SERIES 'AIRWAY MUCUS'

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Airway goblet cell mucin: its structure and regulation of secretion

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Airway goblet cell mucin: its structure and regulation of secretion. K Chul Kim, K. McCracken, C. Young Shin, M. Jo, C. Jae Lee, K. Ho Ko. ©ERS Journals Ltd 1997.

ABSTRACT: Mucociliary clearance is a major function of the airway epithelium. This important function depends both on the physicochemical properties of the airway mucus and on the activity of the cilia. The former, in turn, is dependent mainly on the quality and quantity of mucous glycoproteins or mucins, which are produced by two different cell types, namely, goblet cells of the epithelium and mucous cells of the submucosal gland. Neither the structural nor the functional differences of mucins produced by these two cell types are yet known. The availability of primary airway epithelial cell culture systems, however, has made it possible to study the structure and regulation of airway goblet cells to some extent.

The epithelial mucins are extremely hydrophobic and are associated with various macromolecules, the quality and quantity of which may also affect the physicochemical properties of the mucus. Secretion of epithelial mucins is stimulated by various factors, including a number of inflammatory agents. The recent progress in mucin molecular biological research will allow us to identify different mucin core proteins produced by those different cell types, and, hopefully, the differential functions of these mucins in health and disease.

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In the airways, mucus plays an important role in the defence of the lung against airborne particles. Particles are normally trapped in the luminal mucous layer and constantly removed from the airway by ciliary beating, a process called mucociliary clearance. Maintenance of normal mucociliary function depends on the viscoelastic property of mucus, which is determined mainly by the quality and quantity of mucous glycoproteins or mucins present in the mucus. Therefore, any abnormalities either in the quality or quantity of mucins may result in the development of pathological airways, which often lead to the death of patients, as seen in chronic bronchitis, asthma and cystic fibrosis.

Airway mucins are thought to be derived from periodic-acid-Schiff (PAS)-positive secretory granules ("mucous" granules) found in two different cell types in the airway: goblet cells of the surface epithelium; and mucous cells of the submucosal glands. Therefore, mucins present in the airway lumen are a mixture secreted from the two different cell types. Details of the anatomy of the airway are described in this review series by P.K. Jeffery. In this particular review, we will focus on the goblet cell mucin, and, more specifically, on our current understanding of its structure and secretion. For additional information, refer to other review articles [1–4].

Structure of airway goblet cell mucins

Since airway mucins are a mixture of mucins secreted from the two different cell types, it was practically impossible to purify goblet cell mucins from airway mucus. Therefore, the structure of airway goblet cell mucins was initially defined based mainly on cytochemistry in which the secretory granules are stained with various dyes, depending on the degree of acidity of the mucins [5, 6]. Such studies indicated that goblet cell mucins (or epithelial mucins) contain neutral, sialylated, and sulphated sugars, and that the distribution of these mucins varies greatly depending on animal species. Biochemical characterization of the epithelial mucin was made possible only after successful isolation and culturing of these cells. For details of the tracheal surface-epithelial (TSE) cell culture system, see the review by R. Wu in this series.

Characterization of epithelial cell mucins

Among the various species that have been reported, the hamster TSE cell culture system has been most extensively studied with respect to the biochemistry of epithelial mucins. Details of hamster TSE cell cultures

Previous articles in this series: No. 1: P.K. Jeffery, D. Li. Airway mucosa: secretory cells, mucus and mucin genes. *Eur Respir J* 1997; 10: 1655–1662. No. 2: W.D. Kim. Lung mucus: a clinician's view. *Eur Respir J* 1997; 10: 1914–1917. No. 3: L.D. Martin, L.G. Rochelle, B.M. Fischer, T.M. Krunkosky, K.B. Adler. Airway epithelium as an effector of inflammation: molecular regulation of secondary mediators. *Eur Respir J* 1997; 10: 2139–2146. No. 4: R. Wu, Y.H. Zhao, M.M.J. Chang. Growth and differentiation of conducting airway epithelial cells in culture. *Eur Respir J* 1997; 10: 2398–2403.

have been described previously [7–9]. It is important to mention that production of mucins from cultured TSE cells requires the growth of these cells on a thick matrix, such as collagen gel [10], as well as the presence of vitamin A in the culture medium [8]. TSE cells grown under these culture conditions constitutively secrete mucins at confluency, and the secreted mucins have the following physicochemical characteristics [8, 11]: 1) O-linked glycoproteins, *i.e.* the glycosidic linkage between N-acetylgalactosamine of the oligosaccharides and serine/threonine of the protein backbone; 2) sugars consisting of N-acetylgalactosamine, N-acetylglucosamine, galactose, fucose and sialic acids, but no mannose; 3) the presence of the poly(N-acetylglucosamine) moiety [12]; 4) extreme heterogeneity both in size and charge, the latter being due to the presence of sulphate and sialic acid; 5) resistance to proteoglycan-digesting enzymes; 6) a buoyant density of about 1.5 g·mL⁻¹ [13, 14]; and 7) relatively enriched in serine, threonine and proline [13, 14]. It is important, however, to note that most of the above characteristics are based on the carbohydrate structure, and that lack of structural information regarding the protein backbone has led to serious arguments concerning the identity of these mucins secreted by TSE cells. Mucins produced by these cultures used to be referred to as "high molecular weight mucin-like glycoproteins", and were often confused with one of the proteoglycans, especially type II keratan sulphate proteoglycan. Details of the structural differences between these glycoconjugates have been described previously [1, 3].

Hydrophobicity of epithelial mucins

There are several lines of evidence to suggest that mucins produced from TSE cells are extremely hydrophobic. Firstly, secreted mucins are associated with various kinds of lipids [15, 16]. Secondly, ultrastructural studies of mucins have shown localization not only inside secretory granules but also on the secretory cell surface, and a significant portion of the cellular mucins associated with cell membranes as external glycoproteins [9, 17]. Most (about 97%) of the lipids associated with mucins can be dissociated by a combination of heating and exposure to detergents [13], indicating that they are noncovalently bound. However, about 3% of the lipids require alcoholic potassium hydroxide (KOH) treatment for dissociation; this dissociated lipid has been identified as palmitic acid (unpublished data) suggesting the presence of covalent binding as shown previously in intestinal mucins [18]. Finally, secreted mucins are associated with "small" molecular weight glycoproteins *via* noncovalent hydrophobic interactions [15]. It appears that these molecules are associated with mucins prior to exocytosis, probably within the secretory granules [19]. Such a notion may be supported by the presence of endoperoxidases [20] and protease inhibitors [21] within airway goblet cell secretory granules. Why and how these nonmucin components are packaged together with mucins inside secretory granules is unknown.

Genes encoding epithelial mucins

Seven mucin genes have been identified so far [22, 23]. A major portion of each of the genes consists of

variable numbers of tandem repeats (VNTR) of a defined number of nucleotides. The VNTR are enriched with serine/threonine, which are sites for O-glycosylation of mucin molecules. Four of these mucin genes, namely *MUC1* [24, 25], *MUC2* [26, 27], *MUC4* [28, 29] and *MUC5* [30, 31], have been shown to be expressed in the lung. According to the Human Genome Mapping convention, the mucin gene loci should be designated with the letters MUC, followed by a number reflecting the order in which the genes were cloned. Among the four mucin genes, both *MUC2* and *MUC5* appear to be major candidates for secreted epithelial mucins, since their messenger ribonucleic acids (mRNAs) have been shown to be present in airway epithelial cells [31–35]. Interestingly, the level of *MUC2* expression was low in cultured primary TSE cells [33], and was transcriptionally down-regulated in the presence of vitamin A [32], which is known to cause mucous cell differentiation in cultured TSE cells [8]. In addition, the expression of *MUC2* was upregulated after treatment either of the cultured airway epithelial cells with tumour necrosis factor- α (TNF- α) [33], or the intact airway with products of *Pseudomonas aeruginosa* [34], which suggests that expression of the *MUC2* gene may be associated with airway inflammation or infection, and perhaps secretory cell metaplasia of the airway epithelium. On the other hand, expression of *MUC5* has been shown to be increased by the presence of vitamin A in cultured rat TSE cells [35]. Taken together, airway epithelial mucins are encoded by at least two MUC genes: *MUC5* responsible for mucins normally secreted; and *MUC2* for mucins produced during airway inflammation. However, no direct evidence is yet available. Finally, *MUC1* might be another candidate for the secreted epithelial mucins, since these cell surface mucins have been shown to be released by tumour cells [36].

Regulation of mucin release by goblet cells

The pharmacology of airway mucin secretion has been reviewed previously [1–4, 37–42]. In general, secretion of mucins from airway epithelial cells can be stimulated by three types of secretagogues: irritant gases; inflammatory agents; and others which do not belong to these groups.

Irritant gases

Chemical irritants are well-known for their stimulatory effects on airway goblet cell mucus. Mucous granules of airway goblet cells were released by tobacco smoke in intact rat [43] and guinea-pig [44] airways, and also by sulphur dioxide inhalation in intact canine airways [45]. Ammonia vapour stimulated mucin release from intact cat tracheas [46]. Inhaled irritant gases, such as sulphur dioxide, nitric oxide or ammonia, will be dissolved in airway luminal fluid, changing the pH of the fluid to acidic or alkaline. In cultured hamster TSE cells, a medium of pH <4 or >9 caused mucin release as a result of damage to the plasma membrane [47].

Inflammatory mediators

Airway inflammation is a complex event, which involves a cascade of reactions between inflammatory mediators

and cell types in the lung. For details, see the review by K. Adler in this series. Since airway inflammation is always accompanied by hypersecretion of mucus, any agent which causes inflammation will probably stimulate mucin release from the airway, either directly or indirectly. Availability of airway epithelial cell culture systems has made it possible to study direct effects of individual agents on mucin release at cellular and molecular levels. Use of primary airway epithelial cell culture systems for studying the pharmacology of goblet cell mucin release has been reviewed previously [48]. In this review, we will focus mainly on inflammatory agents which have been shown to stimulate mucin release by acting directly on the goblet cells.

Arachidonic acid metabolites. Prostaglandins E_2 and $F_{2\alpha}$ (PGE_2 and $PGF_{2\alpha}$), and leukotrienes C_4 and D_4 (LTC_4 and LTD_4) did not influence mucin release in cultured hamster TSE cells [47]. However, in cultured guinea-pig TSE cells, mucin release was stimulated by prostaglandin $F_{2\alpha}$ [49]. In intact guinea-pig airways, inhaled LTD_4 caused the release of mucous granules from goblet cells [50].

Platelet-activating factor (PAF). PAF has been shown to induce mucin release in rodent tracheal organ cultures as well as in human tracheal organ explants, seemingly by two different mechanisms: 1) an increased intracellular leukotriene production by mucin-secreting cells, which seem to be responsible for their own mucin release in rodent tracheal organ explants [51]; and 2) extracellular leukotrienes released from other cells by PAF, which in turn act on mucin-secreting cells in human tracheal organ explants [52]. PAF has also been shown to release mucin by stimulation of lipoxygenase metabolism of arachidonic acid to hydroxyeicosatetraenoic acids (HETEs) in guinea-pig TSE cells [53], and by activation of protein kinase C (PKC) in cultured canine TSE cells [54].

Tumour necrosis factor- α (TNF- α). TNF- α has been shown to stimulate mucin release from human airway epithelial cells [33], as well as from cultured guinea-pig tracheal epithelial cells [55], through activation of nitric oxide synthase [55].

Proteases. Proteases released from bacteria which are associated with obstructive pulmonary diseases have been shown to release mucins from cultured tracheal organ explants of rabbits [56], and from guinea-pig tracheal explants [57], via proteolytic damage on the apical cell membrane or an apocrine mechanism [56]. Human neutrophil elastase released mucins from hamster tracheal organ explants [58], and also in cultured hamster TSE cells [17], via proteolytic cleavage of mucins bound to the apical cell surface [17, 59]. Whether or not these cell surface mucins are encoded by the *MUC1* gene remains to be elucidated. Elastase from the porcine pancreas, however, had no such effects [17].

Reactive oxygen species. Superoxide, which is produced by activated neutrophils during airway inflammation, has been shown to release mucins from cultured guinea-pig TSE cells via increased $PGF_{2\alpha}$ production by the

TSE cells [49]. Neither hydrogen peroxide, a major product of superoxide, nor free radicals derived from hydrogen peroxide had any effect on mucin release in the same system. Although there has been no direct demonstration that epithelial mucin release is induced by nitric oxide, ADLER *et al.* [55] have reported that intracellular production of nitric oxide is necessary for the increased mucin release by some inflammatory agents such as histamine, PAF, TNF- α , and superoxide.

Nucleotides. Nucleotides are present in high concentrations inside cells (>5 mM adenosine triphosphate (ATP) in the cytosol) [60]. Therefore, it is likely that the inflamed airway also contains high concentrations of nucleotides, as a result of massive cell injuries. Some purine nucleotides, have recently been shown to stimulate mucin release from cultured hamster TSE cells via a P_2 purinoceptor-mediated mechanism [61–63]. ATP, a prototype agonist of the P_2 purinoceptor, released mucins by activation of phospholipase C (PLC), which is coupled to the receptor, at least in part, via pertussis toxin-sensitive G protein(s) [64]. A downstream pathway of ATP-induced activation of PLC seems to involve activation of PKC via diacylglycerol, but not the inositol 1,4,5-triphosphate (IP_3)- Ca^{2+} pathway [65]. KAI *et al.* [66] also showed that activation of PKC can induce mucin release from hamster TSE cells. In contrast, LARIVÉE *et al.* [54] failed to stimulate mucin release by activation of PKC in canine TSE cells. Finally, activation of PKC by ATP appears to activate phospholipase A_2 , which, in turn, causes mucin release [67]. It is important to note that this PLC-PKC pathway, however, accounts for only 50% of ATP-induced mucin release [65], which suggests the presence of another, as yet unknown, mechanism. Both the binding kinetics of ATP γS^{35} in cultured TSE cells [68], and the comparison of ATP and uridine triphosphate (UTP) in their mucin-releasing activity [69, 70], indicate that mucin release by nucleotides is mediated by the P_{2u} receptor.

Other secretagogues

Neuronal control. Airway epithelium is free of autonomic innervation. Therefore, it is unlikely that neurotransmitters released from these nerve terminals have any direct influence on airway goblet cells. In the isolated cat tracheal epithelial sheet, goblet cell mucin release was not stimulated either by adrenergic or cholinergic drugs [71]. Mucin release from cultured hamster TSE cells was also resistant to virtually all of the neurotransmitters tested [1]. However, in intact guinea-pigs, vagal stimulation of the airway caused the exocytosis of goblet cell granules indicating the presence of a neural control of goblet cell secretion of mucus [72]. In the same system, exocytosis of goblet cell granules was also induced either by capsaicin or substance P, probably through local axonal reflexes, in which capsaicin causes release of neuropeptides from sensory nerves and the released neuropeptides induce discharge of mucus [73]. Both of these pathways seem to be involved in cigarette smoke-induced airway goblet cell secretion in intact guinea-pigs [44]. Substance P, however, could not induce mucin release from cultured hamster TSE cells (unpublished data).

Mechanical strain. In cultured hamster TSE cells, hypo-osmolarity increased mucin release whilst hyperosmolarity decreased it [47]. On the other hand, contraction of the gel upon which TSE cells were cultured induced mucin release without causing cell damage [74]. Since both the change in osmolarity and the gel contraction can cause mechanical strain on secretory cells, the mechanical factor might be a cause of mucin release under the above experimental conditions. Such a situation might exist *in vivo*; in light of the fact that the airway epithelium is physically associated with the underlying smooth muscles, contractility of airway smooth muscles either tonically or under various conditions, including coughs or inflammation, probably causes mechanical strain on the goblet cells, which may result in an increase in mucin release. The former situation, namely, the basal contractility of airway smooth muscles, might be an important regulator of "physiological" secretion of airway goblet cell mucins *in vivo*.

Finally, it is important to note that there is, as yet, no accurate method to quantify mucins, due to the heterogeneous nature of their molecules. The information obtained using one type of assay may be totally contradictory to that obtained by another [3]. In other words, the heterogeneity of the mucin molecules seems to make it necessary for us to understand the function of individual mucins in detail, based both on the protein backbone and carbohydrate structure, and then to focus on the regulation of individual mucins in addition to mucins as a whole. This potentially crucial problem can be resolved only when the structure of epithelial mucins is clearly defined.

Perspectives

Availability of airway epithelial cell culture systems has allowed us to begin to study airway epithelial secretions at the cellular and molecular levels. However, it is important to fully understand the culture system before using it as an *in vitro* model, since these mixed cells grow and differentiate totally differently depending on the culture conditions, such as the matrix, the culture medium, and the polarity. Due to the limited amount of information, it may be premature to develop any molecular model of airway goblet cell mucin secretion at the present time. Nevertheless, there are a number of important questions which may be answered using these culture systems, preferentially the air-liquid biphasic culture system. These include: 1) Which mucin genes encode the secreted mucins, and how these mucins are regulated at the transcriptional and translational levels? 2) What are the conditions and mechanisms for constitutive and regulated (granule) secretions? 3) What substances are present in the secretory granules and what are their roles? 4) What is the role of mucins present on the cell surface? 5) Is the goblet cell membrane polarized in terms of its responsiveness to various modulators of secretion? and 6) Why are there two different types of mucous cells in the airway, which secrete the mucins into a common pool?

It is worth emphasizing that, despite the advantages that the cell culture system can provide in terms of stability and relative homogeneity of the cell population, it

has possible limitations, especially when one uses the cell culture as a model for studying certain functions *in vivo*. This seems to be particularly important in studying the regulation of mucin secretion by the airway goblet cells.

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