

Phenotypes and endotypes of adult asthma: Moving toward precision medicine



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Asthma is a chronic inflammatory disease of the airways that is challenging to dissect into subgroups because of the heterogeneity present across the spectrum of the disease. Efforts to subclassify asthma using advanced computational methods have identified a number of different phenotypes that suggest that multiple pathobiologically driven clusters of disease exist. The main phenotypes that have been identified include (1) early-onset allergic asthma, (2) early-onset allergic moderate-to-severe remodeled asthma, (3) late-onset nonallergic eosinophilic asthma, and (4) late-onset nonallergic noneosinophilic asthma. Subgroups of these phenotypes also exist but have not been as consistently identified. Advances in our understanding of the diverse immunologic perturbations that drive airway inflammation are consistent with clinical traits associated with these phenotypes and their response to biologic therapies. This has improved the clinician's approach to characterizing asthmatic patients in the clinic. Being able to define asthma endotypes using clinical characteristics and biomarkers will move physicians toward even more personalized management of asthma and precision-based care in the future. Here we will review the most prominent phenotypes and immunologic advances that suggest these disease subtypes represent asthma endotypes. (*J Allergy Clin Immunol* 2019;144:1-12.)

Key words: Asthma, heterogeneity, phenotypes, endotypes, biologics

Asthma is now recognized as a heterogeneous disease driven by interactions between epigenetic regulation and environmental exposure. Asthma prevalence has been increasing for decades and now affects 25.7 million persons in the United States and caused approximately 1.8 million emergency department visits in 2010.¹ Multiple biologic pathways underlie the inflammatory responses associated with disease heterogeneity but cause similar clinical symptoms that include shortness of breath, wheezing, and cough.

Abbreviations used

CRTH2: Chemoattractant receptor-homologous molecule expressed on T_H2 lymphocytes
FENO: Fraction of exhaled nitric oxide
ILC: Innate lymphoid cell
ILC2: Type 2 innate lymphoid cell
Rag: Recombination-activating gene
SARP: Severe Asthma Research Program
T2: Type 2
TEA: Transcriptomic endotypes of asthma
TSLP: Thymic stromal lymphopoietin
U-BIOPRED: Unbiased Biomarkers in Prediction of Respiratory Disease Outcome
UK: United Kingdom

This has made it difficult if not impossible to biologically define distinct subgroups by using traditional clinical and physiologic parameters. However, there is now evidence that there are clinical phenotypes “embedded” in the larger context of asthma that are due to underlying differences in biology.

These phenotypes likely reflect specific “endotypes” of disease (defined as a subset of disease with a distinct pathophysiology).² Efforts to subclassify the disease using large multicenter studies conducted in many different environments have consistently identified several phenotypes that support the presence of endotypes. However, it is still unclear how well these phenotypes reflect specific pathobiology because there is heterogeneity within every phenotype cluster, the clusters significantly overlap, and the methodology used and cohorts examined vary widely.

The goal of precision medicine will be difficult to achieve when different phenotypes and endotypes share common symptoms and biomarkers. Novel specific biomarkers must be identified to achieve the precision required to select from the myriad of treatments available to be confident that the best treatment is being chosen for a given patient. Here we will outline what clustering studies have revealed about phenotypes and endotypes of asthma and their relationship to the clinical utility of targeted biologic therapeutics in use for severe uncontrolled asthma.

CLINICAL AND MOLECULAR PHENOTYPES OF ASTHMA

Several large multicenter studies have been conducted that used different clustering techniques to identify asthma phenotypes. The largest studies are from the Severe Asthma Research Program (SARP) in the United States, the Leicester study conducted in the United Kingdom (UK), and the U-BIOPRED study conducted in 11 countries, including the UK,

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There are significant differences in the cohorts examined, the disease features used in each clustering analysis, the computational approaches, and the number of clusters identified among the studies. Despite these differences, the results of all the studies consistently suggest that there are approximately 4 primary phenotypes of asthma. These include (1) early-onset mild allergic asthma, (2) early-onset allergic moderate-to-severe remodeled asthma, (3) late-onset nonallergic eosinophilic asthma, and (4) late-onset noneosinophilic nonallergic asthma (Fig 1). This is consistent with the dogma that age of onset, lung function, and atopy are key discriminators of asthma heterogeneity and is consistent with the recent emergence of the eosinophil as an important biomarker and effector cell in asthmatic patients.³⁻⁵ Other features that are commonly linked to asthma, such as sex, obesity, and smoking, are less consistent across these studies, likely related to differences among the asthma populations examined and the cutoffs used for smoking inclusion for each of the studies.⁶⁻⁸

One of the first multicenter cluster analysis studies was conducted by SARP. In this study of patients with primarily severe disease (approximately 70%), Moore et al⁶ identified 5 dominant phenotypes using an unsupervised cluster analysis of both clinical and physiologic features of disease. In the initial SARP study, demographic data, lung function, and medication use data were collected, and 34 core phenotypic variables were selected to determine whether clinically meaningful phenotypes could be identified by using Ward minimum-variance hierarchical clustering. The 5 phenotypes identified were (1) mild early-onset allergic disease, (2) moderate early-onset allergic disease, (3) late-onset eosinophilic nonallergic disease, (4) severe early-onset eosinophilic allergic disease, and (5) late-onset nonallergic neutrophilic severe asthma with fixed airflow obstruction. Sputum analysis of the SARP clusters showed high eosinophil counts in clusters 3, 4, and 5, with cluster 3 having the greatest percentage of eosinophils and cluster 5 having a mixed population of high eosinophil and neutrophil counts, suggesting a mixed inflammatory response (detailed below).⁶ Clusters 1 and 2 were found to have less than 1% eosinophils.⁶ These results suggest that patients with severe asthma fall into 3 clinically identifiable groups: (1) early-onset allergic patients, (2) late-onset nonallergic eosinophil predominant patients, and (3) sputum neutrophil predominant.⁹ These 3 subgroups also link to 3 predominant immunologically identifiable subtypes of disease that are consistent with differences in the underlying biology of disease and presence of underlying endotypes. Clusters 1, 2, and 4 fall into the spectrum of disease driven by allergic inflammation, whereas clusters 3 and 5 appear to be driven by biologic processes that are nonallergic and most evident in adults. These 2 late-onset clusters can be discriminated by the duration of disease, with cluster 3 being shorter duration and cluster 5 being long-standing disease.⁶

Another important observation is that eosinophilic inflammation is evident in both allergic and nonallergic phenotypes. Of the severe asthma clusters, both the severe allergic cluster 4 and the late-onset cluster 3 (that is more female and obese) have evidence of high eosinophil counts, whereas cluster 5 was more neutrophil predominant. This is consistent with the findings by Wenzel et al¹⁰ that identified 2 distinct inflammatory subtypes of severe asthma when patients with

severe asthma were stratified by high and low eosinophil counts in airway biopsy specimens. Those with high eosinophil counts were found to have type 2 (T2) inflammation with increased eosinophil counts and increased numbers of T cells compared with the low-eosinophil group, in which neutrophil counts were increased.¹⁰ Interestingly, no differences in allergic status were found between the high- and low-eosinophil groups. These findings are consistent with the observation that approximately 50% of patients have T2 inflammation (eosinophils) in the absence of allergy and that there are nonallergic noneosinophilic phenotypes of disease.¹¹

One of the first European efforts to phenotype subgroups of asthma was conducted by Haldar et al (UK clusters).⁷ K-means cluster analysis was used on data from patients in both the primary care setting and in patients with refractory asthma seen by subspecialists. Focusing on the patients with severe uncontrolled disease meeting American Thoracic Society criteria for refractory asthma, 4 distinct clusters were found by clustering demographic data, lung function, sputum eosinophil counts, and medication use. Discriminant function modeling was used to identify inputs (clinical features) that were significant determinants for clustering. Variables chosen included age of onset, atopy, and lung function.⁷ In contrast to the SARP study, this analysis used sputum eosinophil counts in the analysis. Four clusters were identified that overlap with the SARP clusters: (1) cluster 3, early-onset symptoms predominant with minimal eosinophils (similar to SARP clusters 1 and 2); (2) cluster 1, early-onset allergic severe asthma (similar to SARP cluster 4); (3) cluster 4, late-onset eosinophilic inflammation male predominant (similar to SARP cluster 3); and (4) cluster 2, noneosinophilic asthma with greater female predominance (similar to SARP cluster 5).⁷ The early-onset atopic asthma and obese noneosinophilic asthma clusters had high symptom expression compared with low levels of symptomatology present in the late-onset eosinophilic asthma cluster. These results suggest a lack of steroid responsiveness in patients with severe noneosinophilic asthma and those with more severe allergic disease. Other characteristics, such as obesity and sex, were different among the clusters identified in the 2 studies, indicating a modulatory effect on the core phenotypes.

The most recent clustering analysis of asthma is from the Unbiased Biomarkers in Prediction of Respiratory Disease Outcome (U-BIOPRED) cohort published by Leflaudeux et al.⁸ This study differed from the SARP and UK clustering analyses in that it integrated transcriptomic signatures into the analysis to identify subgroups of disease. The 2-step analysis first used clinical and biologic characteristics to identify clusters in the population.

By using demographic data, lung function, medication use, and sputum cell differentials, this study identified 4 clusters that were similar to those seen in the SARP⁶ and Haldar et al⁷ studies. An important difference in this study was that smokers were included in the analysis. Common clinical features, such as atopic status, age of onset, obesity, smoking history, and sputum eosinophil percentages were used to identify the 4 clusters. Cluster 1 included moderate-to-severe asthma with low eosinophil counts and a high proportion of allergic subjects (similar to SARP-2 and UK cluster 3). Cluster 2 included overweight/obese patients with severe nonallergic late-onset asthma with severe airway obstruction, high eosinophil counts, and smoking history (similar to SARP cluster 3 and UK cluster 4). Cluster 3 had

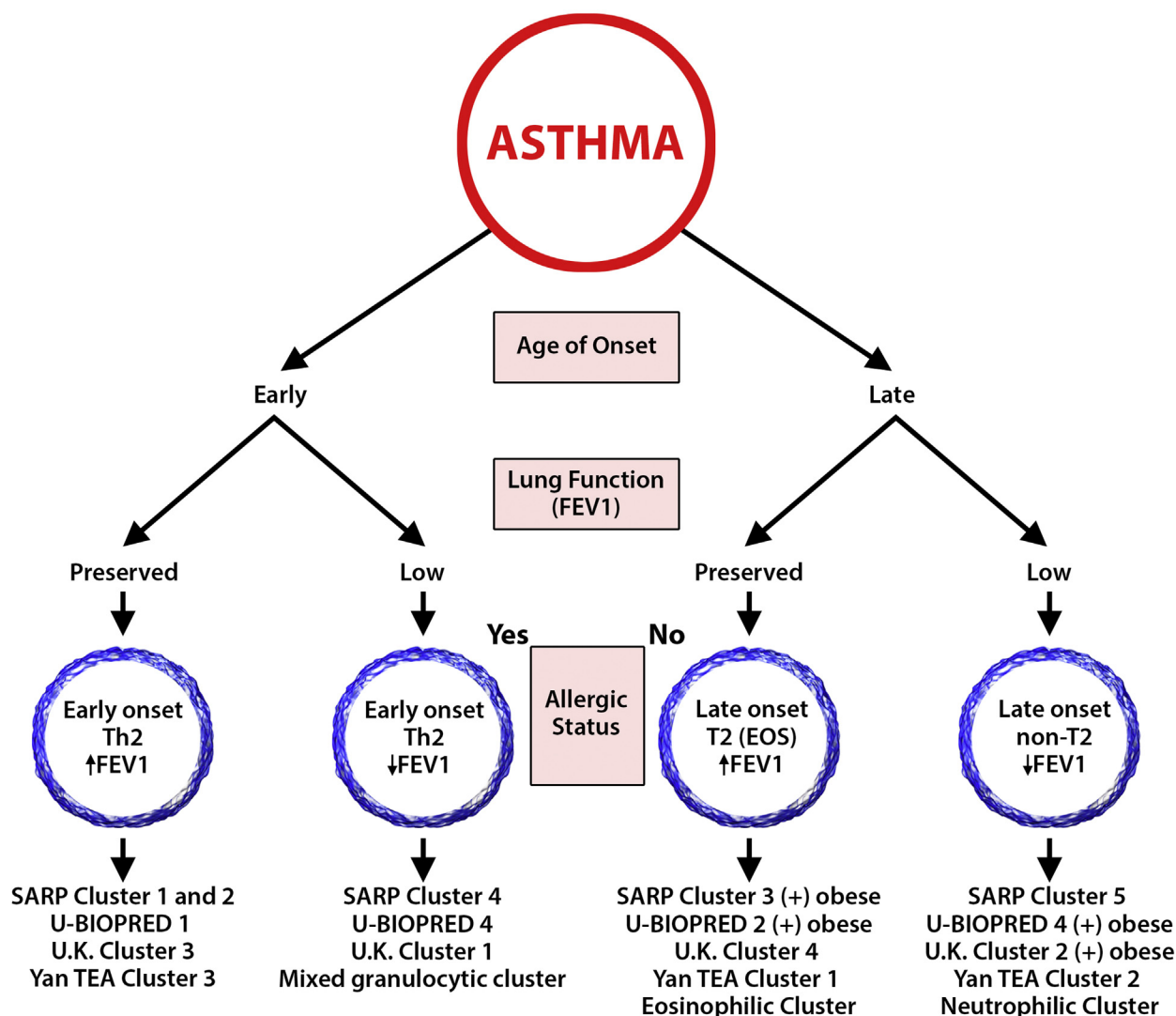


FIG 1. Diagram showing similarities between asthma phenotypes in cluster analysis studies using age of onset and lung function. Inflammatory subtypes: *T_H2*, positive IgE level, positive SPT response, elevated FENO value, elevated eosinophil count; eosinophilic (*T2*), elevated eosinophil count, negative IgE level/SPT response, and elevated FENO value; *non-T2*, normal eosinophil count, negative IgE level/SPT response, and normal FENO value. Disease modifier includes obesity, which is present in both eosinophilic and noneosinophilic late-onset asthma. *EOS*, Eosinophils.

patients with severe late-onset disease similar to cluster 2 but with more allergy and no smoking history. Cluster 4 included obese female patients with normal lung function, allergies, severe asthma, and frequent exacerbations (similar to SARP cluster 5 and UK cluster 2 with overlap into SARP cluster 4). When looking at gene expression in the U-BIOPRED clusters, clusters 2 and 3 had greater expression of IL-16.¹² Cluster 2 also had greater expression of connective tissue-activating peptide III (CTAP-III) and GM-CSF, which was attributed to the effect of smoking.⁸ Cluster 3 had decreased expression of cathepsin G.⁸ Taken together with the other phenotyping studies, this suggests that consistently, there are 4 main asthma phenotypes: (1) early-onset mild allergic asthma, (2) early-onset allergic moderate-to-severe remodeled asthma, (3) late-onset nonallergic eosinophilic asthma, and (4) late-onset noneosinophilic nonallergic asthma. Obesity was found to be a factor in late-onset asthma in both patients with eosinophilic and those

with noneosinophilic disease in all 3 studies, indicating a modulatory effect of obesity on the core phenotypes. Smoking also clearly has a strong effect on phenotypic expression of disease but was not included across all studies.

Evaluation of sputum inflammatory cells has also been used to phenotype and cluster patients with asthma. In the seminal study Simpson et al¹³ evaluated sputum cell differentials to identify inflammatory subtypes of asthma and then compared clinical features among the subtypes of patients. Patients were clustered by using sputum eosinophil and neutrophil proportions based on the 95th percentile of a healthy control group as a cutoff. Four clusters of asthma were identified: (1) eosinophilic (sputum eosinophil cutoff, >1.01%), (2) neutrophilic (sputum neutrophil cutoff, >61%), (3) mixed granulocytic (high eosinophil and neutrophil counts), and (4) paucigranulocytic (low eosinophil and neutrophil counts, similar to SARP cluster 5). The 4 subtypes were similar clinically, except that neutrophil-predominant

patients had a significantly later age of asthma onset. Interestingly, this subgroup was also identified in both the SARP and UK clustering studies, again supporting the existence of a noneosinophilic nonallergic asthmatic subgroup with a later age of onset. The overlap between these sputum cell-defined and clinical feature-defined phenotypes defined by the SARP, UK, and U-BIOPRED studies suggests that in adults asthma might be commonly driven by non-T2 inflammation that approximates an endotype.

Several groups have also pursued clustering analyses of gene expression in an effort to identify molecular patterns associated with clinically meaningful phenotypes of asthma, an effort to move from endotype to phenotype. Yan et al¹⁴ measured gene expression from sputum isolated from a heterogeneous cohort with mostly atopic asthma by using microarrays and found 3 transcriptomic endotypes of asthma (TEA clusters). mRNA was amplified from total RNA purified from cells isolated from mucus plugs induced with hypertonic saline. Three clusters of patients were identified that were clinically distinct. Two clusters of patients had severe disease. TEA cluster 1 had a history of near-fatal asthma attacks, greater fraction of exhaled nitric oxide (FENO) values in the airway, more sputum eosinophils, lower prebronchodilator FEV₁, more bronchodilator reversibility, and the highest level of T_H2 gene expression (IL-4, IL-5, and IL-13). This asthma cluster was similar to the eosinophilic clusters identified by SARP,⁶ Haldar et al,⁷ and U-BIOPRED.⁸ In contrast, TEA cluster 2 had a history of more hospitalizations for asthma compared with the other clusters and the lowest level of atopy. TEA cluster 3 had clinical features consistent with mild atopic disease with the lowest level of ICS dose¹⁴ and was more consistent with the early-onset atopic asthma clusters identified in other studies. Both TEA clusters 2 and 3 had lower levels of T_H2 gene expression. Importantly, these phenotypes were replicated by using a blood-based transcriptomic signature in a large pediatric cohort that suggested it will be possible to define gene signatures in the blood that are associated with specific phenotypes/endotypes of asthma that could be used for clinical management and pharmacogenomic studies.

Machine-based learning and the data-driven approach to cluster analysis have provided more precise identification of phenotypes and endotypes of asthma (Table 1). The clustering studies described above used both conventional statistics and machine-based learning to identify asthma subtypes and identified similar clusters. The SARP,⁶ UK,⁷ U-BIOPRED,⁸ and Yan et al¹⁴ studies all used unsupervised computational modeling of many clinical features and biomarkers to identify patterns in the cohorts. The Ward minimum-variance model uses a hierarchical method that “ranks” clinical features in the analysis, whereas the K-means and partition-around-medoid models partition the data around circles or medoids of means or medians. These methods are less biased compared with the Simpson et al¹³ study, which used a single predefined sputum inflammatory cell cutoff to cluster patients.

In addition, both the SARP⁶ and U-BIOPRED⁸ studies used many sites, including urban and rural areas across the United States and Europe, respectively, for analysis. The importance of using the right level of spatial aggregation and evaluating different regions can be crucial in identifying clusters that are both accurate and reproducible.¹⁵ This might be a limitation of the Haldar et al⁷ and Simpson et al¹³ studies given that only 1 location was used.

There have been few studies evaluating the longitudinal stability of phenotypic clusters. Boudier et al¹⁶ identified 7 clusters using latent transition analysis of asthma symptoms, allergy status, and lung function in adults with asthma. When looking at the phenotypes 10 years later, they found strong similarities among the clusters, especially allergic status. After 20 years, there was persistent low lung function in clusters identified as having low lung function at baseline, indicating a strong correlation over time.¹⁷

Although there are substantial differences in asthma populations examined across these studies, the parameters used to cluster patients, and the computational approaches used to define subgroups (Table 1), several clusters of asthma emerge that are probably biologically driven. Each study identified 3 to 5 severe asthma phenotypes, with the most consistently identified groups being those with (1) mild-to-severe early-onset allergic disease, (2) severe late-onset nonallergic asthma with eosinophils, and (3) nonallergic noneosinophilic severe asthma with irreversible airway obstruction (Fig 1). Although overlap is significant among the clusters, the distinct clinical, physiologic, and immunologic features of the clusters support the concept that unique biologic pathways (or endotypes) underlie these clusters of disease.

MECHANISMS DRIVING THE IMMUNE RESPONSE AND INFLAMMATION IN ASTHMATIC PATIENTS

Recently, multiple pathways have been identified that fit with the clinical heterogeneity seen among patients and the asthma phenotypes that have been defined. We now consider the T2 inflammatory response to result from activation of molecular pathways of both the innate and adaptive immune response. CD4⁺ T cells contribute to the adaptive response, whereas natural killer cells and type 2 innate lymphoid cells (ILC2s) play a role in the innate response.¹⁸

Release of epithelial alarmins, such as IL-25, IL-33, and thymic stromal lymphopoietin (TSLP), activate downstream T cells and ILC2s.¹⁹ IL-33 acts through its transmembrane receptor, ST2, on cell injury or cell death, leading to production of inflammatory cytokines and a T_H2-mediated immune response.²⁰ When IL-25 and IL-33 act on CD4 T cells, they differentiate into T_H2 cells, whereas TSLP primarily activates dendritic cells to induce a T_H2 environment.²¹ T_H2 cells produce several cytokines, including IL-4, IL-5, IL-9, and IL-13. On stimulation by alarmins, resident memory T cells might also be a source of T2 cytokine production.²² IL-4 stimulates B cells and promotes class-switching and specific IgE production.²³

Binding of IgE on the cell surfaces of mast cells and basophils leads to release of inflammatory mediators, such as histamine, serotonin, and tryptase, resulting in airway smooth muscle contraction and mucus hypersecretion.²⁴ Mast cells also release prostaglandin D₂, a lipid mediator, which acts through its receptor chemoattractant receptor-homologous molecule expressed on T_H2 lymphocytes (CRTH2) to amplify this inflammatory cascade.²⁵ CRTH2 is present on the surfaces of T_H2 cells, mast cells, ILC2s, and eosinophils. Activation of CRTH2 leads to increased production and release of T2 cytokines and also increased eosinophil migration and degranulation.²⁵ IL-5 and IL-13 lead to an increase in airway eosinophils and mucus production, as well as airway remodeling.²³ In contrast, ILC2s appear to be preprogrammed to release T2 cytokines in

TABLE I. Asthma heterogeneity revealed by clustering studies

	SARP ⁶	Leicester, UK ⁷	U-BIOPRED ⁸	Yan et al ¹⁴	Simpson et al ¹³
Methodology	Ward minimum-variance unsupervised hierarchical clustering of clinical features	Unsupervised K-means clustering of clinical features and sputum eosinophil percentages	Unsupervised partition-around-medoids clustering of clinical features	K-means unsupervised clustering of sputum gene expression	Manual clustering of patients using sputum inflammatory cell cutoffs
Study size (no.)	726	187	266	100	93
Clusters	(1) Mild early-onset allergic disease (2) Moderate early-onset allergic disease (3) Late-onset eosinophilic nonallergic disease (4) Severe early-onset eosinophilic allergic disease (5) Late-onset nonallergic neutrophilic severe asthma with fixed airflow	(1) Early-onset allergic severe asthma (2) Noneosinophilic asthma with greater female predominance (3) Early-onset symptom predominant with minimal eosinophil counts (4) Late-onset eosinophilic inflammation with male predominance	(1) Moderate-to-severe asthma with allergy and low eosinophil counts (2) Overweight to obese severe late-onset asthma with the least allergy, severe airway obstruction, high eosinophil counts, and smoking history [‡] (3) Severe late-onset disease similar to cluster 2 but more with more allergy and no smoking history [‡] (4) Obese female patients with severe asthma and frequent exacerbations but normal lung function and allergy	(1) Severe asthma with near-fatal asthma attacks, high eosinophil counts, and lower prebronchodilator FEV ₁ (2) Severe asthma with a history of more hospitalizations and least allergic (3) Mild atopic asthma with the lowest inhaled corticosteroid use	(1) Eosinophilic (2) Neutrophilic (3) Mixed granulocytic (high eosinophil and neutrophil counts) (4) Paucigranulocytic (low eosinophil and neutrophil counts) asthma
Shared clinical parameters of clustering studies			Lung function,* atopy [†]		
Unique clinical parameters	Age of onset, obesity	Age of onset, airway inflammation, obesity	Age of onset, airway inflammation, smoking, obesity	Age of onset, airway inflammation, obesity	Airway inflammation

*Pre-FEV₁ ± post-FEV₁, bronchodilator response.

[†]History of atopy or IgE, SPT, or ImmunoCAP positivity.

[‡]Ten or more pack years, current smokers.

response to epithelial alarmins without any antigen presentation (Fig 2).²¹ Therefore both the adaptive and innate immune responses contribute to the overall T2 inflammatory response and are likely differentially expressed among different phenotypes of asthma.

The immunology underlying the T_H2 response is well recognized and widely accepted to underlie early-onset allergic asthma. This subtype is the most prevalent form of the disease and the most common phenotype identified in cluster analyses. Although late-onset asthma is increasingly studied and was consistently identified in the cluster analyses discussed above, the underlying biologic processes that initiate and drive this subtype of asthma is less clear compared with early-onset disease. Late-onset eosinophilic asthma is an emerging subtype that has been of particular interest given the development of biologics targeting the IL-5 pathway. Both the innate and adaptive immune systems can drive eosinophilic inflammation, but the relative contribution of each of these arms of the immune response in any particular patient is unclear. Driven by the adaptive immune

response, patients with allergic asthma are among the easiest to identify in the clinic and can have mild disease with normal lung function or severe disease with evidence of irreversible obstruction suggestive of airway remodeling. Larger contributions of the innate immune response might be responsible for the development of later-onset asthma symptoms and could explain why many eosinophilic patients are nonatopic. A clearer understanding of the relative contribution of these arms of the immune response in patients with early- and late-onset disease will advance the field toward clearer diagnostic criteria, better biomarkers, and more precise selection of advanced therapies in the clinic.

Increased eosinophil counts can be seen in both patients with early-onset allergic asthma and those with nonallergic asthma, indicating that eosinophilia does not arise solely through the adaptive response of the immune system. Patients in the late-onset eosinophilic cluster often have the presence of T2 inflammation without evidence of allergic sensitization.

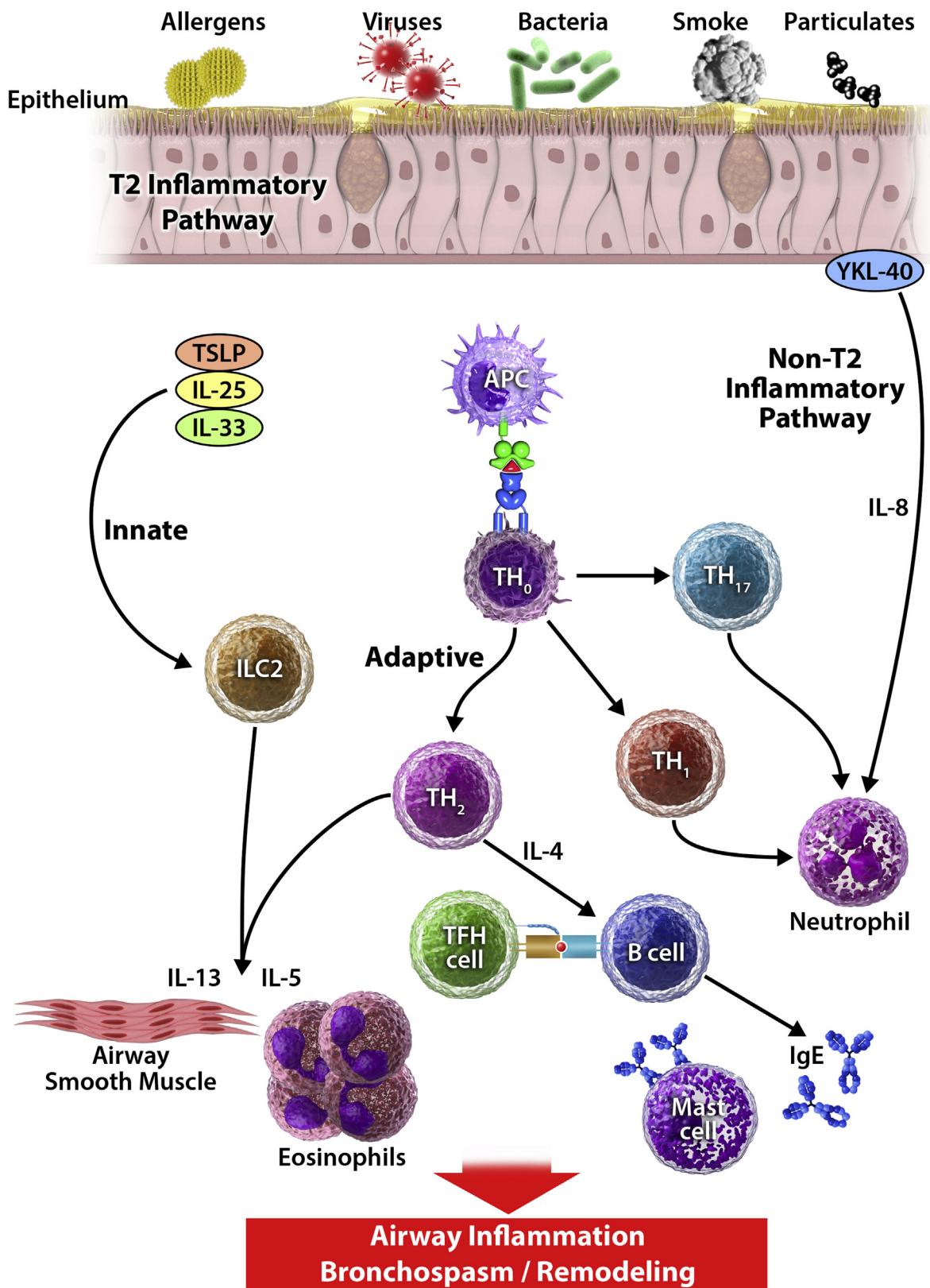


FIG 2. Inflammatory pathways in asthmatic patients. The T2 inflammatory pathway with contributions from adaptive and innate immune responses leads to production of the T2 cytokines IL-4, IL-5, and IL-13. The non-T2 pathway with activation of T_H1 and T_H17 cells leads to neutrophil activation. Both inflammatory pathways lead to asthma symptoms and airway remodeling. *APC*, Antigen-presenting cell; *TFH*, follicular helper T.

In the past decade, innate lymphoid cells (ILCs) have become an area of active research that explain this phenotype in the clinic. ILC2s might be facilitators of eosinophilia independent of a T_H2 -mediated response. ILC2s respond similarly to T_H2 cells in terms of cytokine production (IL-4, IL-5, and IL-13) and activation by epithelium-derived alarmins (IL-25, IL-33, and TSLP).²⁶ IgE levels can be increased but are not specific for any common antigen because of the lack of antigen presentation by antigen-presenting cells.

Prior knowledge of the role of ILC2s in asthmatic patients has mostly been obtained by using mouse models of airway inflammation. In mice that are recombination-activating gene (Rag) deficient, lacking both T and B cells, allergen administration with house dust mites or *Alternaria alternata* induces airway eosinophilia, suggesting the role of a nonadaptive immune response in generating T2 cytokines and eosinophilia.²⁷ Once activated, ILC2s produce IL-5 and IL-13, which could lead to eosinophilia without involvement of the adaptive immune system. ILC2s also produce IL-9, which are involved in mucus production and mast cell accumulation and also perpetuate inflammation by activating ILC2s in an autocrine fashion.²⁸

ILC2s are important producers of T2 cytokines. House dust mite-induced airway inflammation models in mice have shown that up to 50% of the T_H2 cytokines IL-5 and IL-13 and, to a lesser extent, IL-4 are produced by ILC2s.²⁹ Halim et al²⁹ conducted a study using wild-type mice, *Rag1*^{-/-} mice with ILCs present, and *Rag2*^{-/-}*Il2rg*^{-/-} mice without ILCs present to examine the role of innate and adaptive immune responses in asthmatic patients. IL-5 and IL-13 levels, airway eosinophil infiltration, and mucus secretion were increased in papain-treated wild-type and *Rag1*^{-/-} mice. This was not evident in *Rag2*^{-/-}*Il2rg*^{-/-} mice.²⁹ Even without T and B cells, IL-5 and IL-13 levels were still increased, which was not observed in mice without ILC2s, indicating the source of T2 cytokines was the presence of ILC2s. This supports the concept that innate immune mechanisms and ILCs might be the primary drivers of T2 cytokine production and eosinophilic inflammation in patients who are not allergic but have eosinophilia and late-onset disease that tends to be severe.

Smith et al³⁰ examined ILC2s in human asthma and found significantly more ILC2s in the blood and sputum of patients with severe asthma compared with those with mild atopic asthma and control subjects. ILC2s are also less responsive to steroids compared with T_H2 cells.³¹ Several mouse models have also shown the persistence of T2 cytokines that were attributable to ILC2s despite treatment with high-dose steroids.³² Therefore T2 cytokine production by ILC2s might contribute to the persistent inflammation seen in patients with severe asthma who have symptoms despite treatment with steroids.

Jia et al³¹ evaluated whether IL-13⁺ ILC2s circulating in the blood correlate with control of asthma symptoms given that ILC2s produce large amounts of IL-13 and found that patients with uncontrolled or partially controlled asthma had significantly greater percentages of circulating IL-13⁺ ILC2s. These studies support that ILC2s are key contributors of T2 cytokines in patients with severe asthma.

Late-onset eosinophilic asthma has also been shown to have a greater probability of chronic rhinosinusitis and nasal polyposis. There have been studies examining the role of ILC2s in nasal polyps. Miljkovic et al³³ measured ILC2 counts in nasal polyps using flow cytometry in patients with nasal polyps compared with healthy control subjects and found that ILC2 counts were

significantly greater in patients with nasal polyps. Those with chronic rhinosinusitis and nasal polyposis also had increased mRNA expression of IL-5 and IL-13 compared with control subjects. Although it is unclear how much underlying inflammation is caused by ILC2s, these studies demonstrate their importance in the pathobiology of asthma.

There might also be other biologic pathways leading to the development of late-onset eosinophilic asthma. IL-5-producing T_H2 cells might contribute to eosinophil recruitment and activation without any participation from follicular T_H cells, which are involved in class-switching and specific IgE production.

We still have a limited understanding of the biology that underlies the non-T2 asthma phenotype. Non-T2 asthma is often described as asthma without T2 inflammation with either the presence of neutrophilic or paucigranulocytic airway infiltration based on the cluster analyses described above.³⁴ Several mechanisms have been proposed, including neutrophilic inflammation caused by dysregulated innate responses and IL-17-mediated inflammation that promotes airway remodeling.¹⁹ Animal studies have shown that IL-17 can promote fibroblast proliferation, which leads to airway remodeling.³⁵ Non-T2 asthma is often associated with more severe disease that does not respond to steroids.³⁴ Simpson et al³⁶ showed that innate immune mRNA expression of Toll-like receptor 2, Toll-like receptor 4, and CD14 was increased along with increases in levels of the proinflammatory cytokines IL-8 and IL-1 β in patients with neutrophilic asthma compared with those with other sputum subtypes. Patients with neutrophilia have more air trapping, lower lung function, and thicker airway walls.³⁷ Environmental factors, such as cigarette smoke, pollution, and viral illnesses, are also thought to play a role in this subtype of disease.

Macrolides have been shown to be an effective therapy for patients with non-T2 asthma, likely because of their anti-inflammatory properties. Recent studies have demonstrated that macrolides decrease production of cytokines and other inflammatory mediators independent of their antibacterial effects.^{38,39} In one study clarithromycin was compared with placebo in patients with severe noneosinophilic asthma and was found to decrease neutrophil counts and IL-8 concentrations and improve quality-of-life questionnaire scores.⁴⁰ Although it is clear that this subtype of asthma exists, the mechanisms that underlie it and its relationship to other phenotypes of disease remain to be elucidated.

Biomarkers that are reflective of phenotypes and endotypes

Biomarkers can help discriminate patients into the asthma phenotypes and endotypes that have been identified. Several biomarkers exist for T2 inflammation, including blood and sputum eosinophil counts, FENO values, IgE levels, and specific IgE test (skin prick test or ImmunoCAP) results.^{41,42} These biomarkers are useful in determining the presence of T2 inflammation; however, they do not discriminate well between endotypes or precisely predict a patient's response to a T2 biologic.⁴³

Eosinophils have emerged as a key biomarker to identify patients with eosinophilic asthma and are present in both patients with allergic asthma and those with late-onset eosinophilic

asthma but have limitations. Increased eosinophil counts in sputum have been defined as greater than 2% of total cell counts,^{44,45} whereas increased blood eosinophil counts have been classified as either greater than 150 or 300 cells/ μ L in several studies, with no clear consensus.⁴¹ Blood and sputum eosinophil counts correlate, but blood eosinophil counts are not a reliable indicator of eosinophil counts in the sputum or airway wall.^{46,47} In patients with adult-onset asthma, those with high eosinophil counts (classified as blood eosinophils greater than 300) were more likely to be male, have greater FENO values, have more sputum eosinophils, be taking oral steroids, have fixed obstruction, have worse lung function, and have a history of chronic rhinosinusitis and nasal polyposis.^{44,48,49} High eosinophil counts in asthmatic patients have also been associated with a much greater chance of nonfatal (intubated) and fatal attacks.⁵⁰ It is clear from clustering analysis studies and biologic trials that eosinophil counts are increased in at least 2 endotypes of disease that include both innate and adaptive asthma.

To determine the overlap between atopic and eosinophilic asthma, Tran et al⁵¹ determined the frequency of these phenotypes and found that at a greater eosinophil cutoff, a greater percentage of patients are classified as allergic. The current consensus is that in patients with asthma, a blood eosinophil count of greater than 150 cells/ μ L is a good indicator of T2 inflammation and a diagnosis of eosinophilic asthma. Blood eosinophil counts can be used to select patients for T2 biologic therapies, but once treated their value longitudinally is minimal.

FENO is another marker of T2 inflammation that can be increased in patients with eosinophilic airway inflammation.^{52,53} Airway epithelial cells and other airway-resident cells produce nitric oxide synthase that generates FENO, which can be measured in exhaled breath.⁵⁴ FENO is a good but not great indicator of the presence of eosinophils in the airway.⁵⁵ FENO values do not accurately predict sputum eosinophil percentages.⁴⁶ Even with these limitations, given the speed and noninvasive nature of the test, the 2019 Global Initiative for Asthma guidelines recommend using FENO as a marker of residual T2 inflammation in asthmatic patients with a FENO value of greater than 25 ppb, indicating the presence of eosinophilic inflammation.⁵⁶

IgE levels and skin test results are biomarkers of atopy and the standard biomarkers to diagnose allergic disease. Total and specific IgE levels are often increased in patients with allergic asthma. Specific IgE is produced by plasma cells, whereas follicular T_H cells play a role in isotype switching and affinity maturation. Skin testing is also another useful tool to determine whether atopy exists, with a positive result indicating sensitization to environmental allergens. Allergic asthma is a well-recognized subgroup of asthma and was identified in each of the cluster analysis studies. In SARP,⁶ atopy was found in almost 80% of patients with early-onset asthma and about 50% of patients with late-onset eosinophilic asthma.

There are limited biomarkers for non-T2 asthma. A few potential biomarkers include IL-6, YKL-40, and serum and sputum IL-17. Prior studies have demonstrated that IL-6 is mediated by the T_H17 pathway and associated with severe asthma in a group of obese asthmatic patients.⁵⁷

YKL-40 is a chitinase-like protein that is readily measurable in the serum and associated with serum neutrophilia.⁵⁸ Serum YKL-40 levels are increased in asthmatic patients, and greater levels correlate with severity of disease.⁵⁹ Gomez et al⁶⁰ conducted a clustering analysis using the SARP clinical features

and YKL-40 levels and identified 4 clusters of patients. Clusters 1 and 2 had lower YKL-40 levels, and clusters 3 and 4 were found to have the greatest YKL-40 levels. Cluster 1 had lower disease severity, normal lung function, and lower medication requirements. Cluster 3 had earlier disease onset, severe airflow obstruction, and a history of near-fatal exacerbations. Patients in cluster 4 had the greatest serum YKL-40 levels and were found to include those with adult-onset asthma, frequent exacerbations, and moderate airflow obstruction. Clusters 3 and 4 also had more sputum neutrophils, less T_H2 gene expression in sputum, and greater levels of obesity compared with clusters 1 and 2. These studies suggest that serum YKL-40 is a potential biomarker to identify patients with a non-T2 asthma phenotype/endotype.

IL-17 might also be a marker of non-T2 asthma. Chen et al⁶¹ demonstrated that both airway neutrophilia and sputum IL-17 levels were increased in obese asthmatic patients and correlated positively with each other. Plasma IL-17 levels are also increased in patients with severe asthma that can be characterized as a steroid-resistant asthma phenotype.^{62,63} Future research on these biomarkers will determine their utility in characterizing patients with non-T2 asthma.

Target drug approach to phenotypes and endotypes

Given that asthma is a heterogeneous disease, being able to endotype asthma might allow for more precise diagnosis and treatment. The question that remains is how to integrate these clustering approaches with biomarkers into clinical management of patients. Certain biologics, such as anti-IL-5 agents, might be best for those identified as eosinophil predominant, whereas the newer agents against alarmins, such as TSLP, might be useful in targeting both T2 and non-T2 asthma. To find the right biologic to individualize treatment plans, it is imperative to classify asthmatic patients into proper endotypes. In the past decade, several new biologics have become available for the treatment of residual T2 asthma that is present despite use of inhaled steroids and other asthma medications. The targets include cytokines involved in the T_H2 pathway, such as IL-4, IL-5, IL-13, and IgE. One limitation in the use of these newer biologics is the lack of evidence of disease-modifying effects, and therefore for now, they are used chronically. We will discuss which biologics might be useful in the proposed T2 asthma endotypes (Table II).

Omalizumab, which was the first available biologic, has been well recognized as an important option for treating allergic asthma as an add-on therapy for uncontrolled disease. Omalizumab is an mAb that targets the FC portion of free IgE and inhibits IgE binding to the receptor, preventing release of inflammatory mediators and reduction in airway eosinophil counts.⁶⁴ Clinical trials reported significant improvements in asthma control and Asthma Control Test scores that were maintained while on therapy.⁶⁵ Macdonald et al⁶⁶ did a systematic review of 42 studies to evaluate both the short-term and long-term effectiveness of omalizumab and found evidence of improvements in lung function, reductions in exacerbations, decrease in daytime and nighttime symptoms, and reduction in mean dose of inhaled corticosteroids at 1 and 4 years of therapy. Adverse effects of omalizumab include a risk of anaphylaxis.⁶⁴ Initial studies suggested an increased risk of malignancy; however, subsequent studies showed equivalent risk compared with the general population.⁶⁷ Global Initiative for Asthma 2019

TABLE II. Summary of biologics

Biologic	Target	Commonly used criteria	Asthma trials
Omalizumab (Xolair)	IgE	IgE $\geq$ 30 IU/mL, positive SPT response or specific IgE levels to perennial allergens	Significant improvement of asthma control and Asthma Control Test scores
Mepolizumab (Nucala)	IL-5	Blood eosinophil count $\geq$ 300 cells/ μ L	Decrease exacerbations by 39% to 52%
Reslizumab (Cinqair)	IL-5	Blood eosinophil count $\geq$ 400 cells/ μ L	Decrease exacerbations by 50% to 59%
Benralizumab (Fasenra)	IL-5 receptor α	Blood eosinophil count $\geq$ 300 cells/ μ L	Decreased exacerbations by 45% to 51% Improvement in prebronchodilator FEV ₁ by 0.106-0.159 L Improvements in ACQ-5 scores
Dupilumab (Dupixent)	IL-4Ra	Blood eosinophil count $\geq$ 150 cells/ μ L FENO $>$ 25 ppb	Decreased exacerbations by 46% to 48% Improvement in prebronchodilator FEV ₁ by 0.13-0.14 L Improvements in ACQ-5 scores Improved prebronchodilator FEV ₁
Tezepelumab	TSLP	Trial criteria: uncontrolled asthma on ICS/LABA; history of exacerbation FEV ₁ : 40% to 80% of predicted value; post-BDR $\geq$ 12%	Phase 2 trials showed improvement in exacerbation rates by 62% to 71% Reduction in blood eosinophil counts, serum IgE levels, and FENO values

ACQ-5, Asthma Control Questionnaire; BDR, bronchodilator response; ICS/LABA, inhaled corticosteroid/long-acting β -agonist; IL-4R, IL-4 receptor.

guidelines recommend the use of omalizumab in patients with evidence of T_H2 inflammation with an IgE level of greater than 30 IU/mL or positive results on allergy skin or ImmunoCAP tests to any 1 perennial aeroallergen.⁵⁶

Three anti-IL-5 therapies are currently available for the treatment of severe asthma, including mepolizumab, reslizumab, and benralizumab. All of the drugs have different mechanisms of action, pharmacokinetic and pharmacodynamic profiles, and practical considerations after their use in the clinic. All have been shown to be effective in treating patients with eosinophilic asthma based on different eosinophil cutoffs.

Wang et al⁶⁸ conducted a meta-analysis to assess the safety and efficacy of all 3 anti-IL-5 therapies using 20 studies in 7100 patients. Use of anti-IL-5 biologics demonstrated improvement in FEV₁, decreased asthma exacerbations, lower sputum and blood eosinophil counts, and improved quality-of-life questionnaire scores with no increase in adverse events.

In an indirect treatment comparison of anti-IL-5 biologics using randomized controlled trials stratified by eosinophil counts, Busse et al⁶⁹ found that mepolizumab significantly improved asthma control compared with benralizumab and reslizumab. Therefore although each anti-IL-5 therapy had different cutoffs selected for their clinical trial primary end points, the data suggest patient responses to the IL-5 pathway biologics are difficult to distinguish across eosinophil subgroups.

The Mepolizumab for Severe Eosinophilic Asthma trial suggested that a blood eosinophil count of greater than 150 cells/ μ L was the threshold at which patients would benefit most from treatment with mepolizumab.⁷⁰ Ortega et al⁷¹ completed a *post hoc* analysis of the Mepolizumab for Severe Eosinophilic Asthma and Mepolizumab as Adjunctive Therapy in Patients with Severe Asthma trials and also found that at eosinophil counts of less than 150 cells/ μ L, the efficacy of mepolizumab was reduced. Those with eosinophil counts greater than 150 cells/ μ L had a 52% reduction in exacerbations, whereas those with eosinophil counts of greater than 500 cells/ μ L had a 70% reduction.⁷¹

In addition, a supervised cluster analysis to determine which patients would benefit most from mepolizumab found 4 clusters.

Cluster 2, which had patients with a history of nasal polyps and sinusitis, had a 53% reduction in exacerbations, whereas cluster 4 patients with obesity and high airway reversibility had a 67% reduction in exacerbations.⁷² Of the biologics targeting IL-5, benralizumab acts differently than the others by targeting the IL-5 receptor and preventing IL-5 receptor heterodimerization and downstream signal transduction.^{73,74} Benralizumab can also target cells, such as eosinophils and basophils, for antibody-directed cell-mediated cytotoxicity, leading to depletion of cells expressing IL-5R α .⁷⁵ Both the phase 3 clinical trials (CALIMA and SIROCCO) for benralizumab also showed a decrease in exacerbation rates and improvement in prebronchodilator FEV₁^{76,77} in patients with blood eosinophil counts of greater than 300 cells/ μ L.

The newest biologic agent to be US Food and Drug Administration approved for asthma is dupilumab. Dupilumab is a human mAb that targets the α subunit of the IL-4 receptor, which blocks signaling of both IL-4 and IL-13.⁷⁸ Given the presence of the IL-4 and IL-13 pathway in many patients with atopic diseases, dupilumab has been shown to be effective in asthmatic patients, patients with severe atopic dermatitis, and patients with chronic sinusitis with nasal polyposis.^{79,80} Phase 3 clinical trials for dupilumab were conducted in patients aged 12 years and older with a diagnosis of asthma without any criteria for eosinophil cutoffs or markers of T2 inflammation. Patients receiving dupilumab had fewer exacerbations, improved quality-of-life scores, and significant improvements in prebronchodilator FEV₁. In secondary analyses the most improvement was seen in patients with evidence of T2 biomarkers, such as blood eosinophil counts of greater than 150 cells/ μ L and FENO values of greater than 25 ppb.⁷⁸

Weinstein et al⁸¹ examined the effect of dupilumab on perennial allergic rhinitis in patients being treated for concomitant asthma without any history of nasal polyps and found improvement in allergic rhinitis symptoms and Sino-Nasal Outcome Test scores.⁸¹ Examination of phase 3 data on the effect of dupilumab in patients with nasal polyposis showed a reduction in systemic corticosteroid use and nasal polyp surgery compared with placebo and an improvement in lung

function in those with comorbid asthma.⁸² The Quest trial for dupilumab did not use T2 inflammation as a marker for the primary end point, but secondary analyses showed patients with greater biomarkers of T2 inflammation had the greatest level of improvement, indicating similar results to the other T2 biologics.

There are several new biologics in development that are undergoing clinical trials, most notably humanized antibodies directed against epithelial alarmins, such as IL-33 and TSLP. These biologics are directed higher along the inflammatory pathway, raising the possibility that targeting upstream mediators of airway inflammatory cascade will affect both the innate and adaptive immune responses and all phenotypes of disease. Phase 2 trials of tezepelumab showed improvement in exacerbation rates and reduced blood eosinophil counts, serum IgE levels, and FENO values.⁸³ Interestingly, exacerbation rates were lower in both patients with evidence of T2 inflammation (blood eosinophil counts >140 cells/ μ L and serum IgE levels >100 IU/mL) and those without.⁸³ These therapies will most likely benefit patients with T2 asthma; however, they might be useful in those without evidence of T2 inflammation. The usual T2 biomarkers, such as FENO values and eosinophil counts, might help guide treatment; however, newer biomarkers would allow for more precise patient selection. Studies should be conducted to identify biomarkers and determine whether blood or sputum TSLP or IL-33 levels could precisely identify patients who will respond. Nonallergic patients and those with late-onset T2 asthma might especially benefit from these therapeutics given that innate lymphoid cells respond directly to release of alarmins. Tezepelumab has been granted fast-track designation because of the strong phase 2 results. Phase 3 studies are ongoing.

CONCLUSION

Attempts to phenotype and endotype asthma using cluster analysis have identified 4 consistent clusters. These phenotypes of asthma include (1) early-onset allergic asthma, (2) early-onset moderate-to-severe remodeled asthma, (3) late-onset nonallergic eosinophilic asthma, and (4) late-onset nonallergic noneosinophilic asthma. These phenotypes are likely driven by different underlying biologic processes and are suggestive of endotypes that in the future will be identifiable by using specific tests and treated uniquely in the clinic. Being able to identify patients within each cluster using biomarkers and clinical symptoms moves us closer to the goal of precision medicine by allowing clinicians to use the right biologic for the right patient.

What do we know?

- Cluster analysis studies have provided insight into the phenotypes and endotypes that exist in asthmatic patients, moving us closer to the goal of precision medicine.

What is still unknown?

- How these phenotypes and endotypes can guide care in the office and the need for better biomarkers to identify them

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