Airway Mucus
From Production to Secretion

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Mucus hypersecretion is a phenotype associated with multiple obstructive lung diseases. However, in spite of its nefarious reputation under pathologic conditions, there are significant benefits to having low levels of mucus present in the airways at baseline, such as the ability to trap and eliminate inhaled particles and to prevent desiccation of airway surfaces. Mucins are high–molecular-weight glycoproteins that are the chief components that render viscoelastic and gel-forming properties to mucus. Recent advances in animal models and *in vitro* systems have provided a wealth of information regarding the identification of the mucin genes that are expressed in the lungs, the signal transduction pathways that regulate the expression of these mucins, and the secretory pathways that mediate their release into the airways. In addition, the clinical and pathologic literature has corroborated many of the basic laboratory findings. As a result, mucin overproduction and hypersecretion are moving away from being markers of disease and toward being testable as functional components of lung disease processes.

Keywords: epithelium; lung; metaplasia; mucin; secretion

Although mucus hypersecretion has long been observed as a pathologic feature in many obstructive lung diseases, defining the precise mechanisms underlying mucus production and secretion remains a current challenge. Under healthy conditions, few mucous cells reside within the airway epithelium. However, in humans with asthma, cystic fibrosis (CF), or chronic obstructive pulmonary disease (COPD), and in animal models of these diseases, the production and secretion of mucus are markedly upregulated. Recent studies have thus focused on two distinct parameters: (*1*) mucin expression and the signal transduction pathways that lead to its elevated expression during airway inflammation; and (*2*) mucin secretion and the pathways used for its exocytosis by airway epithelial cells. The first of these has been the most widely studied, and this work has identified critical immunologic and growth factor–signaling pathways responsible for elevated mucus production *in vivo* and *in vitro*. The latter has lagged behind until more recently, as progress in understanding the molecular pathways of exocytosis in invertebrate and vertebrate neural organs has provided the bulk of the framework for defining the mechanisms of mucin glycoprotein secretion from epithelial cells. While mucin production and secretion are coordinately regulated, it is important to realize that they are distinct in many respects. For example, a significant increase in mucin production does not necessitate a proportionally significant or coincident increase in mucin secretion *in vivo*. This review will highlight recent advances in our understanding of the cellular and molecular pathways that lead to mucus production and secretion, and it will synthesize these with the historical literature in an attempt to provide an up-to-date context for understanding the protective and pathologic roles of mucus secretion in the lungs.

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MUCUS PRODUCTION

Mucus Composition and Mucin Structure

Airway mucus is a heterogeneous mixture of secreted polypeptides, cells, and cellular debris that are present in the airway surface lining fluid subphase or are tethered together at the fluid surface by oligomeric mucin complexes. Mucins are glycoproteins that due to their heavy glycosylation ($\sim$ 75–90% carbohydrate by mass) can exhibit very high molecular weights (in the MDa range; 1) Taken alone, this physical property suggests functional redundancy among mucin family members. However, differences in their localization, glycosylation, and multimeric complex formation imply that individual mucins have evolved to perform specific physiologic roles within the environments in which they are expressed. Mature mucins fall into two broad classes: the membrane-bound mucins and the secreted mucins. Structurally, membrane-bound mucins differ from secreted mucins in several ways. Most prominently, they have transmembrane and cytosolic domains that permit their localization to the plasma membrane surface, where they participate in such functions as cellular adhesion, pathogen binding, and signal transduction (2, 3). Membrane-bound mucins can also be cleaved proteolytically or alternatively spliced, resulting in their release into the extracellular mucous layer; however, the overall contribution of these mucins to the volume and viscosity of the mucous layer remains undefined (4). By contrast, secreted mucins contribute heavily to the viscoelastic properties of the extracellular mucous layer. After synthesis and post-translational processing, these are stored within intracellular secretory granules until stimulated for release by regulated exocytosis (*see* below and Ref. 5). Most secreted mucins are much larger than the membrane-bound mucins, and these contain cysteine-rich domains located at both the amino and carboxyl termini, most of which are closely related to von Willebrand factor (6, 7). These cysteine-rich domains link covalently as disulfide bonds to form mucin dimers, which are then heavily glycosylated, especially within clustered serine/threonine-rich tandem repeat domains, to from mature mucin dimers. These dimers further multimerize to form the long linear oligomers that provide the adhesive and space occupying properties of the mucous gel layer (8).

Mucin Gene Expression

Since 1992, the number of mucin genes identified in the human genome has increased from 4 to 21. Sixteen mouse orthologs have also been identified. Membrane-bound mucins constitute the majority of mammalian mucin genes (13 in humans and 9 in mice; *see* Table 1). Two conserved clusters of membrane-bound mucin

TABLE 1. MEMBRANE-ASSOCIATED MUCINS

Human Gene	Locus	Mouse Gene	Locus	Tissue Distribution
MUC 1 Aliases: episialin, DF3 antigen, H23 antigen, epithelial membrane antigen, polymorphic epithelial mucin, peanut-reactive urinary mucin	1q21	Muc1 Aliases: epithelial membrane antigen, CD227	3F 1	Cornea, salivary glands, esophagus, stomach, pancreas, large intestine, lung, breast, prostate, ovary, kidney, uterus, cervix, dendritic cells
MUC3A Alias: Muc3	7q22	Muc3	5 G2	Thymus, small intestine, colon, kidney
MUC3B	7q22			Small intestine, colon
MUC4 Aliases: HSA276359	3q29	Muc4	16 B2	Cornea, lung, salivary glands, esophagus, small intestine, kidney, endocervix
MUC11	7q22			Middle ear, thymus, lung, small intestine, Pancreas, Colon, liver, kidney, uterus, prostate
MUC12	7q22			Middle ear, pancreas, colon, uterus, prostate
MUC13 Aliases: downregulated in colon cancer 1	3q13	Muc13 Aliases: Lrrp, NJ-1, 14/A10, lymphocyte antigen 64	16B3	Conjunctiva, stomach, small intestine, colon, lung, kidney
MUC14 Aliases: endomucin	4q24	Muc14 Aliases: endomucin	3 G3	Endothelium
MUC15 Aliases: PAS III, PAS3, GLYCOPROTEIN C, GLYCOPROTEIN 4, COMPONENT II	11p14	Muc15	2 E 3	Conjunctiva, tonsils, thymus, lymph node, breast, small intestine, colon, liver, spleen, prostate, testis, ovary, leukocytes, bone marrow
MUC16 Alias: CA125 ovarian cancer antigen	19q13.2	Muc16	9 A2	Conjunctiva, ovary
MUC17	7q22			Intestinal cells, conjunctival epithelium
MUC18 Aliases: melanoma cell adhesion molecule (MCAM), CD146	11q23.3	Muc18 Aliases: l-gicerin protein, CD146, CD149, s-endo, s-gicerin	9 A5.2	Prostate
MUC20	3q29	Muc20	16 B2	Lung, liver, kidney, colon, placenta, prostate

loci are present in humans and in mice. MUCs 4 and 20 are found in tandem on human chromosome 3q29 and on the syntenic mouse chromosome 16 B2. The second membrane-bound mucin gene cluster—comprised of MUCs 3A, 3B, 11, 12, and 17—is present on human chromosome 7q22. MUCs 3A, 3B, 11, and 12 are located in very close proximity to each other (each within 1 kb of the next), and they show high levels of sequence homology at the cDNA level. Presently, it is not entirely clear whether these represent four individual genes, a variantly spliced single gene, or something in between. Evidence of a mouse ortholog (named Muc3) has only been shown to exist for one of the 7q22 mucins, but comparison of this to all of the other known mammalian syntenic orthologs shows that mouse Muc3 has stronger sequence similarities to rat Muc3 and chimpanzee MUC17 than to human MUC3A, MUC3B, MUC11, or MUC12. Collectively, these data suggest that the 7q22 cluster first evolved by a divergent orthologous evolutionary process that was followed by paralagous duplication events that are different in rodents and primates. Expression of members of the 7q22 cluster members is localized primarily to the intestinal tract, though MUC3 is expressed in human lung cancers (9). Under healthy conditions, MUC1 (which is not part of any mucin gene cluster) and MUC4 are highly expressed in the lungs (10). Of the membrane mucins associated with respiratory diseases but not resident within these clusters, MUC16 (also called cancer antigen 125, CA125) is expressed by epithelial cells in human airways at baseline (11), and MUC18 (also called melanoma cell adhesion molecule, MCAM) is expressed by bronchial epithelial cells and is upregulated in COPD and lung cancer (12, 13).

The gel-forming mucin gene family is comprised of human and mouse orthologs (Table 2), four of which (MUCs 2, 5AC, 5B, and 6) are present in tandem as a conserved cluster on human chromosome 11p15 and the syntenic mouse chromosome 7 F5 (14). MUC19 is a recently identified gel-forming mucin that is also present in mice and humans, but it is not part of the 11p15/7 F5 cluster (15). Analyses of the expression of the gel-forming mucin genes in the lungs of humans and mice have given rise to variable, and sometimes conflicting, data sets. However, the strongest and most frequently reported data support homeostatic roles for MUC5AC and MUC5B in the lungs through their baseline levels of expression (16–21). MUC5AC is the mucin gene most consistently reported to be upregulated during airway inflammation in humans and in animals, though recent studies also point to a role for MUC5B as well (*see below*). Several studies have demonstrated expression of the intestinal mucin MUC2 by airway epithelial cells *in vitro*, and these have been further supported by *in vitro* analysis of the signal transduction pathways that lead to increased MUC2 mRNA and MUC2 promoter-driven reporter expression (22, 23). However, *in vivo* analyses do not consistently support a role for MUC2 expression in obstructive airway diseases. At the protein level, MUC2 accounts for $\sim$ 2% of the total mucin present in sputum from patients with COPD (24). MUC2 is expressed in some lung adenocarcinoma cell lines, and its expression in the airways correlates with the development of certain types of lung cancer, suggesting that a preneoplastic intestinal metaplasia of the lungs akin to intestinal metaplasia in the stomach may be marked by increased MUC2 expression (25, 26). To assess this, we recently analyzed the simultaneous expression of each of the gel-forming mucin genes in antigen challenged mice and found that Muc5ac is the dominant mucin gene induced in this asthma model. Of the five gel-forming mucins, only Muc5ac and Muc5b are abundantly expressed in the lungs at baseline or following antigen challenge. Both are present at approximately the same mRNA levels in unchallenged mice. However, following aerosol antigen challenge, coincident with an increase in histochemically detectable mucin, Muc5ac expression increases $>$ 12-fold and Muc5b increases $<$ 2-fold. Thus, Muc5ac expression is the central event in the development of goblet cell metaplasia in allergic mice (27).

A third group of mucins does not fit well into the above categories. These include human MUCs 7–9, and mouse Muc10 (Table 3). MUC7 and Muc10 map to syntenic regions of human chromosome 4 and mouse chromosome 5 where multiple small secreted oral glycoproteins are encoded. Both MUC7 and Muc10 have putative antimicrobial and immunomodulatory effects. However, high cross-species variation of genes within this region suggests that they have evolved divergently to serve functions

TABLE 2. SECRETED GEL-FORMING MUCINS

Human Gene	Locus	Mouse Gene	Locus	Tissue Distribution
MUC2 Aliases: mucin-like Protein	11p15	Muc2 Aliases: MCM	7 F5	Conjunctiva, middle ear, stomach, small intestine, colon, nasopharynx, lung, prostate
MUC5AC Aliases: HSAPOMUCN, apomucin	11p15	Muc5ac Aliases: mouse gastric mucin	7 F5	Conjunctiva, middle ear, stomach, gall bladder, nasopharynx, lung
MUC5B Aliases: cervical mucin, salivary mucin (high M.W.)	11p15	Muc5b	7 F5	Middle ear, sublingual gland, laryngeal submucosal, glands, esophageal glands, stomach, Duodenum, gall bladder, nasopharynx, lung
MUC6	11p15	Muc6 Alias: gastric mucin-like protein	7 F5	Stomach, duodenum, gall bladder, pancreas, kidney
MUC19	12q12	Muc19 Alias: sublingual apomucin	15 E3	Salivary gland, lung, kidney, liver, colon, placenta, prostate

that are host environment–specific. For example, although these mucins map to syntenic loci, mice have no designated MUC7 ortholog, humans have no Muc10 ortholog, and there is only 35% similarity between them at the peptide level (by contrast, human MUC5B shares $\sim$ 60% similarity with mouse Muc5b). MUC7 and Muc10 lack the cysteine-rich N- and C-terminal motifs of the gel-forming mucins and are thus incapable of disulfide oligimerization. MUC8 mRNA has been demonstrated by RT-PCR to be expressed by respiratory epithelium, and the protein has been localized to both submucosal gland mucous cells (28) and ciliated surface epithelial cells (29). However, neither the function nor the evolutionary conservation of MUC8 are clear at this time. MUC9 (also called Oviductin) was originally defined as a mucin based on its serine- and threonine-rich C-terminal imperfect repeat domain (30). MUC9 and its orthologs map to a cluster of chitinases that are highly conserved (31), and one of these (acidic mammalian chitinase) is important for the development of allergic inflammation in mice (32). Interestingly, both human MUC9 and a mouse Muc9, also known as chitinase 5, have $\sim$ 50% sequence identity at the protein level with acidic mammalian chitinase, and they have the conserved N-terminal glycosyl hydrolase 18 domain that defines these chitinases. However, MUC9 has not yet been shown to be differentially expressed in the lungs.

Localization of Mucin Expression during Airway Inflammation

In healthy humans and animals, few or no mucous cells are visible in the airways distal to the trachea. However, the numbers of visible mucous cells distal to the trachea and the amount of mucin produced within the surface epithelium are greatly increased during airway inflammation (Figure 1). Known stimuli include exposure to inhaled or instilled allergens, parainfluenza virus, bacterial lipopolysaccharide, neutrophil elastase, and various cytokines. The development of the mucous phenotype under these conditions has been described both as mucous cell metaplasia and as mucous cell hyperplasia. The "metaplasia" classification implies that a phenotypic change occurs within an adult cell type, but it makes no direct inference that this is mediated by a proliferative event. By contrast, the "hyperplasia" classification links the phenotypic change not only to cell proliferation but also to an increase in total cell number within a tissue. Mucin production in the airways of allergic mice can occur either in the presence or in the absence of increased epithelial proliferation depending on the nature and frequency of inflammatory stimulation, indicating that a mitotic mechanism may accompany, but is not required, for mucous differentiation. In sensitized mice exposed to a single aerosol antigen challenge, little or no epithelial cell proliferation is induced, whereas in mice exposed to repeated aerosol challenges on multiple days, epithelial cell proliferation is enhanced, but the level of mucous metaplasia is similar (5, 33, 34). Thus, in the case of mucin overproduction by the airway surface epithelium, the "hyperplasia" distinction may be related to chronic inflammation, but there is not a causal role for proliferation in the development of the mucous phenotype. In mice, mucin production by the surface epithelium occurs within Clara cells that are present throughout the conducting airways and can be identified by their expression of CCSP (Clara Cell Secretory Protein). In healthy humans, mucous cells are present within the pseudostratified columnar epithelium of the central bronchial airways and to a lesser extent in the columnar epithelial cells of the peripheral bronchiolar airways. Mucous cells in human bronchioles ($<$ 2 mm diameter airways) also express CCSP, indicating that the induction of mucin expression by Clara cells in humans may be an important mechanism for mucus overproduction in the small airways under diseased conditions (35).

Signal Transduction Pathways and the Regulation of Mucin Expression

Mucin expression is induced in response to a wide variety of inflammatory stimuli that produce a variety of different cytokine expression profiles. The best characterized of these to date is the mucous phenotype associated with allergic airway inflammation. The Type II T-helper (Th2) lymphocyte cytokines IL-4, IL-9, and IL-13 stimulate mucin production *in vitro* and *in vivo*. IL-13, through the activation of signal transducer and activator of

TABLE 3. OTHER MUCINS

Human Gene	Locus	Mouse Gene	Locus	Tissue Distribution
MUC7	4q13–21			Lacrimal glands, salivary glands, lung
MUC8	12q24.3			Nose, Lung
MUC9 Aliases: oviductin, oviductal glycoprotein, oviductal glycoprotein 1, estrogen-dependent oviduct protein	1p13	Muc9 Aliases: oviductin, oviductal glycoprotein 1, oviduct-specific glycoprotein, chitinase 5	3 F3	Oviduct
		Muc10 Aliases: submandibular gland salivary mucin	5 E1	Submandibular gland

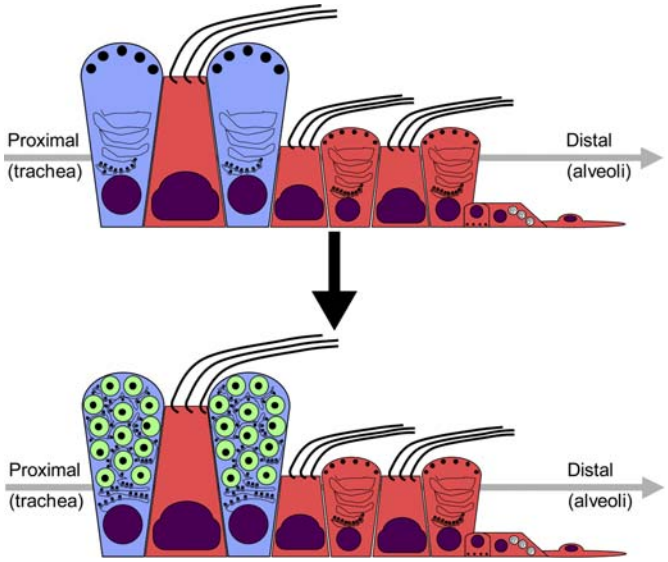

Figure 1. Temporal and spatial regulation of mucin expression in the airways. At baseline (*top*), mouse airways are lined by a tall columnar epithelium consisting of ciliated and nonciliated cells. The nonciliated cells are secretory cells that express the Clara cell marker CCSP and contain abundant smooth endoplasmic reticulum (ER). After allergic stimulation (*bottom*), there is a dramatic alteration in the morphology of nonciliated cells in the proximal airway epithelium (*blue*) characterized by the upregulation of their secretory product contents and rough ER abundance, with the addition of mucin expression occurring as the central event during this differentiation process. Clilated cells, bronchiolar Clara cells, neuroendocrine cells, and type I and II pneumocytes (*red*) are restricted from initiating mucin glycoprotein synthesis.

transcription 6 (STAT6), is the only one of the three Th2 cytokines required for mucous metaplasia in animal models of allergic airway inflammation *in vivo*. Furthermore, since transgenic overexpression of IL-13 in mice with STAT6 signaling intact only in the airway epithelium causes mucous metaplasia in the absence of airway inflammation *in vivo*, direct stimulation of epithelial cells by IL-13 appears to be an important mechanism for mucin production during allergic airway inflammation (36). This is further supported by the finding that IL-13 stimulates Muc5ac promoter-driven luciferase reporter activity *in vitro* (5). What remains unclear, however, is whether the induction of mucin by IL-13 results from direct *cis* activation of Muc5ac gene transcription induced by STAT6 binding to its promoter, since secondary signals mediated by epidermal growth factor (EGF) receptor ligands (37) and transforming growth factor (TGF)-β_2 (38) are also required for IL-13–induced mucin production. These latter signals appear to be mediated by the upregulation of growth factor production, and/or activation by membrane associated proteases, such as TNF-α–converting enzyme (TACE; also called a disintegrin and metalloproteinase domain 17 [ADAM17]; 39). STAT6-dependent signals are also involved in downregulating the expression of Foxa2, a transcription factor that appears to act as a repressor of the MUC5AC promoter, and whose deletion in mouse Clara cells results in spontaneous mucous metaplasia (40). Finally, transcriptional induction of mucin expression may couple with post-transcriptional regulation, as occurs with MUC5AC in adenocarcinoma cell lines stimulated with neutrophil elastase (41), resulting in a further increase in the amount of mucin produced during airway inflammation. Overall, the upregulation of mucin expression by resident secretory epithelial cells is a multifaceted signaling process that may be important for linking mucin production to diverse stimuli, while also contributing to its highly specific tissue and cellular localization.

MUCIN EXOCYTOSIS

The build-up of secretory granules (SGs) within cells, and the ability to acutely stimulate their release with agonists, define a regulated exocytic phenotype. In airway epithelial cells, mucin secretion occurs by a regulated process *in vitro* and *in vivo*, and this involves the sequential initiation of production (i.e., extracellular signal transduction, resulting in the synthesis and storage of mucin in exocytic granules) and secretion (i.e., extracellular signal transduction resulting in exocytosis). Mucous cell exocytosis is rapidly induced by a variety of secretagogues that include cholinergic and purinergic agonists, proteases, arachidonic acid metabolites, secreted inflammatory cell products, and pathogens (for review *see* Refs. 42, 43). Thus, in respiratory diseases, infectious, irritant, and allergic stimuli coordinate elevated mucin synthesis with increased activation of the regulated exocytic pathway to lead to mucus hypersecretion.

Regulated exocytosis in eukaryotes depends upon the trafficking of SGs to the cell surface and fusion of the lipid bilayer surrounding SGs with the plasma membrane (Figure 2). This requires the concerted action of multiple trafficking proteins that mediate SG transport and content release. In the early steps of exocytosis, the GTPase Rab27 localizes to SG membranes and binds to myosin V via the anchoring protein melanophilin, thereby allowing for granule transition from microtubules to the cortical actin network (44–46). Myristoylated alanine-rich C kinase substrate (MARCKS) transiently localizes to SGs upon secretagogue stimulation, where it is necessary for the recruitment of the actin cytoskeleton to mucous SGs and subsequent mucin secretion in polarized airway epithelial cells *in vitro* (47) and in mice *in vivo* (48). These two processes mediate the transition of SGs from microtubule-kinesin–dependent to actin-myosin–dependent transport at the cell periphery (49), and the depolymerization of cortical actin that otherwise serves as a barrier to the later steps of granule fusion (50). After transport, SGs are tethered and then docked to the plasma membrane via protein–protein interactions. The role of Rab3 in the tethering process is especially evident in *Caenorhabditis elegans*, in which deletion of its single Rab3 isoform results in failure to localize synaptic vesicles at the active zone and a $\sim$ 50% decrease in synaptic neurotransmission (51). Mice and humans have four Rab3 isoforms, three of which are present in the secretory cells of the airways (5). The absence of any single isoform has little effect on secretory function of neurons, but a quadruple Rab3A/B/C/D knockout mouse dies of asphyxiation at birth, indicating that neurotransmission is severely impaired (52). Studies focused on gaining an understanding of the role of airway epithelial isoforms of exocytic proteins in mucin granule secretion are underway in several laboratories.

The final steps of vesicular traffic mediate the docking and fusion of SGs with the plasma membrane. Much of the original work identifying the components of this portion of the exocytic machinery and their essential functions have been performed in *Saccharomyces cerevisiae*, *C. elegans*, and *Drosophila melanogaster*. The importance of many of these studies have been confirmed by analyzing the effects of deficiency in their neuronal isoforms in mice. The central components of this process are soluble N-ethylmaliemide sensitive factor attachment receptor (SNARE) proteins that are present on secretory vesicles (v-SNAREs, such as VAMP) and their target membranes (t-SNAREs, such as Syntaxin and SNAP-25). The v- and t-SNAREs contain α-helical domains that interact to form a tightly coiled four-helix bundle (the core complex) that brings together the opposing membranes

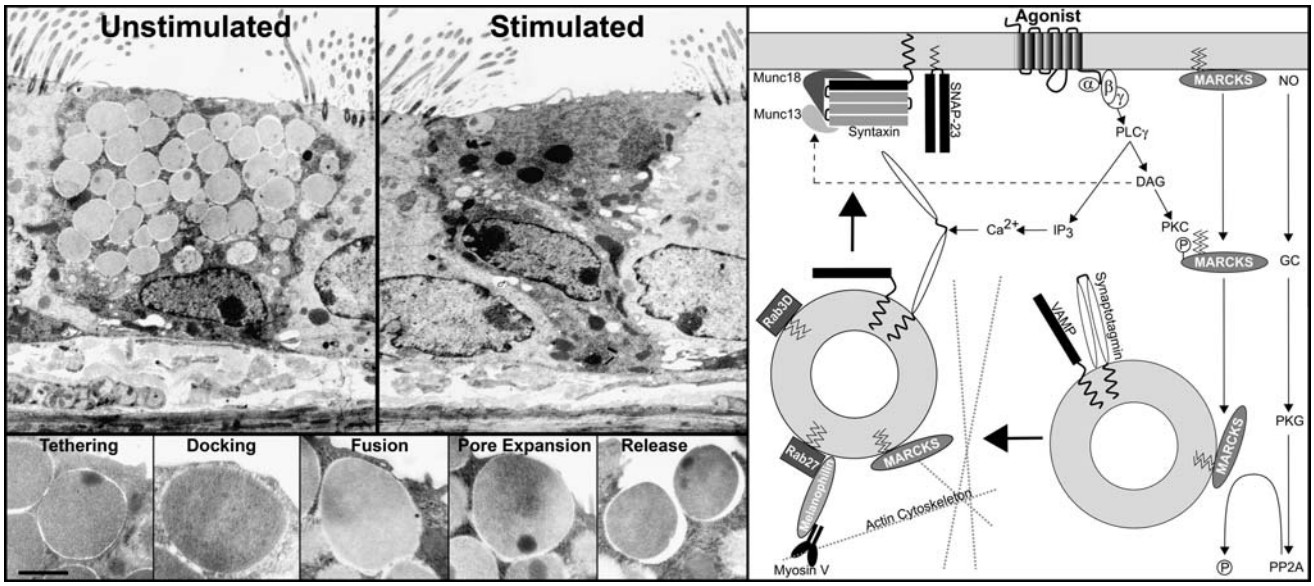

Figure 2. Secretion of mucin from epithelial cells by regulated exocytosis. Left. (*Top*) Transmission electron microscopy (TEM) shows massive release of mucin-containing SGs from metaplastic goblet cells in response to stimulation by ATP aerosol. The remaining SG in the stimulated cell localizes to the apical plasma membrane (PM), suggesting that the default intracellular trafficking pathway for mucin SGs in airway goblet cells positions these in a readily releasable pool for rapid secretion in response to further stimulation. (*Bottom*) Morphologic demonstration of the of late steps of vesicular traffic resulting in close apposition of SGs with the inner leaflet of the apical PM (Tethering and Docking), mixing of the lipid bilayer surrounding mucin SGs with the apical PM (Fusion), and ultimately in mucin content release from the fused SG's (Expansion and Release). *Right*: Schematic demonstration of the molecular machinery involved in mucin SG trafficking and fusion. Agonists (e.g., ATP) activate cell surface receptors (e.g., the purinergic seven transmembrane spanning G protein–coupled P2Y2 receptor) and activate the regulated exocytic machinery thus mediating the docking and fusion of SGs with the apical PM. *Magnification bar* = 1 μm and 500 nm in the upper and lower TEM images, respectively.

and, in conjunction with the calcium binding protein Synaptotagmin, is believed to provide the driving force for membrane fusion. However, in order for efficient fusion to occur *in vivo*, several accessory molecules are required. Prominent among these are Munc18 and Munc13 proteins. Based on structural data, Munc18 proteins promote core complex formation by opening the structure of Syntaxin and allowing it to interact with the other SNARE proteins. Munc13 binds to Syntaxin at a site near Munc18 and dissociates the Munc18–Syntaxin complex, thus allowing for assembly of the core complex (for current reviews of this extensive work *see* Refs. 53, 54). The interactions of Syntaxin with Munc18 and Munc13 constitute a particularly important cascade, since the absence of any of their neuronal isoforms leads to a complete failure of neurotransmission in *Drosophila* (55–57) and in mice (58, 59).

MUCUS IN DISEASE

Airway inflammation, mucus hypersecretion, and impaired mucociliary clearance are characteristics shared by nearly all obstructive pulmonary diseases. In patients with asthma, COPD, or CF, there are two important components of the mucus hypersecretory phenotype. First, elevated mucin production increases the amount of intracellular mucin stores contained within airway secretory cells; and second, elevated mucin exocytosis increases the thickness and viscosity of the extracellular mucous gel positioned above the surface epithelium. Thus, mucus hypersecretion may result from an increase in the steady-state levels of mucin production, mucin SG exocytosis, or both. Indeed, both of these components appear to be upregulated during pulmonary inflammation. Current therapies focus on the use of anti-inflammatory agents such as corticosteroids to reduce mucous metaplasia, aerosolized saline solution and DNA-severing enzymes to reduce sputum viscosity, and β-agonists to stimulate ciliary action. Unfortunately, these treatments have limited efficacy, so effective new therapies are needed. Two pathophysiologic manifestations of mucus hypersecretion, airway obstruction and mucociliary clearance impairment, result in the development of debilitating airflow limitation and particulate/pathogen retention, respectively. Although these may appear inseparable, differences in disease presentations (e.g., between asthma and CF) suggest that they actually are not. Not surprisingly, these differences likely reflect the heterogeneity of mucin-inducing stimuli.

Asthma

Asthma is the most common chronic respiratory disease in the United States, affecting 12.5% of children and 10.4% of adults (60, 61). Physicians often overlook the significance of mucus hypersecretion because patients with asthma generally expectorate little or no sputum. However, widespread occlusion of small and medium-sized airways by mucus and cellular debris is a well-recognized finding in autopsy studies of patients who died from asthma (62, 63). A recent quantitative study of 275 airways from 93 patients who died from asthma in New Zealand found lumenal occlusion ranging from 20–100% of the cross-sectional area in all but five airways (98% of airways; 64). Mucins are the major macromolecular component of mucus plugs (65, 66). Thus, airway lumenal obstruction by mucin appears to be a major contributing cause of fatal asthma.

Even in patients with mild to moderate asthma, the number of mucous cells in the airway surface epithelium is increased (67, 68). As in normal individuals, MUC5AC and MUC5B are the major secreted mucins in the airways of individuals with

asthma, with MUC5AC disproportionately increased (24). MUC5B is primarily produced and secreted by mucous cells of the submucosal glands in humans, but it is also produced by some airway surface goblet cells in humans with asthma (69). These findings are consistent in mouse models of asthma, in which Muc5ac is the predominant gel-forming mucin gene expressed, and Muc5b, which localizes almost exclusively to the submucosal glands at baseline, becomes detectable within the surface epithelium after antigen challenge (18, 19). In humans, MUC5B exists in both low and high charged forms. The low charge form predominates in individuals with asthma but not in individuals without asthma (24), but the clinical significance of this is unclear. The monomeric structures of MUC5AC and MUC5B are extended and stiff, but these monomers polymerize end-to-end *in vivo*, forming long linear branched structures that may vary between normal and diseased states (66, 70). Further studies of clinical specimens and controlled experiments in animals will be essential to better define how these biophysical properties of mucins affect patients with asthma. It is possible that such studies will reveal important differences in the macromolecular makeup of mucus within specific regions of the lung.

Cystic Fibrosis

CF is the most prevalent inherited monoallelic disease affecting the respiratory system in Caucasians, and in the United States it occurs in all major racial groups (71). The pulmonary manifestation of CF is characterized by impaired mucociliary clearance, leading to recurrent respiratory tract infections and chronic airflow obstruction. These phenotypic changes have been linked to the dysfunction or absence of the CF transmembrane regulator (CFTR), a cAMP-dependent chloride channel. In the lungs, CFTR is located on the apical surface of ciliated airway epithelial cells, where it influences airway surface liquid volume by its negative regulatory interaction with the amelioride sensitive apical epithelial sodium (Na) channel (ENaC). In the absence of CFTR-mediated inhibition, excessive activity of ENaC causes airway surface liquid depletion, leading to impaired ciliary function and adhesion of the mucus gel layer to the epithelial surface (72). In support of this model, overexpression of βENaC in the airways of mice depletes airway surface liquid volume, leads to impaired mucus clearance, and results in lethality in 50% of transgene-positive mice. Pronounced goblet cell metaplasia and sterile neutropenia are associated with this phenotype in βENaC transgenic mice (73).

In CF, mucus hypersecretion is associated with airflow obstruction and, in fatal cases, occlusion of small airways (74). Excess mucus in the airways also appears to contribute to CF morbidity by increasing the frequency and severity of pulmonary infections with pathogenic strains of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Burkholderia cepacia* (75–82). These data suggest that mucus hypersecretion is a pre-existing state that increases the susceptibility of patients with CF to prolonged conlonization and infection. However, bacterial products are themselves capable of initiating mucin gene expression *in vitro* (17, 23) and *in vivo* (83), suggesting that bacterial exposure can indeed initiate the development of the mucous phenotype (for review *see* Ref. 84). Notably, however, the mucin-overproducing and neutrophilic phenotypes that occur in βENaC transgenic mice are present in the absence of any detectable infectious pathogens (73), thus setting these two CF characteristics upstream of infectious responses. Collectively, it appears that impaired mucociliary clearance causes worsening of CF lung disease by directly obstructing the airway lumen, by increasing primary colonization and infection, and by perpetuating infectious and inflammatory states in the airways.

MUC5AC and MUC5B are both present in the sputum of patients with CF, and several studies have shown that the levels of MUC5B are increased compared with MUC5AC (24, 85, 86). Interestingly, although total mucus production is greatly increased in patients with CF, the concentrations of MUC5AC and (to a lesser extent) MUC5B in endotracheal tube sputum samples taken from ventilated patients with CF are actually decreased compared to ventilated patients without CF (86). While seemingly at odds with a vast array of prior studies, this finding may indeed highlight the significance of the impairment of mucociliary clearance in the peripheral airways that results in an overrepresentation of the central airway mucin components (e.g., MUC5B released from submucosal glands) and nonmucin components (e.g., DNA and polypeptides released from inflammatory cells) that are efficiently cleared up to the level of the trachea. This is supported by the finding that MUC5B is often found at higher concentrations than MUC5AC in CF sputum samples (24, 85, 86). Biochemically, the airway mucins of patients with CF have a higher degree of sulfated and sialylated carbohydrate sidechains than mucins from normal subjects (87), but similar findings are noted in individuals with chronic bronchitis, suggesting that these changes represent an adaptive mechanism to chronic inflammation that is not an intrinsic aspect of cystic fibrosis (88, 89). This inference is supported by the lack of difference in mucin glycosylation in cell cultures derived from patients with and without CF under noninflammatory conditions (90).

Chronic Obstructive Pulmonary Disease

COPD is the fourth leading cause of death in the United States and Europe, and COPD mortality has more than doubled in the last 20 yr (91). In COPD, mucus production and secretion increase through mucous gland hypertrophy in the trachea and bronchi and through goblet cell metaplasia of the surface epithelium in the bronchi and bronchioles. While chronic expectoration helps to remove excess mucus from the large airways, the impaired mucociliary action in COPD causes ineffective mucus clearance from small airways (i.e., in $\leqslant$ 2 mm diameter bronchioles).

The role of increased mucus in COPD progression and outcome has been debated for a number of years. Early studies in occupational cohorts with mild lung function abnormalities and high prevalence of expectoration showed an association between chronic expectoration and lower respiratory tract infection, but there was no appreciable effect on lung function decline in these patients (92, 93). However, more recent large-scale studies do show a significant association between chronic expectoration and lung function decline, hospital admission, and mortality (93). In a long-term study of 9,435 adults by Prescott and coworkers, excess FEV_1 declines of 22.8 ml/yr in men and 12.6 ml/yr for women were associated with chronic expectoration (94). The same study and others also reported increased hospital admissions and mortality associated with respiratory infections that were also associated with chronic sputum expectoration, thus temporally implicating mucus hypersecretion in the central airways as either a disease marker or a deleterious factor in COPD (93, 94).

In COPD and in other obstructive lung diseases as well, a portion of the ambiguity surrounding the role of mucus hypersecretion is related to sampling difficulties. In contrast to the large airways, where sample collection is relatively uncomplicated, little is known about the role of mucus hypersecretion in small airways, where a lack of reliable noninvasive methods make sample collection much more difficult and risky for both patients and investigators. Instead, most of the data that reliably evaluate the expression of mucin in the small airways are based upon histopathologic findings from surgical specimens. These studies clearly show that mucous cells are increased in small airways

and that increased amounts of gel-forming mucins (MUC5AC and MUC5B) are found in the lumen of small airways in patients with COPD (95, 96). Further, in a subset of patients with COPD with an FEV_1 of $\geq 80\%$ predicted, there is an inverse relationship between goblet cell metaplasia detected histopathologically in surgical specimens and presurgical FEV_1. Thus, patients with higher FEV_1 have less goblet cell metaplasia than patients with lower FEV_1, suggesting that the presence of mucin-producing cells in the airways is related to increased airflow obstruction (97). COPD progression is strongly associated with the accumulation of inflammatory mucous exudates in the lumen of small airways (98), and the presence of a prominent goblet cell phenotype negatively correlates with FEV_1 change after lung volume reduction surgery (99). Collectively, these results show that mucus in the airway lumen may be significant enough to result in measurable mechanical obstruction of the small airways, and it may significantly impact disease pathogenesis and prognosis.

Cigarette smoke itself has been shown to promote mucin synthesis directly *in vitro* by activation of the EGFR cascade (100). Further, it also appears to promote mucous metaplasia indirectly through recruitment and activation of neutrophils and subsequent epithelial cell oxidative stress (101, 102). *In vivo*, neutrophils and neutrophil products (103, 104), which are also seen in respiratory infections (*see below*), can stimulate the upregulation and release of mucin from surface epithelial cells. These effects appear to occur through the activation of a variety of membrane-bound proteolytic enzymes that in turn activate epidermal growth factor signal transduction pathways (for review *see* Ref. 105). Moreover, cigarette smoke may result in mucus stasis by causing release of neutrophil proteases, activation of ENaC, depletion of airway surface liquid, and impaired mucociliary clearance, similar to the mechanism seen in CF (*see* above and Ref. 106).

It has been suggested that mucus can also serve as a suitable medium for adherence and growth of some bacterial pathogens, such as nontypeable *H. influenzae* (NTHi; 107). Both gram-positive and gram-negative bacteria products upregulate MUC5AC and MUC2 gene expression and mucin secretion in human respiratory epithelial cell lines *in vitro* (17), and the same effect can be seen in some animal models *in vivo* (108). Viral infections are also closely associated with COPD exacerbations in humans (109–112). Surgical specimens from smokers with COPD show that these patients have increased goblet cell numbers in the epithelium of peripheral airways compared with nonsmokers, and that this is accompanied by increased macrophages and CD8-positive T-lymphocytes (113), both of which can be indicative of viral infections (reviewed in Ref. 114). *In vivo* IL-6 production is enhanced during the early phase of virus-induced inflammation after an immediate neutrophilic response. Accordingly, IL-6 levels are increased in patients with COPD (115, 116), and during experimental respiratory viral infections in humans (117) and mice (118, 119). *In vitro*, mucin gene expression is increased by IL-6 secretion from differentiated airway epithelial cell cultures via an auto-/paracrine loop (120). *In vivo*, viral infection causes chronic mucous metaplasia in mice that is characterized by sustained Muc5ac expression persisting for the life of the animal (121). However, the role of direct IL-6 action *in vivo* is unclear, since goblet cell metaplasia was not reported to be a prominent histopathologic finding in airway-specific conditional IL-6–overexpressing mice (122).

In summary, the current state of knowledge suggests an important role for mucus hypersecretion in airflow obstruction in COPD. Future studies are needed to establish a causal role for mucus hypersecretion in COPD progression by evaluating effects of abrogated mucin expression and secretion in transgenic animal models of COPD and by evaluating the reversal of mucus hypersecretion on important outcomes such as lung function, dyspnea, quality of life, hospitalization and mortality rates in humans with COPD.

CONCLUSIONS

Over the last decade, a great deal of progress has been made in understanding the role of mucus secretion in health and disease. At baseline, secreted mucus appears intuitively to be a beneficial feature of the airways. However, because mucus overproduction and hypersecretion are closely tied to the development and progression of a variety of airway diseases, and because mucus overproduction and hypersecretion are not yet sufficiently or directly treatable, there is still a need to accurately define the precise functions of airway mucus. Recent advances, such as the completion (or near completion) of the sequencing of several mammalian genomes, the identification of important epithelial cell differentiation and vesicular trafficking processes, and the development of animal models that recapitulate important features of respiratory diseases, are providing investigators with a plethora of opportunities to define the mechanisms of mucus hypersecretion in airway pathologies. With a lot of effort and a bit of luck, the next several years will be fruitful and result in the attainment of these goals.

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