Mucin overproduction in chronic inflammatory lung disease

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Mucus overproduction and hypersecretion are commonly observed in chronic inflammatory lung disease. Mucins are gel-forming glycoproteins that can be stimulated by a variety of mediators. The present review addresses the mechanisms involved in the upregulation of secreted mucins. Mucin induction by neutrophil elastase, bacteria, cytokines, growth factors, smoke and cystic fibrosis transmembrane conductance regulator malfunction are also discussed.

Key Words: *Bacteria; Cytokine; Lung; Mucin; Neutrophil elastase; Overproduction; Smoke*

La surproduction de mucine dans la pneumopathie inflammatoire chronique

La surproduction de mucus et l'hypersécrétion sont courantes en présence d'une pneumopathie inflammatoire chronique. Les mucines sont des glycoprotéines gélifiantes qui peuvent être stimulées par divers médiateurs. La présente analyse traite des mécanismes de régulation positive des mucines sécrétées. Est également exposée l'induction des mucines par l'élastase des neutrophiles, les bactéries, les cytokines, les facteurs de croissance, la fumée et la défaillance du régulateur de conductance transmembraneuse de la fibrose kystique.

Mucus is an important component of both the physiological and pathological processes in airways. It protects, moisturizes and lubricates mucosal surfaces, and traps bacteria and other inhaled irritants for removal by mucociliary clearance. However, excessive production of airway mucus is a feature of chronic inflammatory lung diseases such as bronchial asthma, chronic obstructive pulmonary disease (COPD), bronchiectasis and cystic fibrosis (CF). Mucus hypersecretion results from hyperplasia and metaplasia of mucous cells, which lead to greater numbers of these cells being found throughout the airways, including the distal airways, where they are normally absent (1). The presence of neutrophil elastase, certain bacterial pathogens and altered cytokine patterns all contribute to excess airway mucus production. This overproduction and hypersecretion of mucus, in turn, contributes to airway obstruction and impairment of mucociliary clearance.

An understanding of the mechanisms that lead to mucus overproduction and secretion is therefore of great clinical interest. The present review provides insight into the mechanisms by which excessive mucus production is stimulated in chronic inflammatory lung disease, focusing mainly on enhanced gene expression and protein production of secretory mucins in the airways.

MUCINS

Mucus is formed within the airways by a polymeric matrix of large, oligomeric, gel-forming glycoproteins, called mucins. These gel-forming mucins are primarily responsible for the rheological properties of airway mucus (2). However, in disease states such as CF and chronic bronchitis, polymeric DNA and filamentous actin, two products of leukocyte lysis, contribute greatly to, and are the principal determinants of, the viscoelastic properties of the purulent sputum that is associated with these diseases (3,4). The molecular weights of mucins range between 2×10^6 Da and 40×10^6 Da, and they are composed of 50% to 85% carbohydrate (5-7). Each mucin contains an apomucin core that is enriched by hydroxyamino acids, namely, serine and threonine. These amino acids contain *O*-glycosylation sites for oligosaccharides (8-10). The number and length of these *O*-glycosylation domains vary among mucins, and each domain has a repeated series of sequences (ie, variable number of tandem repeats) (11-14). Thus, a single mucin gene can produce a set of diverse messenger RNA (mRNA) through alternate splicing during transcription.

The amount of protein generated by mucin mRNA can also be varied by mRNA stabilization, which may be observed following the treatment of cells with inflammatory mediators and cytokines (15). Furthermore, a wide diversity of post-translational modifications can produce multiple peptides from each mucin transcript. Oligosaccharides are joined to the mucin protein core through an initial alpha-*O*-glycosidic linkage of *N*-acetyl-galactosamine (GalNAc) to the hydroxyl region of serine or threonine (16), and this linkage provides the starting point for branching oligosaccharide chains. Mucin *O*-glycosylation is accomplished by one of six uridine diphosphate-GalNAc-polypeptide-alpha-*N*-GalNAc-transferases (17,18). Sulfate or neuramic acid moieties produces a polyanionic (ie, acidic) mucin molecule. There is wide mucin heterogeneity among and within individuals, which arises because of the differing lengths of mRNA in a single apomucin gene,

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TABLE 1
Human mucins classified by protein backbone structures encoded by mucin (*MUC*) genes

Classification	Mucins
Membrane-associated, TR+	*MUC1*, *MUC3A*, *MUC3B*, *MUC4*, *MUC11*, *MUC12*, *MUC13*, *MUC16*, *MUC17*, *MUC20*
Secreted, TR+	*MUC2*, *MUC5AC*, *MUC5B*, *MUC6*, *MUC7*, *MUC8*, *MUC9*, *MUC19*
Mucins lacking TR	*MUC15*, *MUC18*

TR+ Tandem repeat present

the varying stability of transcripts and the various combinations of several hundred different carbohydrate chains in each peptide.

At present, 20 human mucin (*MUC*) genes have been identified (*MUC1*, *MUC2*, *MUC3A*, *MUC3B*, *MUC4*, *MUC5AC*, *MUC5B*, *MUC6*, *MUC7*, *MUC8*, *MUC9*, *MUC11*, *MUC12*, *MUC13*, *MUC15*, *MUC16*, *MUC17*, *MUC18*, *MUC19* and *MUC20*) (19-36). The characteristic feature that distinguishes mucins from other glycoproteins is the presence of tandem repeat (TR) domains. However, *MUC15* and *MUC18* do not have TR domains, and it remains to be resolved as to whether a macromolecule indeed requires TR domains or only a significant degree of *O*-glycosylation sites to be classified as a mucin. More than 20 rodent *Muc* genes have been described and confusion arises in the nomenclature; for example, murine *Muc10* is the orthologue of the human *MUC7* gene, and murine *Muc14* is the homologue of a gene that encodes human endomucin-1/2. Mucins are classified based on their protein backbone structure, which is encoded by a *MUC* gene. The genes have been divided into two groups, namely, membrane-associated mucins and secreted mucins (Table 1). The majority of membrane-associated human mucin genes (*MUC3A*, *MUC3B*, *MUC11*, *MUC12* and *MUC17*) are located in a cluster on chromosome 7 at locus 7q22 (29,33,37). Chromosome 3q29 provides the locus for another two genes, namely, *MUC4* and *MUC20*, whereas *MUC13* is at position 3q13.3. The remaining two membrane-associated mucin genes are located on chromosome 1 (*MUC1*) and chromosome 19 (*MUC16*).

Four of the genes encoding secreted mucins are located in a cluster on chromosome 11 at locus 11p15 (*MUC2*, *MUC5AC*, *MUC5B* and *MUC6*) (38,39). The two human mucins that lack TR domains (*MUC15* and *MUC18*) are also located on chromosome 11, at positions 11p14.3 and 11q23.3, respectively (31). The remaining secreted mucins are located on chromosome 12 (*MUC8* and *MUC19*), chromosome 4 (*MUC7*) and chromosome 1 (MUC9) (26-28,35). While the expression of *MUC2* and *MUC5AC* are localized to the surface epithelial cells, *MUC5B* and *MUC7* are mainly produced by submucosal glands.

The promoters of *MUC2*, *MUC5AC* and *MUC5B* contain typical TATA box motifs, and several transcription factor binding sites have been identified, including the following: for *MUC2* – activator protein (AP)-1, AP-2, cyclic AMP response element-binding protein, CCAAT/enhancer binding protein and HC3; for *MUC5AC* – Sp1, AP-2, glucocorticoid receptor element, polyomavirus enhancer activator-3 and nuclear factor-kappa B (NF-κB); and for *MUC5B* – Sp1, NF-κB, c-Myc and N-Myc (39-41). These binding sites provide possible targets for transcriptional regulation of *MUC* gene expression. The present review focuses mainly on *MUC5AC* expression, because it is the predominant mucin secreted in the airways.

INDUCTION OF MUCIN PRODUCTION BY NEUTROPHIL ELASTASE

A neutrophilic pattern of inflammation is typically seen in the bronchoalveolar lavage fluid of patients with chronic inflammatory airway disease, including CF, chronic bronchitis and bronchiectasis (42,43). The presence of neutrophils in the sputum is directly correlated with impaired pulmonary function (44), suggesting a close link between neutrophilic inflammation and airway mucus obstruction.

Neutrophil elastase (EC 3.4.21.37), a serine protease that is released by neutrophils, is found in high concentrations in airway surface fluids of patients with chronic airway diseases (42,43,45,46). This enzyme can impair mucociliary clearance by several mechanisms that ultimately lead to airway obstruction by mucus; for example, the enzyme can cause ciliary injury and impairment of function (47), thereby stimulating mucin production, secretion and release (48-50). Neutrophil elastase also leads to secretory cell metaplasia and hyperplasia within airways, as well as the accumulation of secretory granules (51).

Increased *MUC5AC* mRNA and protein expression due to neutrophil elastase has been demonstrated in vitro (52). Neutrophil elastase enhances *MUC5AC* mRNA stability and, thus, increases mRNA levels (52). Recent studies have also found that neutrophil elastase induces *MUC5AC* gene expression via reactive oxygen species (ROS) (53,54). Conversely, in vitro, antioxidants can inhibit elastase-induced *MUC5AC* gene expression (54). Moreover, there is evidence for the generation of ROS by neutrophil elastase (55), with several studies reporting that ROS increases mRNA stability (56,57).

Neutrophil elastase has also been shown to cause mucin production through activation of the epidermal growth factor receptor (EGF-R) (55). The mechanism by which this process is initiated has recently been elucidated. The first step involves activation of dual oxidase 1 (Duox1) by neutrophil elastase to produce ROS (58); ROS are reported to be generated by NADPH oxidases (Nox) of phagocytes (59). Duox1 is a homologue of the core component of Nox, $gp91^{phox}$ (60), and has been identified in human airway epithelial cells to produce ROS (58). When stimulated by neutrophil elastase, cytosolic components of Nox, $p47^{phox}$ and $p67^{phox}$, are recruited to the plasma membrane to join Duox1 to form an active enzyme complex to generate ROS (58). This process is dependent on protein kinase C (58). ROS activates the latent form of tumour necrosis factor-alpha-converting enzyme (TACE), which, in turn, cleaves protransforming growth factor-alpha (pro-TGF-α) into TGF-α. Soluble TGF-α binds to and activates EGF-R, leading to mucin expression (see section on "Mucin induction by growth factors").

BACTERIA-INDUCED MUCIN PRODUCTION

Several studies have demonstrated that bacteria can induce mucus production. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are potent stimuli in CF patients (61,62). Early studies using cell culture supernatants of *P aeruginosa* revealed that lipopolysaccharides (LPS) (the major outer membrane component of Gram-negative bacteria) could upregulate transcription of the *MUC2* gene in epithelial cells (61). Subsequent experiments showed that the *P aeruginosa*-response element in

the 5′-flanking region of the *MUC2* gene contained overlapping CCAAT/enhancer binding protein and NF-κB sites (63). NF-κB was identified as an important binding site (62), and further studies found that the activation of NF-κB by *P aeruginosa* occurs via a Src-dependent Ras-mitogen-activated or extracellular signal-regulated protein kinase (MEK) 1/2-extracellular signal-regulated protein kinase (ERK) 1/2-90 kDa ribosomal S6 kinase pathway (63).

Despite evidence for the involvement of LPS in the transcriptional activation of mucin by *P aeruginosa*, it is clear that the response induced by LPS does not account for that induced by the whole supernatant (62). The investigation of additional bacterial factors that might stimulate mucin production led to the discovery of a distinct yet overlapping pathway activated by the *P aeruginosa* outer membrane protein flagellin (62). Flagellin is a major structural component of bacterial flagella, and it is required for chemotaxis, motility and nutrition in both Gram-positive and Gram-negative bacteria. *P aeruginosa* flagellin can elicit host cell responses by binding to the glycolipid asialo GM1 (ASGM1) (64). Because ASGM1 lacks transmembrane and intracellular domains, it is not capable of directly interacting with cytoplasmic proteins (62). Using an in vitro system to investigate intracellular signalling, the mechanisms by which ASGM1 ligation stimulates the transcription of the *MUC2* gene was studied. McNamara et al (65) showed that the binding of flagellin to ASGM1 causes the extracellular release of ATP, which then binds to a nucleotide receptor. Downstream events include G-protein activation and the cleavage of phosphatidylinositol 4,5-bisphosphate by phospholipase C, which results in the formation of inositol 1,4,5-trisphosphate. Inositol 1,4,5-trisphosphate triggers mobilization of Ca^{2+} from intracellular stores (65,66), which then binds to an unknown Ca^{2+}-binding protein, leading to phosphorylation of MEK1/2 and ERK1/2, and the subsequent transcription of *MUC2* (65). Although inhibitors of calcium metabolism have been found to inhibit the mucin response, only approximately 50% inhibition was achieved when the mitogen-activated protein kinase (MAPK) pathway was blocked, suggesting alternative ERK-independent downstream events (65).

Gram-positive bacteria such as *S aureus* or *Streptococcus pyogenes* can also induce *MUC2* gene transcription in epithelial cells (62). Teichoic acid and peptidoglycan (PGN) are the major polysaccharides in Gram-positive cell walls. Teichoic acid may also be linked to a lipid moiety such as lipotechoic acid (LTA) (67). Direct administration of PGN and LTA to epithelial cell cultures showed that PGN was a weak mucin stimulus, whereas LTA was shown to specifically stimulate mucin production and was responsible for most of the *MUC2*-inducing activity of Gram-positive bacteria (68). Gram-positive bacteria bind to human cells via the platelet-activating factor receptor (PAF-R) (69,70). LTA, like PAF, causes phosphorylation and internalization of PAF-R, and PAF-R antagonists, such as CV3988 and 1-*O*-hexadecyl-2-*O*-acetyl-sn-glycero-3-phospho-(*N*,*N*,*N*)-trimethyl hexanolamine, block mucin induction by LTA. PAF itself has been shown to stimulate mucin transcription in a concentration-dependent manner (68), supporting the hypothesis that LTA stimulates PAF-R and thereby stimulates *MUC2*. PAF-R, a G-protein-coupled receptor, can potentially act through the transactivation of EGF-R (71-73). The transactivation of EGF-R by certain G-protein-coupled receptors requires a metalloproteinase that cleaves pro-heparin-binding EGF (72). In summary, PAF-R induces mucin via the transactivation of EGF-R, a process that involves the cleavage of pro-heparin-binding EGR by a metalloproteinase (72). The metalloproteinase involved in this process has been identified as ADAM 10 (a disintegrin and a metalloproteinase) (68). Although toll-like receptor (TLR) 2 and/or TLR4 have been implicated in LTA-induced responses of macrophages, dendritic cells and human embryonic kidney 293 cells (74-77), and despite the expression of both TLR2 and TLR4 on epithelial cells, no evidence could be obtained for their involvement in LTA-induced mucin transcription.

The exposure of bronchial explants to *P aeruginosa* supernatants for 6 h in vitro led to an upregulation of *MUC5AC* mRNA. Furthermore, NCI-H292 epithelial cells exposed to *P aeruginosa* exoproducts showed increased levels of mucin protein (78). The presence of *P aeruginosa* in the airways of CF patients, and the elevation of *MUC5AC* mRNA and protein (78,79) pinpoint the need for a better understanding of the transcriptional control of the *MUC5AC* gene by *P aeruginosa*. Induction of *MUC5AC* transcription by cell-free supernatants of *P aeruginosa* or its exoproducts has been demonstrated in a cell culture model (40). This upregulation can be mimicked by LPS and blocked by the tyrosine kinase inhibitor genistein (40). In another study, supernatants from *P aeruginosa* induced EGF-R and *MUC5AC* mRNA and protein expression, as well as p44/42 MAPK phosphorylation in NCI-H292 cells (80). Pretreatment with a selective EGF-R tyrosine kinase inhibitor completely inhibited *MUC5AC* mRNA and protein induction, thus demonstrating the involvement of EGF-R (80). It has also been shown that upregulation of *MUC5AC* gene expression can be induced by exoproducts from Gram-positive bacteria (78).

Nontypeable *Haemophilus influenzae* is frequently reported in COPD exacerbations (81) associated with increased mucus production. Bacterial cytoplasmic proteins from *H influenzae* upregulate *MUC5AC* maximally via p38, whereas surface membrane proteins have only a weak effect on this mucin gene (82). Given the strong inductive effect of bacterial cytoplasmic proteins on *MUC5AC*, one would expect that antibiotics that exert their effects through bacterial lysis would increase mucus production in the short term. Jono et al (83) noted that nontypeable *H influenzae* upregulates the *MUC5AC* gene via activation of the TLR2-MyD88-dependent p38 MAPK pathway.

In microarray expression analysis, *Bordetella pertussis* has been found to induce *MUC2* mRNA in BEAS-2B cells (84). Further experiments showed that *B pertussis* stimulates *MUC2* and *MUC5AC* transcription in both a concentration- and time-dependent manner.

More recently, there have been data emerging suggesting that there are reduced rather than increased amounts of gel-forming mucins (MUC5B and MUC5AC) in the sputum of CF patients compared with normal sputum or the sputum of chronic bronchitis patients. In one study, Henke et al (85) found that the volume/volume concentration of the gel-forming mucins was greatly decreased compared with normal sputum, but that the amount of DNA was greatly increased in CF sputum. The mucins from the different groups were found to have the same apparent size, suggesting that they had not been proteolytically digested in the CF samples. This decreased mucin secretion may actually increase susceptibility to bacterial infection in CF patients by facilitating communication between the epithelial surface and biofilm-causing organisms.

CYTOKINE-INDUCED MUCIN PRODUCTION

A number of studies have shown that cytokines play an important role in mediating mucus overproduction and hypersecretion in lung disease. Specifically, T helper 2 (Th2)-type cytokines (eg, interleukin [IL]-4, IL-9 and IL-13) have been investigated as possible stimulators of mucus production.

IL-4 has been shown to induce *MUC2* gene expression and mucus glycoconjugate production in a human airway cell culture model (86). In vivo, intranasal instillation of IL-4 in mice increased *MUC5AC* mRNA expression and goblet cell metaplasia (86). Transgenic mice overexpressing IL-4 were found to have hypertrophic, nonciliated cells in their conducting airways due, in part, to the accumulation of mucus glycoprotein. An increase in *MUC5AC* gene expression was also reported. In addition, elevated levels of mucus-positive staining material were found in the bronchoalveolar lavage fluid (87).

Exaggerated production of IL-13 is found in both atopic and nonatopic asthma (88,89). IL-13 and IL-4 have overlapping effector profiles due to the sharing of receptor components of the multimeric IL-13 and IL-4 receptor complexes (90,91). Goblet cell hyperplasia and mucus overproduction are observed in transgenic mice that selectively overexpress IL-13 in the lungs (92). In ovalbumin (OVA)-challenged A/J mice, as well as in OVA-challenged BALB/c mice, blocking IL-13 reduces the rise in goblet cells caused by antigens (93,94). On the other hand, the direct delivery of IL-13 to the airways enhances mucus production (94).

Signal transducer and activator of transcription (STAT) 6, which is essential for the development of allergen-induced experimental asthma (95), and which is increased in epithelial cells in asthma (96), is a critical signalling molecule that is activated by the binding of IL-13 to its receptor. STAT6-deficient mice are protected from the pulmonary effects of IL-13, but restoration of STAT6 to epithelial cells alone is sufficient to cause mucus production (and airway hyperresponsiveness) without inflammation, fibrosis or other lung pathologies (97). The direct effects of IL-13 on epithelial cells are therefore involved in mucus overproduction.

IL-13 is reported to mediate a pathway for mucus induction by airway epithelial cells in conjunction with CD4 T cells and IL-9 (98). *MUC5AC* gene expression was found to be reduced after Th2 cell transfer to mice in the absence of IL-13 or IL-4 receptor alpha (IL-4Rα) signalling (98). Interestingly, mucus production could be stimulated in the absence of NF-κB, whereas other studies showed that the inhibition of this transcription factor prevented GATA-3 and Th2-type cytokine expression (99). The IL-4R subunit IL-4Rα, which is shared by IL-4R and IL-13 receptor, plays a key role in Th2-type cytokine-induced mucus production. Cohn et al (100) showed that the induction of airway eosinophilia and mucus were both blocked in IL-4Rα$^{-/-}$ transgenic mice. They also demonstrated that mucus production was not dependent on eosinophils.

The increased expression of IL-9 and its receptor have been reported in the bronchial mucosa of asthmatic patients (101). Longphre et al (102) showed that allergen-induced IL-9 could directly stimulate mucin (*MUC5AC*) transcription in a cell culture model using the mucoepidermoid cell line NCI-H292. Moreoever, IL-9 overexpression in transgenic mice is associated with elevated *MUC2* and *MUC5AC* gene expression, as well as increased mucus in the airways (103). Direct administration of IL-9 to the lungs by intratracheal instillation induced mucin hypersecretion in mice (103), while the Th1-type cytokine interferon-gamma decreased mucin production. Further evidence for the role of IL-9 in mucus overproduction came from a study by Kung et al (104) in which mice were sensitized to and challenged with OVA. As expected, sensitized and challenged mice displayed eosinophilia, epithelial damage and goblet cell hyperplasia. Intraperitoneal administration of an antimouse IL-9 monoclonal antibody reduced pulmonary eosinophilia, serum immunoglobulin E levels, airway epithelial damage, airway hyperresponsiveness and goblet cell hyperplasia (104).

The mechanisms by which IL-9 induces mucus production have been further elucidated. In the lungs of a murine model of asthma, expression of the calcium-activated chloride channel (mCLCA3) was associated with airway hyperresponsiveness and mucus hypersecretion (105). Furthermore, the introduction of mCLCA3, or its human counterpart hCLCA1, into NCI-H292 cells induced *MUC5AC* expression and mucus production in vitro (105). mCLCA3 is involved and has also been shown to be induced in the airway epithelium of IL-9 transgenic mice (106). This induction can be achieved by intratracheal adminstration of IL-9 or other Th2-type cyokines (IL-4 and IL-13), but not by interferon-gamma (106). Antigen exposure increased the mCLCA3 expression in F1 mice, and this effect could be suppressed by treatment with an anti-IL-9 neutralizing antibody (106). In vitro, hCLCA1 could be induced in human primary lung cells by Th2 (IL-9 and IL-4) treatment (106). In the bronchial epithelium of asthmatic patients, an upregulation of hCLCA1 in mucus-producing epithelium was compared with control subjects (107). A strong positive correlation was found between hCLCA1 mRNA and both IL-9 and the IL-9 receptor. Taken together, these data suggest that IL-9 (and other Th2-type cyokines) acts through hCLCA1, and that this protein may be, in part, responsible for mucus overproduction in bronchial asthma. Investigations from our own laboratory have demonstrated increased expression of both IL-9 and its receptor, as well as upregulation of hCLCA1, in patients with CF or COPD (108,109). Recently, cleavage products of hCLCA1 and mCLCA3 were found to be present in bronchoalveolar lavage fluid taken from asthmatic patients and OVA-challenged mice (110). Therefore, hCLCA1 and mCLCA3 may not be ion channels but rather mediator proteins. By blocking hCLCA1 with either niflumic acid (a chloride channel antagonist) or one of its analogues, MSI-2216, mucus expression may be reduced in human airway mucosa (111).

More recent evidence of the role of IL-9 in mucus overexpression comes from a study by Vermeer et al (112), who observed that this cytokine had little effect on fully differentiated, ciliated human airway epithelium. However, during repair processes (eg, after an injury), exposure to IL-9 resulted in goblet cell hyperplasia. The stage of differentiation of airway epithlium is also important in determining the effects of IL-9.

Recent studies have questioned the role of Th2-type cytokines as mucus-inducing factors. Most of the studies were performed on cell lines or in animal models, and the data on primary epithelial cells are minimal. Recently, IL-13 has been shown to decrease *MUC5AC* gene expression and mucin secretion in cultured nasal epithelial cells (113). In another study, Chen et al (114) tested a panel of cytokines on primary human tracheal epithelial cells. IL-6 and IL-17 were found to

induce *MUC5AC* and *MUC5B* gene expression. IL-4 has been observed to decrease *MUC5AC* expression and secretion in normal human nasal epithelial cells (115), and to decrease *MUC5AC* and *MUC5B* expression in human bronchial epithelial cells (116). However, primary airway epithelial cells are typically taken from patients with underlying pulmonary disease, making it uncertain whether the cells have been affected by other cytokines and mediators. On the other hand, it may be possible that Th2-type cytokines increase the number of mucus cells only after longer periods of time.

Other cytokines, such as tumour necrosis factor-alpha (TNF-α), IL-1, IL-5, IL-6, IL-10 and IL-17, have also been shown to increase mucus expression (114,115,117-121). At present, the intracellular signalling pathways by which cytokines upregulate *MUC* gene expression are far from being fully understood. IL-6 induces *MUC5B* gene expression via ERK (114), and IL-17 stimulates mucin mRNA expression partly through IL-6 by acting in an autocrine/paracrine manner, and perhaps through Janus-activated kinase 2 (114). TNF-α and IL-1 have been shown to induce *MUC* gene expression via ERK or the p38 MAPK pathway in vitro (122). The activation of mitogen- and stress-activated protein kinase 1, cyclic AMP response element-binding protein and the cyclic AMP response element signalling cascade are crucial for intracellular mediation of *MUC5AC* gene expression (122). TNF-α may also induce *MUC* gene expression through NF-κB and Sp1 (123). IL-4, IL-13, IL-9 and IL-6 bind to their specific receptors, which, in turn, heteromerize with gamma c chains or glycoprotein 130 molecules to activate intracellular signalling (124). These cytokines act via the Janus-activated kinase/STAT pathway (125-127).

MUCIN INDUCTION BY GROWTH FACTORS

Growth factors are thought to be involved in goblet cell production and mucus overproduction because hypersecretory diseases are associated with abnormal epithelial cell growth and differentiation. Moreover, epithelial damage leads to repair and remodelling processes (128). The activation of EGF-R by its ligands, EGF or TGF-α, increases *MUC5AC* mRNA expression in vitro, and MUC5AC mucin production and goblet cell metaplasia in vivo (129). It has been shown that the induction of MUC2 and MUC5AC via the activation of EGF-R is mediated through an EGF-R-Ras-Raf-ERK pathway (123). TGF-α plays a critical role in EGF-R phosphorylation, leading to MUC5AC production. Pro-TGF-α is cleaved by TACE into its active form (130), which has recently been demonstrated to increase shedding of TGF-α, thereby upregulating *MUC5AC* expression (131). In that study, the knockout of TACE expression with interfering RNA inhibited phorbol-12-myristate-13-acetate-, *P aeruginosa* supernatant- and LPS-induced TGF-α shedding, as well as EGF-R phosphorylation and mucin production.

OTHER MECHANISMS OF MUCIN OVERPRODUCTION

CF transmembrane conductance regulator malfunction is associated with *MUC1* overexpression

The role of the defective CF transmembrane conductance regulator (CFTR) in mucus overproduction in CF has not yet been defined. However, Gonzalez-Guerrico et al (132) reported a link between CFTR failure and *MUC1* overexpression in this disease. Using a cultured cell line (derived from a CF patient) with or without transfection of wild-type CFTR, the tyrosine kinase c-Src was identified as a CFTR-dependent gene (132). c-Src expression was noted to be increased in the absence of functioning CFTR. At present, it is not clear how a channel protein like CFTR might control gene expression. It is possible that chloride transport or phosphorylation induced by CFTR may lead to conformational changes of other membrane proteins, which may ultimately serve as signal transducers (132). Pathways that are sensitive to membrane potential changes may also be present. Moreover, a PSD95/Dlg/ZO-1 (PDZ)-binding domain that recognizes the C-terminal consensus sequence –T(K/R)L has been identified in CFTR, and several other CFTR-associated proteins have been reported (133,134). There is a strong possibility that CFTR may be a control protein, and because c-Src also modulates CFTR activity, this effect may operate in both directions (135). The expression of the *MUC1* gene (136,137) located on chromosome 1 is regulated by c-Src, providing a CFTR-c-Src-MUC1 link.

Smoke-induced mucin expression

Repetitive exposure to irritant stimuli like smoke can cause bronchitis and mucus overproduction. The major aldehydes, including formaldehyde, propionaldehyde and acrolein, have numerous indoor and outdoor sources (138,139).

In Sprague-Dawley rats, exposure to acrolein caused increased *MUC5AC* mRNA expression and mucus hypersecretion in the trachea and lungs, whereas *MUC2* mRNA expression was not significantly altered (140). These findings indicate that acrolein-induced mucus overproduction is due, at least initially, to upregulation of *MUC5AC* mRNA expression, rather than *MUC2* mRNA expression. A concentration-dependent increase in *MUC5AC* mRNA expression has been observed following exposure of human airway cells (NCI-H292) to acrolein in vitro (15), suggesting that acrolein has a direct effect on epithelial cells. However, acrolein can also initiate mucus hypersecretion indirectly via acute and chronic inflammation, which is characterized by neutrophils and macrophages (141,142). Neutrophil elastase can induce goblet cell metaplasia and is a potent secretagogue (51). Macrophages provide another source of mediators, such as IL-1, eicosanoids (eg, prostaglandin E2 and 15-hydroxyeicosatetraenoic acid) and macrophage-derived mucus secretagogue-68, that can initiate mucus secretions (117,143,144). A recent study reported the activation of TACE by cigarette smoke, thereby activating TGF-α-dependent EGF-R phosphorylation and *MUC5AC* expression in NCI-H292 cells (145).

Acrolein can act directly as an electrophilic compound to deplete nonprotein sulfhydryl groups/glutathione in the respiratory epithelium (146,147) or to inactivate metabolizing enzymes (148,149). Despite limited knowledge about cis-activating and trans-activating elements regulating *MUC5AC* expression, electrophiles are known to activate gene expression via a mechanism involving glutathione depletion and the production of ROS (150).

Given the numerous compounds in cigarette smoke, substances other than acrolein may induce mucus overproduction. In fact, inflammation due to smoke may have an important effect on mucus production and secretion. In an animal model, cigarette smoke-induced airway secretory cell hyperplasia was inhibited by the nonsteroidal drug indomethacin, as well as by corticosteroids (151).

CONCLUSIONS

Despite its clinical importance, our knowledge of the pathogenesis of mucus overproduction in chronic inflammatory lung disease remains limited. Although pathomechanisms that contribute to exaggerated mucin expression have been elucidated, the pathogenesis of mucus hypersecretion was not the focus of the present review. Secretory processes have recently been further defined and possible target sites for therapeutic intervention have been identified in animal studies (151-153). If these findings translate to humans, this may ultimately benefit patients by blocking excessive mucus production and mucus hypersecretion in chronic inflammatory lung disease.

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