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Asthma phenotypes: the evolution from clinical to molecular approaches

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Although asthma has been considered as a single disease for years, recent studies have increasingly focused on its heterogeneity. The characterization of this heterogeneity has promoted the concept that asthma consists of multiple phenotypes or consistent groupings of characteristics. Asthma phenotypes were initially focused on combinations of clinical characteristics, but they are now evolving to link biology to phenotype, often through a statistically based process. Ongoing studies of large-scale, molecularly and genetically focused and extensively clinically characterized cohorts of asthma should enhance our ability to molecularly understand these phenotypes and lead to more targeted and personalized approaches to asthma therapy.

The evolving definition of asthma

Asthma affects 5–10% of the population in many developed countries and is associated with a large socioeconomic burden. Yet 'asthma' is a vague term that describes a group of clinical symptoms with reversible expiratory airflow limitation or bronchial hyperresponsiveness. Although international asthma guidelines have added "in the presence of airway inflammation" to the list of criteria for disease, inflammation is almost never measured in practice, and a consistent inflammatory process is rarely confirmed. Thus, the term asthma, like 'arthritis', equates to a definition of grouped clinical and physiological characteristics (**Fig. 1**). These characteristics could identify syndromes, phenotypes or even multiple diseases rather than a single disease. Even leading international clinical journals such as *The Lancet* have suggested that the term asthma is out of date and that the evolution of more detailed clinically and biologically focused definitions of this condition should be encouraged[1]. Yet, most mechanistic studies of asthma focus only on a highly specific process related to allergic airway inflammation, despite the fact that the overall importance of this single process to human disease remains poorly understood.

In the 1990s and early 2000s, this vague clinical definition of asthma led to successful clinical trials of nonspecific anti-inflammatory and bronchodilator medications. At the same time, researchers working with mouse models of allergic asthma and/or inflammation identified the crucial role of T helper (T_H2) immune pathway elements (**Fig. 2**) in both inflammation and airway hyperresponsiveness[2–4]. Thus, asthma was widely believed to be an allergic, eosinophilic and T_H2-mediated (and corticosteroid-responsive) disease[5–7]. Unfortunately, negative initial results from T_H2-focused human clinical trials virtually halted biological approaches to treating asthma.

Simultaneously, a subgroup of people with severe asthma were observed to have refractory disease in the absence of eosinophils, with further studies suggesting that responses of people with asthma to nonspecific anti-inflammatory drugs, such as inhaled corticosteroids, were dependent on the presence and type of airway inflammation[8–11]. It was then shown that an antibody to the allergy-related factor IgE showed efficacy in reducing exacerbations in a targeted population with 'allergic' asthma[12,13]. Thus, 'asthma' began to evolve from a term describing a single disease to one encompassing multiple subgroups or, as they are now termed, phenotypes[14,15].

Definition of 'phenotype'

A phenotype is defined as the "observable properties of an organism that are produced by the interactions of the genotype and the environment"[16]. The concept of the phenotype have been suggested to be the prelude to that of the 'endotype', wherein a specific biological pathway is identified that explains the observable properties of a phenotype[17,18]. Although several endotypes of asthma have been proposed, none has been widely agreed upon; the acceptance even of asthma phenotypes is evolving, and the topic is controversial. Despite these difficulties in agreeing on endotypes, asthma phenotypes based on clinical characteristics, triggers or general inflammatory processes have been proposed, but there have been few attempts to link all of these characteristics together to better define phenotypes[19]. The definition of a true phenotype (or endotype) requires a unifying and consistent natural history, consistent clinical and physiological characteristics, an underlying pathobiology with identifiable biomarkers and genetics and a predictable response to general and specific therapies[18] (**Table 1**). Although both biased and unbiased approaches have begun to link the characteristics of asthma together to form phenotypes, no present system of subgrouping achieves all the requirements for a true phenotype or endotype. In addition, there are a number of co-morbidities and confounders that have been identified that can alter asthma phenotypes (**Box 1**).

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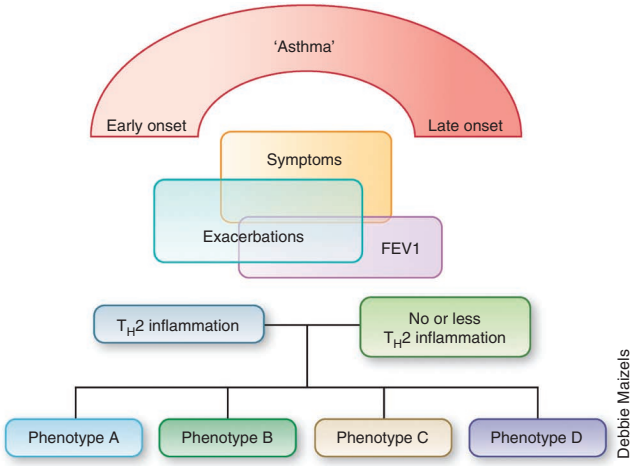

Figure 1 Schematic representation of the umbrella term 'asthma'. The key clinical features of severity (lung function, symptoms and exacerbations), inflammatory characteristics (particularly T_H2 immunity) and their division into associated phenotypes are shown. However, these phenotypes have not yet been fully characterized.

Approaches to identifying phenotypes

Biased approaches. Asthma phenotyping began decades ago with the concepts of extrinsic (allergic) and intrinsic (nonallergic) asthma[14]. People with extrinsic asthma developed the disease early in life, were atopic (they made IgE specific to identifiable allergens) and had identifiable allergic triggers, other allergic diseases such as rhinitis or eczema or a family history of allergic disease. Intrinsic asthma developed later in life (after 40 years of age), was associated with aspirin-exacerbated respiratory disease (AERD) but not with allergic sensitization, and was generally not as well understood. Inflammatory biomarkers other than those related to IgE were not used. When small pathobiological studies in humans suggested that levels of T_H2 cytokines were similar in extrinsic and intrinsic asthma, and treatment with inhaled corticosteroids was found to be effective in the majority of mild to moderate asthma cases, the distinctions between extrinsic and intrinsic asthma fell out of favor[20–23].

Although most asthma is mild to moderate (and heterogeneity is indeed present in individuals with mild to moderate asthma), pathobiological studies in the 1990s of people with severe, refractory asthma reintroduced the concept of asthma heterogeneity with the finding that some of these individuals had neutrophilic inflammation that had not previously been reported in milder asthma[24]. Eosinophils were present in lung tissue from about 50% of people with severe asthma, and this group of individuals had a thicker subepithelial basement membrane (SBM), higher expression of transforming growth factor-β (TGF-β), more frequent and more severe symptoms, and more near-fatal events than individuals who had asthma without eosinophilic inflammation[8,25–27]. Age at onset (using age 12 as a cut-off) was reported to be an important factor in distinguishing atopic and allergic asthma from a less well-defined but more eosinophilic adult-onset phenotype[25]. Exercise-induced, obesity-related, smoking-related, neutrophilic and even 'paucigranulocytic' (the absence of an observable inflammatory process) were all suggested as asthma phenotypes, but few corresponding clinical and biological characteristics were identified.

Unbiased approaches. Because of concerns about clinical bias and the continued lack of specific cellular biomarkers for asthma phenotypes, several groups began to approach phenotyping in a manner that was less biased and more statistically based. Among these were a study in the United Kingdom that involved two groups of patients; a US study that involved a group, enhanced for individuals with severe asthma, from eight centers involved in the National Institutes of Health–sponsored Severe Asthma Research Program (SARP); and an analysis of two large European asthma cohorts[28–30].

The UK and SARP studies used cluster analysis, a method that combines various approaches to group important variables such as those related to asthma exacerbations, which include steroid bursts, emergency-room visits and hospitalization. These grouped variables were then used to produce clustered variables to identify phenotypes. However, the process of choosing variables introduces some bias. For instance, the UK cluster analysis included sputum eosinophils and bronchodilator responsiveness but did not include typical physiological measures of airway obstruction, such as forced expiratory volume in 1 second (FEV1); the SARP analysis included lung function but not inflammatory markers[28,29]. The European analysis was performed on people with asthma from across two European cohorts and used latent class analysis (LCA), which groups variables into latent classes under the statistically based assumption that the variables in a particular latent class are independent of each other. The European analysis had more limited phenotypic information than the other two studies.

Despite statistical variations in their approaches and the wide range of variables that were available and that were analyzed in each study, the results of the three studies are more similar than different, and they overlap with results obtained using the earlier, biased phenotype approaches. All three studies found age at disease onset to be a key differentiating factor. Early-onset disease is consistently associated with a more atopic and allergic condition over a range of severities, whereas later-onset disease is associated with eosinophilic inflammation and obesity, is more common in women and is generally less allergic. Interestingly, despite the association of early-onset disease with atopy and allergy, none of the unbiased approaches found variables associated with these conditions (such as atopy and total IgE) to be key distinguishing features of the subgroups.

A single cluster analysis has also been performed in children with asthma and was primarily limited to one urban center that included a large group of underserved children[31]. These children were predominantly atopic and allergic, as would be expected from a pediatric cohort, but greater severity was not correlated with greater numbers of skin-test reactions, higher IgE or higher exhaled nitric oxide (FeNO, a product of the T_H2-regulated inducible nitric oxide synthase (iNOS) enzyme), which generally tracks with atopy, allergy and the response to inhaled corticosteroids[32–34]. Rather, the determinants of severity were based primarily on asthma duration, medication use and lung function.

Thus, many of the elements needed for pathological and immunological definition of asthma phenotypes were missing from these studies, and only one cluster analysis was generally replicated in a second (albeit milder) cohort[28]. However, in addition to these clinically oriented clusters, a molecular phenotyping analysis in people with mild corticosteroid-naïve asthma has also been published[35]. In this study, the authors analyzed airway epithelial brushings for the expression of three genes upregulated by the T_H2-type cytokine interleukin-13 (IL-13) in epithelial cell cultures—*POSTN*, which encodes periostin; *CLCA1*, which encodes calcium-activated chloride channel regulator 1; and *SERPINB2*, which encodes serpin peptidase inhibitor, clade B, member 2—and used these signature genes to identify 'T_H2-high' individuals. Of those analyzed, approximately 50% of people with

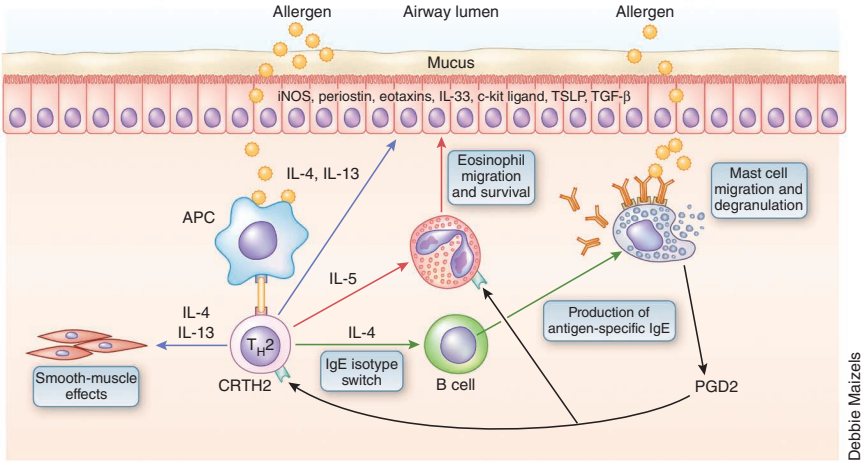

Figure 2 T_H2 immune processes in the airways of people with asthma. The pathway begins with the development of T_H2 cells and their production of the cytokines IL-4, IL-5 and IL-13. These cytokines stimulate allergic and eosinophilic inflammation as well as epithelial and smooth-muscle changes that contribute to asthma pathobiology. APC, antigen-presenting cell; CRTH2, chemoattractant receptor-homologous molecule expressed on T_H2 cells; iNOS, induced nitric oxide synthase; PGD2, prostaglandin D2; TSLP, thymic stromal lymphoprotein.

mild corticosteroid-naïve asthma had a T_H2-high signature in their airway epithelial tissue. Individuals who had asthma but did not have this signature had a similar gene-expression signature (T_H2-low) to that of healthy control subjects. Subjects classified as T_H2 high were subsequently found to have higher amounts of tissue IL-13 and IL-5 mRNA and greater numbers of eosinophils and mast cells, and they showed more atopy and SBM thickening compared to T_H2-low people[36]. Perhaps most importantly, these subjects responded to inhaled corticosteroid therapy, whereas the T_H2-low group did not, suggesting that this distinction may have profound clinical implications. Although clear patterns of clinical phenotype in relation to T_H2 gene expression have emerged from these studies, further long-term studies are needed to assess the stability of the identified phenotypes, integrate clinical clusters with biomarkers and, finally, identify responses to targeted therapies.

T_H2-associated asthma

Almost since the inception of the concept that immunity can be divided into T_H1 and T_H2 type processes, asthma has been considered a T_H2 process that is linked strongly to atopy and allergy, type I hypersensitivity reactions, eosinophilic inflammation and response to corticosteroids. Indeed, data from biased and unbiased studies suggest that the majority of—but, clearly, not all—asthma cases fit this traditional view[28,29]. Current phenotyping approaches support the existence of an early-onset (usually during preadolescence), mostly atopic and allergic asthma phenotype, and most have additionally identified a later-onset (often age 20 or later) eosinophilic phenotype. The molecular and targeted therapy data support an overall T_H2 association with both of these phenotypes, such that these two clinically different yet immunologically overlapping phenotypes may fall into a broader category of T_H2-associated asthma (**Table 1** and **Fig. 3**). Finally, the clinical phenotype of exercise-induced asthma (EIA) is also likely to have a T_H2 component, given its eosinophil- and mast cell–related profile[37–39].

Early-onset allergic T_H2 asthma

Clinical and biological features. Although a specific age cut-off for early-onset asthma has not been determined, most persistent adult asthma that originates in early childhood has an atopic and allergic component, and most people with asthma are likely to have this phenotype. However, the lack of responsiveness to corticosteroids and the lower concentrations of IgE in some children with asthma suggest that not all early-onset asthma is T_H2 associated[31,40].

Studies have suggested that age of onset is a better discriminator of adult asthma phenotypes than allergic factors, consistent with previous reports suggesting that allergic exposure and sensitization in childhood are only modestly associated with asthma development later in life[28,29,41]. Despite this, consistent relationships exist between allergic factors and onset of asthma in childhood. Early-onset asthma is typically associated with other atopic diseases, including allergic rhinitis and atopic dermatitis[14,25,28,29]; for example, 40% of people with early-onset asthma have a history of atopic dermatitis, whereas 4% of people with late-onset asthma do. The amounts of total and allergen-specific IgE are also higher in early-onset asthma than in later-onset asthma. People who have atopic asthma have higher

Table 1 Asthma phenotypes in relation to characteristics

	Natural history	Clinical and physiological features	Pathobiology and biomarkers	Genetics	Response to therapy
Early-onset allergic	Early onset; mild to severe	Allergic symptoms and other diseases	Specific IgE; T_H2 cytokines; thick SBM	17q12; T_H2-related genes	Corticosteroid-responsive; T_H2-targeted
Late-onset eosinophilic	Adult onset; often severe	Sinusitis; less allergic	Corticosteroid-refractory eosinophilia; IL-5		Responsive to antibody to IL-5 and cysteinyl leukotriene modifiers; corticosteroid-refractory
Exercise-induced		Mild; intermittent with exercise	Mast-cell activation; T_H2 cytokines; cysteinyl leukotrienes		Responsive to cysteinyl leukotriene modifiers, beta agonists and antibody to IL-9
Obesity-related	Adult onset	Women are primarily affected; very symptomatic; airway hyperresponsiveness less clear	Lack of T_H2 biomarkers; oxidative stress		Responsive to weight loss, antioxidants and possibly to hormonal therapy
Neutrophilic		Low FEV1; more air trapping	Sputum neutrophilia; T_H17 pathways; IL-8		Possibly responsive to macrolide antibiotics

BOX 1 Comorbidities and confounders that alter asthma phenotypes

Numerous environmental factors including infection, smoking, hormonal factors, obesity (in T_H2 asthma) and others still to be identified can influence an underlying immunoinflammatory process. Although they are sometimes considered as separate phenotypes, these factors may have a more prominent role in altering the existing pathobiology. Innate immune pathways including Toll-like receptors, type II interferons and danger-associated molecular pathways in particular are likely to be important in nonallergic environmental exposures, which can both enhance and dampen T_H2 inflammation[138].

History of smoking or exposure to second-hand smoke[139]
- Worsens any existing asthma, probably through enhanced neutrophilic inflammation and oxidative stress
- Smoking-associated asthma may be a separate phenotype overlapping with chronic obstructive pulmonary disease

Hormonal influences[140]
- Associated with asthma initiation during times of hormonal changes (such as menarche, pregnancy or menopause), perhaps through enhanced T_H2 activation
- Involved in differences in asthma incidence and prevalence between sexes during adolescence
- May worsen or improve existing asthma during pregnancy
- Associated with asthma exacerbations before menses

Viruses and bacteria[141,142]
- Initiate and/or increase likelihood of developing T_H2-related asthma in atopic children
- Exacerbate existing asthma, possibly through type II interferon mechanisms
- May initiate adult-onset asthma in susceptible individuals in conjunction with atypical bacteria

Occupational exposures[143]
- Initiate asthma in susceptible hosts
- Worsen existing asthma

amounts of T_H2 cytokines and greater numbers of cells with receptor-bound IgE than people who have atopy but do not have asthma[20,21]. Within this group of asthma, the recent molecular distinction of mild T_H2-allergic asthma—which is associated with higher levels of eosinophils, mast cells, total and specific IgE as measured by allergy skin testing (atopy) and T_H2 cytokines than another subset of people who have mild asthma without evidence for T_H2 inflammation—supports these earlier studies and probably explains some of the variability in phenotypes observed earlier[35].

Early-onset allergic asthma can present with mild to severe disease, but it is unclear whether mild allergic asthma progresses to severe disease or whether severe allergic asthma arises in childhood and remains severe[31,42]. The SARP cluster analysis showed that people with the most severe early-onset asthma had greater numbers of skin-test reactions and poorer lung function than individuals with mild asthma and that they were more likely to be of African descent. It also linked severe allergic asthma to a longer duration of disease and a history of pneumonia. These results suggest that both genetic and environmental factors are important in asthma pathogenesis[29]. Interestingly, although IgE is one of the strongest risk factors for severe asthma in people of African descent—in whom age of onset is also significantly earlier than in other racial groups—a similar association of IgE with asthma severity has not been observed in people whose racial background is classified as white[43]. It is likely that as severity of allergic early-onset T_H2 asthma increases, non-T_H2 immune pathways—including those related to T_H17 and T_H1—are also engaged (**Fig. 4**), as is innate immunity[44–46].

Clinical phenotyping studies have confirmed the strong family history and probable genetic component to early-onset asthma[25,29]. Genome-wide association studies (GWASs) have shown that there are differences between the genetic factors related to early-onset and later-onset asthma, with stronger genetic links to early age of onset than to atopy and IgE[47,48]. Furthermore, GWASs have indicated that the genes most strongly associated with early-onset asthma, including those encoding IL-33 and its receptor, are epithelial rather than allergy related, although some of them may influence immune function. These results suggest that, despite the relationship between asthma and atopy and allergy, allergy *per se* may not be the pathobiological process that initiates asthma, and that other—perhaps innate—immune and metabolic processes may be important[47] (**Figs. 2** and **4**). In contrast, candidate gene studies carried out by SARP of T_H2 pathway–associated genes (including *IL4*, *IL13*, *IL4Rα* and *GATA3*) reported higher numbers of mutations in T_H2-related genes with greater severity of disease, supporting the idea that a dose response to T_H2-associated gene products is an important factor in high asthma severity[49]. Thus, the relative contribution of allergy- and non-allergy–related genes to both the presence and severity of the early-onset T_H2 phenotype remains to be determined.

Biomarkers and treatment responses. Most crucial to determining a pathway's importance to a disease is the confirmation that inhibition of that pathway or its activity improves disease outcomes. Corticosteroids are the mainstay of asthma therapy, and many of their beneficial effects result from the modulation of T_H2 cytokines and associated inflammation, but their activity is broad and nonspecific[50–52]. Despite this lack of specificity, there is evidence that corticosteroids are most effective in individuals in whom there is evidence of T_H2 inflammation as manifested by high FeNO (usually 30–35 parts per billion or higher), sputum eosinophils (≥2% all sputum inflammatory cells) and elevations in airway periostin[35,40,53]. All of these factors have been proposed as biomarkers for T_H2 asthma. Notably, these biomarkers are not always present in early-onset asthma of any severity; the exact proportion of people with early-onset asthma who have them remains unknown.

Although most pathobiological studies of asthma have not specifically evaluated early-onset allergic asthma, it is likely that many of the previously studied individuals with mild, corticosteroid-naïve asthma were of this phenotype. Thus, the reported basal increases in $CD4^+$ cells and eosinophils and their reported decrease after treatment with inhaled corticosteroids may represent, at least generally, this phenotype[54,55]. Because inhaled and systemic corticosteroids affect this observed pathobiology in most individuals, it can be difficult to identify a T_H2 signature in the airways of corticosteroid-treated people using conventional cellular pathology.

Beyond the nonspecific effects of corticosteroids, treatment with molecules that target components of the T_H2 pathway such as antibodies to IgE, IL-4Rα receptor blockers and anti–IL-13 strategies, combined with allergen challenges in individuals with mild, corticosterioid-naïve asthma, has confirmed the relationship of T_H2 cytokines to specific allergic asthmatic responses[56–58]. An IgE-specific

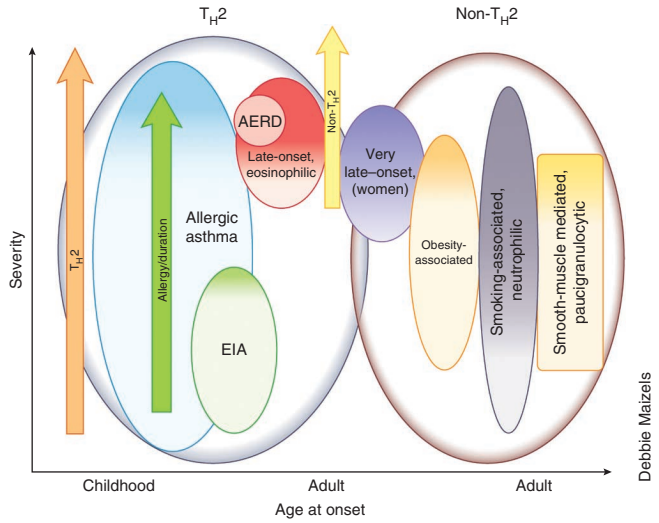

Figure 3 Theoretical grouping of emerging asthma phenotypes based on the distinction between T_H2-high asthma and non-T_H2 asthma. T_H2 asthma consists of both early- and later-onset disease over a range of severities. It is likely that the majority of early-onset allergic asthma is mild but that an increasing complexity of immune processes leads to greater severity. Later-onset eosinophilic asthma without traditional allergic elements is more likely to be severe, whereas EIA is a milder form of T_H2 asthma. Non-T_H2 asthma includes very late–onset, obesity-associated asthma as well as smoking-related and neutrophilic asthma, and asthma in which affected individuals show little inflammation. The intensity of the colors represents the range of severity; the relative sizes of the subcircles suggest relative proportions of affected individuals.

antibody (omalizumab) is the only biological agent now approved for asthma. Although IgE-specific antibody treatment has been targeted toward allergic asthma, this classification is loosely defined as minimal elevations of total IgE in the presence of any IgE specific to a particular allergen. With this definition, IgE-specific antibodies affect both early- and late-phase allergic physiological reactions and eosinophilic inflammation[56].

Even more specifically targeting T_H2 immunity than antibodies to IgE, the four-week administration of an inhaled IL-4Rα antagonist improved physiological responses to allergen inhalation and decreased FeNO in people with mild, corticosteroid-naïve asthma[32,58]. There may also be a pharmacogenetic response to anti–IL-4Rα treatment, as known risk alleles in the gene encoding IL-4Rα identified participants with better treatment responses[59]. In contrast, a monoclonal antibody to IL-5 did not show efficacy in an allergen-challenge model despite causing profound reductions of blood eosinophils[7]. In addition, two weeks of systemic anti–IL-13 treatment affected physiological responses to allergens but not eosinophilic inflammation[57]. Although the reasons for the differing effects of IL-4 and/or IL-13 from those of IL-5 in allergic responses are not known, the observed efficacy of antibodies to IL-13 in the absence of a reduction in eosinophils and the failure of antibodies to IL-5 despite a reduction in eosinophils suggest that noneosinophilic components may be of greater importance than eosinophils in these specific allergic responses.

Specific T_H2 pathway inhibition in nonphenotyped, corticosteroid-treated individuals with chronic asthma has generally been ineffective[5]. In contrast, even modest phenotyping, as shown by the case of antibody to IgE above, improves overall efficacy to some degree, reducing asthma exacerbations and improving symptoms and quality of life[12,13,60]. Yet, as many as 50–60% of individuals did not respond to IgE-specific antibody treatment, particularly those with greater severity of disease, and there are no biomarkers other than IgE to predict response[60,61]. Perhaps the most robust clinical response to IgE-specific antibody therapy was observed in a study of African-American children living primarily in inner-city environments[62], a population enriched for highly T_H2-skewed asthma[43,63]. Thus, it remains to be determined whether using a potential T_H2 biomarker to define T_H2-allergic asthma would improve the likelihood of a response to IgE-specific antibody treatment.

Interestingly, a recent study of treatment with a monoclonal antibody to IL-13, lebrikizumab, showed modest but significant improvements in FEV1 (ref. 64) in people with moderate to severe corticosteroid-treated asthma. Conventional markers of allergic inflammation (IgE, atopy and blood eosinophils) did not define lebrikizumab responders. However, recent studies have suggested that serum periostin, an epithelial protein that is induced by IL-13 and present in greater amounts in the airways of some people with mild asthma, may be a biomarker for a more general T_H2 asthmatic phenotype[65–67]. FeNO has also been proposed as a T_H2 biomarker because it is produced by inducible nitric oxide synthase, an enzyme that is induced in human airway epithelial cells by IL-13 and present in greater abundance in asthma[33,68,69]. In the lebrikizumab study described above, a subgroup of individuals who had asthma with persistent elevations in serum concentrations of periostin showed greater improvements in airway function and fewer exacerbations after treatment than those with lower concentrations of serum periostin[64]. Interestingly, in a *post hoc* analysis, FeNO levels were as helpful as periostin in identifying T_H2-high individuals who would respond to lebrikizumab, but these biomarkers were not compared with the percentages of sputum eosinophils. Although this study suggests that periostin may be an easily obtainable T_H2 biomarker, whether it will be better than FeNO or sputum eosinophils remains to be determined in prospective studies.

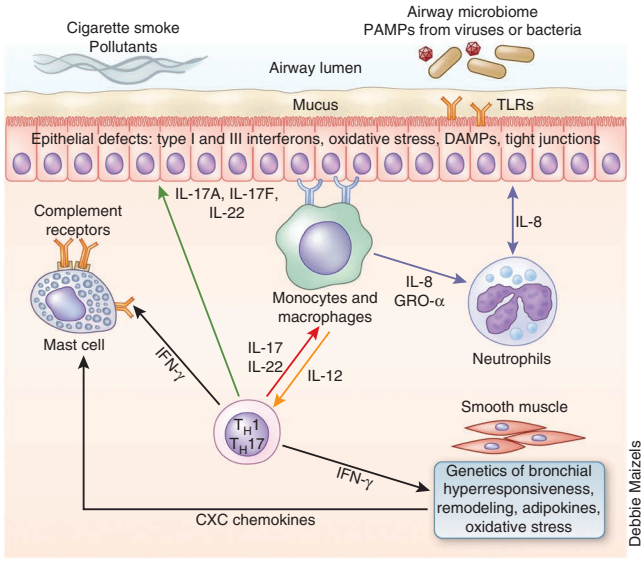

Figure 4 Theoretical range of factors that may be involved in the development of non-T_H2 asthma. These factors include infection-related elements, T_H1 and T_H17 immunity, non-T_H2–associated smooth-muscle changes including genetics and oxidative stress, and the development of neutrophilic inflammation. IFN-γ, interferon-γ; GRO-α, growth-regulated oncogene-α; PAMP, pathogen-associated molecular pathway; DAMP, danger-associated molecular pathway; TLR, Toll-like receptor.

Late-onset persistent eosinophilic asthma

Clinical and biological features. Eosinophilic asthma is characterized by the presence of eosinophils in higher numbers than normal as determined by sputum, bronchoscopic or blood analysis. Although the definition of eosinophilic asthma is not standardized, studies have indicated that a proportion of sputum eosinophils greater than 2% of all sputum inflammatory cells can distinguish individuals with eosinophilic asthma from healthy individuals and those with non-eosinophilic asthma, suggesting eosinophils as a target for therapeutic intervention[9,11,70,71]. The proportion of asthma associated with high numbers of eosinophils is not known, but studies of mild to severe asthma suggest that it may be around 50%[8,35]. In severe asthma, high numbers of eosinophils can persist despite treatment with inhaled and oral corticosteroids and appear to be consistent over at least 5 years[72].

Persistent sputum eosinophilia (≥2%) despite corticosteroid therapy is associated with an adult-onset, less allergic form of asthma[25,28,29]. This form of asthma is often associated with sinusitis, nasal polyps and sometimes AERD, but there is little to suggest that clinical allergic responses are occurring, despite positive allergen skin tests in ~75% of individuals[25]. A family history of asthma is also less commonly observed than in early-onset disease, and the genetics of this phenotype have not been specifically studied[25,29]. This phenotype is often severe from onset.

The lack of clinical allergy in this phenotype suggests that the T_H2 process differs from and is probably more complex than the one associated with the early-onset allergic phenotype. As T_H2 cytokines are also upregulated in cancer, inflammatory bowel disease and interstitial fibrosis, a T_H2 inflammatory process in the lung without mucosal-specific IgE and associated clinical allergic reactions is clearly possible[73–75]. Some individuals show sputum neutrophilia intermixed with their eosinophilic process[76]. This mixed inflammatory process implies that there are interactions of additional immune pathways with T_H2 immunity, including activation of pathways related to IL-33 and IL-17 (refs. 44,77–79).

As noted above, a recognized subphenotype of this late-onset phenotype is AERD, perhaps one of the earliest asthma phenotypes identified. AERD identifies a type of adult-onset, highly eosinophilic asthma with concurrent severe sinusitis, nasal polyps and life-threatening, non-IgE–mediated responses to aspirin and other cyclooxygenase-1 inhibitors[15]. The cysteinyl leukotriene pathway, which is upregulated by T_H2 cytokines and present in cells associated with T_H2 inflammation (eosinophils, basophils and mast cells)[25,80,81] is upregulated in AERD, and some studies have suggested that the AERD phenotype is linked to leukotriene pathway–related genes[80,82,83]. The degree of overlap between late-onset eosinophilic asthma with AERD and late-onset eosinophilic asthma without AERD is not clear.

Biomarkers and treatment responses. Despite the lack of allergy, there is increasing evidence to support a major role for T_H2 immunity in this phenotype. Pathologically, IL-13 and IL-5 are present in the lower airways and in association with nasal polyps. Supporting this link, a gene-expression study identified the T_H2 biomarker periostin in nasal polyps from AERD[67,84–86]. Other downstream T_H2 signature molecules are present in greater amounts in many of these people, including 15-lipoxygenase-1 and its product 15-hydroxyeicosatetraenoic acid, iNOS and eotaxins[67,87,88]. The subset of individuals with AERD and, to some degree, the general late-onset phenotype, is associated with upregulation of the cysteinyl leukotriene pathway, as mentioned above. Finally, the eosinophilia is associated with a thicker SBM and high TGF-β expression, findings that are also observed in early-onset, allergic (T_H2-high) asthma[25]. Elevations in FeNO and urinary cysteinyl leukotrienes have also been reported[25,28,89,90]. Whether distinct biomarkers will identify this phenotype remains to be assessed.

Eosinophils are usually highly responsive to corticosteroids, rapidly undergoing apoptosis in their presence[91,92]. It is surprising, therefore, that lung and blood eosinophils persist in a subset of up to 50% of people with corticosteroid-treated asthma, with increasing percentages of these cells seen with increasing disease severity[8]. As early-onset allergic eosinophilic T_H2 asthma generally responds well to corticosteroid therapy, this persistent eosinophilia in late-onset disease implies that the T_H2 process in this type of asthma is refractory to corticosteroids. The immunobiology underlying the different eosinophil responses in early- and late-onset T_H2-related disease is not clear, but high systemic doses of corticosteroids are generally able to overcome this refractoriness in late-onset asthma[93]. As would be expected given the high concentrations of cysteinyl leukotrienes in these individuals, leukotriene modifiers can have a beneficial impact on lung function and symptoms in the AERD subset, even in the face of systemic corticosteroid therapy, further distinguishing the T_H2-related processes in early-onset disease from those in late-onset disease[94,95].

The most convincing evidence that T_H2 cytokines are present and contribute to the persistent eosinophilia in this phenotype comes from recent drug trials of therapeutics that blocked the IL-4 and IL-13 pathway or the IL-5 pathway[90,96,97]. Results from separate studies of individuals with severe, persistent (and mostly late-onset) eosinophilic asthma treated with a monoclonal antibody to IL-5 showed that this therapy was effective not only in diminishing blood and lung eosinophils but also, importantly, in decreasing exacerbations and systemic corticosteroid requirements[96]. Thus, whether these targeted T_H2 approaches will have greater therapeutic efficacy in mild or severe, allergic or nonallergic asthma will be likely to depend more on the amount of remaining T_H2 inflammation than on the previously used clinical characteristics.

Exercise-induced asthma

Clinical and biological features. For this discussion, EIA refers to asthma whose symptoms are experienced primarily after exercise. Although this phenotype has been described for years, little is known about its immunological and inflammatory underpinnings. People with EIA often have mild asthma and experience reactive bronchoconstriction (a decline in FEV1 of 10–15%) in response to sustained exercise, and this decline is more frequent and severe in cold, dry conditions. Consistent with a relationship to T_H2 processes, this phenotype may be more common in atopic athletes and pathologically associated with high percentages of eosinophils in both sputum and tissue[37,98,99]. However, EIA has also been reported with only low-level eosinophilic inflammation, and the relationship of this form of the disease to T_H2 immunity is less clear[99]. EIA has also been associated with mast cells and their mediators, and these cells are consistently associated with T_H2 processes[38]. No distinct genetic factors or biomarkers for EIA have been described.

Treatment responses. Drugs that modify the cysteinyl leukotrienes also suppress EIA[100,101]. Importantly, monoclonal antibody blockade of the mast cell–promoting cytokine IL-9 (which is linked to T_H2 (and T_H9) immunity) has also recently been shown to inhibit EIA[102],

suggesting that EIA is at least partly T_H2 associated. Whether EIA differs from the other forms of T_H2 asthma beyond its persistence and severity awaits further study.

Non-T_H2 asthma

Non-T_H2 asthma is likely to represent a large proportion of all asthma. However, in comparison to T_H2 asthma, little is understood about this asthma subgroup, the phenotypes underlying it or the molecular elements that control it. Non-T_H2 asthma may affect 50% or more of corticosteroid-naïve individuals, who, although they meet the criteria for asthma, show less airway obstruction and hyperreactivity than people with T_H2-high asthma[35,53]. Many people who have mild to moderate adult-onset asthma and no history of childhood allergic features are likely to fall into this category[103,104]. As these individuals also respond poorly to corticosteroid therapy, in the absence of validated T_H2 biomarkers, the proportion of non-T_H2 asthma in persistently symptomatic, corticosteroid-treated patients is not clear. However, the lack of efficacy of T_H2-targeted therapies in studies of nonphenotyped, corticosteroid-treated patients (even when corticosteroid treatments are tapered) and the existence of multiple other pathways by which airway hyperresponsiveness may develop strongly suggest that a subset of asthma exists with no T_H2 immunity[5,105,106].

Apart from the non-T_H2 phenotypes discussed below, additional ones are likely to be defined in the future (**Fig. 3**). Individuals who have asthma with little to no inflammation of any type may have a more reactive airway, or their smooth-muscle hyperreactivity may not depend on the immune components known to contribute to hyperreactivity[53,107]. Certain genes related to injury and repair have been targeted for their role in the control of FEV1, and single nucleotide polymorphisms (SNPs) in the genes encoding hedgehog-interacting protein and a disintegrin and metalloprotease 33 have been frequently associated with this parameter in the healthy human population and in people with asthma or other lung diseases[108,109]. The role of T_H1 processes is even less understood, although a recent mouse study suggested that interferon-γ (IFN-γ) could enhance mast-cell responses; interestingly, CXC chemokines, induced by IFN-γ, are known mast-cell chemoattractants to smooth muscle[110,111]. Thus, T_H2-independent mast-cell processes could exist in addition to T_H2-dependent ones.

Obesity-related asthma

Clinical and biological features. Obesity has been suggested to have a substantial role in the development, control and severity of asthma. Yet, whether obesity is a driving component in asthma development or a mere confounder or comorbidity of its presence remains controversial. Obesity is associated with greater energy expenditure during breathing, deconditioning, shortness of breath and greater likelihood for gastroesophageal reflux and associated coughing and chest tightness, all of which can lead to misdiagnosis of asthma in obese individuals when specific physiological testing is not used[112–115]. Other studies support an association of obesity with a generalized proinflammatory state involving high expression of certain inflammatory mediators such as TNF-α, IL-6 and leptins, although obesity is consistently associated with lower amounts of FeNO, fewer eosinophils and, importantly, a diminished response to corticosteroid therapy[116–119]. In fact, weight loss was recently shown to enhance T_H2 (and T_H1 and T_H17) cytokine production from peripheral blood lymphocytes[120].

Interestingly, a distinct obesity-related asthma phenotype seems to occur only in non-T_H2 asthma[120,121]. In early-onset allergic T_H2 asthma, the disease duration correlates with body mass index, and a higher body mass index is likely to be related to lower activity levels and more use of corticosteroids; however, no such relationship to duration exists in late-onset, less-allergic asthma that is obesity related[120,121]. Two unbiased studies also supported the existence of a later-onset, non-T_H2 obesity-related phenotype by identifying a group of women, most of whom were obese, who had late adult-onset (mid-40s), minimally allergic asthma with a high burden of symptoms but little of the need for high-intensity healthcare (such as time in an intensive care unit or need for intubations) typically seen in early-onset allergic asthma[28,29]. Despite similarities in the cluster approaches used, each study reported different levels of eosinophilic inflammation, suggesting complexities beyond obesity-related hormonal interactions alone.

Biomarkers and treatment responses. Although adipokines have been proposed, no specific biomarkers have been accepted to define obesity-related asthma, perhaps because the role of obesity differs between T_H2 and non-T_H2 asthma. Although weight-loss programs and bariatric surgery have been reported, anecdotally, to improve the symptoms of asthma (and even cure it), the relationship of obesity to objective measures of inflammation and physiology is unclear[122]. In the most convincing study to date, profound weight loss achieved after bariatric surgery in a group of people who had nonallergic, late-onset (non-T_H2) asthma was associated with improvements in symptoms, quality of life and bronchial hyperresponsiveness[120]. In contrast, similar weight loss in obese individuals with allergic (T_H2) asthma did not improve bronchial hyperresponsiveness, and the high T_H2 cytokine production in these individuals suggests that weight loss may even worsen T_H2 asthma. Thus, weight loss as a therapy for obese-associated asthma seems to be more beneficial when the asthma is not associated with T_H2 inflammation. The poor clinical responses to corticosteroids that have been observed in obesity-related asthma may also be due to the lack of association of this phenotype with T_H2 inflammation, which is traditionally a corticosteroid-responsive process[53,117,119]. Whether this lack of corticosteroid responsiveness supports studies that have suggested that more corticosteroid-refractory pathways—including those involving IL-6 or TNF-α, either alone or in association with increased oxidative stress—are important in driving disease awaits further study[123].

Neutrophilic asthma

Clinical and biological features. Neutrophilia has been inconsistently associated with asthma and severe asthma for several years[24,124]. Data to support neutrophilic asthma as a specific phenotype remain modest, and no consensus exists as to what level of neutrophilia should define the phenotype. Neutrophilia is generally seen in corticosteroid-treated patients. Corticosteroids inhibit neutrophil apoptosis and, in some settings, contribute to neutrophil activation, suggesting that corticosteroid treatment itself is likely to have some role in the development of neutrophilia[125,126]. In affected individuals, lung neutrophilia has been associated with lower lung function, more trapping of air, thicker airway walls (as measured by computed tomography scans) and greater expression of matrix metalloproteinases than are seen in people with non-neutrophilic asthma, but it has not been associated with airway hyperresponsiveness[127–131]. Although lung neutrophils have not been incorporated into any unbiased analysis, in a *post hoc* analysis, sputum neutrophilia was greatest in a SARP cluster of individuals who had generally adult-onset and severely obstructed (and incompletely reversible) asthma and the highest-intensity healthcare usage and

systemic corticosteroid use[29]. The relationship of this phenotype to smoking remains unclear and inconsistent. Although the SARP cluster study did not include smokers, some studies suggest that when the data are controlled for corticosteroid use and smoking, this phenotype does not exist[132,133]. An additional sputum gene-expression profiling study in Australia reported that neutrophilic inflammation was associated with upregulation in IL-1 and TNF-α pathways, but there was little association with any clinical phenotype[133]. Neutrophilia can also co-exist with eosinophilia, and this identifies the people with the most severe asthma and emphasizes the complexity of the immunobiology of severe asthma in which multiple different innate and adaptive immune pathways and cells may have roles[76,134].

Treatment responses. Corticosteroids are less effective in this phenotype, perhaps because of the absence (or suppression) of a T_H2 process[10]. A single study suggested that neutrophilic asthma may be more responsive to treatment with macrolide antibiotics, with a reduction in the expression of neutrophilic markers and improvement in quality of life, but no improvement in asthma control or FEV1 in treated individuals[135]. Whether the observed improvement was caused by the antibiotic or anti-inflammatory effects of macrolides is not clear. The lack of neutrophil-targeted interventions limits the ability to determine whether neutrophilia is a biomarker or a target for therapy. However, the lack of efficacy of anti–TNF-α in severe asthma treated with high doses of corticosteroids raises questions about the importance of TNF-α in neutrophilic asthma[136].

T_H17 inflammation has been strongly linked with neutrophilia and envisioned as a therapeutic target, and this has led to the development of mouse models that overexpress IL-17 and are neutrophilic and corticosteroid resistant[34,44,45]. Expression of both IL-17A and IL-17F has been reported to be high in severe asthma in association with poor lung function, but, interestingly, these cytokines have not been linked to neutrophilic inflammation or clinical parameters[78,137]. Thus, the efficacy of IL-17–targeted therapy in human neutrophilic (or other) asthma phenotypes awaits results from ongoing clinical trials.

Conclusions and future directions

The concept of asthma phenotypes is rapidly evolving from one that was focused on clinical characteristics to one that links underlying biology to phenotype. There is strong evidence supporting a T_H2-high phenotype in up to 50% of people with asthma of any severity, yet 50% show no evidence for this immune process. Even the percentage of early-onset allergic asthma that is truly T_H2 high remains unknown. As the complexity and severity of the disease presentation increases, the accompanying immunopathology becomes more complex and is likely to include additional adaptive immune elements and structural changes in the airways. Asthma of any severity that presents without evidence for T_H2 immunity remains poorly understood. Although there has been substantial interest in the role of T_H17 immunity in neutrophilic (and even obesity-related) asthma, support for this theory in humans is modest. Thus, our understanding of phenotypes remains incomplete.

The future understanding of these phenotypes will require integrating bench and bedside approaches. Data from ongoing genomic and proteomic profiling studies of large, well-characterized asthma populations should allow the development of improved animal models of asthma that mirror more closely the inflammatory (or noninflammatory) environment identified from the human studies. To better represent the non-T_H2 phenotype, studies in animal models will need to focus on infectious and other environmental elements, obesity and hormonal changes in the absence of allergic sensitization and challenge. Genetic studies of mice that are particularly hyperreactive in the absence of inflammation could also provide clues into the biology underlying this phenotype, perhaps for milder asthma in particular. In humans, population studies of a range of asthma severities will require longitudinal components to characterize molecular phenotypes and their stability over time, validate genomics at the protein or lipid level and develop new technologies that enhance the quality of the results that can be obtained from very small samples.

Although our understanding of even mild to moderate asthma remains incomplete, severe and poorly controlled asthma phenotypes remain the great unmet clinical need. To further our understanding of severe asthma, new molecular targets that are first identified using human 'omics' studies will need to be mechanistically evaluated in animal and cell-culture models to better define their functionality, association with accompanying pathways and relationship to specific phenotypes. However, the ultimate test of a phenotype is the efficacy of a targeted molecular approach. It is hoped that these combined bench and bedside approaches will enhance the successful development of personalized and phenotype-specific therapies for asthma.

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