Eosinophilic and Noneosinophilic Asthma

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The term "eosinophilic" asthma (EA) generally refers to the clinical inflammatory phenotype of asthma wherein a significant number of sputum, airway, and/or blood eosinophils are present. Conversely, individuals with "noneosinophilic" asthma (NEA) may still demonstrate low numbers of eosinophils, but the dominant inflammatory cell type may include neutrophils, mixed granulocyte inflammatory cells, or very few inflammatory cells, termed paucigranulocytic inflammation. Although the mechanisms driving EA are becoming clearer, by contrast much less attention has been given to the underlying mechanisms that drive NEA, leading to a lack of available therapies for this patient population.

Although "phenotype" refers to the observable characteristics of disease in an individual, by "endotypes" we mean the specific biological mechanism that causes those observed properties of any given phenotype. Understanding the manifold mechanisms or endotypes contributing to EA and NEA and the resulting clinical phenotypes derived from these endotypes will lead to novel treatment options and provide an opportunity for more precise and tailored treatments. This review highlights the mechanisms currently believed to underlie the recognized subphenotypes of EA and NEA and briefly discusses their clinical presentations as well as implications for treatment.

Definition of Eosinophilic and Noneosinophilic Asthma

Asthma can be broadly classified as eosinophilic or noneosinophilic on the basis of airway or peripheral blood cellular profiles, with approximately half of individuals with asthma falling into each category (1–5). Sputum cellular profiles are believed to directly reflect lung inflammation and therefore are the preferred method used in asthma research to determine EA (6). Sputum eosinophils should be reported as a percentage of total cells from either a whole expectorate or sputum plug. Sputum eosinophil levels of greater than 2 to 3% have been used to define EA (7). However, technical requirements for sputum processing and cell counting may limit the feasibility of using sputum eosinophil counts in all clinical centers.

Eosinophils may also be detected in bronchial wall biopsies. Bronchial wall biopsy samples must be obtained via bronchoscopy, which, as a relatively invasive procedure, is not routinely performed in patients with asthma outside of the research setting. There is no actual cutoff for numbers of eosinophils in the airway wall that defines EA *per se*. Wenzel and colleagues demonstrated two distinct phenotypes on the basis of presence or absence of bronchial wall eosinophils in a severe asthma cohort, wherein those subjects with asthma with eosinophils demonstrated an average of 20 eosinophils/mm^2 of tissue (interquartile range, 16–31 eosinophils/mm^2) (5). In 2007, Berry and colleagues described compartmental inflammatory characteristics of asthma in a group of patients not using controller therapy. In the group of patients with EA, defined as sputum eosinophils greater than 2%, bronchoalveolar wash eosinophil count was 2.0% (geometric mean, 0.2), and the mean number of submucosal eosinophils detected was 23 eosinophils/mm^2 (interquartile range distance, 29 eosinophils/mm^2) (8). Supporting the use of these findings as endotypically meaningful, Jia and colleagues compared serum periostin, a biomarker produced by airway epithelial cells and shown to be elevated in T-helper 2 or type 2 (Th2/T2) asthma (9), to sputum and lower airway eosinophils (10). They demonstrated that serum periostin was significantly elevated in subjects with asthma who demonstrated airway wall eosinophilia (defined as >22 eosinophils/mm^2) and elevated sputum eosinophils (defined as >3%). However, the use of inhaled corticosteroids, which is first-line therapy for asthma, can blur this classification and reduce eosinophils, as demonstrated by Cowan and colleagues (11).

By comparison, blood eosinophil count is a convenient biomarker, because blood is easy to obtain and cell counting is standardized and/or mechanized in commercial laboratories. However, it is not available as a true point-of-care test, and

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values can fluctuate over time (12–14). Recently, the recommended range of peripheral blood eosinophilia considered consistent with EA has been derived from pharmacologic studies of biologics that target eosinophils (15–17). The range of peripheral eosinophilia that corresponds to treatment with anti–IL-5 biologics is in the range of 300 to 400 cells/μl, but data also suggest that response to these therapies can be seen with peripheral eosinophils as low as 200 cells/μl (18). Studies of the anti–IL-5 mepolizumab required only peripheral blood eosinophilia greater than or equal to 150 cells/μl at screening or 300 cells/μl in the preceding year (15, 16). However, blood eosinophil counts do not necessarily reflect sputum eosinophil counts. For example, Hastie and colleagues showed that sputum and blood eosinophils do not always correlate well, with an *R* value of 0.19 with approximately 40% misclassification (19). The extremes of blood eosinophil levels may be more sensitive and specific for identifying sputum eosinophils (20, 21). For example, patients with blood eosinophil counts less than 90 cells/μl are highly unlikely to have airway eosinophilia; alternately, almost all patients with more than 400 cells/μl can be expected to have significant sputum eosinophils (22).

In light of the challenges underlying comprehensive evaluation of compartmentalized eosinophilia, use of other biomarkers in combination with or in lieu of eosinophil counts may help to identify eosinophilic and noneosinophilic disease. For example, sputum and serum periostin levels, which reflect IL-13–related inflammation, are elevated in EA as compared with NEA, and this may also serve to help clinicians distinguish between these two subtypes (23, 24). The gene for dipeptidyl peptidase-4 is also induced by IL-13, expressed by eosinophils (25), and can predict individuals who will benefit from anti–IL-13 treatments for severe asthma (26). The fraction of exhaled nitric oxide may predict sputum eosinophilia (27). Levels of sputum, oropharyngeal, or nasal eosinophil peroxidase correlate strongly with airway eosinophilia in asthma (28) and may signal uncontrolled disease (29). Newer technology, including that of noninvasive exhaled breath analysis (i.e., eNOSE), can also identify lower airway eosinophilia in asthma (30). Although these biomarkers do not yet replace sputum eosinophilia as the gold standard of diagnosis, when and if clinically available they could be used as noninvasive markers of eosinophilic inflammation in asthma.

The lack of eosinophilia, by default, has been used to define NEA (31, 32). Those considered to have NEA can be further differentiated into paucigranulocytic (<61% neutrophils), mixed granulocytic, or neutrophilic asthma, with defining values of sputum neutrophilia ranging from as low as >40% to as high as ⩾76% (33, 34). Wenzel and colleagues demonstrated that in a cohort of subjects with severe asthma receiving high-dose inhaled and oral corticosteroids, some subjects with severe asthma had very few or no airway eosinophils (median 0; interquartile range, 0–2/mm^2) (5). Pavord and colleagues identified a group of subjects with asthma with very few eosinophils and a poor response to corticosteroids (4). The same group later demonstrated in a cohort of patients with asthma who were not taking controller therapy that NEA is a distinct pathological entity with sputum eosinophils less than 2% and relatively few submucosal eosinophils (median, 4.4/mm^2; interquartile range distance, 7.9/mm^2) (8). Blood neutrophil levels have not been strongly correlated with airway neutrophil counts, however, and other reliable biomarkers of airway neutrophilia have not yet been established (19, 21, 35).

Clinical Characteristics of EA and NEA

Although stratification by blood eosinophils is relatively easy, this does not create robust clinical phenotypes. Therefore, cluster analysis has been used to identify groups of patients with asthma who share specific clinical characteristics. For example, using unsupervised cluster analysis, which used clinical characteristics of age of asthma onset, lung function, bronchodilator reversibility, and demographics, the Severe Asthma Research Program (SARP) cohort identified five clinical clusters of adult asthma, in which four demonstrated eosinophilia of varying degrees (36, 37), and four clusters of pediatric asthma, all of which included eosinophilia (38). A recent investigation evaluating the ADEPT (Airways Disease Endotyping for Personalized Therapeutics) cohort identified four phenotypes, which were also present in the UBIOPRED (Unbiased Biomarkers for the Prediction of Respiratory Disease Outcome Consortium) cohort (39). Three of the four asthma clusters were associated with eosinophilia. A representative comparison of the clinical clusters from the three mentioned cohorts as examples of the cluster analysis approach to defining clinical characteristics in asthma is presented in Table 1. Although cluster analysis can define groups of patients with asthma with specific shared clinical characteristics, it does not define specific endotypes, as it is likely that several endotypes drive the phenotypes present in each cluster.

To engage the concept of endotyping to shed light on mechanisms driving clinical phenotypes, histopathology, genetics, and treatment response of asthma, in addition to the variables used by cluster analysis, Lötvall and colleagues (40) proposed several endotypes driving multiple clinical phenotypes, emphasizing that a clinical phenotype may be driven by more than one endotype. Wenzel and colleagues (41) proposed grouping asthma into Th2/T2 and non-Th2/T2, referring to the type of inflammation present, with many subendotypes leading to the commonly observed clinical phenotypes with and without eosinophils. The endotypes associated with clinical phenotypes characterized by eosinophilia included: early-onset allergic asthma with or without obesity, aspirin-sensitive asthma, late-onset EA, exacerbation-prone asthma, and exercise-induced asthma (Table 2, Figure 1). Clinical phenotypes of asthma not associated with eosinophils, and for which the pathobiologic pathways are not yet defined, include those patients with obesity-related late-onset asthma, asthma with fixed airflow obstruction and very little inflammation (paucigranulocytic), and asthma associated with neutrophilia (Table 2, Figure 1). Other noneosinophilic clinical phenotypes that are less well defined, but which may share similar endotypes, include smoking-related asthma, occupational asthma due to low molecular weight agents (42) or environmental pollution (43), and respiratory infections (44).

Mechanisms of EA

For clinicians and researchers, the implications of recognizing EA have two main facets. When assessing patients with

Table 1. Comparison of Clinical Cluster Analyses in Three Large, Well-phenotyped Asthma Cohorts

SARP Clusters	UBIOPRED Clusters	ADEPT Clusters
1: Early onset, mild, atopic, eosinophilic	US1: Mild asthma, good lung function, early onset, low inflammation	A1: Mild, normal lung function, early onset, low inflammation
2: Early onset, moderate, atopic, eosinophilic	US2: Moderate, hyperresponsive, eosinophilic	A2: Moderate, atopic, mild reversible obstruction, hyperresponsive, eosinophilic
3: Late onset, nonatopic, eosinophilic, female, obese	US3: Mixed severity, mild reversible obstruction, noneosinophilic, neutrophilic	A3: Mixed severity, mild reversible obstruction, noneosinophilic, neutrophilic
4: Early onset, severe, atopic, reversible obstruction, eosinophilic	US4: Severe uncontrolled, severe reversible obstruction, mixed granulocytic	A4: Severe uncontrolled, severe reversible obstruction, mixed granulocytic
5: Late onset, long duration, severe, fixed airflow obstruction		

Definition of abbreviations: ADEPT = Airways Disease Endotyping for Personalized Therapeutics; SARP = Severe Asthma Research Program; UBIOPRED = Unbiased Biomarkers for the Prediction of Respiratory Disease Outcome Consortium.

mild, moderate, or severe disease, eosinophilia can identify patients who may benefit more significantly from inhaled corticosteroids (45, 46). For patients with severe asthma, particularly among those not well controlled with high-dose inhaled steroids or requiring systemic steroids for disease control, the presence of eosinophilia can identify those patients appropriate for the biologic therapies that target eosinophils or related inflammatory pathways. With the observation that eosinophilia is identified across multiple clusters of asthma and disease severity, the focus is shifting toward defining the mechanisms underlying eosinophilia rather than simply identifying eosinophilia as present or absent. Indeed, multiple mechanisms have been identified and implicated in the initiation or potentiation of EA (47), particularly among the proposed asthma endotypes (Table 2). Among these emerging patterns are elevated IL-4 and IL-13 expression in early-onset atopic asthma and predominant IL-5 expression in later-onset, or less-atopic asthma (47, 48) (Figure 2).

The eosinophil is a terminally differentiated granulocyte with a bilobed nucleus and characteristic acidophilic granules. The presence of large specific granules, also known as secondary granules, is a characteristic feature that distinguishes eosinophils from other granulocytes, such as neutrophils and basophils. Specific granules consist of a dense crystalline core and a matrix, surrounded by a membrane (5, 49). They contain a large number of mediators capable of inducing inflammation and/or tissue damage, including basic proteins, cytokines, chemokines, growth factors, and enzymes. The main mediators are proteins, including major basic protein (MBP) located in the core, and eosinophil cationic protein, eosinophil peroxidase, and eosinophil-derived neurotoxin, which are located in the matrix (50). Primary granules are also present and contain Charcot-Leyden crystal protein, which exhibits lysophospholipase activity lipids. Lipid bodies, which are in the cytoplasm, are a key site of arachidonic acid esterification and eicosanoid production because of their high concentrations of relevant enzymes such as cyclooxygenases, 5-lipoxygenase, and leukotriene C4-synthase (51). Eosinophils also express a significant number of receptors, including cytokine receptors, pattern recognition receptors, Siglec 8, Fc receptors, lipid receptors, and major histocompatibility II receptors (49).

Eosinophil development in the bone marrow is stimulated by IL-3, IL-5, and granulocyte-macrophage colony-stimulating factor, and tissue recruitment through IL-5–induced upregulation of selectins and integrins acting synergistically through tissue expression of the eotaxins (eotaxin-1 [CCL11], -2 [CCL24], and -3 [CCL26]). IL-5 is potently expressed by type 2 innate lymphoid cells (ILC2s) and Th2 cells; therefore, blood and tissue eosinophilia can serve as evidence of inflammation driven by both cell types.

Increased numbers of $CD34^+$/IL-$5Ra^+$ eosinophil precursors have been identified in the bronchial biopsies of patients with asthma compared with control subjects without asthma (52). More recently, eosinophil progenitors isolated from the blood of patients with severe EA have been shown to have an exaggerated response to IL-5 *in vitro*, compared with eosinophil precursors from subjects with mild asthma, suggesting that *in situ* eosinophilopoiesis may have a clinically relevant role in severe EA (53). The differentiation of eosinophils is regulated by the transcription factors GATA-binding protein 1 (GATA-1), PU.1, and the CCAAT-enhancing binding protein family. GATA-1 and PU.1 synergistically promote transcription of MBP (54). GATA-1 is believed to have the most important role, as disruption of the GATA-1 gene in mice results in a strain completely devoid of eosinophils (55).

The proteins contained in eosinophil granules (i.e., MBP, eosinophil peroxidase, eosinophil cationic protein, and eosinophil-derived neurotoxin) have direct toxic effects on host tissues and can promote inflammation (56, 57). Host factors, such as innate immune molecules like surfactant protein-A, may interact with eosinophils to promote or inhibit their antimicrobial function (58). Furthermore, eosinophils synthesize and release type 1 and type 2 inflammatory cytokines and chemokines, which can further modulate or stimulate the immune response (57). Therefore, far from being a passive biomarker, eosinophils play a significant role in disease pathogenesis and guide phenotypic characteristics in asthma.

Mechanisms of Allergic EA

The association between allergy and asthma is widely recognized. As a group, individuals with the "allergic asthma" endotype have earlier-onset disease that is triggered predominantly by exposure to aeroallergens and commonly seen with comorbid allergic rhinitis or other atopic disorders.

Table 2. Clinical Phenotypes and Proposed Endotypes of Eosinophilic and Noneosinophilic Asthma

Phenotype	Clinical Characteristics	Biomarkers	Lung Physiology	Targeted Treatments	Possible Mechanism (Endotype)
Early-onset, allergic asthma	Childhood onset, allergen triggers, allergic rhinitis	Aeroallergen-specific IgE, elevated total IgE, elevated $F_{E_{NO}}$	Bronchospasm with allergen exposure	ICS, anti-IgE, inhibition of IL-4/13 pathways	IgE-mediated Th2/T2 inflammation
Early onset, obesity exacerbated	Obesity, childhood onset, allergy	Allergen-specific IgE, eosinophilia	Airway hyperresponsiveness	Weight loss	Eosinophil recruitment, airway remodeling
Aspirin-exacerbated respiratory disease	Adult onset, severe asthma, nasal polyposis	Eosinophils, leukotrienes	Bronchospasm with aspirin/NSAID exposure	Aspirin desensitization, leukotriene inhibitors, systemic steroids	Eicosanoids
Allergic bronchopulmonary mycosis	Adult onset, severe asthma, pronounced mucus production	High IgE, eosinophils in blood and sputum, specific IgE to mold	Fixed airflow obstruction can develop with bronchiectasis and/or fibrosis	Systemic steroids, antifungals, anti-IgE	Fungal colonization with Th2/T2 and mixed responses
Asthma-predictive index-positive preschool wheezer	>3 episodes of wheeze per year, 1 major or 2 minor atopy characteristics	Eosinophils, aeroallergen-specific IgE	Potential increased risk of loss of lung function	ICS	Th2/T2 inflammation, eicosanoids
Severe late onset hypereosinophilic	Adult onset, severe exacerbations, less atopic	Eosinophils in blood and sputum, sinusitis	Variably steroid-sensitive obstruction	Systemic or inhaled steroids, anti–IL-5 agents	ILC2 activation; dual-positive Th2/Th17 inflammation
Exacerbation prone	Frequent exacerbations, sinusitis, GERD	Eosinophils in blood	Lower lung function	Antieosinophil	Eosinophil-driven inflammation
Exercise-induced asthma	Symptoms develop with or after exercise, more so in cold, dry air	Some aeroallergen-specific IgE, variable eosinophils in blood and sputum	Bronchospasm with sustained exercise	ICS, systemic steroids, leukotriene antagonists, reduction of exercise intensity	Th2/T2 in some, but not clear in others
Neutrophilic	Adult onset, variable severity	Elevated neutrophils in blood and sputum	Reduced lung function, less bronchodilator reversibility	Steroids are variably effective; antibiotics	Lack of Th2/T2 inflammation; Th17, infection, inflammasome
Obesity induced	Adult onset predominantly female, very symptomatic	Lack of Th2 biomarkers, IL-6, leptin	Variable restriction, bronchial hyperreactivity	Weight loss, lower-fat diet, antioxidants	IL-6, oxidative stress pathways
Paucigranulocytic	Mild and severe	Lack of airway inflammation	Fixed airflow obstruction, bronchial hyperreactivity	Antimuscarinics, β_2 antagonists, bronchial thermoplasty	Airway remodeling, increased smooth muscle tone, thickened basement membrane
Asthma with smoking	Current or former tobacco smoke exposure, worse quality of life and more symptoms, corticosteroid insensitivity	Less eosinophilic, more neutrophilic	Fixed airflow obstruction	Smoking cessation, steroids, antimuscarinic antagonists	Epithelial dysfunction, altered histone deacetylase

Definition of abbreviations: $F_{E_{NO}}$ = fraction of exhaled nitric oxide; GERD = gastroesophageal reflux disease; ICS = inhaled corticosteroids; ILC2 = innate lymphoid cells type 2; NSAID = nonsteroidal antiinflammatory drug; T2 = type 2; Th2 = T-helper type 2 cell; Th17 = T-helper type 17 cell.
Adapted by permission from Reference 40; some data from Reference 137.

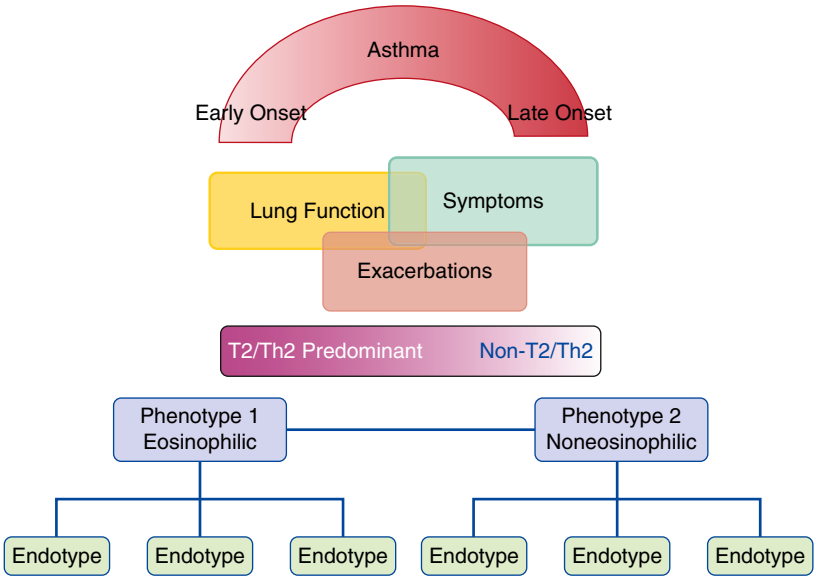

Figure 1. Schematic representation of asthma and the spectrum of inflammation type and endotypes underlying clinical phenotypes. T2 = type 2; Th2 = T-helper type 2 cell. Adapted by permission from Reference 41.

Characteristic of atopic disease, the type 2 inflammation seen in this asthma endotype is mediated by antigen-specific Th2 cells; secretion of IL-4, IL-5, and IL-13 from these and other cells; IgE-mediated mast cell and basophil degranulation; and eosinophilia (Figure 2). IL-5 recruits eosinophils to the site of antigen exposure, and the eotaxins establish a powerful chemotactic gradient inducing eosinophil influx into airways. Murine and *ex vivo* human experiments support that IL-13, also an eosinophil product (49, 57), contributes to the bronchial hyperreactivity characteristic of asthma (47, 59). Over time, these pathways may no longer require external stimulation and can become self-perpetuating (60). Airway remodeling may contribute to the persistent changes in lung function, which may be in part attributed to the presence of eosinophils through their expression of transforming growth factor-β (61). Inhibition of IgE-mediated pathways through treatment with omalizumab can reduce the prominent viral-induced inflammation related to asthma exacerbations in atopic individuals (62).

Obesity is believed to exacerbate preexisting T2, EA through speculated mechanisms, including altered type 2 inflammation, suppressed CD4 effector cell function, and immune aging, as well as via remodeling and metabolic mechanisms (63–65). For example, a high-fat diet may increase bone marrow eosinophilia, with subsequent alteration in eosinophil trafficking (63, 66), and contribute to airway structural changes associated with increased collagen deposition (63). Lipid profiles consistent with metabolic syndrome negatively correlated with blood eosinophil counts in patients with atopic asthma, suggesting, type 2 inflammation in asthma may be modulated by lipids or related metabolic pathways not previously appreciated (67). In addition, inhibition of tumor necrosis factor (TNF)-α, which is elevated in the airways of obese individuals, can lead to improvements in airway hyperresponsiveness and increased eosinophil apoptosis in obese mice and may function through mechanisms involving the antiinflammatory effects of surfactant protein-A (68, 69). Adipokines such as leptin, which is elevated in obesity, increased airway hyperresponsiveness and serum IgE in allergic mouse models (63, 70), whereas adiponectin reduces allergic inflammation and hyperresponsiveness, particularly at elevated serum levels (63). Additional mechanisms connecting obesity to increased type 2 and EA continue to be discovered, and additional work will be necessary to clarify these pathways (71, 73).

Allergic bronchopulmonary mycosis (ABPM) is a distinct disease entity related to allergic EA, characterized by a pronounced eosinophilic, neutrophilic, and lymphocytic response to mold exposure in the lung, most commonly to *Aspergillus fumigatus* (74). Although mold-specific IgE and markedly elevated total IgE levels are characteristic of this disease, patients with ABPM can have pronounced peripheral blood and sputum eosinophilia (75). The factors contributing to disease development are not well understood. However, *A. fumigatus* proteases have been shown to directly cause bronchial epithelial cell damage and stimulation of cytokines and chemokines (76). *Aspergillus* proteins are processed by dendritic cells, particularly those with HLA (human leukocyte antigen)-DR2/DR5, which induce a potent type 2 inflammatory response (77). IL-4 receptor polymorphisms (78) and IL-10 promotor polymorphisms (79) increase sensitivity to type 2 inflammation and together may drive the development of ABPM. Furthermore, polymorphisms in toll-like receptors (80) and pulmonary surfactant protein-A (81) have been identified and likely participate in susceptibility to disease.

Mechanisms of Nonallergic EA

Allergy is not a prominent mechanism in some subphenotypes of EA. When asthma is distinguished by expression of IL-4, IL-5, and IL-13 from sputum cells, those with levels higher than healthy control subjects have higher peripheral blood eosinophil counts than those with low Th2 gene expression (9, 82). It is noted, however, that patients with asthma with lower sputum Th2 gene expression may still have significant peripheral blood eosinophilia (82). The presence of activated type 2 innate lymphoid cells (ILC2) in the airways of patients with asthma correlates with airway eosinophilia, asthma development, and severe asthma, and these cells seem to be an important local source of the IL-5 and IL-13 driving the nonatopic subgroup of EA (83). Indeed, a lack of peripheral blood ILC2s can also be used to distinguish NEA from EA (84).

The subphenotype of late-onset EA is characterized by severe asthma with persistently elevated sputum and peripheral blood eosinophilia that is relatively corticosteroid insensitive (40, 85, 86). The nonatopic eosinophilic airway inflammation in this subgroup of asthma is likely induced by innate immune pathways

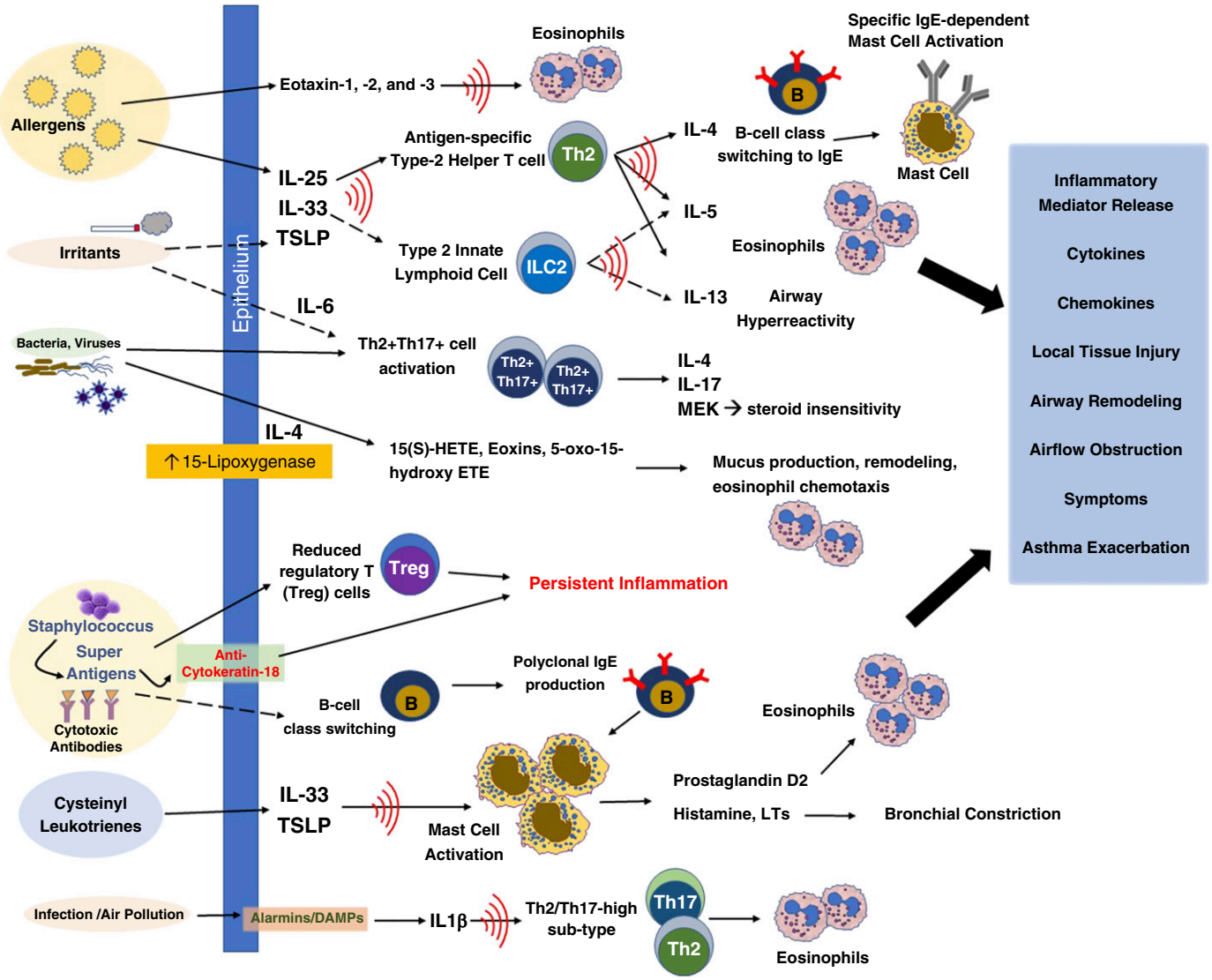

Figure 2. Mechanisms of eosinophilic asthma. DAMP = damage-associated molecular patterns; ETE = eicosatetraenoic acid; HETE = hydroxyeicosatetraenoic acid; LT = leukotriene; MEK = MAPK/ERK kinase; Th = T-helper cell; TSLP = thymic stromal lymphopoeitin.

that include type 2 inflammatory pathways but in which IgE-mediated signals are not prominently contributing (Figure 2). Briefly stated, murine and human data suggest that irritants/pollutants and microbes induce airway epithelial injury, which in turn releases IL-25, IL-33, and TSLP (thymic stromal lymphopoeitin), stimulating ILC2s to release IL-5, promoting eosinophilia, and IL-13, thereby inducing airway hyperreactivity (47) (87). The lack of IL-4 production minimizes B cell class switching to IgE, and therefore antigen-specific IgE is not prominent in these patients (47).

The presence of Th17-type inflammation may be contributing to the more severe, late-onset eosinophilic subphenotype. Irvin and colleagues reported the presence of helper T cells expressing both Th2 and Th17 cytokines (IL-4 and IL-17) in a subgroup of patients with asthma, in whom airway cells are predominantly eosinophilic and elevated blood eosinophil level is common (88). These patients with dual-positive asthma were clinically consistent with the late-onset eosinophilic phenotype, with more significant airway hyperresponsiveness, relative corticosteroid insensitivity, and no increased atopy when compared with the groups with only Th2 or neither Th2- nor Th17-predominant inflammation. This group then examined inflammatory markers in the BAL fluid and cells from patients with Th2, Th17, and Th2/Th17 dual-positive asthma (89). The dual-positive group expressed more IL-6, IL-1β, and IL-23 as well as other molecules related to Th17 pathways, and *in vitro* experiments suggested the induction of Th17 cells was subsequent to IL-1β–mediated inflammation.

The exacerbation-prone phenotype of asthma describes individuals who have more than three exacerbations of asthma per year. Denlinger and colleagues (90) described a phenotype of exacerbation-prone adults with asthma, which was not associated with asthma severity, duration, age of onset, race, socioeconomic background, or allergy. However, blood eosinophilia as well as elevated body

mass index, sinusitis, and gastroesophageal reflux disease are prominent clinical features of this patient group. Although mechanisms underlying this phenotype are not well defined, the antiexacerbation benefit of eosinophil-targeted biologic drugs suggests the eosinophil contributes to the disease pathology (15, 16).

Although not extensively studied, mechanisms related to staphylococcal enterotoxins may also be important in the production of superantigens driving nonatopic eosinophilic disease (91). Superantigen exposure can drive class switching of local B cells, leading to polyclonal IgE production in the airways, resulting in non–antigen-specific mast cell activation. This leads to T-cell activation and suppression of regulatory T cells. Production of cytotoxic autoantibodies directed against epithelial proteins (i.e., cytokeratin-18) can also be detected. Superantigens can also contribute to loss of corticosteroid sensitivity in asthma leading to severe symptoms and may contribute to the steroid insensitivity seen in late-onset severe EA.

Aspirin-exacerbated respiratory disease (AERD) is a distinct clinical subphenotype of asthma that is also characterized by a specific endotype involving exposure to cyclooxygenase-1 inhibitors such as aspirin or nonsteroidal antiinflammatory drugs. Patients have severe disease presenting as persistent asthma symptoms, refractory nasal polyposis, and chronic eosinophilic rhinosinusitis, which can be complicated by acute respiratory reactions on exposure to cyclooxygenase-1 inhibitors. Inflammation in these individuals is characterized by the overproduction of cysteinyl leukotrienes through the 5-lipoxygenase pathway, upregulation of cysteinyl leukotriene receptors, and increased production of prostaglandin D2 (92). Despite histologic and chemical evidence of ongoing mast cell activation, eosinophil recruitment, and eosinophil activation, individuals with AERD, although they have normal or elevated levels of serum IgE, often have no detectable antigen-specific IgE against common aeroallergens (93). Although these patients manifest notable EA, they are clearly distinct from those with classic type 2 allergic inflammation and may require disease-specific targeted interventions, including aspirin desensitization and blockade of leukotriene effect through 5-lipoxygenase inhibitors or leukotriene receptor antagonists.

Lipid mediators also contribute to asthma pathogenesis and inflammation in non-AERD eosinophilic and noneosinophilic phenotypes. The 15(s)-hydroxyeicosatetraenoic acid metabolite of the 15-lipoxygenase pathway has been strongly linked to severe asthma with airway eosinophilia and associated subepithelial remodeling (94). Furthermore, these 15-lipoxygenase pathways, which can be induced by infection or cell injury (95), have also been shown to affect intracellular airway epithelial cell signaling (96) and chemokine and cytokine production by lung macrophages (97) and to induce airway mucus production (98).

Mechanisms of NEA

Less is known about NEA. Similar to the case in EA, the literature supports multiple molecular pathways underlying each phenotype. Emerging subphenotypes of NEA include neutrophilic asthma, obesity-induced asthma, paucigranulocytic asthma, and asthma related to environmental exposures. Although atopy can be present in some of these patients, asthma with predominant neutrophilic inflammation or the paucigranulocytic subtype represent a relatively Th2/T2-low inflammatory milieu. Instead, pathways inducing neutrophilic inflammation and airway dysfunction are implicated.

Mechanisms of Neutrophilic Asthma

Corticosteroids are known to inhibit neutrophil apoptosis, which in turn may prolong or promote airway neutrophilia, potentially leading (or at least contributing) to the diagnosis of neutrophilic asthma (99). This calls into question whether the continued use of corticosteroids to treat severe neutrophilic asthma may promote or complicate diagnosis of the neutrophilic phenotype in patients using inhaled or systemic corticosteroids. Despite this, many potential molecular pathways may be implicated in the development of the neutrophilic asthma subphenotype independent of corticosteroid use (Figure 3). Clinically, patients with neutrophilic asthma develop asthma at an older age and demonstrate impaired lung function, less bronchodilator reversibility, and less atopy (Table 2) (39, 100).

Differential inflammation through both the acquired and innate immune pathways distinguishes neutrophilic asthma from EA. The innate immune pathway is activated during infection (viral, bacterial) or during irritant environmental exposure, such as endotoxin, ozone, and particulate matter exposures. Instead of triggering the aforementioned ILC2 or T2 pathways, these agents can activate toll-like receptor 4 and CD (cluster of differentiation)-14 on epithelial cells and macrophages leading to nuclear factor (NF)-κB activation, which in turn establishes a highly proinflammatory state (101, 102). This then leads to heightened production of IL-8, which recruits activated neutrophils into the airways (Figure 3).

Neutrophilic inflammation in asthma is related to markers of systemic inflammation and metabolic dysfunction, including elevation in biomarkers such as IL-6, C-reactive protein, and TNF-α (103, 104). The extent to which IL-6 is pathogenic in neutrophilic asthma is not yet clear; however, *in vitro* studies of human airway smooth muscle suggest that trans-signaling of IL-6 through the IL-6 receptor may contribute to airway remodeling and smooth muscle cell proliferation in asthma (105).

It is very likely that other pathways and mechanisms contribute to airway neutrophilia either alone or in combination. One potential explanation for an increase in sputum neutrophil levels in neutrophilic asthma may be the result of impaired alveolar macrophage phagocytosis of apoptotic cells, termed "efferocytosis." In a study of patients with stable asthma undergoing sputum induction, macrophage efferocytosis was impaired in patients with neutrophilic asthma leading to persistent airway neutrophilia but was not impaired in those with EA (106). The mitogen-activated protein kinase family, including the p38 kinases, is activated in various inflammatory responses, including eosinophilia and neutrophilia. The p38 kinase is also induced by environmental triggers (e.g., endotoxin, oxidative stress, cigarette smoke, air pollution) relevant to asthma and in particular to NEA. Inhibition of p38/mitogen-activated protein kinase mitigates neutrophilic inflammation, which may thus play a role in neutrophilic asthma (107).

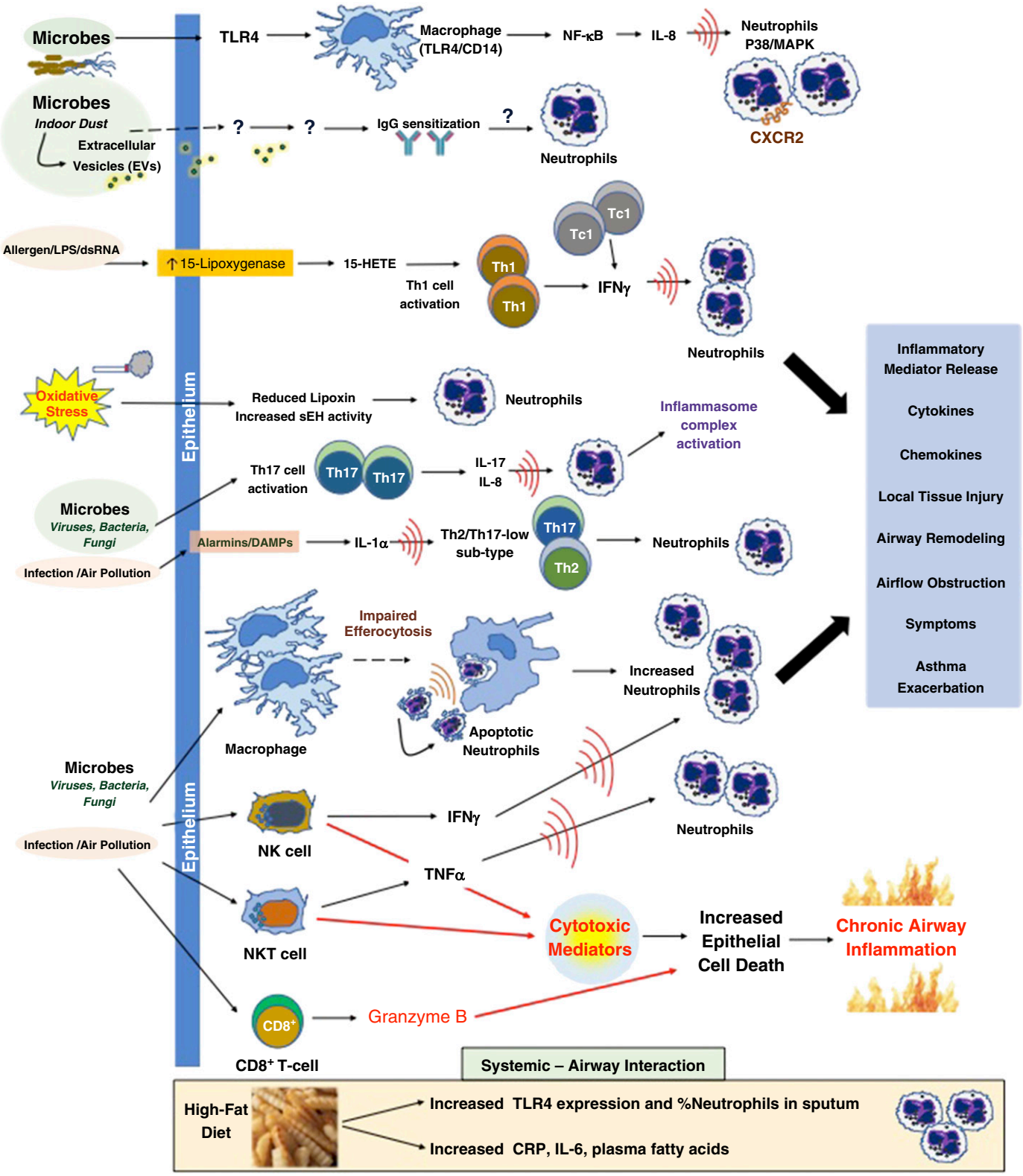

Figure 3. Mechanisms of noneosinophilic asthma. CRP = C-reactive protein; CXCR2 = chemokine receptor 2; DAMP = damage-associated molecular patterns; dsRNA = double-stranded RNA; HETE = hydroxyeicosatetraenoic acid; MAPK = mitogen-activated protein kinase; NF-κB = nuclear factor κB; NK = natural killer; sEH = soluble epoxide hydrolase; Tc1 = cytotoxic T-cell subset 1; Th = T-helper cell; TLR4 = toll-like receptor 4; TNFα = tumor necrosis factor-α.

The lipoxins are endogenous arachidonate-derived proresolution mediators that decrease inflammation and are reduced in severe asthma. Lipoxins inhibit eosinophil trafficking and neutrophil chemotaxis, transcellular migration, and toxic degranulation, and may support natural killer cell function in severe asthma (108). Oxidative stress in asthmatic airways is associated with reduced lipoxin levels and with activity of the enzyme-soluble epoxide hydrolase. Inhibition of this enzyme increases lipoxin levels that mediate antiinflammatory pathways relevant to asthma (109). The antiinflammatory club cell secretory protein CC16 (approved symbol SCGB1A1) has been directly correlated with lung function (110) and may function as an antiinflammatory mediator through effects at the lipoxin A4 receptor, such as IL-8 and serum amyloid A inhibition (111). Treatments that enhance lipoxin levels or CC16 or inhibit soluble epoxide hydrolase may be beneficial in neutrophilic asthma, given the prominent cellular effects of lipoxins on lung neutrophils.

In neutrophilic asthma, there is evidence of upregulated nucleotide-binding domain, leucine-rich repeat-containing family protein-3 inflammasome activity (112). In a recent study using transcriptome-associated clusters, the cluster that was characterized by IFN-, TNF-, and inflammasome-associated genes had the greatest sputum neutrophilia as compared with other clusters, which were, by contrast, associated with type 2 inflammation or metabolic/mitochondrial genes and sputum eosinophilia (113). This suggests a role for inflammasome mechanisms in neutrophilic asthma not previously appreciated.

NK and NKT-like cells are effector lymphocytes that are a major source of proinflammatory and cytotoxic mediators with relative corticosteroid insensitivity, which may contribute to the development of neutrophilic asthma. Evidence supporting this notion includes increased expression of cytotoxic markers in poorly controlled neutrophilic asthma. For example, in one cohort, subjects with poorly controlled neutrophilic asthma had higher expression of granzyme B by $CD8^+$ T cells, greater IFN-γ production by NK cells, and greater TNF-α production by NKT-like cells than healthy control subjects (114). Cytotoxic effects of granzyme B may lead to increased epithelial cell death and chronic inflammation, which contribute to persistent symptoms despite therapy with corticosteroids (115).

Because IL-17 can induce prominent neutrophilic inflammation, the IL-17 axis is gaining significant attention for its contribution to both neutrophilic (116) and mixed granulocytic airway inflammation. In asthma, Th17 inflammation is related to a severe phenotype, frequent exacerbations, steroid resistance, bronchial wall remodeling, mucus hypersecretion, and smooth muscle hypertrophy (117, 118). IL-17 may also contribute to severe asthma by inducing relative steroid unresponsiveness (119). Irvin and colleagues discovered that patients who lack significant Th2/Th17 inflammation have more significant airway neutrophilia than those with Th2 or Th2/Th17 dual-positive markers (88). The presence of type 2 cytokines may inhibit production of IL-8 and thus abrogate the development of neutrophilia despite the presence of Th17-type inflammation (89, 120). In the cohort by Liu and colleagues, many of those patients with neutrophilic asthma also had evidence for bacterial lung infection (89).

Chronic infection may be a key underlying stimulus for this Th17-type inflammatory pathway in asthma, given that Th17 cells produce IFN-γ and IL-17 in addition to other proinflammatory cytokines, in response to bacterial and fungal infection (121, 122). Indeed, neutrophilic asthma could be a manifestation of individuals with asthma with underlying chronic subclinical infection or inhaled exposures (123). Biological contaminants in indoor air originating from microorganisms can lead to immune dysfunction and inflammation leading to or exacerbating lung disease. For example, extracellular vesicles originating from microorganisms in indoor dust can cause neutrophilic inflammation in asthma (124). In murine models, there is evidence suggesting that 15-lipoxygenase metabolites, such as 15-hydroxyeicosatetraenoic acid, are important mediators in the development of Th1 inflammation induced by ovalbumin and double-stranded RNA, as well as in the development of EA. This suggests that virus-associated neutrophilic asthma with high IFN-γ, an exacerbation-prone clinical phenotype, may be explained by a Th1 response via a 15-lipoxygenase mechanism (125).

A significant proportion of patients with asthma are current smokers, at rates similar to the general population (126). Multiple cohorts have described smokers with asthma as suffering from more severe disease and having fewer sputum eosinophils; ex-smokers and current smokers have predominant neutrophilic inflammation (127, 128). Steroid insensitivity in this phenotype may be due to alterations in histone deacetylase activity (129). Cigarette smoke is toxic to the respiratory epithelium (130) and in fact may induce type 2 inflammation from the subepithelial dendritic cells, even through *in utero* exposure (131, 132). Occupational asthma due to low-molecular-weight agents (42) and secondary to environmental pollution (43) may be induced by similar pathways.

Paucigranulocytic asthma represents up to 40% of NEA (133). Although paucigranulocytic asthma is associated with well-controlled or mild intermittent asthma in the SARP cohort (34), it can be severe and may not respond well to standard asthma medications, such as inhaled corticosteroids. The mechanisms underlying paucigranulocytic asthma are believed to be related to abnormalities or dysfunction of structural cells, including airway smooth muscle, nerves, and vascular tissue. Airway remodeling, such as airway smooth muscle hypertrophy and hyperplasia, subbasement membrane fibrosis, and mucus gland hypertrophy, may occur as a result of aberrant repair mechanisms (134). Airway bronchospasm can induce transforming growth factor-β release and subbasement membrane fibrosis in the airway (135). Increased bronchomotor tone from muscarinic and adrenergic pathways can contribute to paucigranulocytic asthma symptoms and may be successfully treated with muscarinic and β_2 receptor antagonists (136, 137). Airway smooth muscle is abnormal in asthma, with even patients with mild asthma demonstrating hyperplasia (138). This abnormal airway smooth muscle can not only contract to cause airflow obstruction but also actively secrete chemokines and cytokines that promote and mediate inflammation (139, 140). Bronchial thermoplasty can reduce airway smooth muscle mass and collagen deposition and thereby reduce the risk of acute exacerbations (141). Therefore, bronchial thermoplasty may be very useful in this population of more severe paucigranulocytic asthma. However, its benefit in other asthma

phenotypes and endotypes is not yet known and is currently under investigation.

Mechanisms of Obesity-induced NEA

Obesity and diet are increasingly recognized as important and interrelated variables in the development and severity of asthma. Obesity can complicate early-onset allergic asthma, as previously described. However, asthma can also be consequent to obesity, in a late-onset, female-predominant phenotype (142, 143). This obesity-induced asthma is associated with neutrophilic or paucigranulocytic airway inflammation with low eosinophil counts (66, 104). In these patients, peripheral lung tissue elastance and airway resistance are abnormally elevated, but the increased peripheral airway sensitivity to closure can be reversed with significant weight loss (142, 144). The mechanisms underlying these changes may relate to inflammation in the adipose tissue mediated by activated macrophages, causing increases in IL-6, TNF-α, and leptin that are also reversible with weight loss (68). Interestingly, abnormal IL-6 inflammation can be detected in even nonobese patients with asthma (104), suggesting that metabolic pathways can be aberrant independent of body mass index. Patients with obesity-induced asthma may be more sensitive to environmental pollutants and irritants than those who are nonobese (63). For example, ozone exposure can decrease lung function, increase airway neutrophilia, and induce IL-6 in obese women with asthma (71).

Dietary consumption of fat, antioxidants, and lycopene may impact this obese-asthma airway inflammation and lung function. Nonobese patients with asthma eating a high-fat meal developed a decrease in lung function and increase in sputum neutrophilia and toll-like receptor 4 mRNA expression 4 hours post meal as compared with the low-fat group (72, 73). The genes involved in this high-fat meal mechanism in asthma include those important in immune system processes, including calcium ion binding/signal transduction, calcium binding signaling, T-cell differentiation, apoptosis, antimicrobial glycoproteins and mucosal protection, and noncanonical NF-κB activity, to name a few (70). In a high-fat diet mouse model of obese asthma, airway hyperreactivity was found to be independent of adaptive immunity, instead characterized by IL-17A released by ILC3 cells and macrophage-derived IL-1β (145). Consumption of a low antioxidant diet is associated with worse asthma symptoms, reduced lung function (FEV_1, FVC), and increased percentage sputum neutrophils. Treatment with lycopene-rich supplements reduces sputum neutrophil elastase activity and may be useful in the treatment of NEA (76). Therefore, obesity is a complex modulator of inflammation that affects EA by perpetuating T2/Th2 inflammation and NEA as a distinct subphenotype driven by several mechanisms or endotypes.

Implications for the Treatment of Asthma

Although eosinophils contribute to inflammation in asthma and may be present because of more than one molecular mechanism, the depletion of eosinophils can improve asthma control and exacerbation risk in many patients with asthma. Specific biologics evaluated

Table 3. Representative Biologic Therapies in Use or under Investigation for Eosinophilic Asthma

Target	Treatment	Studies	Patient Population	Outcomes
IL-5	Anti–IL-5 IgG mepolizumab*	Bel *et al.*, 2014 (15); Ortega *et al.*, 2014 (16)	Severe asthma, peripheral blood eosinophilia ⩾ 150 cells/μl at screening or 300 cells/μl in the preceding year	Steroid sparing, reduced exacerbations, improved control
	Reslizumab*	Castro *et al.*, 2015 (146)	Uncontrolled moderate to severe asthma with peripheral blood eosinophilia ⩾ 400 cells/μl and ⩾1 exacerbation in the past year	Reduced exacerbations
IL-5 receptor	Benralizumab	Bleecker *et al.*, 2016 (17); FitzGerald *et al.*, 2016 (153)	Severe asthma, peripheral blood eosinophilia > 300/μl	Reduced exacerbations, improved lung function and symptom control
IgE	Omalizumab*	Cochrane Review 2014 (154)	FDA approved for treatment of moderate to severe allergic asthma, age ⩾ 6 yr	Improved asthma symptom control, reduced exacerbations, reduced eosinophil counts
IL-4 receptor, α subunit	Dupilumab	Wenzel *et al.*, 2016 (149)	Phase IIb: Uncontrolled moderate to severe asthma (n = 769)	Improved lung function, reduced exacerbations
Prostaglandin D2 receptor 2	Fevipiprant	Gonem *et al.*, 2016 (155)	Phase II: Moderate to severe asthma with sputum eosinophils ⩾ 2%	Reduced sputum eosinophils, improved quality of life and lung function

Definition of abbreviation: FDA = U.S. Food and Drug Administration.
*FDA approved at time of article submission.

as treatment for EA have helped to define what is "eosinophilic" and perpetuate the phenotypic distinction of EA versus NEA (15–17). Two IL-5 inhibitors, reslizumab (146) and mepolizumab (16), are available for clinical use in the United States for treatment of severe EA. One IL-5 receptor antagonist, benralizumab, has at the time of this review just received U.S. Food and Drug Administration approval for the treatment of severe EA (147). Furthermore, the presence of sputum or blood eosinophilia is a biomarker of response to systemic and inhaled corticosteroids (4, 8, 148, 149). EA may also benefit from non–IL-5–specific biologic therapies such as dupilumab, which inhibits both IL-4 and IL-13 signaling through blocking the IL-4 receptor alpha chain. However, benefits from dupilumab are not limited to those with eosinophilia (150). Biologic treatments in development for the treatment of severe EA also target type 2 inflammatory pathways and are listed in Table 3.

Table 4. Representative Therapeutics in Use or under Investigation for Noneosinophilic Asthma

Target	Treatment	Studies	Patient Population	Outcomes
Muscarinic receptors	Tiotropium* (long-acting muscarinic antagonist)	Iwamoto *et al.*, 2008 (137)	Severe asthma ($n = 17$)	Improvement in lung function with increasing sputum neutrophils
Bacteria	Clarithromycin (macrolide antibiotic)	Simpson *et al.*, 2008 (156)	Severe refractory asthma ($n = 45$)	Improved QOL scores in NEA
	Azithromycin (macrolide antibiotic)	Brusselle *et al.*, 2013 (157)	Severe asthma ($n = 109$)	Fewer exacerbations and lower respiratory infections in NEA only
HMG-CoA reductase	Atorvastatin	Braganza *et al.*, 2011 (158)	Smokers with asthma with NEA ($n = 71$)	Improved QOL at 4 wk but not 8 wk
PPARγ	Rosiglitazone (PPARγ agonist)	Spears *et al.*, 2009 (159)	Smokers with mild to moderate asthma ($n = 46$)	Improvement in lung function at 28 d
	Pioglitazone (PPARγ agonist)	Dixon *et al.*, 2015 (160)	Obese patients with asthma ($n = 23$)	No improvement; weight gain
PDE	Theophylline* (nonselective PDE inhibitor)	Spears *et al.*, 2009 (161)	Smokers with asthma with NEA ($n = 68$)	Improved symptoms and peak flow at 4 wk
	Roflumilast (PDE4 inhibitor)	Gauvreau *et al.*, 2011 (162)	Mild allergic asthma ($n = 25$)	Inhibits allergen-induced increase in sputum neutrophils and eosinophils
		Meltzer *et al.*, 2015 (163)	Pooled analysis of 9 randomized studies on moderate to severe asthma ($n = 4{,}873$)	Improved asthma symptom control and lung function
CXCR2	SCH527123 (CXCR2 antagonist)	Nair *et al.*, 2012 (164)	Severe asthma with sputum neutrophilia > 40% ($n = 34$)	Fewer mild exacerbations
IL-17 receptor	Brodalumab (anti–IL-17RA antibody)	Busse *et al.*, 2013 (165)	Moderate to severe asthma ($n = 302$)	No improvement
TNF	Etanercept (soluble TNF-α receptor blocker)	Berry *et al.*, 2006 (166)	Mild to severe asthma ($n = 30$)	Improvement in QOL, lung function, and bronchial hyperreactivity
		Holgate *et al.*, 2011 (167)	Moderate to severe asthma ($n = 132$)	No benefit
	Golimumab (anti–TNF-α antibody)	Wenzel *et al.*, 2009 (168)	Severe asthma ($n = 309$)	Discontinued early; unfavorable risk
IL-1	Anakinra (IL-1R antagonist)	www.clinicaltrials.gov		—
IL-6	Sirukumab (anti–IL-6 antibody)	www.clinicaltrials.gov		—
Protein kinases	Phosphoinositide 3 kinase inhibitors	Preclinical	—	—
	Tyrosine kinase inhibitors			

Definition of abbreviations: CXCR2 = chemokine receptor 2; HMG-CoA = 3-hydroxy-3-methylglutaryl-coenzyme A; NEA = noneosinophilic asthma; PDE = phosphodiesterase; PPARγ = peroxisome proliferator-activated receptor-γ; QOL = quality of life; TNF = tumor necrosis factor.
*U.S. Food and Drug Administration approved at time of article submission for treatment of asthma.

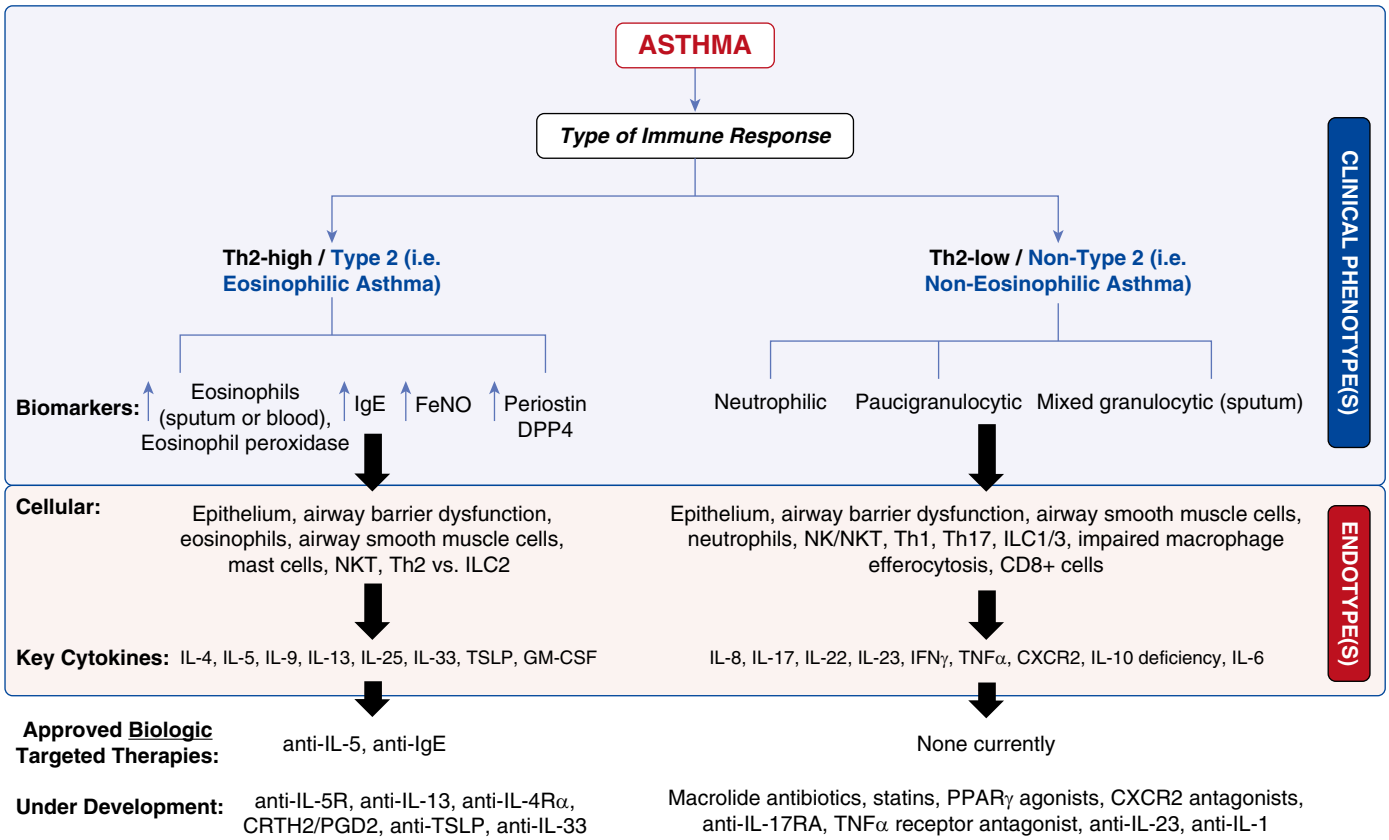

Figure 4. Clinical phenotypes, endotypes, and therapeutic options in eosinophilic and noneosinophilic asthma. CRTH2 = chemoattractant receptor-homologous molecule expressed on T-helper type 2 cells; CXCR2 = chemokine receptor 2; DPP4 = dipeptidyl peptidase-4; $F_{E_{NO}}$ = fractional exhaled nitric oxide; GM-CSF = granulocyte–macrophage colony–stimulating factor; ILC = innate lymphoid cell; NK = natural killer; NKT = natural killer-T; PGD2 = prostaglandin D2; PPARγ = peroxisome proliferator-activated receptor-γ; Th = T-helper cell; TNFα = tumor necrosis factor-α; TSLP = thymic stroll lymphopoeitin.

Given our limited understanding of what drives NEA, and the underlying mechanistic complexity of this phenotype, our therapies are likewise limited. Nonpharmacologic prevention and treatment of NEA include smoking cessation, avoidance of environmental and occupational pollutants, dietary changes, and treatment of comorbid conditions that could lead to neutrophilic asthma, such as infection and obesity. Targeting the structural airway cells, through techniques such as bronchial thermoplasty, may prove to be useful in paucigranulocytic asthma or other phenotypes (141, 151).

Although the mainstay of asthma treatment is corticosteroids, clinical evidence indicates that NEA is generally poorly responsive to corticosteroid treatment in terms of symptoms and lung function (4, 8, 11, 152) and, furthermore, corticosteroid treatment could potentially worsen the disease. Off-label use of medications such as macrolide antibiotics, PDE (phosphodiesterase)-4 inhibitors, or statins in NEA appears to have limited or selective efficacy and/or they currently lack the clinical data to support their wider use in NEA. Additional focused studies are needed to evaluate several classes of these and novel small-molecule inhibitors and biologic agents that can be targeted to treat NEA, and some of these treatments are highlighted in Table 4.

Conclusions

Asthma is a complex syndrome that requires an integrated and holistic approach before major advances can lead to truly effective therapies for all patients (Figure 4). There is an urgent need to uncover new knowledge about the mechanisms and molecular phenotypes underlying the endotypes for both EA and NEA. We need to reconcile this phenotyping with the more descriptive cytokine-based or endotypic nomenclature.

Looking beyond our present focus on the mechanisms of EA and NEA, such cellular and molecular pathways must be contextualized and linked to the clinical scenario unique to each patient or cohort. The future of this discussion should also extend to an improved understanding of how each of these endotypes develops. We predict that different combinations of gene polymorphisms, epigenetic modifications, and early-life exposures will confer risk of development of each of the asthma endotypes.

Most important for effective patient care is the development of novel therapies that target these relevant molecular phenotypes in carefully designed individual or synergistic combination therapies that will lead to improved clinical outcomes. Such an approach should ultimately be guided by the identification and use of multiple biomarkers, which can reliably predict responses to therapy while also revealing emerging endotypes. The greatest need for novel therapies is in noneosinophilic phenotypes, such as neutrophilic or paucigranulocytic asthma, where no targeted biologic or other therapies are yet available and outcomes studies are still lacking. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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