PERSPECTIVE

Emergence of Biomolecular Pathways to Define Novel Asthma Phenotypes

Type-2 Immunity and Beyond

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Abstract

Asthma is increasingly recognized as a heterogeneous entity, encompassing a variety of different subgroups, or phenotypes, which share clinical and inflammatory characteristics. However, it is only recently that molecular pathways have been both identified and successfully targeted in association with these clinical–inflammatory phenotypes. This integration of clinical–inflammatory and molecular pathways has enabled the broad differentiation of "asthma" into those with and without type-2 inflammation, on the basis of elevations in pathways downstream of type-2 cytokines, such as periostin, exhaled nitric oxide, and blood eosinophils. Although these rather general downstream biomarkers can identify patients more likely to respond to novel type 2–targeted therapies, they may have limited ability to determine which patients respond best to which type 2–targeted therapy, or even who will respond less than optimally, despite elevation in the biomarkers. In addition, new biomarkers and targets are required for the 50% of patients with asthma without elevations in these type-2 biomarkers. The path forward will require integrated 'omics approaches, development of more complex mouse models of asthma, as well as identification and validation of novel biomolecular pathways.

Keywords: asthma; type-2; eosinophils; phenotypes

Although clinicians have appreciated differences in the mix of characteristics associated with asthma for many years, in the last 25–30 years, asthma has too often been approached as a single disease with a single therapeutic pathway. In fact, the term "asthma" much more strongly resembles the medical terms "arthritis" and "anemia," which represent clinically defined patient presentations associated with swelling in a joint or low red blood cell counts. These terms are well appreciated by the medical community to be umbrella terms, which include multiple additional subtypes (or phenotypes), including such contrasting entities as rheumatoid arthritis and osteoarthritis, or iron deficiency anemia or B12 deficiency. Each of these presents with its own specific clinical and, importantly, molecular characteristics that mandate vastly different treatment approaches. Likewise, asthma represents disease entities associated with episodic wheeze, chest tightness, cough, and/or shortness of breath, typically associated with reversible airflow limitation. Although differences in molecular underpinnings are not yet confirmed, it is apparent that adding other clinical characteristics and, increasingly, inflammatory, molecular, and treatment responses to the core characteristics identified previously here, strongly supports the concept that asthma, like arthritis and anemia, is also an umbrella term overarching different "asthmas."

Clinical to Inflammatory to Molecular Phenotyping

Phenotypes are defined as the observable characteristics of an organism that result from a combination of hereditary and environmental influences. By its nature, phenotyping requires an approach that integrates a broad range of characteristics to develop patterns of associated traits common to groups of identifiable individuals. Clinicians treating asthma have long recognized patterns of allergic/early-onset, extrinisic disease, Samter's Triad (aspirin sensitivity), and, more recently, adult-onset, eosinophilic disease (1–3). These clinician-recognized patterns are now being validated in less biased, clustering approaches of well characterized populations of patients. Early attempts at clustering used primarily small numbers of clinical and limited inflammatory variables, which recognized the importance of age at onset, sputum eosinophils, and degree of airflow limitation (4–6). Even with differing approaches, these analyses were generally able to identify a typically early-onset, allergic group of patients whose

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disease ranged from mild to severe and was relatively stable over time. However, the identification of clusters beyond those patients with mild to more severe allergic asthma remained somewhat disparate, especially in relation to late-onset disease. Adding more variables (>100) to the clustering, including a broader range of inflammatory variables than sputum eosinophils alone, increased the granularity of the clusters, especially in relation to inflammatory patterns and specific associated characteristics, but continued to support the importance of age at onset/allergy and severity markers (7). Combining these approaches led to consistent confirmation of a late-onset, less allergic, highly eosinophilic, and relatively severe phenotype, in addition to the early-onset allergic disease of varying severity.

These early clustering studies supported the concept that, although clinical–inflammatory patterns in asthma were indeed variable, consistent patterns could be identified, which, in some cases, even appeared to be stable over time. However, they did not identify specific molecular pathways for therapeutic approaches.

Type-2 High Asthma and Its Molecular Subphenotypes

The ultimate goal for management of any disease is to identify underlying molecular pathways associated with the disease or its phenotypes, and then to target those pathways to improve treatment. Since the identification of a Th2 immune process, associated with a GATA-3–driven, IL-4–, -5–, and -13–producing Th2 cell, asthma has been considered a Th2 disease (8–10). This was increasingly accepted, given the Th2 bias of most mouse models and the few differences in reported cytokine expression between atopic and nonatopic asthma (11–13). Although early failures of "Th2-targeted therapy" in all comers with asthma eventually led to doubts regarding this pathway's importance, the overarching concept that all asthma was molecularly similar likely contributed to these early failures. However, between 2007 and 2009, four studies substantially changed the course of the asthma world. First, a recombinant/mutant IL-4, which blocked both IL-4 and IL-13 signaling through IL-4 receptor α (IL-4Rα), was shown to be efficacious in a specific asthma setting; namely, in the prevention of an allergic asthmatic response to allergen challenge in patients with mild allergic asthma (14). This study was followed by an epithelial gene expression–based profiling study of mild, corticosteroid naive patients with asthma compared with healthy control subjects, which revealed that previously recognized "Th2" signature genes were present and elevated when compared with those in healthy control subjects, but in only 50% of the evaluated patients with asthma (15). When present, the signature was associated with more eosinophils, greater airway hyperresponsiveness, and a robust response to inhaled corticosteroids (CSs) as compared with those without the signature. In the same year (2009), mepolizumab, a monoclonal antibody to the proeosinophilic cytokine, IL-5, was targeted to patients with more severe asthma, who had evidence of eosinophilic inflammation (16, 17). In contrast to previous negative trials of all comers with mild–moderate asthma, mepolizumab effectively reduced asthma exacerbations when added to high-dose CSs and long-acting β-agonists. In a second study, mepolizumab decreased the need for oral CS treatment, further confirming the link between eosinophilic asthma and the Th2/type-2 cytokine, IL-5. Interestingly, in these studies, mepolizumab had very little impact on lung function and clinical symptoms, supporting a specific biomolecular role for IL-5/eosinophils in asthma exacerbations. Although these four studies identified patients with asthma who differentially responded to both nonspecific and specific targeted therapies, the studies were widely disparate in the severity of the patient cohorts. In addition, IL-5 is only one of at least three recognized type-2 cytokines. However, these were the first steps toward identifying a subgroup (or subgroups) of asthma with an underlying Th2 or type-2 process. Interestingly, although links between allergy and Th2/type-2 inflammation were clear in two of the studies, the studies with anti–IL-5 did not link efficacy with allergic biomarkers, such as atopy or IgE levels, thus beginning to "disconnect" traditional allergy from Th2/type-2 immunity (Table 1).

In addition to numerous successful anti–IL-5/anti–IL-5R studies, anti–IL-13 and anti–IL-4Rα approaches have also

Table 1. Asthma Phenotypes Associated with Type 2 Signatures

	Age at Onset	Corticosteroid Responsiveness	IgE/Atopy	Cellular Inflammation	Additional Characteristics
Mild–moderate allergic asthma	Childhood	Good to excellent	High	Low level, corticosteroid-responsive eosinophilia	Seasonal allergic symptoms
Severe allergic asthma	Childhood	Modest to poor	High	Low level, less corticosteroid-responsive eosinophilia and neutrophiliia	Fewer allergic symptoms
Highly eosinophilic (blood), despite ICS	30–40 yrs of age	Typically requires (but responds to) systemic corticosteroids	Generally low	Persistent high eosinophilia possible ILC2 involvement	Nasal polyps, sinusitis, aspirin sensitivity
Type 2 plus additional immune pathways (type 1, 17, etc.)	Middle age	Poor	Low	Persistent eosinophils	Systemic connective tissue symptoms, familial autoimmunity

Definition of abbreviations: ICS, inhaled corticosteroids; ILC2, innate lymphoid cell type 2.

shown clinical efficacy in broad, but mostly severe, asthma populations, particularly in association with a biomarker for Th2/type-2 inflammation, such as blood eosinophils, serum periostin, or fractional exhaled nitric oxide (Fe_{NO}) (18–21). Similarly, using IgE to guide therapy with any of these newer monoclonal antibodies, including anti-IgE itself, has been disappointing, again disassociating Th2/type 2 from traditional measures of allergy/atopy (19). It is not yet clear from these studies whether: (*1*) all Th2/type-2 inflammation is the same; (*2*) all Th2/type-2 inflammation is associated with allergy/atopy; and (*3*) these different Th2/type 2–directed therapies will be similarly effective in all type-2 biomarker–identified patients. Thus, it is critical to discern whether different patterns of type-2 inflammation may be present among different clinically recognizable phenotypes, or whether some type-2 biomarkers may better identify different type-2 molecular phenotypes. For example, is the eosinophilia identified in early-onset allergic disease driven by the same factors as the eosinophilia in late-onset, less-allergic disease associated with aspirin sensitivity and nasal polyps? Although the answer is unclear, it is unlikely that the immune-inflammatory processes are the same. As part of this question, it is important to recognize that IL-5, IL-13, and, perhaps to a lesser degree, IL-4, may arise from cells other than Th2 cells, most particularly from innate lymphoid cell (ILC) 2 cells (22). These lineage-negative cells are believed to respond directly to stimulation with cytokines, such as IL-33, thymic stromal lymphopoietin, and even lipid mediators, such as lipoxins and prostaglandin D2 (23, 24). Whether these cells and their mediators are likely to be more or less important in certain asthma phenotypes remains to be proven, but there may be a suggestion that these ILC2s are more important in late-onset eosinophilic asthma (25). If so, then differences in approaches to therapy among early-onset allergic and late-onset eosinophilic asthma could evolve.

Finally, it does not appear that absolute levels of type-2 cytokines drive disease severity or that disease severity is only driven by type-2 cytokines. A recent mepolizumab study showed efficacy of the drug in allowing a 75% or greater reduction in systemic CSs in the majority of patients with severe, eosinophilic asthma studied (26). However, 35–40% of these patients with eosinophilic asthma failed to respond better than placebo, suggesting that factors beyond the type-2 cytokine, IL-5, were important. Whether these cytokines include IL-4 and -13 or other non–type-2 cytokines is not currently understood.

Non–Type 2 Asthma

Similarly, 50% of asthma and severe asthma patients have shown little evidence of any type-2 process. The biologic factors driving these patients without type-2 disease are very poorly understood, but may include factors related to comorbidities, such as obesity/associated oxidative stress, smoking, infection, and underlying poorly understood smooth muscle abnormalities (27–30). It is also likely that some non–type-2 asthma may be identified as such only because CS therapy lowered the type-2 signal below the threshold for our current means of detection. Considerable effort is needed to better identify, understand, and treat these patients.

The Path Forward

At this point, we are able to identify relatively simple biomarkers that have both been associated with type-2 inflammation and appear to decrease in response to therapies targeted toward the type-2 cytokines. Thus, we can simply divide patients with asthma into those with and without evidence for type-2 inflammation. However, as in most complex human diseases, this division is likely overly simplistic. Moving forward toward a precision medicine approach to asthma will require a combination of four elements: (*1*) improved molecular phenotyping; (*2*) tracking stability/plasticity of molecular phenotypes over time and in association with specific clinical situations; (*3*) development of more complex animal models; and (*4*) discovery of new molecular targets based on these approaches. As noted earlier, Th2/type-2 asthma, like asthma itself, encompasses a number of rather distinct clinical phenotypes, ranging in severity from mild to severe. Most inflammatory-based clustering identifies a group of patients with very severe disease with a mixed complex immune process, associated with type-2 biomarkers, such as Fe_{NO} and eosinophils, but also with evidence of neutrophilic inflammation (5, 7, 31). To begin to address the first point, a gene expression clustering analysis was performed on freshly brushed airway epithelial cells from a range of patients with asthma and healthy control subjects, which were analyzed by microarray (32). Given the association of Fe_{NO} with a wide range of asthma phenotypes, 589 genes were identified that correlated strongly with Fe_{NO}. These genes were then clustered using a K-means approach to identify five different patient clusters—three with "high" Fe_{NO} and two with "low" Fe_{NO} levels—each with differing clinical characteristics. Interestingly, in addition to the high-Fe_{NO} clusters being associated with type-2 signature genes, the two most severe high-Fe_{NO} clusters were also associated with IFN-related genes. This suggests that the presence of type-2 inflammation may be accompanied by additional innate and adaptive immune pathways, which lead to less robust responses to CSs and even type 2–directed therapy. These results led to further analyses of severe asthma bronchoalveolar lavage cells, which identified the presence of factors, such as IFN-γ and IL-27 (33–35). The association of high levels of these factors was associated with the most severe disease. Newer animal models are being developed based on these observations, which incorporate a more complex immune response, using combinations of infectious and allergic stimuli. These animal models demonstrate one of the hallmark features of severe asthma; specifically, corticosteroid-refractory inflammation and airway hyperresponsiveness, with evolving data to support a critical role for the type-1 cytokine, IFN-γ, in the process (35). Additional complex animal models incorporating obesity, infection, and activated ILC2 cells (perhaps in the absence of allergen) will be needed to complement and reflect the findings from the various 'omics/clustering approaches. Finally, the integration of these human and animal studies will be necessary to more granularly define type-2 asthma phenotypes, and also to begin to tease out molecular phenotypes of asthma not associated with type-2 inflammation. These more granular approaches to existing phenotypes and the development of novel molecular phenotypes, tested in animal models, should lead to new therapeutic targets, which can be tested in more rigorously defined asthma phenotypes, ultimately allowing the promise of precision medicine. ■

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