

# Up-regulation of *MUC5AC* and *MUC5B* Mucin Genes in Chronic Rhinosinusitis

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**Background:** Excess mucus production and hypersecretion characterize upper airway diseases. The primary mechanisms leading to mucus hypersecretion in chronic rhinosinusitis inflammation are not well understood. Mucus hypersecretion is commonly accompanied by goblet cell and submucosal gland cell hyperplasia. It is important to identify which mucin gene messenger RNAs (mRNAs) are expressed in the sinus mucosa.

**Objectives:** To investigate the expression of *MUC5AC* and *MUC5B* mRNAs and localization of these proteins in human sinus mucosa and to compare the expression of *MUC5AC* and *MUC5B* mRNAs in normal and in chronic sinus mucosa.

**Design:** Twenty chronic maxillary sinusitis mucosa samples and 20 normal maxillary sinus mucosa samples were obtained; RNAs were extracted from sinus mucosa, and semiquantitative reverse transcription-polymerase chain reaction was performed for *MUC5AC*

and *MUC5B*. Localization of these proteins was sought by using immunohistochemical analysis.

**Results:** The levels of *MUC5AC* and *MUC5B* mRNA in chronic rhinosinusitis were significantly increased compared with those in normal sinus mucosa ( $P=.02$ ). In inflamed sinus mucosa, *MUC5AC* protein was expressed in the cytoplasm of the goblet cell in the surface epithelium, and *MUC5B* expression was restricted to mucous cells of the submucosal glands and to the epithelium of sinus mucosa. However, in the normal sinus mucosa, *MUC5AC* and *MUC5B* proteins were expressed at low levels in the sinus epithelium and submucosal glands, respectively.

**Conclusion:** These results suggest that up-regulation of *MUC5AC* and *MUC5B*, which are major components of respiratory secretion in chronic rhinosinusitis, may play important roles in the pathogenesis of sinus hypersecretion in chronic rhinosinusitis.

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**M**UCINS HAVE BEEN POSTULATED to be important molecules in maintaining epithelial homeostasis in inflammatory diseases. The mucous glycoproteins (mucins) are the major component responsible for the gellike properties of mucus secretion. Cellular production of mucin is controlled by a number of mucin genes. To date, 18 distinct mucin genes have been identified (*MUC1-MUC4*, *MUC5AC*, *MUC5B*, *MUC6-MUC13*, and *MUC15-MUC18*). At least 8 different *MUC* genes are expressed in the respiratory epithelium of humans (*MUC1*, *MUC2*, *MUC4*, *MUC5AC*, *MUC5B*, *MUC7*, *MUC8*, and *MUC13*).<sup>1</sup>

The mucins are produced primarily by 2 different airway cell types in the respiratory tract: goblet cells and glandular cells. *MUC1* is expressed to serous cells of the submucosal glands and to apical

membranes of the surface epithelium. *MUC4* is localized to both surface and glandular cells. While *MUC2* and *MUC5AC* are located inside cells of surface epithelium of the respiratory tract and are exclusively expressed in mucus-secreting goblet cells, *MUC5B* and *MUC8* are expressed to mucous cells of the submucosal glands in the lower airways. *MUC7* is restricted to submucosal serous cells. *MUC5AC* and *MUC5B* are now accepted as the major constituents of airway mucus.<sup>2-4</sup>

Airway mucociliary clearance depends on the properties and volume of the mucus, on ciliary function, and on mucociliary interaction.<sup>5</sup> However, clinically it is unclear whether the extra mucus seen in rhinosinusitis is due to overproduction of mucus or failure of the cilia to move normally produced mucus. Respiratory mucus overproduction with an up-regulation of mucin gene expression is one

of the major manifestations of chronic airway diseases such as allergic rhinitis, asthma, and cystic fibrosis.<sup>6,7</sup>

Chronic rhinosinusitis has a feature of mucus hypersecretion of the upper respiratory tract. Currently, contrary to the data on mucin gene and protein expression in the lower airway disease, little is known about the regulation of mucins in chronic rhinosinusitis. We postulated that mucus hypersecretion of the upper airway in chronic rhinosinusitis is commonly caused by the up-regulation of mucin expression as is mucus overproduction in the lower respiratory tract. Because mucus hypersecretion is commonly accompanied by goblet cell hyperplasia and submucosal gland hyperplasia, it is important to identify which mucin gene messenger RNAs (mRNAs) and mucin proteins are expressed in the sinus mucosa in the human airway. Herein, we investigate the expression of *MUC5AC* and *MUC5B* mRNA and compare our findings with the localization of *MUC5AC* and *MUC5B* in sinus mucosa of human chronic rhinosinusitis.

## METHODS

### SUBJECTS

We defined chronic rhinosinusitis in our patients as an inflammation of the nasal and sinus mucosa with a persistent mucoid or mucopurulent nasal discharge for longer than 3 months that resisted repeated antimicrobial therapy and antral irrigation. Diagnosis was made on the basis of clinical history, rhinoscopic findings, and computed tomographic scan of paranasal sinuses. Chronic sinusitis was confirmed by computed tomographic examination that showed diffuse mucosal thickening in both the ethmoid and maxillary sinuses bilaterally with scores higher than 12 by the Lund-Mackay staging system.<sup>8</sup>

Twenty subjects (10 men and 10 women, aged 28 to 40 years) undergoing surgery for chronic rhinosinusitis and 20 healthy controls (10 men and 10 women, aged 35 to 50 years) were recruited for this study. Inflamed mucosal tissue samples of 1 maxillary sinus were removed during functional endoscopic sinus surgery through the extended middle meatal antrostomy. As controls, nasal maxillary sinus mucosa samples were obtained from 20 patients with blowout fracture who were undergoing endoscopic endonasal reconstruction of the orbital walls. Patients with nasal polyposis or immunodeficiency and individuals who had undergone prior sinus surgery were excluded from the study. No patients had received any antibiotics, steroids, antihistamines, or mucokinetic agents in the previous month. The study protocols was approved by the Review Board for Human Studies of the Korea University College of Medicine, and informed written consent was obtained from the subjects or their surrogates as required by the institutional review board.

### SAMPLES OF SINUS MUCOSA

Tissue samples were cut into 2 portions. One portion was immediately flash frozen in liquid nitrogen and stored at  $-70^{\circ}\text{C}$  for subsequent RNA studies. Another portion was fixed with 4% paraformaldehyde in 0.1M phosphate-buffered saline (pH, 7.4) and stored overnight at  $4^{\circ}\text{C}$  and then embedded in paraffin for immunohistochemical staining.

### REVERSE TRANSCRIPTION-POLYMERASE CHAIN REACTION

Total RNAs from sinus mucosa specimens were prepared using TRIzol (Gibco BRL, Gaithersburg, Md) according to the

manufacturer's suggestion. Total RNA from each sample was reverse-transcribed in 20 mL of reaction mixture containing 2.5 U of Moloney murine leukemia virus reverse transcriptase (RT) (Gibco BRL) and 50 pM of random hexanucleotides at  $42^{\circ}\text{C}$  for 60 minutes. Based on the published sequences, oligonucleotides for polymerase chain reaction (PCR) were synthesized commercially at Bioneer Company (Daejeon, South Korea).

For *MUC5AC*, the forward primer is 5'-TGA TCA TCC AGC AGC AGG GCT-3', and the reverse primer is 5' CCG AGC TCA GAG GAC ATA TGGG-3' (nucleotide positions 2897-2917 and 3284-3305, respectively; accession No. AJ001402). For *MUC5B*, the forward primer is 5'-CTG CGA GAC CGA GGT CAA CATC-3', and the reverse primer is 5'-TGG GCA GCA GGA GCA CGG AG-3' (nucleotide positions 9057-9078 and 10108-10127, respectively; accession No. Y09788). The primers 5'-GTG GAT ATT GTT GCC ATC AAT GAC C-3' and 5'-GCC CCA GCC TTC ATG GTG GT-3' for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were run simultaneously as an internal control.

The PCR product was analyzed by gel electrophoresis. *MUC5AC*, *MUC5B*, and *GAPDH* PCR product sizes are 409, 415, and 271 base pairs (bp), respectively. Amplification of the complementary DNA was carried out using 35 cycles at  $94^{\circ}\text{C}$  for 45 seconds,  $55^{\circ}\text{C}$  for 30 seconds, and  $72^{\circ}\text{C}$  for 1 minute followed by a final extension cycle of  $72^{\circ}\text{C}$  for 7 minutes. As negative controls, *MUC5AC* and *MUC5B* primers or RTs were omitted from some RT-PCR reactions.

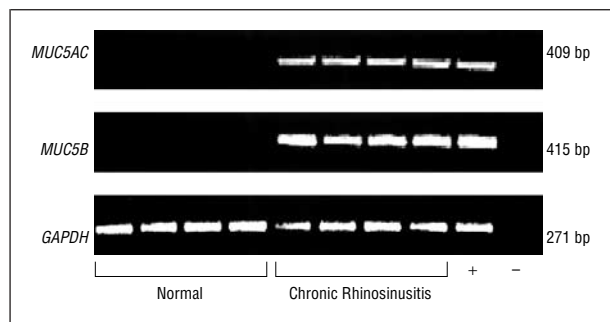
The PCR products obtained from sinus samples were cut out from the agarose gel, subcloned with the TA Cloning Kit (Invitrogen, Carlsbad, Calif), and sequenced. To analyze semiquantitatively the result of RT-PCR, we scanned the gel images and measured the intensity of the PCR product through use of NIH Image software (National Institutes of Health, Bethesda, Md). We determined the relative intensity of individual bands on a gel image as the ratio of the intensity of *MUC5AC* and *MUC5B* to the intensity of *GAPDH*. The negatives were scanned with a Molecular Dynamics Densitometer (Sunnyvale, Calif), and the signal was analyzed using ImageQuant software (Amersham Biosciences, Sunnyvale, Calif).

### IMMUNOHISTOCHEMICAL STAINING

Immunohistochemical analysis was performed by the avidin-biotin-peroxidase method. Paraffin-embedded 6- $\mu\text{m}$  sections were deparaffinized through 100% xylene and rehydrated. For immunostainings of *MUC5AC* and *MUC5B*, we used monoclonal antihuman *MUC5AC* antibody (Neo Markers, Union City, Calif) and monoclonal *MUC5B* antibody (Biotechnology Inc, Santa Cruz, Calif). Distribution of peroxidase was revealed by incubating the sections in a solution containing 3,3'-diaminobenzidine tetrahydrochloride (Sigma, St Louis, Mo). For the negative control, nonimmune serum IgG or a phosphate-buffered saline, instead of primary antibody, was used. The slides were counterstained with hematoxylin and mounted. The immunoreactivity within the different cells was scored for immunoreactivity as strongly positive (+++), moderately positive (++), weakly positive (+), or negative (0).

### STATISTICAL ANALYSIS

The Mann-Whitney *U* test was used to test for differences in each of *MUC5AC/GAPDH* and *MUC5B/GAPDH* ratios between the 2 groups. A probability of less than .05 was accepted as a statistically significant difference.



**Figure 1.** Expression of *MUC5AC* and *MUC5B* in human sinus mucosa by reverse transcription–polymerase chain reaction (RT-PCR). Ethidium bromide–stained agarose gel shows the presence of 409–base pair (bp) and 415–bp RT-PCR product using specific primer for *MUC5AC* and *MUC5B*. Shown are examples of RT-PCR products of *MUC5AC*, *MUC5B*, and *GAPDH* in 2% agarose gel with ethidium bromide stain. The plus sign indicates positive control; minus sign, negative control.

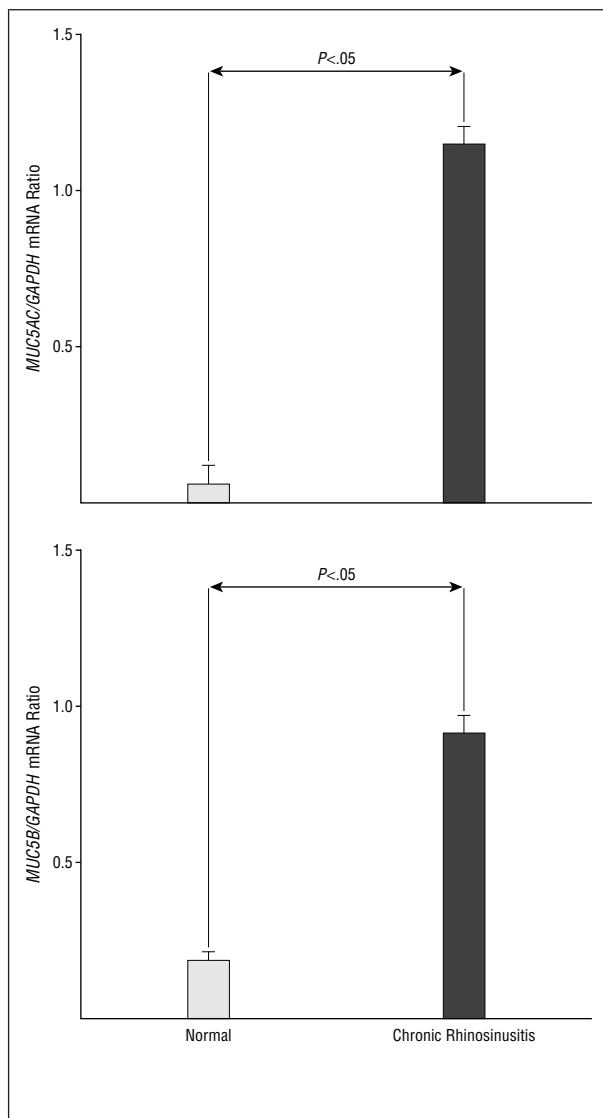
## RESULTS

### RT-PCR OF *MUC5AC* AND *MUC5B*

The RT-PCR examination showed that the sinus mucosa specimens contained mRNA encoding for *MUC5AC* and *MUC5B*. On RT-PCR screening, *MUC5AC* and *MUC5B* mRNAs were amplified weakly in normal sinus mucosa (**Figure 1**). However, the inflamed sinus mucosa showed strong expression of *MUC5AC* and *MUC5B* mRNA. *MUC5AC*/*GAPDH* and *MUC5B*/*GAPDH* mRNA ratios in chronic rhinosinusitis were significantly increased compared with those in normal sinus mucosa ( $P<.05$ ) (**Figure 2**). The PCR products extracted from the chronic rhinosinusitis mucosa were 409 bp for *MUC5AC*/*GAPDH* and 415 bp for *MUC5B*/*GAPDH*, which was expected, given the selected primers. There was no significant difference in the amount of *MUC5AC* and *MUC5B* expression between the 20 chronic sinusitis tissue samples examined. For the negative control RT-PCR, *MUC5AC* and *MUC5B* mRNA were not expressed.

### IMMUNOHISTOCHEMICAL LOCALIZATION OF *MUC5AC* AND *MUC5B* PROTEINS

The tissue distribution of *MUC5AC* and *MUC5B* proteins was analyzed by immunohistochemical staining. Positively immunostained cells appeared brown. In sections of chronic rhinosinusitis tissues, staining for *MUC5AC* immunoreactivity was abundant in goblet cells of the surface epithelium, while there was no staining of submucosal glandular cells (**Table; Figure 3**). There was scanty *MUC5AC* immunoreactivity in normal sinus mucosa. Immunohistochemical analysis for *MUC5B* in sections of sinus mucosa showed abundant signaling in chronic rhinosinusitis (Table). Next to staining of epithelial goblet cells, submucosal gland exhibited *MUC5B* immunoreactivity (**Figure 4**). Within the glandular structures, *MUC5B* immunoreactivity was restricted to mucous cells. No specific localization occurred with negative controls, which confirmed the specificity of the *MUC5AC* and *MUC5B* antibodies.



**Figure 2.** Comparison of the *MUC5AC*/*GAPDH* and *MUC5B*/*GAPDH* messenger RNA (mRNA) ratios between the tissue samples of normal sinus mucosa and chronic rhinosinusitis. Expression was significantly increased in the chronic rhinosinusitis mucosa compared with that in the normal sinus mucosa ( $P<.05$ ).

## COMMENT

Chronic inflammatory upper airway diseases such as allergic rhinitis and chronic rhinosinusitis have the common pathologic findings of secretory cell hyperplasia and mucus hypersecretion. Causes of these pathogenic events in chronic rhinosinusitis are not well understood. One possible explanation for this cascade of events is metaplasia or hyperplasia of the 2 mucin-producing types of cells, goblet cells and glandular mucous cells, by increased or altered expression of mucin genes. Thus, an important issue to be resolved is the level of expression of mucin genes and proteins in normal and chronic rhinosinusitis mucosae.

The present study shows that normal healthy sinus mucosa reveals very low expression of *MUC5AC* mRNA and *MUC5B* mRNA, whereas strong expression of *MUC5AC* mRNA and *MUC5B* mRNA takes place in si-

nus mucosa with chronic rhinosinusitis. In addition, we found that MUC5AC protein is exclusively expressed in the mucus-secreting goblet cells in the sinus epithelium, and the expression of MUC5B protein is restricted mainly to mucous cells of the submucosal glands and weakly to the goblet cells of sinus mucosa. In immunohistochemical analysis, more intense staining for MUC5AC and MUC5B proteins was displayed in the chronic sinusitis specimens than in the healthy controls. This study demonstrates that MUC5AC and MUC5B proteins are expressed in sinus mucosa, indicating increased number of MUC5AC- and MUC5B-positive staining cells due to goblet cell hyperplasia and metaplasia. These results suggest that MUC5AC and MUC5B are ex-

pressed in sinus mucosa by different mucus-producing cell types, including goblet cells and mucous cells of submucosal gland, which confirms a recent report that MUC5AC is absent in nasal glandular cells.<sup>9</sup>

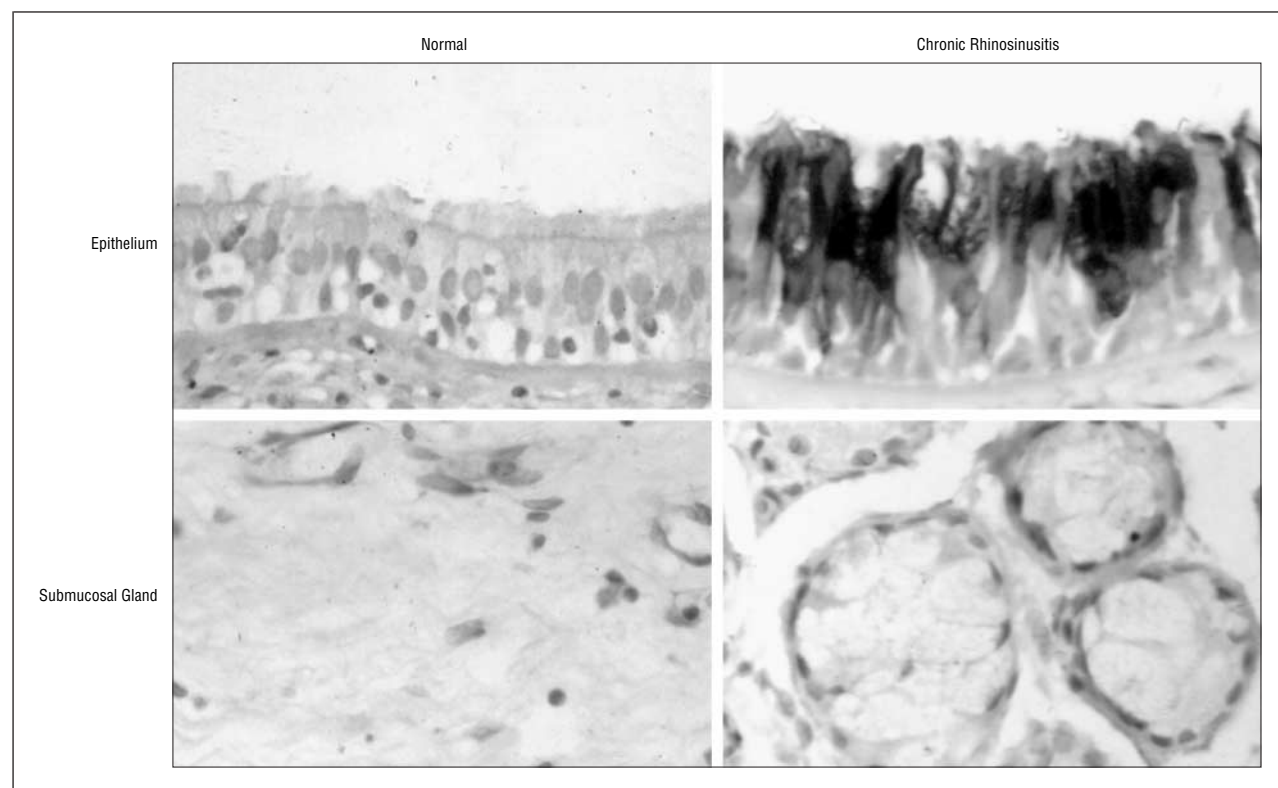
Although our current understanding of the relationships between other airway mucins and chronic rhinosinusitis is not well documented, we may infer 2 conclusions from our present results. First, altered post-translational modification may be the result of differences in the profiles of *MUC5AC* and *MUC5B* genes expressed in chronic rhinosinusitis in that different mucin protein backbones can be glycosylated differently in the same tissues. Second, various inflammatory mediators and bacterial products may regulate specific *MUC5AC* and *MUC5B* gene expression.<sup>10,11</sup> Other mucins such as *MUC2*, *MUC7*, and *MUC8*, which belong to the secretory mucin protein group, may also contribute to the secretion of mucus from the sinus mucosa with chronic rhinosinusitis. Kim et al<sup>13</sup> reported that only a portion of the goblet cells in the human nasal mucosa expressed *MUC5AC* mRNA, suggesting that surface goblet cells might have other mucin genes in addition to *MUC2* and *MUC5AC*.<sup>12</sup>

We investigated the expression of *MUC5AC* and *MUC5B* because these 2 mucins are the major gel-forming components of respiratory mucus in normal respiratory tract secretion. To date the other mucins, except mucins found in secretions (*MUC2*, *MUC5AC*, *MUC5B*, *MUC7*, and *MUC8*), are membrane-bound, incompletely characterized, or non-gel-forming mucins.<sup>13</sup> Davies et al<sup>14</sup> reported that *MUC5AC* and *MUC5B* but not *MUC2* were identified to be the main matrix-forming

Immunohistochemical Localization of MUC5AC and MUC5B Proteins*		
Structures	Normal/CRS	
	MUC5AC	MUC5B
Epithelial cells		
Ciliated	0/0	0/0
Goblet	0/+++	0/0
Basal	0/0	0/0
Epithelial surface/lumen	0/+++	0/+
Glandular cells		
Mucous	0/0	0/+++
Serous	0/0	0/0

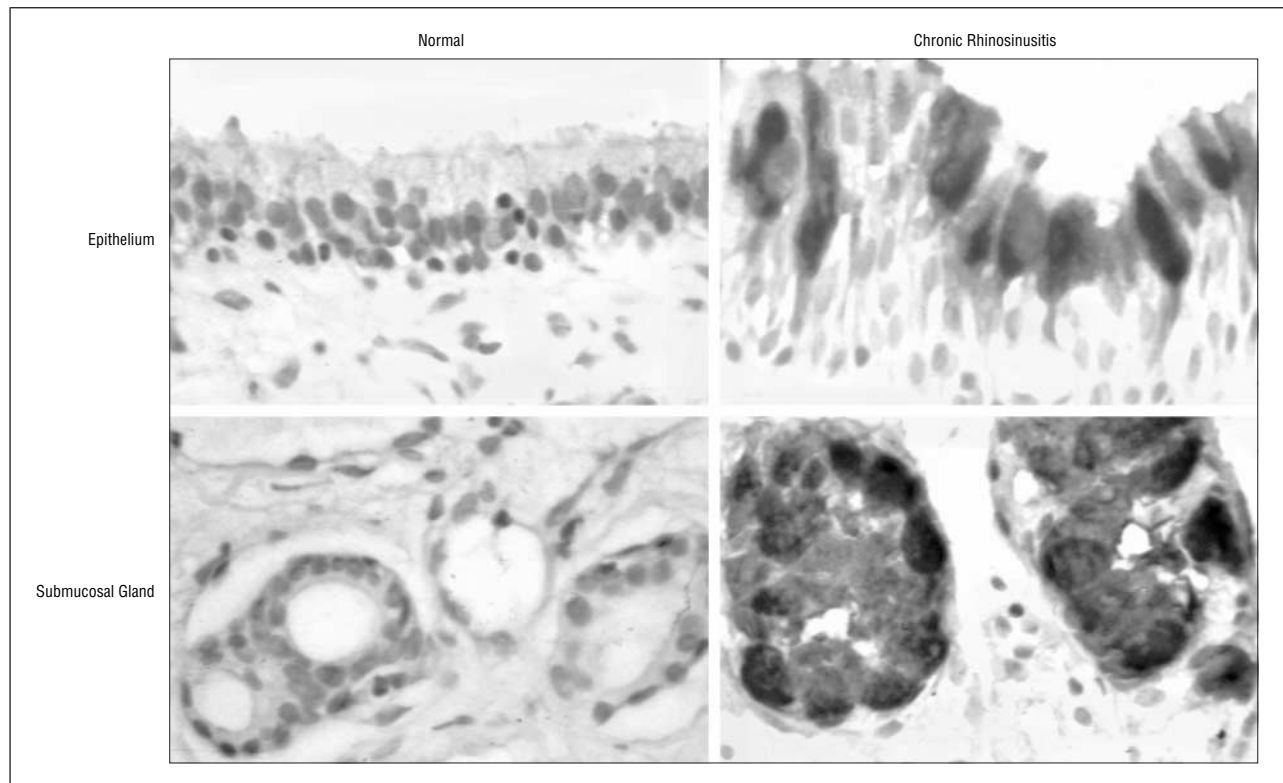
Abbreviation: CRS, chronic rhinosinusitis.

\*0 indicates a negative finding; +++, strongly positive; +, weakly positive.



**Figure 3.** Cellular localization of MUC5AC immunoreactivity in human sinus mucosa. MUC5AC immunoreactivity is present in sinus epithelial goblet cells but not in submucosal glands. There are increased numbers of goblet cells positive for MUC5AC in chronic rhinosinusitis compared with normal sinus mucosa (immunohistochemical stain, original magnification  $\times 400$ ).





**Figure 4.** Cellular localization of MUC5B immunoreactivity in human sinus mucosa. MUC5B immunoreactivity is localized to the goblet cells and mucous cells of submucosal glands. There are increased numbers of goblet cells and mucous cells of submucosal glands positive for MUC5B in chronic rhinosinusitis mucosa compared with normal sinus mucosa (ABC method; original magnification  $\times 400$ ).

components of the gel phase in cystic fibrosis airway secretion. MUC5AC is reported as a main mucin in respiratory secretion, and MUC5B is a predominant gel-forming oligomeric mucin in the human salivary gland and respiratory tract.<sup>2,15</sup>

Following the characterization of MUC5AC and MUC5B as major components of human nasal epithelial secretions, we examined the expression of MUC5AC and MUC5B genes and MUC5AC and MUC5B proteins in chronic rhinosinusitis and normal sinus tissues by using semiquantitative RT-PCR and immunohistochemical analysis. Our RT-PCR results of the sinus mucosa coincided with immunostaining data of MUC5AC and MUC5B, indicating that mucin mRNA expression is in accordance with mucin protein expression in normal sinus mucosa and chronic rhinosinusitis mucosa. MUC5AC is up-regulated by inflammatory mediators (eg, tumor necrosis factor  $\alpha$  and interleukin 9),<sup>11,16</sup> acrolein, prostaglandin E<sub>2</sub>, 15-hydroxyeicosatetraenoic acid, oxidative stress, and activated neutrophils via activation of the epidermal growth factor receptor.<sup>17</sup>

MUC5B is one of the tracheobronchial mucin genes, and its expression was localized to the mucous cells from submucosal glands, which suggests that MUC5B is a major mucin of submucosal glands.<sup>9,18</sup> The MUC5B mucin gene and its product were detected in the middle ear secretory cells of patients with chronic otitis media, which suggests that inflammatory cell products are involved in the production of MUC5B.<sup>19</sup> Sinus mucus contains MUC5B as its major mucin, which indicates an up-regulation of this mucin in inflammatory disease of the

sinuses.<sup>20</sup> MUC5AC and MUC5B mucin have been identified in secretions from patients with asthma<sup>21</sup> and in even mild cases of asthma, goblet cell hyperplasia and increased expression of MUC5AC are detected.<sup>22</sup> Jung et al<sup>23</sup> reported that MUC4, MUC5AC, MUC5B, and MUC8 mRNA are main mucins in the ethmoid mucosa and are up-regulated by chronic inflammation.

In summary, the present study indicates that sinus mucosal cells express MUC5AC and MUC5B proteins and genes, which suggests the potential for mucus production in sinus epithelium. Increased expression of MUC5AC and MUC5B are associated with secretory cell hyperplasia and metaplasia in the sinus. Further studies of other mucins, as well as MUC5AC and MUC5B, involved in upper airway diseases may help to define their role in mucus production in response to toxins, infection, and inflammation. The study of mucin gene and protein expression in chronic rhinosinusitis can enhance the understanding of the pathologic processes underlying this disease.

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