

Clinical reviews in allergy and immunology

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IL-13 in asthma and allergic disease: Asthma phenotypes and targeted therapies

Jennifer L. Ingram, PhD, and Monica Kraft, MD Durham, NC

Decades of research in animal models have provided abundant evidence to show that IL-13 is a key T_H2 cytokine that directs many of the important features of airway inflammation and remodeling in patients with allergic asthma. Several promising focused therapies for asthma that target the IL-13/IL-4/signal transducer and activator of transcription 6 pathway are in development, including anti-IL-13 mAbs and IL-4 receptor antagonists. The efficacy of these new potential asthma therapies depends on the responsiveness of patients. However, an understanding of how IL-13-directed therapies might benefit asthmatic patients is confounded by the complex heterogeneity of the disease. Recent efforts to classify subphenotypes of asthma have focused on sputum cellular inflammation profiles, as well as cluster analyses of clinical variables and molecular and genetic signatures. Researchers and clinicians can now evaluate biomarkers of T_H2 -driven airway inflammation in asthmatic patients, such as serum IgE levels, sputum eosinophil counts, fraction of exhaled nitric oxide levels, and serum periostin levels, to aid decision making in clinical trials and drug development and to identify subsets of patients who might benefit from therapies. Although it is unlikely that these therapies will benefit all asthmatic patients with this heterogeneous disease, advances in understanding asthma subphenotypes in relation to clinical variables and T_H2 cytokine responses offer the opportunity to improve the efficacy and safety of proposed therapies for asthma. (J Allergy Clin Immunol 2012;■■■:■■■-■■■.)

Key words: IL-13, asthma, phenotypes, biomarkers, periostin, IgE, eosinophils, fraction of exhaled nitric oxide, T_H2 inflammation

Nearly 15 years ago, 2 landmark articles demonstrated IL-13 to be a “central mediator of allergic asthma.”^{1,2} Although it has long been recognized that T_H2 lymphocytes and cytokines play an

Abbreviations used

BAL:	Bronchoalveolar lavage
BMI:	Body mass index
CLCA1:	Chloride-channel regulator 1 gene
FENO:	Fraction of exhaled nitric oxide
ICS:	Inhaled corticosteroid
IL-4R:	IL-4 receptor
IL-13R:	IL-13 receptor
POSTN:	Periostin gene
SARP:	Severe Asthma Research Program
SERPINE2:	Serpin peptidase inhibitor clade B, member 2, gene
siRNA:	Small interfering RNA
STAT:	Signal transducer and activator of transcription

important role in directing the allergic response in asthmatic patients, the mechanisms by which these cytokines mediate responses in these patients were not fully understood until the development of allergic murine models of IL-13 blockade and overexpression. The data to support the title statement at the time and since then have shown that IL-13 alone is sufficient to induce responses in murine models of allergen challenge that have a striking resemblance to human asthma. Since that publication, the field has made great strides in understanding how this potent T_H2 cytokine directs airway inflammation, airway remodeling, and asthma pathogenesis. Support for IL-13 as a central mediator of human asthma has accumulated because we now know that *IL13* and IL-4 receptor (*IL4R*) polymorphisms are associated with asthma susceptibility³ and that a variety of IL-13-induced pathways are also associated with allergy and airway responses in asthmatic patients.^{4,5}

Despite progress in the understanding of the basic biology of IL-13 in asthma, our field is just beginning to see rewards in the development of promising therapies that target IL-13 and its signaling pathway in asthma. One potential reason for the delay in the development of IL-13-targeted therapies is the complex heterogeneity of asthma, an aspect of the disease that has become increasingly appreciated by clinical scientists. Although IL-13 is a critical mediator of allergic asthma, it might not be the only important mediator driving asthmatic responses in all patients; thus progress in recognizing patient subphenotypes is essential to our understanding of how to direct treatment for asthma. In this review we will describe the gains that have been made in understanding the role of IL-13 in allergic asthma and developing targeted therapies toward IL-13 and its pathway in patients with airways disease and in how knowledge of specific patient phenotypes can expand efficacy for these therapies.

From the Division of Pulmonary, Allergy and Critical Care Medicine, Duke University Medical Center.

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Corresponding author: Jennifer L. Ingram, PhD, Division of Pulmonary, Allergy and Critical Care Medicine, Department of Medicine, Duke University, Box 2641, Durham, NC 27710. E-mail: Jennifer.ingram@duke.edu.

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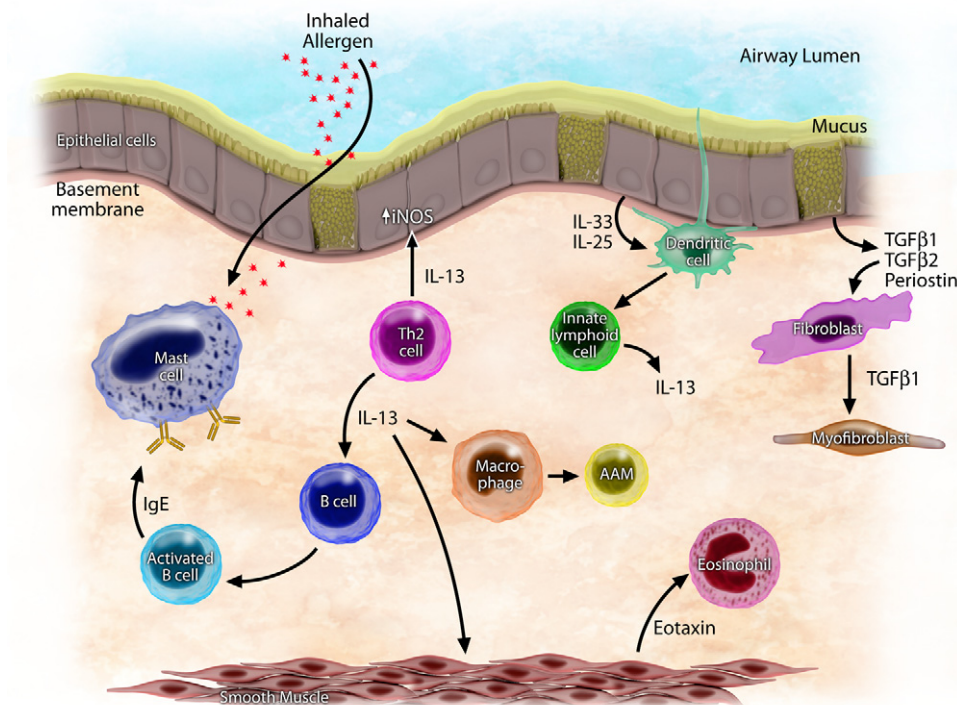


FIG 1. Overview of the cellular effects of IL-13 on airway inflammatory and structural cells in asthmatic patients. Mast cells, T_H2 cells, and innate lymphoid cells secrete IL-13 on inhaled allergen stimulation. IL-13 triggers macrophage and eosinophil activation to accelerate airway inflammation, IgE production by B cells, smooth muscle cell activation contributing to airways hyperresponsiveness, mucus and growth factor production in airway epithelial cells, eotaxin production to stimulate eosinophil recruitment to the airway, and activation/proliferation/migration of airway fibroblasts to promote airway remodeling. Adapted from Kraft.⁶ AAM, Alternatively activated macrophage; *iNOS*, inducible nitric oxide synthase; *MCP-1*, monocyte chemoattractant protein; *PGD₂*, prostaglandin D₂; *SCF*, stem cell factor; *Treg*, regulatory T; *TSLP*, thymic stromal lymphopoietin.

IL-13/IL-4 PATHWAY IN ASTHMA

Allergic asthma is the most common and well-characterized manifestation of the syndrome. The immune response to allergen exposure in the asthmatic airway is complex and involves many cell types. These processes have been reviewed elsewhere.^{6,7} As stated above, abundant evidence has shown that IL-13 directs many of the processes involved in the allergic asthmatic response (Fig 1). In the current model of allergic airway inflammation, inhaled allergen initiates activation of mast cells. T_H2 cells secrete IL-4 and IL-13, driving IgE isotype switching in B cells.⁸ Airway epithelial cells also produce IL-33 and IL-25, which activate dendritic cells and promote release of abundant IL-13 and IL-5 from innate lymphoid cells.⁹⁻¹⁴ IL-13 production in the airway promotes the survival and migration of eosinophils,¹⁵ activation of macrophages,¹⁶ increased permeability of and mucus production by airway epithelial cells,^{17,18} production of inducible nitric oxide synthase by airway epithelial cells,¹⁹ and transformation of airway fibroblasts to myofibroblasts,¹⁸ leading to collagen deposition.¹⁸ IL-13 also stimulates airways hyperresponsiveness.²⁰ Therefore, considering the importance of T_H2 cytokines, specifically IL-13, in the complex immune response mounted by susceptible patients with allergic asthma, this cytokine is certainly a relevant target for asthma therapy. However, many other airway cell types and pathways are involved in the allergen immune response, and these pathways might be key in the expression of the different asthma phenotypes.

A complex receptor system mediates the signaling of IL-13 and IL-4 (Fig 2). Four heterodimeric receptor complexes have been identified that bind to IL-4, IL-13, or both. The IL-4R α subunit is a component of both the type I and type II receptors. Type I receptors are composed of the IL-4R α subunit complexed with the common γ chain. These type I receptors bind to IL-4 on cells of hematopoietic stem cell origin. The common γ chain does not bind IL-4 or IL-13, but it is important for signaling of the type I receptor. In this complex IL-4 first binds to the IL-4R α subunit, and then the common γ chain is recruited to form the complex. Both IL-4 and IL-13 bind to and signal through the type II receptor, a heterodimer comprised of the IL-4R α subunit and the IL-13 receptor (IL-13R) α 1 subunit, which is found on cells of non-hematopoietic stem cell origin. In this complex IL-4 binds to the IL-4R α subunit, which then recruits the IL-13R α 1 subunit to form the heterodimer. Alternatively, IL-13 first binds to the IL-13R α 1 subunit and recruits the IL-4R α subunit to form the stable heterodimer.²¹

Both the type I and type II receptor complexes signal through a tyrosine kinase (Tyk)/Janus kinase (JAK)–signal transducer and activator of transcription (STAT) cascade. Activation of these proteins by IL-13 phosphorylates intracellular tyrosines, resulting in recruitment of STAT6 to the receptor complex. STAT6 is then phosphorylated and activated, resulting in translocation of STAT6 to the nucleus for binding to specific DNA elements on the promoter regions of downstream genes for initiation of transcription.²² Although STAT6 is the well-recognized primary mediator

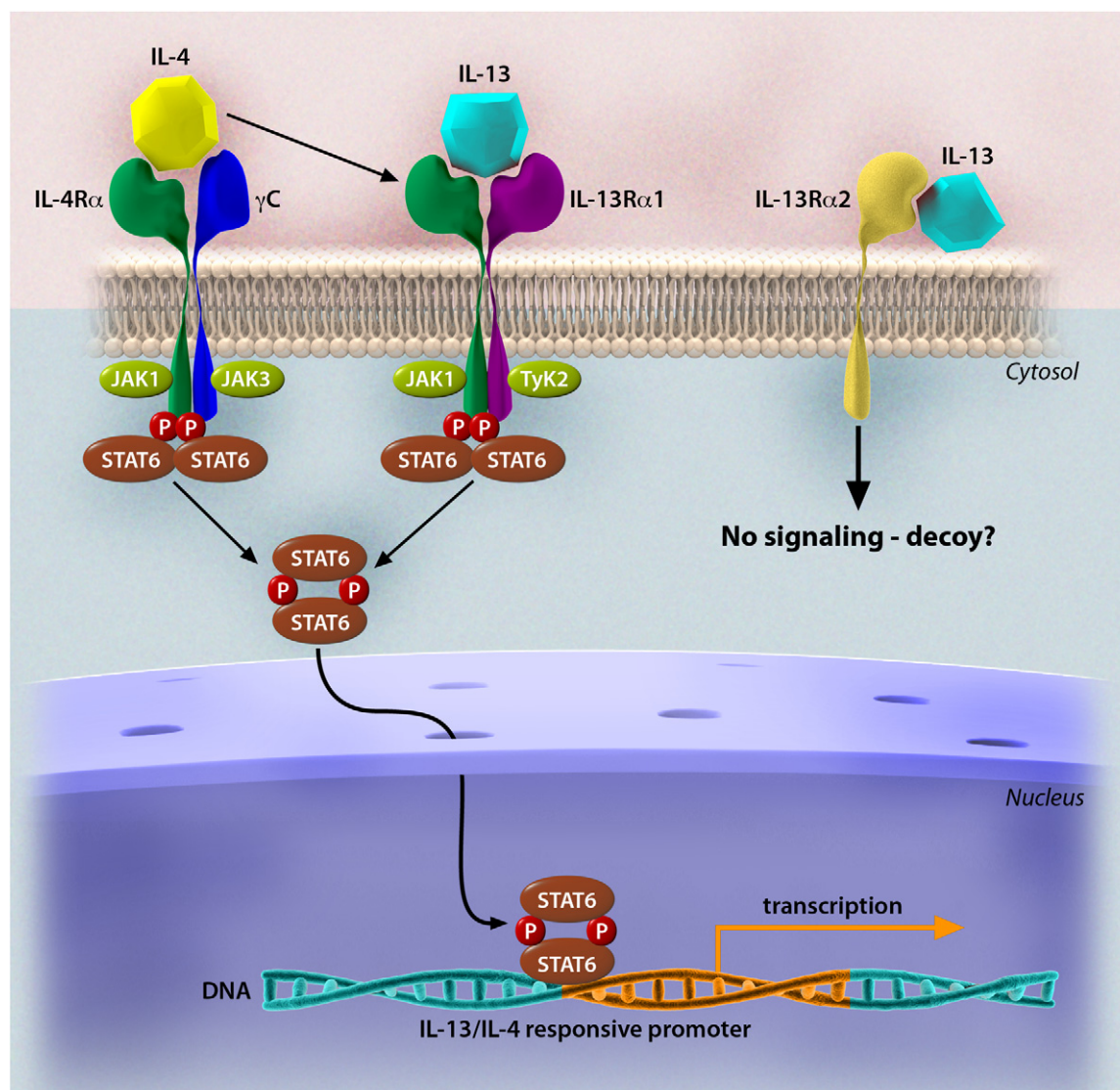


FIG 2. Schematic representation of the IL-4/IL-13 receptors and signaling pathways. IL-4 binds to either the type I receptor, which is composed of the IL-4R α and common γ chain heterodimer, or to the type II receptor, which is composed of the IL-4R α and IL-13R α 1 heterodimer. IL-13 binds to either the IL-13R α 1 subunit or to the high-affinity IL-13R α 2 subunit. IL-13R α 2 has been hypothesized to act as a decoy receptor, but reports of signaling by IL-13R α 2 have challenged this notion. IL-13/IL-4-induced gene expression is mediated predominantly through a phosphorylation cascade initiated on IL-4R α with signaling through the Janus kinase (JAK)/STAT6 pathway.

of IL-13- and IL-4-induced gene expression, studies of gene-deficient mice have revealed that other transcription factors, such as STAT1,^{23,24} STAT3,²⁵ and early growth response 1,^{23,26} also mediate IL-13-induced functions.

A third IL-13 receptor is the IL-13R α 2 subunit. This subunit binds only to IL-13 and in mice exists in both membrane-bound and soluble forms.^{27,28} A soluble form of IL-13R α 2 has not been detected in human subjects.^{27,28} IL-13 binds with much higher affinity to IL-13R α 2 than to the IL-13R α 1 subunit.^{29,30} The high affinity of IL-13 for binding to IL-13R α 2 and the lack of evidence for signaling activity for this receptor subunit have led to the hypothesis that IL-13R α 2 is a decoy receptor. Studies of lung-targeted IL-13 transgenic mice with IL-13R α 2 null loci indicated that IL-13R α 2 deficiency significantly augmented IL-13- or ovalbumin-induced pulmonary inflammation and remodeling.³¹

IL-13R α 2 effectively attenuated IL-13-induced eotaxin and STAT6 phosphorylation in human bronchial fibroblasts. Furthermore, although IL-13R α 2 does not directly bind to IL-4, it has been shown to physically interact with IL-4R α in an IL-4-dependent manner in human bronchial fibroblasts, thus potentially suppressing IL-4-mediated functions in the airway.³² We have reported that cell-surface expression of IL-13R α 2 on human asthmatic airway fibroblasts was reduced compared with expression on normal control airway fibroblasts, supporting the hypothesis that IL-13R α 2 is a negative regulator of IL-13-induced responses in asthmatic patients.³³

However, the concept of IL-13R α 2 as a decoy receptor has lately come under question because recent studies have reported that IL-13R α 2 has the capacity to mediate IL-13 signaling.³⁴⁻³⁶ In a murine model of bleomycin-induced fibrosis, *in vivo* silencing

of IL-13R α 2 with gene-specific small interfering RNAs (siRNAs) resulted in significantly reduced production of TGF- β 1 and deposition of collagen in the lungs of mice.³⁴ Also, an intriguing recent study of IL-13 signaling in colorectal cancer lesions demonstrated that IL-13R α 2 can serve dual functions as both a decoy receptor and a mediator of mitogen-activated protein kinase signaling.³⁵ Thus these studies call into question the dogma of IL-13R α 2 as a decoy receptor and suggest that IL-13R α 2 can serve as a signaling receptor in some cell types. All 3 of the IL-13 receptor subunits are targets for directed therapies in the treatment of asthma and allergy.

Pioneering and comprehensive studies of targeted and inducible pulmonary overexpression of IL-13 transgenic mice by Elias et al^{18,37} have defined the role of IL-13 in directing cellular and molecular responses that might be relevant to airway inflammation and remodeling in human allergic asthma. These mechanistic studies have shown that IL-13 expression alone stimulates many of the key inflammatory, remodeling, and physiologic features of human asthma, including airway eosinophilia, mucus hypersecretion, subepithelial airway fibrosis, and methacholine airway hyperresponsiveness.¹⁸ Furthermore, these studies revealed signaling pathways and specific mechanisms by which IL-13 directs its effects in the lung.^{18,37} In addition, a study of lung-specific transgenic IL-9-expressing mice led to the conclusion that airway epithelial cells were a significant source of IL-13 expression.³⁸ All of these IL-13-driven processes and mechanisms have subsequently been found to be important to human asthma and lung disease; thus these studies of IL-13 transgenic mice have been an essential tool to lay the foundation for ongoing work to further investigate the effects of IL-13 in directing human airway inflammation.

To this end, investigations of human cells and tissues have demonstrated that IL-13 is a key cytokine associated with asthma, correlating IL-13 expression with physiologic measurements of lung function and severity in asthmatic patients. Table I* summarizes key features of human asthma that can be attributed to increased IL-13 expression in the airway or *in vitro* responses of human airway cells to IL-13 and how these relate to observations made using pulmonary-targeted IL-13-overexpressing transgenic mice.

First, IL-13 expression was demonstrated to be increased in bronchoalveolar lavage (BAL) fluid and cells in patients with atopic mild asthma after allergen challenge.^{39,40} Second, genome-wide association studies have identified multiple polymorphisms of *IL13* and genes encoding the IL-13 receptors as associated with asthma susceptibility, bronchial hyperresponsiveness, and increased IgE levels.^{3,60-62} IL-13 has been demonstrated to induce human epithelial cell proliferation, mucus secretion, and production of TGF- β 2.^{41,42} In patients with mild asthma, IL-13 has been demonstrated to stimulate airway fibroblast proliferation, an effect that was augmented and not suppressed by dexamethasone.⁴³ Furthermore, airway fibroblast invasion in patients with mild asthma was increased in response to IL-13, an effect that was significantly associated with methacholine airway hyperresponsiveness.³³ Taken together, these human investigations and others have demonstrated that asthma is associated with T_H2 inflammation and that IL-13 is a key cytokine involved in directing allergen-induced airway inflammation and remodeling.

TABLE I. Summary of some of the most important pathophysiologic features and signaling mediators observed in pulmonary-targeted overexpression of IL-13 in transgenic mice, *in vitro* IL-13-induced responses in human airway cells, and airway expression of IL-13 in human asthma

	References
Murine models	
Eosinophil-, lymphocyte-, and macrophage-rich inflammation	18
Methacholine airways hyperresponsiveness	18
Subepithelial fibrosis	18
Airway epithelial cell hypertrophy	18
Airways obstruction	18
Soluble and membrane-bound IL-13R α 2	27
Compliance alterations	45
Alveolar remodeling	46
Goblet cell metaplasia and mucus hypersecretion	18
Leukotriene pathway activation	47
Inhibition of α 1 antitrypsin	48
Surfactant phospholipid and surfactant accumulation	49
Type II cell hypertrophy	49
Increased mediators: TGF- β 1, MMPs, adenosine, CC chemokines, VEGF, cathepsins, Egr1, PDGF-AA and PDGF-CC, AMCase, eotaxin, MCP-1, hyaluronic acid	18, 23, 26, 37, 45, 46, 50-53
Human asthma	
IL-13 produced by innate lymphoid cells	14
Macrophage activation	16
Increased airway epithelial cell permeability	17
Survival and migration of airway eosinophils	54
IL-13 levels increased in BAL fluid after allergen challenge	39, 40
Mucus hypersecretion	41, 55
Airway epithelial cell, fibroblast, and smooth muscle cell proliferation	42, 43, 56, 57
Induction of inducible nitric oxide synthase in bronchial epithelial cells	19
Airway fibroblast invasion, smooth muscle cell migration	33, 58
No soluble IL-13R α 2	27
Reduced cell-surface IL-13R α 2 on airway fibroblasts	33
Production of TGF- β 2, TGF- β 1, eotaxin, periostin, and IgE	8, 33, 42, 44, 59
IL-13 and IL-4R polymorphisms associated with asthma susceptibility, AHR, and increased IgE levels	3

AHR, Airways hyperresponsiveness; MCP-1, monocyte chemoattractant protein 1; MMP, matrix metalloproteinase; PDGF, platelet-derived growth factor; VEGF, vascular endothelial growth factor.

ASTHMA PATHOBIOLOGY AND ASSOCIATED HETEROGENEITY

Despite the considerable body of knowledge that has accumulated regarding the role of IL-13 in allergic asthma, asthma is a chronic inflammatory disease that presents in patients with marked heterogeneity in regard to severity, responses to medications, symptoms, and disease management. Thus, not all

*References 3,8,14-19,23,26,27,33,37,39,40-59.

pathways active in human asthma are IL-13 driven. In addition, the current clinical guidelines for asthma diagnosis and management do not recognize that different phenotypes of asthma exist that exhibit variations in the manifestation of airway inflammation driven by differences in the types and expression levels of cellular and genetic mediators. A classification of asthma severity based on symptoms, lung function, exacerbations, limitation of physical activity, and need for rescue medications divides the syndrome into 2 management groups, intermittent and persistent, with the latter group further subclassified into mild, moderate, and severe.⁶³

In general, achieving long-term control of persistent asthma involves a coordinated approach in patients based on severity and control. Specifically, treatment involves use of controller medications, such as inhaled corticosteroids (ICSs), in patients with mild persistent asthma and combined at higher doses with a second controller, such as long-acting β -agonists or leukotriene modifiers, in patients with moderate asthma. Treatment of severe asthma involves high-dose ICSs or oral corticosteroids with additional controllers, including omalizumab for allergic patients with severe asthma.⁶³

The pathogenesis of asthma includes an increase in the number of inflammatory cells within the airways. Recruitment of eosinophils has long been recognized to be a key feature of the inflammatory response in the allergic asthmatic airway.⁶⁴ Still, a subset of patients with asthma persistently exhibit no eosinophils in their sputum (nearly 50% of asthmatic patients), and these patients with noneosinophilic asthma generally have a poor response to ICSs.^{65,66} Noneosinophilic severe asthma is also associated with fewer inflammatory cells in the airway, lower FEV₁, and reduced airway subbasement membrane thickness and subepithelial collagen deposition.⁶⁷

On the basis of analyses of airway inflammatory cell profiles in induced sputum samples, 4 subtypes of asthma have emerged: (1) eosinophilic; (2) neutrophilic or noneosinophilic; (3) mixed granulocytic; and (4) paucigranulocytic asthma, with normal levels of neutrophils and absent eosinophils.⁶⁸⁻⁷⁰ Eosinophilic asthma is noted in atopic patients with allergen-mediated, T_H2 cytokine-driven inflammation and generally responds well to corticosteroids, except in patients with severe asthma.^{66,67} Neutrophilic asthma is associated with obesity,⁷¹ smoking,⁷² infections,^{73,74} exposure to pollutants,^{75,76} and subsets of severe asthma.⁷⁷ Neutrophilic asthma has also been shown to correlate with markers of T_H1 cytokine-induced pathways.⁷⁸ The stability of this phenotype might be somewhat controversial based on a recent report by Cowan et al.⁷⁹ in which patients with moderate asthma and sputum neutrophilia underwent a tapering of their ICSs. Subsequent sputum differential analysis did not reveal significant neutrophilia but rather eosinophilia when the corticosteroids were discontinued. In addition, McGrath et al.,⁶⁵ in collaboration with the Asthma Clinical Research Network, recently reported, in a longitudinal assessment of sputum differentials, that sputum neutrophilia was not a consistent phenotype. Asthmatic patients with mixed granulocytic inflammation were identified as having the lowest lung function, increased daily asthma symptoms, and increased health care use in a study of nearly 250 asthmatic patients.⁷⁰ These approaches to classify asthma based on cellular inflammation phenotypes highlight the heterogeneity of the disease and reveal that a variety of mechanisms and drug targets might be available in the treatment of asthma.

ASTHMA SUBPHENOTYPES

Recognizing the inherent heterogeneity within asthma and the benefits of classifying asthma by phenotype to potentially target asthma therapy, several groups have used multivariate approaches to define clusters or clinical phenotypes of asthma.^{80,81} The studies used cluster analyses to identify similarities between patients based on multiple specified variables and then group the patients into clusters that would support the similarities of patients in the same cluster and distinguish patients in different clusters.

First, Haldar et al.⁸⁰ used principle components analysis to aid in selection of 16 commonly measured clinical variables for characterization of asthma, including age of onset, body mass index (BMI), FEV₁, sputum eosinophil count, and others. Cluster analysis of patients with mild-to-moderate asthma using these variables identified 3 clusters: one with early-onset atopic asthma, including eosinophilic airway inflammation; a second cluster of primarily female obese patients with noneosinophilic asthma; and a third subgroup of patients with "benign" asthma with well-controlled symptoms. In a population with severe refractory asthma,⁸² 4 clusters were identified. Two of these clusters were very similar to those identified in the population with mild-to-moderate asthma. However, 2 other distinctive clusters were identified in the refractory population, including dissociation between eosinophilic inflammation and asthma symptoms. One of these clusters exhibited early onset of atopy and symptoms with absence of eosinophilia, and the other cluster exhibited late onset of symptoms with eosinophilic inflammation.⁸⁰

A larger study conducted by the multicenter Severe Asthma Research Program (SARP) determined clinical phenotypes across a broad spectrum of asthma severity in adults.⁸¹ This study used an unsupervised hierarchic cluster analysis in which 628 initial variables were reduced to 34 core variables before analysis. These variables for analysis included age of onset, medication use, health care use, infection and smoking history, atopy, BMI, and lung function measurements. Five clusters were identified: cluster 1 consisted of patients characterized by early-onset/atopic asthma with normal lung function who were taking 2 or fewer controller therapies and required minimal health care use; cluster 2 consisted of patients characterized by early-onset/atopic asthma with normal lung function who were taking 2 or more controller therapies and required significant health care use; cluster 3 consisted of patients characterized by adult-onset asthma, obese status, predominant female sex with low lung function, and high medication requirements and health care use; cluster 4 consisted of patients with early-onset/atopic asthma and severe obstruction with some reversibility and high health care use; and cluster 5 consisted of patients characterized by early-onset asthma and severe obstruction with less reversibility and high health care use. Although sputum eosinophil counts were not evaluated in this large study, cluster 3 apparently corresponds to the obese, female-predominant, nonatopic asthma, late-onset, noneosinophilic inflammation cluster observed by Haldar et al.⁸⁰ Furthermore, a follow-up study conducted by SARP to investigate the heterogeneity of severe asthma in children⁸³ identified 4 clusters. The authors concluded that the observed pediatric phenotypes were similar to the adult clusters identified in the previous SARP cluster analysis⁸¹ in that all of the clusters of childhood asthma included patients with atopy and that asthma duration and lung function were key

phenotypic features but that no observed cluster in childhood asthma corresponded to published guidelines for definitions of severe asthma.⁶³

Finally, unsupervised hierarchic cluster analysis has been conducted in 2 separate studies to investigate induced sputum samples. First, Baines et al⁸⁴ analyzed gene expression profiles of induced sputum to reveal 3 distinct transcriptional asthma phenotypes. The first phenotype consisted of patients with low FEV₁ and high Asthma Control Questionnaire scores, exhaled nitric oxide levels, and sputum eosinophil numbers, and the second phenotype consisted of patients with lower FEV₁ and higher sputum neutrophil numbers compared with a third phenotype that resembled healthy control subjects.⁸⁴

Second, a large multinetwork study was conducted by the Asthma Clinical Research Network to characterize noneosinophilic asthma and to determine rates of incidence of this phenotype among patients with mild-to-moderate asthma.⁶⁵ Analysis of repeated induced sputum samples revealed 3 phenotypes of eosinophilic asthma: persistent eosinophilia, intermittent eosinophilia, and persistent noneosinophilia. Cluster analysis of the persistently noneosinophilic asthma subgroup revealed 3 clusters that described clinical characteristics of each phenotype within the group. One cluster represented 42% of the 157 subjects studied and was comprised of younger predominantly female subjects who were lean and experienced moderate-to-severe bronchial hyperresponsiveness. A second identified cluster is similar to those described in other studies^{80,81,85} and represents the older female subjects with late-onset asthma, high BMI, and high sputum neutrophil numbers. The third cluster represented 36% of the subjects and was characterized by younger and predominantly male subjects who were lean and had low FEV₁ with early-onset asthma. Thus comprehensive analysis of large patient cohorts and analysis of inflammation and gene expression has led to the identification of phenotypes that can be useful in identifying responders to specific therapies.

T_H2 SIGNATURE AND USE OF BIOMARKERS TO PHENOTYPE ASTHMA

A different approach for defining subphenotypes of asthma is to use knowledge of molecular pathways to distinguish patient subsets based on distinct inflammatory phenotypes. An elegant study was conducted by Woodruff et al⁸⁶ in which a 3-gene signature composed of periostin (*POSTN*), chloride-channel regulator 1 (*CLCA1*), and serpin peptidase inhibitor clade B, member 2 (*SERPINE2*), in airway epithelial cells was used as a surrogate marker of T_H2 inflammation. In a cohort of 70 subjects, 42 asthmatic patients and 28 healthy control subjects, the authors found that expression of the gene signature was highly correlated within individual subjects and that some but not all of the asthmatic subjects overexpressed the genes of the signature, suggesting that a subset of patients overexpressed markers of T_H2 inflammation.

The group performed unsupervised hierarchic cluster analysis of the 70 subjects based on the microarray expression levels of the genes in the gene signature. Two clusters emerged from the analysis: a T_H2-high cluster, comprising asthmatic patients with consistently high expression of T_H2 cytokine-induced gene expression, and a T_H2-low cluster, comprising asthmatic subjects

whose gene expression analysis was indistinguishable from healthy control subjects. The T_H2-high cluster phenotype was characterized by increased serum IgE levels and airways hyperresponsiveness, eosinophilic inflammation, increased reticular basement thickening, and increased epithelial expression of mucins. Finally, patients in the T_H2-high cluster showed significant improvements in FEV₁ in response to inhaled fluticasone compared with placebo, whereas patients in the T_H2-low cluster did not improve.⁸⁶ T_H2 gene signatures have also been identified in bronchial biopsy specimens of patients with mild-to-moderate asthma⁸⁷ and in nasal epithelial cells of atopic children.⁸⁸ Both of these studies found that expression of the T_H2 signature correlated with clinical measures of T_H2 inflammation and allergy.

Biomarkers for disease diagnosis and classification have been used for years to investigate asthma and its association with T_H2-predominant immune responses. These indicators include induced sputum eosinophils, measurement of the fraction of exhaled nitric oxide (FENO), peripheral blood eosinophil counts, serum IgE measurements, and, more recently, measurement of periostin expression levels. Each of these biomarkers has been associated, with some variability, with T_H2 inflammation in the airway. Indeed, Woodruff et al⁸⁶ noted an association between expression of the T_H2-high signature and BAL fluid eosinophils and serum IgE levels. Therefore reproducible measurements of these biomarkers can be used as a means to identify subsets of patients who might or might not respond to therapies targeting T_H2 cytokines and receptors or to the inflammatory process, such as corticosteroids and leukotriene inhibitors.

Collection of induced sputum is a relatively noninvasive technique, and analysis of eosinophil and neutrophil counts obtained in sputum induction are similar to those obtained from the more invasive bronchoscopic techniques.⁸⁹ Primary information obtained from induced sputum comprises differential cell counts of inflammatory cells; a secondary assay of the induced sputum supernatant might offer information relevant to molecular markers of inflammation, including levels of mediators of T_H2 inflammation.⁹⁰ Several clinical trials have used induced sputum cellularity in patients with mild-to-moderate asthma to show that increased sputum eosinophil counts can predict asthma exacerbations and guide recommendations for use of corticosteroids in management of exacerbation rates.⁹¹⁻⁹³ However, a recent study of severe asthma in children demonstrated that the inclusion of sputum eosinophil control into a management algorithm did not significantly reduce overall asthma exacerbations or improve asthma control.⁹⁴ In addition, another recent study has demonstrated that repeated measures of differential sputum cell counts must be analyzed over time to gain a true understanding of the heterogeneity in eosinophil phenotypes, including both intermittent and persistent eosinophilic asthma.⁶⁵ A recent study of pediatric severe refractory asthma found that sputum and BAL eosinophil levels were not correlated with BAL T_H2 cytokines or tissue IL-5⁺ and IL-13⁺ cell counts.⁹⁵ These data suggest that in patients with pediatric asthma, sputum eosinophilia is not a biomarker of T_H2-driven inflammation. Still, sputum cell counts can be a useful tool to guide therapy because cluster analysis using sputum eosinophil counts as a variable has demonstrated that within the various clusters of asthma phenotypes, patients with sputum eosinophilia respond best to corticosteroids, whereas subjects characterized by noneosinophilic asthma can benefit from reduction of corticosteroid therapy and management of symptoms.⁸⁰

Significant correlations between sputum eosinophil counts and FENO levels in asthmatic patients have prompted clinicians and researchers to use the reasonably easy-to-perform measurement as a surrogate marker for airway inflammation.^{96,97} In addition, the report that IL-13 stimulated inducible nitric oxide synthase mRNA and protein expression in differentiated human airway epithelial cells lent support to an association between FENO levels and T_H2 inflammation.¹⁹ Accurate reproducible measurements of FENO are available for both children⁹⁸ and adults,⁹⁹ but FENO levels have been reported to increase in atopic subjects¹⁰⁰ and decrease in current smokers.¹⁰¹ FENO levels were responsive to corticosteroids in asthmatic patients¹⁰² and increased after allergen challenge in atopic asthmatic patients.¹⁰³ Evidence that increased FENO levels were predictive of asthma exacerbations¹⁰⁴ and that FENO levels might be used to guide corticosteroid and anti-IL-13 therapies^{105,106} suggests its use in identifying phenotypes of asthma.

In a large cohort of asthmatic patients spanning all severity levels as part of SARP, Dweik et al¹⁰⁷ characterized 2 asthma subphenotypes of high (>35 ppb) and low (<35 ppb) FENO values. Patients with high FENO levels were characterized by longer duration of disease, atopy with eosinophilic airway inflammation, and increased airway hyperreactivity and obstruction. Low-FENO asthmatic patients were characterized by noneosinophilic inflammation with high BMI. However, FENO level is a nonspecific marker of airway inflammation because FENO levels can be altered in patients with lung conditions other than asthma, such as pulmonary fibrosis,¹⁰⁸ lung transplantation,¹⁰⁹ pulmonary hypertension,¹¹⁰ and cystic fibrosis.¹¹¹ Therefore use of FENO as a biomarker to guide therapy should be applied with caution when assessing inflammatory phenotypes present in severe asthma, including a general correlation with T_H2 inflammation.

Peripheral blood eosinophil counts have served for decades as an indirect assessment of airway inflammation. Nearly 40 years ago, Horn et al¹¹² used peripheral blood eosinophil counts in 52 patients with active asthma to show that eosinophilia is an important diagnostic feature of asthma. The study further suggested the use of total eosinophil counts to aid in determining steroid dosages and to predict exacerbations. Furthermore, Ulrik and Frederiksen¹¹³ determined that blood eosinophil counts were highly significantly related to asthma-related deaths.

In the Childhood Asthma Management Program, researchers found that the Arg130Gln *IL13* polymorphism was significantly associated with peripheral blood total eosinophil counts in children with mild-to-moderate asthma. Moreover, both peripheral blood eosinophil counts and serum IL-13 levels were significantly reduced after 16 weeks of omalizumab treatment.¹¹⁴ Finally, mepolizumab (anti-IL-5) therapy significantly decreased blood eosinophil counts in patients with severe refractory eosinophilic asthma.^{115,116} Thus, peripheral blood eosinophil counts can be an indirect indicator of T_H2 inflammation.

IgE is one of the most important mediators of allergic disease and asthma. Increased serum IgE levels have been associated with asthma and atopy in both adults and children.^{117,118} The total serum IgE level has been used for decades as a biomarker of atopy and T_H2-induced inflammation. In a large multicenter trial, patients with mild-to-severe asthma were followed for 2.5 years, with one third of the patients receiving ICSs. While correcting for FEV₁, sex, and age, the total serum IgE level was the only independent variable tested that predicted corticosteroid-induced improvement in airways hyperresponsiveness.¹¹⁹ In

addition, total serum IgE levels inversely correlated with asthma severity and FEV₁ percent predicted values in a study of 562 patients with mild-to-severe allergic asthma.¹²⁰ Moreover, variants of *IL13* contributed to increased total IgE levels and sensitization to food allergens in young atopic children.¹²¹ Anti-IgE therapy has been approved for use in human subjects (omalizumab), and a review of the efficacy, safety, and evaluation of this drug for use in the treatment of asthma is described elsewhere.^{122,123}

Periostin is an extracellular matrix protein that is induced by IL-4 and IL-13 in airway epithelial cells¹²⁴ and lung fibroblasts.⁴⁴ In the asthmatic airway, periostin plays a role in cell adhesion, TGF- β production, and subepithelial fibrosis.¹²⁵⁻¹²⁷ The importance of periostin as a biomarker for T_H2-induced airway inflammation was brought to the forefront by an innovative study conducted by Woodruff et al.¹²⁸ These authors used microarray analysis of airway epithelial cells collected from asthmatic patients to investigate effects of corticosteroids on gene expression. They demonstrated that expression of 3 genes, *POSTN*, *SERPINB2*, and *CLCA1*, were upregulated in asthma and that IL-13 specifically increased expression of these genes in airway epithelial cells. Corticosteroid treatment suppressed expression of the 3 genes in asthmatic patients and airway epithelial cells.¹²⁸ As described, *POSTN* is among the 3-gene signature that has been used successfully by this group to identify T_H2-high and T_H2-low phenotypes in asthmatic patients, which were validated in bronchial biopsy specimens. A significant improvement in FEV₁ in response to corticosteroids was reported for the T_H2-high group but not for the T_H2-low group.⁸⁶ Finally, *POSTN* expression was recently demonstrated to be upregulated in bronchial and nasal epithelial cells of children with asthma compared with that seen in atopic or healthy children, with increased expression observed in response to IL-4 or IL-13.¹²⁹ Thus evaluation of *POSTN* expression in both patients with adult and those with childhood asthma has potential in serving to determine patient responsiveness to specific therapies, including corticosteroids and IL-13 blockade.

The use of single inflammatory and molecular markers has potential to allow for phenotyping of patients and to inform therapy decisions in asthmatic patients, but overall, weaknesses exist in relationships between these markers, and T_H2 inflammation in asthmatic patients might be more effectively identified by using combinations of biomarkers. For instance, the correlation between FENO levels and sputum eosinophil counts has been reported to be 0.59 ($P < .001$),⁹⁷ that with blood eosinophil counts has been reported to be 0.51 ($P < .001$),¹³⁰ and that with IgE levels has been reported to be 0.38 to 0.48 ($P < .001$).^{130,131} A recent study found the sensitivity and specificity of FENO measurements to be poor when discriminating patients with sputum eosinophilia from those with noneosinophilic asthma.⁶⁵ Furthermore, a meta-analysis of the efficiency of the use of FENO values or sputum eosinophil counts versus clinical symptoms or spirometry for asthma-related outcomes in children and adults found that FENO measurements offered no significant benefit in number of exacerbations when adjusting daily dose of corticosteroids based on FENO values. In children the number of exacerbations was reduced based on FENO values, but the reduction required increased daily doses of corticosteroids. In contrast, a treatment strategy based on sputum eosinophil counts significantly reduced asthma exacerbations in adults.¹³² Another recent study found that FENO levels during exacerbation did not predict robust clinical and

spirometric responsiveness to corticosteroids in patients with moderate-to-severe asthma.¹³³ Little et al¹³⁴ found that only the combination of sputum eosinophilia and increased FENO levels significantly improved the sensitivity and specificity of the inflammatory markers to predict responses to oral steroids. Each biomarker alone was insufficient. In addition, a study of 80 asthmatic subjects has suggested that peripheral blood eosinophil counts are superior to FENO levels in predicting sputum eosinophilia, and the authors suggest combining this marker with the patient's clinical history and FEV₁ measurements to practically assess airway eosinophilia.¹³⁵

The relationships between the relatively new biomarker periostin and other markers of inflammation are not very clear, and we have little information on the interactions of periostin with IgE and FENO levels beyond what has been reported in Corren et al.¹⁰⁶ Significant correlation (0.63, $P < .0001$) has been noted between periostin expression and eosinophil levels in biopsy specimens of patients with eosinophilic esophagitis.¹²⁵ However, the strength of using periostin as an inflammatory biomarker alone or in combination with other markers should be further investigated.

ASTHMA PHENOTYPES AND RESPONSE TO T_H2 CYTOKINE INHIBITION

Phenotyping human asthma has demonstrated that approximately 50% of asthmatic patients demonstrate a T_H2-driven phenotype.⁸⁶ Therefore therapies that target this type of inflammation will not be uniformly effective in asthmatic patients. Recently, an innovative study was conducted to prove the efficacy of lebrikizumab (Genentech, San Francisco, Calif), a humanized mAb that specifically binds to and inhibits the activity of IL-13, in patients with uncontrolled asthma.¹⁰⁶ This study was unique in that patients with moderate-to-severe asthma (prebronchodilator FEV₁, 40% to 80% of predicted value) taking ICSs were stratified before randomization according to an IL-13 signature surrogate comprised of total serum IgE levels, peripheral blood eosinophil counts, and baseline serum periostin levels. Measurements of FEV₁, peak expiratory flow, FENO levels, and Asthma Control Daily Diary questionnaire scores were obtained for all patients and patient subgroups at baseline and after lebrikizumab. High T_H2 was defined as a total IgE level of greater than 100 IU/mL and an eosinophil count of 0.14×10^9 cells/L or greater; low T_H2 was defined as a total IgE level of 100 IU/mL or less or an eosinophil count of less than 0.14×10^9 cells/L. A high-periostin subgroup consisted of patients with periostin levels that were greater than the median for the 212 subjects in the study; the low-periostin subgroup consisted of patients whose periostin levels were less than the median value.

After stratification by these criteria of high and low T_H2, patients were randomized to receive lebrikizumab or placebo over a 24-week period. Patients in the T_H2-high stratification group receiving lebrikizumab therapy improved significantly in baseline prebronchodilator FEV₁ over 12 weeks and exhibited 60% reduced exacerbations compared with those receiving placebo. A significant increase in baseline FEV₁ was noted with lebrikizumab compared with placebo, and this increase in FEV₁ was most evident in the high-periostin subgroup (Fig 3). Changes in FEV₁ were noted within 1 week after treatment with the antibody, and they were sustained throughout the study, indicating that blockade of IL-13 quickly affected airway obstruction. In addition, lebrikizumab was associated with a significant reduction in FENO levels

compared with those seen in the placebo group, especially in the high-periostin subgroup. These data suggest that serum periostin levels might be a useful tool in identifying patients with asthma who would be most responsive to anti-IL-13 therapies. However, as promising as the lebrikizumab study might be, this report is a single study, and the relative effect of periostin as a biomarker for T_H2 inflammation for asthma interventions is somewhat limited until these results are replicated in independent evaluations to confirm the association of periostin with other hallmarks of asthmatic inflammation and remodeling, such as airway eosinophilia and subepithelial fibrosis.

Another strategy to help guide therapy in the lebrikizumab study was stratification of the patients based on baseline FENO measurements. Lebrikizumab affected FENO levels over the course of the study, with a 19% mean decrease in FENO levels by week 12. The greatest improvements in FENO levels were observed in the high-periostin subgroup (34%) compared with the low-periostin subgroup (4%). Furthermore, the high-periostin subgroup exhibited an increased mean baseline FENO level compared with the low-periostin subgroup. These data suggest that FENO levels are related to T_H2 inflammation in the airway and that anti-IL-13 therapy has the potential to modify FENO measurements in asthmatic patients. High baseline FENO levels compared with low baseline FENO levels were associated with reduced rates of severe exacerbations and improved FEV₁ in patients receiving the drug; however, greater intrapatient variability was observed in baseline FENO levels compared with periostin levels (mean coefficient of variation, 19.8% vs 5%).¹⁰⁶ Taken together, IgE levels, peripheral blood eosinophil counts, periostin levels, and FENO levels are promising biomarkers for anti-IL-13 therapies that have been demonstrated to have association with T_H2 inflammation, but independent validation and replication of this study is needed to confirm the utility of these and/or combinations of these biomarkers for regular clinical use. We also suggest that combination of biomarkers might ultimately identify patients with T_H2 inflammation.

The Corren et al¹⁰⁶ study of lebrikizumab is just one example that provides convincing evidence that classification of asthmatic patients into inflammatory, clinical, molecular, or genetic subphenotypes has the potential to guide therapeutic decision making. In addition, 2 independent studies have confirmed increased responsiveness to mepolizumab (anti-IL-5) therapy in severe asthma patients selected to demonstrate airway eosinophilia. In these studies, inclusion of patients based on sputum eosinophil counts allowed for greater drug efficacy, reduced asthma exacerbations, and reduced dosage of corticosteroids.^{115,116} Similarly, Busse et al¹³⁶ have found that patients with increased FENO levels and blood eosinophil counts responded to omalizumab (anti-IgE) with significantly reduced rates of exacerbations compared with patients treated according to standard guidelines. Thus these studies provide proof of concept for the heterogeneity of asthma and highlight the benefits of tailoring therapies to specific subgroups of asthmatic patients.

SPECIFICALLY TARGETING THE IL-13 PATHWAY IN ASTHMATIC PATIENTS

In addition to the Corren et al¹⁰⁶ study, several approaches have been used to target IL-13, IL-13/IL-4 receptors, and STAT6 in clinical trials of both acute and chronic asthma. These approaches include an IL-4 variant, mAbs to IL-4R α or IL-13, or inhibitory RNAs and peptides specific for STAT6. The results gathered

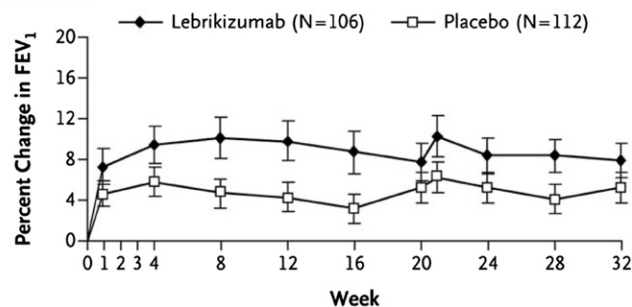
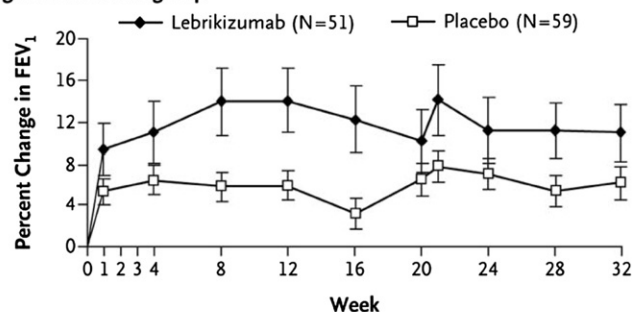
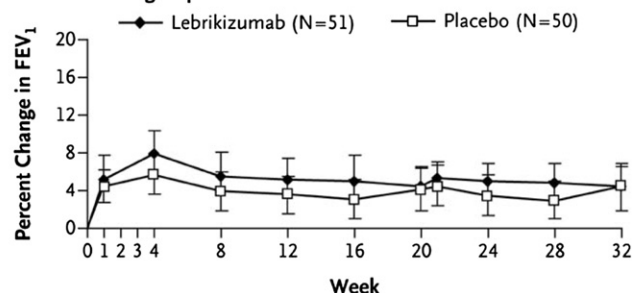
A Total Cohort**B High-Periostin Subgroup****C Low-Periostin Subgroup**

FIG 3. Serum periostin levels inform efficacy of anti-IL-13 therapy to treat airway obstruction. In a study by Corren et al,¹⁰⁶ patients were stratified before treatment with lebrikizumab by using baseline serum periostin levels. After 12 weeks of treatment with the antibody, the increase in FEV₁ was higher in the lebrikizumab group compared with that seen in the placebo group (A), but an even greater increase in FEV₁ with lebrikizumab was observed in the high-periostin subset of patients (B). In the low-periostin subgroup the change in FEV₁ was not significant (C). Excerpted from Corren et al.¹⁰⁶

from these studies significantly contribute to our understanding of the roles of IL-13 and its receptors in the pathobiology of human asthma.

First, 2 groups performed clinical trials using allergen challenges to identify the effect of IL-13 or IL-4R α antagonists on allergen-induced asthmatic responses. Using an experimental design that tested effects on IL-4- and IL-13-induced acute asthmatic responses, Wenzel et al¹³⁷ reported the results of 2 clinical trials of a recombinant human IL-4 variant (pitrakinra; Aerovance, Berkeley, Calif) administered after inhaled allergen challenge. Pitrakinra is a recombinant form of IL-4 that blocks signaling of both IL-4 and IL-13 through IL-4R α . Atopic asthmatic patients were enrolled for both studies, and for one study, pitrakinra or placebo was injected subcutaneously once daily; for the other study, the drug was delivered through a nebulizer. Treatment of patients with inhaled pitrakinra for 4 weeks attenuated the decrease in FEV₁ after allergen challenge and also

decreased resting state FENO levels. The effect of pitrakinra on change in FEV₁ compared with placebo was only observed in the late phase of the asthmatic response (ie, 4-10 hours after allergen challenge). No significant effect of pitrakinra was seen in the early phase of the asthmatic response, likely because of the short 4-week duration of the pitrakinra treatment.

Recently, a pharmacogenetics study confirmed that several *IL4RA* nonsynonymous SNPs were associated with a reduction in FEV₁ that occurred during the late antigen response in the pitrakinra-treated group.¹³⁸ A follow-up study consisted of patients with moderate-to-severe asthma who participated in a placebo-controlled, dose-ranging clinical trial of pitrakinra over a course of 12 weeks to evaluate safety and efficacy of the drug. No significant differences were noted in the frequency of exacerbations in patients receiving pitrakinra compared with placebo; however, analysis of *IL4RA* genotype association with asthma exacerbations stratified by treatment group revealed that the

rs8832GG common allele was associated with reduced asthma symptoms compared with the rs8832A minor allele randomized to active pitrakinra.¹³⁹ Thus, in this study genetic variation was used as biomarker to identify a subgroup of patients with moderate-to-severe asthma who would be more responsive to anti-IL-4R therapy.

Another study of acute allergen-induced airway responses in patients with mild atopic asthma evaluated 2 fully humanized neutralizing mAbs that block IL-13 activity (IMA-638 and IMA-026; Pfizer, New York, NY). IMA-638 specifically prevents recruitment of the IL-4R α subunit to complex with IL-13R α 1 after initial binding of IL-13 to the IL-13R α 1 subunit. IMA-026 inhibits the initial interaction between IL-13 and the IL-13R α 1 and IL-13R α 2 subunits. Gauvreau et al¹⁴⁰ reported results of clinical trials of each of the antibodies with allergen challenge across a time course of 35 days in patients with mild asthma who had exhibited an early and late allergen response. IMA-638 significantly attenuated the allergen-induced, early-phase maximum percentage decrease in FEV₁ and late-phase area under the curve measurements, suggesting that blocking binding of IL-13 to the IL-4R α subunit abrogates allergen-induced bronchoconstriction. No significant effect on early- or late-phase responses was observed for IMA-026, and neither antibody had an effect on airways hyperresponsiveness or eosinophil recruitment. Although both antibodies target human IL-13, the mechanism of action of each of the antibodies is distinct, and each inhibits binding of IL-13 to different IL-13 receptor subunits. Much higher titers of circulating IL-13 (free plus bound) were detected after treatment with IMA-026 than with IMA-638. A follow-up study of allergen-challenged patients with mild asthma and healthy control subjects demonstrated that the differential inhibition of IL-13R α 2 by IMA-026 interferes with the normal role of cell-surface IL-13R α 2 in scavenging extracellular IL-13 in human cells.¹⁴¹ Therefore the effect of IL-13R α 2 on IL-13 clearance and the differences in the abilities of IMA-026 and IMA-638 to block this process might have significant clinical implications for evaluation of responses to these potential therapies and others targeting the IL-13/IL-13 receptor pathways in human asthma.

When compared with other interventions, such as leukotriene inhibitors and ICSs, these studies of the effects of anti-IL-13 or anti-IL-4R on allergen-induced asthmatic responses show that these drugs affect the late asthmatic response to varying degrees. In allergen challenge studies pitrakinra inhibited the maximum percentage decrease in FEV₁ to a similar degree compared with montelukast but not as well as budesonide on late-phase asthmatic responses.¹⁴² The anti-IL-13 antibodies IMA-638 and IMA-0926 did not perform as well in inhibition of maximum percentage decrease in FEV₁ compared with the other interventions (Table II).^{137,140,142}

Models of inhalation allergen challenge in patients with mild asthma provide a proved method to evaluate potential new drugs for asthma, but as pointed out by O'Byrne et al,¹⁴³ not all drugs that have attenuated allergen-induced responses in these challenge models have become confirmed effective asthma therapies. These studies focus only on a feature of asthma pathobiology and cannot take into account the full complexity of this disease. Therefore, although beneficial for drug development, allergen challenge models are limited in scope.

Hodsmen et al¹⁴⁴ recently reported further evidence of the potential for anti-IL-13 mAb therapy in asthmatic patients. GSK679586 is a humanized anti-IL-13 antibody that blocks

TABLE II. Comparison of selected interventions and effects on the maximum percentage decrease in FEV₁ in the late phase of allergen-induced asthmatic response

	Placebo	Treatment	P value	Reference
Montelukast	17.8	7.8	.01	142
Budesonide	17.8	7.8	<.001	142
Pitrakinra	27.6	16.1	.0009	137
IMA-638	19.3	12.8	.09	140
IMA-026	20.9	16.4	.31	140

binding of IL-13 to both IL-13R α 1 and IL-13R α 2, a mechanism similar to IMA-026. In a 2-part study of placebo-controlled dose escalation in which healthy subjects were administered single intravenous infusions of 5 doses of antibody or patients with mild asthma were administered 2 repeat intravenous infusions (4 weeks apart) of 3 doses of antibody, the safety and tolerability of the drug were the primary measured end points, and the antibody was well tolerated. Secondary measured outcomes demonstrated that infusion of the antibody in patients with mild asthma resulted in a dose-dependent reduction in FENO levels. Also, total serum IL-13 levels increased in patients with mild asthma after infusion of GSK679586 at the highest doses, presumably because of binding of free IL-13 to form an antibody/cytokine complex.¹⁴⁴ These results are similar to those observed with IMA-026 in which reduced IL-13 clearance can result from neutralization of IL-13R α .¹⁴¹ The natural inhibitory role of IL-13R α 2 must be considered when developing anti-IL-13 therapies.

Corren et al¹⁴⁵ reported the results of a large clinical trial of patients with moderate-to-severe atopic asthma on ICSs treated with AMG 317, a humanized mAb that binds IL-4R α . Patients received the antibody treatment or placebo once weekly for 12 weeks. No significant change in Asthma Control Questionnaire scores was noted in response to treatment with AMG 317, but a significant dose-response decrease in serum IgE levels was observed, and fewer exacerbations were seen in patients receiving the highest dose of AMG 317.

CAT-354 (MedImmune, Gaithersburg, Md) is a human mAb directed against IL-13.¹⁴⁶ The efficacy of CAT-354 was first demonstrated in a murine model in which IL-13-induced lung eosinophil recruitment and airways hyperresponsiveness were blocked after pretreatment of CAT-354.¹⁴⁷ In human cells CAT-354 inhibited IL-13-stimulated mast cell proliferation, Fc ϵ RI expression, and histamine release.¹⁴⁸ In a cynomolgus monkey model of *Ascaris* species allergen challenge, CAT-354 successfully inhibited airway eosinophil recruitment and airways hyperresponsiveness.^{149,150}

Because STAT6 is an essential mediator of IL-4 and IL-13 signaling²² and STAT6-deficient mice exhibit attenuated inflammatory responses to allergens,¹⁵¹ STAT6 is an obvious target for blockade therapies. The most promising approach for inhibition of STAT6 for drug development is the use of siRNAs. The efficacy of siRNA inhibition of STAT6 has recently been demonstrated in a murine model of allergen-induced airway inflammation. After identification and testing of a potent inhibitory and specific STAT6 siRNA in murine and human cells *in vitro*, STAT6 siRNA was administered intranasally to BALB/c mice that were subsequently sensitized and challenged with ovalbumin. Mice treated with the STAT6 siRNA exhibited significantly reduced airway eosinophilic inflammation and airways hyperresponsiveness.¹⁵² McCusker et al¹⁵³ made similar

observations using STAT6 inhibitory peptides in a murine model of allergen-induced airway disease. Intranasal delivery of the peptides to murine airway epithelial cells inhibited ovalbumin-induced increase of IL-13 levels in BAL fluid, mucus production, airway eosinophilia, and airways hyperresponsiveness. These preclinical studies suggest that development of inhibitory molecules to target STAT6 has potential as a therapeutic approach for treating human airway inflammation and asthma, but more research is needed.

CONCLUSIONS

Strong experimental evidence has accumulated over the years to demonstrate that IL-13 directs many of the important features of airway inflammation and remodeling in patients with allergic asthma. Yet an almost equally rich body of evidence has shown that asthma is a complex heterogeneous disease characterized by different severities, cellular inflammation subtypes, and molecular subphenotypes. Biomarkers have now been identified that correlate IL-13 expression and airway inflammation in asthmatic patients. Indeed, these biomarkers have already been used to define subsets of patients who might be responsive to corticosteroids or anti-IL-13 therapies. There is now increased awareness that despite our increased knowledge of the IL-13 pathway in asthmatic patients, not all patients will be responsive to therapies targeting this pathway. More studies focusing on identifying clinical characteristics/phenotypes associated with response and prestratifying subjects to study responders is critical if we are to move our field forward and personalize medicine.

What do we know?

- Asthma is a complex heterogeneous disease that can be characterized by different severities and cellular inflammatory and molecular phenotypes.
- IL-13 is a T_H2 cytokine that directs most of the key features of airway inflammation and remodeling in asthmatic patients.
- Total serum IgE levels, sputum eosinophil counts, $FENO$ levels, and serum periostin levels might serve as biomarkers of T_H2 inflammation in asthmatic patients.
- Therapies in development that target the IL-13/IL-4/STAT6 pathway have demonstrated effectiveness in abrogating airway inflammation and obstruction but not asthma control.
- Directed patient selection based on a cellular inflammation profile or a T_H2 gene signature allows for improved efficacy of corticosteroids or anti-IL-13 therapies in specific subsets of patients.

What is still unknown?

- What is the role of IL-13 in innate immunity in human allergic asthma?
- Will a simple easy-to-perform biomarker test or combinations of biomarker tests be available for routine use in the clinic to inform physicians of the T_H2 inflammation status of patients and guide therapy?
- What cytokines and signaling pathways are important in the noneosinophilic, low- T_H2 subphenotype of asthma, and can targeted therapies be developed to benefit these patients?

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