

***Staphylococcus aureus* enterotoxin B, protein A, and lipoteichoic acid stimulations in nasal polyps**

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Background: Increasing evidence points toward a modifying role of *Staphylococcus aureus* and its products in the pathogenesis of nasal polyposis.

Objective: The aim of this study was to investigate cytokine and mediator production after stimulation with *S aureus*-derived proteins enterotoxin B, protein A, and lipoteichoic acid in nasal polyp and control inferior turbinate tissue.

Methods: Tissue fragments were stimulated with RPMI (negative control), enterotoxin B, protein A, and lipoteichoic acid for 30 minutes and 24 hours. Supernatants were measured by multiplex for proinflammatory cytokines (IL-1 β , TNF- α) and T-cell and subset-related cytokines (IFN- γ , IL-2, IL-4, IL-5, IL-8, IL-10, IL-12p70, IL-13). Histamine, TGF- β 1, cysteinyl leukotrienes, and prostaglandin D₂ were analyzed by ELISA.

Results: Thirty minutes of protein A stimulation resulted in a significant increase of histamine, leukotrienes, and prostaglandin D₂. Enterotoxin B stimulation over a period of 24 hours induced a significant increase of IL-1 β , TNF- α , IFN- γ , IL-2, IL-4, IL-5, IL-10, and IL-13 in both groups, with this increase significantly higher in nasal polyps compared with controls.

Conclusion: We here show that *S aureus* products have various effects on mucosal tissues: surface protein A induces mast cell degranulation, whereas enterotoxins induce the release of cytokines, with a T_H2-skewed pattern in nasal polyps, supporting the stimulatory role of superantigens in the development of this inflammatory disease.

Clinical implications: *S aureus* products affect inflammation in nasal polyps by inducing mast cell degranulation and T-cell activation. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Nasal polyps, chronic rhinosinusitis, *Staphylococcus aureus*, protein A, lipoteichoic acid, tissue stimulations

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Abbreviations used

LTA:	Lipoteichoic acid
NP:	Nasal polyposis
PGD ₂ :	Prostaglandin D ₂
SAE:	<i>Staphylococcus aureus</i> -derived enterotoxin
SEB:	<i>Staphylococcus aureus</i> enterotoxin B
sIgE:	Specific IgE
SpA:	Protein A
SPT:	Skin prick test
TSST-1:	Toxic shock syndrome toxin
TCR:	T-cell receptor

Chronic rhinosinusitis with nasal polyposis (NP) is a chronic inflammatory disease of the paranasal sinuses, associated with T_H2-biased inflammation,¹ an increase of tissue eosinophils,² and polyclonal IgE production, not related with the allergic status of the patients.³ The mast cells in the stroma of nasal polyps are often degranulated.^{4,5} In the general population, the prevalence of NP ranges from 1% to 4%, and the precise mechanism underlying the pathogenesis of NP is unknown and probably multifactorial.^{2,6}

The colonization rate with *Staphylococcus aureus* in the middle meatus is increased in patients with NP versus controls.⁷ These bacteria express a number of surface proteins such as lipoteichoic acid (LTA) and protein A (SpA) that have the potential to interfere with host defense mechanisms. LTA has been suggested to be essential for nasal colonization and interaction with human nasal epithelial cells,⁸ and SpA has been demonstrated to increase histamine release from human basophils⁹ and human heart mast cells.¹⁰ SpA appears to activate basophils by interacting through its alternative binding site with IgE V_H3⁺ bound to the high-affinity IgE receptor (Fc ϵ RI).^{9,10} In addition, SpA has been proposed to have B-cell superantigenic effects.^{11,12}

Moreover, *S aureus* secretes several toxins with superantigen activity, namely the *S aureus*-derived enterotoxins (SAEs) and the toxic shock syndrome toxin (TSST-1). Superantigens for T lymphocytes have the ability to cross-link the class II major histocompatibility complex of antigen-presenting cells and the T-cell receptor (TCR) β -chain variable regions. This cross-linking takes place outside the conventional antigen-binding groove and may lead to the stimulation of as much as 20% to

25% of the T-cell population in a nonspecific way, compared with stimulation of only about 0.1% via the conventional allergen-specific way.¹³ Once activated, T cells may produce ILs including IL-4, IL-5, IL-13, eotaxin, and many others, which may lead to an eosinophilic inflammation and local IgE production.

Specific IgE (sIgE) against SAEs is found more frequently in NP versus controls and correlates with higher levels of IL-5, eotaxin, and eosinophil cationic protein.² Moreover, an increased number of T cells expressing the TCR β -chain variable region, known to be induced by microbial superantigens, was detected in NP and correlated with the presence of sIgE against SAEs.¹⁴ A recent study of our group demonstrated that NP was predominantly characterized by increased T_H2 cytokines such as IL-5, eotaxin, IL-2 soluble receptor α , and IgE compared with controls.¹

The current study sought to elucidate the modulatory effects of the *S aureus* surface proteins SpA, LTA, and the *S aureus* enterotoxin B (SEB) in nasal polyp tissue and to determine possible differences from normal nasal (control) tissue. The following cytokines were measured: proinflammatory cytokines (IL-1 β , TNF- α), T-cell and subset-related cytokines (IFN- γ , IL-2, IL-4, IL-5, IL-8, IL-12p70, IL-13), and immunoregulatory cytokines (IL-10, TGF- β 1). Furthermore, mediators such as histamine, cysteinyl leukotrienes, and prostaglandin D₂ were analyzed to determine the response of local mast cells.

METHODS

Patients

Nasal tissue was obtained from 25 patients at the Department of Otorhinolaryngology of the Ghent University Hospital. The ethical committee of the Ghent University Hospital approved the study, and all patients gave their written informed consent before inclusion in the study. None of the subjects received intranasal corticosteroids, antihistamines, antileukotrienes, oral and intranasal decongestants, or intranasal anticholinergics within 1 week before surgery, and none of the subjects received oral and/or intramuscular corticosteroids within 4 weeks before surgery. For female subjects, pregnancy or lactation was excluded.

Nasal polyp samples were collected during functional endoscopic sinus surgery from 12 patients (median age, 43 years; range, 24–67 years; 10 men and 2 women). Nasal polyposis was diagnosed on the basis of symptoms, clinical examination, nasal endoscopy, and sinus computed tomography scan according to the European Position Paper on Rhinosinusitis and Nasal Polyps guidelines.¹⁵

Samples were collected from inferior turbinates (controls) from 13 patients undergoing septal surgery and/or turbinotomy because of nasal obstruction (median age, 29 years; range, 22–62 years; 8 men, 5 women).

The atopic status of all patients was evaluated by skin prick tests (SPTs) with a standard panel of 14 inhalant allergens. The reaction to a SPT was considered positive if the wheal area caused by the allergen was greater than 7 mm² (diameter >3 mm). Negative and positive controls (10 mg/mL histamine solution) were included with each SPT. Five inferior turbinates and 5 NPs were obtained from patients with positive SPT for at least 1 of the most common aeroallergens.

Two patients with NP reported mild asthma in history, and all patients were free of aspirin intolerance. Three control patients and 1 patient with NP reported smoking cigarettes.

The nasal tissue collected during surgery was immediately transported to the laboratory and divided into 2 parts. One part was immediately snap-frozen in liquid nitrogen and stored at –80°C until analysis for immunohistochemistry and until homogenization. The remaining tissue was used for the *ex vivo* stimulations.

Mechanical disruption and stimulations of human nasal tissue

The human nasal mucosa and submucosa were cut thoroughly in tissue culture medium consisting of RPMI 1640 (Sigma-Aldrich, Bornem, Belgium) containing 2 mM L-glutamine (Invitrogen, Merelbeke, Belgium), antibiotics (50 IU/mL penicillin and 50 μ g/mL streptomycin; Invitrogen), and 0.1% BSA (Sigma-Aldrich). The tissue was passed through a mesh to achieve comparable fragments. The tissue fragments (± 0.9 mm³) were weighed and resuspended as 0.04 g tissue/1 mL tissue culture medium.

Because SpA interacts with IgE V_H3⁺,¹⁰ the tissue was preincubated for 1 hour at 37°C 5% CO₂ with 1 μ g/mL human myeloma IgE (Calbiochem; VWR International, Leuven, Belgium). After 3 washing steps, the tissue fragments were resuspended in the appropriate amount of culture medium, and then the fragments were divided into a 48-well plate (BD Falcon; VWR International) filled with 0.5 mL tissue fragment suspension in each well.

In a following step, the tissue fragments (inferior turbinates, n = 13; NP, n = 12) were stimulated with culture medium (negative control) and 0.5 μ g/mL SEB (Sigma-Aldrich) for 30 minutes and 24 hours. A subgroup of patients (inferior turbinates, n = 8; NP, n = 8) also was stimulated with 10 μ g/mL SpA (Sigma-Aldrich) and 10 μ g/mL LTA (Sigma-Aldrich), both for 30 minutes and 24 hours.

After that, tissue fragments and supernatants were separated by centrifugation. Aliquots of the supernatants were taken and stored immediately at –20°C until analysis of cytokines and histamine, leukotriene (LT) C₄/D₄/E₄, and PGD₂.

Measurements of mediators in supernatants of stimulated tissue fragments

Concentrations of IL-1 β , TNF- α , IFN- γ , IL-2, IL-4, IL-5, IL-8, IL-10, IL-12p70, and IL-13 (2.4–10,000 pg/mL) were measured on tissue supernatants obtained after the *ex vivo* stimulations using Multi-spot assays (Meso Scale Discovery, Gaithersburg, Md) following the instructions of the manufacture. The plates were analyzed by using a Sector Imager 6000 (Meso Scale Discovery).

Concentrations of histamine (2.7–219 ng/mL), LTC₄/D₄/E₄ (0.0313–2 ng/mL), PGD₂ (2–250 pg/mL), and TGF- β 1 (7.8–1000 pg/mL) were measured using ELISA kits: histamine (IBL, Hamburg, Germany), LTC₄/D₄/E₄ (Oxford Biomedical Research, Nuclilab BV, Ede, The Netherlands), PGD₂ (Cayman Chemicals, Ann Arbor, Mich), and TGF- β 1 (R&D Systems Europe Ltd, Abingdon, United Kingdom) following the instructions of the manufacturer.

Measurement of IgE to SAEs mix in tissue homogenates

Snap-frozen tissue specimens were weighed, and 1 mL 0.9% NaCl solution was added per every 0.1 g tissue. The tissue was then homogenized with a mechanical homogenizer (B. Braun, Melsungen, Germany) at 1000 rpm for 5 minutes on ice as described previously. After homogenization, the suspension was centrifuged at 3000 rpm for 10 minutes at 4°C, and the supernatants were separated and stored at –80°C until analysis. All samples were assayed for IgE to SAEs (*Staphylococcus enterotoxin A*, C, and TSST-1; 0.35–100 kUA/L) by the UNICAP system (Pharmacia, Uppsala, Sweden).

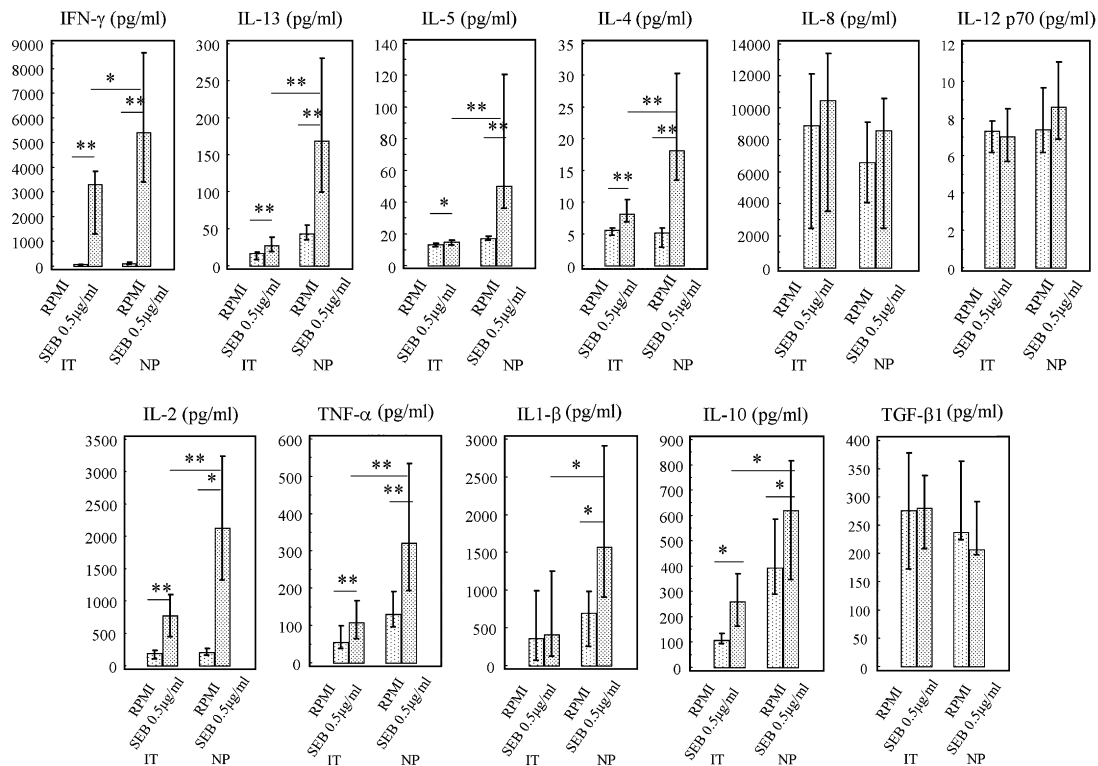


FIG 1. Effect of 24 hours SEB (0.5 μg/mL) stimulation compared with RPMI on IFN-γ, IL-13, IL-5, IL-4, IL-2, TNF-α, IL-1β, IL-10, IL-8, IL-12p70, and TGF-β1 release. Comparison between nasal polyps (NP; n = 12) and inferior turbinates (IT; n = 13). **P* < .05; ***P* < .01.

Immunohistochemistry

Cryostat sections were prepared (6 μm) and mounted on SuperFrost Plus glass slides (Menzel Glaeser, Braunschweig, Germany), packed in aluminium paper, and stored at -30°C until staining. Sections were immunohistochemically stained with the mouse mAb CD3 clone UCHT1 Z (Dako, Glostrup, Denmark) to compare the number of T cells in inferior turbinates and NP.

For immunohistochemical stainings, specimens were fixed in acetone. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in TBS containing 0.1% sodium azide for 20 minutes. Primary antibody or negative control, consisting of the corresponding isotype control, was incubated for 1 hour and detected by using the LSAB+ technique conjugated with peroxidase according to the manufacturer's instructions (labeled streptavidin-biotin, Dako). The peroxidase activity was detected using AEC Substrate chromogen (Dako), which results in a red-stained precipitate. Finally, sections were counterstained with hematoxylin and mounted.

The number of positive cells was analyzed by using a magnification of 400× and scored by 2 independent observers who did not know the diagnosis and clinical data. A grading scale from 0 to 3 was applied, ranging from absent to numerous stained cells. Score 0 represented no positive cells; score 1, <10 positive cells/field; score 2, 10 to 100 positive cells/field; and score 3, >100 positive cells/field. The analysis included all areas of the biopsy, and for each sample, 10 fields were scored.

Statistical analysis

Statistical analysis was performed by using the Wilcoxon test (for paired comparisons). The Mann-Whitney *U* test was used for between-group (unpaired) comparisons. *P* values of less than .05

were considered statistically significant. Results are expressed as medians ± interquartile ranges.

RESULTS

SEB stimulations

The inferior turbinates (n = 13) and the nasal polyp explants (n = 12) were stimulated for 30 minutes and for 24 hours with culture medium alone (RPMI) and SEB (0.5 μg/mL). SEB stimulation for 30 minutes did not increase the release of cytokines in comparison with culture medium alone in controls or NP (results not shown).

Twenty-four-hour SEB stimulation demonstrated a significant increase of T_H1 and T_H2 cytokines (IFN-γ, IL-2, IL-4, IL-5, IL-10, and IL-13) in inferior turbinates and NP compared with RPMI, but not for IL-8, IL-12p70, and TGF-β1. The release of these cytokines in NP was significantly higher compared with inferior turbinates for all measured cytokines, except for IL-8, IL-12p70, and TGF-β1. The proinflammatory cytokine IL-1β only demonstrated a significant increase in NP, and TNF-α showed a release pattern similar to the aforementioned cytokines (Fig 1).

After 24 hours in RPMI, a significantly higher release was noticed for IL-13, IL-5, TNF-α, and IL-10 in NP tissue (Table I). No difference in release could be found between the patients with and without allergy.

TABLE I. Concentrations of cytokines in supernatants after 24 hours in RPMI (spontaneous release)*

Cytokine	Inferior turbinates	Nasal polyps	P value
IFN- γ (pg/mL)	57.0 (44.3-68.2)	84.3 (61.5-136.0)	.1
IL-13 (pg/mL)	16.5 (8.6-18.2)	42.4 (35.1-54.9)	<.0001
IL-5 (pg/mL)	13.0 (12.0-14.1)	17.0 (16.1-18.7)	.0003
IL-4 (pg/mL)	5.5 (4.8-5.9)	5.1 (2.9-5.9)	.36
IL-2 (pg/mL)	186.2 (101.6-235.2)	202.5 (164.2-265.8)	.34
TNF- α (pg/mL)	54.2 (38.9-98.9)	129.0 (95.6-191.5)	.007
IL-1 β (pg/mL)	355.7 (67.4-989.2)	692.0 (253.5-981.5)	.29
IL-10 (pg/mL)	104.7 (93.5-134.8)	390.9 (288.6-584.7)	.0001
IL-8 (pg/mL)	8860.2 (2458.5-12,122.2)	6563.8 (4082.1-9105.4)	.27
IL-12p70 (pg/mL)	7.3 (6.2-7.9)	7.4 (6.2-9.7)	.41
TGF- β 1 (pg/mL)	275.5 (172.6-377.9)	236.7 (224.0-363.2)	.67

*Inferior turbinates (n = 13) versus nasal polyps (n = 12). Data are expressed as medians $\pm$ interquartile ranges.

TABLE II. Ratio of cytokine concentrations after SEB (0.5 μ g/mL) stimulation and RPMI for 24 hours*

Cytokine	Inferior turbinates	Nasal polyps	Ratio (nasal polyps/inferior turbinates)
IFN- γ	54.1	70	1.29
IL-13	3.6	4.5	1.25
IL-5	1.2	4.3	3.58
IL-4	2	5.8	2.9
IL-2	5.6	12.3	2.2
TNF- α	1.8	2.7	1.5
IL-1 β	1.5	2.3	1.53
IL-10	2.6	1.5	0.58
IL-8	1.3	1.2	0.92
IL-12p70	1.2	2	1.67
TGF- β 1	1.1	0.8	0.73

*Inferior turbinates (n = 13) versus nasal polyps (n = 12).

The cryostat sections were stained for CD3 and semi-quantitatively scored. No difference in the number of T lymphocytes could be found between inferior turbinates and NP (results not shown).

A ratio was calculated between the concentrations of cytokines in culture medium and after SEB stimulation, both for NP and inferior turbinates, and compared with each other (Table II). Strikingly, the relative increase in cytokine release in NP was highest (above 2) for IL-5, IL-4, and IL-2, but lowest (0.58) for IL-10 and TGF- β 1 (0.73).

S aureus enterotoxin B did not show an effect on mast cell-derived cytokines; after 30 minutes (Fig 2, A) and 24 hours of stimulation (Fig 2, B), no increase could be found for histamine, LTC₄/D₄/E₄, and PGD₂ in either group. None of the samples were positive for IgE to SAEs (results not shown).

SpA and LTA stimulations

Thirty-minute SpA stimulation induced a significant increase of histamine, LTC₄/D₄/E₄, and PGD₂ compared with culture medium in inferior turbinates and NP (Fig 2, A). However, no increase of T_H1/T_H2 cytokines or proinflammatory cytokines was measured after SpA and

LTA short-time stimulation in comparison with culture medium alone. After 24-hour stimulation with SpA, the production of cysteinyl leukotrienes (cysLTs) remained significantly increased in inferior turbinates and NP compared with culture medium (Fig 2, B). Furthermore, IL-5 was significantly increased in NP, and IL-13 demonstrated an increasing trend that did not reach significance (Fig 3).

Stimulation with LTA for 30 minutes and 24 hours did not induce any increase in T_H1/T_H2 or proinflammatory cytokines (results not shown), nor in histamine, LTC₄/D₄/E₄, and PGD₂ (Fig 2, A and B).

DISCUSSION

We show here that staphylococcal products have different effects on nasal mucosal samples: SpA after 30 minutes resulted in the early release of mast cell mediators including histamine, LTC₄/D₄/E₄, and PGD₂, whereas SEB after 24 hours induced a late-phase release of numerous immunoregulatory and proinflammatory cytokines, favoring T_H2 cytokines and disfavoring IL-10 and TGF- β 1 in nasal polyps.

There is increasing evidence that the colonization of *S aureus* and the release of its cell products may be linked to the inflammation in NP.^{3,7} In NP, increased rates of *S aureus* colonization were found,⁷ and sIgE against SAEs was more frequently present in NP versus controls and correlated with higher levels of IL-5, eotaxin, and eosinophil cationic protein.² With the role of *S aureus* as disease modifier suggested, a number of diagnostic and therapeutic approaches such as antibiotic treatment or *S aureus* vaccination may be considered. The potential effect of *S aureus* eradication in sinus disease has not been studied yet, but large-scale, double-blind, placebo-controlled studies are currently ongoing. However, in atopic dermatitis, the role of *S aureus* and the use of antibiotic treatment have already been established. Antimicrobial treatment leads to a significant, albeit temporary, improvement of atopic dermatitis in patients who are colonized with *S aureus*.¹⁶

S aureus secretes several enterotoxins with superantigen activity. Superantigens induce large-scale stimulation

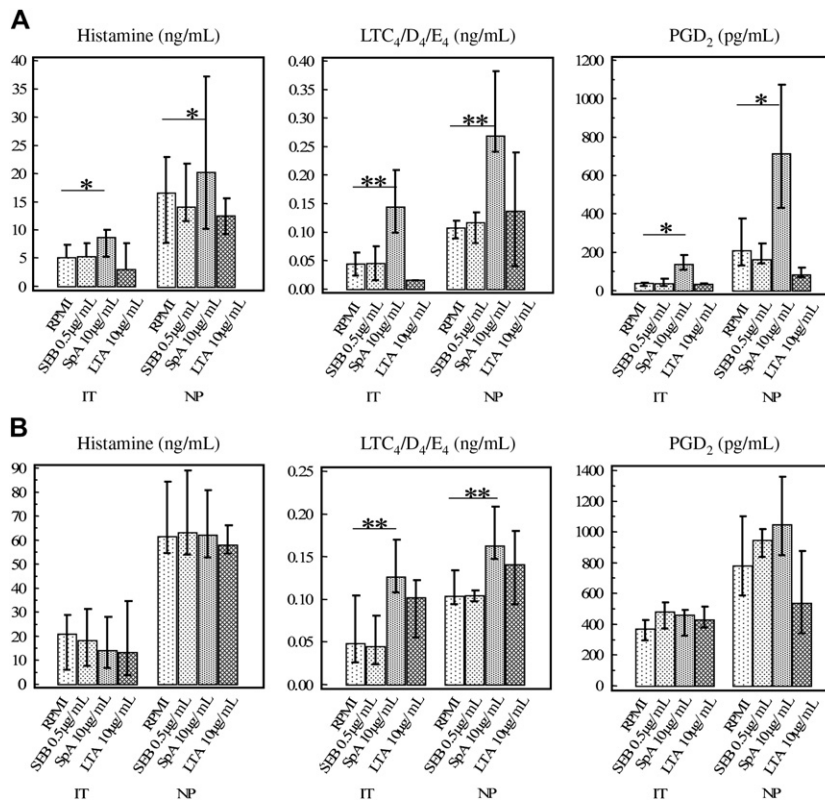


FIG 2. A, Effect of 30-minute stimulation with RPMI (baseline), SEB (0.5 μ g/mL), SpA (10 μ g/mL), and LTA (10 μ g/mL) on histamine, LTC₄/D₄/E₄, and PGD₂ release in nasal polyps (NP; n = 8) and inferior turbinates (IT; n = 8). **B**, Effect of 24-hour stimulation with RPMI (baseline), SEB (0.5 μ g/mL), SpA (10 μ g/mL), and LTA (10 μ g/mL) on histamine, LTC₄/D₄/E₄, and PGD₂ release in nasal polyps (NP; n = 8) and inferior turbinates (IT; n = 8). * P < .05; ** P < .01.

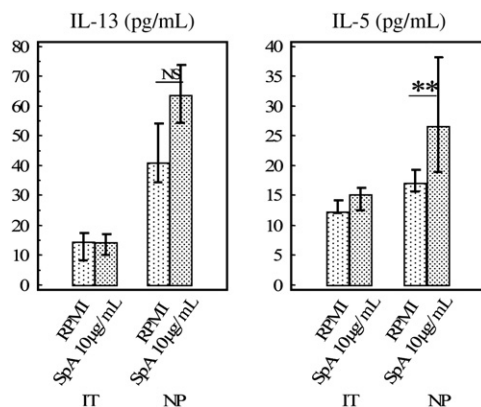


FIG 3. Effect of 24-hour stimulation with SpA (10 μ g/mL) compared with RPMI on IL-13 and IL-5 in inferior turbinates (IT; n = 8) and nasal polyps (NP; n = 8). ** P < .01; NS, not significant.

of T lymphocytes by a mechanism distinct from conventional antigen presentation, involving direct class II major histocompatibility complex binding and stimulation of TCR families based on V β gene usage.^{17,18} In this study, 24-hour stimulation with SEB induced a remarkable mean increase of IFN- γ (54 times and 70 times more for inferior

turbinates and NP, respectively, compared with culture medium) and a substantial mean increase of IL-2 (5.6 times for inferior turbinates and 12.3 times for NP). However, this release is not reflected in tissue concentrations of patients with chronic nasal polyposis. A recent study of our group revealed significantly higher IL-5 protein concentrations (used as a T_H2 marker) in NP homogenates versus controls, whereas IFN- γ (a T_H1 marker) did not demonstrate any difference.¹ In line with these results, we have demonstrated, in the supernatants of tissue fragments cultured for 24 hours with medium alone, a significantly higher expression of IL-5 in NP compared with inferior turbinates (P = .0003), but no difference in IFN- γ expression (P = .1). Furthermore, when stimulated with SEB, the cytokine production was further skewed to IL-5, IL-4, and IL-2, but not to IFN- γ , in NP. Concomitantly, the production of IL-10 and TGF- β 1 decreased, indicating a possible lack in T-cell regulation induced by SEB.

In other inflammatory diseases, similar effects of SEB were described. PBMCs from patients with active atopic eczema/dermatitis syndrome or asthma and non-atopic controls secreted increased levels of IL-5, IL-4, IL-13, and IFN- γ in response to SEB. Only IL-5 and IL-13 were significantly higher in active atopic

eczema/dermatitis syndrome or asthma compared with nonatopic controls.^{19,20}

S aureus enterotoxin B not only has effects on T lymphocytes but also may affect directly the eosinophil activity by upregulating cell surface expression of antigens and by inhibiting the eosinophil apoptosis.²¹ Furthermore, SEB induced IL-12p40 production in peritoneal mice macrophages,²² and a culture of corneal epithelial cells has been shown to release IL-8 after treatment with SEB.²³ In this study, neither IL-12p70 nor IL-8 was upregulated after SEB stimulation in inferior turbinates and NP. However, similar *ex vivo* studies are required because results of animal studies cannot necessarily be projected onto human beings.

By measuring mediators such as histamine, LTC₄/D₄/E₄, and PGD₂, the responses of specific cells, such as mast cells, were analyzed. No increase was measured for these mediators in inferior turbinates and NP after 30 minutes and 24 hours of stimulation, which may demonstrate the lack of direct effect of SEB in releasing mast cell mediators. In line with our results, SEB was not shown to release histamine from a human mast-cell line (HMC-1) and led to a dose-dependent inhibition of IL-4 release.²⁴ Other studies, however, demonstrated opposite results. Peripheral blood basophils from patients with atopic eczema stimulated with SEB secreted significantly higher amounts of histamine and leukotriene C₄ than peripheral blood basophils from healthy controls,²⁵ and in rodent mast cell cultures, serotonin was released after SEB stimulation.²⁶ Because none of the patients were positive for sIgE against SAEs, we could not demonstrate here the conventional allergen-mediated reaction in mast cells, basophils, and FcεR-bearing cells after SEB stimulation. In a previous report, it was demonstrated that isolated basophils released histamine in response to SEB only when patients had sIgE against SEB.²⁷ The role of IgE and its functionality need to be further studied.

In contrast, stimulations with SpA, which is a surface protein on *S aureus*, demonstrated an increase of histamine, LTC₄/D₄/E₄, and PGD₂. Marone et al⁹ described an increased histamine release after SpA stimulation in basophils and in human heart mast cells.¹⁰ SpA has a classic site that binds to Fcγ, a constant region of IgG,²⁸ and an alternative site that binds the Fab portion of human polyclonal IgM, IgA, IgG, and IgE.²⁹ SpA's releasing activity is mediated by interaction with the commonly expressed V_H3 region of IgE, bound to the FcεRI.¹⁰ The concept of the classic superantigens (SAEs and TSST-1) applied to the pathophysiology of allergic disorders led to the definition of "superallergens" to indicate proteins of various origins able to activate FcεRI⁺ cells by interacting with membrane-bound IgE.²⁹ Our results support the "allergenic" effect of SpA, because, in contrast with SEB, inferior turbinate and nasal polyp tissue stimulated with SpA did give a significant increase of histamine, LTC₄/D₄/E₄, and PGD₂ after 30 minutes. Because colonization of *S aureus* is present in 63.6% of subjects with NP, with rates as high as 66.7% and 87.5% in the subgroups with asthma and aspirin sensitivity compared with rates of 33.3% in

controls,⁷ not only SEB but also SpA may be relevant in the contribution of the ongoing inflammation in NP.

No increase of T_H1/T_H2 cytokines or proinflammatory cytokines was measured after SpA stimulation in comparison with culture medium alone, except for IL-5 in NP after 24 hours, again emphasizing the limited influence of SpA on T cells.

Finally, stimulations with LTA were performed, because LTA is known to be important in the interaction of *S aureus* with human nasal epithelium cells.⁸ LTA stimulation on nasal tissue did not induce any increase of T_H1/T_H2 cytokines, proinflammatory cytokines, or mast-cell-derived mediators, clearly reflecting the limited role of LTA in the inflammatory scene in NP. In future, similar experiments may be performed to approach the role of other agents such as viruses, fungi, and atypical bacteria in inducing inflammation in nasal mucosal tissue.

In conclusion, these results support the hypothesis that *S aureus* may be linked to the inflammation in NP. Its enterotoxin SEB is able to induce the release of T_H1/T_H2-derived and proinflammatory cytokines in nasal tissue, with significantly higher release in NP compared with controls. The production is in favor of T_H2 cytokines such as IL-5, IL-4, and IL-2 and disfavored IL-10 and TGF-β1. SEB does not appear to have any allergenic effect in nasal tissue. In contrast, SpA, a surface protein of *S aureus*, has clearly different properties and is able to increase histamine, LTC₄/D₄/E₄, and PGD₂ release in nasal tissue, which demonstrates its allergenic effect and may therefore be an additional factor in causing or exacerbating the inflammation in NP.

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