

Staphylococcus aureus increases cytokine and matrix metalloproteinase expression in nasal mucosae of patients with chronic rhinosinusitis and nasal polyps

Jong Hwan Wang, M.D., Hyun Ja Kwon, M.Sc., and Yong Ju Jang, M.D.

ABSTRACT

Background: *Staphylococcus aureus* is a common bacterial pathogen associated with chronic rhinosinusitis with/without nasal polyps (CRSw/sNP). We investigated the effect of *S. aureus* on the secretion of eotaxin, interleukin (IL)-5, IL-8, IL-13, matrix metalloproteinase (MMP) 2, MMP-9, and tissue inhibitor of MMP (TIMP) 1 in nasal mucosae from CRSwNP patients to assess the roles of these materials in NP pathogenesis.

Methods: We infected organ cultures of NP and inferior turbinate (IT) mucosae taken from patients with CRSwNP with *S. aureus* ATCC 25923 for 24 hours and incubated the cultures for an additional 48 hours at 37°C. *S. aureus* infection and staphylococcal enterotoxins were confirmed by real-time polymerase chain reaction. Eotaxin, IL-5, IL-8, IL-13, MMP-2, MMP-9, and TIMP-1 protein levels were measured by ELISA.

Results: *S. aureus* infection significantly increased the concentrations of eotaxin, IL-5, IL-8, and IL-13 in the IT and NP groups ($p < 0.01$ for all comparisons). *S. aureus* infection also significantly increased the concentrations of MMP-2, MMP-9, and TIMP-1 in both groups ($p < 0.001$ for all comparisons). After *S. aureus* infection, the relative increases in eotaxin (6.42 versus 3.56), IL-5 (15.29 versus 8.89), MMP-2 (1.95 versus 1.58), MMP-9 (2.34 versus 1.95), and TIMP-1 (1.45 versus 1.31) were greater in the NP group than in the IT group.

Conclusion: *S. aureus* infection enhances the secretion of cytokines, MMP-2, MMP-9, and TIMP-1 by both NPs and IT mucosae from patients with CRSwNP. *S. aureus* may play an important role in the pathogenesis of NP via tissue remodeling as well as eosinophilic inflammation.

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Staphylococcus aureus is a common bacterial pathogen associated with chronic rhinosinusitis with/without nasal polyps (CRSw/sNP),^{1–4} a chronic inflammatory state characterized by tissue infiltration of lymphocytes and eosinophils^{4,5} and chronic bacterial colonization or infection.³ The rate of *S. aureus* isolation from sinonasal mucus of CRSw/sNP patients varies from 11.1 to 55%. The effect of *S. aureus* on CRSwNP is associated with its secretion of several enterotoxins, such as staphylococcal enterotoxin A (SEA), staphylococcal enterotoxin B (SEB), and toxic shock syndrome toxin 1.^{4,6–8} These enterotoxins, which function as superantigens, bind to the α -chain of class II major histocompatibility complexes of antigen-presenting cells and next to the variable region of the T-cell receptor β -chain.⁹ This superantigen–lymphocyte interaction may lead to the activation of as many as 20% of T lymphocytes; in comparison, conventional peptide antigens activate only ~0.01% of these lymphocytes.¹⁰ Activation of such T cells may result in the secretion of T-helper 1 (Th1) and Th2 cytokines, including interferon (IFN) γ , interleukin (IL)-2, IL-4, IL-5, IL-10, and IL-13 in nasal mucosae.¹¹ *S. aureus* infection also stimulates airway epithelial cells to secrete chemokines such as IL-8 and eotaxin, which promote the local chemotaxis of neutrophils and eosinophils, respectively.¹² NP are characterized by eosinophilic inflammation, and IL-5, IL-13, and eotaxin have been shown to play roles in pathogenesis.^{13,14}

Recently, matrix metalloproteinases (MMP) and tissue inhibitors of MMP (TIMP) have been found to play important roles in the formation of NP.^{15,16} MMP are a family of Ca^{2+} -activated, Zn^{2+} -dependent endopeptidases that are involved in the degradation of extracellular matrix (ECM) components, and their extracellular activities are regulated by TIMP. Imbalances between MMP and TIMP activity may lead to variations in tissue remodeling. *S. aureus* peptidoglycan (PEG) has been shown to increase the level of MMP-2 and/or MMP-9 in rat

lungs, liver, and blood, as well as to elevate MMP-9 secretion by human neutrophils.^{17,18} Lipoteichoic acid (LTA) of *S. aureus* also increased macrophage secretion of MMP-9.¹⁹ Patients with hospital-acquired pneumonia attributable to high-risk bacteria such as *S. aureus*, *Pseudomonas aeruginosa*, and *Stenotrophomonas maltophilia* have been found to have higher concentrations of MMP-8, MMP-9, and TIMP-1; elevated MMP-9 activity; and a higher MMP-9/TIMP-1 molar ratio in bronchoalveolar lavage than control patients.²⁰ These findings suggest that *S. aureus* infection may be involved in the expression and secretion of MMP and TIMP. Little is known, however, about the effects of *S. aureus* infection on the expression of MMP and TIMP in CRSwNP patients. We hypothesized that *S. aureus* infection enhanced not only the inflammatory response but also tissue remodeling in patients with CRSwNP, thereby playing a role in the pathogenesis of NP formation. To address this hypothesis, we investigated the *S. aureus*-induced secretion of eotaxin, IL-5, IL-8, IL-13, MMP-2, MMP-9, and TIMP-1 in both NP and inferior turbinate (IT) tissues.

MATERIALS AND METHODS

Subjects

Eight NP and eight IT mucosal tissue samples were harvested from eight patients, of mean age 37 years (range, 16–67 years; seven men, one woman), who underwent endoscopic sinus surgery and turbinate reduction for the treatment of CRSwNP and turbinate hypertrophy between April 2008 and July 2008. CRSwNP was diagnosed based on rhinosinusitis consensus research definitions: (i) symptoms present for ≥ 12 weeks; (ii) requires two or more of the following symptoms, such as anterior and/or posterior mucopurulent drainage, nasal obstruction, and decreased sense of smell; and (iii) endoscopy to confirm presence of bilateral polyps in middle meatus and imaging by computed tomography with confirmation of bilateral mucosal disease.²¹ No subject was prescribed intranasal and/or oral corticosteroids, antihistamines, antileukotrienes, oral or intranasal decongestants, or intranasal anticholinergics within 1 month before surgery. No patients were positive for allergy to 11 common aeroallergens, as determined by skin-prick tests (Bencard; Bradford, England); no patient was aspirin intolerant.

From the Department of Otolaryngology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, South Korea

Address correspondence and reprint requests to Yong Ju Jang, M.D., Department of Otolaryngology, Asan Medical Center, University of Ulsan College of Medicine, Pungnap-2-dong, Songpa-gu, 138–736 Seoul, Korea

E-mail address: jangyj@amc.seoul.kr

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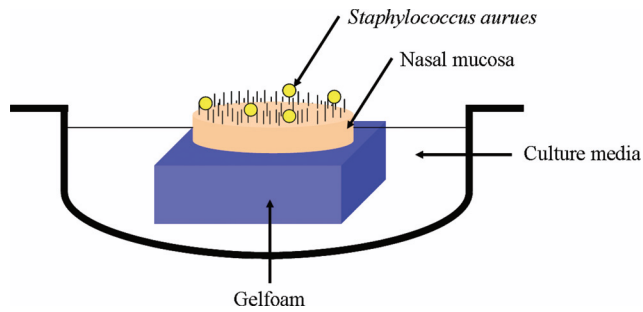


Figure 1. Schematic drawing of the organ culture model used in the study. A mucosal sample was placed on a 5 × 5-mm piece of gelfoam that had been hydrated with culture medium, followed by addition of *Staphylococcus aureus* (30 μ L; 10^7 CFU/mL) to the apical surface.

ant or had a history of asthma. This study was approved by the Institutional Review Board of the Asan Medical Center, and all subjects provided written informed consent.

Bacterial Stock Preparation

S. aureus ATCC 25923 was seeded onto blood-agar plates and grown for 24 hours at 37°C. Bacteria were next grown overnight at 37°C in Mueller-Hinton broth (Difco, Detroit, MI),²² and bacterial concentration was measured using a Densitometer turbidity photometer (bioMérieux, Lyon, France) and assessed using the McFarland scale. Colonies were harvested and resuspended in phosphate-buffered saline (PBS) to a concentration of 10^7 CFU/mL.

Organ Culture and *S. aureus* Infection

The NP and IT mucosal tissues collected during surgery were immediately transported to the laboratory and washed three times in PBS to remove blood cells and mucus. Each tissue sample was divided into two small fragments with a 4-mm biopsy punch (Stiefel, Offenbach, Germany) and placed in minimal essential medium (GIBCO, Grand Island, NY), supplemented with 2 mM of L-glutamine, 20 mM of HEPES, penicillin (100 U/mL), streptomycin (100 μ g/mL), and fungizone (0.25 μ g/mL) for 4 hours at 4°C. Then, each fragment was implanted at the air-liquid interface, with the epithelium up and the submucosa down, on a 5 × 5-mm support of gelfoam (Johnson & Johnson, Gargrave, U.K.), prehydrated for at least 24 hours with 1.5 mL of culture medium in a well of a 24-well plate (Nunc; Roskilde, Denmark; Fig. 1).²³

In the control group, each tissue sample (eight NP and eight IT samples) was cultured for 24 hours, washed eight times with PBS, and incubated for an additional 48 hours at 37°C in fresh medium. Absence of *S. aureus* organisms in all control tissue samples and supernatants were confirmed by blood-agar plate culture. In the *S. aureus* infection group (*S. aureus*), 30 μ L of *S. aureus* (10^7 CFU/mL) was applied to the top of each sample (eight NP and eight IT samples) for 24 hours, after which samples were washed eight times with PBS. The *S. aureus*-infected mucosae were transferred to a second set of identical plates and incubated for an additional 48 hours at 37°C, after which time each culture supernatant was removed and stored at -70°C.

Real-Time Polymerase Chain Reaction Analysis for *S. aureus*, SEA, and SEB

Each tissue sample was lysed, and total RNA was extracted with the TRIzol reagent (Life Technologies, Rockville, MD) according to the manufacturer's protocol. Total RNA was digested with DNase (DNA-free; Ambion, Inc., Austin, TX), as per the manufacturer's protocol, and quantified by spectrophotometry. Two micrograms of total RNA was converted to cDNA using Superscript II reverse transcriptase (Invitrogen,

Carlsbad, CA), according to the manufacturer's instructions. The cDNA samples were stored in aliquots at -80°C until required. Real-time quantitative polymerase chain reaction (PCR) was performed in triplicate in 96-well plates; each 25- μ L reaction contained 12.5 μ L of 2× SYBR I Mix (Applied Biosystems, Foster City, CA), 0.5 μ L of 10 μ M-forward and reverse primers, and 2 μ L of cDNA. Oligonucleotide PCR primer pairs were designed as follows: *S. aureus*, 5'-GATACGCCTCTCCACGCATA-3' (forward) and 5'-GCCTGCA-CAAGGACGCTTAC-3' (reverse); SEA, 5'-AAAATACAGTACCTTT-GGAAACGGTT-3' (forward) and 5'-TTTCTGTAAATAACGTTCTT-GCTTGA-3' (reverse); SEB, 5'-CACCCAACGTTTATGACAGAGAG-3' (forward) and 5'-CCATCAAACCAGTGAATTTACTCG-3' (reverse); β -actin, 5'-TTCTACAATGAGCTGCGTGGCT-3' (forward) and 5'-GCTTCTCCTTAATGTCACGCACGA-3' (reverse). PCR reactions were performed using an ABI7900HT real-time sequence detection system programmed for 35 cycles of denaturation for 5 seconds at 95°C, annealing for 20 seconds at 60°C, and extension for 30 seconds at 72°C. Primers for human β -actin were used as loading controls.

Quantification of Eotaxin, IL-5, IL-8, IL-13, MMP-2, MMP-9, and TIMP-1

The concentrations of eotaxin, IL-5, IL-8, and IL-13 were assayed using dual-antibody ELISA kits (Biosource, Nivelles, Belgium) and immunoreactivity for MMP-2, MMP-9, and TIMP-1 was quantified using other dual-antibody ELISA kits (R&D Systems, Minneapolis, MN) according to the manufacturers' protocols. Data were expressed in pg/mL and were obtained by extrapolation from a standard curve generated in parallel with each experiment.

Statistical Analysis

Between-group comparisons were analyzed using the Mann-Whitney *U* test with a two-sided *p* < 0.05 used to define statistical significance. Results are expressed as medians $\pm$ interquartile ranges (IQRs). All analyses were performed using SPSS for Windows, Version 15.0 (SPSS, Inc., Chicago, IL).

RESULTS

Confirmation of *S. aureus* Infection and Detection of SEA

S. aureus ATCC 25923 infections were confirmed by real-time PCR in all tissue samples infected with *S. aureus* (mean C_t 30.7; range, 26.7–33.6). In contrast, none of the control samples yielded a detectable PCR product characteristic of *S. aureus*. Using real-time PCR to detect the SEA and SEB genes in the *S. aureus*-infected samples, we found that all samples were positive for SEA (mean C_t 27.9; range, 24.3–31.1) but none was positive for SEB. None of the control samples showed detectable PCR products for SEA or SEB.

S. aureus Infection Increased the Secretion of Eotaxin, IL-5, IL-8, and IL-13

By ELISA, we found that *S. aureus* infection significantly increased the secretion of eotaxin (Fig. 2 A), IL-5 (Fig. 2 B), IL-8 (Fig. 2 C), and IL-13 (Fig. 2 D) in both IT and NP tissues, compared with noninfected control tissue samples. In the IT group, *S. aureus* infection significantly increased the median concentrations of eotaxin, from 1.5 (IQR, 1.4–1.6) to 5.4 (IQR, 4.7–5.9) pg/mL; of IL-5, from 12.6 (IQR, 10.2–14.6) to 112.3 (IQR, 95.7–144.9) pg/mL; of IL-8, from 1048.8 (IQR, 954.4–1190.5) to 1583.0 (IQR, 1260.9–1691.0) pg/mL; and of IL-13, from 17.3 (IQR, 16.5–17.8) to 27.8 (IQR, 25.4–29.7) pg/mL (*p* < 0.01 for each comparison). In the NP group, *S. aureus* infection also significantly increased the median concentrations of eotaxin, from 3.8 (IQR, 3.6–4.0) to 24.2 (IQR, 19.5–25.9)

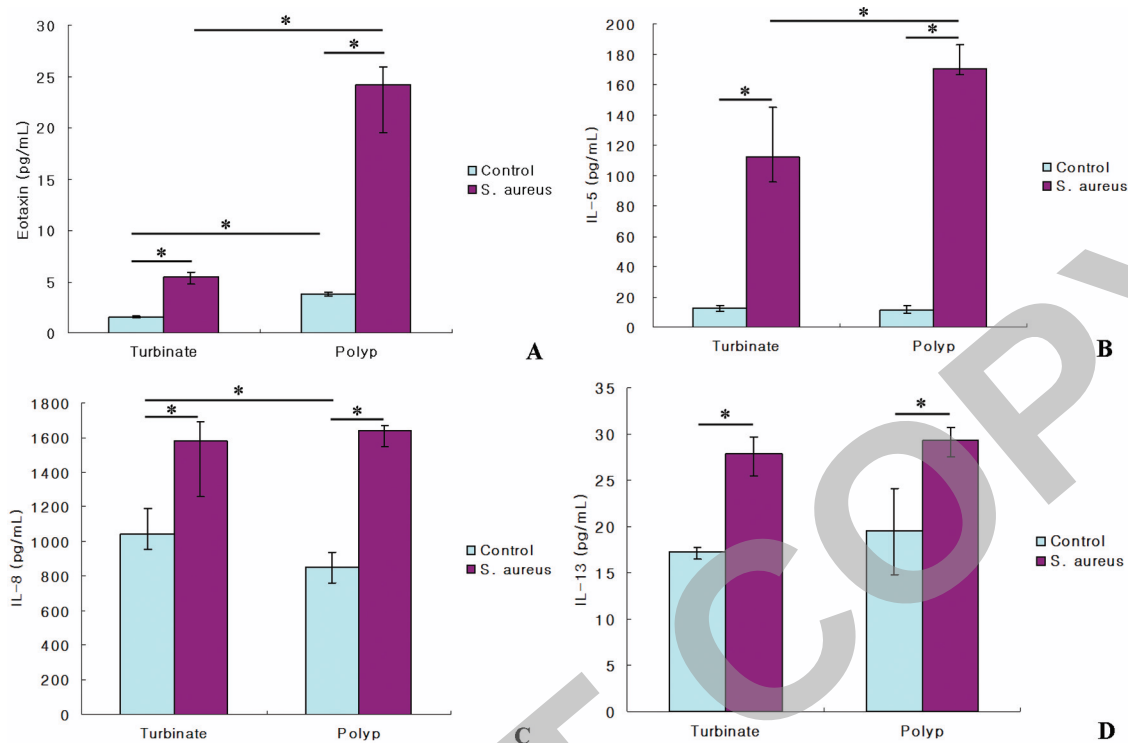


Figure 2. Effects of *Staphylococcus aureus* infection on the secretion of (A) eotaxin, (B) interleukin (IL)-5, (C) IL-8, and (D) IL-13, by the nasal mucosae of patients with chronic rhinosinusitis and nasal polyps. *S. aureus* infection significantly increased the secretion of eotaxin, IL-5, IL-8, and IL-13 by both turbinate and polyp mucosae, relative to uninfected controls. Each bar represents the median $\pm$ interquartile range of eight independent experiments involving nasal polyps and inferior turbinates from eight patients (* $p < 0.01$).

Table 1 Comparison of concentration ratios between groups

Parameters	IT (<i>Staphylococcus aureus</i> /Control)	NP (<i>Staphylococcus aureus</i> /Control)	Control (NP/IT)	<i>Staphylococcus aureus</i> (NP/IT)
Eotaxin	3.56	6.42	2.48	4.48
IL-5	8.89	15.29	0.88	1.52
IL-8	1.52	1.93	0.82	1.04
IL-13	1.60	1.50	1.13	1.06
MMP-2	1.58	1.95	1.39	1.71
MMP-9	1.95	2.34	1.22	1.46
TIMP-1	1.31	1.45	1.46	1.62

IT = inferior turbinate; NP = nasal polyp; IL = interleukin; MMP = matrix metalloproteinase; TIMP = tissue inhibitor of matrix metalloproteinase.

pg/mL; of IL-5, from 11.2 (IQR, 9.4–14.1) to 170.7 (IQR, 166.4–186.5) pg/mL; of IL-8, from 852.1 (IQR, 757.2–937.2) to 1641.8 (IQR, 1549.1–1667.8) pg/mL; and of IL-13, from 19.5 (IQR, 14.8–24.1) to 29.3 (IQR, 27.5–30.7) pg/mL ($p < 0.01$ for each comparison).

Table 1 shows the concentration ratios of eotaxin, IL-5, IL-8, and IL-13 for IT and NP groups relative to the control group, and between the IT and NP groups before and after *S. aureus* infection. After

S. aureus infection, the relative increases in eotaxin (6.42-fold versus 3.56-fold) and IL-5 (15.29-fold versus 8.89-fold) were significantly greater in the NP group than in the IT group. In the control group, the baseline eotaxin concentration was 2.48-fold higher in NPs than in IT mucosae, whereas the IL-8 concentration was higher in IT tissue samples (by 0.82-fold). In the *S. aureus* infection group, the concentrations of eotaxin (4.48-fold) and IL-5 (1.52-fold) were higher in NPs than in IT samples.

S. aureus Infection Increased the Secretion of MMP-2, MMP-9, and TIMP-1

S. aureus infection also significantly increased the secretion of MMP-2 (Fig. 3 A), MMP-9 (Fig. 3 B), and TIMP-1 (Fig. 3 C) in both IT and NP tissues, compared with noninfected control samples. In IT samples, *S. aureus* infection significantly increased the median concentrations of MMP-2, from 182.4 (IQR, 170.5–206.3) to 288.1 (IQR, 241.6–311.5) pg/mL; of MMP-9, from 449.6 (IQR, 433.1–482.0) to 877.5 (IQR, 813.8–891.9) pg/mL; and of TIMP-1, from 235.5 (IQR, 200.0–259.5) to 309.0 (IQR, 297.0–333.7) pg/mL ($p < 0.001$ for each comparison). In the NP samples, *S. aureus* infection also significantly increased the median concentrations of MMP-2, from 252.8 (IQR, 232.1–272.8) to 492.7 (IQR, 484.3–540.2) pg/mL; of MMP-9, from 546.5 (IQR, 502.5–609.5) to 1277.0 (IQR, 1129.5–1447.0) pg/mL; and of TIMP-1, from 344.0 (IQR, 327.4–

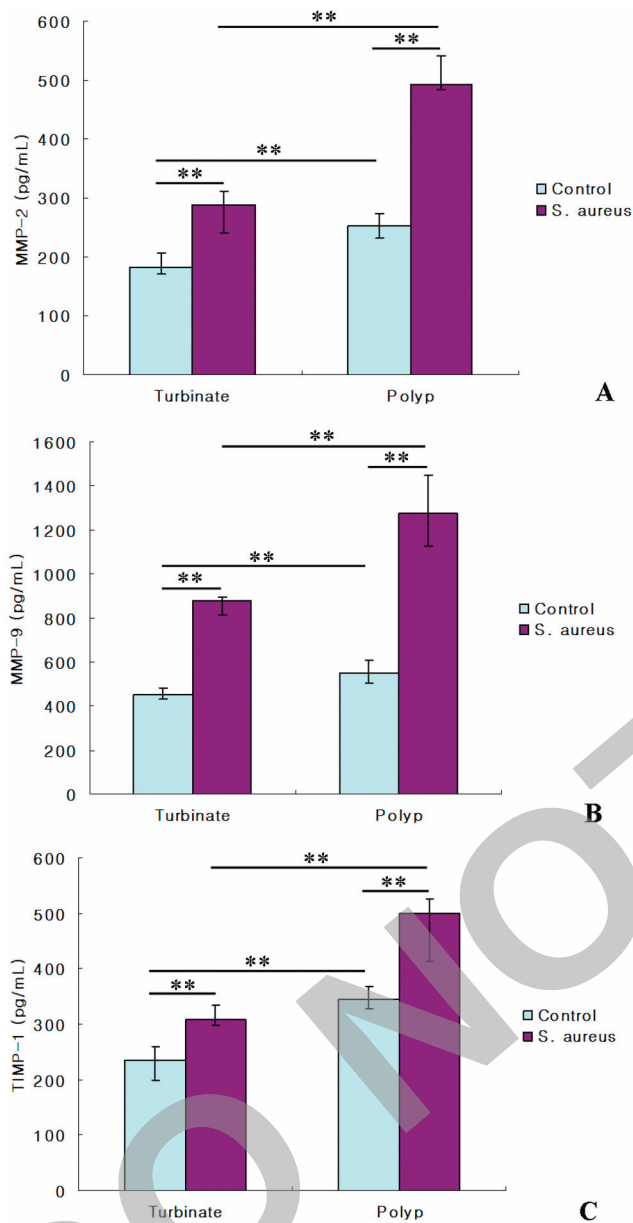


Figure 3. Effects of *Staphylococcus aureus* infection on the secretion of (A) matrix metalloproteinase (MMP) 2, (B) MMP-9, and (C) tissue inhibitor of MMP (TIMP) 1 by the nasal mucosae of patients with chronic rhinosinusitis and nasal polyps. *S. aureus* infection significantly increased the secretion of MMP-2, MMP-9, and TIMP-1 by both turbinate and polyp mucosae, relative to uninfected controls. Each bar represents the median $\pm$ interquartile range of eight independent experiments involving nasal polyps and inferior turbinates from eight patients (** $p < 0.001$).

367.9) to 499.7 (IQR, 412.5–525.9) pg/mL ($p < 0.001$ for each comparison).

After *S. aureus* infection, the relative increases in MMP-2 (1.95-fold versus 1.58-fold), MMP-9 (2.34-fold versus 1.95-fold) and TIMP-1 (1.45-fold versus 1.31-fold) were greater in NPs than in IT samples (Table 1). In the control group, baseline MMP-2 (1.39-fold), MMP-9 (1.22-fold), and TIMP-1 (1.46-fold) concentrations were higher in NPs than in IT tissue. In the *S. aureus* infection group, the concentrations of MMP-2 (1.71-fold), MMP-9 (1.46-fold), and TIMP-1 (1.62-fold) were higher in NPs than in IT tissues.

DISCUSSION

There are at least four potential inflammatory processes active in NPs: (i) late-phase allergic inflammation in response to airborne allergens; (ii) T-cell activation with secretion of IL-5, IL-13, and IFN- γ in response to fungal antigens in the sinus mucus; (iii) T-cell activation, cytokine secretion, and local IgE production in response to bacterial superantigens; and (iv) dysregulation of the sinus epithelium with oversecretion of chemokines, including RANTES.²⁴ We used an organ culture model to assess the effects of *S. aureus* infection on the inflammatory response and tissue remodeling in NPs and ITs by measuring the *S. aureus*-induced secretion of eotaxin, IL-5, IL-8, IL-13, MMP-2, MMP-9, and TIMP-1.

We found that *S. aureus* infection significantly increased the release of eotaxin, IL-5, IL-8, and IL-13 by both IT and NP samples from patients with CRSwNP. Moreover, the baseline eotaxin concentration and the *S. aureus*-induced increases in eotaxin and IL-5 secretion were higher in NP than in IT mucosae. In this study, we used *S. aureus* ATCC 25923 and confirmed the presence of not the *SEB* gene but the *SEA* gene in infected tissue samples by real-time PCR. However, these findings are consistent with results showing that *SEB* stimulation leads to significant increases in the secretion of IL-1 β , tumor necrosis factor (TNF) α , IFN- γ , IL-2, IL-4, IL-5, IL-10, and IL-13 by both normal IT and NP samples, with the increases being significantly higher in NP.¹¹ In addition, *SEB* was found to change the cytokine secretion pattern in NP to Th2 cytokines including IL-2, IL-4, and IL-5, but reduced the secretion of IL-10 and transforming growth factor β_1 . *SEB* significantly increased the secretion of IL-5 and granulocyte macrophage colony-stimulating factor in human nasal epithelial cells.²⁵ Local production of IL-5, IL-13, and granulocyte macrophage colony-stimulating factor has been found to decrease the apoptosis of eosinophils, thus increasing the survival of tissue eosinophils in NPs.¹³ The term “chemokine” is an abbreviation of “chemotactic cytokines”; ~50 human chemokines have chemotactic activity.¹⁴ The CC chemokine eotaxin attracts eosinophils, basophils, and Th2 lymphocytes via CCR3, the eotaxin receptor. In this study, *S. aureus*-induced eotaxin and IL-5 production was significantly higher in the NP group than in the IT group. This finding shows that NP tissues are more likely to be influenced by *S. aureus* infection than IT tissues via local chemotaxis of eosinophils and the extension of eosinophil survival. Accumulation of large quantities of eosinophils in NP and their secretion of major basic protein led to significant sodium flux into the interior of the epithelial cells without a significant net flux of chloride.¹³ Thus, water will flow into the cells and lamina propria and may lead to edema. A previous study reported that *SEB* stimulation significantly enhanced the secretion of IL-13 in both NP and normal IT samples, and the IL-13 concentration ratio after *SEB* treatment was similar between the two groups.¹¹ We also found that *S. aureus*-induced release of IL-13 was significantly increased in both NP and IT groups, and the IL-13 concentration ratio after *S. aureus* infection was similar between the two groups. This result suggests that *S. aureus* infection may induce similar inflammatory effect on NPs and IT by IL-13 production.

The CXC chemokine IL-8 is chemotactic for neutrophils, the only cells that have the IL-8 receptors CXCR1 and CXCR2. *S. aureus* enterotoxins have shown varying effects on IL-8 secretion by nasal epithelial cells. Stimulation with *SEA* or *SEB* (1.0 μ g/mL) has been shown to significantly induce IL-8 secretion by normal nasal epithelial cells,²⁶ whereas *SEB* stimulation (at 10 ng/mL or 0.5 μ g/mL) enhanced IL-8 release by both normal IT and NP tissues, but between-tissue differences were not statistically significant.^{11,27} These discrepancies may be attributable to the different concentrations of *S. aureus* enterotoxins used. In this study, baseline IL-8 concentration of the IT group was higher than that of the NP group, whereas *S. aureus* infection significantly increased IL-8 secretion to similar concentrations in both IT and NP groups. This finding suggests that local chemotaxis of neutrophils are greater in control IT tissues, but local chemotaxis of neutrophils are similar in *S. aureus*-infected IT and NP

tissues. Our findings were consistent with a recent study, which reported that direct *S. aureus* infection up-regulated the basolateral and apical secretion of IL-8 and eotaxin by human airway epithelial cells.¹²

MMP are major ECM-degrading proteinases that participate in various normal biological processes, including embryonic development, ovulation, cervical dilatation, postpartum uterine involution, endometrial cycling, hair follicle cycling, bone remodeling, and wound healing.²⁸ In humans, the extracellular activities of MMP are regulated by four TIMP isoforms: TIMP-1, -2, -3, and -4. Tissue remodeling is a process that leads to transient or permanent changes in tissue architecture, by both degrading and repairing basement membranes and interstitial stroma. This process is mediated by MMP released from epithelial cells, fibroblasts, and infiltrating inflammatory cells.²⁹ These MMP may also be responsible for microvascular permeability, causing edema and cell transmigration, and ECM remodeling in asthmatic airways.³⁰ NP are characterized by tissue remodeling, including basement membrane thickening, gland modifications, ECM accumulation, and edema.³⁰ The concentrations of MMP-2 (gelatinase A; type IV collagenase)¹² and MMP-9 (gelatinase B; type IV collagenase)^{15,30,31} were recently found to be significantly higher in NP than in normal controls, whereas any role for TIMP-1, which inhibits both MMP-2 and MMP-9 by forming 1:1 enzyme-inhibitor complexes, is unclear in NPs.^{15,16,31} MMP-9 staining inflammatory cells were significantly increased in antrochoanal polyps and NP.³²

We found that the baseline concentrations of MMP-2 (1.39-fold), MMP-9 (1.22-fold), and TIMP-1 (1.46-fold) were higher in NP than in IT mucosae. This finding suggests that control NP tissues seems to be in a tissue remodeling state, a balance between MMP and TIMP-1, similar to control IT tissues.

SEB was recently reported to increase the production of interstitial collagenase (MMP-1) and stromelysin 1 (MMP-3) in human mesenchymal cells.³³ In addition to the *S. aureus* enterotoxins PEG and LTA, the main components of the cell walls of Gram-positive bacteria, have been shown to contribute to inflammatory responses.³⁴ Moreover, *S. aureus* PEG and LTA have been found to up-regulate the release of MMP-2 and/or MMP-9 in rat and human neutrophils and macrophages.^{17–19} We found that *S. aureus* infection significantly increased the secretion of MMP-2, MMP-9, and TIMP-1 by both IT and NP tissue samples from patients with CRSwNP. The *S. aureus*-induced increases in MMP-2, MMP-9, and TIMP-1 were higher in NP than in IT. However, the relative increases in MMP-2 (1.58-fold and 1.95-fold) and MMP-9 (1.95-fold and 2.34-fold) induced by *S. aureus* infection were greater than those of TIMP-1 (1.31-fold and 1.45-fold) in both IT mucosae and NP, respectively. These findings suggest that *S. aureus* infection affects tissue remodeling in both IT and NP from patients with CRSwNP by inducing an imbalance between MMP and TIMP-1, and that this process may be greater in NP than in IT.

In conclusion, we found that *S. aureus* infection enhances the release of eotaxin, IL-5, IL-8, IL-13, MMP-2, MMP-9, and TIMP-1 by both NP and IT mucosae from patients with CRSwNP. Moreover, the *S. aureus*-induced secretion of eotaxin, IL-5, MMP-2, and MMP-9 was significantly greater in NP than in IT. These results suggest that *S. aureus* may play a key role in the pathogenesis of NP via tissue remodeling as well as eosinophilic inflammation.

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