AIRWAY GOBLET CELL MUCIN. ITS STRUCTURE AND REGULATION OF SECRETION

K. Chul Kim and Kevin McCracken
Department of Pharmaceutical Sciences, University of Maryland School of Pharmacy, Baltimore, Maryland, U.S.A.

Chan Young Shin, Mijeong Jo, Choong Jae Lee, and Kwang Ho Ko
Department of Pharmacology, Seoul National University College of Pharmacy, Seoul, and Center for Biofunctional Molecules, POSTECH, Korea.

INTRODUCTION

Mucus in the airway plays an important role in the defense of the lung against airborne particles. Particles normally are trapped in the luminal mucus layer and constantly removed from the airway by ciliary beating, a process called mucociliary clearance. Maintenance of the 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 in either the quality or the quantity of mucins may result in the development of pathologic airways which often lead to deaths of patients, as seen chronic bronchitis, asthma and cystic fibrosis. Airway mucins are thought to be derived from PAS-positive secretory granules ("mucous" granules) found in two different cell types in the airway; goblet cells of surface epithelium and mucous cells of submucosal glands. Therefore, mucins present in the airway lumen are a mixture of mucins secreted from the two different cell types. Details of the anatomy of the airway are described in this review series by Dr. Peter Jeffery. In this particular section, we will focus on the goblet cell mucin, more specifically, 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 the 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 mucins (5,6). Such studies indicated that goblet cell mucins (or epithelial mucins) contain neutral, sialylated, and sulfated sugars, and that the distribution of these mucins vary 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 TSE cell culture system, see a review by Dr. Reen Wu in this series.

A. Characterization of epithelial cell mucins

Among various species that have been reported, the hamster tracheal surface epithelial (TSE) cell culture system has been most extensively studied for the biochemistry of epithelial mucins. Details of hamster TSE cell cultures were described in references #7-9. It is important to mention that production of mucins from cultured TSE cells requires growing 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): (a) O-linked glycoproteins, i.e., the glycosidic linkage between N-acetylgalactosamine of the oligosaccharides and serine/threonine of the protein backbone, (b) sugars consisting of N-acetylgalactosamine, N-acetylglucosamine, galactose, fucose and sialic acids, but no mannose, (c) the presence of poly(N-acetyllactosamine) moiety (12), (d)

the extreme heterogeneity in both size and charge, the latter being due to the presence of sulfate and sialic acid, (e) resistance to proteoglycan-digesting enzymes, (f) a buoyant density of about 1.5 g/ml (13,14), and (g) 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 led to serious arguments over 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 often confused with one of the proteoglycans, especially type II keratan sulfate proteoglycan. Details of the structural differences between these glycoconjugates are described elsewhere (1, 3).

B. Hydrophobicity of epithelial mucins

There are several lines of evidence that suggest that mucins produced from TSE cells are extremely hydrophobic. First, secreted mucins are associated with various kinds of lipids (15,16). Second, ultrastructural studies of mucins showed localization not only inside secretory granules but also on the secretory cell surface, and a significant portion of the cellular mucins was 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 detergents (13), indicating that they are non-covalently bound. However, about 3% of the lipids require alcoholic KOH treatment for dissociation; this dissociated lipid has been identified as palmitic acid (unpublished data) suggesting the presence of covalent binding as previously shown in intestinal mucins (18). Lastly, secreted mucins are associated with "small" molecular weight glycoproteins via non-covalent hydrophobic interactions (15). It appears that these molecules are associated with mucins prior to exocytosis, most likely 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 non-mucin components are packaged together with mucins inside secretory granules are unknown.

C. Genes encoding epithelial mucins

Seven mucin genes have been identified so far (see Ref #22,23 for review). 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. In the lung, 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. According to 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 mucins appear to be major candidates for secreted epithelial mucins since their 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 downregulated 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 of either cultured airway epithelial cells with TNF_{α} (33) or the intact airway with products of *Psudomonas aeruginosa* (34), which suggests that expression of MUC2 gene may be associated with airway inflammation or infection, 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 at least by two MUC genes - MUC5 responsible for mucins normally secreted and MUC2 for mucins produced during airway inflammation. No direct evidence, however, is yet available. Finally, MUC1 mucins might be another

candidate for the secreted epithelial mucins, since these cell surface mucins have been shown to be released by tumor cells (36).

REGULATION OF GOBLET CELL MUCIN RELEASE

Pharmacology of airway mucin secretion has been reviewed elsewhere (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 those which do not belong to the formers.

A. 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 sulfur dioxide inhalation in intact canine airways (45). Ammonia vapor stimulated mucin release from intact cat tracheas (46). Inhaled irritant gases such as sulfur dioxide, nitric oxide, or ammonia will be dissolved in airway luminal fluid changing the fluid pH to acidic or alkaline. In cultured hamster TSE cells, medium pH of either below 4 or above 9 caused mucin release as a result of plasma membrane damage (47).

B. Inflammatory mediators

Airway inflammation is a complex event which involves a cascade of reactions between various inflammatory mediators and various cell types in the lung. For details, see a review by Dr. Kenneth Adler in this series. Since airway inflammation is always accompanied with mucus hypersecretion, any agent which causes the inflammation will likely 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 was reviewed previously (48). In this section, we will focus mainly on inflammatory agents which have been shown to stimulate mucin release by acting directly on the goblet cells.

(a) arachidonic acid metabolites

Prostaglandins E_2 and $F_{2\alpha}$, and leukotrienes C_4 and D_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 leukotriene D_4 caused release of mucous granules from goblet cells (50).

(b) 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 by seemingly two different mechanisms: (a) an increased intracellular leukotriene production by mucin-secreting cells which seems responsible for their own mucin release in rodent tracheal organ explants (51), and (b) 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 HETEs in guinea pig TSE cells (53) and by activation of protein kinase C in cultured canine TSE cells (54).

(c) tumor necrosis factor alpha (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).

(d) 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 MUC1 gene remains to be elucidated. Elastase from the porcine pancreas, however, had no such effects (17).

(e) 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) has 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.

(f) nucleotides

Nucleotides are present in high concentrations inside cells (>5 mM 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 protein kinase C (PKC) via diacyglycerol, but not the IP_3-Ca^{++} pathway (65). Kai et al. (66) also showed that activation of PKC can induce mucin release from hamster TSE cells. In contrast, Larivee 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 for ATP-induced mucin release. Both binding kinetics of $ATP\gamma S^{35}$ in cultured TSE cells (68) and the comparison of ATP and UTP in their mucin releasing activity (69,70) indicate that mucin release by nucleotides is mediated by P_{2u} receptor.

C. Others

(a) neuronal control

Airway epithelium is free of autonomic innervation. Therefore, it is not likely that neurotransmitters released from these nerve terminals have any direct influence on airway goblet cells. In the isolated cat tracheal epithelium sheet, goblet cell mucin release was not stimulated by either adrenergic or cholinergic drugs (71). Mucin release from cultured

hamster TSE cells was also resistant to virtually all the neurotransmitters tested (1). However, in intact guinea pigs, vagal stimulation of the airway caused goblet cell granule exocytosis indicating the presence of neural control of goblet cell mucus secretion (72). In the same system, goblet cell granule exocytosis was also induced by either capsaicin or substance P, most likely through local axonal reflexes in which capsaicin causes release of neuropeptides from sensory nerves and the released neuropeptides induce mucus discharge (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).

(b) mechanical strain

Hypo-osmolarity increased mucin release while hyper-osmolarity decreased it in cultured hamster TSE cells (47). On the other hand, contraction of the gel upon which TSE cells were cultured induced mucin release without causing cell damage (73). Since both the osmolarity change 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 situation might exist *in vivo*; in light of the fact that airway epithelium is physically associated with underlying smooth muscles, contractility of airway smooth muscles either tonically or under various conditions including coughs or inflammation likely causes mechanical strain on 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 for "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 the mucins due to the heterogeneous nature of the molecules. The information obtained using one type of assay may be totally contradictory to that obtained by another type of assay (see Ref #3 for review). In other words, the heterogeneity of the mucin molecules seems to make it necessary for us to understand in detail the function of individual mucins - based on both the protein backbone and carbohydrate structure - and then to focus on the regulation of individual mucins in addition to the total mucins. 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 this moment. Nevertheless, there are a number of important questions which may be answered using one of these culture systems, preferentially the air-liquid biphasic culture system. These include: (1) which MUC genes encode the secreted mucins and how are these mucins regulated at the transcriptional and translational levels? (2) What are the conditions and mechanisms for the constitutive and the 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 secretion modulators? (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 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 especially important in studying the regulation of airway goblet cell mucin secretion.

ACKNOWLEDGMENTS

The preparation of this manuscript was supported by a grant from National Institute of Health, RO1-HL47125.

REFERENCES

1. Kim KC (1991) Biochemistry and pharmacology of mucin-like glycoproteins produced by cultured airway epithelial cells. *Exp Lung Res* 17:533-545.
2. Kim KC (1993) Cellular and biochemical mechanisms of airway epithelial mucin release. In *Signal Transduction in Lung Cells*. JS Brody, V Tkachuk and D Center (eds). Lung Biology of Health and Disease, Marcel Dekker Inc., New York, pp. 309-321.
3. Kim KC (1994) Regulation of airway goblet cell mucin secretion. In *Airway Secretion: Physiological Bases for the Control of Mucus Hypersecretion*. T Takishima and S Shimura (eds). Lung Biology of Health and Disease, Marcel Dekker Inc., New York, pp. 433-449.
4. Rogers DF (1994) Airway goblet cells: responsiveness and adaptable front-line defenders. *Eur Respir J* 7:1690-1706.
5. Spicer SS, Mochizuki I, Setser ME, Martinez JR (1980) Complex carbohydrates of rat tracheobronchial surface epithelium visualized ultrastructurally. *Am J Anat* 158:93-109.
6. Plopper CG, St. George JA, Nishio SJ, Etchison JR, Nettesheim P (1984) Carbohydrate cytochemistry of tracheobronchial airway epithelium of the rabbit. *J Histochem Cytochem* 32;209-218.
7. Lee TC, Wu R, Brody AR, Barrett JC, Nettesheim P (1984) Growth and differentiation of hamster tracheal epithelial cells in culture. *Exp Lung Res* 6:27-45.
8. Wu R, Nolan E, Turner C (1985) Expression of tracheal differentiation functions in serum-free hormone-supplemented medium. *J Cell Physiol* 125:167-181
9. Wasano K, Kim KC, Niles RM, Brody JS (1988) Membrane differentiation markers of airway epithelial secretory cells. *J Histochem Cytochem* 36:167-178.
10. Kim KC (1985) Possible requirement of collagen gel substratum for production of mucin-like glycoproteins by primary rabbit tracheal epithelial cells in culture. *In Vitro* 21:617-621.
11. Kim KC, Rearick JI, Nettesheim P, Jetten AM (1985) Biochemical characterization of mucous glycoproteins synthesized and secreted by hamster tracheal epithelial cells in primary culture. *J Biol Chem* 260:4021-4027.
12. Rearick JI, Kim KC, Nettesheim P, Jetten AM (1984) Hamster tracheal mucin secreted in vitro contains poly-N-acetllyactosamine oligosaccharides. *Fed Proc* 43:1696.
13. Kim KC (1991) Mucin-like glycoproteins secreted from cultured hamster tracheal surface epithelial cells: Their hydrophobic nature and amino acid composition. *Exp Lung Res* 17:65-76.
14. Wu R, Plopper CG, Cheng PW (1991) Mucin-like glycoproteins secreted by cultured hamster tracheal epithelial cells. Biological and immunological characterization. *Biochem J* 277:713-718.
15. Kim KC, Opaskar-hincman H, Bhaskar KR (1989) Secretions from primary hamster tracheal surface epithelial cells in culture: Mucin-like glycoproteins, proteoglycans, and lipids. *Exp Lung Res* 15:299-314.
16. Kim KC, Singh BN (1990) Hydrophobicity of mucin-like glycoproteins secreted bycultured tracheal epithelial cells: Association with lipids. *Exp Lung Res* 16:279-292.

17. Kim KC, Wasano K, Niles RM, Schuster JE, Stone PJ, Brody JS (1987) Human neutrophil elastase releases cell surface mucins from primary cultures of hamster tracheal epithelial cells. *Proc Natl Acad Sci USA 84*:9304-9308.
18. Slomiany A, Witas H, Aono M, Slomiany BL(1983) Covalently linked fatty acids in gastric mucus glycoprotein of cystic fibrosis patients. *J Biol Chem* 258: 8535-8538.
19. Kim KC, Singh BN (1990) Association of lipids with mucins may take place prior to secretion: Studies with primary tracheal epithelial cells in culture. *Biorheology* 27:491-501.
20. Christensen TG, Hayes JA (1982) Endoperoxidase in the conducting airways of hamsters: Morphological evidence of synthesis and secretion. *Am Rev Respir Dis* 125:341-346.
21. Water RD, Willems LNA, Van Muijen GNP, franken C, Fransen JAM, Dijkman JH, Kramps JA (1986) Ultrastructural localization of bronchial antileukoprotease in central and peripheral human airways by a gold-labeling technique using monoclonal antibodies. *Am Rev Respir Dis* 133:882-890.
22. Gum JR (1992) Mucin genes and the proteins they encode : structure, diversity and regulation. *Am J Respir Cell Mol Biol* 7:557-564.
23. Gendler SJ, Spicer AP (1995) Epithelial mucin genes. *Annu Rev Physiol* 57:607-634.
24. Hollingsworth MA, Batra SK, Qi WN, Yankaskas JR (1992) MUC1 mucin mRNA expression in cultured human nasal and bronchial epithelial cells. *Am J Respir Cell Mol Biol* 6:516-520.
25. Park H, Hyun SW, Kim KC (1996) Expression of MUC1 mucin gene by hamster tracheal surface epithelial cells in primary culture. *Am J Respir Cell Mol Biol* (in press)
26. Gerard C, Eddy RL, Shows TB (1990) The core polypeptide of cystic fibrosis tracheal mucin contains a tandem repeat structure. Evidence for a common mucin in airway and gastrointestinal tissue. *J Clin Invest* 86:1921-1927.
27. Jany BH, Gallup MW, Yan P, Gum JR, Kim YS, Basbaum CB (1991) Human bronchus and intestine express the same mucin gene. *J Clin Invest* 87:77-82.
28. Porchet N, Van Cong N, Dufosse J, Audie JP, Guyonnet-Duperat V, Gross MS, Denis C, Degand P, Bernheim A, Aubert JP (1991) Molecular cloning and chromosomal localization of a novel human tracheo-bronchial mucin cDNA containing tandemly repeated sequences of 48 base pairs. *Biochem Biophys Res Commun* 175:414-422.
29 Aubert JP, Porchet N, Crepin M, Duterque-Coquillaud MD, Vergnes G, Mazzuca M, Debuire B, Petitprez D, Degand P (1991) Evidence for different human tracheobronchial mucin peptides deduced from nucleotide cDNA sequences. *Am J Respir Cell Mol Biol* 5:178-185.
30. Meerzaman D, Charles P, Daskal E, Polymeropoulos MH, Martin BM, Rose MC (1994) Cloning and analysis of cDNA encoding a major airway glycoprotein, human tracheobronchial mucin (MUC5). *J Biol Chem* 269:12932-12939.
31. Audie JP, Janin A, Porchet N, Copin MC, Gosselin B, Aubert JP (1993) Expression of human mucin genes in respiratory, digestive, and reproductive tracts ascertained by in situ hybridization. *J Histochem Cytochem* 41:1479-1485.
32. An G, Luo G, Wu R (1994) Expression of MUC2 gene is down-regulated by vitamin A at the transcriptional level in vitro in tracheobronchial epithelial cells. *Am J Respir Cell Mol Biol* 10:546-551.
33. Levine SJ, Larivee P, Logun C, Angus CW, Ognibene FP, Shelhamer JH (1995) Tumor necrosis factor - alpha induces mucin hypersecretion and MUC-2 gene expression by human airway epithelial cells. *Am J Respir Cell Mol Biol* 12:196-204.
34. Dohrman A, Young C, Gallup M, Ohmori H, Massion P, Nadel J, Escudier E, Chapelin C, Coste A, Basbaum C (1995) MUC2 mucin gene expression is upregulated in human airways by products of *Pseudomonas aeruginosa*. *Am J Respir Crit Care Med* 151:A160.
35. Guzman K, Bader TH, Hill EM, Nettesheim P (1995) Regulation of mucin gene expression by retinoic acid in rat tracheal epithelial cells correlates with mucous secretory differentiation. *Am J Respir Crit Care Med* 151:A337.

36. Boshell M, Lalani EN, Pemberton L, Burchell J, Gendler S, Taylor-Papadimitriou J (1992) The product of the human MUC1 gene when secreted by mouse cells transfected with the full-length cDNA lacks the cytoplasmic tail. *Biochem Biophys Res Commun* 185:1-8.
37. Widdicombe JG (1978) Control of secretion of tracheobronchial mucus. *Br Med Bull* 34:57-61.
38. Reid L, Bhaskar K, Coles S (1983) Control and modulation of airway epithelial cells and their secretion. *Exp Lung Res* 4:157-170.
39. Spicer SS, Martinez JR (1984) Mucin biosynthesis and secretion in the respiratory tract. *Environ Health Perspect* 55:193-204.
40. Nadel JA (1985) Regulation of airway secretions. *Chest* 87(suppl): 11S-113S. 1985.
41. Adler KB (1986) Mucin secretion by explants of respiratory tissue in vitro. *In In vitro models of Respiratory Epithelium.* LJ Schiff (ed). CRC Press, Boca Raton, Florida, pp.27-50.
42. Marin MG (1986) Pharmacology of airway secretion. *Pharmacol Rev* 38:273-289.
43. Jones R, Boldue P, Reid L (1973) Goblet cell glycoprotein and tracheal gland hypertrophy in rat airway: the effect of tobacco smoke with or without the anti-inflammatory agent, phenylmethyloxidiazole. *Br J Exp Pathol* 54:229-239.
44. Kuo HP, Rohde JAL, Barnes PJ, Rogers DF. Cigarette smoke-induced airway goblet cell secretion: dose-dependent differential nerve activation. *Am J Physiol* 1992; 263: L161-167.
45. Spicer SS, Chakrin LW, Wardell JR, Jr. (1974) Effect of chronic sulfur dioxide inhalation on the carbohydrate histochemistry and histology of the canine respiratory tract. *Am Rev Respir Dis* 110:13-24.
46. Gallagher JT, Hall RL, Phipps RJ, Jeffrey PK, Kent PW, Richardson PS (1986) Mucus-glycoproteins (mucins) of the cat trachea: characterisation and control of secretion. *Biochim Biophys Acta* 886:243-254.
47. Kim KC, Nassiri J, Brody JS (1989) Mechanism of airway goblet cell mucin release: Studies with cultured tracheal surface epithelial cells. *Am J Respir Cell Mol Biol* 1:137-143.
48. Kim KC, Brody JS (1989) Use of primary cell culture to study regulation of airway surface epithelial mucus secreion. In *Mucus and Related Topics.* EN Chantler and NA Ratcliffe (eds). Cambridge, U.K., Company of Biologists Limited, pp. 231-239.
49. Adler KB, Holden-Stauffer WJ, Repine JD (1990) Oxygen metabolites stimulate release of high molecular-weight glycoconjugates by cell and organ cultures of rodent respiratory epithelium via an arachidonic acid-dependent mechanism. *J Clin Invest* 85:75-85.
50. Houdret N, Perini JM, Galabert C, Scharfman A, Humbert P, Lamblin G, Roussel P (1986) The high lipid content of respiratory mucins in cystic fibrosis is related to infection. *Biochim. Biophys. Acta* 880:54-61.
51. Adler KB, Schwarz JE, Anderson WH, Welton AF (1987) Platelet activating factor stimulates secretion of mucin by explants of rodent airways in organ culture. *Exp Lung Res* 13:25-43.
52. Goswami SK, Lhashi M, Stathas P, Marom ZM (1989) Platelet-activating factor stimulates secretion of respiratory glycoconjugate from human airways in culture. *J Allergy Clin Immunol* 84:726-734.
53. Adler KB, Akley NJ, Glasgow WC (1992) Platelet-activating-factor provokes release of mucin-like glycoproteins from guinea pig respiratory epithelial cells via a lipoxygenase-dependent mechanism. *Am J Respir Cell Mol Biol* 6:550-556.
54. Larivee P, Levine SJ, Martinez A, Wu T, Logun C, Shelhamer JH (1994) Platelet activating factor induces airway mucin release via activation of protein kinase C: Evidence for translocation of protein kinase C to membranes. *Am J Respir Cell Mol Biol* 11:199-205.

55. Adler KB, Fisher BM, Li H, Choe NH, Wright DT (1995) Hypersecretion of mucin in response to inflammatory mediators by guinea pig tracheal epithelial cells in vitro is blocked by inhibition of nitric oxide synthase. *Am J Respir Cell Mol Biol* 13:526-530.
56. Klinger JD, Tandler B, Liedke CM, Boat TF (1984) Proteases of Pseudomonas aeruginosa evoke mucin release by tracheal epithelium. *J Clin Invest* 74:1669-1678.
57. Adler KM, Hendley DD, Davis GS (1986) Bacteria associated with obstructive pulmonary disease elaborate extracellular products that stimulate mucin secretion by explants of guinea pig airways. *Am J Pathol* 125:501-514.
58. Niles RM, Christensen TG, Breuer R, Stone PJ, Snider GL (1986) Serine proteases stimulate mucous glycoprotein release from hamster tracheal ring organ culture. *J Lab Clin Med* 108:489-497.
59. Breuer R, Christensen TG, Niles RM, Stone PJ, Snider GL (1989). Human neutrophil elastase causes glycoconjugate release from the epithelial cell surface of hamster trachea in organ culture. *Am Rev Respir Dis* 139:779-782.
60. Gordon JL (1986) Extracellular ATP: effects, sources and fate. *Biochem J* 233:309-319.
61. Kim KC, Lee BC (1991) P_2 purinoceptor regulation of mucin release by airway goblet cells in primary culture. *Br J Pharmacol* 103:1053-1056.
62. Kim KC, Wilson AK, Lee BC (1992) Nucleotides and mucin release from cultured airway epithelial cells. *Chest* 101:68S-69S.
63. Davis CW, Dowell ML, Lethem M, Van Scott M (1992) Goblet cell degranulation in isolated canine tracheal epithelium: response to exogenous ATP, ADP, and adenosine. *Am J Physiol* 262 (*Cell Physiol* 6): C1313-C1323.
64. Kim KC, Zheng QX, Van Seuningen I (1993) Involvement of a signal transduction mechanism in ATP-induced mucin release from cultured airway goblet cells. *Am J Resp Cell Mol Biol* 8:121-125.65.
65. Kim KC, McCracken K, Young T (1995) Mechanism of ATP-induced mucin release from airway goblet cells: presence of another pathway separate from the PLC-PKC pathway. *Am J Resp Crit Care Med* 151:A337.
66. Kai H, Yoshitake K, Isohama Y, Hamamura I, Takahama K, Miyata T (1994) Involvement of protein kinase C in mucus secretion by hamster tracheal epithelial cells in culture. *Am J Physiol* 267:L526-L530.
67. Jo M, Ko KH, Kim KC (1995) Possible involvement of phospholipase a in ATP-induced mucin release from airway goblet cells. Mol Biol Cell 6:222a, 1995.
68. Kim KC, Zheng QX, Wilson AK, Lee BC, Berman JS (1994) Binding kinetics of $ATP\gamma S^{35}$ on cultured primary tracheal surface epithelial cell. *Am J Resp Cell Mol Biol* 10:154-159.
69. Lethem MI, Dowell ML, Van Scott M, Yankaskas JR, Egan R, Boucher TC, and Davis CW. -- Nucleotide regulation of goblet cells in human airway epithelial explants: Normal exocytosis in cystic fibrosis. *Am. J. Resp. Cell Mol. Biol.* 1993;
70. Kim KC, Park HR, Shin CY, Akiyama T, Ko KH (1996) Nucleotide-induced mucin release from primary hamster tracheal surface epithelial cells involves the P_{2u} purinoceptor. *Eur Resp J* 9:542-548.
71. Sherman JM, Cheng PW, Tandler B, Boat TF (1981) Mucous glycoproteins from cat tracheal goblet cells and mucous glands separated with EDTA. *Am Rev Respir Dis* 124:476-479.
72. Tokuyama K, Kuo HP, Rohde JA, Barnes PJ, Rogers DF (1990). Neural control of goblet cell secretion in guinea pig airways. *Am J Physiol* 259:L108-L115.
73. Kuo HP, Rohde JAL, Tokuyama K, Barnes PJ, Rogers DF (1990) Capsaicin and sensory neuropeptide stimulation of goblet cell secretion in guinea-pig trachea. *J Physiol* 431:629-641.
74. Kim KC, Lee BC, Brody JS (1993) Effect of floating a gel matrix on mucin release in cultured airway epithelial cells. *J Cell Physiol* 156:480-486.