a role in initiating Th2-cell differentiation, as human thymic stromal lymphopoietin (TSLP), a novel epithelial-cell-derived cytokine, activates DCs to prime naïve T cells to produce the proallergic cytokines IL-4, IL-5, IL-13 and TNF- α , and down-regulates IL-10 and IFN- γ [61,62]. These studies suggest that stimuli that enhance TSLP production might be involved in directing Th-cell development

away from T_R-cell development towards Th2-cell development.

Innate immunity

Although exposure to antigen in the mucosal tissues in the absence of inflammation may induce protective T_R-cell development, this process may be enhanced by co-activation of the innate immune system. It is known that the establishment of tolerance mechanisms at mucosal surfaces requires the presence of commensal bacteria in the gastrointestinal tract [63], which may activate the innate immune system. Gastrointestinal microorganisms express pathogen-associated molecular patterns (PAMPs), such as endotoxin, flagella, lipoproteins or CpG oligodeoxynucleotides, which may activate the innate immune system through Toll-like receptors, thereby enhancing the development of tolerance or some form of protective immunity. *Mycobacterium vaccae* contains bacterial components that appear to induce CD4⁺CD45RB^{lo} regulatory T cells, which inhibit the development of airway eosinophilia. This specific inhibition was mediated through IL-10 and TGF- β [64]. In addition, exposure to high levels of endotoxin may enhance the development of Th1 responses, which may protect against the development of allergy and asthma [65]. Furthermore, *Bordetella pertussis* filamentous hemagglutinin protein stimulates the development of T_R1 cells that produce IL-10 and suppress Th1 cytokine production [66]. Finally, infection with hepatitis A virus, which is associated with protection against asthma and allergy [67], may directly induce protective immunity by binding to its receptor, TIM1 (T-cell Ig-domain mucin-domain), which regulates IL-4 production and AHR in murine models [68]. Infections with these pathogens may therefore result in protection against allergy and asthma, and perhaps may explain the beneficial effects of probiotics [69], and provide immunological mechanisms for the hygiene hypothesis [4].

NKT cells and asthma

Other cell types that can affect the differentiation of Th2 cells include NKT cells. Upon activation, NKT cells rapidly produce large quantities of cytokines, resulting in the recognition of self-glycolipid antigens. In the case of asthma, self-glycolipid antigens that are normally concealed in the respiratory tract may be exposed or expressed when allergens enter the lungs. These self-glycolipids would be recognized by NKT cells and cause their activation. Indeed, NKT cells are present in the lungs of asthmatic individuals and produce high levels of IL-4 and IL-13, which appear to regulate the development of AHR in mice [70].

The precise mechanisms by which NKT cells induce AHR are not clear. Previous studies have indicated that Th2 responses can occur in NKT-cell deficient mice [71,72]. However, Th2 responses alone are not sufficient

for the development of AHR (not all individuals with allergic rhinitis develop asthma), indicating that NKT cells must regulate additional elements in the lower respiratory tract that are required for the development of AHR. We suggest that NKT cells in the lung become activated by antigens encountered in the lung and provide a necessary prerequisite for the development of AHR, perhaps by localizing the Th2 response to the lungs or 'licensing' Th2 cells that enter the pulmonary compartment to induce AHR. Thus, IL-4 and IL-13 produced by NKT cells potentiate the development and action of Th2-biased T cells in the lungs, which drive the development of asthma.

Which regulatory T cells control asthma and allergy?

Although it is likely that tolerance and regulatory T cells are involved in controlling the development of asthma and allergic disease, it is not yet clear which of the various regulatory T cells are most important in controlling the development of these disorders [73]. It is possible that Th3 cells are primarily involved in regulating gastrointestinal disease, whereas T_R1 cells are more involved in respiratory disease [74], as DCs from the respiratory tract produce IL-10, required for T_R-cell development, whereas DCs from the gastrointestinal tract produce both IL-10 and TGF- β , which may be required for the development of Th3 cells [55]. T_R1 cells inhibit Th2 responses as well as Th1 responses [75], and other regulatory T cells suppress the development of Th2 responses and AHR [11*]. CD4⁺CD25⁺ cells inhibit the development of airway eosinophilia in mice [76], but appear not to block the induction of AHR [77]. In humans, conventional allergen immunotherapy may induce the development of CD25⁺ regulatory cells [78], although it is not clear whether these cells that express the CD25 marker are truly regulatory cells or merely activated Th cells. Finally, NKT cells appear to be essential for the induction of AHR in mice, but their importance in human asthma has not yet been studied. Clearly, a great deal more study of regulatory cells in asthma and allergy is warranted.

Conclusions

Our understanding of the mechanisms that protect against the development of allergic disease and asthma is still limited. Th1 responses, which are generally pro-inflammatory rather than anti-inflammatory, are unlikely to provide the primary mechanism of protection against allergy and asthma. Rather, a wide variety of regulatory T cells may be involved in actively suppressing the development of allergy and asthma by providing anti-inflammatory responses and protective immunity. Therapies that enhance the development of regulatory T cells and inhibit the function of NKT cells in patients with allergic disease and asthma may be very effective in the prevention and cure of these disorders.

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