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T cells in asthma: Influences of genetics, environment, and T-cell plasticity

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Asthma is classically considered the archetypal T_H2 disease, with increased circulating IgE levels and eosinophilic inflammation being caused by increased levels of T_H2 -type cytokines. However, this paradigm has been challenged because of the realization that strategies designed to suppress T_H2 function are not effective for all patients. The clinical phenotype of asthma is notoriously heterogeneous and is affected by genetic and environmental exposures in addition to interactions between airway structural cells, including epithelial cells, and the immune system, as well as contributions from cells other than T_H2 cells. A combination of genetic and environmental factors is thought to influence whether inflammation resolves or progresses, and the pulmonary epithelium is increasingly recognized to play a key role in this process. This complex interplay has made it increasingly apparent that immune responses are tailored to the individual patient and determined by the weight of each influence, and thus the label of asthma as a T_H2 disease is too conservative. Indeed, an important concept that needs to be addressed, both in animal models and clinically, is that of T-cell plasticity and how lymphocytic responses are determined by environmental influences. (J Allergy Clin Immunol 2013;■■■:■■■-■■■.)

Key words: T cells, asthma, plasticity, environment, genetics, epigenetics

Asthma is a heterogeneous chronic inflammatory disease of the airways influenced by interactions between genetic and environmental factors. Disease pathology is characterized by airway inflammation that is classically eosinophilic in nature concomitant with changes in lung function, typically airways hyperresponsiveness (AHR). In addition, structural changes within the lung termed airway remodeling are present. Airway remodeling is a collective term that encompasses subepithelial fibrosis, increased smooth muscle mass, angiogenesis, and mucous gland

Abbreviations used

AHR: Airway hyperresponsiveness
BAL: Bronchoalveolar lavage
FoxP3: Forkhead box protein 3
miRNA: MicroRNA
PRR: Pattern recognition receptor
STAT: Signal transducer and activation of transcription
Treg: Regulatory T

hyperplasia. The physiologic consequences of these changes remain uncertain, in part because they are not fully reversed by current asthma therapy. However, airway remodeling is postulated to be a determinant of AHR, as well as the accelerated loss of lung function over time that is documented in many asthmatic patients. In the allergic lung multiple cycles of allergen-driven T_H2 inflammation have been proposed to drive a series of reactions in the pulmonary tissue that are akin to a classic wound-healing response. The normal response to harmless airborne allergen is tolerance, and this is achieved in the lung by a complex network of cells and molecules. In contrast, asthmatic patients respond with an acute inflammatory response that can either resolve or become chronic.

A wide variety of *in vitro* and *in vivo* model systems have been established to investigate the molecular and cellular pathways involved in the progression and resolution of airway inflammation and remodeling and have outlined roles for a diverse range of leukocytes in disease development.

EFFECTOR T-CELL SUBSETS

Although asthma has classically been considered a disease of T_H2 immunity, the complex clinical heterogeneity of the disease is increasingly recognized. T_H2 cells and the cytokines that they secrete are a distinct feature of the allergic response in atopic asthmatic patients; however, other effector T-cell populations are also observed in the lung. Thus the molecular mechanisms that drive the underlying pathology in the allergic lung are likely to involve a more diverse panel of cytokines than the classical T_H2 family. This might indicate contribution from different immune pathways in eliciting disease.

T_H2 cells

A large body of experimental data, both mouse and human, document the expression of T_H2 -type cytokines, such as IL-4, IL-5, and IL-13, in the allergic lung.¹ Experimental models of asthma indicate that these cytokines, IL-13 in particular, are critical in

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driving key pathologic features of the allergic response, including eosinophilic inflammation, airway mucus production, airway remodeling, and the cardinal trait of the disease, AHR. Moreover, T_H2 blockade is very effective in suppressing these features of allergic disease in mice.² However, clinical trials of drugs designed to reduce the activity of these cytokines have not been universally successful in asthmatic patients. Although initial trials with neutralizing antibodies targeting IL-5 had no effect on lung function,³ anti-IL-13 antibody was found to temper disease in only a subgroup of patients.^{4,5} Although recent data have shown that the anti-IL-5 antibody mepolizumab was effective in reducing exacerbation risk in patients with severe eosinophilic asthma, it is clear that this is not going to be a treatment applicable to all patients.⁵

The classical asthmatic phenotype is one of eosinophilia concomitant with high circulating IgE levels. However, a proportion of patients are not atopic and do not have eosinophilic inflammation. In fact, it is estimated that as many as 50% of adult patients are encompassed by this nonatopic, non-eosinophilic, non-IgE-dependent subgroup. Current phenotyping approaches in adults support an early-onset (childhood onset), mostly atopic and allergic asthma phenotype, and most have identified a later, adult-onset eosinophilic phenotype. Molecular and targeted therapy data support an overall T_H2 association with both of these phenotypes, such that they might satisfy a definition of T_H2 -associated asthma.⁶ However, even these distinctions are too simple, especially when disease severity is considered. Although children with severe asthma have eosinophilic inflammation, high-dose steroids effectively suppress T_H2 -type cytokines, such as IL-13 and IL-5, but symptoms remain with persistent eosinophilia,⁷ thus raising the importance of identifying other less steroid-sensitive, non- T_H2 mediators driving disease.

A growing number of studies have used sophisticated bioinformatics tools to look more closely at the clinical features of asthma to categorize patients according to symptoms. Unbiased statistical cluster analysis techniques have identified multiple distinct asthma phenotypes that differ with respect to lung function, age of asthma onset, disease duration, atopy, sex, symptoms, medication use, and health care use.⁸ Others have been able to classify asthmatic patients into phenotypic groups exhibiting clinically relevant differences in outcome, focusing on eosinophilic inflammation as an indicator for titrating corticosteroid therapy.⁹ Overall, it is apparent that asthma can no longer be considered simply a T_H2 -mediated disease but involves a more complex interplay between different T-cell subsets determined in each patient by genetic and environmental influences.

T_H17 cells

Effector CD4 cells expressing IL-17A, IL-17F, or both were first described in 2005^{10,11} and were thought to represent a distinct T-cell lineage that promoted the first revision of the T_H1/T_H2 paradigm of immunity. Differentiation of naive effector T cells in the presence of IL-6 and TGF- β results in IL-17 expression through the transcription factors Smad 2/3, signal transducer and activation of transcription (STAT) 3, and nuclear factor κ B. The T_H17 phenotype is marked by the expression of retinoic acid-related orphan receptor γ as a transcription factor.

Mechanistic studies in mouse models have shown that airway sensitization with allergen primes strong T_H17 cell responses that

promote lung neutrophilia and AHR and that T_H17 cell transfer promotes steroid-resistant airway inflammation and AHR in mice, outlining a possible association between T_H17 cells and severe asthma.^{12,13} Moreover, T_H17 cells have been found to promote airway remodeling independent of the T_H2 response.¹⁴ In support of a role for T_H17 cells in airway function, mice with a specific defect in $\alpha\beta8$ integrin on their dendritic cells did not generate T_H17 cells in lung tissue after allergen exposure and were protected from AHR. This lack of T_H17 cells had no effect on airway inflammation or epithelial morphology, but there was an obvious absence of airway narrowing. T_H17 cytokines were subsequently shown to exert direct effects on airway smooth muscle cells, implying that this group of cytokines might directly drive development of AHR.¹⁵ In contrast, blocking studies *in vivo* reveal a complex role for IL-17 in the allergic lung. Although abrogation of IL-17 function during allergic sensitization is protective in mouse models of asthma, delivery of exogenous IL-17A during allergen rechallenge decreases airway inflammation, suggesting that there is also a regulatory role for IL-17 in the allergic lung.¹⁶ In addition, IL-17-secreting $\gamma\delta$ T cells are able to promote resolution of acute and chronic airway inflammation and remodeling in mouse models.¹⁷

The role of IL-17 family cytokines in human atopic disease is similarly intricate. Studies in patients with allergic rhinitis have suggested an association between disease severity and serum levels of IL-17,¹⁸ but this has not been replicated in asthmatic patients.¹⁹ Numbers of IL-17A⁺ and IL-17F⁺ cells were increased in lungs taken from patients with mild asthma, but only IL-17F⁺ cell numbers were increased in patients with severe asthma. Interestingly, there was a positive correlation with IL-17F⁺ cells and eosinophils in the submucosa rather than neutrophils.¹⁹ Another study found that the percentage of CD4⁺IL-17⁺ cells in peripheral blood of patients with allergic rhinitis or asthma was found to be higher compared with that in healthy control subjects. Moreover, the percentage of T_H17 cells increased in the blood of allergic patients after bronchial challenge with allergen.²⁰ In contrast, fluorescence-activated cell sorting analysis of bronchoalveolar lavage (BAL) fluid samples from patients with mild-to-moderate or severe asthma showed that there was no significant difference in the proportion of T_H17 cells compared with nonasthmatic control subjects.²¹ Although transfer of antigen-specific T_H17 cells to allergic mice resulted in steroid-resistant neutrophilia, no clear correlation between IL-17 levels in general or T_H17 cells specifically has been observed in BAL fluid, lung tissue, or peripheral blood taken from patients.^{19,21,22}

Therefore the role that IL-17 plays in human asthma is still unclear. Multiple cell types in the lung can secrete IL-17, including both hematopoietic cells and structural cells, such as epithelial cells, vascular endothelial cells, and fibroblasts, and therefore the precise function within the airway might be dependent on the cellular source.²³ However, it is clear that T_H17 -associated cytokines are able to contribute to the development of a variety of different asthma pathologies, including remodeling and increase in smooth muscle mass, shown in both *in vivo* mouse models and *in vitro* culture models by using human cells.^{24,25} At present, it is not clear whether IL-17 is playing a pathogenic or proresolution role in the allergic lung. Increased levels might be observed due to underlying subclinical infections, and it could be argued that targeting IL-17 as a therapeutic agent should be approached with caution because IL-17 might be important for effective resolution of lung inflammation.

T_H22 cells

T_H22 cells are characterized by the secretion of IL-22, and expression of the skin-homing receptors CCR4 and CCR10 and are generated *in vitro* from naive cells by means of culture with IL-6 and TNF.²⁶⁻²⁸ Although T_H17 cells can also secrete IL-22, T_H22 cells are thought to represent a distinct memory T-cell subset with a skin-homing function and have, as yet, only been described in human subjects. IL-22 is thought to be important at mucosal surfaces, particularly for epithelial homeostasis and maintenance of barrier function.²⁷ This property has been demonstrated in the skin, where IL-22 receptor is expressed on keratinocytes, and IL-22 can promote keratinocyte proliferation and activation of innate immune defense pathways, such as secretion of antimicrobial peptides. T_H22 cells are increased in biopsy specimens from patients with atopic dermatitis, and interaction with T_H17 cells is thought to be critical in driving disease. T_H22 cells have not been documented in the human lung, but IL-22 is expressed in peripheral blood of asthmatic patients.²⁹ Mechanistic studies in murine disease models have shown that IL-22 is important in the initiation of allergic sensitization but might be protective at later stages.³⁰ This protective effect exerted by IL-22 during allergen challenge might occur as a result of inhibition of IL-25 from mouse lung epithelial cells.³¹ IL-22 was also found to be able to regulate the inflammatory properties of IL-17, suggesting that these cytokines play a synergistic role in the development and modulation of allergic immune responses.

T_H9 cells

IL-9-secreting CD4 T cells have been proposed as a distinct T-cell subset after *in vitro* experiments determined that culture of naive T cells with TGF- β in conjunction with IL-4 resulted in generation of an IL-9-secreting population of cells rather than forkhead box protein 3 (FoxP3)-positive regulatory T (Treg) cells.^{32,33} Subsequently, it has been shown that T_H9 cells lack the defining transcription factors for T_H1 or T_H2 cells, T-bet and GATA-3, but express the transcription factors PU.1 and interferon regulatory factor 4.^{34,35} However, these are also expressed by other myeloid lineages, and therefore at present, there is no defining signature transcription factor. Indeed, because of this, it has been argued that T_H9 cells cannot be regarded strictly as a separate lineage. House dust mite exposure in mice promotes rapid T_H9 differentiation and proliferation, with much faster kinetics than T_H2 cell differentiation.³⁶ Moreover, adoptive transfer of T_H9 cells resulted in enhanced pathology, most notably with the recruitment and activation of mast cells. IL-9 secretion has been localized to lymphocytes in the lungs of allergic patients³⁷ and in nasal polyps.³⁸ T_H9 cell numbers are increased in the peripheral blood of atopic subjects compared with nonatopic volunteers, and numbers correlated with increased IgE levels.³⁶ Functionally, T_H9 cells promote allergic responses, resulting in enhanced pathology mediated by the specific recruitment and activation of mast cells in the lungs.

Treg cells

Allergic inflammation has been postulated to represent an abnormal response to harmless airborne particles. These reactions are normally suppressed by a complex series of mechanisms, including regulatory cells and cytokines, which maintain airway tolerance. Treg cells are central to the maintenance of immune homeostasis in the airway, and the anti-inflammatory cytokines

IL-10 and TGF- β likely play a central role. There is emerging evidence that early-life events, including exposure to allergen and infections, are critical in programming effective regulatory pathways to maintain pulmonary homeostasis. In asthmatic patients there is a breakdown in these regulatory mechanisms, leading to development of pulmonary inflammation on exposure to innocuous proteins, such as pollens or animal dander.³⁹ Thus a greater understanding of how immune responses are regulated in the allergic lung will enable us to exploit these regulatory mechanisms to develop novel and improved therapies for asthma.

Animal model studies have determined that manipulation of the Treg cell population in the allergic lung by either transfer of CD4⁺CD25⁺FoxP3⁺ regulatory cells or blockade of CD25 results in abrogation or enhancement, respectively, of allergic inflammation and AHR.^{40,41} In patients evidence shows that CD4⁺CD25⁺ T cells isolated from PBMCs from patients with allergic rhinitis were less effective in suppressing allergen-specific T-cell responses (proliferation and T_H2 cytokine production) compared with Treg cells from nonatopic donors.⁴² Indeed, the effect was even more pronounced if cells were taken during the pollen season. Similarly, when looking at pulmonary lymphocytes, although asthmatic patients have increased levels of CD4⁺CD25^{bright} cells expressing high levels of FoxP3 after bronchial allergen challenge, they still exhibit enhanced levels of T_H2 cytokines in the lung, which might indicate defective suppression of T_H2 responses by Treg cells.⁴³ This defective suppression is also shown in asthmatic children. Treg cells isolated from BAL fluid or peripheral blood of asthmatic children were less effective in suppressing proliferation and production of T_H2 cytokines and chemokines by CD4⁺CD25⁺ responder T cells than Treg cells isolated from control subjects.⁴⁴

Strategies designed to increase the production of regulatory cells might be an effective treatment for asthma. Proof of this concept comes from studies of nonallergic beekeepers, who are continually exposed to high doses of bee venom antigens, which weakened T cell-related cutaneous late-phase swelling to bee stings in parallel with suppressed allergen-specific T-cell proliferation and T_H1/T_H2 cytokine secretion. This phenomenon was found to be associated with the rapid expansion of IL-10-secreting Treg cells. Allergen-specific immunotherapy is also associated with the development of allergen-specific Treg cells, which are able to control allergic immune responses, and suppressive cytokines, such as IL-10 and TGF- β are thought to play a pivotal role.⁴⁵⁻⁴⁸

T-CELL PLASTICITY

The original T_H1/T_H2 hypothesis provided a useful model for delineating T-cell function. However, the overall concept has been expanded to incorporate other effector T-cell subsets, such as T_H17, T_H22, T_H9, and Treg cells. Contrary to this rigid model of T-cell programming, recent evidence indicates that T cells do not become fully committed to a specific subset after encounter with antigen but might retain flexibility, which is dictated by the particular microenvironmental context.^{49,50} This plasticity of phenotype is dictated by environmental influences. In the context of allergic disease, allergen exposure/atopy, genetic background, age, infection history, pollution, or diet can all affect the nature of the T-cell phenotypes that develop after allergen exposure. Moreover, it is likely that this phenotype is not rigid, but there is fluidity in phenotypes that might fluctuate according to changing circumstances (Fig 1).

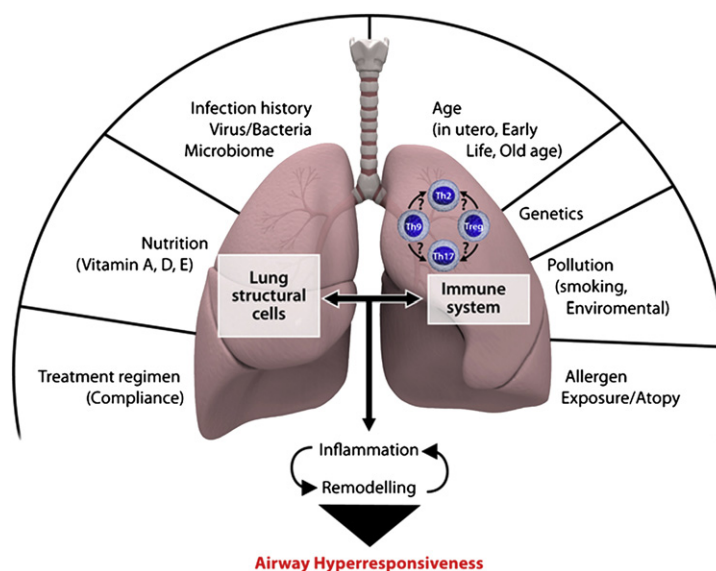


FIG 1. The interplay between environmental exposures, lung structural cells, and immune cells in determining asthma pathophysiology. The lungs are continuously exposed to numerous environmental exposures, which in a susceptible asthmatic patient determine responses from both lung structural cells (primarily epithelial cells) and immune cells. The microenvironment seems critical in determining T effector cell function and likely contributes to alterations in T-cell phenotype and determines development of T_H lineage plasticity.

The description of a novel subset of T_H2 -type memory/effector cells that express the T_H2 transcription factor GATA-3 and the T_H17 transcription factor retinoic acid–related orphan receptor γ while producing both T_H2 and T_H17 cytokines provides evidence for T-cell plasticity in the context of allergic immune responses. Compared with classical T_H2 and T_H17 cells, these antigen-specific IL-17⁺ T_H2 cells promoted recruitment of a more diverse, inflammatory infiltrate incorporating both neutrophils and eosinophils concomitant with enhanced lung pathology.⁵¹ Numbers of dual producing IL-17⁺ T_H2 cells were also found to be increased in the circulation of patients with allergic asthma. Whether these cells develop from classical T_H2 or T_H17 cells is not clear, but they might be an indicator of fluctuating disease severity. Lineage fate mapping reporter mice have shown that during chronic inflammation, IL-17–secreting cells can be converted into T_H1 -type cells.⁵² It will be interesting going forward to determine whether a similar phenomenon occurs during allergic responses.

In support of the concept of *in vivo* T-cell plasticity, a comprehensive analysis of transcription factor expression in circulating CD4 cells showed that healthy subjects, as well as those with mild-to-moderate asthma, show T_H cells expressing either single or several transcription factors. These data support the notion of T-cell plasticity and suggest that although T cells can be defined by a single transcription factor during stable disease after allergic stimulation or during exacerbations, they might express multiple transcription factors.

Whether expression represents a state of transition between different subsets or stable dual expressing subsets has not been confirmed. However, it is interesting to note that transcription factor expression can be associated with key features of disease. For example, T-cell receptor stimulation increased the GATA-3⁺ population, and expression of GATA correlated with FEV₁ reversibility.⁵³ Longitudinal analysis of patients is required to map fluctuations in T-cell phenotypes and correlate with changes in clinical disease.

Antigen-specific T_H2 cells can be manipulated to express FoxP3 by TGF- β , and this expression can be enhanced by

addition of retinoic acid or rapamycin, indicating that it is possible to deviate T_H2 cells toward a Treg cell phenotype.⁵⁴ Thus the drug regimen might be able to promote effective and safe Treg cell–based immunotherapy. Similarly, numbers of IL-10–secreting CD4⁺ cells can be enhanced *in vivo* by ingestion of vitamin D, indicating that nutritional status might be an influencing factor in the development of T-cell subsets.⁵⁵

Significant technical advances, such as next-generation sequencing and ChIPSeq, have facilitated unbiased genome-wide mapping to be undertaken. This comprehensive transcriptome analysis has enabled investigators to assess the effect of epigenetic modifications on development of T-cell phenotypes.⁵⁶ Epigenetic changes can arise at the DNA level through methylation or through posttranslational modifications of histones and chromatin remodeling. Alternatively, they can occur at the mRNA level through small noncoding RNAs, including microRNAs (miRNAs) that interfere with translation. Genetic approaches in mice have provided evidence for functional effects of epigenetic modifications. For example, deletion of chromatin-modifying/remodeling proteins has identified a variety of abnormalities in T-cell differentiation, which alter the expression of IFN- γ or T_H2 -type cytokines, as well as affecting the ability of T_H1 or T_H2 cells to effectively suppress the expression of opposing cytokine genes (reviewed by Kanno et al⁵⁷). Similarly, miRNAs are able to regulate transcriptional networks, and evidence for their importance in T-cell function was shown by the fact that selective deletion of miRNA in Treg cells results in profound autoimmunity.⁵⁸ It is thought that STAT proteins, which are intimately involved in the development of particular effector T-cell subsets, function as sensors of the external cytokine milieu and are able to facilitate the acquisition of epigenetic changes (reviewed by Hirahara et al⁵⁶). This feature is clearly exhibited by the contrasting effects of STAT4 and STAT6 on epigenetic modifications at the IL-18r1–IL-18rap locus. Although both STAT4 and STA6 bind at the IL-18rap locus in T_H1 and T_H2 cells, respectively, STAT4 is required for permissive epigenetic changes in T_H1 cells, whereas STAT6 is vital for repressive

epigenetic changes at the same locus in T_H2 cells.⁵⁹ For this and other loci, STAT4 and STAT6 were shown to have opposing actions on the same genes, thereby providing a mechanism for maintaining gene expression in a lineage-specific manner.

Environmental factors, such as cigarette smoke, pollution, and nutrition, can all alter the epigenome, which will influence T-cell phenotype and affect the development of asthma.⁶⁰ An example of this is the effect of pollutants, such as cigarette smoke or diesel particles, on DNA methylation, which in turn has been shown to modify the risk of asthma conferred by genetic variants for particular asthma phenotypes. Similarly, miRNA patterns have been shown to differ between smokers and nonsmokers.⁶⁰ Considerable evidence suggests that multiple environmental factors affect DNA methylation, which in turn influences asthma risk (reviewed by Karmaus et al⁶⁰). Importantly, exposure can occur prenatally and affect ensuing generations. Maternal influences, such as nutrition, alcohol intake, stress in the final trimester, infections, and smoking, can all change DNA methylation patterns, which affect asthma susceptibility in offspring. This developmental programming has been investigated mechanistically in mouse models of asthma transmission. Intrauterine exposure to bacteria commonly found in cowsheds was associated with a decreased risk of allergic sensitization and asthma-like disease in pups.⁶¹ In contrast, feeding pregnant mice with methyl donors, such as folate, was found to enhance allergic disease throughout several generations of pups.⁶² The diet rich in methyl donors affected DNA methylation at specific CpG sites and resulted in altered gene expression and enhanced disease. Although none of these *in vivo* studies have specifically examined the effect of epigenetic changes on T cells, T_H2 responses were enhanced. In the future, it will be interesting to determine the specific effect of environmental exposures on epigenetic marks and determine the particular influence on T-cell subsets.

INTERACTION BETWEEN LUNG STRUCTURAL CELLS AND IMMUNE CELLS

Although a large amount of data from animal models provides functional evidence for a role for immune cells and T_H2 cells in particular in driving allergic inflammation, recent advances indicate that asthma is unlikely to be solely driven by adaptive immune responses. More likely, cross-talk between the innate and adaptive immune system facilitates initiation and propagation of immune responses to allergens.⁶³ However, the molecular mechanisms underlying these interactions are not well understood. Communication between airway structural cells and cells of the immune system are now thought to be instrumental in dictating the nature and magnitude of allergic immune responses. In particular, the epithelium might be central because this surface is the first contact for allergen within the lung. The airway epithelium represents a first line of defense for the lung. Apart from functioning as a physical barrier, pulmonary epithelial cells sense the local environmental milieu because airborne particles are able to interact with receptors on the cell surface, such as pattern recognition receptors (PRRs) and protease-activated receptors. PRRs recognize pathogen-associated molecular patterns (PAMPs) on the surface of microbes or damage-associated molecular patterns, which are released by cell death, tissue damage, or oxidative stress.⁶⁴ Allergens such as house dust mite and cockroach contain enzymatic activity that triggers protease-activated receptors. Cytokines such as IL-25, IL-33, and thymic stromal lymphopoietin are all secreted by airway epithelial cells after these interactions and have been

shown to promote T_H2 differentiation, as well as typical features of the allergic response, such as AHR.⁶⁴ Intriguingly, IL-25 and IL-33 have also been shown to elicit production of lineage-negative non-T/non-B innate lymphoid cells, which are also able to secrete classical type 2 cytokines, such as IL-13 and IL-5.⁶⁵ The interaction between these innate-type lymphocytes and cells more traditionally involved in allergic responses, such as T_H2 cells and eosinophils, will affect the extent of allergic responses and affect the type and severity of pathologic symptoms. For example, IL-25 derived from airway structural cells rather than IL-17 from T_H17 cells was shown to be critical for induction of local inflammation through activation of lung epithelial cells and eosinophils in the elicitation phase of T_H2-type/eosinophilic airway inflammation. In contrast, it was not required for antigen-specific T_H2 cell differentiation in the sensitization phase of this mouse model.⁶⁶ It is clear then that the properties of these epithelial cells are vital in determining how allergic immune responses are propagated. Genome-wide microarray screening of bronchial epithelial cells from asthmatic patients identified a set of genes that were IL-13 responsive and predicted the response to corticosteroids.⁶⁷ The set of genes *POSTN* (encoding perlestin), *SERPINB2* (encoding Serpin peptidase inhibitor clade B, member 2), and *CLCA1* (encoding chloride channel accessory 2) was proposed as an asthma-specific mRNA signature for epithelial cells, and subsequent studies have shown that this signature defines patients with a T_H2/eosinophilic asthma profile who are responsive to corticosteroids.⁶⁸ The environment of allergen challenge must be taken equally into consideration: age, obesity, sex differences, and exposure to pollution and different infections are all likely to influence epithelial-immune interactions after allergen exposure. Gene-environment interactions will affect the epithelium, and the type of molecular pathway triggered will likely influence the nature of the ensuing immune response. An example of this is the exacerbation of asthma after viral infections. Viruses, such as rhinovirus, normally trigger interferon release by pulmonary epithelial cells, but in asthmatic patients this response is defective, which results in less effective viral clearance and an increase in disease severity.⁶⁹ Because IL-13 is known to suppress double-stranded RNA-induced IFN- λ production from airway epithelial cells, this would explain why asthmatic patients are less able to clear respiratory viruses.⁷⁰ Coupled with the antiviral immune response, which would include interferon-producing T cells, infection with virus would change the nature of the leukocyte infiltrate in the lung to incorporate neutrophil influx.

CONCLUSION

The concept of functional T-cell plasticity helps to explain disease heterogeneity and the variation in clinical phenotypes that are seen in patients with asthma as individual immune responses adapt to environmental exposures. It also suggests that to progress with personalized patient therapies, our clinical assessments of inflammatory and immune responses to determine therapy need to be more sophisticated. It is apparent that restricting measurements of inflammation to neutrophils and eosinophils is inadequate. Numbers of cells might vary within the same patient and between occasions,^{71,72} and using eosinophils to guide management is not useful in children with severe disease.⁷³ Future phenotype-specific approaches to therapy need to incorporate an assessment of lymphocytic inflammation, specifically of T-cell subsets. This seems justified at least in patients with severe

disease, in whom novel and expensive steroid-sparing agents, such as mAb therapies, are being considered. Of course, what remains uncertain and needs to be investigated is whether peripheral blood T cells or pulmonary T cells should be measured.

An increasing number of mAbs are being developed as novel asthma therapies; however, it is important to consider the most appropriate target for each patient and not adopt a blanket target for all. Experimental studies have highlighted that the cellular source of a mediator is as important as the mediator itself in determining function and downstream effects. IL-17 is a typical cytokine to which this applies; when it is produced by CD4⁺ T cells (T_H17 cells), it appears to have a proinflammatory proneutrophilic role,¹² but if secreted by $\gamma\delta$ T cells, it is likely to have a regulatory, proresolution function.¹⁷ Thus prescribing anti-IL-17 antibody to all patients with high levels of IL-17 might result in very different effects.

Another important example of cytokine function being determined by its cellular source is IL-10. Initially thought to be a T_H2 cytokine, IL-10 is now known to be made by T_H1, T_H2, and Treg cells, as well as a variety of innate immune cells, and its function is determined by the cellular source.⁷⁴ Thus future approaches to phenotypic characterization of asthmatic patients, especially patients with severe asthma in whom novel steroid-sparing agents are considered, have to include assessments of both cytokines and also their cellular source to determine whether blocking the action of mediators might be therapeutic or indeed potentially harmful. An alternative approach to therapy might be to manipulate the fate of T cells and thus their function rather than searching for downstream targets, such as their cytokines. It seems prudent for future studies to investigate the molecular mechanisms underlying T-cell plasticity in different asthma phenotypes and thus find targets that alter or modulate cellular fate and function.

What do we know?

- Asthma is a heterogeneous disease that is driven by numerous T-cell subsets, including T_H2, T_H17, and T_H9 cells.
- Interactions between genetic susceptibility and the environment determine the pathophysiology of disease.
- Blocking the action of the T_H2 cytokines IL-5 and IL-13 is only effective in a small subgroup of patients.

What is still unknown?

- The role of T-cell plasticity in determining individual asthma phenotypes
- Whether systemic or pulmonary immune profiles best determine therapeutic responses
- How best to characterize T-cell profiles and their mediators in patients
- The molecular mechanisms underlying T-cell plasticity in asthma and whether they can be manipulated to modulate T-cell fate and function

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