Mucins MUC5AC and MUC5B in the Airways: MUCing around Together

Chronic obstructive pulmonary disease (COPD) and chronic asthma clinically manifest with sputum production and cough, which correlate with pathologic features such as mucin hypersecretion and airway luminal mucus accumulation (1, 2). Increased mucin and mucus concentration is closely related to cigarette smoke and chronic bronchitis symptoms; for example, cough and sputum production, slowed mucociliary clearance (MCC), and COPD severity (3, 4).

Two major secreted airway mucins, MUC5AC and MUC5B, constitute the airway mucus layer, and the presence of two “similar” gel-forming mucins in the lungs has been a conundrum for decades that can be summarized by several major questions: 1) What are the macromolecular, structural differences that may couple with biophysical properties of their gels? 2) What are the individual roles and functions of these two in the airways, and what are the consequences of not having either one of them? 3) What is their distribution throughout the airways, and how are they regulated? 4) Are they being made in the same cells, and if so, are they stored in the same or in separate secretory granules? The more we reveal the answers to these questions, the closer we will get to finding strategies to manipulate mucins therapeutically to treat mucus abnormalities.

First, although MUC5AC and MUC5B share domain similarities as different gene products, recent studies indicated that their multimeric organization could be distinct and linked to their structural differences (5). Individually, MUC5AC and MUC5B form a distinct network infrastructure on the epithelial surfaces: MUC5AC forms tightly organized and branched networks, whereas MUC5B forms linear and occasionally branched networks (5). Furthermore, MUC5AC binds significantly more to hydrophobic surfaces, whereas MUC5AC layers are more rigid and viscoelastic than those of MUC5B (5).

Second, MUC5AC is essential for lung homeostasis and defense (6), whereas MUC5B is a necessary player in allergic airway response and is responsible for mucus plugging and impaired MCC (7, 8). MUC5B is the dominant mucin in health; and in disease, both MUC5B and MUC5AC increase, but MUC5AC increases disproportionately (3) and becomes closely associated to pathologic airway measures such as small airway abnormalities, airway obstruction, and increased exacerbation frequencies (9). In addition to MUC5AC’s potential as a sensitive biomarker for the initiation and progression of chronic bronchitis and COPD (9), genomewide association studies highlighted the causative role of increased MUC5AC expression in the pathogenesis of moderate and severe asthma (10, 11). Therefore, selectively targeting MUC5AC production and secretion has been of therapeutic interest.

Third, studies on the airway regional distribution of MUC5AC and MUC5B indicated that MUC5B is expressed in both the superficial epithelium and the glands. In contrast, MUC5AC is only expressed in the superficial epithelium (12). MUC5B is expressed in the trachea, bronchi, and bronchioles, whereas MUC5AC expression is concentrated in relatively large airways, including the trachea and bronchi, but not in the distal bronchioles in healthy lungs. It is also notable that neither mucin is expressed in the terminal bronchioles in healthy lungs (12), suggesting that the presence and accumulation of these mucins in this region is a manifestation of airway disease. The difference in regional distribution between MUC5B and MUC5AC could also provide an insight into the distinct properties of the two mucins.

Last, we have had no information as to whether they are made in the same airway cells and packaged together or separately in the secretory granules. In this issue of the Journal, a detailed, elegant study by Hoang and colleagues (pp. 1081–1095) addresses this fundamental information gap by observing both mouse and human airway tissues and primary airway cell cultures using state-of-the-art, high-resolution light microscopy techniques (13). The authors aimed to understand the packaging of MUC5AC and MUC5B mucins in the secretory granules in the secretory cells of mouse and human airways. They stimulated mouse airways with either IL-1β or IL-13, representing type 1 and type 2 inflammation, respectively. After IL-1β stimulation, approximately half of the cells in the mouse airway bronchus had muc5b and muc5ac together, whereas the other half had only muc5b expression. After IL-13 stimulation, approximately three-fourths of the cells had both muc5b and muc5ac together, whereas the others had either only muc5b or muc5ac. When the authors quantitated the secretory granule populations after these challenges, the vast majority of the granules contained either both muc5s or muc5b alone, whereas muc5ac-only granules were not more than 15%.

Similar results were obtained in the tissues from human airways; the majority of the cells in the proximal and distal airways expressed both MUC5B and MUC5AC. The amount of MUC5B-only cells in the distal airways was three times higher than in the proximal airways. This observation is consistent with a previous study that found MUC5B predominantly expressed in the distal airway superficial epithelium (12). Most (three-fourths) of the cells in the distal airways expressed both mucins. Although the amount was much lower, MUC5AC-only cells were also present in the distal
airways. In addition, approximately one-third of the granules in both proximal and distal airways contained both mucins. These observations regarding the distal airways were surprising or unexpected, because previous studies found no MUC5AC expression in healthy, nonsmoker, human small or distal airway tissues (12). Because of its adhesive, more viscoelastic nature, MUC5AC expression, even in low amounts, in the distal airways could be a pathologic factor rather than essential (5, 9). Because cigarette smoke causes remodeling in the airways and drives MUC5AC overexpression, this observation could be due to the tissue having been obtained from a donor (or donors) who smoked; as the authors mentioned, they had limited information about the donors of the lung samples. Figure 1 summarizes the finding from Hoang and colleagues (13) and from previous studies (5, 14).

Technically, using sophisticated microscopy and morphometric techniques, the authors showed the cellular distribution of secretory mucin protein in airway cells and notably mapped their intracellular granular distribution. Although the approaches used in the human tissues and cultures are highly dependent on antibodies that may have limited access to their respective mucin epitope(s), especially in tightly packaged secretory granules, the authors also used fluorescently tagged mucins in mice to reproduce and confirm the results. How MUC5AC and MUC5B are packed together in the same granule—for instance, whether they are made as mixed (hetero)multimers or separate multimers—and what factor (or factors) determines and regulates this complex process remain unanswered. Future mechanistic studies to address these questions are warranted.

Mucin biosynthesis is a multistep and complex process involving MUC gene transcription; sugar addition by means of O-glycosylation; dimerization; dense glycan decoration; multimerization; selective terminal sugar addition; and finally, tightly packaging all these into the secretory granules. Some of these steps can be targeted for therapeutic purposes to prevent mucin hypersecretion. Indeed, the regulation of the secretory machinery of mucin granules has been one of the strategies to control mucus release from goblet cells to prevent airway obstruction (15). This report clearly indicates that selectively targeting either MUC5B or MUC5AC granular secretion for therapeutic purposes is much more complicated than we thought.

**Figure 1.** An illustration of the mucociliary apparatus of human (large) airways with ciliated and secretory (goblet) cells covered with a sheet of mucus. As reported by Hoang and colleagues (13), MUC5AC (red) and MUC5B (green) are packaged/stored mostly together but also separately in secretory granules (SG). After (or, perhaps, before) secretion, they interact and interpenetrate with each other to make a netlike gel framework for the formation of an effective airway mucus barrier. Surely, they are both essential macromolecular components of an effective protective mucus gel layer of healthy human proximal and large airways where the foreign particles, microbes, and so forth are trapped and cleared before they advance to the more distal and small airways and cause complications.

**References**

1. Hogg JC, Chu F, Utokaparch S, Woods R, Elliott WM, Buzatu L, et al. The nature of small-airway obstruction in chronic obstructive pulmonary disease. *N Engl J Med* 2004;350:2645–2653.
2. Thomson NC, Chaudhuri R, Messow CM, Spears M, MacNee W, Connell M, et al. Chronic cough and sputum production are associated with worse clinical outcomes in stable asthma. *Respir Med* 2013;107:1501–1508.
3. Kesimer M, Ford AA, Ceppe A, Radicioni G, Cao R, Davis CW, et al. Airway mucin concentration as a marker of chronic bronchitis. *N Engl J Med* 2017;377:911–922.
4. Anderson WH, Coakley RD, Button B, Henderson AG, Zeman KL, Alexis NE, et al. The relationship of mucin concentration (hydration) to mucus osmotic pressure and transport in chronic bronchitis. *Am J Respir Crit Care Med* 2015;192:182–190.
5. Carpenter J, Wang Y, Gupta R, Li Y, Handl P, Subramani DB, et al. Assembly and organization of the N-terminal region of mucin MUC5AC: indications for structural and functional distinction from MUC5B. *Proc Natl Acad Sci USA* 2021;118:e210490118.
6. Costain G, Liu Z, Mennella V, Radicioni G, Goczi AN, Albulescu A, et al. Hereditary mucin deficiency caused by biallelic loss of function of MUC5B. *Am J Respir Crit Care Med* 2022;205:761–768.
7. Bonser LR, Zock L, Finkbeiner W, Erle DJ. Epithelial tethering of MUC5AC-rich mucus impairs mucociliary transport in asthma. *J Clin Invest* 2016;126:2367–2371.

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

Mehmet Kesimer, Ph.D.
Marsico Lung Institute
and
Department of Pathology and Laboratory Medicine
The University of North Carolina at Chapel Hill
Chapel Hill, North Carolina

ORCID ID: 0000-0003-3867-1873 (M.K.).
Obesity, Insulin Resistance, and Asthma

Many pulmonologists, although familiar with the consequences of different respiratory diseases coexisting in the same individual, may be less aware of the potential interactions between these respiratory diseases and metabolic disease (1, 2). In 1976, the forerunner of the Journal published novel work by Schuyler and colleagues showing that young nonsmoking adults with diabetes exhibited lower total lung capacities and poorer elastic recoil (3). Since then, evidence of connections between lung health and diabetes or insulin resistance (IR) has grown (1, 2), including studies linking lower spirometry values to diabetes (4) and to IR in the absence of diabetes (5), even after accounting for coexisting obesity. More recently, IR has been linked to the rate of lung function decline among older individuals (6). A particular role for IR in the “obese asthma” phenotype, which can be challenging to treat, has been suggested by the finding that the relationship between obesity and current asthma is stronger with increasing IR (7). This has stimulated the hypothesis that for some patients, the inflammation driving their asthma may be driven partly by systemic metabolic abnormalities (8). This could result from shared immunological mechanisms that underlie the different disease processes, or from metabolically induced modification of inflammatory responses, raising the possibility that drugs aimed at treating IR may be of benefit to the control of coexisting asthma (8). Analysis of health records has already shown an association between prescription of GLP1R (glucagon-like peptide-1 receptor) agonists for type 2 diabetes and lower asthma exacerbation rates (9). The global rise in obesity, poor metabolic health, and chronic airway disease adds impetus to both understanding their interrelatedness and determining how best to manage or prevent them.

In this issue of the Journal, Peters and colleagues (pp. 1096–1106) explore how IR among subjects with asthma relates to lung function trajectories and lung function responses to β adrenergic agonists and corticosteroids (10). They sampled data from the NHLBI’s Severe Asthma Research Program III, which characterized a cohort of late middle-aged individuals with severe asthma in detail and then followed them longitudinally. A major study strength is the inclusion of reliable biochemical markers of metabolic dysfunction and asthma-related characteristics. Their measure of IR was the homeostatic model assessment of IR (HOMA-IR), calculated by multiplying fasting plasma glucose (mg/dl) by the serum insulin value (mIU/ml) and dividing by 405. One hundred sixty-seven patients without IR had HOMA-IR < 3, 63 with moderate IR had HOMA-IR between 3 and 5, and 77 patients had severe IR with HOMA-IR > 5. In keeping with prior studies, obesity and IR appeared common among patients with asthma, and IR was associated with lower lung function at enrollment and a steeper rate of FEV1 decline across 5 years of follow-up. The authors also show a blunting of the bronchodilator and corticosteroid response among those with IR, and these individuals exhibited features of previous described type 2 inflammation, namely, lower sputum eosinophil counts (11). On the basis of these findings, the authors suggest that IR may have led to the cross-sectional decrements and accelerated the longitudinal decline in lung function observed within this asthma cohort.

Although it provides stimulating data, an important caveat of this study is that it shows association, not causation (12). As the authors infer, IR could be driving lung function loss, but it is also possible that this association instead reflects the clustering of susceptibility factors in the same individuals (1). Exposures we encounter across our whole life course, and even prenatally, help shape our respiratory and metabolic health in adulthood. To list some relevant examples, gestational diabetes and obesity appear to be risk factors for the development of childhood asthma, while insulin appears to impair surfactant production, and childhood environmental exposures influence both adult lung function and BMI (2, 13–15). The direction of the relationships underlying the cross-sectional associations observed among lung function, obesity, and IR remains unclear. Similar uncertainty may also apply when interpreting the reported association between IR and...