ASTHMA HAS BEEN CLINICALLY RECOGNIZED as a heterogeneous disease for decades, including descriptions of patients with aspirin-exacerbated respiratory disease, allergy-associated disease, and exercise-induced asthma. Despite this recognition, treatment approaches were similar, involving the use of non-specific agents, such as inhaled corticosteroids (CS) and β2-agonists. These drugs, which were generally effective in the majority, also failed in many. In each case, the molecular underpinnings of the heterogeneity or the varied responses to treatment were unknown.

Clinical Phenotypes

Early efforts to more objectively define human asthma heterogeneity involved identifying and grouping primarily clinical, physiological, and cellular parameters to loosely identify phenotypes of asthma. The initial descriptions of phenotypes, a term defined as “the integration of multiple observable characteristics of an organism which arise from the interaction of genetic and environmental influences,” typically utilized clinical characteristics of asthma, such as age at asthma onset, allergic features, degree of airway obstruction and reversibility, or relationships to obesity and tobacco smoking. Unfortunately, in many cases, these characteristics were overlapping or nonspecific enough, such that identifying and grouping these characteristics alone did not identify the importance of specific pathological processes or, importantly, improve the treatment of the patient. An important example is the allergic characteristic of asthma, which is often solely defined by the presence of specific IgE to known allergens (atopy), without incorporating physiological/clinical responses to them (allergy). Although this propensity to making specific IgE is linked to Th2 immune processes, epidemiological, pathological, and specific biological treatment trials contribute to the observations that atopy can be present without systemic evidence for Th2 immunity and that Type 2 (or Th2) immunity can be present without elevations in IgE or accompanying allergic symptoms. Thus traditional clinical characteristics of asthma may be unlikely to provide mechanistic insights, even when approached in an unbiased/statistical manner (77, 94).

The Transition to Molecular Phenotyping

Transitioning the clinical heterogeneity of human asthma to a mechanistic understanding of its pathobiology will require incorporation of both human and animal studies. Profound ethical limitations exist to invasive studies in humans, and, unfortunately, it is impossible to recapitulate all cellular and molecular phenotypes of asthma in a single animal model in any species (17, 122). Therefore, it is imperative that bench-to-bedside and bedside-to-bench approaches are constantly utilized.

Much of the understanding of immune pathways relevant or potentially relevant to human asthma phenotypes has arisen...
from studies in the mouse. The mouse has been the animal of choice in asthma studies because of the ability to exploit mouse genetics and the availability of mouse-specific reagents. However, it is worth noting that there are key differences between human and mouse airway physiology (51). Importantly, no strain of mouse has been identified or generated that develops asthma-like symptoms spontaneously. Furthermore, whereas in humans both nasal and oral breathing are possible, mice are obligate nose breathers. And their quadruped habitus influences the structure of the lungs as well as the dynamics of airflow (17). Airway branching in mice extends to only six to eight generations but to >30 in humans. Unlike human airways, there is no evidence for smooth muscle bundles in mouse airways (51, 105). Importantly, epithelial structure, composition, and function are markedly different between mouse and human airways, as are differences in peribronchiolar vs. reticular basement membrane fibrosis (59). Finally, in general, mouse models of asthma have been heavily reliant on allergic sensitization and reactions, whereas the central relationship of atopy/allergy to human asthma (and even its phenotypes) is becoming less clear (23, 83).

Despite these profound differences, mouse models have been critical in our mechanistic understanding of allergic inflammation, particularly the role of Th2 immunity in some asthma phenotypes (86, 87). In fact, the Type 1/Type 2 (Th1/Th2) immune response paradigm was initially established in mice in the mid-1980s (78). Subsequently, the presence of Type 2 cytokines was described in the airways of asthmatics (88, 101), and multiple laboratories became engaged in developing mouse models of asthma harboring a Type 2 immune response. These models largely involved use of the egg allergen ovalbumin (OVA), initially because of the availability of OVA-specific T cell receptor transgenic mice. However, many other allergens have been used, including house dust mite (16, 31, 53, 58), ragweed (26), cockroach (10), and even papain (95), a prototype for protease-expressing allergens first suggested in the 1970s (73, 80). The molecular regulator for these Th2 processes in both mice and humans, GATA-3, is common between the two species, and targeting GATA-3 can reduce allergic airway disease in mouse models, with preliminary evidence to support efficacy in an allergen challenge model in humans as well (32, 79, 90, 118, 121).

Thus mouse models clearly linked allergic inflammation and physiological changes to Th2 cytokines and were critical for the emergence of biological therapies targeting IL-4, -5, and -13 for treatment of human disease (42, 110). However, initial studies of these therapies in humans failed, and these therapies were nearly abandoned (34, 61). Although the reasons for these failures are likely complex, heterogeneity of the initiating factors involved in initiation and perpetuation of disease, immune, inflammatory, and structural features associated with asthma certainly contributed.

Immunologically, although it is clear that transcription factors like GATA-3 control development of Th2 cells, in chronic disease, activation and proliferation of many other cell types, including eosinophils, are observed. Whereas those reactions, driven by reactivation of Th2 cells by the cognate allergen(s), blunt the allergen-induced inflammatory response, other pathways arising in parallel may not be inhibited. Similarly, if airway hyperresponsiveness is due to other factors (including factors related to obesity or infection, which may not activate Th2 responses), inhibition of Th2 pathways will have little effect. Furthermore, structural changes established over time may not be influenced by the dampening of inflammation alone in established disease, even if the disease was initiated by Th2 cells. Thus a recognition of complexity of both murine and human phenotypes (specifically broadening of mouse model systems beyond allergen alone and identification of human asthma phenotypes with biomarkers suggestive of Th2/Type 2 inflammation) was required before clinical efficacy would be achieved.

Mouse models of asthma have also been instrumental in increasing our understanding of the innate immune system, including dendritic cells, and the recently described group 2 innate lymphoid cells (ILC2s) (25). However, translating the importance of these cell types to human asthma phenotypes requires further study.

The emergence of an eosinophilic asthma (inflammatory) phenotype. Although lung eosinophils have long been associated with asthma and mouse models of asthma, the presence of eosinophils alone does not define a molecular (or even inflammatory) phenotype. Identification of a true molecular phenotype requires an integrated approach that incorporates clinical, physiological, genetic, and, importantly, molecular characteristics to identify underlying disease mechanisms (Fig. 1). However, in that regard, eosinophils, particularly lung eosinophils, have been associated with symptomatic exacerbation-prone disease (23, 58). Reducing eosinophils through titration of nonspecific anti-inflammatory (CS) therapy also decreased exacerbations (38, 41, 108). However, an early human study targeting the eosinophil through a monoclonal antibody directed at IL-5, although profoundly decreasing blood eosinophils (and linking IL-5 to eosinophils), failed to impact physiological responses to allergen challenge, which, in mouse models, is highly Type 2 dependent (61). Similarly, differing approaches to abolishing eosinophils in mice had mixed impact on clinically relevant characteristics, such as airway hyperresponsiveness (AHR) and mucus production, likely attributable to the different models used, such that mouse models were also not definitive regarding the importance of eosinophils (50, 62). Thus the early data from both mouse and humans did not confirm the importance of eosinophils in human asthma or whether it identified a specific phenotype.
Asthma has been associated with eosinophilic inflammation for years. However, as early as the 1950s, it was observed that eosinophilic inflammation was not present in all asthma patients but appeared to link to identifiable clinical characteristics, including response to systemic CS therapy (13). Although Type 2 cytokines, including IL-4, -13, and specifically the proeosinophilic cytokine IL-5, were being reported in human asthma, there were wide ranges in levels and no attempt to relate the expression to particular characteristics beyond atopy and eosinophils (43, 49).

One of the first studies to do this compared patients with severe asthma with and without evidence of tissue eosinophilia on endobronchial biopsies (108). This eosinophilic phenotype, seen in about 50% of patients with severe asthma, was associated with more symptoms, air trapping, and a history of severe exacerbations. Importantly, this eosinophilic phenotype was “molecularly” associated with higher numbers of transmural tissue eosinophils reported earlier, the Type 2 signature was then evaluated in freshly brushed epithelial cells (111). The expression of this Type 2 molecular signature was present in only about 50% of patients with severe asthma, there were wide ranges in levels and no attempt to relate the expression to particular characteristics beyond atopy and eosinophils (43, 49).

The transition to a Th2-like/Type 2-Hi molecular phenotype. As discussed previously, mouse models of asthma were integral to identifying a potential role for Th2 inflammation and the canonical Type 2 cytokines IL-4, -5, and -13 in human asthma. However, initial human studies blocking these pathways failed to show efficacy, likely at least partially attributable to their use in general asthma populations, ineffective/low-potency therapeutic agents, or inappropriate model systems.

To address whether differences in molecular pathways involved in asthma might explain the insufficient responses to molecularly targeted therapies (111), asthmatic patients were evaluated for the presence or absence of a Type 2 cytokine molecular signature. This Type 2 molecular signature was first identified in vitro, in epithelial cell cultures, where three genes, periostin, CLCA1, and SERPINEB2, were found to be upregulated by IL-13 (111). The expression of this Type 2 molecular signature was then evaluated in freshly brushed epithelial cells obtained from mild, CS naïve asthmatics, where clear differences were observed in the presence or absence of this Type 2 signature. The Type 2 signature was present in only about 50% of the mild asthmatics and was not seen in the nonatopic healthy controls. Whereas the absence of this Type 2 signature in many mild asthmatics was perhaps surprising, the presence of the signature was associated with relevant clinical and biological phenotypic characteristics, including more lung eosinophils and mast cells, higher IgE levels, more bronchial hyperresponsiveness, and, importantly, higher tissue expression of IL-5 and -13 (28, 111). Similar to the association with tissue eosinophils reported earlier, the Type 2 signature was associated with a thicker subepithelial basement membrane, supporting considerable overlap between a Type 2 cytokine molecular phenotype and the eosinophilic phenotypes. Importantly, the presence of this Type 2 molecular signature also predicted a robust response to moderate-dose inhaled CS, whereas no response was seen in those without the Type 2 cytokine signature. Thus this study began to link the traditional allergic clinical phenotype to an underlying Th2 signature (Fig. 2A).

As noted, in addition to tissue eosinophils, mast cells have also been reported in airway tissue in relation to Type 2 inflammation (28). Importantly, there is an increase in epithelial mast cells expressing the enzyme hematopoietic prostaglandin (PG) D synthase and its product PGD2. PGD2 activates several receptors, one of which, chemoattractant receptor-homologous molecule expressed on Th2 cells, is expressed on eosinophils, Th2 lymphocytes, and, more recently, ILC2 cells, suggesting that mast cells/their products could integrate several cell types/pathways, including those related to innate immunity (30). ILC2 cells are of particular interest for their role in Type 2 asthma, as they could both initiate and perpetuate Type 2 cytokine responses, with some evidence for their existence in asthmatic airways; their importance in Type 2 asthma, however, remains unknown (7, 45). Importantly, however, the IL-33 pathway has been strongly linked to ILC2 cells. Polymorphisms in both IL-33 and its receptor, IL1RL1, have been consistently linked to early-onset allergic asthma in genome-wide association studies, consistent with a role in some Type 2 phenotypes (75, 98).

BIOMARKERS IDENTIFYING TYPE 2 CYTOKINE-ASSOCIATED ASTHMA. The initial identification of Type 2 cytokine-associated asthma relied on an invasive bronchoscopic approach. For this molecular phenotype to become more widely applicable, identification of more easily measured biomarkers was needed. Numerous less invasive biomarkers have now been reported in relation to a Type 2 asthma phenotype. Serum levels of peristatin, the protein associated with one of the three epithelial Type 2 signature genes, have been reported to better predict sputum eosinophilia than other biomarkers (52). Interestingly, the relation of serum peristatin to epithelial expression of the Type 2 molecular signature has not yet been reported. The predictive value was maintained even in a multivariate approach, which included fractional exhaled nitric oxide (NO) (FeNO) and was seen in both CS-treated and -untreated patients. It also was reported to predict response to treatment with anti-IL-13 (lebrikizumab) molecularly targeted therapy (23).

Sputum assessment has also been used to less invasively identify this Type 2 Hi phenotype. The mean expression of the Type 2 cytokines IL-4, -5, and -13 in sputum was reported to strongly correlate with the Type 2 epithelial signature genes, CLCA1 and peristatin (but not SERPINEB2), which are thought to be arising from epithelial cells in sputum and to predict both blood and sputum eosinophilia, at least in patients with milder asthma. The utility of this sputum Type 2 signature in more severe CS-treated patients or as a predictor of response to Type 2-targeted therapies remains to be determined (84).

Elevated levels of FeNO have been associated with asthma and allergy for many years. Inducible NO synthase (iNOS), the enzyme primarily responsible for generation of NO in the airway epithelium, is strongly induced by IL-13 in vitro, and FeNO is consistently decreased following treatment with anti-IL-4/-13 therapies (19, 23, 104, 106). It is also known to be upregulated by the Type 1 cytokine interferon-γ, such that elevated FeNO is not specific to Type 2 inflammation (39). This may explain the observation that FeNO can be elevated in...
Fig. 2. A: traditional view of Type 2/Th2 inflammation in early-onset, allergic asthma. iNOS, inducible nitric oxide synthase; PGD, prostaglandin D; TSLP, thymic stromal lymphopoietin; CCR, CC chemokine receptor; MUC5AC, mucin 5AC. B: possible underlying pathobiology in patients with nontraditional Type 2 inflammation associated with adult-onset, eosinophilic disease. ILC2, innate lymphoid cells. C: possible underlying pathobiology in complicated/mixed Type 2 severe asthma. APC, antigen-presenting cell; GCSF, granulocyte colony-stimulating factor; DUOX2, dual oxidase 2.
atopic healthy controls as well as severe CS-treated patients (35, 48, 116). Although it has been associated with eosinophilic inflammation, selective reduction in blood and sputum eosinophils following anti-IL-5-targeted therapy does not decrease FeNO, supporting important differences between these two Type 2 cytokine-associated biomarkers (40, 82). Whether differences in identification of Type 2 subphenotypes or prediction of responses to different Type 2-targeted biological therapies will emerge remains to be investigated.

The presence of Type 2 molecular phenotypes is supported by responses to Type 2-targeted therapies. Determining the clinical significance of any molecular phenotype requires evidence that the identified molecular pathway responds to targeted molecular therapies and that clinically meaningful responses occur. Trials of Type 2-targeted therapies for asthma were generally not successful in nonphenotyped populations (22, 23, 34). However, studies of Type 2 cytokine-directed therapies have confirmed the importance of these molecular pathways in predefined patients with Type 2 biomarker-associated asthma and suggest that clinically identifiable phenotypes may be driven by common underlying molecular pathways. When a Type 2 biomarker is utilized to identify a Type 2 asthma phenotype, all recent studies of Type 2 cytokine-targeted therapies have been clinically successful. Thus confirmation of biological importance of a molecular pathway to a clinically and molecularly identifiable disease is the first step toward identifying disease endotypes (Fig. 1) (64).

**Type 2 Asthma: Confirming the Role of IL-5.** Mouse models have been extremely beneficial to our understanding of Type 2 immunoinflammatory processes, primarily because of the ability to over- or underexpress elements of the process in mice and evaluate the effect on AHR and inflammation. The emergence of specific, targeted biological therapies for human use parallels the use of gene knockdown approaches but accomplishes this in the species of ultimate interest, the human.

In this regard, IL-5, a canonical Type 2 cytokine, is one of the most proeosinophilic cytokines yet identified (reviewed in Ref. 57). When mepolizumab, a monoclonal antibody to IL-5 that had failed in previous nonselective asthma studies, was targeted to patients with moderate to severe asthma with historical sputum eosinophilia, a significant reduction in asthma exacerbations occurred over a period of a year (40). There were no effects on other phenotypic characteristics, such as symptoms or lung function. There was a small effect on airway wall thickening measured by CT scan observed, in line with the previously observed effect of reductions in extracellular matrix components, eosinophils, and TGF-β expression in bronchoscopic biopsy specimens collected from patients with milder asthma (33). Clinical characteristics of this eosinophilic IL-5-responsive phenotype included the presence of late-onset disease, nasal polyps, and sinus disease, clinical characteristics known to be related to eosinophilic inflammation (3, 41, 74). This initial study was followed by a larger study of patients recruited on the basis of blood/sputum eosinophils or FeNO, where reduction in blood eosinophils was highly predictive of reduction in asthma exacerbations but not of changes in lung function (82). The drug was most effective in patients with the highest numbers of circulating eosinophils and exacerbations.

A similar antibody to IL-5 (resilizumab) was evaluated in moderate to severe asthma with persistent high levels of sputum eosinophilia (≥3% on 2 occasions). There was a tendency for improvement in symptoms and lung function, but the study was not of long enough duration to address exacerbations (15). Most recently, anti-IL-5 was targeted to eosinophilic systemic CS-dependent severe asthmatics (8). Forty percent of these primarily adult-onset patients, eosinophilic despite systemic CS, were able to reduce their CS dose by >75% while maintaining asthma control and decreasing exacerbations compared with placebo. Interestingly, the remaining subjects had little overall difference compared with placebo, suggesting that IL-5 plays a critical role in some, but perhaps not all, eosinophilic asthmatic patients. Overall, these studies confirm the molecular relationship of the Type 2 cytokine IL-5 to an eosinophilic inflammatory process, with some suggestions that responsive patients (later onset, highly eosinophilic) may differ from the traditional early-onset allergic phenotype (Fig. 2B).

**A Type 2 Molecular Phenotype: Confirmation of a Role for IL-4/-13.** Like IL-5, IL-4 and -13 are reported to be increased in asthma (49, 89). However, associations with clinical characteristics other than disease severity or atopy have not been reported. Therefore, it was perhaps not surprising that studies targeting IL-4, alone or in combination with IL-13, although efficacious in early small trials, were not successful in larger studies (11, 22, 23) (Fig. 3).

Despite these negative studies, and unlike anti-IL-5, which had failed in an allergen challenge model in humans, a mutant IL-4 (pitrakinra) that blocked the IL-4 receptor complex and a monoclonal antibody to IL-13 showed efficacy in inhaled allergen challenge studies. These challenge studies suggested that targeting IL-4/-13 could be efficacious in the presence of a prototypical allergic/Type 2 inflammatory process (36, 106).

These allergen challenge studies were followed by studies of patients with chronic asthma, in which Type 2 status was not initially addressed. An antibody to the IL-4 receptor was not effective in unphenotyped moderate asthma (22). Anti-IL-13 (lebrikizumab) was also only marginally effective in all enrolled patients with moderate to severe asthma (23). However, lebrikizumab was more effective in improving lung function/
forced expiratory volume in 1 s (FEV1) in patients with high serum periostin levels, a biomarker of a Type 2 phenotype, compared with those with low levels. Greater improvements in lung function were also seen in those patients with high compared with low FeNO levels, with the greatest improvements seen in those with both high periostin and FeNO (23, 117). Although there was a suggestion of marginal effects on asthma exacerbations, there were no effects on symptoms or asthma control, supporting a selective role of IL-13 on the phenotypic characteristic of airflow limitation. Similar results were seen with the anti-IL-13 antibody tralokizumab, which was minimally effective in a general asthma population but had greater efficacy in improving FEV1 in those patients with detectable sputum IL-13 levels (85).

Blood and sputum eosinophils (as Type 2 biomarkers) were used to prospectively identify an eosinophilic moderate to severe asthma population for a trial of a monoclonal antibody to the IL-4 receptor-α (dupilumab) (104). Most patients were included on the basis of blood eosinophils ≥300/mm³ despite use of moderate- to high-dose inhaled CS and long-acting β2-agonists (LABA). Dupilumab or placebo was added to background-inhaled CS/LABA for 4 wk, followed by a taper of the LABA and inhaled CS, such that patients were on no other asthma medications for the last 3 wk of the trial. The primary outcome was reduction in protocol-defined exacerbations compared with placebo, with secondary outcomes including asthma control, FEV1, and symptoms. In this population of generally severe asthmatics, treatment with dupilumab resulted in an 87% reduction in protocol-defined exacerbations while improving asthma control, FEV1, asthma and rhinitic symptoms compared with placebo both when added to background therapy and when that therapy was withdrawn. The clinical improvements on top of inhaled CS/LABA suggested that CS had not completely suppressed the Type 2 cytokine immune process in these patients with persistent eosinophilia. This study suggests that combined inhibition of IL-4/-13 modifies symptoms and FEV1, perhaps through effects to inhibit epithelial mucins/MUC5AC and smooth muscle activity (5, 91). Confirming that dupilumab impacted pathways downstream of IL-4/-13, reductions in serum and plasma levels of numerous Type 2 biomarkers, including thymus and activated regulated chemokine/CCL17, eotaxin-3/CCL26, and IgE, were observed. As had been seen with anti-IL-13 therapy, FeNO was decreased by dupilumab, and the decrease in FeNO inversely correlated with the improvement in FEV1. Of importance, and despite these profound clinical and biological effects, there were no improvements in blood (or sputum) eosinophil numbers. Thus these “human knockout” studies confirm the importance of IL-4/-13 to biological, physiological, and clinical characteristics in an eosinophilic/Type 2 phenotype. However, the eosinophilia associated with this molecular phenotype in mouse and humans does not appear to be controlled by IL-4 or IL-13.

**TYPE 2 CYTOKINE-ASSOCIATED MOLECULAR SUBPHENOTYPES** (FIG. 2). Elements of a Type 2 signature can be identified over a range of asthma severity levels and treatments (52, 108, 111, 114). However, the characteristics associated with a Type 2 signature may differ by severity level and clinical phenotype, such that a Type 2 signature alone may not predict disease severity or associated clinical phenotype. Whereas it identifies a traditional early-onset allergic asthma phenotype in patients with milder/CS-naïve asthma, it may also associate with a late-onset, severe-CS-dependent clinical phenotype (29, 111, 114, 116). Previous studies suggested that lung (tissue and sputum) eosinophils, as a Type 2 (IL-5) biomarker, were more prominent in adult-onset, less allergic, and more severe asthma, a group where anti-IL-5 appears to be quite effective (3, 41, 74) (Fig. 2B). Interestingly, this adult-onset Type 2 molecular phenotype was also reported to be specifically associated with higher epithelial levels of eotaxin-2/CCL24, one of the potent proeosinophilic chemokines compared with early-onset allergic asthma, potentially suggesting a mechanism for the increased eosinophilia (21). In contrast, an anti-IL-5 was ineffective in an allergen challenge model, whereas inhibitors of IL-4/-13 significantly impacted these responses (36, 61, 106). Thus, although the contributions of different Type 2 cytokines to disease may vary by clinical phenotype, an improved understanding of which phenotype is more strongly linked to which anticytokine will clearly advance our understanding of molecular phenotypes.

The distribution of Type 2-associated characteristics across different clinically recognizable phenotypes was further supported by a recent unbiased clustering analysis of over 300 patients with asthma and healthy controls who had undergone bronchoscopy and bronchoalveolar lavage (BAL) as part of the Severe Asthma Research Program (SARP) (112). With the use of 112 clinical, physiological, immunological, and lung/blood inflammatory characteristics, five asthmatic and one healthy control cluster were identified. BAL eosinophilia, as a marker of Type 2-associated inflammation, was greatest in a cluster of mostly severe, later (adult)-onset asthma with nasal polyps, sinusitis, and lesser allergy skin test reactivity. Success of anti-IL-5 in eosinophilic adult-onset asthma with nasal polyps, polyposis suggests that this Type 2 molecular subphenotype may do better with anti-IL-5-directed approaches (37, 40).

The presence of Type 2 molecular subphenotypes is also supported by in vitro lymphocytic expression studies, which report differences in expression of Type 2 cytokines by cellular phenotype. IL-4, -5, and -13 may arise from different cell types, with IL-5 expression being associated with both a more differentiated (“super”) Th2 lymphocyte (99). Alternatively, it (as well as IL-13) can be expressed by ILC2 cells (97). Thus a subphenotype identified by high levels of IL-5 with lesser association with IL-4 (and perhaps IL-13) potentially exists, which could selectively respond to IL-5-targeted therapy (Fig. 2C). However, this molecular pathobiology has not yet been confirmed in vivo.

In contrast, BAL eosinophilia was less in two clusters of early-onset asthmatics but more allergic mild to severe asthmatics than in the late-onset cluster. The efficacy of biologics targeted to IL-4/-13 in allergen challenge studies suggests that IL-4/-13-targeted therapies may be more effective in this Type 2 subphenotype, where eosinophilia is less central to disease outcomes (Fig. 2A).

Importantly, however, a fourth patient cluster identified the most severe patients with the most systemic CS use. In these patients, BAL eosinophils and neutrophils were persistently elevated, similar to previously reported associations of some patients with severe asthma with a mixed granulocytic inflammation (44, 76, 77). FeNO (as an additional Type 2 biomarker) was also highest in this severest patient cluster, despite more modest elevations in BAL eosinophils and low levels of blood
tissues, including obesity, sinus disease, gastroesophageal reflux, and vocal cord dysfunction may make it appear more severe (41, 70, 112). Interestingly, one of the unifying characteristicsof these non-Type 2 phenotypes, even in milder disease, is their relative CS unresponsiveness, perhaps attributable to lack of CS-responsive inflammatory elements (13, 70, 81).

**POTENTIAL MOLECULAR PHENOTYPES RELATED TO OBESITY.** Obesity in Western societies has become epidemic, with several epidemiological studies suggesting that obesity predisposes to asthma (67). Additionally mouse studies suggest that obese mice (obese because of leptin or leptin receptor deficiency) develop more AHR to ozone or allergen challenge, but the relationship to inflammatory mediators and cellular inflammation is less clear (54, 65, 93). The effect on AHR does not appear to be leptin dependent, and, similar to obese humans with asthma, the reasons for the increase in AHR are not clear (1, 92).

Whether obesity causes a distinct molecular asthma phenotype or whether it worsens existing disease in humans is controversial. One study reported that obesity in childhood-onset asthma is directly proportional to duration of disease, suggesting that it is a comorbidity but not a driver of disease (46). In contrast, the degree of obesity in asthma that develops later in life is not related to disease duration. Rather, it associates strongly with symptoms, health care utilization, and molecularly with low FeNO, eosinophils, and IgE levels (4, 46, 56), supporting a lack of Type 2 immune background. Supporting this, weight loss through bariatric surgery had a greater therapeutic effect on late-onset/non-Type 2 obese asthma compared with early-onset/Type 2-associated severe asthma (27).

Although highly preliminary, these studies suggest that obesity, with its associated metabolic and oxidative stress, may contribute to the development of a late-onset obese asthma molecular phenotype. Although there are few specifics regarding the oxidative stress pathway in obese asthma, obesity in general induces a higher oxidative stress level (9, 55). If there is an underlying, perhaps genetic increase in AHR, the addition of oxidative stress could make the condition worse. Preliminary studies have suggested that a compound increased in obesity and metabolic syndrome, asymmetric dimethylarginine (ADMA), is increased in late-onset obese asthma (47). ADMA is a known inhibitor of iNOS, which can switch iNOS from metabolizing arginine to NO to generating superoxide, which could then contribute to oxidative stress observed in late-onset obese asthma (103). Confirming the importance of this or other oxidative pathways to obese, non-Type 2 molecular (perhaps metabolic) phenotype of asthma will require specific antioxidant/metabolic pathway approaches.

**DO IL-17 OR TNF-A CONTRIBUTE TO MOLECULAR PHENOTYPES?** The lack of CS response in association with neutrophilia has suggested that the IL-17 pathway may be important in some poorly controlled non-Type 2 molecular phenotypes (2, 60, 71). IL-17 has been related to neutrophilia and steroid resistance, as well as enhanced allergic/complement-associated responses in mouse models. Although one study suggested a relation to smoking-associated asthma, with its generally neutrophilic phenotype, other recent data suggest that an active Type 17 (and neutrophilic) immune process may actually contribute to less severe disease (68, 69, 96). In fact, a monoclonal antibody to the IL-17 receptor, which inhibits all isoforms of IL-17, including the Type 2-associated cytokine IL-13, MOLECULAR PHENOTYPES OF ASTHMA

Persistent elevations of FeNO despite systemic CS in patients with severe asthma have also been seen in association with elevations of concomitant Type 1 cytokines (interferon-γ in particular). IL-4 and/or IL-13 in combination with interferon-γ synergistically enhance iNOS expression (39). This combination contributes to a high degree of nitrooxidative stress in the airway epithelium and, potentially, to severe disease (100). The specific contribution of Type 1 inflammation to certain Type 2 subphenotypes remains to be determined but may make this very severe Type 2 subphenotype more refractory to both CS and Type 2-directed therapy than other phenotypes. This complex immunity may explain the inability of another monoclonal antibody to IL-13 to improve outcomes in patients with more severe asthma than those previously studied (24).

**Non-Type 2 cytokine-associated asthma.** Although substantial strides have been made in identifying molecular markers and even nascent molecular subphenotypes of Type 2 cytokine-associated asthma, the same cannot be said for non-Type 2 asthma. In fact, the very definition of non-Type 2 asthma is the absence of any evidence for activated Type 2 pathways (low levels of FeNO, eosinophils, or Type 2 signature genes), without links to other molecular pathways/phenotypes. Nonetheless, there is considerable interest in this group of patients. Clinical phenotypes have been identified, as they relate to adult-onset obese asthma and smoking-related asthma. Less well defined phenotypes include paucigranulocytic asthma as well as neutrophilic asthma (with or without eosinophils). In some cases, statistical clustering approaches have identified these groups, but the relationship to molecularly defined inflammation has been weak (6, 41, 70).

Early suggestions that these non-Type 2 phenotypes/clusters may be more unstable over time than those related to Type 2 cytokines support the lack of a constantly active specific molecular pathway (12). Thus identification of these non-Type 2 phenotypes is limited by an overall lack of association between clinical characteristics, responses to therapy, and underlying pathology, including the presence of lung neutrophils.

Except for the poorly studied smoking-associated asthma, the underlying disease present in most non-Type 2 asthmatics may be less severe on the whole than those with persistent Type 2-related inflammation (41, 70, 112). Rather, comorbidi-
IL-25 (broludalumab), was modestly efficacious only in patients with asthma with a robust bronchodilator response (14). There was no relation to neutrophilic or eosinophilic inflammation.

Similarly, TNF-α and related Toll-like receptor pathways have been suggested to contribute to neutrophilic asthma by gene expression array profiling (6). However, the confirmation of the relevance of these pathways to a clinically identifiable phenotype that responds to selective molecularly targeted therapies does not yet exit. The few studies that have evaluated anti-TNF-α approaches in larger studies of moderate to severe asthma were unable to demonstrate efficacy in the total population and unable to identify neutrophilia (or eosinophilia) as a specific responder phenotype. Interestingly, subgroup analyses of the efficacy of anti-TNF-α identified robust bronchodilator responsiveness, later-onset disease, and sinusitis as elements of a responder phenotype in very severe asthma (107). However, despite the association of obese asthma with increases in TNF-α, the efficacy of the anti-TNF-α, golimumab, was not modified by obesity (66). Thus the relevance of specific immunological factors to some phenotypes of non-Type 2 asthma remains unknown.

The molecular underpinnings of smoking-related asthma are also not clear. It is likely that, in some cases, a Type 2 immune process was and still is present, perhaps exacerbated by effects of oxidative stress, neutrophilic inflammation, epithelial damage, and infection, particularly in those with early-onset disease (18). Interestingly, active smoking lowers FeNO levels although whether the mechanisms include inhibition of Type 2 cytokine pathways, increased levels of nitrative stress (as opposed to FeNO), or simply activation of non-Type 2 cytokine pathways, including neutrophilic processes, is not clear (72).

Conclusions

The view that asthma is a single disease is rapidly disappearing. The integration of clinical and statistically based phenotyping with improved understanding of molecular pathology and the confirmation of the relevance of a given pathway to clinically important outcomes has confirmed the existence of a broadly defined Type 2 cytokine molecular phenotype. This Type 2 phenotype generally responds better to nonspecific anti-inflammatory medications, like inhaled CS, as well as targeted biological therapies, than non-Type 2 phenotypes, establishing the clinical importance of this phenotyping. Further human and animal molecularly targeted studies are likely to refine this broad Type 2 molecular phenotype, improving the ability to therapeutically treat many patients with severe disease. However, uncertainties remain with this approach, as well. Although early studies with newer biologies are encouraging and in some cases even spectacular, with improvements in many relevant disease outcomes, the cost effectiveness of this approach is not clear. Whether single, reasonably inexpensive biomarkers will be able to adequately identify Type 2 patients whose disease is improved enough to justify the costs of these expensive biological therapies remains to be proven. Initial precision/personalized medicine approaches, especially if more than a single biomarker is needed, will be more feasible in patients with more severe asthma phenotypes, where costs are more easily justified. This approach may best be thought of as a proof-of-concept approach, confirming the importance of certain potentially critical pathways but also opening the door to development of less expensive drugs or technologies to treat these molecular phenotypes of asthma.

Molecular phenotypes beyond those related to Type 2 cytokine pathways remain uncharacterized. Integrated approaches using animal and human systems are needed to better understand those patients who appear to lack an underlying Type 2 process, evaluate the impact of comorbidities, and define their molecular, perhaps metabolic, underpinnings. Ultimately, it is hoped that these investigations will influence the approach to evaluation of asthma and its phenotypes and lead to improvements in their treatment.

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AUTHOR CONTRIBUTIONS

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