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Airway Mucus: Its Components and Function

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The airway surface liquid (ASL), often referred to as mucus, is a thin layer of fluid covering the luminal surface of the airway. The major function of mucus is to protect the lung through mucociliary clearance against foreign particles and chemicals entering the lung. The mucus is comprised of water, ions, and various kinds of macromolecules some of which possess the protective functions such as anti-microbial, anti-protease, and anti-oxidant activity. Mucus glycoproteins or mucins are mainly responsible for the viscoelastic property of mucus, which is crucial for the effective mucociliary clearance. There are at least eight mucin genes identified in the human airways, which will potentially generate various kinds of mucin molecules. At present, neither the exact structures of mucin proteins nor their regulation are understood although it seems likely that different types of mucins are involved in different functions and might also be associated with certain airway diseases. The fact that mucins are tightly associated with various macromolecules present in ASL seems to suggest that the defensive role of ASL is determined not only by these individual components but rather by a combination of these components. Collectively, mucins in ASL may be compared to aircraft carriers carrying various types of weapons in defense of airborne enemies.

Key words: Airway surface liquid, Mucus, Defensive function, Mucus glycoprotein, Proteoglycan

INTRODUCTION

The airway surface liquid (ASL), often referred to as mucus, is a thin layer of fluid covering the luminal surface that plays an important defensive role against foreign particles and chemicals entering the lung. Particles are trapped in this viscous layer of mucus and removed from the airways by the constant beating of cilia present on the surface of underlying epithelial cells. This process of mucociliary clearance is crucially important and controlled by a number of factors (for reviews see Cole, 2001; Houtmeyers *et al.*, 1999). In addition to the active involvement in mucociliary clearance, airway mucus contains various macromolecules that contribute to its defensive function either alone or in combination. This review will focus on the macromolecules present in airway mucus and their potential roles in airway defense.

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MACROMOLECULES IN AIRWAY MUCUS

Airway mucus consists of water, salts and various macromolecules including mucins, proteoglycans, lipids and other proteins. It is thought that the optimum function of mucus is performed when individual components are present in proper concentrations. Pathology may occur when the optimum function of mucus is distorted by altered quality or quantity of its individual components.

Mucins

Mucins are high molecular weight glycoproteins containing variable numbers of tandem repeats (VNTR) in which Ser, Thr and Pro are highly enriched. The presence of VNTR with these amino acids is responsible for heavy glycosylation and thus polydispersity in both size and charge of the mucin molecules. Glycosylation within the VNTR takes place between the Ser/Thr moieties of the peptide backbone and N-acetylgalactosamine of the oligosaccharides, characteristic of O-linked glycoproteins. However, a small number of N-glycosidic linkages (between an Asn moiety of the protein backbone and N-acetylglu-

cosamine of the oligosaccharides) also are present in the non-repeat region of the molecule.

Thirteen mucin-encoding genes (designated MUC in human, Muc in nonhumans) have been identified, eight of which (MUC1, MUC2, MUC4, MUC5AC, MUC5B, MUC7, MUC8 and MUC13) are expressed in lung tissues. Three of these are membrane-associated mucins whereas the others are secreted mucins present in the ASL. The structures of various MUC genes were recently reviewed in great detail (Moniaux et al., 2001). Although the roles of individual mucins are not known, the diversity as well as tissue specificity of these mucins seems to suggest the unique roles of individual mucins. In this section, we will focus on mucins produced by airways and discuss their structure and potential roles.

Membrane-associated mucins

Three membrane-associated mucins are expressed in the airways: MUC1, MUC4 and MUC13. The general structural features of membrane-associated mucins consist of an N-terminal large molecular weight, heavily O-glycosylated extracelluar (EC) domain containing the VNTR, a transmembrane (TM) domain and a C-terminal cytoplasmic (CT) domain. The EC domain is often cleaved by proteolysis allowing it to be shed from the cell. Although the exact functions of these mucins are not known, their cellular locations and peptide sequences suggest possible roles as signaling molecules.

MUC1. MUC1 is the prototype membrane-bound mucin (Gendler *et al.*, 1990; Lan *et al.*, 1990; Ligtenberg *et al.*, 1990; Park *et al.*, 1996). The MUC1 EC domain contains a 20 amino acid VNTR that may be replicated over 100 times, the actual number being a polymorphic trait (Hilkens *et al.*, 1992). MUC1 is initially synthesized as a single polypeptide chain but intracellular proteolysis generates two subunits. The larger (>250 kDa) is derived from most of the EC domain while the smaller (20-30 kDa) contains a juxtamembrane region of the EC domain, the TM domain and the CT domain (Ligtenberg *et al.*, 1992). The MUC CT domain contains 72 amino acids, is highly conserved in different species and contains a relatively high percentage of Tyr, Ser and Thr residues that are potential phosphorylation sites (Park *et al.*, 1996). Some of the Ser (Baruch *et al.*, 1999; Li *et al.*, 1998) and Tyr (Zrihan-Licht *et al.*, 1994; Meerzaman *et al.*, 2000; Quinn and McGuckin, 2000; Li *et al.*, 2001a) were shown to be phosphorylated under different experimental conditions. Unlike other membrane-tethered mucins, the MUC1 CT domain possesses four phosphorylation sequence motifs postulated to interact with signaling proteins (Zrihan-Licht *et al.* 1994; Pandey *et al.*, 1995; Li *et al.*, 1998).

In the bronchus, MUC1 is expressed on ciliated epithelial and serous gland cells (Gendler and Spicer, 1995). While it is well-established that airway epithelial cells abundantly express MUC1 mucin mRNA and protein (Park *et al.*, 1996; Paul *et al.*, 1998; Copin *et al.*, 2000; Lopez-Ferrer *et al.*, 2001), the exact role of MUC1 in the airways remains to be clarified. Due to its long and extended conformation, MUC1 may destabilize cell-cell and/or cell-matrix interactions thus aiding in epithelial morphogenesis (Braga *et al.*, 1992; Hilkens *et al.*, 1992). Alternatively, MUC1 has been shown to promote cell-cell interactions, possibly by cross-linking intercellular adhesion molecules on adjacent cells (Hayashi *et al.*, 2001). However, the best clue for MUC1ís function in the airways comes from its characteristic receptor-like structure, particularly with respect to signal transduction motifs in its CT domain. Based on this structure, we proposed that MUC1 functions as a receptor on airway epithelial cells for inhaled particles, particularly *Pseudomonas aeruginosa*, and proposed an important role for MUC1 in the clearance of inspired bacteria (Kim *et al.*, 2001). Our studies demonstrated that: (1) hamster Muc1 mucins expressed on the surface of Chinese hamster ovary cells bind to *P. aeruginosa* (Lillehoj *et al.*, 2001), (2) adhesion was mediated by the direct interaction of bacterial flagellin with Muc1 (Lillehoj *et al.*, 2002) and (3) Muc1 CT domain phosphorylation was stimulated following interaction of its EC domain with *P. aeruginosa* or purified flagellin (Lillehoj *et al.*, unpublished observations). Collectively, these results have led us to conclude that *P. aeruginosa* binding to the EC region of Muc1 results in phosphorylation of its CT domain and activation of one or more signaling pathways ultimately augmenting host defensive mechanisms responsible for bacterial clearance from the airways.

MUC4. MUC4 is a large molecular weight glycoprotein heterodimer consisting of an EC mucin subunit with a 16 amino acid VNTR and a smaller subunit containing part of the EC region, the TM domain and the CT domain (Moniaux *et al.*, 2001). The rat homologue of MUC4 is referred to as sialomucin complex (SMC) and the individual subunits as ascites sialoglycoprotein-1 (ASGP-1) and ASGP-2 (Carraway *et al.*, 2000). Whereas MUC1 and MUC4 both are expressed by bronchial ciliated epithelial cells, their expressions in airway glandular epithelial cells differ. MUC4 is present on mucous cells but absent from serous cells while MUC1 is absent from mucous cells but present on serous cells (Gendler and Spicer, 1995). Using in situ hybridization, Buisine *et al.* (1999) observed that MUC4 is the earliest mucin gene expressed in the human fetal respiratory tract being first detected at 6.5 weeks of gestation. The small subunit of MUC4 contains two epidermal growth factor (EGF)-like domains and acts as a ligand for the receptor tyrosine kinase ErbB2. Unlike the MUC1 CT domain (72

amino acids), the CT region of MUC4 is relatively short (22 amino acids) and does not contain any known sequence motifs for signaling pathways. Rather, MUC4 binding to ErbB2 stimulates receptor (ErbB2) phosphorylation and association with the Src tyrosine kinase as well as individual components of the Ras-mitogen-activated protein (MAP) kinase pathway. Thus, by way of its interaction with ErbB2, the MUC4 CT region modulates epithelial cell signaling.

MUC13. Recently, Williams *et al.* (2001) described MUC13, a novel human cell surface mucin expressed by epithelial and hemopoietic cells. Northern blotting demonstrated that MUC13 mRNA expression in the trachea was very high and second only to the colon. Sequence analysis of the MUC13 gene indicated that it encoded a 512 amino acid protein containing EC, TM and CT domains. The EC domain consisted of a VNTR, three EGF-like sequences and a SEA module. The SEA module is a region common to different O-glycosylated cell surface proteins that contain putative proteinase cleavage sites potentially involved in shedding of the EC domain. Indeed, analysis of the MUC13 protein demonstrated that it was cleaved by proteolysis to generate a large, heavily glycosylated subunit (>500 kDa) and a smaller subunit containing the EGF, TM and CT domains. While the CT domain of MUC13 (69 amino acids) is similar in size to that of MUC1, it does not possess the any of the MUC1 Tyr-containing sequence motifs implicated in signal transduction. However, one consensus phosphorylation motif for protein kinase C is present in the MUC13 CT region. Future studies examining the signaling function of the MUC13 CT domain should shed light on its physiologic role in the airways.

Secreted mucins

Five secreted mucins are expressed in the airways: MUC2, MUC5AC, MUC5B, MUC7 and MUC8. Genes for three of these (MUC2, MUC5AC and MUC5B) along with MUC6 are contained in a four gene cluster on chromosome 11p15.5 (Pigny *et al.*, 1996). In general, the genes and corresponding proteins of secreted mucins are much larger than those of cell-associated mucins. Characteristic of secreted mucins is the presence of Cys-rich domains, referred to as D domains, homologous to those of von Willebrand factor and possibly involved in mucin oligomerization, a property essential for their mucus gel-forming ability.

MUC2. The MUC2 gene product is a very large protein, greater than 5,100 amino acids in length and containing two different VNTRs (Gum *et al.*, 1994). The first repeat domain contains 50-100 Thr/Pro-rich 23 amino acid continuous repeats while the second is composed of a 347 residue irregular and discontinuous Thr/Ser/Pro-rich repeat. MUC2 possesses four D domains, three located at the N-terminus and the fourth at the C-terminus of the protein. This D domain organization is similar to that seen in von Willebrand factor. The MUC2 D domains contain a characteristic Cys-X-X-Cys sequence that appears to mediate mucin oligomerization through disulfide bonding. As reported by Karlsson *et al.* (1996), the oligosaccharides of MUC2 contain an equal fraction of neutral (40%) or sialylated (40%) residues with the remainder being sulfated. Mass spectrometry identified the sulfate group attached to C-6 of the N-acetylglucosamine moiety.

MUC2 levels are normally low in the airways, comprising approximately 2.5% by weight of the total secreted mucins (Kirkham *et al.*, 2002). Its expression has been shown to be down-regulated by Vitamin A in tracheobronchial epithelial cells (An *et al.*, 1994) as well as by dexamethasone in NCI-H292 lung cancer cell line (Kai *et al.*, 1996). In contrast, Guzman *et al.* (1996) as well as Gray *et al.* (2001) showed an increase in the level of MUC2 mRNA by retinoids in normal human tracheobronchial epithelial (NHTBE) cells. The expression of MUC2 mRNA is dramatically up-regulated during inflammation or bacterial infection (Li *et al.*, 1997; Dohrman *et al.*, 1998). For example, IL-4 and IL-9 cytokines that play an important role in allergic diseases, increased MUC2 expression both in cultured primary and established human airway cells (Dabbagh *et al.*, 1999; Louahed *et al.*, 2000). In a guinea pig model of allergic asthma, Li *et al.* (2001b) recently demonstrated that levels of Muc2 in lungs of ovalbumin-sensitized animals increased significantly shortly after acute allergen exposure. These studies suggested that MUC2 likely plays an important role in airway inflammation and mucin overproduction, both hallmark pathophysiologic features of asthma. Similarly, IL-1β, a major pro-inflammatory cytokine, was shown to increase expression levels of MUC2 by a transcriptional mechanism in cultured airway cell lines (Kim *et al.*, 2000). Interestingly, however, MUC2 mRNA levels in nasal epithelial cells from cystic fibrosis (CF) patients were not significantly different from those of normal individuals (Voynow *et al.*, 1998). Since CF patients demonstrate constitutively high levels of pro-inflammatory cytokines, including IL-1β (Noah *et al.*, 1997), further studies are needed to clarify the relationship between MUC2 expression and cytokines in normal and disease states.

MUC5AC. Numerous genetic clones were described for MUC5. From these, three genes initially were thought to encode unique mucins and were designated MUC5A, MUC5B and MUC5C. Subsequent studies demonstrated that MUC5A and MUC5C were identical and its designa-

tion was changed to MUC5AC (Guyonnet *et al.*, 1994). Molecular cloning based on the sequences of tryptic digests of a secreted human tracheal mucin preparation led to the identification of MUC5 which turned out to be identical to MUC5AC (Meerzaman *et al.*, 1994). MUC5AC is expressed in the ciliated epithelium and mucous glands of the bronchus (Gendler and Spicer, 1995). Along with MUC5B, it constitutes the major airway mucin (>95% by weight) (Kirkham *et al.*, 2002). Structurally, the MUC5AC VNTR is relatively short, 8 amino acids of which 6 are Ser or Thr. These VNTRs alternate along the peptide core with ~30 amino acid D domains each with 10 conserved Cys residues. As with MUC2, the Cys-rich regions exhibit sequence homology to von Willebrand factor in areas required for tail-to-tail dimerization. The N-terminus of MUC5AC contains a putative leucine zipper motif not found in any other mucin identified so far but its function is unknown (van de Bovenkamp *et al.*, 1998).

MUC5AC gene expression is regulated both transcriptionally and post-transcriptionally and its aberrant up-regulation contributes to airway diseases (Rose *et al.*, 2000). In the case of asthma, IL-4 and IL-9 were shown to up-regulate MUC5AC protein expression *in vitro* and *in vivo* (Dabbagh *et al.*, 1999; Longphre *et al.*, 1999). Intratracheal instillation of IL-13 was also shown to increase the expression of Muc5ac in mouse airways (Zuhdi Alimam *et al.*, 2000). Bacteria such as *Pseudomonas aeruginosa* and *Haemophilus influenzae* that are responsible for most of the morbidity and mortality associated with CF and chronic obstructive pulmonary disease (COPD) respectively have also been shown to augment MUC5AC production. Dohrman *et al.*, (1998) demonstrated that *P. aeruginosa* lipopolysaccharide (LPS) stimulated MUC5AC mRNA and protein levels both in bronchial explants and cultured airway epithelia cells. Wang *et al.* (2002) recently identified *H. influenzae* cytoplasmic proteins as transcriptional activators of MUC5AC gene expression by a complex mechanism involving both positive and negative regulation. Activation of p38 MAP kinase was required for H. influenzae-induced MUC5AC expression whereas activation of a phosphoinositide 3-kinase signaling pathway led to down-regulation of MUC5AC transcription via negative cross-talk with the p38 pathway.

MUC5B. MUC5B mucin is a major respiratory mucin (Wickstrom *et al.*, 1998) normally expressed and secreted by submucosal gland mucous cells and very little, if any, by surface epithelial cells (Sharma *et al.*, 1998). During airway disease, however, MUC5B also is expressed by epithelial cells. For example, Chen *et al.* (2001) demonstrated that induction of asthma in mice by treatment with ovalbumin stimulated expression of Muc5B by surface epithelia cells. MUC5B is unique in the mucin superfamily in that its repeat region is degenerate and non-tandem (Dufosse *et al.*, 1993). Due to numerous amino acid insertions and deletions, only 22 of possible 55 complete repeats are present. Nevertheless, the sequence remains mucin-like with a high percentage of Ser, Thr and Pro residues and is heavily O-glycosylated. The amounts and glycosylated variants of MUC5B were reported to be substantially altered in airway diseases, for example an increase in its low-charge form in CF and COPD (Kirkham *et al.*, 2002). Similar to other secreted mucins, the large size of the MUC5B gene necessitated isolation of partial clones that were later combined to deduce its overall genomic organization (Desseyn *et al.*, 1997). The central region of MUC5B contains a single large exon of 10,713 base pairs (3,570 amino acid) that may be the biggest exon described for a vertebrate gene. The deduced full-length MUC5B protein contains 19 subdomains with so-called super-repeats of 528 amino acids, the largest ever determined in mucin genes. Each super-repeat contains a 108 amino acid Cys-rich region that is replicated 7 times in MUC5B.

MUC7. MUC7 was originally described as a salivary mucin but recent studies have shown conclusively that it also is expressed in the airways (Sharma *et al.*, 1998; Bernacki *et al.*, 1999). Primary normal human nasal epithelial (NHNE) cells and primary NHTBE cells expressed detectable levels of MUC7 mRNA (Yoon *et al.*, 2000; Gray *et al.*, 2001). Developmental gene expression studies demonstrated that MUC7 mRNA was present in serous, but not mucous cells of the fetal respiratory tract and this pattern of expression was unique compared with those of MUC2, MUC5AC and MUC5B (Buisine *et al.*, 1999). Structurally, the MUC7 protein is relatively small (120-150 kDa) but does contain a region of 6 almost perfect mucin tandem repeats of 23 amino acids. While two Cys residues are present, they are likely to mediate intramolecular rather than intermolecular disulfide bonds. Thus, MUC7 is unique in that it is the only airway mucin that is neither considered as a gel-forming mucin nor membrane-associated. It is expressed by glandular serous cells (Sharma *et al.*, 1998) as well as surface epithelial cells (Gray *et al.*, 2001). What function it serves remains to be elucidated.

MUC8. The human MUC8 gene was originally cloned by Shankar *et al.* (1994) from a normal tracheal expression library. Sequence analysis revealed an almost perfect 41 base pair tandem repeat nucleotide sequence that encoded two mucin consensus peptide repeats of 41 and 13 amino acids. By Northern blot analysis, the MUC8 gene was expressed at high levels in both normal and CF airways as well as 2 non -small cell lung cancer cell lines. Increased expression of MUC8 mRNA levels was seen

following treatment of primary NHNE cells with IL-1β alone or in combination with TNF-α (Yoon *et al.*, 1999). Similar cytokine treatments had no effect on expression of MUC5AC or MUC5B. The effects of retinoic acid on MUC8 gene expression also has been investigated, but here the results are more controversial. When cultured in the presence of retinoic acid, primary NHNE cells at an air-liquid interface underwent differentiation and secreted several mucins, including MUC8 (Yoon *et al.*, 2000). The cells became squamous and mucin secretion decreased when retinoic acid was deleted from the culture media. In contrast, Gray *et al.* (2001) found that mucous differentiation and expression of MUC2, MUC5AC and MUC5B, but not MUC8, were retinoic acid dependent in cultures of primary NHTBE cells. Further studies are needed to determine the effects of transcriptional activators such as retinoic acid on expression of MUC8.

Proteoglycans

Proteoglycans are large molecular weight glycoconjugates characterized by variable numbers of disaccharide repeats, the structures of which are used to classify the types of proteoglycans. The presence of both glucuronic or iduronic acid residues and high degree of sulfation renders these molecules highly acidic in physiologic conditions. Bhaskar *et al.* (1985) first demonstrated the presence of proteoglycans in human bronchial aspirates. Three types of proteoglycans were shown to be secreted by cultured primary airway epithelial cells: hyaluronic acid containing proteoglycans (Kim, 1985; Wu, 1985; Kim *et al.*, 1987; Paul *et al.*, 1988), chondroitin sulfate containing proteoglycans (Kim *et al.*, 1987; Paul *et al.*, 1988) and heparan sulfate containing proteoglycans (Wu, 1985; Kim *et al.*, 1987). Baraniuk *et al.* (1996) showed that hyaluronan was present in both airway "epithelial" cells and glandular serous cells, but neither in surface goblet cells nor submucosal mucous cells. Secretion of chondroitin sulfate proteoglycans from bovine tracheal gland serious cells was shown to be stimulated by mast cell chymase (Sommerhoff *et al.*, 1989), neutrophil elastase and cathepsin G (Sommerhoff *et al.*, 1990). The exact roles of proteoglycans in the ASL remain largely unknown. Suggested functions, however, include airway development (Zhao *et al.*, 1999), remodeling (Huang *et al.*, 1999), inflammation (Ohkawara *et al.*, 2000) and mucosal host defense (Forteza *et al.*, 2001).

Lipids

The ASL contains neutral lipids, phoshpholipids and glycolipids (Bhaskar *et al.*, 1987). The lipid profile of mucus, however, is different between normal individuals and those with mucus hypersecretory conditions. The former contained mainly cholesterol and some phospholipids but no glycolipids, whereas the latter contained glycolipids, often as the predominant species, in addition to the other two lipid species. This has suggested that mucus glycolipids may serve as markers of disease (Bhaskar *et al.*, 1987). It has been shown that the amount of lipids in purulent sputum increases with the degree of bacterial infection (Houdret *et al.*, 1986) and most of the lipids in purulent sputum are associated with mucin glycoproteins (Nadziejko *et al.*, 1993). Lipids in the ASL are derived not only from alveolar epithelial cells but also directly from airway epithelial cells. Using tracheal mucosa explants, Bhaskar *et al.* (1986) demonstrated that the explants synthesize and secrete various lipids. A similar result was also observed in a primary tracheal epithelial cell culture (Kim *et al.*, 1989), which is highly enriched in goblet cells (Wasano *et al.*, 1988).

Lipids secreted by the airway epithelial cells are tightly associated with purified mucins (Kim *et al.*, 1989). Dissociation of mucins and lipids requires both heat denaturation and detergent treatment (Kim, 1991) but is not affected by 4 M guanidinium hydrochloride alone (Kim and Singh, 1990a) indicating that airway mucins are extremely hydrophobic. The lipid profile associated with secreted mucins appears to be virtually identical to that of cell-associated mucins suggesting that the association of mucin with lipids might occur prior to exocytosis and might not be a result of molecular aggregation following exocytosis (Kim and Singh, 1990b). A molecular model of goblet cell mucin secretion has been proposed in conjunction with lipid association (Kim, 1993). Recently, Sims and Horne (1997) provided morphological evidence of lipid association with tracheal mucus at the ultrastructural level.

In addition to the major lipid species, the ASL also contains various lipid metabolites most notably prostaglandins and leukotrienes which are involved in airway inflammation. Elevated airway leukotriene levels have been suggested to reflect airway epithelial damage (Sara *et al.*, 1991). Leukotriene B4 was shown to be the predominant eicosanoid in the CF airway (Konstan *et al.*, 1993). The exact roles of individual lipids in the ASL remain poorly understood. However, their functions may include modification of mucus rheology, an effect on ciliary beating and mucociliary clearance, modification of mucus adhesiveness, an action on bacterial invasion and lessening of the tendency of small airway collapse (Widdicomb, 1987).

Other proteins

While the macromolecules described above play crucial roles mainly in mucociliary clearance, the ASL also contains a number of other proteins mainly associated with airway defense (Jacquot *et al.*, 1992). These include antiproteases, anti-oxidants, anti-microbial proteins, secretory immunoglobulin A (IgA) and cytokines. The cytokines are

involved in airway repair and remodeling during inflammation and reviewed elsewhere in great detail (Renauld, 2001; Holgate, 2000; Lukacs *et al.*, 1996). In this section, we will focus on anti-proteases, anti-oxidants and anti-microbia proteins.

Proteases and anti-proteases. A number of proteases were identified in the ASL, all of which known to be associated with inflammation and derived from inflammatory cells. Among these were elastase and various cathepsins from neutrophils and chymase and tryptase from mast cells. Neutrophil elastase has been shown to cause destruction of elastin leading to a pathology characteristic of emphysema (Snider *et al.*, 1984), stimulate mucin release from goblet cells (Kim *et al.*, 1987; Breuer *et al.*, 1989; Lundgren *et al.*, 1994), contribute to antigen-induced mucociliary dysfunction (OíRiordan *et al.*, 1997) and induce chemotaxis via production of IL-8 by the underlying epithelial cells (Nakamura *et al.*, 1992). Surplus elastase released from neutrophils during lung injury and inflammation is balanced mainly by α1-protease inhibitor (anti-trypsin) in alveoli (Perlmutter and Pierce, 1989, for review) and by two acid-resistant protease inhibitors in the respiratory tract (Vogelmeier *et al.*, 1991). The latters are soluble leukocyte protease inhibitor (sLPI) or mucus protease inhibitor (MPI) secreted by airway epithelial cells as well as neutrophils (Thompson *et al.*, 1986; De Water *et al.*, 1986; Grutter *et al.*, 1988) and elafin (elastase-specific inhibitor) secreted mainly by Clara cells (Sallenave *et al.*, 1993). It was also shown that purified airway mucins also have an anti-protease activity (Nadziejko and Finkelstein, 1994).

Other proteases are also found in the ASL. For example, a trypsin-like protease produced by Clara cells, referred to as Tryptase Clara, activates influenza A virus (Kido *et al.*, 1992) and is possibly responsible for pneumopathogenicity of the virus. Both tryptase and chymase are also produced by mast cells, the former being responsible for disruption of the epithelial cell barrier allowing both antigens and inflammatory mediators to enter the mucosa and cause inflammation as seen in asthma whereas the latter is responsible for mucus secretion from serious cells (Sommerhoff *et al.*, 1989) as well as the release of TGF-β from the matrix possibly contributing to the accumulation of connective tissue in inflammation (Taipale *et al.*, 1995).

Anti-oxidants. It has been shown that the airway mucosa secretes a peroxidase probably active in preventing infection of the airway (Christensen *et al.*, 1981). Salathe *et al.* (1997) isolated and characterized an airway peroxidase from sheep airway secretions that is similar to lactoperoxidase and constitutes about 1% of the soluble proteins in airway secretions. The airway lactoperoxidase was microbiocidal and able to facilitate bacterial clearance from the airway (Gerson *et al.*, 2000). In light of it relatively high concentration in the ASL and ability to scavenge H_2O_2, it has been suggested that this anti-oxidant may be responsible for the majority of H_2O_2 scavenging activity in airway secretions (Salathe *et al.*, 1995).

Anti-microbial agents. Human ASL contains several anti-microbial factors, the most abundant being lysozyme and lactoferrin. Both were secreted mainly by glandular serous cells. Although mRNA of lysozyme was strictly limited to glandular serious cells in human bronchus tissues (Dohrman *et al.*, 1994), its secretion was demonstrated also in human tracheobronchial epithelial cells (Gray *et al.*, 1996). The levels of both lysozyme and lactoferrin were up-regulated in the respiratory tract of chronic bronchitis patients (Thompson *et al.*, 1990) as well as in patients with stable asthma (van de Graaf *et al.*, 1991). Lactoferrin secretion from nasal and bronchial tissues was modulated by various inflammatory cytokines (Boca-Ferrer *et al.*, 2001) and has been shown to act synergistically with other anti-microbial agents such as lysozyme and sLPI (Singh *et al.*, 2000). Lactoferrin also has been shown to be a potent inhibitor of tryptase and abolish late-phase airway responses in allergic sheep (Elrod *et al.*, 1997).

Human β-defensins (hBDs) are cationic anti-microbial peptides that may play a role in mucosal defense. Since their activities have been shown to be salt-sensitive (Smith *et al.*, 1996), diminished activity of these peptides has been implicated in the pathogenesis of CF. There are two isoforms in human: hBD-1 and hBD-2. hBD-1 is expressed constitutively whereas hBD-2 is induced in inflammatory lung diseases such as CF (Singh *et al.*, 1988). In contrast, ASL also contains anionic anti-microbial peptides (Ellison *et al.*, 1985; LaForce *et al.*, 1984). Overall, airway anti-microbial peptides have broad-spectrum activity against both Gram-positive and Gram-negative bacteria (Boman, 1995).

PERSPECTIVES

Given the crucial role of airway mucus as the first line of defense against various ambient stimuli, it is not surprising not only that it contains a diverse array of macromolecules exhibiting a variety of functions, but also that the concentrations of these molecules should be tightly regulated by the underlying epithelial cells. Among all these molecules, however, the most important are the mucins judging from their content in mucus, physicochemical properties and the highly heterogeneous nature of their structure. In addition to conferring the characteristic viscoelastic and adhesive properties on airway mucus,

mucins neutralize proteases through charge interactions and capture bacteria through tight adhesion. Furthermore, the fact that secreted mucins are strongly associated with other macromolecules through ionic and hydrophobic interactions strongly suggests that the structural organization of these macromolecules with mucins might be of paramount importance in maintaining the proper function of airway mucus.

Another important aspect of airway mucus concerns cellular economy. In light of the enormously large size of mucins and their extensive glycosylation, it would not be economical to "waste" these "expensive" molecules through constant mucociliary clearance. Therefore, it is conceivable that there might be at least two types of mucins, one with a "minimum" structure to perform "minimum" functional roles and the other for "heavy-duty" function. The former might be used for normal maintenance of the airway whereas the latter for "emergency" situations such as airway infection and inflammation. The existence of multiple MUC gene products seems to support this hypothesis. It also is likely that the types of accessory molecules associated with mucins may vary depending on the particular types of mucins involved. In summary, understanding the interactions of mucins with these accessory macromolecules should help us better understand normal physiological process of the airways as well as the pathophysiology of airway diseases.

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