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Pathobiology of Severe Asthma

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Abstract

Severe asthma (SA) afflicts a heterogeneous group of asthma patients who
exhibit poor responses to traditional asthma medications. SA patients likely
represent 5–10% of all asthma patients; however, they have a higher eco-
nomic burden when compared with milder asthmatics. Considerable re-
search has been performed on pathological pathways and structural changes
associated with SA. Although limitations of the pathological approaches,
ranging from sampling, to quantitative assessments, to heterogeneity of dis-
ease, have prevented a more definitive understanding of the underlying
pathobiology, studies linking pathology to molecular markers to targeted
therapies are beginning to solidify the identification of select molecular phe-
notypes. This review addresses the pathobiology of SA and discusses the
current limitations of studies, the inflammatory cells and pathways linked to
emerging phenotypes, and the structural and remodeling changes associated
with severe disease. In all cases, an effort is made to link pathological findings
to specific clinical/molecular phenotypes.

INTRODUCTION AND CLINICAL BACKGROUND

In the United States, severe asthma (SA) represents 5–10% of all asthma cases. Yet per asthma patient, asthma-related costs attributed to SA patients were at least six times higher than costs attributed to mildly asthmatic patients (1). Despite the large number of patients (conservatively at least 2 million in the United States alone), SA and its pathobiology remain poorly understood and treated. The reasons for this are several and often linked.

Severe Asthma Has Been Poorly Defined

Although many pathobiological studies of SA have been conducted, definitions of the disease have varied widely, from those based purely on airway obstruction (2), to those related to corticosteroid resistance (3, 4), to those based on life-threatening (or life-ending) SA. In 2000, the American Thoracic Society (ATS) published the first comprehensive definition of SA, which has been recently updated to describe SA as (*a*) asthma requiring treatment with high doses of inhaled corticosteroid plus a second controller (long acting β_2-agonists, leukotriene modifier, or theophylline) and/or systemic corticosteroids to achieve symptom control or (*b*) asthma that remains uncontrolled despite therapy. This updated definition, which has been endorsed by the European Respiratory Society (ERS) and is hereafter referred to as the ATS-ERS definition, is utilized across developed countries with general access to inhaled corticosteroids (5, 6).

The first step in identifying a SA patient is to confirm that they meet the criteria for asthma itself. Unfortunately, the definition of asthma is equally vague, including nonspecific criteria such as reversible airway obstruction or bronchial hyperresponsiveness in the face of appropriate symptoms. Many people diagnosed with SA fail to meet those criteria, including patients with chronic obstructive pulmonary disease and vocal cord dysfunction, among others. However, once a diagnosis of asthma is confirmed, the second step is to determine whether the asthma (*a*) requires high-dose inhaled and/or systemic corticosteroids, with an additional controller medication (usually a long-acting beta agonist or leukotriene modifier), to achieve asthma control, or (*b*) remains poorly controlled despite that therapy. Poor control is defined as poor symptom control, i.e., having a history of severe or frequent exacerbations or airway obstruction on pulmonary function testing. A definition of SA that requires the demonstration of poor responsiveness to corticosteroids excludes, as examples of SA, cases in which evaluation of the pathobiology is based on measurements of lung function alone. Thus, moving forward, a reasonably specific but clinical definition of SA exists that should allow a more refined understanding of the pathobiology. Most pathological studies quoted here focus on those studies that used an approximation of the current ATS-ERS definition. However, in many cases (especially those involving distal lung analyses), additional studies, particularly from autopsies of patients who died of asthma, are included as well.

Severe Asthma Is Not a Single Disease

Asthma has long been appreciated as an umbrella term identifying patients who meet the criteria for asthma but who may have distinct clinical, biological, and therapeutic characteristics. These distinct groups of patients may be more easily identified in the broad group of SA, such that studies of SA, without background characterization, often gave widely varying results. Although asthma is considered an eosinophilic disease, it was recognized as early as the mid-1950s that not all patients with asthma had eosinophilic inflammation and that those without eosinophilic

inflammation responded less well to corticosteroid therapy (7). This concept was largely lost for many years, as inhaled corticosteroids became the gold-standard therapy for all asthma patients.

The advent of targeted biological therapies for asthma and the application of unbiased statistical clustering approaches rejuvenated the concept of asthma phenotypes, or groups of patients identified by a range of characteristics. In 1999, SA patients were divided into those with and without tissue eosinophilia. Pathologically, patients with tissue eosinophilia had a thicker subepithelial (reticular) basement membrane and more evidence of cells positive for transforming growth factor-β (TGF-β) (8). Clinically, these patients were more likely to have had a severe exacerbation of asthma and to have physiological changes suggestive of airway collapse (i.e., ratio of lower forced vital capacity to slow vital capacity). A follow-up study reported that patients with later-onset SA were more likely to be eosinophilic but less likely to be atopic (9). Less-biased statistical clustering approaches followed these clinical approaches, generally supporting the earlier studies and confirming the importance of age at onset, gender, airway obstruction, and lung eosinophilia (as well as neutrophilia) (10–14). Genetic studies further support differences in genetic risk in early-onset compared with later-onset asthma (15). Longitudinal studies also support the stability of the more severe phenotypes but additional studies are needed (16).

Most of these early clustering approaches relied primarily on clinical, physiological, and cellular characteristics without integration of molecular characteristics. However, Woodruff et al. (17) advanced the field of phenotypes by identifying patients with corticosteroid-naïve, mild asthma who exhibited a T-helper (Th2)/Type-2 molecular signature in their airway epithelial cells. These patients, who made up about 50% of this mild asthma population, differed from those without this molecular signature owing to a greater degree of atopy, tissue eosinophilia, and reticular basement membrane thickening, and, importantly, to a greater response to inhaled corticosteroids. Their study essentially began the concept of molecular phenotyping, in which certain molecular pathways are linked to clinical and physiological characteristics, with a Type-2 inflammatory signature observed at all severity levels.

Further supporting the significance of molecular phenotyping, biological therapies are now targeted to specific asthma phenotypes. Treatments with monoclonal antibodies to interleukin (IL)-5, IL-13, and IL-4 receptor (alpha chain) demonstrate efficacy, primarily in moderate asthma to SA, when used in patients identified by Type-2 cytokine-related biomarkers (see **Table 1**), such as blood eosinophils, fractional exhaled nitric oxide (FeNO), and serum periostin (18–22). In all cases, these therapeutic approaches work better in Type-2-associated phenotypes. Although increasing data support a Type-2-related signature in about 50% of asthma and SA cases, the molecular phenotype(s) of patients without evidence for Type-2 pathways remains poorly understood. These earlier studies emphasize the importance of developing a review of SA pathobiology in light of this heterogeneity.

FeNO: fractional exhaled nitric oxide

Table 1   Biomarkers of Type-2 inflammation in severe asthma

Biomarker
FeNO
Increases in total IgE
Serum periostin
Sputum/blood eosinophilia
Sputum expression of IL-4/IL-5/IL-13

Abbreviation: FeNO, fractional exhaled nitric oxide.

Severe Asthma Pathobiology Is Based Primarily on Very Small Biopsies of Superficial Airway Walls

Very little whole-lung pathology or surgical biopsy tissue has been collected and evaluated in relation to asthma. This dearth is likely due primarily to the concept that asthma is a large airway disease with little involvement from the alveolated lung parenchyma. Unlike diseases such as interstitial pulmonary fibrosis, high-resolution computed tomography (CT) imaging studies of SA rarely reveal parenchymal abnormalities. It may also relate to the concept that SA pathology is assumed to look like mild asthma. Therefore, the majority of distal lung pathology is from autopsy specimens of patients who died of an acute asthma exacerbation, while few surgical biopsy studies are reported (23–28). In autopsy cases, the background phenotype of patients is often poorly understood, and these fatal asthma cases are often patients who were not prescribed corticosteroid therapy or who were not taking the corticosteroid prescribed for their asthma. Thus, the pathobiology of these exacerbations of SA may differ from that of patients with severe and treated asthma.

Sampling of the airways in asthma for morphological examination is typically conducted via fiber-optic bronchoscopy. Two main types of specimens are used for examination, endobronchial biopsies (EBBs) and transbronchial biopsies (TBBs). In contrast to bronchoalveolar lavage or induced sputum, direct biopsies provide histological information on airway morphology, allowing investigators to examine the different compartments of the bronchial wall, such as epithelium, submucosa, smooth muscle, and glands, as well as their structural relationships. EBBs are the most common specimens pathologists encounter. Nevertheless, they are extremely small ($\sim$1 mm^3), sample only the proximal airway and thus have the potential for sampling bias, and do not include peribronchial tissue or lung parenchyma. TBBs allow investigators to examine smaller airways and lung parenchyma but carry a substantially higher morbidity/mortality risk than EBBs do and generally require the use of fluoroscopy. Thus, there are few TBB studies in the field of asthma research. In comparison, EBBs performed as part of a bronchoscopy in SA are safe, well tolerated, and common (29).

Tissue obtained thoracoscopically is rare because of the invasiveness and increased morbidity and mortality of the procedure. Nevertheless, video-assisted thoracic surgery (VATS) is an important diagnostic tool in the workup of some SA patients, further aiding to characterize such patients and exclude cofounding conditions and clinical mimickers. VATS enables a more representative examination of the distal/small airways, the blood vessels, and the alveolated parenchyma. VATS sampling helps rule out diseases such as Churg-Strauss syndrome (also known as eosinophilic granulomatosis with polyangiitis), constrictive bronchiolitis, hypersensitivity pneumonia, and autoimmune-associated airway disease. This approach led to the first description of asthmatic granulomatosis, a disease with features of SA but that includes alveolar septal mononuclear inflammation and poorly formed non-necrotizing granulomas (26).

Pathology of Severe Asthma Is Reported from Luminal Assessments, Including Bronchoalveolar Lavage and Sputum

Analyses of bronchoalveolar lavage fluid and sputum help researchers understand the pathobiology of asthma and SA, but they do not include tissue analyses. Not surprisingly, the correlations between bronchoalveolar lavage fluid and tissue are rather poor likely because of the different compartments being sampled (30). Recently, analysis of induced sputum has also been integrated into pathobiological studies of asthma. Sputum analysis enables investigators to collect samples from patients of various ages (including children, on whom few bronchoscopic studies have been

performed) and repeat analyses to address the stability of the findings over time. Unfortunately, although sputum has provided a wealth of new information regarding inflammatory processes in SA, sputum does not allow the assessment of any structural features. Although it likely represents primarily an integration of intraluminal processes in the larger airways, the precise location of the origin of the sputum and its relation to structural pathology are poorly understood.

Animal Models of Asthma Only Modestly Reflect Human Severe Asthma Pathobiology

Many insights into the pathobiology of human disease have been derived from animal models. However, in the case of asthma, the available models only modestly reflect the asthmatic/allergic inflammatory process at least partly because mouse lungs bear little resemblance to human lungs (31). Important differences include the lack of small airways and smooth muscle in mouse airways, as well as marked differences in the oxidative/nitrative pathways in the epithelium and mono-cytes/macrophages of mice. Finally, most murine allergic asthma models have a strong parenchy-mal component that is not seen in human disease. Although newer murine models have been developed with the hope of better reflecting SA (or its phenotypes), the relation to human SA has not yet been confirmed (32, 33).

Variation in Methodological Approaches and Pathological Analysis

SA is traditionally diagnosed by clinicians. A pathological examination of asthmatic airways is performed only when response to treatment is minimal, other diagnoses are entertained, or when data are needed for research. Nevertheless, advances in our understanding of SA phenotypes/endotypes may encourage the use of bronchoscopic and VATS sampling in selected patients with SA.

Light microscopy with or without immunohistochemical analysis is the most common patho-logical approach used to examine airway disease. Approaches such as general descriptions and semi-quantitative scales, counts per high-power field, computer-assisted image analyses, and stereology-based methods have been used for qualitative, semiquantitative, and quantitative analysis. The diversity of these methods contributes to the variability in reported pathological outcomes. Some investigators suggest that stereological approaches, which use an unbiased method for the quan-titation of three-dimensional geometric characteristics of objects normally studied in a two-dimensional histology, are the gold standard for quantitative morphological evaluation of the respiratory tract (34, 35). For example, stereology-based microscopy provides the number of cells (e.g., mast cells) per volume of epithelium or a more precise three-dimensional measurement of basement membrane thickness. Although this technique has been used in SA research, it requires special sampling, instruments/software, and considerable experience, which have limited its appli-cation (36–38). Ultimately, these various approaches to quantification further limit comparisons between studies. Thus, despite the large number of patients worldwide who suffer from SA, its pathology or pathologies remain poorly understood, with few attempts made by researchers to categorize asthma phenotypes and their location.

PATHOBIOLOGY OF SEVERE ASTHMA

Despite the limitations discussed above, a pathobiological picture of human SA is emerging. The pathobiology must be viewed in the context of the heterogeneity of the disease, in relation to both inflammation and structural changes. The following sections address how heterogeneity

relates to specific biological pathways, clinical phenotypes, and, when available, airway remodeling. However, much work remains to be done to link heterogeneity to disease processes.

ILC: innate lymphoid cell

iNOS: inducible nitric oxide synthase

Inflammatory Processes: Pathobiological Evidence for Type-2 Inflammation

Type-2 immunoinflammatory pathways have long been associated with asthma, and emerging evidence supports their role in at least a subtype of SA. Type-2 inflammation is associated with increased expression of the canonical Type-2 cytokines, IL-4, IL-5, and IL-13; with Th2 lymphocytes; and with increases in certain cell types, including eosinophils, basophils, and mast cells. Immunohistochemical evaluations of CD3 and CD4 antigens on EBBs from SA patients have indirectly shown that T-helper lymphocytes are elevated in SA, particularly in patients with lung eosinophilia (8). Th2 cells are considered the main source of IL-4, IL-5, and IL-13 in SA, but data support their release by other cell types, including basophils, mast cells, and the recently identified innate lymphoid cell, ILC2 (39). Unlike milder asthma, which is strongly associated with allergic/IgE-mediated inflammation, the mechanisms driving Type-2 inflammation in SA remain unclear. Type-2 inflammation has also been associated with allergic/specific IgE production, although many patients with SA, especially those with later onset of disease, appear to have eosinophilic inflammation without increases in serum IgE (9). Type-2 inflammation has been linked to goblet cell metaplasia/hyperplasia (see below) and expression of inducible nitric oxide synthase (iNOS), the primary enzyme responsible for generating nitric oxide. **Figure 1** depicts the complexity of the Type-2 inflammatory processes in SA and includes relatively well-described pathways and their biomarkers and downstream pathways.

Current clinical-pathobiological studies of human asthma suggest that only about 50% of SA patients present evidence for ongoing Type-2 inflammation, as measured by blood/lung/airway eosinophilia, mast cells, or high levels of FeNO (8, 40, 41). However, although studies of milder asthma have associated these downstream cells with increased expression of Type-2 cytokines in the lung tissue, data on the expression of Type-2 cytokines in SA are sparse, with some studies suggesting lower levels of expression (17, 42, 43).

Despite low levels of Type-2 cytokines, numerous studies support downstream activation of Type-2 pathways, as evidenced by increases in certain Type-2 biomarkers. Currently, there is no gold-standard biomarker for Type-2 inflammation, although FeNO, blood/sputum eosinophils, and periostin have all been suggested (see **Table 1**). Periostin was identified in cultured bronchial epithelial cells as a protein upregulated by IL-4 and IL-13; it is also measureable in serum. Its expression is elevated in human bronchial epithelial cells in association with increased expression of IL-5 and IL-13 in the submucosa (44, 45). Serum periostin is a better predictor of airway eosinophilia in SA than FeNO is, although blood eosinophils are also reasonably predictive (46, 47). Therefore, it is not yet clear which biomarker will best identify Type-2 immune-inflammation in SA.

Finally, studies using antibodies to IL-13 or IL-4Rα (see **Table 2**) in patients with SA confirm a link between patients with pathobiological evidence for Type-2 inflammation and clinical outcomes with such therapies. These targeted biological approaches in patients with elevated blood eosinophils, sputum IL-13, serum periostin, or FeNO have, for example, improved lung function, reduced symptoms, and reduced numbers of exacerbations (18, 20, 48, 49). Thus, despite the low levels of Type-2 cytokines measured in SA, they apparently play an active role in the pathobiology.

Type-2-associated SA may be divided into at least two subtypes/phenotypes. The first is a more severe form of early-onset (usually preadolescence) disease, commonly associated with atopic/allergic conditions and higher IgE levels but less often with eosinophilia. The second
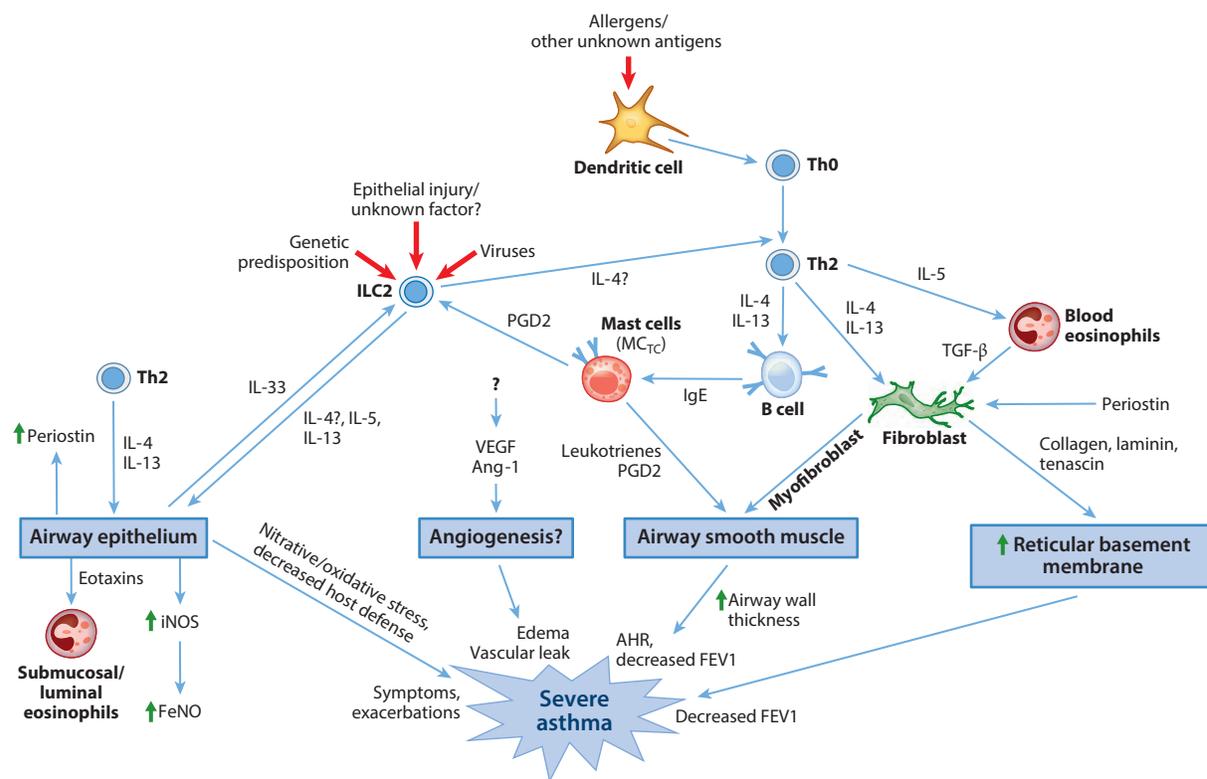
Figure 1

Type-2 inflammation and its relationship to the structural changes of severe asthma. Type-2 cytokines are generated by Th2 lymphocytes and ILC2 cells, which activate various downstream cells, inducing airway remodeling through RBM thickening, goblet cell metaplasia/hyperplasia, mucus hyperproduction, airway smooth muscle hyperplasia/hypertrophy, and angiogenesis. Note the several potentially self-maintaining loops. Abbreviations: AHR, airway hyperresponsiveness; Ang-1, angiopoietin-1; FeNO, fraction of expired nitric oxide; FEV1, forced expiratory volume in 1 second; IL, interleukin; ILC2, innate lymphoid cell-2; iNOS, inducible nitric oxide synthase; MC_{TC}, mast cell, tryptase and chymase positive; PGD2, prostaglandin D2; RBM, reticular basement membrane; TGF-β, transforming growth factor-β; VEGF, vascular endothelial growth factor.

subtype is a later-onset (usually after 20 years of age), non- or less-allergic disease with striking systemic and airway eosinophilia (9, 22). Although both phenotypes manifest airway/lung eosinophilia (to some degree), the mechanisms for eosinophilia likely differ.

Eosinophils. Airway eosinophilia occurs in about 50% of SA patients (8). Eosinophilia has been examined by sputum, bronchoalveolar lavage, and EBBs, although the correlations among them are weak (50). Traditionally, recruitment of eosinophils into the large airway wall and lumen is one of the histological findings characteristic of asthma, particularly fatal (likely severe) asthma (**Figure 2**), along with marked mucus plugging and epithelium shedding/denudation (see below). The amount of eosinophils in the airways is suggested to be a marker of disease severity, with lung eosinophilia (as measured by sputum or EBB) being strongly associated with the severity of asthma symptoms (51), worsened lung function, and near-fatal events (9).

Airway eosinophilia is strongly associated with increases in reticular basement membrane (see later sections on airway structure), as well as increases in TGF-β isoforms, probably at least in part

Table 2   Inflammatory mediators and biomarkers in severe asthma with targeted therapy

Mediator	Biomarker	Inhibitor	Clinical outcome	Biological outcome
IL-4/IL-13	Blood/sputum eosinophils, serum periostin, eotaxin-3, specific IgE or FeNO	Dupilumab (IL-4 receptor blocker) (48) Pitrakinra (IL-4 mutant) (49)	Prevented loss of asthma control Improved lung function (FEV1) and symptoms	No changes in blood eosinophils Decreased FeNO, eotaxin-3, TARC, and IgE
IL-5	Sputum/blood/lung tissue eosinophils	Reslizumab (61) Mepolizumab (21, 22, 52) Benralizumab (62)	Decreased exacerbations	Marked decrease in blood eosinophils Modest decrease in lung eosinophils (tissue/sputum) Decreased airway thickness Decreased RBM
IL-13	Total IgE levels FeNO Blood eosinophils Serum periostin Sputum IL-13	Lebrikizumab (18) Tralokinumab (20)	Improved lung function (FEV1) No effect on symptoms	Decreased FeNO, periostin, and IgE Blood eosinophils tend to increase
IgE	IgE levels	Omalizumab (74, 76, 209)	Decreased exacerbations	Decreased tissue eosinophils and mast cells
IL-17(A, F, E)	Sputum/blood neutrophilia	Brodalumab (101)	For the overall study population, no differences between treatment and placebo Symptoms and lung function (FEV1) improved in the high-reversibility group	No change in any measured biomarker
TNF-α	TNF-α expression on peripheral mononuclear cells	Golimumab (105) Etanercept (104)	For overall study population, no differences between treatment and placebo Significant decrease in exacerbations in a subset of patients with bronchodilator response	No change in any measured biomarker

Abbreviations: FeNO, fractional exhaled nitric oxide; FEV1, forced expiratory volume in 1 second; RBM, reticular basement membrane; TARC, thymus and activation-regulated chemokine; TNF-α, tumor necrosis factor-α.

from the eosinophil itself (52). Eosinophilia has also been linked to increases in 15-lipoxygenase (15-LO1) and its product 15-hydroxyeicosatetraenoic acid (15-HETE) in the airway epithelium (53). 15-LO1 is induced by IL-4/IL-13 in both epithelial cells and monocyte/macrophages, supporting its relevance to Type-2 inflammation. Through its binding to the novel protein phosphatidylethanolamine binding protein-1, 15-LO1 may play an important role in activating early response kinase (ERK)-related gene pathways, including increased expression of the mucin
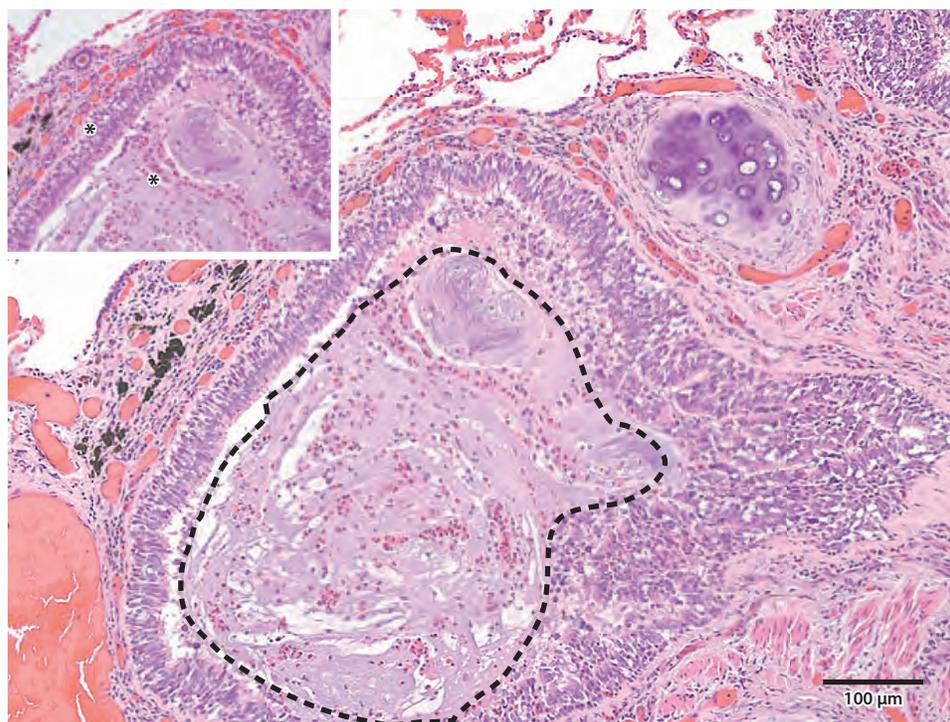
15-LO1:
15-lipoxygenase

Figure 2

Airway eosinophilia, goblet cell metaplasia (clear cells within epithelium), and mucus plugging (*dashed line*) in a case of fatal asthma. The inset highlights eosinophils (*asterisks*) within the mucus plug and in the epithelium. Magnification 200×; scale bar = 100 μm.

glycoprotein MUC5AC (54, 55). Thus, eosinophilia may be indirectly related to enhanced mucin expression as well.

Airway eosinophilia in SA is correlated with increased expression of the CCL chemokines CCL24 and CCL26 (eotaxin-2 and eotaxin-3, respectively) in the epithelium (56). CCL24 and CCL26 (including CCL11, or eotaxin-1) are upregulated by IL-4 and IL-13 in vitro (57, 58). Their upregulation is associated with lower lung function and more exacerbation-associated disease. Evidence suggests that CCL24/eotaxin-2 may be preferentially upregulated in adult-onset SA, potentially explaining the differences associated with adult- versus childhood-onset eosinophilic SA (56). CCL11/eotaxin-1 is expressed not in airway epithelial cells, but in the submucosa by fibroblasts or epithelial cells. Thus, the three eotaxins may promote an eosinophilic gradient from submucosa to epithelium to lumen. In addition, expression of eotaxin-1 is markedly synergistically enhanced when stimulated with TGF-β1 and IL-4/IL-13 combined, suggesting a mechanism by which eosinophilia in SA may become self-sustaining (59); eotaxin-1 is a potent chemoattractant for eosinophils, particularly in SA patients (60).

However, the most critical evidence confirming the importance of eosinophils in SA comes from therapeutic trials of antibodies to IL-5 in SA patients (see **Table 2**). In repeated studies, monoclonal antibodies to IL-5 and its receptor have decreased exacerbations by nearly 50%, compared with placebo, while decreasing blood and EBB tissue eosinophilia to a lesser degree (21, 22, 61, 62). In addition, these decreases in eosinophils are associated with reduced airway wall

thickness, as measured by CT imaging, as well as reductions in extracellular matrix deposition, supporting a role for eosinophils in airway remodeling (52, 63). No data suggest that inhibition of IL-4/IL-13 decreases systemic or lung eosinophil levels.

PGD2: prostaglandin D2

AHR: airway hyperresponsiveness

MC_T: mast cell, tryptase positive

MC_{TC}: mast cell, tryptase and chymase positive

Mast cells. Mast cells are also linked to Type-2 (and allergic) inflammation (38). This finding is further supported by a study of polymorphisms in the IL-4 receptor gene, *IL4RA*, which was associated with increased mast cells in the airways (64). Moreover, the polymorphisms were associated with both increases in tissue mast cells and an exacerbation-prone phenotype.

Mast cells are classically activated by allergenic proteins when IgE is bound to FcεRI (the high-affinity receptor for IgE). IgE-mediated mast cell degranulation and activation in the large airways result in the release of histamine, tryptase, and cysteinyl leukotrienes and in the generation of prostaglandin D2 (PGD2), which contribute to bronchospasm, airway hyperresponsiveness (AHR), and perhaps additional inflammatory cell influx. Mast cells, identified by IgE binding to their surface, are increased in SA, primarily in association with eosinophilic inflammation (28). Mast cells in humans are generally divided into those expressing tryptase (MC_T) and those expressing both tryptase and chymase (MC_{TC}) (65). The predominant mast cell in healthy lung submucosa is MC_T. However, in patients with SA, there apparently is a shift in the submucosa (and epithelium) and MC_{TC} predominates (66), while mast cells of the MC_T phenotype are present in highest amounts in the epithelium in corticosteroid-naïve mild asthma.

Similarly, mast cells expressing the synthesizing enzyme for PGD2, hematopoietic prostaglandin-D synthase, are increased in the airway epithelium in SA patients, in association with increases in PGD2 (40). PGD2-generating mast cells are linked to Type-2 inflammation and, importantly, to exacerbation-prone, poorly controlled asthma. PGD2 released from mast cells could further activate Th2 cells (as well as ILC2 cells, see below) to increase release of Th2 cytokines through activation of the DP2 receptor (the PGD2 receptor). Thus, mast cells could also initiate and perpetuate inflammation in SA (66).

In addition to their presence in the epithelium and submucosa, mast cells are elevated in the smooth muscle in patients with milder asthma (67). Whether a similar increase occurs in SA patients remains unclear. However, the recently reported increase in interferon-γ (IFN-γ) in SA (42) could support a further migration of mast cells into smooth muscle or other cell regions because of increased expression of CXC chemokines. Chemokines such as CXCL9 and CXCL10 increase mast cell migration through the activation of the type-1 chemokine receptor, CXCR3, in smooth muscle cells in vitro (68). Murine mast cells, which express the receptor for IFN-γ, can exhibit enhanced cell activation in the presence of IgE. Mast cell expression of both the receptor for IFN-γ and the signaling gamma chain of the FcεRI was required for optimal mast cell–dependent enhancement of features of airway allergic inflammation, AHR, and airway remodeling in a chronic model of ovalbumin-induced allergic airway inflammation (69). These studies imply that the role for mast cells in SA may go beyond the presence of a Type-2 inflammatory process.

Mast cells could play a role in regulating airway smooth muscle activity and perhaps remodeling through a positive feedback loop. As noted above, activated smooth muscle cells can release chemoattractant factors for mast cells, which, through tryptase and CXC chemokine release, could further promote recruitment and activation of myofibroblasts (70). In addition, activated mast cells in airway smooth muscle could contribute to acute bronchoconstriction following generation of leukotrienes/prostaglandins.

Mast cells are elevated in the small airways and alveolar attachments in patients with SA, again with a specific increase in the MC_{TC} phenotype. The greatest increase occurred in the alveolar attachments and outer airway walls. Although mast cells are abundant in the alveolar parenchyma, there does not appear to be a rise in alveolar mast cells in SA (28, 29).

Other inflammatory cells involved in Type-2 inflammation. Wakahara et al. (71) proposed that basophils, via IgE and the activation of FcεRI, could amplify or even mediate the development of Type-2 inflammation in the lungs. However, the specific role of basophils in SA remains elusive, although a report suggested that basophils are elevated in fatal asthma lungs compared with control and nonfatal asthma lungs (72). A cluster analysis of children with SA identified a particular cluster with more allergic asthma, blood eosinophilia, and basophilia (73).

Omalizumab is a monoclonal antibody that binds to IgE, preventing it from activating FcεRI, present on both mast cells and basophils (and perhaps other cells). It decreases asthma exacerbations in some severe allergic asthmatics and improves asthma quality of life, likely by affecting mast cell or basophil activation (74). Basophils were reduced following omalizumab therapy in a pediatric population of SA patients, supporting a link between IgE levels and circulating basophils (75). Although not all patients with atopy/allergy appear to respond to anti-IgE therapy, data suggest the presence of an underlying and active Type-2 immune process, as increases in blood eosinophils, FeNO, or serum periostin may help predict a response (76) (see **Table 2**).

Increasing data support a role for ILC2 cells in asthma. These innate immune cells have been proposed to be a major source of IL-5 and IL-13, in an allergen-independent manner, during acute inflammation in the mouse lung (77). In humans, ILC2 cells are characterized by expression of the CRTH2/DP2 (PGD2) and ST2L (IL-33) receptors, which upon engagement by their ligand appear to markedly increase IL-5, IL-13, and perhaps even IL-4 expression (78). Research linking ILC2 cells to SA is sparse. A study reported a possible rise in ILC2 cells in nasal polyp tissue, an abnormality often associated with SA (79). However, the ability to definitively detect these cells in tissue is limited owing to the large panel of antibodies needed for detection. Nevertheless, the consistent increases in PGD2, the ligand for CRTH2/DP2 in Type-2-prominent SA, suggest that mast cell activation could also enhance ILC2 cell migration and activation (40, 78). Moreover, SA patients present lower levels of lipoxin-4, a potent inhibitor of ILC2 cells, in serum and bronchoalveolar lavage, which could also promote ILC2-associated responses in SA (80).

Finally, infiltrates of CD8^{+} T cells in the lungs and elevated levels in blood have been related to Type-2 inflammation, AHR, and, in the case of blood CD8+ cells, asthma severity and eosinophilia (81, 82). CD8^{+} cells can release the Th1 cytokine IFN-γ (82) (see below). In this regard, tissue from fatal asthma lungs had more CD8^{+} cells compared with tissue from control or mild asthma lungs with associated increases in IL-18, an inducer of IFN-γ (83). Thus, it is conceivable that IL-18 from CD8^{+} T cells may contribute to severe or fatal asthma through the induction of both Th1 and Th2 cytokines (in the absence of IL-12), markedly increasing the complexity of the inflammatory process.

Non-Type-2 Immunoinflammatory Processes in Severe Asthma

Non-, or low-, Type-2 asthma is identified primarily by the lack of evidence for Type-2 immunoinflammatory processes, i.e., lack of elevation in Type-2 biomarkers, as there are currently no well-defined non-Type-2 biomarkers. There is considerably less knowledge of the underlying pathobiology and molecular pathways that control non-Type-2 asthma when compared with Type-2-predominant asthma, which can be identified by elevated Type-2 biomarkers; however, data on the role of Type-1 (Th1) and Type-17 (Th17) pathways in SA, both alone and in combination with Type-2 inflammation, are emerging. In general, SA patients lacking Type-2 biomarkers are a heterogeneous group of patients. Non-Type-2 patients are also consistently observed in statistical cluster analyses. The clusters are linked to neutrophilic or paucigranulocytic inflammation, but associated molecular pathways are poorly understood (84, 85). Actual disease may in fact be less severe on the whole in non-Type-2 asthmatics than in patients with persistent Type-2-related
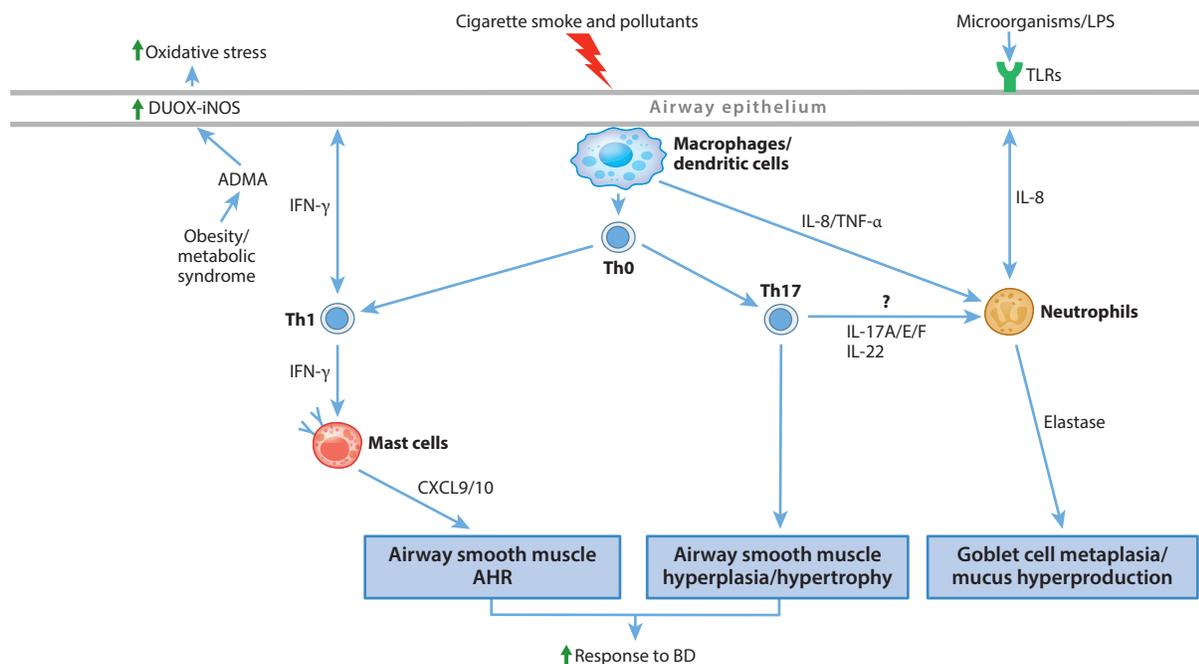
Figure 3

Non-, or low-, Type-2 inflammation and its relationship to structural changes in severe asthma. Factors involved in the development of non-Type-2 inflammation in asthma include pollutants, cigarette smoke, and microorganisms. These factors can activate innate immune-mediated pathways, as well as Th1 and Th17 inflammatory processes. Abbreviations: ADMA, asymmetric dimethylarginine; AHR, airway hyperresponsiveness; BD, bronchodilator; CXCL, (C–X–C motif) ligand; DUOX, dual oxidase; IFN-γ, interferon-γ; IL, interleukin; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; TLRs, Toll-like receptors; TNF-α, tumor necrosis factor-α.

inflammation, although comorbidities, including obesity and smoking history, may be more common (12, 86). **Figure 3** depicts the best-characterized non-Type-2 inflammatory pathways in SA and focuses on the structural changes.

Pathobiological evidence for Type-1 inflammation in severe asthma. Increasing evidence demonstrates that SA (and its phenotypes) is associated with and even driven by a complex immune pathology. Th1 lymphocytes and their canonical cytokine IFN-γ are elevated in SA. Tissue biopsies of airway submucosa and bronchoalveolar lavage cells from SA patients show increased IFN-γ-expressing cells, compared with that from moderate asthmatics and healthy controls (42, 87). Increased IFN-γ was associated with elevations in both iNOS and dual oxidase-2 (DUOX2), key nitrative- and oxidative-stress-related enzymes, respectively (42). In vitro, the combination of Type-1 (IFN-γ) and Type-2 cytokines (IL-13) synergistically increased both iNOS and DUOX2 expression and activation, in association with evidence for enhanced nitrative stress. Although FeNO levels have been strongly related to Type-2 inflammation (epithelial iNOS is increased by IL-13 stimulation) and generally decrease in response to corticosteroid use, FeNO was recently described to be a strong independent predictor of chronic systemic corticosteroid use in patients with SA (88). Persistent elevations despite systemic corticosteroids use suggest elements of corticosteroid resistance, perhaps more common in mixed immune processes. Thus, Type-1 and Type-2 may contribute to both oxidative and nitrative stress in some patients with SA.

A genome-wide association study, performed by the National Heart Lung and Blood Institute's Severe Asthma Research Program on patients of European descent, identified four single nucleotide polymorphisms in Th1/IL-12 pathway genes (*IL12A*, *IL12RB1*, *STAT4*, and *IRF2*) significantly associated with FEV1% predicted and asthma severity (89). Data from this study and others imply that although Th2 pathway genes (*IL13*, *TSLP*, *IL33*, and *IL1RL1*) may modify asthma susceptibility, they are not associated with lung function. Therefore, the authors proposed a two-step approach in which genetic variants in Th2-related genes, combined with environmental factors, increase overall susceptibility to asthma. However, additional genetic variants in Th1/IL-12 genes, together with variants of airway structure/remodeling genes, are needed to worsen asthma severity. Further pathological studies are needed to address the role of Type-1/Th1 immunity in SA.

Th17 and TNF-α pathways: relation to neutrophilic asthma. Some SA patients present with sputum neutrophilia without (or with) eosinophilia, suggesting involvement of non-Type-2-mediated mechanisms. Nearly 20 years ago, marked distal lung neutrophilia was identified in a subgroup of SA patients on chronic oral corticosteroid therapy, whereas eosinophils were present in more limited numbers (90). Following that study, other investigators have reported that the degree of airway neutrophilia correlates with asthma severity and lung function, with Sur et al. (91) suggesting that some asthma deaths are associated with neutrophilic inflammation, especially if the interval between onset of attack and death is short (87, 92). Neutrophilic SA is less responsive to corticosteroids (51), perhaps not surprising given that corticosteroids can prevent neutrophil apoptosis (93). Some asthmatics with elevated airway neutrophils and reduced eosinophils express high levels of CXCL1, CXCL10, and IL-6, as well as matrix metalloproteinases (MMPs), particularly neutrophilic MMP-9. The presence of these neutrophilic chemokines/cytokines was recently associated with better clinical outcomes (32).

Th17 cells are a distinct population of CD4+ lymphocytes that secrete IL-17A, IL-17E, IL-17F, and IL-22, which can mediate neutrophil migration through an IL-8 mechanism (94). Because of this property, IL-17 pathways have been proposed to play important roles in so-called neutrophilic asthma. These cytokines also increase airway smooth muscle proliferation (95) and contraction (96), suggesting that they could play a role in smooth muscle hyperplasia and AHR.

Studies of mouse models have delineated pathways by which Th17 cells could play a role in asthma pathobiology (97). For instance, transfer of Th2 cells to mice challenged with ovalbumin resulted in the expected eosinophilic inflammation, which was highly corticosteroid responsive, whereas transfer of Th17 cells resulted in increased CXC chemokine secretion and neutrophilic inflammation, which were corticosteroid resistant (97). An allergy-driven mouse model also demonstrates the involvement of Th17 pathways. In this model, IL-17A mediated AHR via Type-2 (IL-13)-dependent mechanisms, involving complement proteins (C3 and C5) and IL-23 (98). IL-17 alone failed to induce AHR but required concomitant Type-2 inflammation. This model, through which neutrophilic inflammation is suggestive of SA, supports the combined importance of Th17 and Th2 inflammation in the pathobiology of SA. The responsiveness of this process to corticosteroids was not evaluated, nor were there human data to support the joint expression of Type-2 and Th17 pathways.

Results from immunohistochemistry and mRNA levels report increased IL-17 in lung tissue (2) and sputum (99), respectively, from moderate asthmatics and SA patients, compared with milder asthmatics and control subjects. However, no evidence demonstrates a direct relationship between Th17 cytokines and neutrophilic inflammation, although Doe et al. (100) reported a weak relationship to eosinophilic inflammation. A monoclonal antibody against IL-17 receptor (brodalumab) failed to improve asthma outcomes in a mixed population of moderate asthmatics

FEV1: forced expiratory volume in 1 second

MMP: matrix metalloproteinase

and SA patients (see **Table 2**). Although lung neutrophilia was not addressed, subtyping by the presence of blood neutrophils did not identify a responder group, nor were blood neutrophils affected. Some effect was observed in a subgroup with high reversibility (postbronchodilator FEV1 improvement ≥20% (101), indicating an impact on airway smooth muscle (96). However, the nature of the antibody that blocks the IL-17 receptor does not allow us to determine whether the effect is through IL-17A or IL-17F or potentially through IL-25 (IL-17E), which has little to no neutrophilic effects but may affect eosinophilic inflammation (102).

These findings indicate that Th17-associated inflammation may be present in a small subset of patients with SA; some data suggest it may actually have protective effects, perhaps through important host defense mechanisms (32). Further studies of IL-17 and its family members in SA are needed.

Tumor necrosis factor-α (TNF-α) has also been proposed to play a role in neutrophil-rich SA (85). In vitro and in vivo models have shown that TNF-α increases AHR, mucus production, and expression of adhesion molecules. Further, it may synergize with IL-17 to enhance neutrophil recruitment (103). A small study suggested that a soluble TNF-α receptor (etanercept) improved outcomes in patients with SA (104). However, a larger study of golimumab (an anti-TNF-α monoclonal antibody) failed to show efficacy in an unphenotyped population of SA patients and was associated with substantial complications ranging from sepsis to cancer (105). As with brodalumab, asthma exacerbations improved in patients who responded to bronchodilators. Whether these similar effects occur through a common overlapping pathway requires further investigation (see **Table 2**).

Comorbidities associated with non-Type-2 immunoinflammatory pathological processes and severe asthma.

Smoking. Numerous studies suggest that a smoking history worsens asthma and is associated with worse clinical and healthcare outcomes, as well as a poor response to corticosteroids (106). This decreased response to corticosteroids may be attributable to fewer lung eosinophils and more neutrophils, although the mechanisms for neutrophilia are not clear (107). These worsened outcomes were also associated with increases in mast cells (108). Despite this, FeNO is consistently reported to be decreased in asthmatics who smoke. It is not clear whether this is evidence for inhibition of Type-2 inflammation.

Obesity. Considerable literature supports an association (not causality) between obesity and severity of asthma (10, 109, 110). It is currently unclear whether obesity is a comorbidity that worsens asthma symptoms primarily because of excessive weight or whether obesity is a pathobiological driver of asthma. It is conceivable that it could be both. In patients with early-onset asthma, obesity is associated with more airway obstruction, bronchial hyperresponsiveness, and asthma duration (111). This finding may suggest that when asthma, particularly SA, is present since childhood and associated with limited activity and/or increased systemic corticosteroid use, BMI (body mass index) is a comorbidity rather than a pathological driver. Although there is no consistent relationship to FeNO in early-onset asthma, an active Type-2 process could still explain the rise in IL-5 and submucosal eosinophils observed in some obese SA patients (112). In contrast, obesity in late-onset asthma is strongly and inversely associated with FeNO. In obese, late-onset, non-Type-2 asthmatics, oxidative stress, metabolic syndrome, and mitochondrial dysfunction might all be contributors. In obese SA patients, elevated plasma levels of asymmetric dimethylarginine (ADMA) relative to plasma L-arginine, an imbalance that can also be seen in metabolic syndrome, could contribute to iNOS uncoupling and increased oxidative stress (113). Finally, a recent study suggests that most

symptoms experienced by non-Type-2 obese asthmatics result from mechanical compression of their small airways, owing to increased body mass in the absence of any inflammation (114). Thus, additional pathobiological, physiological, and perhaps radiological or other imaging studies are needed to identify specific characteristics of this particular group of SA patients.

Innate Immunity and Severe Asthma

Pattern recognition receptors (PRRs), such as Toll-like (TLRs) and NOD-like (NLRs) families of receptors, are key receptors of the innate immune system engaged in response to environmental stimuli, including foreign microbial proteins. Upon PRR activation in the lung, chemokines and cytokines are released from a variety of cells to initiate host defense. Expression of TLRs (particularly TLR4) (115) and epithelial cell–derived thymic stromal lymphopoietin (116), a potent, presumably innate, activator of dendritic cells, mast cells, and invariant natural killer T cells linked to Th2 cell differentiation, is reported to be elevated in both small and large airways of fatal asthma cases compared with mild asthmatics and control subjects.

Natural killer T cells are innate, non-MHC-class II-restricted, CD4$^+$ T lymphocytes that respond to lipid antigens through CD1d and are often associated with fungal and bacterial antigens (117). They can express Type-2 cytokines and IFN-γ, giving them the ability to link innate and adaptive immune systems (118). However, their role in SA has been highly controversial, with an initial study suggesting natural killer T cells made up a large percent of all CD4 cells in asthmatic airways (118), but subsequent studies were unable to find such a relationship (119, 120), making their role in SA unclear.

Viral infections also play a major role in exacerbations of asthma and SA. Some studies have suggested that airway epithelial cells from asthmatic patients have a deficiency in innate responses, particularly in Type-I and Type-III interferons, which could promote adaptive immune responses that lead to asthma exacerbations (121, 122). Similarly, ILC2 cells are innate-responding, non-CD4-expressing cells that can enhance expression of Type-2 cytokines (see above). IL-33, which is produced by epithelial and other cell types in response to lytic cell death, is believed to be one of the strongest activators of ILC2 cells (78). Thus, stimuli that cause lytic cell death, including viral infections, might serve as the initial activators of ILC2 cells. However, the role of any of these pathways in SA remains unclear.

Airway Structural Changes/Remodeling in Severe Asthma

The pathology of SA represents a mix of inflammatory and remodeling elements. Airway remodeling generally refers to structural changes in the airway walls caused by repeated cycles of injury, inflammation, and presumably abnormal repair or remodeling. Although SA has been prototypically associated with airway remodeling, in fact, how these remodeling changes relate to SA and its phenotypes remains poorly studied. Typically, inflammation itself is thought to contribute to these structural changes, but recent studies have questioned the critical nature of that relationship and indicated that perhaps physical forces alone (i.e., bronchoconstriction) can trigger airway remodeling (123). Histopathological findings seen in association with SA in the large airways include goblet cell metaplasia/hyperplasia; subepithelial fibrosis, particularly reticular basement membrane (RBM) thickening and extracellular matrix (ECM) deposition; increased airway smooth muscle mass (hyperplasia and hypertrophy); and vascular changes (124–127). These structural changes are discussed in detail below; we first define the structural compartments of the lung and then correlate them to well-known inflammatory pathways (see **Table 3**). **Figures 1** and **3** summarize the complex relationship between inflammation and the structural changes in SA.

TLR: Toll-like receptor

RBM: reticular basement membrane

ECM: extracellular matrix

Table 3 Structural changes in severe asthma with described associated mediators and other factors

Structural changes	Associated mediators and other factors
Subepithelial fibrosis/thickened RBM	Type-2 canonical cytokines (IL-4 and IL-13) TGF-β (from eosinophils and epithelial cells) MMPs and TIMPs Periostin (through TGF-β) IL-5 (through eosinophils) Mechanical stretch/contraction
Airway smooth muscle hyperplasia/hypertrophy	No direct in vivo evidence in humans In vitro evidence for TGF-β, HBEGF, angiotensin-II, cardiotrophin-I
Goblet cell metaplasia/hyperplasia Increased MUC5AC expression	Type-2 canonical cytokines (IL-4 and IL-13) through 15-LO1 and FOXA2 TGF-β2 Elastase (from neutrophils)
Angiogenesis	No direct in vivo evidence in humans
Increased epithelial permeability and loss of barrier function	Loss of occludins and claudins EGF (inducing remodeling via TGF-β pathways)

Abbreviations: 15-LO1, 15-lipoxygenase; EGF, epidermal growth factor; HBEGF, heparin-binding epidermal growth factor; MMPs, matrix metalloproteinases; RBM, reticular basement membrane; TGF-β, transforming growth factor-β; TIMPs, tissue inhibitors of metalloproteinases; TNF-α, tumor necrosis factor-α.

Airway anatomy. For this review, we divide the structural changes observed in SA into those that involve the large airways, which are airways that contain smooth muscle and cartilage and have a luminal diameter greater than 2 mm (bronchi up to terminal bronchioles), and those involving the small airways, which include all peripheral membranous bronchioles less than 2 mm in diameter. The large airways are composed of a mucosal surface epithelium; an underlying submucosa containing submucosal glands followed by a circular layer of smooth muscle; a cartilaginous plate; and finally the adventitia, which is near the pulmonary vasculature and the lung parenchyma. In contrast to the large airways, the small airways lack a cartilaginous plate and typically a smooth muscle layer. Using the smooth muscle layer as a landmark, we can divide airways into an inner layer, which includes the epithelium and the submucosa, and an outer layer, which includes the smooth muscle up to the adventitia. The alveolar attachments attach to the outer layer. The alveolar attachments, the boundary between airways and parenchyma, also serve as important tethers to maintain airway patency and prevent airway collapse (128) (see below).

Epithelial changes. The normal bronchial mucosa is a pseudostratified epithelium composed mainly of columnar ciliated epithelial cells, intermixed mucus secreting goblet cells, and a pool of basal cells responsible for epithelial regeneration. Also present in rare numbers are neuroendocrine cells, intermediate cells, and club cells (i.e., Clara cells). Goblet cell hyperplasia/metaplasia is the most significant histological finding identified in the epithelium of asthmatics regardless of disease severity. It is more significant in severe and fatal asthma (129–131), contributing to increased mucus production, airway narrowing, and perhaps increased overall airway wall thickness. Goblet cells produce mucin glycoproteins (MUC); MUC5AC is the dominant mucin in asthmatic airways (132). Although normally expressed, MUC5AC is upregulated in asthmatics, particularly in those with evidence for a Type-2 immune process. IL-13 induces goblet cell hyperplasia and mucus production through several pathways, including TGF-β2, FOXA2, and 15-LO1 (133, 134). IL-13 induces expression of both TGF-β2 and 15-LO1 in vitro, which subsequently contributes to

MUC: mucin glycoprotein

MUC5AC expression, suggesting that the effect of IL-13 to induce mucus is not direct. Their expression in relation to MUC5AC ex vivo has also been reported. Neutrophil elastase can induce mucus secretion (135), which could contribute to mucus plugging in neutrophil-rich SA. There appear to be disease-specific differences in mucin production in human airways, with MUC5B produced under basal conditions (nonasthmatics) in the submucosal glands and in epithelial cells (without mucus metaplasia) of the small and large airways. In contrast, MUC5AC is likely the most important mucin produced primarily by goblet cells (not in submucosal glands) in response to Type-2 cytokines of asthmatic airways (132). Recent murine studies find that MUC5B is a critical regulator of innate host defense (136), suggesting that the switch to MUC5AC in asthma may also impair normal innate immunity.

In many instances, abrupt or progressive obstruction of the airway lumen is believed to be the main cause of death in fatal SA. In cases of fatal asthma, there is often extensive luminal mucus plugging in both large and small airways, frequently admixed with inflammatory cells, predominantly eosinophils, and detached epithelial cells (see **Figure 2**), which can be seen as Creola bodies in sputum cytological preparations, although these do not constitute a specific finding of asthma (25, 137). It is also possible that more exaggerated bronchoconstriction occurs in the presence of luminal mucus and inflammatory infiltrate, further contributing to the fatal event (138).

There has been little research on the pathobiological role of the submucosal glands in SA. There is evidence of increased submucosal glands, associated with increased mast cells and neutrophil infiltration, in fatal asthma, but this was also seen in nonfatal asthma cases (139, 140). Submucosal glands in fatal asthma demonstrate elevated myoepithelial cells, which could regulate mucus secretion, although the type of mucin secreted from the glands in SA is unknown (141); moreover, increased submucosal glands can be seen in other chronic lung diseases such as chronic bronchitis.

Ciliary dysfunction, based partially on the replacement of ciliated cells by goblet cells, has been reported for SA epithelia. However, actual ciliary dysfunction with decreased ciliary beat frequency, dyskinesia, and ciliary disorientation has also been reported (142). Ultrastructural microtubule defects, mitochondrial damage, and cytoplasmic webbing could also contribute to ciliary dysfunction (142). Impaired ciliary function further impacts mucociliary clearance and, together with increased goblet cells and submucosal gland hyperplasia, could lead to increases in bacterial infections and bronchiectasis (143).

Bronchial epithelial shedding has been observed in autopsy lungs of fatal asthma. In addition, sputum and bronchoalveolar lavage fluid from SA patients contain elevated amounts of epithelial cells (128). Epithelial denudement has also been identified in healthy controls, raising the possibility that epithelial sloughing is an artifact of tissue sampling. Therefore, the extent of airway epithelial denudation, at least in bronchoscopic biopsies, should not be used as an indicator of asthma severity (127, 144).

Bronchial epithelial cell hyperplasia is believed to be one of the components responsible for mucosal thickening in SA, although the mechanisms underlying hyperplasia are less clear. The airways of SA patients show increased epithelial cell proliferation, as evidenced by increased Ki-67 proliferation index and decreased hypophosphorylated retinoblastoma protein, with Bcl-2 down-regulation, indicating lower levels of apoptosis (127, 145). Dysregulated epithelial cell proliferation could lead to a loss of epithelial cell contact with the basement membrane and increased apoptosis, a process also called anoikis (146).

Early data suggest that in addition to decreases in apoptosis, autophagy pathways may be abnormal. Macroautophagy normally aids in the removal of oxidized proteins or organelles, contributing to cell survival and reductions in tissue damage. However, autophagy can contribute to cell death.

Activation of autophagic pathways in the epithelium has been associated with increasing asthma severity in adults, but further studies are needed to clarify their role in the pathobiology of SA (147).

Under physiological conditions the airway epithelium acts as a protective barrier against pathogens and toxins, mainly via tight and adherens junctions, which regulate epithelial permeability. SA bronchial epithelial cells express fewer occludins and claudins, important components of tight junctions, and thus are more permeable (148). This increased permeability may also involve redistribution of E-cadherin and p120 catenin, important components of the adherens junctions, and possibly be mediated by TNF-α (149). Other studies have proposed that, even when cultured, airway epithelial cells from children and adults who died of fatal asthma are differentiated, with fewer adherens junction proteins, such as E-cadherin, increased numbers of basal cells, and phosphorylation of p38 mitogen–activated protein kinase, compared with epithelial cells from nonasthmatic controls. Thus, the asthmatic epithelium may be phenotypically different and more susceptible to injurious external stimuli (150). Moreover, in vitro data have shown in cultured bronchial epithelial cells that IL-4 can suppress E-cadherin via TGF-β1-dependent mechanisms, supporting a role for Type-2 inflammation in this process. However, the relationship of these changes to severe disease is less clear.

Injury and loss of epithelial barrier function can also lead to the generation of growth factors that, when interacting with the underlying mesenchyme, can promote airway remodeling responses (see below). Epidermal growth factor (EGF) receptor, as a marker of epithelial injury, is increased in bronchial biopsies from patients with SA (151, 152), and it is believed to be a marker of ongoing epithelial activation in response to injury. Enhanced EGF expression in the airway mucosa implies a constant state of repair, which may mediate airway remodeling by regulating TGF-β signaling (151) and inducing mucus production (153). This constant repair state could also contribute to neutrophilic inflammation through enhanced production of IL-8 (152). However, it remains unclear which pathways regulate EGF signaling in SA.

Cigarette smoke also affects asthmatic epithelial cells. EBBs have shown more goblet cells and increased epithelial thickness, as well as a higher proliferation rate of intact and basal epithelium in current smokers when compared with ex-smokers and never-smokers (108).

Subepithelial fibrosis.

Reticular basement membrane thickening. One striking histological finding of airway chronic inflammation and remodeling in SA is subepithelial fibrosis of the large/conducting airways, which occurs concomitant with the epithelial structural changes described above. Histological sections demonstrating marked RBM thickening in a case of fatal asthma can be seen in **Figure 4**. VATS tissue demonstrating RBM thickening in an SA patient is shown in **Figure 5**. Subepithelial fibrosis includes two distinct pathological findings, thickening in the region of the basement membrane and an increase in ECM deposition in the submucosal space below the basement membrane. The basement membrane consists of a superficial layer (basal lamina) and a deeper layer (lamina reticularis, or RBM). In asthma, thickening of the basement membrane occurs just below the basal lamina and is restricted to the lamina reticularis. The basal lamina is composed of fine filaments of collagen type-IV approximately 0.1 μm thick that cannot be resolved by light microscopy. In contrast, the RBM can be observed by light microscopy and its thickness is measured by various methods. Direct visual observation by light microscopy is acceptable if the tissue has been properly embedded and oriented. However, different measurement approaches yield different results, likely explaining some of the discrepancies (154–156). Typically, specific immunostains for different ECM components (e.g., collagens, tenascin, laminin) provide additional information regarding
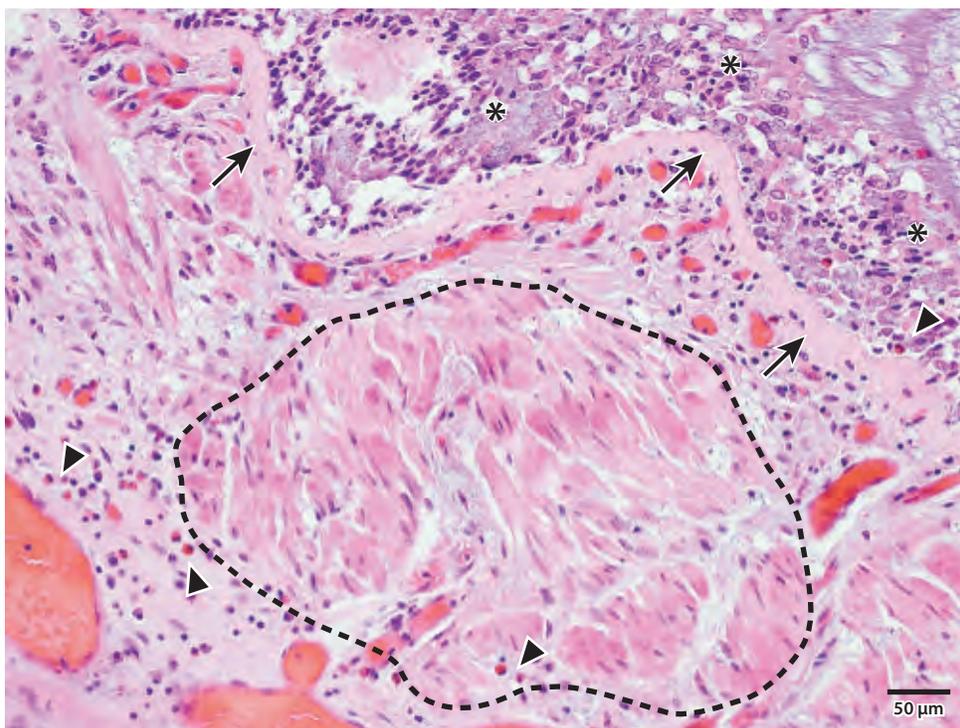
Figure 4

Epithelial sloughing (*asterisks*), thickened reticular basement membrane (*arrows*), eosinophilia (*arrowheads*), and smooth muscle hyperplasia/hypertrophy (*dashed line*) in a case of fatal asthma. Magnification 400×; scale bar = 50 μm.

the quality (and quantity) of the RBM (157). However, no studies to date report differences in these components between severe and milder asthma, or how they relate to any phenotypes.

Thickening of the RBM is due primarily to a plexiform deposition of immunoglobulins, collagen-I and collagen-III, tenascin, and fibronectin (158). It has been extensively studied as a marker of remodeling in asthma and SA, but the reported studies are conflicting. Some investigators have concluded that thickened RBM is the most discriminative histological finding of SA, generally not occurring in other chronic obstructive lung diseases (159, 160), whereas others have suggested that it may be unrelated to asthma severity (157). Regardless, the reported thickness of the RBM in asthmatic patients varies considerably. Whereas normal values in healthy controls range from 3 to 7 μm (155), measurements in asthmatics range from 7 to 23 μm (161). Additional confounders include the heterogeneity of SA definitions and phenotypes, as well as the relationship of RBM thickening to specific inflammatory responses.

Mechanisms for RBM thickening. The thickness of the RBM in asthma is determined by the proportion of ongoing deposition and degradation of proteins, including collagen-I, collagen-III, collagen-V, fibronectin, tenascin, lumican, and biglycan. These components are likely secreted by activated fibroblasts and myofibroblasts, primarily because of TGF-β signaling (162, 163). However, unlike diseases such as interstitial pulmonary fibrosis, RBM thickening in asthma does not progress to an overall fibrotic airway. The mechanisms for this limited fibrosis are not clear, yet
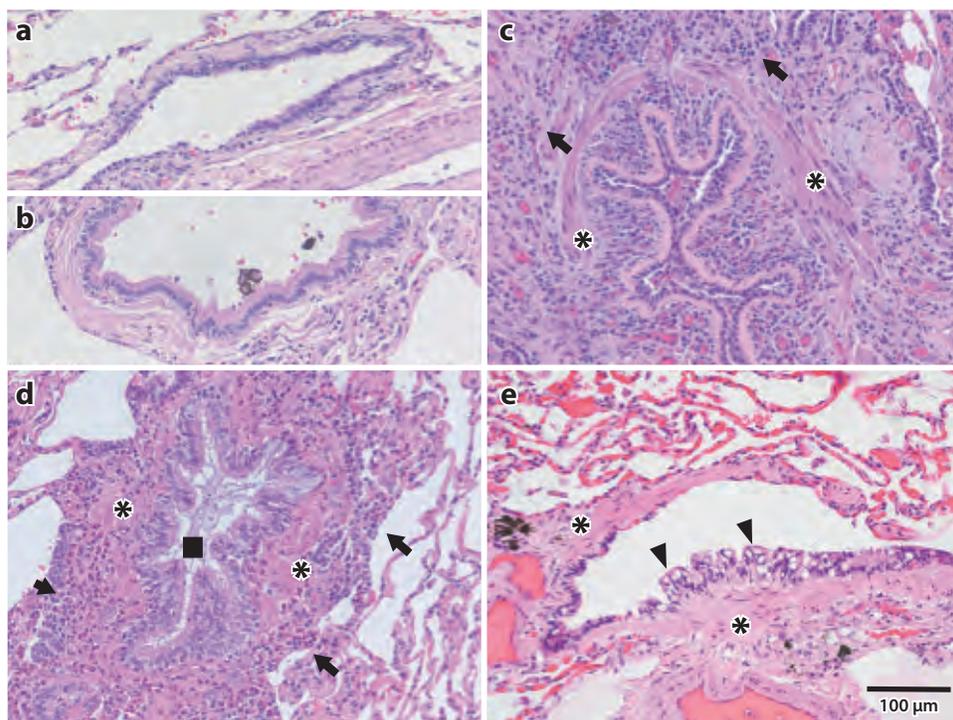
Figure 5

Small airway disease in severe asthma. (*a,b*) VATS tissue from nonasthmatic subject showing two
unremarkable small airways. (*c,d*) VATS tissue from a severe asthmatic showing small airways with epithelial
hyperplasia (*black square*), prominent smooth muscle hyperplasia/hypertrophy (*asterisks*), and adventitial
eosinophilic/lymphocytic inflammation extending into areas of alveolar attachments (*arrows*). (*e*) VATS
tissue from a fatal asthma specimen showing a significantly muscularized small airway (*asterisks*) with goblet
cell metaplasia (*arrowheads*). Magnification 200×; scale bar = 100 μm. Abbreviation: VATS, video-assisted
thoracic surgery.

some reports support active turnover of the ECM proteins through interactions with various pro-
teases, including the MMPs and their inhibitors (tissue inhibitors of metalloproteinases, TIMPs)
(154, 164). The balance of MMPs and TIMPs, in association with TGF-β expression, has been
linked to the overall thickness of the RBM (92, 165), indicating that neutrophilic inflammation
may help limit thickness.

An eosinophilic/Type-2 cytokine-associated process appears to be critical for RBM thickening.
The RBM is thickest in SA patients with high tissue eosinophils and in milder asthmatics with
evidence of a Type-2 gene signature in the epithelium (53). The importance of this association
was confirmed by the ability of a monoclonal antibody to the proeosinophilic Type-2 cytokine
IL-5 to substantially decrease specific ECM components of the RBM (tenascin/laminin), perhaps
through diminished eosinophil-related TGF-β (8). Activated eosinophils are suggested to be one
of the main sources of TGF-β in the asthmatic airway (166).

Type-2 cytokines such as IL-4 and IL-13 are also likely important modulators of ECM de-
position in asthma. Both can modulate the recruitment of eosinophils and induce collagen de-
position from fibroblasts (167, 168). IL-4 and IL-13 can induce secretion of epithelia-derived
periostin, which can activate TGF-β signaling pathways, leading to type-1 collagen deposition.

TIMPs: tissue
inhibitors of
metalloproteinases

Turnover through MMP-2 and MMP-9 pathways may also be involved (169). Further, in addition to contributing to goblet cell hyperplasia, IL-13-induced TGF-β_2 could affect collagen deposition through either an epithelial or an eosinophil source (134, 170).

Finally, in vitro and ex vivo studies have suggested that mechanical stretch and contraction, which might be seen during an asthma exacerbation, could contribute to ECM deposition (123, 171). However, the relative contribution of mechanical stretch to inflammatory responses, especially in SA, is not clear.

In summary, RBM thickening is a major histological finding in asthma and particularly SA. Although many factors likely contribute to overall thickness, RBM thickening occurs primarily in SA patients with ongoing Type-2 inflammation, likely through TGF-β pathways.

Submucosal fibrosis. Remodeling of the submucosal ECM in SA is much less characterized and understood than remodeling of the RBM. Although the RBM is generally thicker in asthma and certain subtypes of asthma, ECM remodeling in the submucosal space is even more controversial, with some studies showing an increase, whereas others have not (124, 157). The importance of submucosal fibrosis in SA compared with milder asthma is not yet clear.

Increased airway smooth muscle mass. Airway smooth muscle is one of the main structural components of the conducting airways and a prominent histological indicator of remodeling in an asthmatic airway. It is believed that airway smooth muscle thickening in asthma is a consequence of both hypertrophy (increased size of airway smooth muscle cells) and hyperplasia (increase proliferation of airway smooth muscle cells), both of which correlate with asthma severity (172, 173). Benayoun et al. (145) suggested that hypertrophic airway smooth muscle thickening is a better histological indicator of asthma severity than RBM thickening or airway inflammation. They observed a correlation between increased numbers of fibroblasts/myofibroblasts within the airway smooth muscle and degree of asthma severity. This finding could indicate that increased airway smooth muscle area in SA may be due to migration and proliferation of fibroblasts in the muscle bundles (145).

Others have suggested that hyperplasia of airway smooth muscle cells plays a more important role, with in vitro evidence implying that many cytokines, including TGF-β and heparin-binding epidermal growth factor (HBEGF), are involved (172, 174). Despite this, airway smooth muscle hyperplasia in asthma is controversial; one study reported an increase in markers of proliferation such as Ki-67 and proliferating cell nuclear antigen in SA (174), whereas another did not (175). The remaining studies are indirect, with various factors, including FGF-2 (fibroblast growth factor-2) and cysteinyl leukotrienes, increasing airway smooth muscle cell proliferation in vitro without relation to markers of cell proliferation in vivo (174, 176–178).

Airway smooth muscle cells could also modulate airway remodeling by secreting inflammatory mediators (e.g., TGF-β) and matrix proteins and by expressing cell adhesion molecules and other costimulatory molecules involved in further migration and activation of inflammatory cells. In particular, airway smooth muscle cells express chemokines for mast cells, including CXCL10, which may contribute to the reported increase in mast cells in airway smooth muscle (68). However, how these processes relate to either SA in general or, more interestingly, Type-2 inflammatory phenotypes of SA is not known.

Increased airway smooth muscle mass is a feature of airway remodeling in SA. Nevertheless, years of research have not clarified whether this is due to increased proliferation, myocyte mass, or migration of fibroblasts/myofibroblasts, as many cytokines are involved in this process. Examples of airway smooth muscle thickening in SA can be seen in **Figures 4** and **5**.

Angiogenesis. Research in the field of angiogenesis in SA is limited, owing to the generalization of findings in mild-to-moderate asthma and SA, a lack of uniformity in the SA definition used, and an overall paucity of human studies. Some human studies have demonstrated increased submucosal vascularity both in total number of vessels and vasodilation in EBBs of mild asthmatics and SA patients and fatal asthma lungs (179–181). Reports of corticosteroids inhibiting vascular changes in asthma have emerged (182), but the relevance of this approach to SA, in which patients are already treated with high doses of corticosteroids (and perhaps are refractory to them), is unclear and requires further investigation. Others failed to demonstrate increased vascularity or angiogenesis in mild asthma, moderate asthma, or SA (183). Methodological issues may contribute to these discrepancies. Measuring of vasculature density in different areas of the mucosa (anywhere from subepithelial to deep submucosal) has been performed; stereology has rarely been applied; and different antibodies, including CD31, CD34, factor VIII, and collagen IV, among others, have been used to highlight the vasculature in tissue sections. Moreover, none of these antibodies is entirely specific, and cross-reactivity with inflammatory cells and other mesenchymal components ultimately makes comparisons difficult. A small study of SA patients proposed that increased submucosal vascularity was associated with increases in intercellular adhesion molecule-1, which could also mediate migration of inflammatory cells in the airways of SA patients (180).

Considerable interest has focused on vascular endothelial growth factor (VEGF) and angiopoietin-1 (Ang-1) as factors triggering neovascularization in asthma (184, 185). Both are elevated in sputum from SA patients, but their expression in relation to vascular remodeling in SA is unknown (186, 187). Although various murine studies suggest strong interactions between VEGF and Type-2 inflammation, there are no reports on the relationship between Type-2 inflammation and vascular remodeling in human asthma or SA (188, 189).

Finally, although changes in the number or size of bronchial vessels have been reported, vasculitis per se is not a feature identified with asthma. The obvious exception is Churg-Strauss syndrome, in which vasculitis and asthma are key features. However, this syndrome represents a small percent of SA (190).

Distal Lung Pathology: Small Airways and Alveoli

Inflammatory and remodeling events occurring at the level of the small airways in asthma and SA remain poorly understood, as most research has used EBBs targeted to the central airways. However, the small airways are likely important contributors to the functional impairment in SA. Small airways have a larger combined cross-sectional area than the larger airways do, and inflammation can extend into the alveolar parenchyma (24). Thus, inflammation and remodeling in this compartment can be detrimental to SA patients, as some reports suggest asthma severity increases in proportion to the involvement of the distal lung (191, 192). Unfortunately, most data on the distal lung in asthma provide little to no histological information or association with computed tomographic assessments of air trapping and regional ventilation/perfusion (193), exhaled gases (nitric oxide) (194), or physiological measures (195). Alternatively, autopsy distal lung tissue from fatal asthma specimens often has limited associated clinical information (138).

Early autopsy data indicated that eosinophilic infiltration was not restricted to the large airways; rather, these inflammatory cells distributed throughout the bronchial tree, including the smaller airways in both fatal and nonfatal asthma lungs. Overall, inflammatory cells were more abundant in both large and small airways of SA lungs than in control nonasthmatic lungs (128). Early studies of resected lung tissue confirmed that asthmatic small airways have higher numbers of activated eosinophils but not T cells when compared with larger airways, possibly indicating that a more severe inflammatory process takes place in the smaller airways of asthmatics (24).

Likewise, eosinophils and macrophages accumulate largely in the alveolar tissue of nocturnal asthmatics (196). Unfortunately, these studies did not include SA patients, but they did highlight the importance of small airways and prompt for further exploration. Examples of SA with small airway eosinophilic inflammation in VATS tissue from SA patients can be seen in **Figure 5c,d** (note the inflammatory infiltrate extending into the areas of alveolar attachments).

The only quantitative data from living SA patients comes from TBBs and occasional VATS. Sampling of alveolar tissue using these techniques showed higher numbers of neutrophils in SA patients than in milder asthmatics or in the larger airways sampled by EBBs (90). However, that study did not evaluate small airway tissue. A later study demonstrated that inflammatory cell counts were higher in SA small airways (predominantly) and alveolar tissue than in larger airways, with increased inflammation in both the inner and outer airway walls as measured on TBBs and VATS tissues (27, 197). Finally, mast cells in the small airways and alveolar tissue of SA patients have also been evaluated. Mast cells are more prominent in the small airways (inner and outer walls) and in the alveolar attachments of SA patients, than in large/conducting airways (28). There was no increase in mast cells in the nonairway-associated alveolar tissue. Whether the relative decrease in mast cells in the large airways is due to deposition of inhaled corticosteroids is not known (198).

Data on fatal asthma lungs also support regional differences in composition of the ECM, with the small airways showing decreases in decorin and lumican and increased versican in the outer wall in asthmatics compared with control subjects (138). However, evidence supporting overall subepithelial fibrosis in the small airways in SA is controversial (2, 157, 199). Of note, central and distal lung fibroblasts are phenotypically different, as distal fibroblasts are more proliferative and stellate in appearance and express more α-smooth muscle actin (more myofibroblast-like) (200–202). Regional differences in fibroblast phenotype could contribute to structural differences between small and large airways. However, the clinical relevance of these differences to SA remains unknown. Finally, airway smooth muscle is increased in the small airways of fatal asthma cases. Smooth muscle should typically not be found in the small airways (203). Examples of smooth muscle hyperplasia/hypertrophy in small airways (**Figure 5c–e**) can be compared with nonasthmatic small airways (**Figure 5a,b**), which have almost no smooth muscle.

Small airway pathology due to alterations in airway-parenchyma uncoupling might also play a role in SA. Small airways of the tenth generation and beyond are directly connected to the alveolar parenchyma (204). Fatal asthma lungs have shown loss of airway-alveoli attachments, which could decrease the mechanical tethering forces of the parenchyma. Fatal asthma lungs also show loss of elastic fibers in the adventitial wall of the small airways and peribronchial alveolar septae, with no effect on the distal alveoli (23). These mechanical changes could result in excessive contraction of the smooth muscle upon stimulation, contributing to early airway closure during expiration (23, 205). In addition, these changes have been proposed to lead to physiological loss of elastic recoil in patients with SA (206), suggestive of a pseudoemphysematous component. Studies of older people who died of asthma showed adventitial thickening with deposition of fibronectin, collagen-I, and collagen-III but with increased expression of MMPs, which could also contribute to loss of airway-parenchyma tethering (191, 207). Of note, there are no descriptions of broader anatomic emphysema in SA in the absence of a smoking history (208). The processes controlling the loss of alveolar attachments are not known.

These data highlight the importance of understanding the pathological relevance of the small/distal airways in SA, as these airways are less accessible to inhaled delivery routes for anti-inflammatory medications such as corticosteroids. In addition, the large and small airways are not independent of each other, at least physiologically, and thus should be viewed as a complex interrelated network. Further studies of the distal lung in asthma are needed.
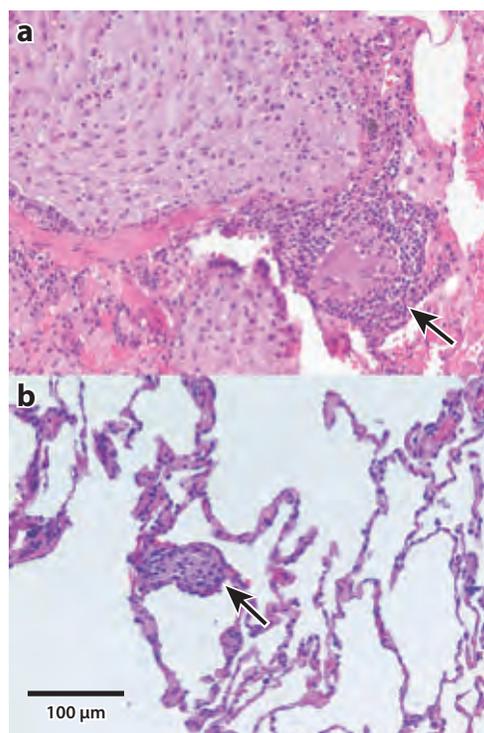
Figure 6

VATS tissue from a subject with asthmatic granulomatosis showing poorly formed non-necrotizing granulomas (*arrows*) (*a*) in close proximity to an airway and (*b*) in the interstitium. Magnification 200×; scale bar = 100 μm. Abbreviation: VATS, video-assisted thoracic surgery.

Use of VATS sampling in severe asthma. As noted above, distal lung pathology in living asthmatic patients is poorly described. However, in many cases, SA remains a debilitating set of phenotypes or diseases with few treatment options besides systemic corticosteroids. Thus, in patients with a clinical asthma diagnosis, but who require high doses of systemic corticosteroids, a pathological approach may be justified. In this regard, we recently reported 10 asthma cases in whom extensive small airway disease (eosinophilia and goblet cell hyperplasia) was present in association with interstitial, poorly formed non-necrotizing granulomas of epithelioid histiocytes with or without giant cells. This newly observed entity was named asthmatic granulomatosis (26). Histological sections from VATS tissue showing asthmatic granulomatosis are presented in **Figure 6**; note the poorly formed non-necrotizing granulomas distributed predominantly in the bronchioles but also occasionally in the interstitium. This particular subset of SA patients often has a family history of autoimmune diseases and can improve with nonsteroidal immunomodulatory therapy. Of note, asthmatic granulomatosis is not observed in all patients undergoing VATS sampling for asthma, with additional cases showing little to no inflammation, caseating granulomas, microaspiration, or predominant lymphocytic bronchiolitis in the absence of granulomas, suggesting that tissue biopsy could help personalize treatment and support for multiple asthma phenotypes. Further studies are needed to characterize the immunological pathways involved and to develop biomarkers that would more easily identify and then treat this newly described disease.

SUMMARY POINTS

1. The pathobiology of the lung in SA remains poorly understood for reasons ranging from poor definitions to lack of adequate tissue sampling.

2. Severe asthma pathology is best addressed in relation to the clinical heterogeneity of disease.

3. There is strong evidence for ongoing Type-2 immune processes despite the use of high-dose inhaled (and even systemic) corticosteroids, although the importance of other adaptive immune pathways is not clear.

4. The large airway epithelium in SA likely contributes substantially to disease and associated clinical symptoms through abnormalities in innate immunity and mucus secretion.

5. Although the RBM is thickened in a Type-2 subset of SA, its role as a pathological marker for SA is less clear.

6. Increased airway smooth muscle mass is a consistent feature of airway remodeling in SA. It remains unclear to what extent this is due to increased proliferation, myocyte hypertrophy, and/or migration of fibroblasts/myofibroblasts, as many cytokines are implicated in this process.

7. VATS sampling can aid in the pathological evaluation of SA, potentially improving therapeutic approaches based on better characterization of the underlying disease process.

DISCLOSURE STATEMENT

ACKNOWLEDGMENTS

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