

Clinical reviews in allergy and immunology

Series editors: Donald Y. M. Leung, MD, PhD, and Dennis K. Ledford, MD

The role of the T cell in asthma

Douglas S. Robinson, MD, FRCP *London, United Kingdom, and Madrid, Spain*

Since the initial detection of T_H2 cytokines in asthmatic airways, our understanding of the complexity of T-cell subtypes and flexibility and of the potential role of airway structural cells in the immunopathology of asthma has increased. Cytokines derived from airway epithelium, including IL-25, IL-33, and thymic stromal lymphopoietin, might be important drivers of T_H2-type inflammation in asthma. The balance between effector T_H2 cells and suppressive regulatory T cells is skewed toward a proinflammatory T_H2 response in atopy and asthma, and there is much interest in how to redress this equilibrium. Novel T-cell subsets, including T_H17, T_H9, and T_H22, have been described, although their role in asthma remains unclear. Other T cells, including natural killer T cells, $\gamma\delta$ T cells, and CD8 T cells, have also been implicated in asthma, although their importance remains to be confirmed. Therapeutic strategies aimed at T_H2 cytokines are beginning to bear fruit in patients with asthma, although like many biologic agents, these might need specific targeting at subgroups of patients. Strategies directed specifically at the T cells are currently being evaluated, including novel forms of allergen immunotherapy. T cells remain an exciting potential target for new treatments in patients with asthma. (*J Allergy Clin Immunol* 2010;■■■:■■■-■■■.)

Key words: T lymphocyte, T_H2, cytokine, regulatory T cell, asthma, natural killer T cell, $\gamma\delta$ T cell

It is now more than 20 years since the seminal description by Mosmann et al¹ of 2 functional subsets of murine T_H clones termed T_H1 and T_H2. It has been almost 20 years since our group, among others, published evidence that atopic asthma was associated with activation of a T_H2 type of T cell in the airway.² Expression of T_H2 cytokines could be related to activity of disease, symptom scores, airway eosinophilia, and bronchial hyperresponsiveness³; increased after allergen challenge⁴; and decreased with corticosteroid treatment of asthma.⁵ Additional clinical studies, genetic studies, and many studies of animal models of allergic

Abbreviations used

AHR: Airways hyperresponsiveness
FoxP3: Forkhead box protein 3
NK: Natural killer
EB: Eosinophilic bronchitis
T-bet: T-box transcription factor
Treg: Regulatory T
TSLP: Thymic stromal lymphopoietin

airways disease have supported the T_H2 hypothesis of asthma, namely that allergen-specific T_H2 cells drive many of the immunopathologic features of asthma (Fig 1). In addition, a T_H2 cytokine profile was also demonstrated in patients with nonatopic asthma.⁶

As further studies reveal the heterogeneity of asthma phenotypes and response to treatment, the complexity of the immunology of both T cells and asthma, and the contribution of structural airway tissue cells to pathophysiology, it has become fashionable to question the relevance of the T_H2 hypothesis. Therefore where does the T_H2 hypothesis of asthma stand today, and what is the current understanding of the role of T cells in asthma?

DEFINING AND UNDERSTANDING THE HETEROGENEITY OF ASTHMA

Initial studies of T cells in asthma were performed when classification of subtypes of asthma went little further than separating out atopic allergic disease from nonatopic or intrinsic asthma, with possible overlapping subgroups of occupational and aspirin-sensitive asthma. By means of analysis of cytokine gene expression and airway inflammation, these subtypes appeared similar, with eosinophilic inflammation and T_H2 cytokines prominent. Indeed, detection of local airway IgE synthesis, together with identification of autoallergens, raises the possibility that nonatopic asthma might be a form of autoallergy with a T_H2 response to self-antigens. *Autoallergens* is a term suggested for self-antigens recognized by IgE, such as Hom s 1.⁷ Thus the concept arose of a unifying T_H2-type inflammation in all forms of asthma, providing an attractive rationale for T_H2-targeted therapy.

A series of investigations of asthma's immunopathology have focused on comparison of asthma with eosinophilic bronchitis (EB).⁸⁻¹¹ EB was initially characterized by Gibson et al⁸ as eosinophilic airway inflammation (detected in sputum eosinophilia) with cough but no other physiological features of asthma, such as airway obstruction or hyperreactivity. The group in Leicester, United Kingdom, have shown that EB and asthma share many of what were previously thought to be cardinal features of asthma, including eosinophilic inflammation and features of airway remodeling. The distinguishing immunopathologic feature between EB

From the Leukocyte Biology Section, NHLI, Imperial College London, and Laboratorios Leti SL, Madrid.

Disclosure of potential conflict of interest: D. S. Robinson is employed by Leti Laboratories; is a consultant for MedImmune, Inc; and is a research investigator for Imperial College.

Received for publication April 19, 2010; revised June 23, 2010; accepted for publication June 25, 2010.

Reprint requests: Douglas S. Robinson, MD, FRCP, Laboratorios Leti SL, Calle del Sol, 5, 28760 Tres Cantos, Madrid, Spain. E-mail: dsrobinson@leti.com.
0091-6749/\$36.00

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doi:10.1016/j.jaci.2010.06.025

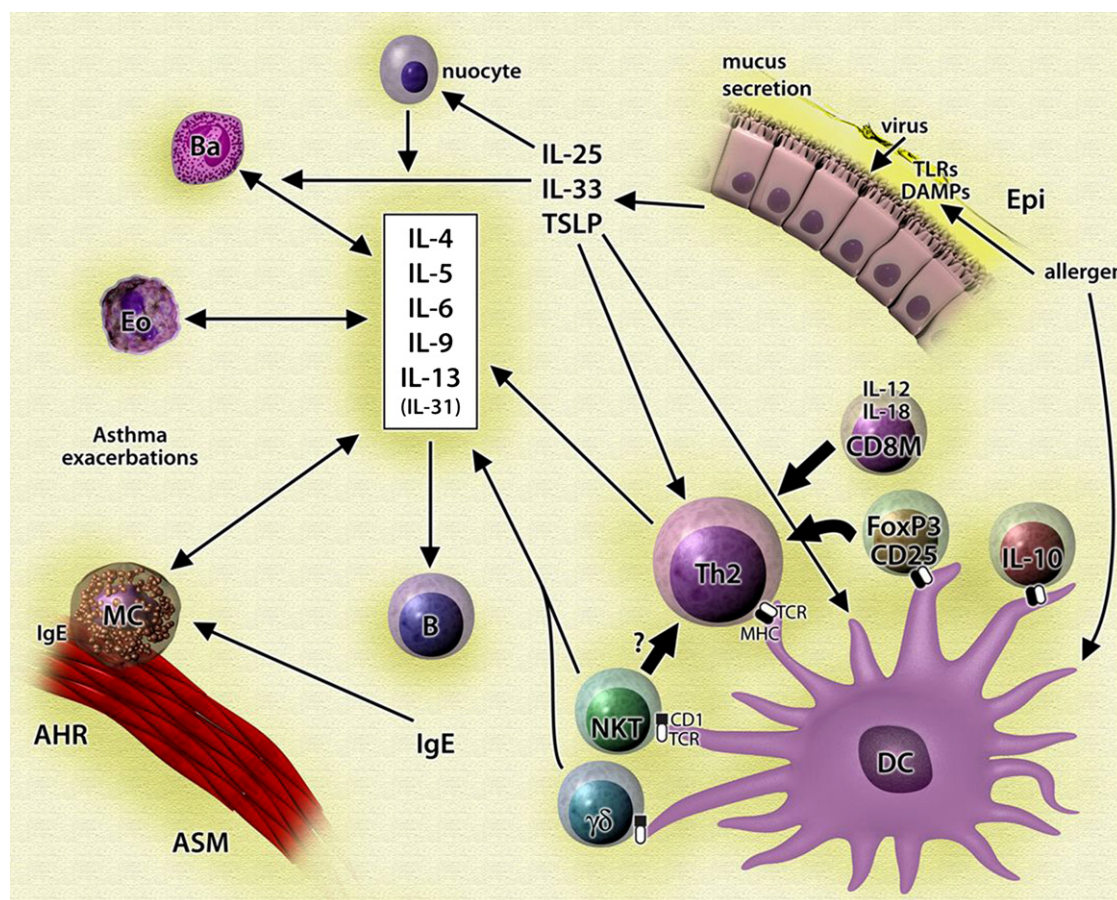


FIG 1. Immunologic interactions of T cells in patients with asthma. Activation of airway epithelium (Epi) by allergens and viruses through Toll-like receptors (TLR) and damage receptors (DAMPs) leads to release of the pro- T_H2 cytokines IL-25, IL-33, and TSLP, which act through dendritic cells (DC), which present allergen peptides through the MHC/T-cell receptor (TCR) complex, and directly on T cells and non-T cells to favor T_H2 cytokine production (IL-4, IL-5, IL-6, IL-9, IL-13, and possibly IL-31). Some of these cytokines act on (and are produced by) eosinophils (Eo), which might act in asthma exacerbations, and basophils (Ba) and mast cells (MC), and IL-4 and IL-13 switch B cells to IgE production. T_H2 cytokines also act on structural cells, such as epithelium, to increase mucus production, and MC interaction with airway smooth muscle (ASM) might induce AHR. NKT cells, $\gamma\delta$ T cells (both activated through T-cell receptor and CD1), and CD8 $^+$ T cells can also produce T_H2 cytokines but might also suppress T_H2 responses in certain settings. Treg cells, including FoxP3 $^+$ CD25 $^+$ and IL-10-producing Treg cells, have the potential to suppress T_H2 cells.

and asthma was infiltration of mast cells into the airway smooth muscle in patients with asthma, which was not seen in patients with EB.⁹ Analysis of bronchoalveolar lavage T cells by means of intracellular cytokine staining showed higher percentages of CD4 $^+$ T cells staining for IL-4 in both patients with EB (7.2%) and those with asthma (5.3%) than in control volunteers (2.8%), whereas IFN- γ -producing T cells were not different in numbers among the 3 groups.¹⁰ By means of immunohistology, IL-4 $^+$ and IL-5 $^+$ cells were increased in both patients with EB and those with asthma, although these were mostly non-T cells. IL-13 levels were increased in the bronchial mucosa in patients with asthma compared with those seen in patients with EB but was mostly localized to eosinophils by using the staining techniques used.¹¹ Thus these studies suggest that T_H2 T cells might contribute to many of the immunopathologic features of asthma, but because these are also seen in patients with EB, other factors must act in concert with T_H2 cells to produce the characteristic pathophysiology of asthma or act to prevent asthma in patients with EB. Further studies of patients with EB and asthma will be of interest.

Recent studies have aimed to subtype asthma by using a cluster analysis of multiple variables measured in large cohorts with differing disease severities.^{12,13} The aim here is to perform an unbiased analysis to examine how these variables cluster to define subgroups rather than predefine types of asthma. Clearly an important first step is to confirm the diagnosis of asthma and consider adherence with treatment.¹⁴ Two large studies have shown broadly similar clusters, including a large group with early-onset atopic asthma (which can be subdivided by response to treatment), a cluster of female subjects with older-onset nonatopic asthma, a cluster of patients who have persistent eosinophilic disease as detected by sputum eosinophilia but few symptoms, and a subgroup with persistent symptoms but relatively little airway inflammation.^{12,13} Defining these subgroups within severe asthma cohorts will be particularly important for targeting immunologically based therapy (see below).¹⁵ In addition, a small percentage of subjects with severe asthma have steroid-resistant asthma and do not respond well to inhaled or oral steroid treatment. It is not yet clear how these different clusters fit within

the T_H2 hypothesis of asthma: response to anticytokine treatment (considered below) suggests there might be subgroups of patients with severe asthma in which eosinophilia is relevant, particularly to asthma exacerbations, despite corticosteroid therapy, whereas other factors, as yet less well defined, might play a role in clusters with persistent symptoms without much airway inflammation. Are other types of T cells involved in some of these subtypes? Let us first consider the increasing complexity of T cells and cytokines that might play a role in asthma.

T-CELL COMPLEXITY AND FLEXIBILITY

Since the initial description of T_H1 and T_H2 cells, an increasing array of cytokines and chemokines has been described, both produced by and influencing differentiation of T_H1 and T_H2 cells. In addition, new proinflammatory and suppressive (or regulatory) T-cell subsets have been defined, and our understanding of T-cell programming and flexibility has increased at the level of the transcription factors acting to augment or repress cytokines genes and the resulting epigenetic changes involved in determining the stability of T_H cell phenotypes. How does this increased knowledge affect our understanding of asthma?

CYTOKINES

In addition to the canonical T_H2 cytokines IL-4 and IL-5, additional cytokines relevant to asthma have been described. In particular, IL-13, which is clustered with *il4*, *il5*, and *gm-csf* genes on chromosome 5q and is coordinately expressed with IL-4 and IL-5 in murine and human T_H2 cells,^{16,17} is of great current interest. First, studies of mRNA and protein expression showed increased IL-13 levels in the airways in both patients with atopic and those with nonatopic asthma, with preferential expression of IL-13 in airway eosinophils and blood T cells from asthmatic patients when compared with patients with EB.^{11,18} Second, the biology of IL-13 makes it another clear candidate for driving features of asthma: it shares activity with IL-4 in switching B cells to IgE production and activating monocytes and macrophages and increases airway mucus production through goblet cell hyperplasia.¹⁹ IL-13 shares a receptor with IL-4 and has its own IL-13 receptor α chain, which is not expressed by naive T cells. Hence IL-13 (unlike IL-4) does not drive T_H2 differentiation. Blocking the IL-13 receptor α chain (with a fusion protein that also blocked the IL-4 receptor α chain) dramatically reduced airway hyperresponsiveness in a murine model of allergic airways disease and interestingly did so without affecting either airway eosinophil infiltration or allergen-specific IgE.²⁰ Initial results with agents that block the IL-13 receptor in clinical trials are mixed.^{21,22}

IL-6 is produced by T_H2 cells (as well as many other cells types, including airway epithelium), and in addition to being an acute-phase proinflammatory cytokine, it is implicated in enhancing T_H2 differentiation and proliferation of memory T_H2 cells. In addition, IL-6 inhibits suppression by CD4⁺CD25⁺ regulatory T (Treg) cells.²³ In a murine model blockade of the soluble IL-6 receptor α chain reduced airway inflammation, whereas blockade of membrane-bound IL-6 receptor α chain increased Treg cell numbers.²⁴ Unless targeted to the airway blocking, IL-6 might adversely affect the ability to mount acute-phase responses.

IL-9 is a T_H2 cytokine that is also a current drug target (antibodies are in phase II development). It acts to enhance T_H2

cytokine production, airway mucus production, and eosinophil and basophil differentiation among other actions, at least in part by induction of IL-13 production.²⁵ In murine models blockade of IL-9 reduced airways hyperresponsiveness (AHR) and airway mast cell numbers. IL-9 has been linked to asthma in genetic studies, and its expression is increased in the airways of asthmatic patients.²⁶ Recent reports of T_H9 cells raise the question of whether IL-9 might be produced by these cells in asthma (see below).

IL-31 is a member of the IL-6 cytokine family also produced by T_H2 cells. Although it has principally been linked to atopic dermatitis, increased expression was detected in airway tissues from sensitized mice after allergen challenge, and serum IL-31 concentrations were increased in asthmatic patients, as were IL-31 transcripts in PBMCs.^{27,28} How IL-31 might act in patients with asthma is unclear, although it might affect epithelial cell functions, and intervention studies are not yet reported in mice or human subjects.

Increasingly, the interplay between tissues and the adaptive immune system is recognized as an important factor in inflammation, including asthma. A group of cytokines derived from a variety of cell types might act in initiating, amplifying, and maintaining T_H2-type inflammation in patients with asthma. These include thymic stromal lymphopoietin (TSLP), IL-25, IL-33, and osteopontin.^{29,30} TSLP, which is related to IL-7 and derived from epithelial cells in response to infectious agents, damage, and allergens, acts on dendritic cells to upregulate surface OX40 ligand and hence favors T_H2 differentiation. It also directly activates mast cells. TSLP expression is increased in the airways in asthma, and blocking TSLP can reduce inflammation and AHR in murine models.³¹ IL-25 was initially described as a murine T_H2 cell product and can be produced by mast cells and epithelial cells, but in human patients with asthma, it appears to be produced predominantly by eosinophils and basophils.³² Murine epithelial cells produced IL-25 in response to allergen exposure.³³ Instillation or overexpression of IL-25 in the airways of mice caused T_H2-type inflammation and AHR, and blockade of IL-25 at both the sensitization and challenge phases reduced inflammation and AHR in a murine allergic airways model.³² IL-25 acted on human memory T_H2 cells to increase GATA-3 and c-maf expression and thus increased cell numbers and cytokine production.³² In the initial article describing IL-25, it was shown that it still induces T_H2 cytokines in mice lacking T cells, raising the possibility of a T cell-independent pathway for T_H2-type cytokine production.³⁴ More recently, IL-25 has been shown to induce T_H2 cytokine production from hematopoietic lineage cells recently identified as multipotent progenitors and a lineage termed nuocytes^{35,36}; whether this action amplifies airway inflammation in asthma or acts independently of T-cell activation is as yet unknown.

IL-33 is an IL-1 family cytokine that acts to increase cytokine production from polarized T_H2 cells (through the receptor termed T1/ST2) in a manner analogous to the IL-1 family member IL-18, which enhances cytokine production from polarized T_H1 cells.²⁹ Neither cytokine acts on naive T cells. Unlike other IL-1 family members, IL-33 does not require caspase activation. Indeed, caspases from apoptotic cells acted to reduce its activity.³⁷ IL-33 is produced by airway epithelial cells, fibroblasts, and smooth muscle cells and therefore might also act as link between tissue cells and the adaptive immune response in asthma. Recently, IL-33 was reported to act on murine bone marrow-derived dendritic cells to

induce a dendritic cell phenotype that activated IL-5 and IL-13, but not IL-4 or IFN- γ , production from naive CD4⁺ T cells.³⁸ Whether this is relevant to human asthma remains unclear. IL-33 also acts on mast cells and basophils to increase survival and cytokine production and on eosinophils to prime for degranulation. IL-33 expression was increased in the airways of patients with severe asthma.³⁹ What activates IL-33 production from airway epithelium in asthmatic patients remains to be determined. Both IL-33 and IL-25 induce TSLP production from epithelial cells.²⁹ This triad of cytokines might represent a potential target for intervention in asthma and a way of intervening in epithelial cell–T-cell cross-talk.

Osteopontin is produced from a variety of cell types, including epithelial cells. Its expression was increased in the airways in human asthma (both in epithelial cells and submucosal cells), and osteopontin knockout mice had reduced airway T_H2 inflammation, AHR, and airway remodeling in a chronic challenge model.³⁰ However, administration of osteopontin to the airways of sensitized wild-type mice at the time of allergen challenge reduced AHR and T_H2 inflammation. Osteopontin transcripts were increased in peripheral blood CD4⁺ T cells from asthmatic subjects when compared with those seen in control subjects.³⁰ Because osteopontin is thought to be involved in other diseases, therapeutic agents might be developed: their effects in human asthma will be of interest.

T_H1, T_H2, T_H17, T_H22, T_H9, AND MORE?

As understanding of the factors acting in the differentiation of T_H1 and T_H2 cells increased, evidence for T_H2 involvement in asthma accumulated. For example, mice deficient in T-box transcription factor (T-bet), a key T_H1 transcription factor that controls expression of IFN- γ , spontaneously showed features of allergic airways disease, including AHR and T_H2/eosinophilic airway inflammation, and features of airway remodeling.⁴⁰ Furthermore, T-bet expression was reduced in the airways of human subjects with asthma compared with that seen in control volunteers. Similarly, GATA-3 expression, which acts in T_H2 differentiation, was increased in the airways in patients with asthma,⁴¹ and knockdown of this transcription factor prevented development of AHR and inflammation in a murine allergen challenge model.^{42,43} Some reports have suggested that T_H1 cells might have a role in asthma, with detection of IFN- γ in bronchoalveolar lavage T cells by means of intracellular cytokine staining.⁴⁴ However, this might reflect the technique used, and most reports do not support a role for T_H1 cytokines in patients with asthma.^{2,10} Since their initial description, it has been clear that T_H1 and T_H2 cells represent polarized effector cells and that T cells with different cytokine patterns also occur.¹ More recently, several different T_H types have been described, including T_H17, T_H22, and T_H9.

T_H17 cells, which produce IL-17 and IL-22, were initially described as developing from naive T cells stimulated in the presence of IL-23 and having the ability to induce autoimmune inflammation. Their biology was recently reviewed in the *Journal*.⁴⁵ TGF- β , together with IL-1 β and IL-6, is also important in their development (probably in both mice and human subjects) through induction of the transcription factor retinoic acid receptor–related orphan receptor γ t.⁴⁶ Human T_H17 cells can be recognized by means of surface expression of CCR6 together with CD161. Although *IL17* mRNA expression is increased in human

asthmatic airways, it is not confirmed whether this is from T_H17 T cells because many other cell types can make IL-17. T_H17 cells were reported to induce a neutrophilic, steroid-resistant inflammation and AHR in one murine model,⁴⁷ another murine model showed enhanced T_H2-mediated airway inflammation by means of coadministration of polarized T_H2 and T_H17 cells to the airway,⁴⁸ and in another model T_H17 cells reduced eosinophilic airway inflammation and AHR.⁴⁹ Murine T_H17 cells expressed IL-13 receptors, and IL-13 downregulated IL-17 production, raising the possibility that blocking IL-13 in patients with asthma might switch to a T_H17 response.⁵⁰ A variant of T_H17 clones that also makes IL-4 (T_H17/T_H2 cells) was recently derived from human CD4⁺CCR6⁺CD161⁺ peripheral blood T cells and could also be derived from naive T cells when IL-4 concentrations were high.⁵¹ A role for T_H17 cells or T_H17/T_H2 cells in human asthma remains to be demonstrated, although it has been suggested that these cells might contribute to neutrophilic, steroid-resistant severe asthma.

TGF- β was shown to induce an IL-9–producing T-cell subset, termed T_H9 cells, from T_H2 cells,⁵² and this phenotype could also be driven from naive CD4⁺ T cells by TGF- β combined with IL-4. TGF- β also increased IL-9 production from T_H17 cells. However, whether these cells really represent a stable T_H subset or tell us that the *IL9* gene can be activated by TGF- β in multiple T_H cell types is unclear,¹⁶ as is whether this mechanism might act to increase IL-9 levels in the asthmatic airway.

Recently, a subset of human T cells was identified from human epidermis that produce IL-22 and TNF- α but not IL-17, IL-4, or IFN- γ . These T_H22 cells enhanced wound healing and were increased in a variety of skin diseases, including atopic dermatitis.⁵³ There is as yet no information on whether they exist in the airway. Further studies of T_H subsets in different subtypes of human asthma are required.

Clearly, the spectrum of potential T-cell phenotypes that might contribute to airway inflammation in patients with asthma is more complex than was previously appreciated. One might speculate that different T-cell subtypes might be involved in different clusters of asthma or that T-cell cytokine production might have a degree of flexibility (possibly at the epigenetic level) depending on allergen/viral interactions with the epithelium, treatments such as inhaled corticosteroids, or both. For example, T_H2- or T_H9-type T cells might be important in more benign atopic asthma, whereas T_H17- or T_H17/T_H2-type T cells might be important in at least some patients with therapy-resistant severe disease. Immunologic assessment of different asthma subtypes will be of fundamental importance in optimizing biologic agents in asthma treatment. On the other hand, fixed epigenetic remodeling of memory T-cell phenotypes in patients with chronic asthma might also engender therapy resistance in some settings.

TREG CELLS AND ASTHMA

In addition to proinflammatory T_H cell subsets, it is now clear that tolerance to self-antigens and many harmless environmental antigens is maintained, at least in part, by means of active suppression by Treg cells. Treg cell subsets include naturally occurring thymically derived CD4⁺CD25^{hi} T cells characterized by expression of the transcription factor forkhead box protein 3 (FoxP3), which controls their function, and inducible FoxP3⁺ Treg cells and IL-10–producing Treg cells (which can be FoxP3 positive or negative). The biology of these cells and their

relation to asthma has been extensively reviewed.⁵⁴ In brief, there is evidence that in atopic allergic disease suppression of T_H2 responses to allergens by both $CD25^{hi}FoxP3^+$ and IL-10-producing cells is deficient.^{55,56} Furthermore, $CD25^{hi}FoxP3^+$ T-cell numbers and function were reduced in bronchoalveolar lavage samples from children with asthma compared with those seen in control subjects,⁵⁷ and FoxP3 expression by circulating $CD4^+CD25^{hi}$ T cells was reduced in asthmatic patients.⁵⁸ In a murine model of allergic airways disease, transfer of allergen-specific $CD4^+CD25^{hi}$ T cells could reduce AHR and T_H2 inflammation after airway inflammation had been established and prevent, but not reverse, airway remodeling changes in a chronic challenge model.^{59,60} Why might Treg cell function be deficient in asthmatic patients? In part this might be due to actions of signals that reduce Treg cell function and expand effector cells. These include mediators released in response to activation of danger and cell damage receptors on dendritic cells and epithelial cells by factors such as allergens (house dust mite) and infectious agents. For example, IL-10 Treg cell function is reduced by engagement of Toll-like receptor 9,⁶¹ and it is of note that experimental rhinovirus challenge of asthmatic patients reduced airway IL-10 expression, which might in part explain virus-induced asthma exacerbations.⁶²

It is an attractive idea that augmentation of Treg cell function might control T_H2 -mediated inflammation in asthmatic patients. Various experimental protocols have been described for inducing Treg cells *in vivo* in experimental animals, including low-dose and high-dose inhaled tolerance (which induce TGF- β -dependent and IL-10-producing Treg cells, respectively),^{63,64} histamine receptor 4 agonists,⁶⁵ and prostanoid DP-1 receptor agonists,⁶⁶ but these all act before induction of airways disease. In addition, IL-2/anti-IL-2 antibody complexes could selectively expand Treg cells *in vivo* and reduce AHR in a murine model.^{67,68} TGF- β and activin-A induce distinct Treg cell populations,^{69,70} can suppress allergic airway inflammation in animal models, and might be important in suppression of T_H2 responses in the airway. Of note, expression of receptors for these TGF family cytokines and the related bone morphogenic proteins was reduced in asthmatic airways, raising the possibility that this control loop might be deficient in asthmatic patients.^{70,71}

Treg cell biology has opened several encouraging areas for understanding and manipulating the development of allergic asthma. Vitamin D3, acting with corticosteroids, can induce an IL-10-producing Treg cell population from murine and human naive T cells *in vitro*.⁵⁴ Epidemiologic studies suggest widespread vitamin D3 deficiency in Western populations, and it is hypothesized that this might reduce Treg cell differentiation, thus predisposing to asthma (and autoimmune diseases).⁵⁴ Oral vitamin D3 supplementation in steroid-resistant asthmatic patients restored *in vitro* IL-10 production after steroid treatment of their peripheral blood $CD4^+$ T cells.⁷² Vitamin D3 supplementation in murine models of subcutaneous and sublingual immunotherapy enhanced both IL-10 production and response to therapy.^{73,74} It will be of great interest to determine whether vitamin D3 supplementation can improve or even prevent asthma or enhance the response to allergen immunotherapy. Of note, IL-10 is also produced by T_H1 and T_H2 cells relatively late after activation, as well as by T_H9 cells, $CD8^+$ T cells, natural killer (NK) cells, macrophages, B cells, eosinophils, and mast cells: in this context IL-10, which is a potent inhibitor of antigen-presenting cell

function and proinflammatory cytokine production (although it is a mast cell growth factor), might act as a natural brake on inflammation. What controls IL-10 production from these cell types and whether the balance between IL-10 and proinflammatory T_H2 cytokine cytokines is altered in asthmatic patients remains uncertain.

The influence of neonatal development on subsequent risk for asthma is controversial. Exposure of lactating mice to inhaled allergens transferred the allergen in breast milk and induced a TGF- β -dependent Treg cell-induced tolerance to induction of allergic airways disease in the young.⁷⁵ In experimental models persistent allergen exposure is required to maintain Treg cell-dependent tolerance.⁷⁶ For bee keepers who induce tolerance to bee venom through multiple stings during the months of tending their bees, tolerance wanes rapidly during the winter months.⁷⁷ Whether long-term preventive tolerance can be achieved by inducing memory Treg cells remains to be established, although data from studies of allergen immunotherapy (see below) suggest that long-term immune modulation might be possible.⁷⁸

OTHER T CELLS: CD8 T CELLS, NKT CELLS, AND $\gamma\delta$ T CELLS

In addition to further complexity among $CD4^+$ T-cell subsets, there is increasing interest in other T-cell types in asthma. In particular, the role of $CD4^+$ NKT cells is controversial. These T cells recognize glycolipids presented by the MHC class I-like molecule CD1d and have an invariant V α 24J α 18 T-cell receptor in human subjects. They can produce a similar variety of cytokines to conventional $CD4^+$ T cells, including T_H1 , T_H2 , T_H17 , and IL-10-predominant patterns. An initial report suggested that up to 60% of $CD3^+CD4^+$ T cells in the airways of patients with moderate-to-severe asthma were NKT cells.⁷⁹ However, subsequent studies did not support such high numbers, nor did all agree that NKT cell numbers were increased in asthmatic patients.⁸⁰ Thus whether NKT cells are important in asthma remains unclear. If they do have a role, what might they do, and how might their activity be reduced? A CD1d-binding lipid antagonist prevented AHR in a murine model of allergen challenge but had no effect on T_H2 cells.⁸¹ Interestingly, CD1d-restricted NKT cell clones recognized phospholipids from cypress pollens, although more phospholipid-responsive clones were conventional $CD4^+$ T cells or $\gamma\delta$ T cells.⁸²

$\gamma\delta$ T cells use a T-cell receptor comprising γ and δ chains as opposed to the classical $\alpha\beta$ T-cell receptor found on most $CD4^+$ T cells. They recognize small organic molecules, alkylamines, and lipids presented by CD1 on dendritic cells. These cells can also produce an array of different cytokines, and an increase in $\gamma\delta$ T cells expressing T_H2 -type cytokines is reported in bronchoalveolar lavage fluid after allergen challenge of asthmatic patients.⁸³ These cells can have both proinflammatory and regulatory functions. Indeed, in murine models they have been shown to be required for T_H2 -type eosinophilic inflammation and AHR to allergen challenge⁸⁴ and to exert a suppressive role on the T_H2 response to allergen challenge, depending on the model.⁸⁵ The relative importance of $\gamma\delta$ T cells in human asthma remains to be determined, and it is clear that, as for $CD4^+$ T cells, they might have various, possibly opposing roles.

$CD8$ lymphocytes react to antigens presented by MHC class I molecules. Although some, but not all, studies have identified a

minority population of CD8⁺ T cells in the asthmatic airway that express IL-4 and IL-5,⁸⁶ it is not clear how exogenous antigens, such as allergens, would be recognized by CD8⁺ T cells. Recently a potential regulatory role for cytokine-activated CD8⁺ memory T cells was described in an allergic airways animal model.⁸⁷ CD8⁺ memory T cells can be activated without antigen by the cytokines IL-12 and IL-18 to proliferate and produce IFN- γ and TNF- α . This can occur early in infections as Toll-like receptor activation of dendritic cells triggers IL-12 release and might thus program subsequent adaptive immune responses toward a T_H1 response. Indeed, in a murine model early nonspecific activation of memory CD8⁺ T cells could reduce AHR and T_H2 inflammation in an allergic airways disease.⁸⁷ Because CD4⁺ lymphocytes can also be activated by IL-12 and IL-18, they might have the same modulatory capacity. Thus CD8⁺ (or possibly CD4⁺) memory cells in the airways might be a potential brake on the development of T_H2 responses, which might be one immunologic explanation of how early infections are thought to protect against development of allergic diseases and asthma (ie, the hygiene hypothesis).

OTHERS CELLS PRODUCING T_H2 CYTOKINES: ARE T CELLS NEEDED FOR DEVELOPMENT AND MAINTENANCE OF ASTHMA?

From very soon after the initial description of T_H1 and T_H2 T cells, it became clear that many other cell types have the capacity to produce T_H1- or T_H2-associated cytokines. These include cells of hematopoietic lineage, such as eosinophils, basophils, mast cells, and macrophages, and structural cells of mesenchymal origin, such as epithelial cells, fibroblasts, and smooth muscle cells.⁸⁸ Cytokine production from these cells might be triggered as part of an allergic response (eg, through IgE receptors) or through activation of Toll-like receptors and other danger signals by infectious agents or some allergens.⁸⁸ Recently, house dust mite extract was shown to directly activate Toll-like receptor signaling, and this might be a mechanism for activation of epithelial cells, mast cells, basophils, eosinophils, and other cell types in asthmatic patients.⁸⁹ These cells might thus amplify T_H2-dependent allergic inflammation, or because basophils, eosinophils, and other cells can act as antigen-presenting cells, they might even initiate or perpetuate T_H2 cell activation. The relative importance of these cells varies in different animal models, although it is of note that AHR and inflammation in murine models are dependent on airway CD4⁺ T cells⁹⁰ and dendritic cells⁹¹ for both initiation and maintenance, suggesting a central place for T-cell activation and the adaptive immune response.

Whether epithelial cell activation can induce AHR independently of T cells is unclear. One theoretical pathway would be through IL-25 because this cytokine can activate T_H2-type cytokine production from non-T cells.³⁴⁻³⁶ However, it seems most likely that interaction between the innate immune response, structural cells, and an adaptive T_H2 response acts to produce asthma. Development of asthma might require abnormalities in airway epithelium and other components of the airway, as well as a T_H2 response.

T-cell migration to and maintenance in the airways is an important determinant of airway inflammation in patients with asthma. Chemokines are discussed in another review in this issue of the *Journal*,⁹² but it is of note that although specific chemokine receptor signatures might allow selective ingress of specific T-cell subsets, both T_H2 cells and Treg cells express CCR4 and CCR8 so

that selective blockade of either receptor in an asthmatic patient might both alleviate and exacerbate disease. In addition, chemokines can act to alter T-cell function, including cytokine production and Treg cell function.

TREATMENT OF ASTHMA: WHAT DOES IT TELL US ABOUT THE ROLE T CELLS, AND DOES UNDERSTANDING THE ROLE OF T CELLS IN ASTHMA OFFER NEW THERAPIES?

The mainstay of asthma therapy is inhaled corticosteroids. Corticosteroids reduce T_H2 cytokine production in the asthmatic airway and inhibit many aspects of T-cell activation.⁵ More recently, it was shown that corticosteroids also upregulate FoxP3 and IL-10 in Treg cells.^{93,94} Thus many of the beneficial effects of corticosteroids in asthma might be due to effects on T cells. Corticosteroids have many other actions, and more direct support for the importance of T-cell activation in patients with chronic severe asthma came from studies showing clinical improvement in lung function in patients with severe asthma with cyclosporin A,⁹⁵ although this agent also has actions on basophils and mast cells.⁹⁶ This treatment has not found widespread use in the clinic, partly because of side effects but also because responses seem variable. It should be re-evaluated in light of recent cluster subtypes of severe asthma.

A more direct proof-of-concept study showed improved peak expiratory flow in patients with severe asthma after treatment with a blocking CD4 mAb (keliximab).⁹⁷ This treatment was not developed further because of safety concerns, but the findings suggest that specific targeting of CD4⁺ T cells in the asthmatic airways would be beneficial (see Table I).

Daclizumab, which blocks the IL-2 receptor α chain and thus targets activated T cells, was effective in asthma, improving FEV₁ and symptoms and prolonging time to exacerbation, presumably through an effect on activated T_H2 cells.⁹⁸ The IL-2 receptor is also expressed on other cell types.⁹⁹

Another approach to target T cells is to block the second signal required in T-cell activation through antigen-presenting cells (the first is antigen peptides presented by MHC molecules to the T-cell receptor). Blockade of a variety of costimulators has been studied in animal models of asthma, including CD28, CD86, inducible costimulator, CD40L, OX40, and 41-BB (CD137).¹⁰⁰ The fusion protein cytotoxic T lymphocyte-associated antigen 4-Ig, which blocks CD28 interactions with CD80 and CD86, is now licensed for use in patients with rheumatoid arthritis (abatacept), and a modified version of this molecule binds preferentially to CD86, which might be particularly relevant to T cells in asthmatic patients.¹⁰⁰ A problem with this approach might be potential toxicity, either because the targets are expressed on other cell types or because of immunosuppressive effects. It has been suggested that OX40 (which might be important for the actions of TSLP, see above) and inducible costimulator are the most promising targets for asthma.¹⁰¹

Data from studies of T_H2 cytokine blocking antibodies in asthmatic patients have been reviewed elsewhere¹⁵: anti-IL-5 antibodies were effective in reducing exacerbation rates in selected patients with severe asthma and persistent eosinophilia^{102,103} but not in those with unselected moderate/severe asthma.¹⁰⁴ A combined IL-4/IL-13 receptor blocking agent inhibited the allergen-induced late asthmatic response.²¹ A study of an mAb blocking the IL-4 receptor α chain, hence targeting both IL-4

TABLE I. Current and potential T cell-directed treatments or asthma

Treatment	Immunologic effect (T cells)	Clinical status	Clinical effects in asthma	Reference nos.
Corticosteroids	Reduced T-cell activation and T _H 2 cytokines increased FoxP3 and IL-10 levels (Treg cells)	In clinic	Improved PFT responses, S scores, AHR	5, 83, 84
Cyclosporin A	Reduced T-cell activation and T _H 2 cytokines	In clinic	Improved FEV ₁	85
Anti-CD4 (keliximab)*	Reduced T-cell activation	Phase II	Improved PEF	86
Anti-CD25 (daclizumab)*	Reduced T-cell activation	Phase II	Improved FEV ₁ and S score	87
Anticytokines				
Anti-IL-5 (mepolizumab)	Reduced eosinophilia	Phase III	Reduced exacerbation rate (subgroup)	90, 91, 93
Anti-IL-4/IL-13 (pitakinra/AMG)	Reduced IgE, mucus, AHR	Phase II	Reduced LAR, improved ACQ (subgroup)	19
Anti-IL-9	Reduced mucus, AHR	Phase II (in progress)	Unknown	13
Anti-IL-25, anti-IL-33, anti-TSLP	Decreased T _H 2 cytokine levels	Not yet tested	Unknown	26
T-cell recruitment				
Chemokine receptor antagonists	Reduced T _H 2 recruitment	Not yet tested	Unknown	This issue
CrTh2 antagonists	Reduced T _H 2 recruitment	Phase II (in progress)	Unknown	95
T-cell signaling	Reduced T-cell activation	Not yet tested	Unknown	94
Costimulation				
OX40L, CD137, ICOS	Reduced T-cell activation	Not yet tested	Unknown	89
CTLA4-Ig (abatacept)	Reduced T-cell activation	In clinic for RA	Unknown	88
Immunotherapy				
Conventional	Reduced T _H 2 activation Treg cells, IgG4	In clinic	Improved S score	
Allergoids	? Same	In clinic	Improved AHR and S score	100
Peptides	? Same	Phase II	Unknown	101

ACQ, Asthma Control Questionnaire score; AMG, Amgen; CTLA4, cytotoxic T lymphocyte-associated antigen; ICOS, inducible costimulator; LAR, late asthmatic response; OX40L, OX40 ligand; PEF, peak expiratory flow; PFT, pulmonary function test; RA, rheumatoid arthritis; S score, symptom score.

*Development discontinued.

and IL-13, failed to meet its primary end point of a reduction in the Asthma Control Questionnaire score, although there was a reduction in exacerbation rates.²² Of note, recent gene array analysis has suggested only approximately 50% of asthmatic subjects have increased IL-13-induced gene signatures, so that targeting to this subgroup might be required.¹⁰⁵ Although omalizumab is principally targeted at IgE-effector cell interactions, it also blocks IgE-mediated antigen uptake and presentation by dendritic cells, thus reducing T-cell activation in the airway.¹⁰⁶ Current data suggest that anti-TNF therapy is not effective in patients with severe asthma, although a subset of patients might benefit.¹⁵ Of T_H2-related cytokines, TSLP, IL-25, and IL-33 are all currently being evaluated as therapeutic targets in asthma. Small-molecule antagonists have been developed for a number of chemokine receptors, including CCR3 and CCR4, which might be relevant for targeting T-cell recruitment to the airway in asthma. Of particular interest is CRTh2, which is the receptor for prostaglandin D₂ and is expressed by T_H2 cells, mast cells, and eosinophils and acts both in chemotaxis and increasing T_H2 cytokine production.¹⁰⁷ Antagonists of CRTh2 are in phase IIb clinical trials in asthmatic patients. By contrast, agonists of the other receptor for prostaglandin D₂, DP-1, modulated dendritic cell function and reduced allergic airway inflammation in an animal model by inducing Treg cells.⁶⁶

A variety of signaling molecules (kinases) and transcription factors (eg, nuclear factor κ B and GATA-3, which is inhibited by peroxisome proliferator-activated receptor [PPAR] γ inhibitors in use in diabetes), either expressed in T cells or important in T-cell activation, have been suggested as targets to reduce T_H2 inflammation in asthmatic patients.^{108,109} Another potential target might

be Notch signaling.¹¹⁰ As yet, none of these targets has shown efficacy in clinical trials, and these agents might lack the specificity required to prevent side effects through immune suppression.

Allergen immunotherapy (through either the subcutaneous or sublingual route) is effective treatment for allergic asthma and is thought to act at least in part by inducing allergen-specific Treg cells producing IL-10 (and possibly TGF- β).¹¹¹ These Treg cells might then reduce T_H2-mediated allergic inflammation and, through IL-10, induce B-cell switching to IgG4 production and hence production of blocking antibodies. However, present allergen immunotherapy is restricted by the potential for IgE cross-linking and thus adverse events, including anaphylaxis and severe asthma. A number of approaches have been suggested to reduce or avoid this problem. Currently in clinical use, chemically modified allergen extracts, or allergoids, with reduced IgE binding but preserved T-cell activity have shown efficacy in patients with allergic asthma.¹¹² Novel approaches to immunotherapy have been described elsewhere. Of particular relevance here is the use of short peptides derived from the allergen molecule that do not cross-link IgE but retain activity for T cells. Peptide immunotherapy has been evaluated in patients with asthma for the major cat allergen Fel d 1. In proof-of-concept studies the peptide treatment could downregulate allergen-induced late cutaneous responses and T_H2 cytokine production by allergen-stimulated PBMCs.¹¹³ This was accompanied by increased IL-10 levels and induction of the regulatory CD4⁺ T-cell subset. As mentioned above, use of vitamin D as an oral supplement improved outcomes in animal models of allergen immunotherapy, although a recently reported clinical trial showed negative results.

CONCLUSIONS

There is good evidence that T-cell activation is critical to initiation and maintenance of airway inflammation in patients with asthma. Interactions with cells of the innate immune system and airway structural cells, such as epithelium, fibroblasts, and smooth muscle, are increasing the focus for understanding asthma immunopathology. Accurate phenotyping of severe asthma will be essential in selections for appropriate investigation of new biological therapies aimed at the T cell.

What do we know?

- Different subtypes of asthma exist with different degrees and types of airway inflammation.
- T_H2 cytokine levels and activated T_H2 cell numbers are increased in the airways of asthmatic patients.
- Patients with EB also have T_H2 inflammation in the airway, but IL-13 levels might be increased in asthmatic patients.
- Atopy and asthma are associated with ineffective suppression of T_H2 cells by Treg cells.
- Corticosteroids reduce T_H2 cytokine levels, and other T cell-directed treatments, such as cyclosporin A and anti-CD4 antibodies, validate the $CD4^+$ T cell as a therapeutic target in asthmatic patients.
- Anti-IL-5 antibodies reduce exacerbation rates in some patients with severe asthma.
- Allergen immunotherapy can be effective in asthmatic patients, possibly by inducing Treg cells.

What is still unknown?

- The role of NKT cells in asthma (including different subtypes)
- The role of $\gamma\delta$ T cells in asthma (including different subtypes)
- The role of CD8 T cells in asthma (including different subtypes)
- The relative importance of T cell-derived and non-T cell-derived T_H2 cytokines in different subtypes of asthma
- The effect of anticytokine treatments aimed at IL-9, IL-13, IL-25, IL-33, and TSLP
- The effect of novel T cell-directed therapies, including costimulation blockade, signaling blockade, and CrTh2, and chemokine receptor antagonists and novel immunotherapy strategies

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