Increased Expression of Glycoprotein 340 in the Ethmoid Sinus Mucosa of Patients With Chronic Sinusitis

Tae Hoon Kim, MD; Sang Hag Lee, MD; Heung Man Lee, MD; Hak Hyun Jung, MD; Seung Hoon Lee, MD; Woo Sung Cho, MD; Young Gi Cinn, MD; Hwan Choe, MD; Moo Pil Kim, MD; Ik One Yoo, MD; Ho Yeon Hwang, MD

Objectives: To investigate the expression and distribution of glycoprotein 340 (gp340), a secretory glycoprotein, in normal human sinus mucosa and inflammatory sinus mucosa and evaluate the possible effects of gp340 on the development of chronic sinusitis. Glycoprotein 340 was identified as a key element in the innate host defense mechanism on many mucosal surfaces and is directly involved in defense functions aimed at clearing gram-positive and gram-negative bacteria.

Design: Prospective study.

Setting: Tertiary academic institution.

Patients: Normal sinus mucosa was obtained from the ethmoid sinus mucosa of 8 patients with blowout fractures undergoing endoscopic reduction. Inflammatory sinus mucosa was taken from 25 patients with chronic polypoid sinusitis during endoscopic sinus surgery.

Intervention: Semiquantitative reverse transcription–polymerase chain reaction (RT-PCR), immunohistochemical analysis, and Western blotting were performed.

Main Outcome Measures: The expression level and distributional pattern of gp340 in normal and inflammatory sinus mucosa were analyzed.

Results: Transcripts of the gp340 gene were detected in all human sinus tissues analyzed by RT-PCR. Immunohistochemical analysis revealed that gp340 is mainly localized in submucosal gland of both normal and inflammatory sinus mucosa. Semiquantitative RT-PCR and Western blot analysis showed the increased expression levels of gp340 in the inflammatory sinus mucosa compared with the normal sinus mucosa.

Conclusion: These results suggest that gp340 may play a constitutive role in nasal defense and may be upregulated in response to inflammation, participating in antimicrobial defense in chronic sinusitis.


INONASAL MUCOSA IS CONSTANTLY EXPOSED TO NOXIOUS FUMES, PATHOGENS, AND ADVERSE ENVIRONMENTAL CONDITIONS AND PROVIDES A PHYSICAL BARRIER TO CHEMICAL AND INVASIVE BIOLOGICAL AGENTS. THESE MUCOSAL DEFENSE MECHANISMS OF THE SINONASAL MUCOSA DEPEND ON NASAL SECRETIONS AND THEIR CONSTITUENT PROTEINS DERIVED FROM EPITHELIAL CELLS, SUBMUCOSAL GLANDS, AND BLOOD VESSELS IN THE MUCOSA.1 These proteins are vital to the sterility of the nasal airways and act to destroy bacteria or prevent their colonization of the mucosal surface and to protect against host and bacterial proteinase. These components include lysozyme, lactoferrin, transferring proteinase, proteinase inhibitors, and secretory IgA.1,2

Glycoprotein 340 (gp340) is a secreted glycoprotein, first purified from human bronchoalveolar lung washings from a patient with alveolar proteinosis.3 Sequence analysis revealed gp340 to be a member of the scavenger receptor family closely related to the putative tumor suppressor gene DMBT1.4 Glycoprotein 340 has been identified as the salivary agglutinin5 that mediates specific adhesion to and aggregation of streptococcus mutans and other bacteria.6 More recently, gp340 was found to be present ubiquitously on many mucosal surfaces, including the lung, trachea, salivary gland, small intestine, and stomach.6 Furthermore, it has been shown that gp340 is directly involved in defense functions aimed at clearing gram-positive and gram-negative bacteria and influenza A virus, contributing to defend mucosal surfaces.7

In this respect, gp340 may serve an important role as the innate defense system in the sinonasal mucosa. However, little attention has been paid to the expression and distributional patterns of gp340 in human mucosal surfaces.
sinus mucosa, despite its importance in mucosal defense system. The present study was undertaken to investigate the expression and distributional patterns of gp340 in normal and inflammatory sinus mucosa and evaluate its possible effects on the development of chronic sinusitis.

## METHODS

### SAMPLE PREPARATIONS

Normal ethmoid sinus mucosa (25 sinus mucosa) was removed from the ethmoid sinus during endoscopic reduction in 8 patients (6 men and 2 women; age range, 22-26 years) with blowout fracture. The subjects had no history of nasal infection, allergy, smoking, or ongoing drug treatment. During operation, the sinus mucosa with normal appearance, which was not injured by fracture, was removed and used as normal controls. Inflammatory ethmoid sinus mucosa (25 mucosa) were taken from 25 patients (18 men and 7 women; age range, 25-45 years) undergoing endoscopic sinus surgery for chronic polyoid sinusitis. None of these patients had a history of nasal allergy, asthma, aspirin sensitivity, or ongoing drug treatment. Prior to obtaining the tissue specimens, the protocols and informed consent form were approved by the institutional review boards for humans at our institution. Protocols and informed consent form were approved by the institutional review boards for humans at our institution.

Tissue samples (20 normal sinus mucosa and 20 inflammatory sinus mucosa) were cut into 2 portions; one portion was dissected, frozen in liquid nitrogen, and stored at −80°C for subsequent RNA isolation, and another portion was used for protein isolation. For immunohistochemical analysis, other samples (5 normal sinus mucosa and 5 inflammatory sinus mucosa) were fixed overnight in a freshly prepared fixative containing 4% paraformaldehyde in phosphate-buffered saline (PBS) (pH 7.4) and then immersed in 20% sucrose solution in PBS. The frozen tissues were homogenized in TRIZol reagent (GIBCO BRL, Grand Island, New York), and equal amounts of total RNA from each sample were reverse transcribed in 20 µL of a reaction mixture containing 2.5 U of Moloney murine leukemia virus reverse transcriptase (GIBCO BRL) and 50 pmol of random hexanucleotides at 42°C for 60 minutes. The following primers were used at 57°C annealing temperature: gp340 (542 base pairs [bp]) (sense: 5'-AAATTCTACCTTATGGTCTA-3', and antisense: 5'-AGAGGGAACTCGGGTAGAC-3'), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (302 bp) (sense: 5'-ATCTTCCAGGAGGAGGACTCC-3', and antisense: 5'-ACCACCTGACAGTGGCCAGT-3'). Amplified polymerase chain reaction (PCR) products were resolved in 2% agarose gels, stained with ethidium bromide, and photographed under UV light. Semiquantitative reverse transcription–PCR (RT-PCR) was performed on the samples to assess for differential expression of the gp340 messenger RNA (mRNA) in normal and inflammatory sinus mucosa. The optimum number of PCR cycles for each complementary DNA (cDNA) species was determined by plotting the PCR product yield of different cycles on a semilogarithmic graph and the cycle number representing exponential amplification was chosen for the final amplification. For quantification of the PCR products of all samples, the expected products were analyzed by densitometry and the data were expressed as the ratio of gp340 cDNA to the corresponding GAPDH cDNA. The data are presented as mean±SD. The statistical significance of differences between groups was evaluated using the t test, and the level of significance was set at P<.05.

### IMMUNOHISTOCHEMICAL AND WESTERN BLOT ANALYSES

For immunohistochemical study, tissues were embedded, cryosectioned, and stored at −80°C prior to use. The sections were air dried, rehydrated in PBS, and blocked with normal horse serum. Endogenous peroxidase was inactivated by incubation with 3% hydrogen peroxidase for 15 minutes at room temperature. After incubation with mouse monoclonal gp340 antibody (Abcam, Cambridge, England) at a dilution of 1:500 overnight at room temperature, a biotinylated secondary antimouse antibody was applied, followed by the avidin-biotin-peroxidase complex. The color was developed using 3,3′-diaminobenzidine.

For Western blot analysis, equal amounts of total protein were separated on 7.5% sodium dodecyl sulfate–polyacrylamide gels and transferred to Immobilon, a polyvinylidene fluoride membrane (Millipore, Bedford, Massachusetts). The blots were blocked for 1 hour at room temperature with PBS containing 1% skim milk and then incubated with anti-gp340 antibody overnight at room temperature. As an internal control, β-actin expression was analyzed in parallel blots using the β-actin antibody (Sigma-Aldrich Inc, St Louis, Missouri). Intensity of detected bands was quantified using densitometry. Relative intensities of each protein signal were obtained by dividing intensities of each protein band by β-actin signal. Statistical significance of differences was evaluated by the t test.

## RESULTS

The frozen tissues were homogenized in TRIZol reagent (GIBCO BRL, Grand Island, New York), and equal amounts of total RNA from each sample were reverse transcribed in 20 µL of a reaction mixture containing 2.5 U of Moloney murine leukemia virus reverse transcriptase (GIBCO BRL) and 50 pmol of random hexanucleotides at 42°C for 60 minutes. The following primers were used at 57°C annealing temperature: gp340 (542 base pairs [bp]) (sense: 5'-AAATTCTACCTTATGGTCTA-3', and antisense: 5'-AGAGGGAACTCGGGTAGAC-3'), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (302 bp) (sense: 5'-ATCTTCCAGGAGGAGGACTCC-3', and antisense: 5'-ACCACCTGACAGTGGCCAGT-3'). Amplified polymerase chain reaction (PCR) products were resolved in 2% agarose gels, stained with ethidium bromide, and photographed under UV light. Semiquantitative reverse transcription–PCR (RT-PCR) was performed on the samples to assess for differential expression of the gp340 messenger RNA (mRNA) in normal and inflammatory sinus mucosa. The optimum number of PCR cycles for each complementary DNA (cDNA) species was determined by plotting the PCR product yield of different cycles on a semilogarithmic graph and the cycle number representing exponential amplification was chosen for the final amplification. For quantification of the PCR products of all samples, the expected products were analyzed by densitometry and the data were expressed as the ratio of gp340 cDNA to the corresponding GAPDH cDNA. The data are presented as mean±SD. The statistical significance of differences between groups was evaluated using the t test, and the level of significance was set at P<.05.
GAPDH and 542 bp for gp340 were detected in normal and inflammatory ethmoid sinus tissues examined and showed 100% sequence homology with published sequence. These results indicate that normal and inflammatory sinus mucosae express gp340 mRNA. Negative control reactions without RT in the cDNA synthesis process showed no bands, indicating that there was no contamination by genomic DNA in the RNA samples (data not shown). The relative abundance of gp340 with respect to GAPDH was significantly increased in inflammatory sinus mucosa compared with those in normal sinus mucosa (Figure 1B) ($P < .05$).

In immunohistochemical staining, a homogenous pattern was observed on all samples of normal and inflammatory sinus mucosa examined in the present study. In both normal and inflammatory sinus mucosae, gp340 was detected in submucosal glands, where intense staining was found in serous glands but not in mucous glands (Figure 2). Goblet cells in the superficial epithelial layer do not show the immunoreactivity for gp340 (Figure 2). In comparison with normal sinus mucosa, the submucosal glands with gp340 immunopositivity were markedly increased in inflammatory sinus mucosa, suggesting the increased expression levels of gp340. Western blot analysis with the gp340 antiserum was able to detect gp340 protein in normal and inflammatory ethmoid sinus mucosa. The expression level of gp340 expression was increased in inflammatory ethmoid sinus mucosa compared with that in the normal ethmoid sinus mucosa (Figure 3) ($P < .05$).

In the present study, we have characterized the expression and distribution pattern of gp340 in normal and inflammatory sinus mucosa. At the mRNA and protein levels, gp340 was expressed in both normal and inflammatory sinus mucosae tested in the present study. Findings from immunohistochemical examination demonstrated the expression of gp340 in the submucosal gland of normal and inflammatory sinus mucosa. Furthermore, our results show that the expression levels of gp340 mRNA and protein in inflammatory sinus mucosa are increased compared with those in normal sinus mucosa. Therefore, our data imply that gp340 is constitutively expressed in normal human sinus mucosa and is up-regulated in chronic sinusitis, playing a key role in the host defense like other antimicrobial substances in nasal secretions. To our knowledge, this is the first detailed analysis of the expression and distributional patterns of gp340 mRNA and protein in normal and inflammatory sinus mucosa.

In various organs repeatedly exposed to a variety of foreign substances and organisms, a differential localization pattern of gp340 was revealed by immunohistochemical analysis. In the small intestine, gp340 was located in the epithelial cells, while staining in the trachea was observed mainly in the submucosal glands. In parotid gland tissue, gp340 was present only in striated duct cells, while the findings for serous acini were negative. In the submandibular gland, staining for gp340 was found in serous acinar cells and demilune cells but not in the duct cells. Glycoprotein 340 was also abundant in lacrimal gland tissues where only acinar cells were labeled. On the other hand, the epithelial cells lining the nasal airways and submucosal glands play a vital role in nasal health. In addition to serving as an important physical barrier, 2 major functions of these cells are the production and modification of nasal surface liquid and the manufacture and secretion of a number of host defense factors. These factors act in a broad-spectrum fashion, in some cases exerting antimicrobial effects against bacteria, fungi, and viruses. Many of these factors are produced in more abundance by submucosal gland epithelia and some are also produced by the surface epithelia. In the present study, gp340 in sinus mucosa was localized mainly in the submucosal gland of both mucosae (3,3'-diaminobenzidine, original magnification ×100).
cated in the submucosal gland but not in goblet cells of superficial epithelium, suggesting that the major source of gp340 in nasal secretion is the submucosal gland of sinus mucosa. The submucosal gland is the primary source of secretory cells of the sinonasal mucosa and hence may secrete gp340 in addition to a battery of other antimicrobial proteins that were detected in human nasal secretion. In this way, gp340 may be a candidate for antibacterial activity in nasal secretion and contribute to the mucosal defense of the sinonasal mucosa with other antibiotic proteins reported previously.1,3

One study evaluated the constituents of the nasal secretions that were obtained from patients with recurrent sinusitis. Baseline nasal secretions of patients with recurrent sinusitis were relatively elevated with the glandular proteins lysozyme and lactoferrin as compared with that of secretions in normal subjects, suggesting that these antimicrobial factors were up-regulated in patients with chronic inflammation.12 In the mucosa from patients with chronic sinusitis, an increased immunoreactivity for lysozyme and lactoferrin was noted in epithelial cells and newly formed atypical glands.13 Besides the aforementioned antimicrobial factors, the study using RT-PCR and immunohistochemical analysis revealed that the expression levels of some defensin isootypes, a prominent group of antimicrobial peptides, are increased in the nasal mucosa of patients with chronic sinusitis.11 Recently, cathelicidin, another antimicrobial peptide, originally isolated from neutrophil granule, is also identified in normal nasal mucosa and is up-regulated during inflammation.10 Our results also demonstrated the increased expression of gp340 in the sinus mucosa of patients with chronic sinusitis and are consistent with previous studies in which the expression of gp340 was up-regulated in inflammation status.10 Therefore, it can be considered that alteration in the activity or distribution of these antimicrobial factors has some association with the affinity of pathogens to sinonasal mucosa. Furthermore, the synergistic effects of these antimicrobial substances, such as lysozyme, lactoferrin, and defensins, may be effective to make these factors more potent against infectious pathogens.

This study has established that gp340 is expressed in normal human sinus mucosa and is up-regulated in the inflammatory sinus mucosa of patients with chronic sinusitis, suggesting that gp340 is constitutively expressed in normal sinus mucosa and may play a key role in the nasal host defense like other antimicrobial substances in chronic sinusitis. In this study, we did not reach any conclusions regarding the regulation of gp340 in normal and inflammatory sinus mucosa. This remains an area for further investigation.

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Correspondence: Sang Hag Lee, MD, Department of Otorhinolaryngology—Head & Neck Surgery, College of Medicine, Korea University, 126-1, 5 Ka, Anam-Dong, Sung-Buk-Ku, Seoul 136-705, South Korea (sanghag@kumc.or.kr).

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REFERENCES