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Mucin gene expression in rat airways following infection and irritation

Berthold Jany†, Marianne Gallup§, Tohru Tsuda§, and Carol Basbaum §,*

†Medizin Poliklinik der Universität, Klinikstrasse 6-8, D-8700 Würzburg, Germany.
§Department of Anatomy and Cardiovascular, Research Institute, University of California, San Francisco, San Francisco, California 94143

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Summary: Airway mucus hypersecretion occurs in response to infection and irritation and poses an important and poorly understood clinical problem. In order to gain insight into its pathogenesis, we have focused on an mRNA encoding the major mucus glycoprotein, mucin. Northern blots showed that mucin mRNA was abundant in the intestine of specific pathogen free rats whereas it was undetectable in the airways of these rats until pathogen-free conditions were suspended and rats acquired Sendai (Parainfluenza I) virus infections. Airway mucin hybridization signals in rats that were both infected with Sendai virus and exposed to SO2 were more intense than those in rats with infection alone. These results suggest that pathogen-and irritant-induced hypersecretion may be partly controlled at the level of mucin mRNA. © 1991 Academic Press, Inc.

Mucus hypersecretion is a common response of the pulmonary airways to infection or injury and is a hallmark of cystic fibrosis, chronic bronchitis, and asthma [1]. In these conditions, excessive mucus accumulates in the air passages, compromising airflow to the lung, causing respiratory insufficiency, and sometimes death [2], [3], [4]. The pathophysiology of hypersecretion is likely to involve abnormalities at control points regulating the production of the major mucus glycoprotein, mucin. These abnormalities could occur at the level of transcription, processing, or stability of RNA transcripts, as well as translation, processing, or stability of the protein. Some of the most common mechanisms modulating protein biosynthesis act by regulating RNA steady state, either by altering the transcription or degradation rate of specific mRNAs [5]. To determine whether mucin mRNA steady state varies under conditions of infection and irritation, we monitored airway mucin mRNA steady state by Northern blot in specific pathogen-free (SPF) rats, rats acquiring respiratory tract infections, and rats exposed to the irritant SO2. The results clearly showed that airway mucin mRNA steady state increases in the presence of infection and irritation. A preliminary report has appeared [6].

Materials and Methods
Animal model: Experiments approved by the UCSF Animal Care Committee were performed on specific pathogen-free male, 12 week old Sprague-Dawley rats. Food and water were

*To whom correspondence should be addressed at Department of Anatomy & CVRI, Box 0130, HSE 1331, 513 Parnassus, San Francisco, CA 94143.
accessible ad libitum. SO2-exposed rats were exposed in plexiglass chambers to 400 ppm sulfur dioxide gas in air for 3 h/d, 5 d/week, for 1 to 3 wk [7]. SO2-concentrations were measured in the chamber outflow by a colorimetric assay [8]. Sham-exposed control rats were placed in an exposure chamber into which air was administered in place of SO2. Non-exposed time control rats were sacrificed immediately after arrival from the vendor or after living for three weeks in the animal colony.

Rats were assigned to experimental and control groups for RNA analysis as follows: unexposed 0 time=5 rats (Fig. 3, Group D); unexposed 3 weeks=4 rats (Fig. 3, Group B); SO2 exposed 1 week=2 rats (Fig. 3, Group C); SO2 exposed 2 weeks=2 rats (Fig. 3, Group C); SO2 exposed 3 weeks=3 rats (Fig. 3, Group C; sham exposed 3 weeks=2 rats (Fig. 3, Group A). In addition, rats used for electron microscope analysis were as follows: unexposed 0 time=2 rats (Fig 1, upper panel); unexposed 3 weeks=4 rats; SO2 exposed 1 week =3 rats; SO2 exposed 2 weeks=4 rats; SO2 exposed 3 weeks=5 rats (Fig. 1, lower panel).

Electron microscopy: Electron microscopy was performed on tissues from rats that had been fixed by intracardiac perfusion with 2.5% glutaraldehyde in 0.08 M Na cacodylate buffer, pH 7.4. Osmication was in 2% OsO4 in 0.14 M Na veronal and block staining was in uranyl acetate in 0.2 M Na maleate buffer, pH 5.2. Tissue was dehydrated in graded ethanol, passed through propylene oxide, and embedded in Medcast resin. Silver sections were stained with uranyl acetate and lead citrate and photographed in a Zeiss EM 10 electron microscope.

Serum antibody titer determination: Serum was taken from rats upon sacrifice and was assayed by ELISA (Enzyme Linked Immunosorbent Assay) for antibodies against M. pulmonis, Corona and Sendai (Parainfluenza 1) viruses, PVM, KRV, Toolan H-1, and CAR Bacillus by Microbiological Associates, Inc. (Bethesda, MD).

Northern blotting and hybridization: For RNA analysis, the tracheobronchial tree, as well as the intestine and heart, were removed from anesthetized rats. RNA was extracted as previously described [9]. Ten micrograms of total RNA per lane were electrophoresed through denaturing agarose/formaldehyde gels, transferred to GeneScreen membranes, and hybridized to the [32P]-labeled human intestinal mucin cDNA, SMUC 41 [10]. Washing conditions were 0.2 x SSC (2 x SSC = 0.3 M sodium chloride, 0.03 M sodium citrate), 1% SDS, 63°C, 30'. After washing, blots were exposed to X-OMAT film (Kodak). Blots were stripped and re-probed with a [32P]-labeled beta-actin cDNA.

Results

As shown in Figure 1, the airways of rats exposed to SO2 for three weeks contained numerous mucous (goblet) cells. These were similar to those seen in human chronic bronchitis [2]. Secretions adhering to the apical surfaces of airway epithelial cells were also present in SO2-exposed, but not SPF control rats (Fig. 1).

In order to monitor mucin mRNA levels in the airways of experimental and control rats, it was necessary to identify a mucin cDNA that hybridized with rat mucin mRNA. At the time we began our studies, mucin cDNAs had been isolated from cDNA libraries constructed from the human intestine [10], pig submaxillary [11], and human mammary [12, 13, 14] glands. The similarity between the amino acid compositions of intestinal and airway mucins [15, 16, 17, 18] led us to test the feasibility of using an intestinal cDNA for our studies.

Our initial studies revealed a high degree of homology between mucin mRNAs in the intestine and the airway of man [19]. Using the intestinal mucin cDNA SMUC 41 as a probe, we isolated HAM-1, a close homologue of SMUC 41 containing threonine- and proline-rich tandem repeats, from a human airway cDNA library [19]. Although we initially expected the human airway mucin cDNA to be more favorable for probing rat airways than the human intestinal mucin cDNA, the larger size of SMUC-41 made it more favorable.
Fig. 1. Electron micrographs of rat small bronchus. Upper panel: Pathogen-free rat airways contain no mucous cells or visible secretion. Lower panel: After exposure to SO$_2$ (6), mucous cells filled with electron-lucent secretory granules appear and secretion is visible in the lumen. M = mucous cell, S = serous cell, C = ciliated cell, CT = connective tissue. Magnification 350 x.

Using SMUC-41 in Northern blots, we probed RNA from two rat mucin-secreting organs (intestine and airways), and used RNA from a non-mucin secreting organ (heart) as a negative control. In Northern blots, the intestinal (Fig. 2) and airway (Fig. 3) signals were polydisperse, a distinguishing feature of mucin mRNA [10] [19] [20] corresponding to polydispersity in the size of nascent [21] and deglycosylated [18] mucin peptides. The integrity of the RNA was confirmed by reprobing blots with a beta actin cDNA (Figs. 2 and 3, lower panel). RNA from the heart and airways of specific pathogen-free rats was negative (Figure 2, and D lanes, Fig 3). The airway
Fig. 2. RNA from pathogen-free rat small intestine, airways and heart hybridized with the mucin cDNA SMUC 41. RNA was extracted as described (6). &approx; 10 μg total RNA per lane was electrophoresed through a denaturing agarose/formaldehyde gel, transferred to GeneScreen membrane and hybridized to 32P-labeled SMUC 41. Washing conditions were 0.2 x SSC (2 x SSC = 0.3 M sodium chloride, 0.03 M sodium citrate), 1% SDS, 63°C, 30'. Positions of ribosomal RNAs are indicated as size markers. Blots were stripped and re-probed with a beta-actin cDNA.

negativity is consistent with studies showing the paucity of mucous cells and mucin glycoprotein in the airways of specific pathogen-free rats (Fig 1, control, [22], [23]) as well as in healthy dogs [24], [25] and humans [26].

The failure of SMUC-41 to hybridize to RNA from SPF rat airways was conspicuously reversed when rats were housed in the Animal Colony under non-pathogen-free conditions and exposed for 1-3 weeks to SO2 (Fig. 3, C lanes) or air alone (Fig. 3, A lanes). Serum from each rat was tested (see Table) for the presence of antibodies directed against PVM, Sendai virus, Corona virus, KRV, Toolan H-1, M. pulmonis, and CAR Bacillus. We found that all rats with significant antibody titers for Sendai virus [27], [28], [29] (Fig. 3) showed mucin hybridization signals. SPF rats (Fig. 3, D lanes and the bronchus lane in Figure 2) as well as those infected by another pathogen (i.e. Corona virus, Fig. 3, B lanes) did not. Mucin hybridization signals obtained from airway RNA of Sendai virus-infected rats that were exposed to SO2 for 1 wk showed signals more intense than those from rats with infection alone (Fig. 3, C lanes).

Discussion
We have shown that a human intestinal mucin cDNA, SMUC-41, is homologous to mucin mRNA in the intestine and airways of the rat. This homology enabled us to use SMUC-41 to monitor rat mucin mRNA and to discover that a SMUC mucin gene analogue is constitutively
expressed in the intestine of SPF rats, but requires activation by infection or injury in order to be expressed in the airways.

As in human tissues [10] [19], the rat intestine and airway yielded large, polydisperse signals when hybridized with SMUC 41. The polydispersity could not be explained by overall RNA degradation since reprobing with a beta actin probe consistently produced crisp hybridization bands (Fig 3). Polydispersity in the size of deglycosylated mucin polypeptides [18] suggests that polydisperse transcripts are functional. Such transcripts could arise through alternative splicing [30], although partial degradation of message during RNA turnover [31] cannot be excluded.

The constitutive expression of mucin mRNA in the rat intestine (Fig 2) is not surprising in light of the necessity in that organ for continuous lubrication. On the other hand, mucin and mucin mRNA may be only minimally produced in airways except in specific response to insult or injury. Mucin appears to be present in low abundance in the airways of healthy dogs [25] and humans [26] but is present in high abundance in the airways of chronic bronchitics [26] or dogs exposed to tobacco smoke [25]. Our results are consistent with these data as well as with data showing that mucous cells are rare in the airways of SPF rats [23], [22] but increase when airways become injured [7], [32] or infected [22]. The particularly high abundance of mucin mRNA observed after one week SO2 exposure is intriguing; the diminution at two and three weeks occurs while mucous (goblet) cell numbers remain elevated. This may indicate that during the early stages of injury, goblet cells rapidly discharge and resynthesize mucin, but in the later stages, tolerance occurs and cells are relatively quiescent.
The results of our study suggest that one of the molecular events responsible for converting a low-mucin-secreting to a high-mucin-secreting epithelium is the induction of mucin mRNA. The increased mucin mRNA steady state does not seem to occur secondary to mucous cell mitosis because mucous cells (containing electron lucent secretory granules) do not incorporate $[3^\text{H}]$-thymidine during the period of mucin mRNA induction [33]. Further, mucous cells have not been observed to incorporate $[3^\text{H}]$ thymidine during the period of rapid mucous cell increase following cigarette smoke exposure [34]. We suggest that the increased numbers of mucous cells in injured airways arise through environmental stimulation of mucin gene transcription, presumably an early event in mucous cell differentiation.

Our studies revealed that the airways of SPF rats contained undetectable mucin mRNA, as determined by lack of hybridization with SMUC-41. Under the housing conditions used in our studies, rats spontaneously became infected with Sendai virus, Corona virus, or CAR bacillus. Those infected by Sendai virus were the only rats to show mucin hybridization signals, and all rats with significant antibody titers against Sendai virus showed conspicuous signals (Figure 3 and Table 1). Although correlation does not prove causality, these data strongly suggest that Sendai virus is responsible for the marked induction seen in our experiments. Although we cannot rule out potential roles of other pathogens, the rarity of rat pathogens other than those screened for makes it unlikely that an undetected pathogen accounted for the induction.

Although Sendai virus-infected rats showed conspicuous hybridization signals, they were much less intense than those seen in two animals simultaneously infected with Sendai virus and exposed for 1 wk to the irritant SO$_2$ (Fig 3, C lanes, 1 week exposure). The relative intensities of the signals suggest that mucin mRNA induction by Sendai virus may be potentiated by SO$_2$.

### TABLE 1

|             | A          | B          | C          | D          |
|-------------|------------|------------|------------|------------|
| Sendai v.   | 18         | 20         | 0.02       | 0.01       |
| (Parainfl.v.) |           |            | 0.09       | >20        |
|             | *          | *          | *          | *          |
| Corona v.   | 0.02       | 0.04       | >20        | 1.74       |
|             | *          | *          | *          | *          |
|             | 0.01       | 0.01       | 0.06       | 0.01       |
|             | 0.01       | 0.01       | 0.01       | 0.01       |
| Mycoplasma  | --         | --         | 0.2        | --         |
| (M.Pulm.)   |            |            | --         |            |
| PVM         | 0.01       | 0.01       | 0          | 0          |
| H-1         | --         | --         | 0          | 0          |
| CAR         | 0.05       | 0.03       | 0          | 0          |
|             | *          | *          | 0.16       | 0.08       |
|             | 0.21       | 0.27       | 0.07       | 0          |
| KRV         | --         | --         | 0          | 0          |
|             |            |            |            |            |

Groups A, B, C, D correspond to rats described in legend Fig.3. Individual values correspond to experimental animals as in Fig.3. Values are ELISA absorbance units. Significant pathological antibody titers (*) correlate to values >0.17 absorbance units. 0 = Not determined; (--) = Not detectable.
Highly prevalent in rodent colonies [27], Sendai virus is known to induce a variety of structural and functional alterations in the respiratory tract [27]. Based on our results, these may include mucin mRNA induction. Although the specific mechanisms by which pathogens (and possibly irritants) induce mucin mRNA are unknown, such mechanisms would likely act to increase the transcription rate or decrease the degradation rate of mucin mRNA. Viruses may activate the expression of multiple gene products through induction of interferons [35]. Further, irritation can introduce macrophage-, neutrophil-, and lymphocyte-derived mediators to the environment [2] [36] [37]. These could potentially stimulate epithelial cells to synthesize or activate transcription factors [31] or could influence the processing of specific mRNAs to affect their stability [30].

Although infection and irritation have long been known to initiate mucus hypersecretion in animals and humans [38], the underlying mechanisms have remained obscure. In demonstrating that pathogens and irritants are capable of affecting mucus secretion at the level of mucin mRNA, our results provide one of the first clues to the pathogenesis of hypersecretion.

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References

[1] A.T.S.; Chronic bronchitis, asthma, and pulmonary emphysema. Am. Rev. Respir. Dis. 1962; 85:762-768
[2] Reid LM.; Pathology of chronic bronchitis. Lancet 1954; 1:275-278
[3] Aikawa T, Shiruma S, Sasaki H, Takishima T, Yaegashi H, Takahashi T.; Morphometric analysis of intraluminal mucus in airways in chronic obstructive pulmonary disease. Am. Rev. Respir. Dis. 1989; 140:477-482
[4] Hogg JC, Macklem PT, Thurlbeck WM.; Site and nature of airway obstruction in chronic obstructive lung disease. New England Journal of Medicine 1968; 278:1355-1360
[5] Darnell JE.; Variety in the level of gene control in eukaryotic cells. Nature 1982; 297:365-371
[6] Jany B, Gallup M, Basbaum C.; Parainfluenza I virus: a possible trigger for mucin gene expression in rat airways. Am. Rev. Respir. Dis. 1991; 143:A135
[7] Lamb D, Reid L.; Mitotic rate, goblet cell increase and histochemical changes in mucus in rat bronchial epithelium during exposure to sulphur dioxide. Journal of Pathology & Bacteria 1968; 96:97-111
[8] Occupational exposure to sulfuric acid. U.S. Department of Health, Education and Welfare, Publication # (NIOSH) 1974; 74-128
[9] Chomczynski P, Sachi N.; Single step methods of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. 1987; 162:156-159
[10] Gum JR, Byrd JC, Hicks JW, Toribara NW, Lampert DTA, Kim YS.; Molecular cloning of human intestinal mucin cDNAs. J. Biol. Chem. 1989; 264:6480-6487
[11] Timpte CS, Eckhardt AE, Abernethy JL, Hill RL.; Porcine submaxillary gland apomucin contains tandemly repeated identical sequences of 81 residues. J. Biol. Chem. 1988; 263:1081-1088
[12] Gendler SJ, Burchell JM, Duhig T, White R, Parker M.; Cloning of partial cDNA encoding differentiation and tumour-associated mucin glycoproteins expressed by human epithelium. Proc. Natl. Acad. Sci. USA 1987; 84:6060-6064
[13] Siddiqui I, Abe M, Hayes D, Shani E, Yunis E, Kufe D.; Isolation and sequencing of a cDNA coding for the human DF3 breast carcinoma-associated antigen. Proc. Natl. Acad. Sci. USA 1988; 85:2320-2323
[14] Gendler S, Taylor Papadimitriou J, Duhig T, Rothbard J, Burchell J.; A highly immunogenic region of a human polymorphic epithelial mucin expressed by carcinomas is made up of tandem repeats. J. Biol. Chem. 1988; 263:12820-12823

[15] Neutra MR, Forster JF. Gastrointestinal mucus: Synthesis, secretion, and function. New York: Editor, Raven Press, 1987

[16] Woodward H, Horsey B, Bhavanandan P, Davidson EA.; Isolation, purification, and properties of respiratory mucus glycoproteins. Biochem. 1982; 21:694-701

[17] Chace KV, Flux M, Sachdev GP.; Comparison of physicochemical properties of purified mucus glycoproteins isolated from respiratory secretions of cystic fibrosis and asthmatic patients. Biochem. 1985; 24:7334-7341

[18] Perini JM, Marianne T, Lafitte J-J, Lamblin G, Rousset P, Mazzuca M.; Use of an antiserum against deglycosylated human mucins for cellular localization of their peptide precursors: Antigenic similarities between bronchial and intestinal mucins. J. Histochem. Cytochem. 1989; 37:869-875

[19] Jany B, Gallup M, Yan PS, Gum J, Kim Y, Basbaum C.; Human intestine and airways express the same mucin gene. J. Clin. Invest. 1991; 87:77-82

[20] Gum JR, Hicks JW, Swallow DM, Lagace RL, Byrd JC, Lamport DTA, Siddiki B, Kim YS.; Molecular cloning of cDNAs derived from a novel human intestinal mucin gene. Biochem. & Biophy. Res. Comm. 1990; 171:407-415

[21] Marianne T, Perini J-M, Lafitte J-J, Houdret N, Pruvot F-R, Lamblin G, Slayter HS, Rousset P.; Peptides of human bronchial mucus glycoproteins. Biochem. J. 1987; 248:189-195

[22] Huang HT, Haskell A, McDonald DM.; Changes in epithelial secretory cells and potentiation of neurogenic inflammation in the trachea of rats with respiratory tract infections. Anat. Embryol. 1989; 180:325-341

[23] Plopper CG, Mariassy AT, Wilson DW, Alley JL, Nishio SJ, Netteheim P.; Comparison of nonciliated tracheal epithelial cells in six mammalian species: Ultrastructure and population densities. Exp. Lung Res. 1983; 5

[24] Koshino T, Bhaskar R, Reid LM, Gerard C, Warner A, Shore SA, Anderson K, Butler G, Iijima H, Drazen MM.; Recovery of an epitope recognized by a monoclonal antibody from airway lavage during experimental induction of chronic bronchitis. American Journal of Respiratory Cell & Molecular Biology 1990; 2:453-462

[25] Bhaskar KR, Drazen JM, O'Sullivan DD, Scanlon PM, Reid LM.; Transition from normal to hypersecretory bronchial mucus in a canine model of bronchitis: Chances in yield and composition. Exp. Lung Res. 1988; 14:101-120

[26] Bhaskar KR, O'Sullivan DD, Seltzer J, Rossing TH, Drazen JM, Reid LM.; Density gradient study of bronchial mucus aspirates from healthy volunteers (smokers and nonsmokers) and from patients with tracheostomy. Exp. Lung Res. 1985; 9:289-308

[27] Castileman WL.; Respiratory tract lesions in weanling outbred rats infected with Sendai virus. Am. J. Vet. Res. 1983; 44:1024-1031

[28] Castileman WL.; Alterations in pulmonary ultrastructure and morphometric parameters induced by para-influenza (Sendai) virus in rats during postnatal growth. Am. J. Pathol. 1984; 114:322-335

[29] Giddens WE, Hoosier GL, Garlinghouse LE.; Experimental Sendai virus infections in laboratory rats II. Pathology and immunohistochemistry. Lab. Animal Sci. 1987; 37:442-448

[30] Smith CWJ, Patton JG, Nadal-Ginard B.; Alternative splicing in the control of gene expression. Annu. Rev. Genet. 1989; 23:527-577

[31] Ross J.; Messenger RNA turnover in eukaryotic cells. Mol. Biol. Med. 1988; 5:1-14

[32] Lamb D, Reid L.; Goblet cells increase in rat bronchial epithelium after exposure to cigarette and cigar smoke. Brit. Med. J. 1969; 1:33-35

[33] Royce FA, Basbaum CB.; Analysis of thymidine incorporation by airway epithelial cells in a rat model of chronic bronchitis. Am. Rev. Respir. Dis. 1990; 141:A107

[34] Ayers MM, Jeffery PK. Cell division and differentiation in bronchial epithelium. In: Cellular Biology of the Lung, New York: G. Bonsignore and G. Cummings, Plenum Press, 1982;

[35] Shan B, Lewis JA.; Interferon-induced expression of different genes is mediated by distinct regulatory pathways. 1989; 170:277-281

[36] Harkema JR, Hotchkiss JA, Harmsen AG, Henderson RF.; Am. J. Pathol. 1988; 130:605

[37] Christensen TG, Korth N, Snider GL, Hayes JA.; Irreversible bronchial goblet cell metaplasia in hamsters with elastase-induced panacinar emphysema. J. Clin. Invest. 1977; 59:397-404

[38] Basbaum C, Jany B.; Plasticity in the airway epithelium. Am. J. Physiol. 1990; 259:L38-L46