

## Autopsy and Imaging Studies of Mucus in Asthma

### Lessons Learned about Disease Mechanisms and the Role of Mucus in Airflow Obstruction

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#### Abstract

Autopsy studies in fatal asthma have clearly documented the central role of airway plugging with pathologic mucus in the pathophysiology of death from asthma, but the role of mucus plugs in chronic severe asthma has been less well understood. Recently, multidetector computerized tomography imaging of the lungs has emerged as a valuable method to visualize mucus plugs in asthma. These multidetector computerized tomography data have revealed mucus plugs as a common occurrence in severe forms of asthma. In

addition, an image-based mucus plug scoring system shows that mucus plugs are strongly associated with measures of airflow obstruction and with biomarkers of type 2 cytokine and eosinophilic inflammation. These data provide a rationale for treating airflow obstruction in severe asthma with mucolytics, and they also raise the possibility that treatments that target type 2 inflammation may decrease mucus plugs in asthma.

**Keywords:** eosinophils; eosinophil peroxidase; type 2 inflammation; mucins

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Maintaining airway patency is essential to life, and, consequently, sophisticated mucociliary machinery has evolved in the airway to balance mucus secretion, transport, and clearance to keep the airways healthy and patent (1). In health, mucus is a lightly crosslinked gel that is continually transported by the cilia to the upper pharynx, where it is cleared. In inflammatory airway disease, alterations in the volume, composition, and viscoelastic properties of mucus, can combine in varying degrees to form abnormal or “pathologic mucus.” This pathologic mucus is not as easily cleared by the cilia and can accumulate in the airway, leading to mucus plugging and airflow obstruction. In this article, we review the role of pathologic mucus plugs in the pathophysiology of asthma, with an emphasis

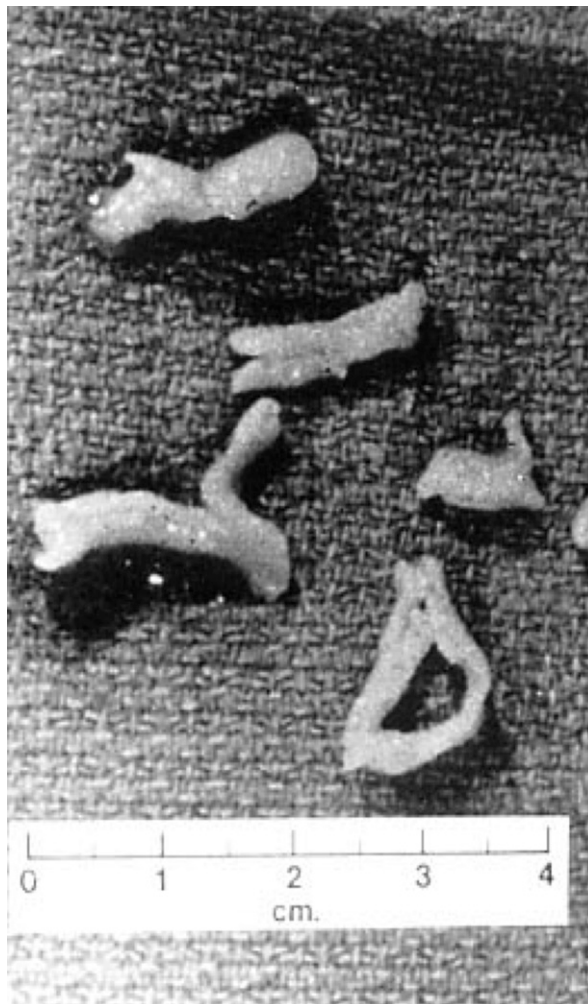
on how studies have transitioned from a historical reliance on autopsy studies to more recent studies that have taken advantage of multidetector computed tomography (MDCT) imaging of the lungs.

#### Autopsy Studies of Mucus Plugs in Asthma

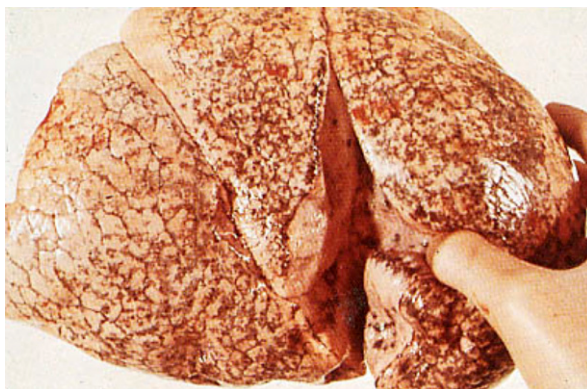
Airway mucus casts are frequently recovered from bronchoalveolar lavage from patients with asthma who are experiencing an acute exacerbation (Figure 1) (2). In addition, accumulation of pathologic mucus is a key feature of fatal asthma, where autopsy studies clearly show extensive mucus occlusion of the airways (1, 3). More than 50 years ago, Dunnill provided graphic

descriptions in 20 cases of fatal asthma noting “a striking picture with numerous gray, glistening, mucous plugs scattered throughout the airway passages” (3). The consequences of these mucus plugs are also manifestly apparent at autopsy. The lungs are markedly hyperinflated, ballooning to occupy the whole thoracic cavity and “failing to collapse once the negative intrathoracic pressure is released” (Figure 2) (3, 4). Dunnill summarized that “pathologically, the outstanding feature of the asthmatic lung lies in the failure of clearance of the bronchial secretions” (3).

Despite longstanding awareness of the prominence of airway mucus plugs in the pathophysiology of acute fatal asthma, the role of mucus occlusion of the airways in chronic asthma has been less well



**Figure 1.** Bronchial casts in asthma. Bronchial casts recovered from the bronchoalveolar lavage of a patient experiencing an acute exacerbation. High concentrations of albumin are found in the airway mucus in acute severe asthma. Reprinted by permission from Reference 2.



**Figure 2.** Mucus plugs contribute to airflow obstruction in acute severe asthma. Gross specimen of lungs removed from a patient with fatal asthma. The lungs fail to remain inflated despite loss of negative intrathoracic pressure and fail to deflate when pressed as a result of air trapping from intraluminal mucus plugs. Reprinted by permission from Reference 4.

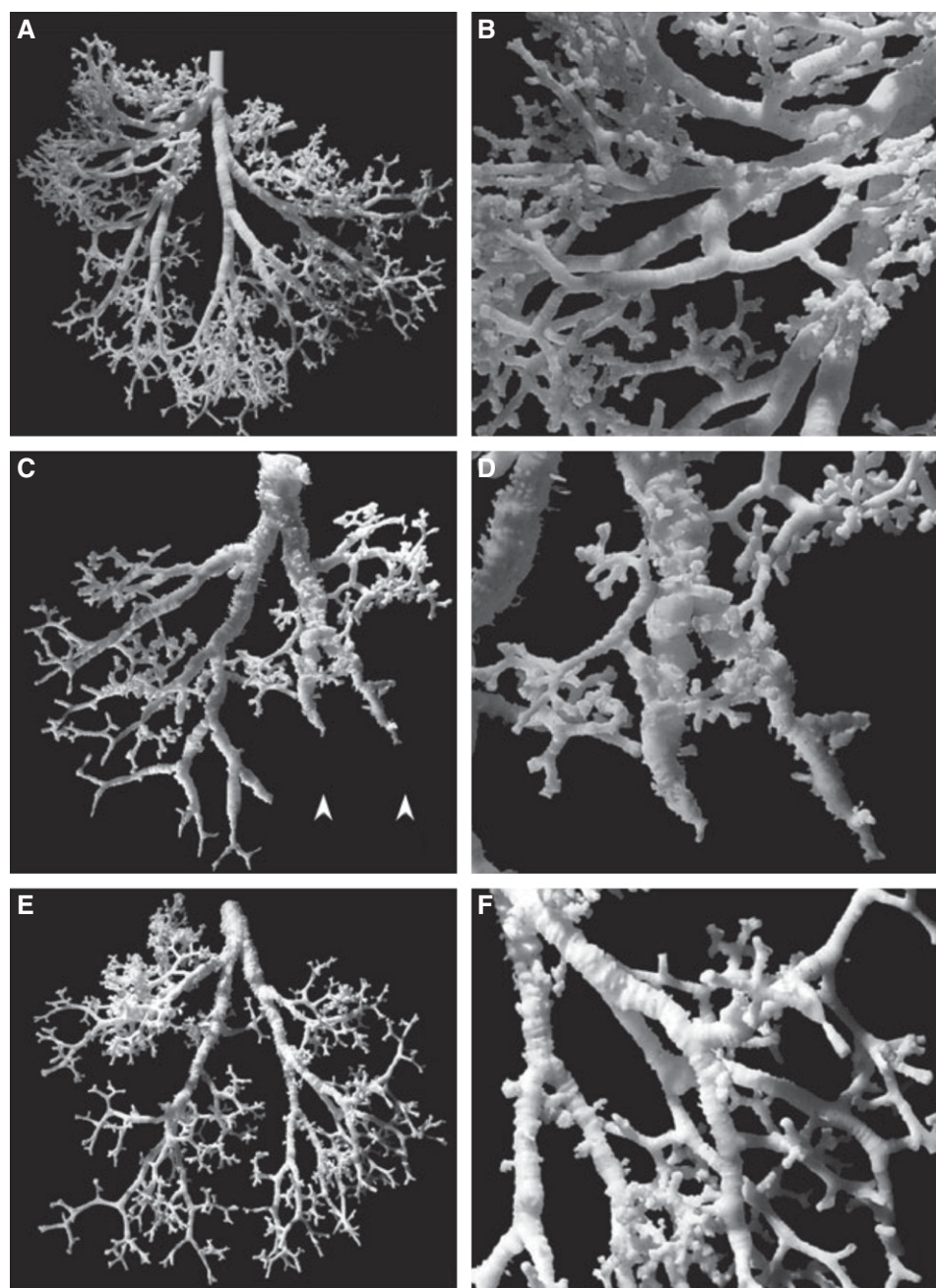
understood. Using silicon casting of the airways postmortem, a recent study showed that mucus plugging occurs in chronic asthma as well as fatal asthma (Figure 3) (5). Specifically, in subjects without asthma, silicone casts delineate the airways all the way to the alveolar duct level, whereas in subjects with fatal asthma, there is widespread truncation and loss of airways as a result of mucus plugging (6). In subjects with asthma who died of nonpulmonary causes (nonfatal asthma), there is also evidence of significant mucus plugging, although not as extreme a phenotype as fatal asthma. Notably, smaller distal airways tended to be lost in nonfatal asthma, rather than the larger proximal airways lost in fatal asthma. These silicone cast studies were not able to evaluate the effects of airway mucus plugs on lung physiology in asthma. Such studies require quantification of mucus plugs using MDCT imaging in patients with well-characterized asthma.

### Imaging Mucus Plugs *In Vivo* Using MDCT Lung Images

MDCT of the lungs has emerged as the imaging modality of choice for noninvasive assessment of airway anatomy, regional lung mechanics, and associated lung function (7). MDCT has been used in chronic asthma to study airway remodeling (8, 9), and CT measurements correlate with pathologic changes on biopsy, measures of disease severity, and airflow obstruction (10). These studies demonstrate that CT is a highly reproducible method for studying structure–function relationships in asthma.

In recently published studies, we used MDCT of the lungs to directly visualize and quantify airway mucus plugging in patients with asthma. Mucus plugs are discernible as low-attenuation areas of opacification within the airway lumen, contiguous with patent airway across sequential CT slices (Figure 4) (11). In asthma, these mucus plugs are predominantly found in nondilated subsegmental airways and appear as focal or branching opacities. We developed a visual scoring system on the basis of presence or absence of mucus plugs in each of the 20 bronchopulmonary segments and applied this mucus score (ranging from 0–20) to the MDCTs of participants enrolled in the Severe Asthma Research Program (SARP)-3 (11).

The SARP network was established to characterize the mechanisms underlying



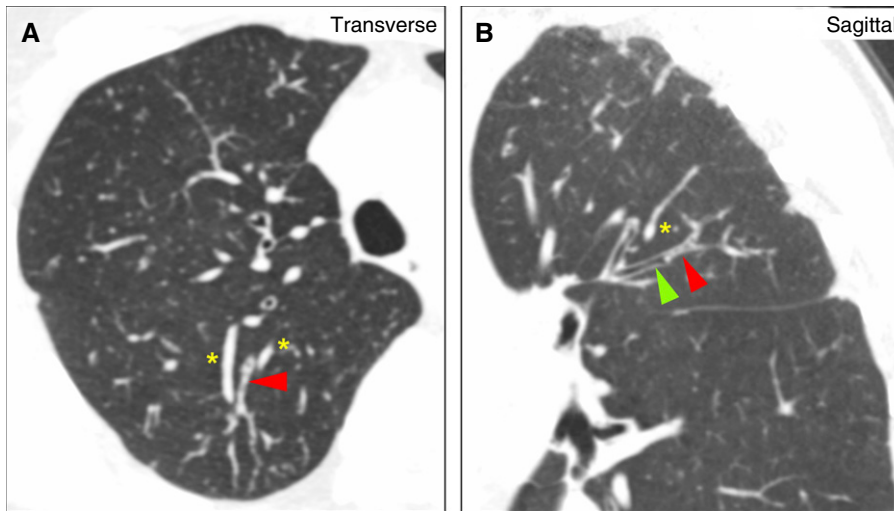
**Figure 3.** Negative pressure silicone casts of the airways. (A) Cast of the apical segment left lower lobe from a healthy control subject shown in greater detail in B. (C) Cast of a patient who died of asthma showing widespread loss of large airways (arrows) and (D) enlarged mucus glands on higher power. (E) Cast of a patient who died with asthma from nonasthma cause, showing loss of smaller airway, with (F) enlarged mucus glands on higher power. Reprinted by permission from Reference 5.

severe asthma, which affects 5% to 10% of patients with asthma (12, 13). SARP-3 was a longitudinal study in which 60% of patients had severe disease. We found that mucus plugging was present in the majority of patients with asthma (58%) and in less than 5% of healthy control subjects. SARP-1 and SARP-2 were earlier cross-sectional studies

in which approximately 40% of patients had severe disease. Data from a subgroup of patients who had an earlier CT scan as part of SARP-1 or SARP-2 showed that, in the majority of instances, bronchopulmonary segments with a mucus plug on the earlier scan had a plug in the same segment on the SARP-3 scan.

### Mucus Plugging Is Associated with Airflow Obstruction

Three categories of mucus score were used for analysis of the MDCT images from the SARP-3 cohort. Patients with no plugging on CT (score = 0) were assigned to the zero-mucus group, and patients with plugging on

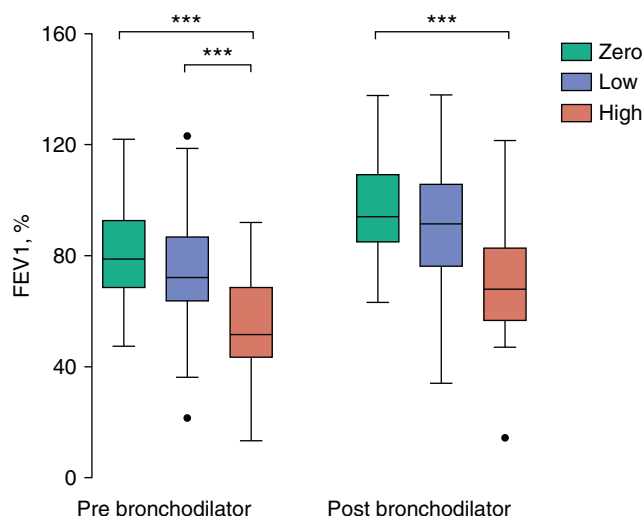


**Figure 4.** Mucus plugs shown in different planes on multidetector computed tomography. (A) Intraluminal mucus plug (red arrow) in longitudinal section on transverse plane. The accompanying bronchopulmonary vessels are indicated with yellow asterisks. (B) The same mucus plug seen on the sagittal plane (red arrow) demonstrates continuity with a patent airway lumen (green arrow) visible proximally. Reprinted by permission from Reference 11.

CT (score > 0) were assigned to the low-mucus group (score >0 to 3.5) or high-mucus group (score 4–20) on the basis of the median score in the “plugged” group. We found that patients with a high mucus score have much lower FEV<sub>1</sub> values than patients without mucus plugs, with a median forced expiratory volume in 1s (FEV<sub>1</sub>) of 50% in the high-mucus group, compared with 80% in the zero-mucus group (Figure 5) (11). Forced vital capacity was also lower in the

high-mucus group, reflecting a variable degree of air trapping in this group.

The relationship between mucus plugs and low FEV<sub>1</sub> persisted despite treatment with inhaled bronchodilators and systemic corticosteroids (Figure 5) (11). Although our data cannot prove a causal relationship between the presence of mucus plugs and low FEV<sub>1</sub>, such a causal relationship is highly plausible, because the plugs that were scored were ones that completely

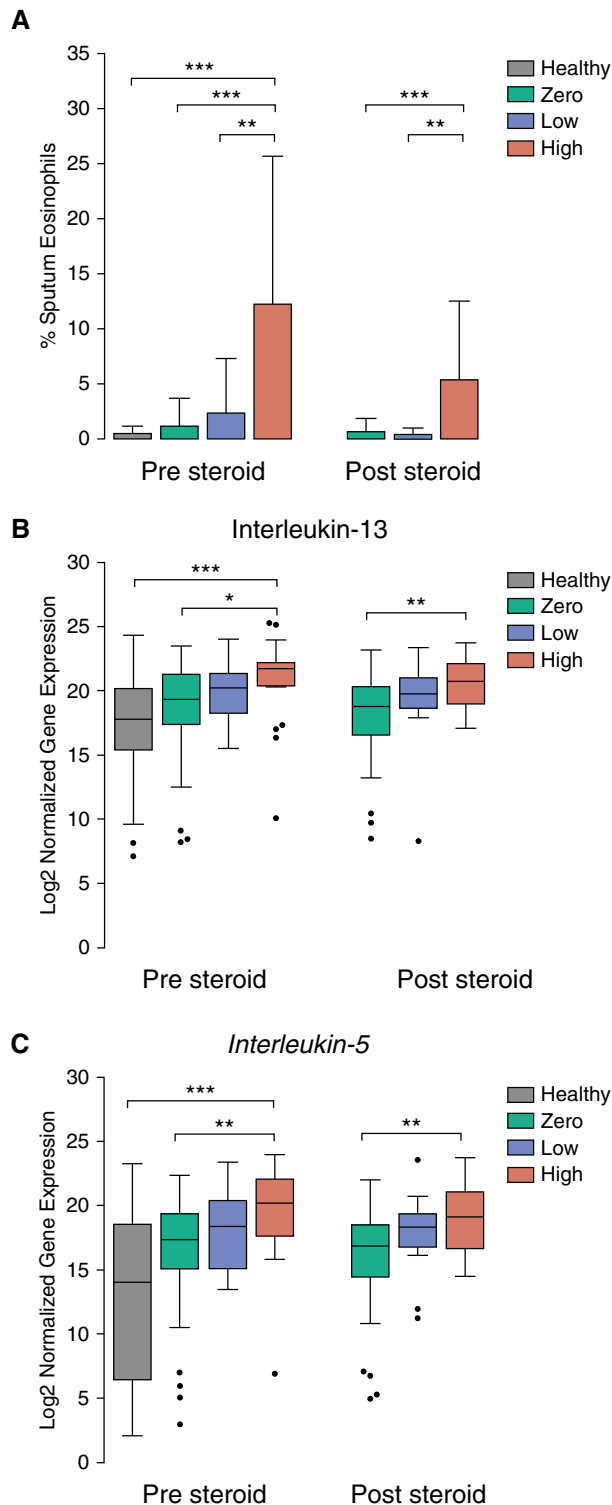


**Figure 5.** High mucus score is associated with airflow obstruction. Forced expiratory volume in 1 second (FEV<sub>1</sub>)% predicted in patients with a high mucus score was significantly lower than in subjects with a low mucus score and patients with a zero mucus score before and after maximal bronchodilation reversibility testing. \*\*\*Indicates  $P < 0.001$ . Reprinted by permission from Reference 11.

occluded airways. Thus, standard asthma therapies fail to normalize a low FEV<sub>1</sub> in the setting of a high mucus score. It is possible that mucolytic treatment would restore FEV<sub>1</sub> to the normal range in these patients.

## Mucus Plugging Is Associated with Persistent Type 2 Inflammation

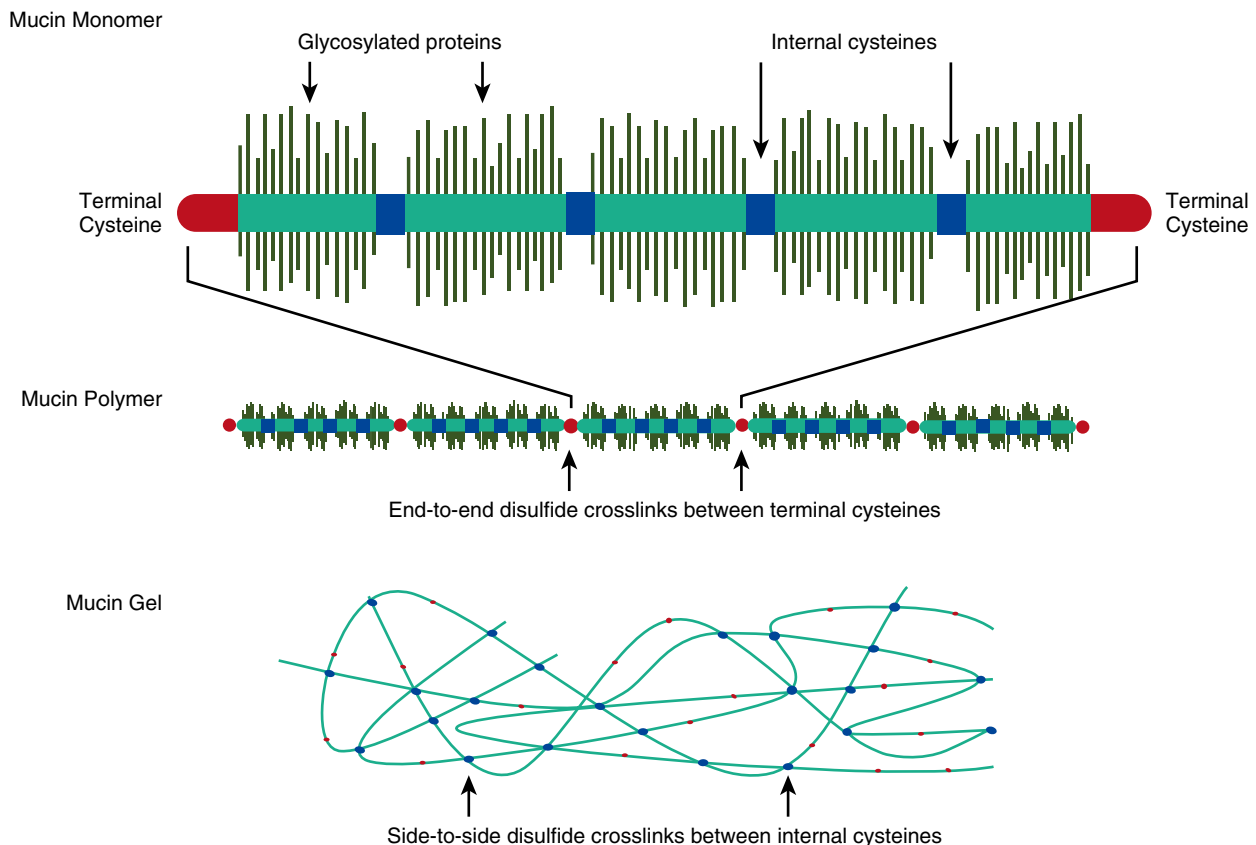
Blood and airway eosinophilia are strongly correlated with airflow obstruction in asthma (14, 15), and we examined the relationship between eosinophils and mucus plugs. We found that mucus plugging is strongly associated with airway eosinophilia and other markers of type 2 inflammation (Figure 6) (11). Specifically, among subjects with high mucus scores, 71% had sputum eosinophilia (sputum eosinophils > 2%) and 66% had systemic eosinophilia (blood eosinophils >  $300 \times 10^{-9}/L$ ). Remarkably, 90% of the high-mucus group were on high-dose inhaled corticosteroids and 23% were on systemic corticosteroid therapy at time of assessment, which would be expected to suppress type 2 inflammation (16). In addition, sputum eosinophilia persisted in the high-mucus group after intramuscular triamcinolone acetonide treatment (Figure 6) (11). Furthermore, the high-mucus group showed markedly increased sputum cell gene expression of type 2 cytokines, such as interleukin (IL)-5 and IL-13, which remained increased in the high-mucus group after triamcinolone treatment (Figure 6) (11). IL-13 induces club cells to transition into goblet cells through the coordinated actions of forkhead box A2 (FoxA2), thyroid transcription factor-1 (TTF-1), SAM pointed domain containing ETS transcription factor (SPDEF), and  $\gamma$ -aminobutyric acid A receptor (GABA<sub>A</sub>R) (17). Activation of these pathways leads to upregulation of mucin (MUC) gene expression (in particular MUC5AC) and increased mucin production and release. Consistent with these IL-13 effects, we see a relative increase in MUC5AC gene expression over MUC5B in the high-mucus group. Cysteine domains are more prevalent in MUC5AC than in MUC5B (18), and this alteration in relative abundance of MUC5AC could represent an important qualitative difference in airway mucins in the high mucus group.



**Figure 6.** High mucus score is associated with airway type 2 inflammation. (A) Percent sputum eosinophils were significantly higher in patients with a high mucus score before and after treatment with intramuscular triamcinolone. (B) Sputum cell gene expression of IL-13 was significantly higher in patients with a high mucus score before and after treatment with intramuscular triamcinolone. (C) Sputum cell gene expression of IL-5 in patients was significantly higher in patients with a high mucus score before and after treatment with intramuscular triamcinolone. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ . Reprinted by permission from Reference 11.

We have previously proposed that cysteine residues in the mucin apoprotein play an important role in pathologic mucus formation in cystic fibrosis, and we showed that oxidation arising from neutrophilic inflammation can convert cysteines to their oxidized disulfide products (cystines) (19). Disulfide interactions between internal cysteines in adjacent mucin polymers have the effect of crosslinking mucins to stiffen the mucus gel (Figure 7) (19). More recently, we showed that in asthma there is a similar role for eosinophil-driven oxidation in mucin crosslinking (11). Specifically, eosinophil peroxidase (EPO) is an eosinophil-derived enzyme that catalyzes reactions of  $H_2O_2$  with bromide, chloride and thiocyanate to generate potent oxidants (hypobromous acid, hypochlorous acid, and hypothiocyanous acid, respectively). In experimental systems, we found that cysteine crosslinking was greatest when thiocyanate (rather than bromide or chloride) was present as the substrate for EPO and  $H_2O_2$ . Using a thiolated hydrogel as a model for the airway mucus gel, we found that hypothiocyanous acid can oxidize the internal cysteines in the thiolated hydrogel to convert it from a liquid form to a solid form, mirroring what we see in sputum from subjects with asthma (11).

Thiocyanate ( $SCN^-$ ) is a pseudohalide (20), ubiquitously present in extracellular fluids at varying concentrations (21). *In vitro* experiments in human airway epithelial cells have shown that  $SCN^-$  is transported across the epithelium and concentrated 10-fold at the apical surface (22) by apical anion channels, such as cystic fibrosis transmembrane conductance regulator,  $Ca^{2+}$ -activated  $Cl^-$  channel, and pendrin. Notably, pendrin (also known as SLC26A4) is one of the most upregulated genes in asthma (23). Moreover, epithelial cell activation with IL-13 leads to increased expression of pendrin and potentially increased  $SCN^-$  in the airway surface fluid (24). Overall, we suggest that type 2 cytokines, such as IL-5 and IL-13, could orchestrate multiple pathologies to cause mucus plug formation. IL-13 increases  $H_2O_2$  production and MUC5AC-rich mucus secretion (25, 26) by the epithelium and increases expression of pendrin, an exchanger that transports thiocyanate into the airway lumen. IL-5 brings in EPO-laden eosinophils and promotes their accumulation and survival in this cysteine-rich mucin environment (Figure 8).



**Figure 7.** Schematic representation of the components of a generic mucin glycoprotein. The mucin monomer typically consists of cysteine-rich NH<sub>3</sub>- and -COOH termini (red) and one or more central domain(s) (green/blue). The central domains contain heavily glycosylated proteins (green) interspersed with internal cysteine domains (blue). The N- and C-terminal domains form disulfide bonds with other mucin monomers end-to-end to form long polymers. Internal cysteines form disulfide bonds with other mucin polymers side-to-side to form a complex entangled three-dimensional mesh, which is the basis of the elastic properties of mucus gel.

## Mucus Plugging Is a Distinct Phenotype from Chronic Mucus Hypersecretion

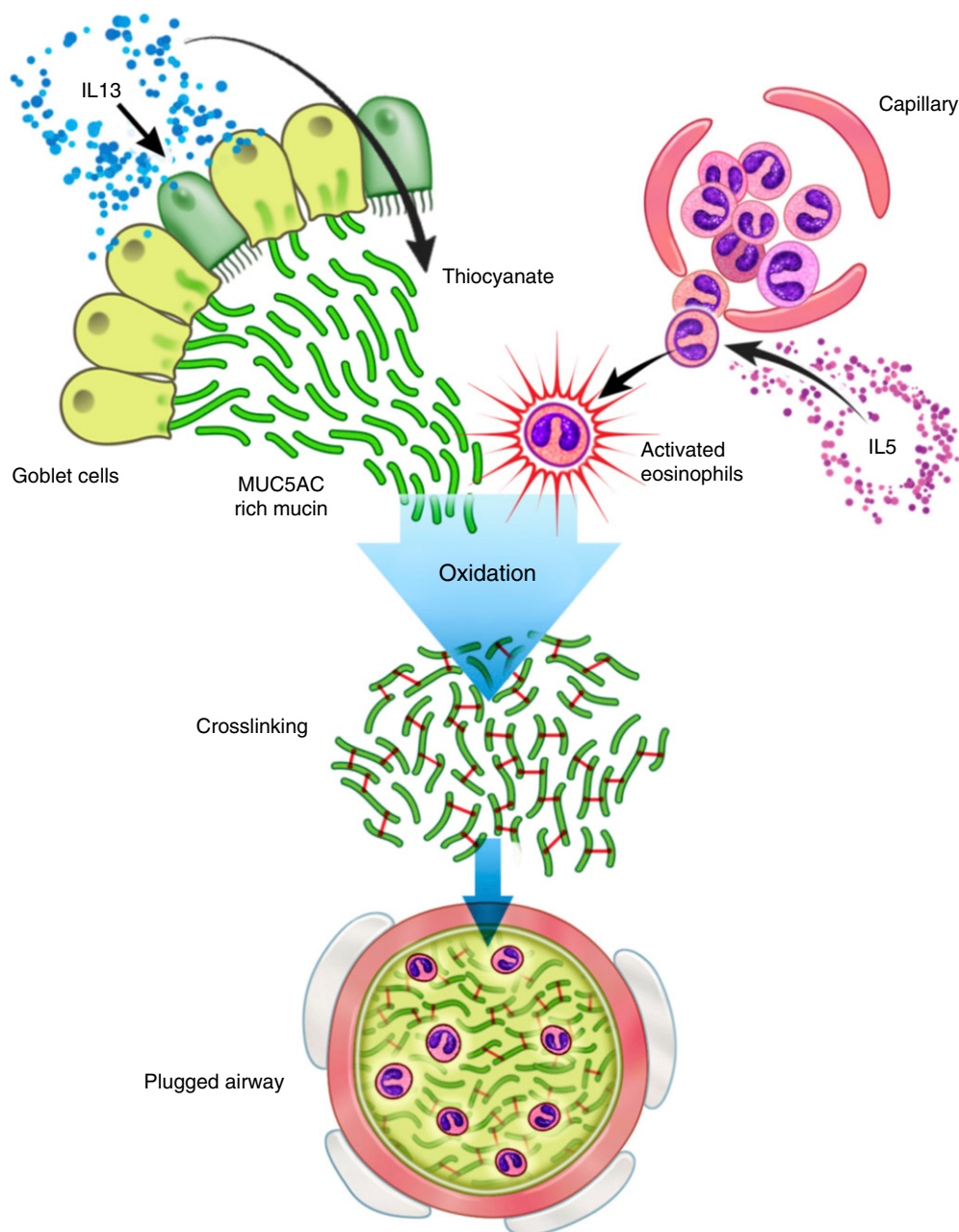
A striking finding from this MDCT imaging study in asthma is that mucus symptoms are neither sensitive nor specific for predicting mucus plugging (11). Specifically, the majority of patients with mucus plugs did not have symptoms of chronic mucus hypersecretion, and patients with mucus symptoms often had no mucus plugs. To date, studies of mucus pathology in asthma have relied on chronic cough and sputum production, a symptom complex known as chronic mucus hypersecretion (CMH) (27, 28), as a surrogate marker for mucus pathology within the lung. However, reliance on symptoms of CMH to identify patients with airway mucus plugs has been

questioned, because studies in chronic obstructive pulmonary disease show that symptoms of CMH are often absent in patients with mucus occlusion of small airways (4). A lack of cough receptors in the small subsegmental airways (29–31) may account for the lack of association seen between mucus plugs and mucus symptoms. We also show that the airway inflammation features of the CMH phenotype are different from the features of the mucus plug phenotype. Subjects with CMH did not differ from subjects without CMH in blood or sputum cell differentials or in sputum cell gene expression of cytokines or mucin genes (11). Thus, not all mucus phenotypes in asthma are the same, and we demonstrate the unique ability of MDCT lung imaging to identify patients with a mucus plug phenotype.

## Conclusions

Efforts to prevent airway mucus stasis and pathologic mucus accumulation have, to date, focused on understanding and targeting mucus hypersecretion. An alternative and easier approach would be lyse mucus plugs to restore airway patency and improve airflow. The discovery in the 1950s that streptokinase could lyse fibrin clots in the coronary arteries and reestablish arterial patency and flow changed the management of myocardial infarction from palliation to cure (32). We propose that lysing mucus plugs could similarly benefit patients with asthma suffering a “lung attack” as a result of mucus occlusion of their airways. ■

**Author disclosures** are available with the text of this article at [www.atsjournals.org](http://www.atsjournals.org).



**Figure 8.** Conceptual model for eosinophils promoting mucus plug formation in asthma. Epithelium, stimulated by IL-13, secretes high concentrations of mucin, particularly cysteine-rich MUC5AC mucin, into the airway lumen. IL-13 also increases transport of thiocyanate into the airway lumen through the ion exchanger pendrin. High levels of IL-5 promote survival of these eosinophils. On activation, eosinophils release eosinophil peroxidase and hydrogen peroxide that react with thiocyanate to promote crosslinking of mucins and mucus gel stiffening through cysteine oxidation and disulfide bond formation.

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