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Mucin gene expression in rat airways following infection and irritation

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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 SO_2 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.

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 SO_2. 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

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accessible ad libitum. SO_2-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]. SO_2-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 SO_2. 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); SO_2 exposed 1 week=2 rats (Fig. 3, Group C); SO_2 exposed 2 weeks=2 rats (Fig. 3, Group C); SO_2 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; SO_2 exposed 1 week =3 rats; SO_2 exposed 2 weeks=4 rats; SO_2 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% OsO_4 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 ethanols, 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 [^{32}P]-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 [^{32}P]-labeled beta-actin cDNA.

Results

As shown in Figure 1, the airways of rats exposed to SO_2 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 SO_2-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.

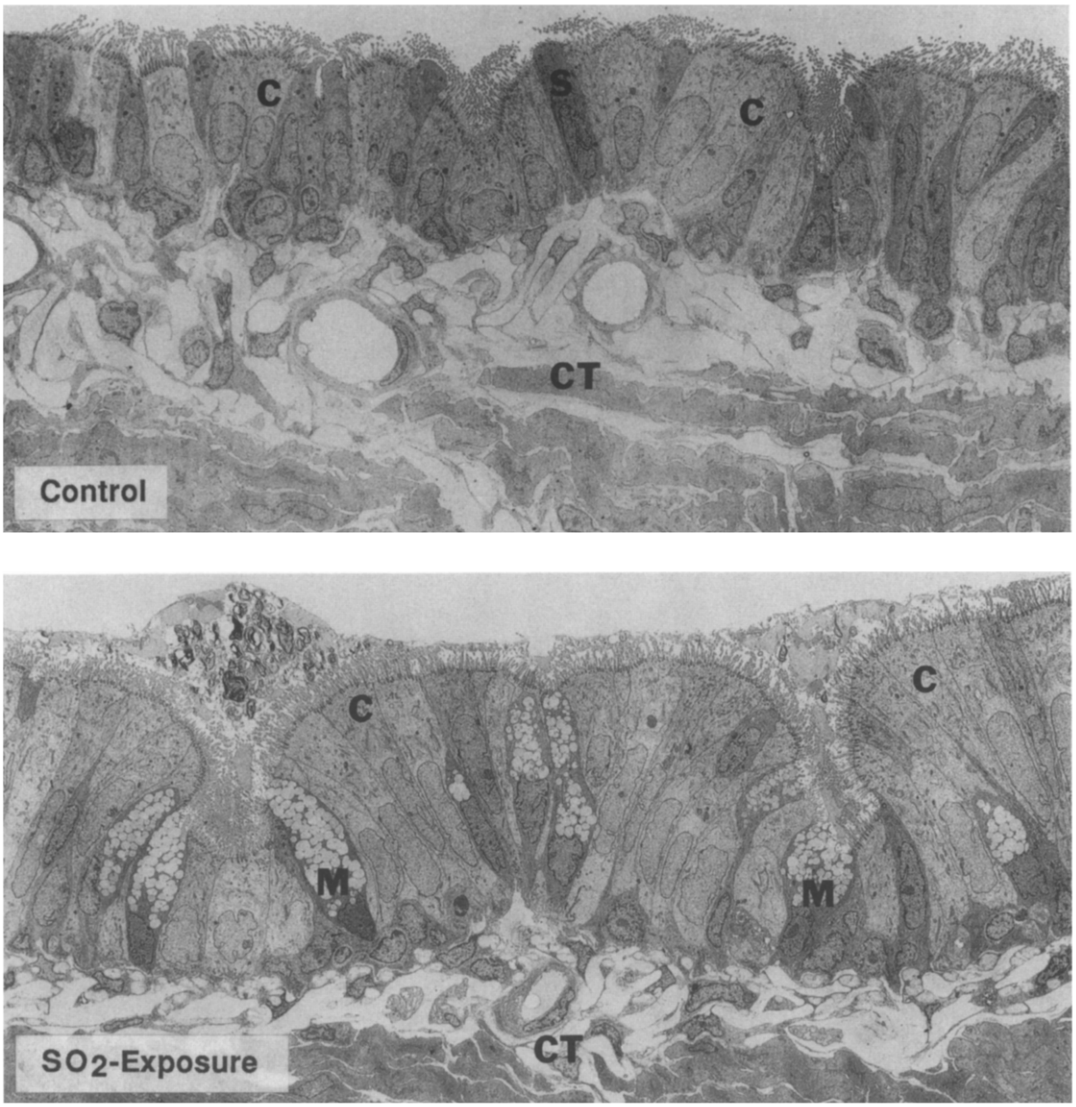

Fig. 1. Electron micrographs of rat small bronchus. Upper panel: Pathogen-free rat airways contain no mucous cells or visible secretion. Lower panel: After exposure to SO_2 (6), mucous cells filled with electron-lucent secretory granules appear and secretion is visible in the lumen. M = mucous cell, 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

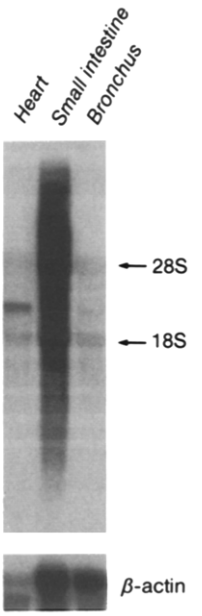

Fig. 2. RNA from pathogen-free rat small intestine, airways and heart hybridized with the mucin cDNA SMUC 41. RNA was extracted as described (6). ≈ 10 µg total RNA per lane was electrophoresed through a denaturing agarose/formaldehyde gel, transferred to GeneScreen membrane and hybridized to 32P-labeled 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 SO_2 (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 SO_2 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

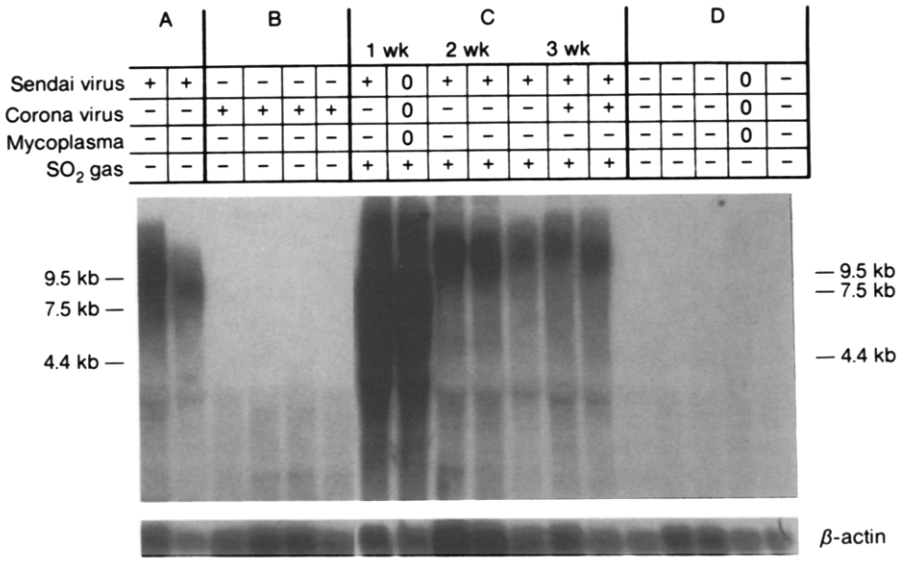

Fig. 3. Rat airway RNA hybridized with mucin cDNA SMUC 41. (A)Rats sham-exposed to air, housed in laboratory 3 wk; (B) rats housed in animal colony 3 wk; (C) rats exposed to SO_2, 1, 2, or 3 wk, housed in laboratory, (D) rats sacrificed immediately upon arrival from vendor (D). Each lane represents an individual animal (6, 17). "+" = significant antibody titer or SO_2-exposure; "—" = no significant antibody titer or SO_2-exposure; "O" = antibody titer not determined. For a complete list of pathogens assayed, see Table 1. ≈ 10 µg total RNA was loaded per lane. Blots were hybridized to [32P]-labeled SMUC 41 as described above, exposed to X-OMAT film (Kodak), stripped, and reprobed with a beta-actin cDNA.

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 SO_2 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 reponsible 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}H]-thymidine during the period of mucin mRNA induction [33]. Further, mucous cells have not been observed to incorporate [^{3}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

SERUM ANTIBODY DETERMINATIONS

	A		B				C							D				
Sendai v. (Parainfl.v.)	18 *	20 *	0.02	0.01	—	0.09	>20 *	0	>20 *	>20 *	>20 *	>20 *	>20 *	0.06	0.04	—	0	0.03
Corona v.	0.02	0.04	>20 *	1.74 *	>20 *	1.4 *	0.01	0	0.06	0.01	—	0.73 *	1.50 *	—	—	0.07	0	0.01
Mycoplasma (M.Pulm.)	—	—	—	—	0.2	—	—	0	—	—	—	—	—	—	—	—	0	—
PVM	0.01	0.01	0	0	0	0	0.01	0	—	—	—	—	—	0	0	0	0	0
H-1	—	—	0	0	0	0	—	0	—	—	—	—	—	0	0	0	0	0
CAR	0.05	0.03	0	0	0	0	0.1	0	0.16	0.08	0.21 *	0.27 *	0.07 *	0	0	0	0	0
KRV	—	—	0	0	0	0	—	0	—	—	—	—	—	0	0	0	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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