Staphylococcus aureus Enterotoxin B Regulates Prostaglandin E$_2$ Synthesis, Growth, and Migration in Nasal Tissue Fibroblasts

Claudina A. Pérez-Novo,[1] **Anouk Waeytens,**[2] **Cindy Claeys,**[1] **Paul Van Cauwenberge,**[1] **and Claus Bachert**[1]

[1]Upper Airways Research Laboratory, Department of Otorhinolaryngology, and [2]Department of Pathology, Ghent University Hospital, Belgium

Background. Superantigens and eicosanoids are important amplifiers and regulators of inflammation in airway diseases. We therefore studied the possible influence of *Staphylococcus aureus* enterotoxin B (SEB) on the cyclooxygenase (COX) pathway and basic functions of airway structural cells.

Methods. Fibroblasts were isolated from nasal inferior turbinate tissue and cultured in the presence of different concentrations of SEB. Preincubation with interferon (IFN)−γ was performed to induce expression of major histocompatibility complex (MHC) class II receptors. Prostaglandin E2 (PGE$_2$) production was assayed by enzyme-linked immunosorbent assay, and levels of COX-2 and prostanoid E receptors 1−4 (EP$_{1-4}$) were assayed by real-time polymerase chain reaction. Migration and growth tests were performed, and SEB was localized within the cells by confocal microscopy.

Results. Stimulation with IFN-γ and SEB significantly down-regulated PGE$_2$, COX-2, and EP$_2$ expression but not EP$_1$, EP$_3$, or EP$_4$ expression. The enterotoxin blocked cell growth but increased the fibroblast migration rate. SEB was localized within the cell in the presence and absence of MHC-II, suggesting that mechanisms other than conventional binding may allow the enterotoxin to enter the cell.

Conclusions. These findings may have major implications for our understanding of the role played by bacterial superantigens in regulating the inflammatory and remodeling mechanisms of upper airway diseases and hence may help elucidate the pathophysiology of these diseases.

Fibroblasts are important sentinel cells of the immune system, playing a critical role in chronic inflammation and tissue repair. However, the implication of these cells in the regulation of immune responses has long been neglected. Recently, several studies have demonstrated that human lung fibroblasts activated through the CD40-CD40L bridge may up-regulate prostaglandin E2 (PGE$_2$) production [1] and, hence, hematopoietic cells infiltration in damaged tissue [2]. This interaction activates the transcription factor NF-κB, resulting in the synthesis of high levels of cytokine mediators (e.g., interleukin [IL]−6 and IL-8), adhesion molecules (e.g., intercellular and vascular cell adhesion molecules), and cyclooxygenase (COX) metabolites [3]. In the airways, fibroblasts play a crucial role in the remodeling process. These cells are a primary cellular source of collagens and growth factors, which play a crucial role in epithelial regeneration and tissue remodeling processes [4].

The potential role played by superantigens in the pathogenesis of airway diseases has become a relevant issue during the last years [5]. Furthermore, it is known that prostanoids, metabolites of arachidonic acid, are crucial molecules in the control of inflammatory and remodeling processes in airways [6, 7]. We have previously demonstrated that immune response to *Staphylococcus aureus*, in terms of IgE specific to *S. aureus* enterotoxins, correlates with total IgE levels and severity of inflammation in patients with nasal polyposis [8, 9]. Additionally, this immune response to *S. aureus* enterotoxins was associated with an amplification of the eosinophilic inflammation

and an up-regulation of leukotrienes and lipoxins within nasal polyp tissue [9], which is also characterized by reduced PGE_2 concentrations [7].

Additionally, in vitro experiments have shown that *S. aureus* enterotoxins can regulate the secretion of interstitial collagenase and stromelysin in human fibroblastlike synoviocytes [10, 11], and these cells can function as accessory cells for enterotoxin-mediated T cell activation [12]. More recently, it was demonstrated that *S. aureus* enterotoxins can induce IL-6 and IL-8 gene expression on human fibroblastlike synoviocytes isolated from patients with rheumatoid arthritis [13]. Production of PGE_2 can also be enhanced by these superantigens in bovine mononuclear cells [14]. Until now there have been no studies regarding the role played by *S. aureus* superantigens on structural cells from the upper airways. Given the previous findings, we aimed to study the capacity of *S. aureus* enterotoxin B (SEB) in regulating the COX pathway and vital cell processes, such as growth and migration, in nasal tissue fibroblasts.

MATERIALS AND METHODS

Samples. Fibroblasts were isolated from inferior turbinate tissue obtained from patients ($n = 5$) who were undergoing septoplasty or rhinoseptoplasty because of anatomical variations and were not suffering from any sinus disease. Surgery was performed in the Department of Othorinolaryngology at Ghent University Hospital. All patients gave informed consent before their participation, and the study was approved by the ethical committee of Ghent University Hospital.

Reagents. Dulbecco's PBS, penicillin-streptomycin (penicillin, 5000 IU/mL; streptomycin, 5000 μg/mL), and trypan blue (0.4% solution in PBS) were obtained from Invitrogen. Minimum essential medium (MEM), Opti-MEM I reduced serum medium, L-glutamine (200 mmol/L), trypsin-EDTA ($1\times$), and fetal bovine serum (FBS) (qualified; origin, United States) were purchased from Gibco (Life Technologies). Ultroser G (5%) was obtained from Biospera. Recombinant human interferon (IFN)–γ was obtained from R&D Systems, and SEB was obtained from Sigma Chemicals. SYBR Green I Master mix, Aurum Total RNA, and Script cDNA synthesis kits were obtained from Bio-Rad Laboratories.

Fibroblast isolation from nasal tissue. Nasal tissues obtained during surgical operations were rinsed several times with Opti-MEM I supplemented with 5% FBS, 5% Ultroser G, 2 mmol/L glutamine, and penicillin (50 IU/mL)–streptomycin (50 μg/mL) and cut into small pieces ($\sim$1 mm^2). Diced specimens were then plated (density, 9 pieces/6-well tissue culture dish) and incubated in a humidified atmosphere containing 5% CO_2 at 37°C, until a monolayer of fibroblastlike cells was observed to be confluent. Then the explanted tissues were removed, and cells were trypsinized and replated into 250-cm^2 tissue culture Falcon tubes at a final volume of 5 mL. The medium was changed every 3 days for 2–3 weeks until 90% confluence was obtained. Subsequently, the cells were split and passaged.

Cell stimulation. Cells were stimulated with 0.01, 0.1, or 1 μg/mL SEB or 10 ng/mL IL-1β for 24 h at 37°C in 5% CO_2. To induce receptors for major histocompatibility complex (MHC) class II, cells were pretreated with IFN-γ (10 ng/mL) for 24 h at 37°C in 5% CO_2 and were then stimulated with 0.01, 0.1, or 1 μg/mL SEB for 24 h at 37°C and 5% CO_2. For each sample in each experiment, we used a negative control that consisted of cells incubated only with culture medium (MEM).

The cells were characterized with antibodies against vimentine, cytokeratin, and α–smooth muscle actin, using the peroxidase–anti-peroxidase technique. Stimulated cell cultures were centrifuged at 4°C, and the supernatants were collected and stored at -20°C until use. Subsequently, cells were trypsinized, washed, resuspended in Opti-MEM I, and then centrifuged. After the supernatants were removed, a portion of the cells were resuspended in lysis buffer for posterior RNA extraction, and another portion was stored at -80°C.

Enzymatic immunoassays. Concentration of PGE_2 were measured by ELISAs in cell culture supernatants according to the manufacturer's instructions (Oxford Biomedical); the intra- and interassay coefficient of variation for all ELISAs was <10%.

Quantitative real-time polymerase chain reaction (PCR). Cell cultures were centrifuged, and pellets containing 1×10^6 cells were disrupted and homogenized in lysis solution. Total RNA was extracted using the Aurum Total RNA Mini Kit, and cDNA was synthesized from 1 μg of RNA using the Script cDNA Kit, in accordance with the manufacturer's instructions. Amplification reactions were performed with the iCycler iQ Real-Time PCR Detection System, with the specific primer sets (table 1). PCRs included 20 ng of cDNA from cultured cells, $1\times$ SYBR Green I Master mix, and 300 nmol/L each primer. The PCR protocol consisted of 1 cycle at 95°C for 10 min followed by 40 cycles at 95°C for 30 s and at 62°C for 1 min. Ubiquitin C and hypoxanthine phosphoribosyltransferase 1 were used as internal control genes for normalization. Primer sequences for these genes were obtained from the Real-Time PCR Primer and Probe Database of the Department of Medical Genetics, University of Ghent (http://medgen.ugent.be/rtprimerdb). The relative number of molecules for each gene was expressed in relative expression units quantified per 20 ng of cDNA sample and was determined by the $\Delta\Delta$Ct value method.

Cell growth assay. Confluent cells in 25-cm^2 Falcon dishes were trypsinized with preheated trypsin-EDTA and resuspended in 5 mL of Opti-MEM I. Living cells were counted with a Neubauer counting chamber, seeded (10,000 cells/well) in a 24-well plate, and incubated with Opti-MEM I, IFN-γ (10 ng/mL), or SEB (0.1 μg/mL) at 37°C and 5% CO_2 for 24, 48, and 72 h. At each time point, cells were again trypsinized with preheated trypsin-EDTA and resuspended in 1 mL of Opti-MEM I, and the

Table 1. Primer sequences used for real-time polymerase chain reaction amplification.

Gene	Primer (5'→3')		Amplicon size, bp	GenBank accession no.
	Forward	Reverse		
HLA-DR1α	CATCAAGGGAGTGCGCA	CTCCATGTGCCTTACAGAGGC	64	NM_019111
COX-2	GCTGGAACATGGAATTACCCA	CTTTCTGTACTGCGGGTGGAA	88	XM_051899
EP$_1$	GATGGTGGGCCAGCTTGTC	GCCACCAACACCAGCATTG	73	NM_000955
EP$_2$	GACCGCTTACCTGCAGCTGTAC	TGAAGTTGCAGGCGAGCA	73	NM_000956
EP$_3$	AAGGCCACGGCATCTCAGT	TGATCCCCATAAGCTGAATGG	76	NM_000957
EP$_4$	ACGCCGCCTACTCCTACATG	AGAGGACGGTGGCGAGAAT	63	NM_000958

NOTE. COX-2, cyclooxygenase 2; EP$_{1-4}$, prostanoid E receptors 1–4.

number of living cells at each time point was quantified with trypan blue using the Neubauer counting chamber.

Cell migration assay. Fibroblasts were seeded in a 35-mm-diameter 6-well plate, incubated at 1×10^5 cells/well in serum-free MEM, and allowed to grow until confluency for 7 days. Then the medium was replaced by 1× Dulbecco's PBS, and cells were incubated at 37°C in 5% CO_2 for 5 min. The monolayer was wounded by pressing a sterile razor blade down onto the well, making a sharp visible demarcation at the wound edge. The blade was then gently moved to one side to remove a part of the cell monolayer. Two separated 15–20-mm wounds were made in the same well. The wounded layers were washed 2 times with Opti-MEM to remove the cell debris and incubated with Opti-MEM I, IL-1β (10 ng/mL), IFN-γ (10 ng/mL), or SEB (0.1 μg/mL) at 37°C and 5% CO_2. Migrating cells reaching a line (L2) situated 240 μm from the culture wound after 24 (L2D1) and 48 h (L2D2) were counted using a counting eyepiece with grid graticule (10 × 10 mm). The first line of the grids falls together with the sharp beginning demarcation of the wound. Ten places were counted in each wound.

Cellular localization of SEB. Cellular localization of SEB was assayed by immunofluorescence confocal microscopy. Nasal tissue fibroblasts were cultured with 0.1 μg/mL SEB, with or without IFN-γ (10 ng/mL), on poly-D-lysine–coated glass slides for 24 h at 37°C in 5% CO_2. The slides were then air dried for 24 h at room temperature and stored at −20°C. At the time of staining they were fixed in acetone for 10 min at 4°C and blocked using 0.4% fish-skin gelatine/1× PBS for 2 h at room temperature. The cells were then incubated with rabbit polyclonal antibody anti-SEB (1:500; Sigma) for 1 h and with the secondary antibody anti-rabbit Alexa Fluor 526 (1:2000; Molecular Probes) for 1 h at room temperature; both primary and secondary antibodies were diluted in 0.4% fish-skin gelatine/1× PBS. After nuclear staining, 4',6-diamidino-2-phenylindole (DAPI; Molecular Probes) was added for 5 min, and the slides were mounted in Vectashield mounting medium (Vector Laboratories). Cellular localization of the enterotoxin was evaluated using a Bio-Rad Radiance 2100 laser scanning confocal microscope system with a 4-line argon primary laser, a green helium-neon laser, and a blue laser diode. Image analysis was performed using Adobe Photo-shop (version 7.0) and Confocal Assistant (version 4.02, Todd Clark Brelje) software programs.

Statistical analysis. All data were analyzed using MedCalc software (version 6.0) and are presented as medians and inter-quartile ranges. Data were compared within different patient subgroups by use of the Kruskal-Wallis test (*H* test). The Wilcoxon test for unpaired samples (or Mann-Whitney *U* test) was applied to evaluate the statistical differences between patient groups. Our criterion for significance was $P \le .05$.

RESULTS

Effect of SEB on PGE$_2$ production. Nasal tissue fibroblasts cultured in MEM in the absence of stimulus produced significant baseline concentrations of mRNA of COX-2 and PGE$_2$. Stimulation with SEB without preincubation with IFN-γ did not induce any change in PGE$_2$ production or COX-2 mRNA expression. Induction of MHC-II molecule expression in fibroblasts by stimulation with IFN-γ was verified by real-time PCR, demonstrating HLA-DR1α gene expression in all IFN-γ–stimulated cells compared with nonstimulated cells (figure 1). Furthermore, preincubation with IFN-γ led to a significant decrease
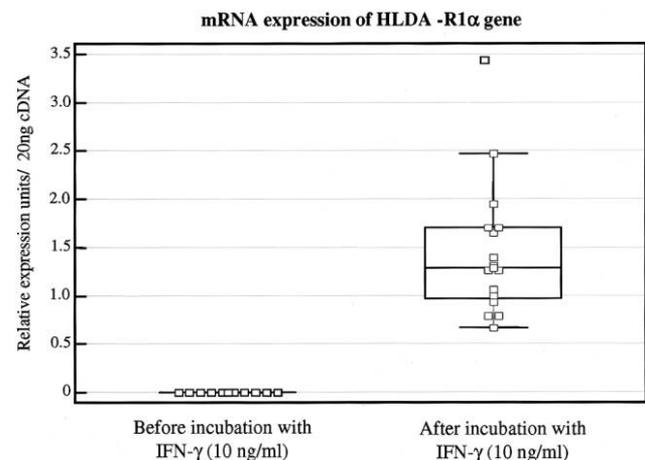

Figure 1. Box-and-whisker plots representing the expression levels of the HLA-DRα gene in nasal tissue fibroblasts before and after incubation with interferon (IFN)–γ (10 ng/mL).

a

COX-2 mRNA
in inferior turbinate nasal tissue fibroblasts

b

PGE$_2$ concentrations
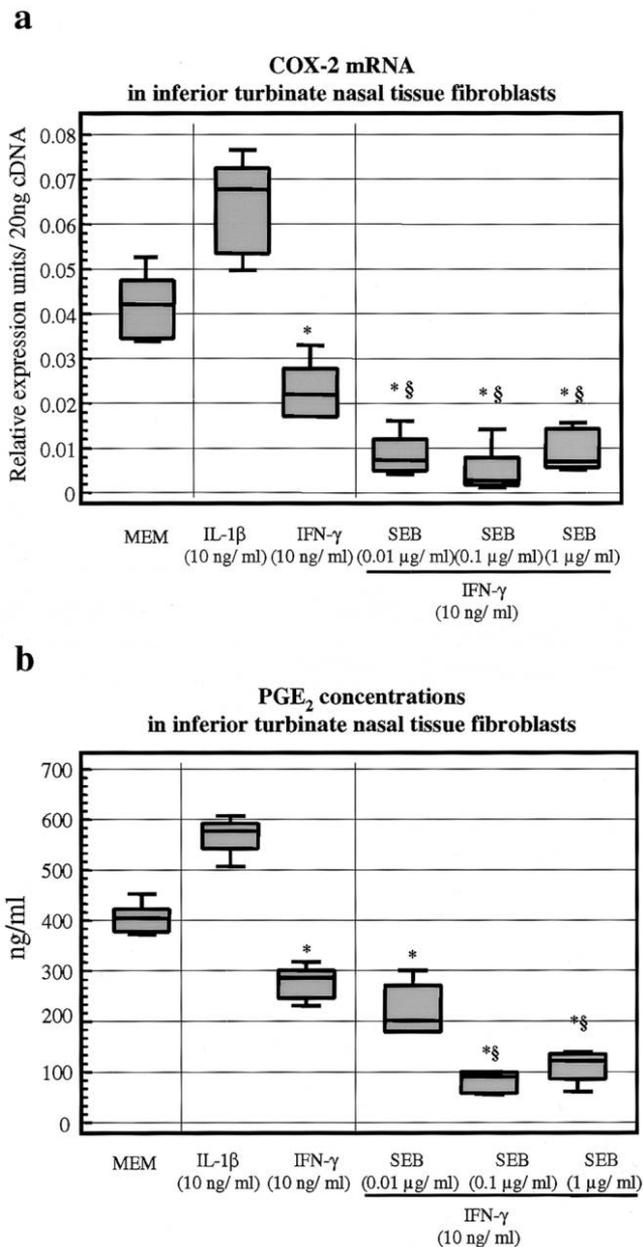**in inferior turbinate nasal tissue fibroblasts**

Figure 2. mRNA levels of cyclooxygenase (COX)–2 *(a)* and concentrations of prostaglandin E2 (PGE$_2$) *(b)* in nasal tissue fibroblasts after stimulation with interleukin (IL)–1β (10 ng/mL), interferon (IFN)–γ (10 ng/mL), or *Staphylococcus aureus* enterotoxin B (SEB; 0.01, 0.1 or 1 μg/mL). *Significant difference compared with cells incubated with minimum essential medium (MEM); §significant difference compared with cells stimulated with IFN-γ (10 ng/mL) ($P < .05$, Mann-Whitney *U* test for independent samples).

in COX-2 mRNA (figure 2*a*) and endogenous PGE$_2$ (figure 2*b*) compared with nonstimulated cells. Interestingly, this down-regulation was amplified after incubation with SEB at 0.1 and 1 μg/mL in a dose-independent manner. In addition, incubation with IL-1β significantly increased the expression of COX-2 and PGE$_2$ release. Furthermore, expression of the transcripts expressing the genes encoding for prostanoid E receptors (EP$_1$,

EP$_2$, EP$_3$, and EP$_4$) was analyzed. Real-time PCR results showed no expression of EP$_4$ receptor in nasal fibroblasts (data not shown). SEB did not induce any change in the expression of EP$_1$ (figure 3*a*) and EP$_3$ (figure 3*c*) receptors; however, the enterotoxin down-regulated the EP$_2$ receptor after preincubation with IFN-γ, and this effect was dose dependent (figure 3*b*).

Effect of SEB on growth and migration. Results of the growth test were expressed as numbers of living cells after 24, 48, and 72 h. The number of cells per stimulus was compared with the number of cells cultured only in MEM, which were considered to be controls for normal growth. Cells in MEM showed a significant increase in proliferation after 72 h. The same pattern was observed in cells stimulated with IFN-γ or IL-1β (figure 4). When cells were incubated with SEB, however, cell proliferation was inhibited. This process was IFN-γ independent and was counterbalanced by coexposure with the cytokine. Furthermore, the cell migration capacity was not changed by incubation with IFN-γ or SEB. After preincubation with IFN-γ, however, SEB significantly induced this process (figure 5).

Cellular localization of SEB. Cellular localization of SEB was assayed by confocal microscopy. Confocal images of nasal fibroblasts after incubation with 0.1 μg/mL SEB for 24 h showed that the enterotoxin was located mainly in the cytoplasm but also partly in the nucleus of the cell (figure 6*c* and 6*d*), indicating spontaneous penetration into the cell. A different distribution was observed after preincubation with IFN-γ followed by stimulation with 0.1 μg/mL SEB (figure 6*e* and 6*f*). The enterotoxin was located mainly in the periphery of the cell cytoplasm and around the nucleus, suggesting the binding of SEB to MHC-II molecules on the cell surface.

DISCUSSION

Eicosanoid production is differentially regulated and correlates with the degree of inflammation in patients with nasal polyps with and without an immune response (specific IgE antibodies) to *S. aureus* enterotoxins [9]. PGE$_2$ plays an important role in the regulation of tissue repair events during airway diseases, and this mechanism may be influenced by triggers such as viral or bacterial infections. Therefore, we aimed to investigate whether staphylococcal enterotoxins may affect the upper airway remodeling process and influence the eicosanoid pathway of nasal tissue fibroblasts.

In this study, we were able to demonstrate that the levels of endogenous PGE$_2$, COX-2 mRNA, and EP$_2$ receptor significantly decreased after stimulation with SEB in IFN-γ–pretreated cells but not in cells cultured without the cytokine, suggesting that this effect may be mediated by cross-linking with the MHC-II receptor. Incubation with IFN-γ was performed to induce the MHC-II molecules, which are normally not expressed in fibroblasts, and mimic the classic binding of superantigens to the cell. This cytokine has been demonstrated to induce the syn-
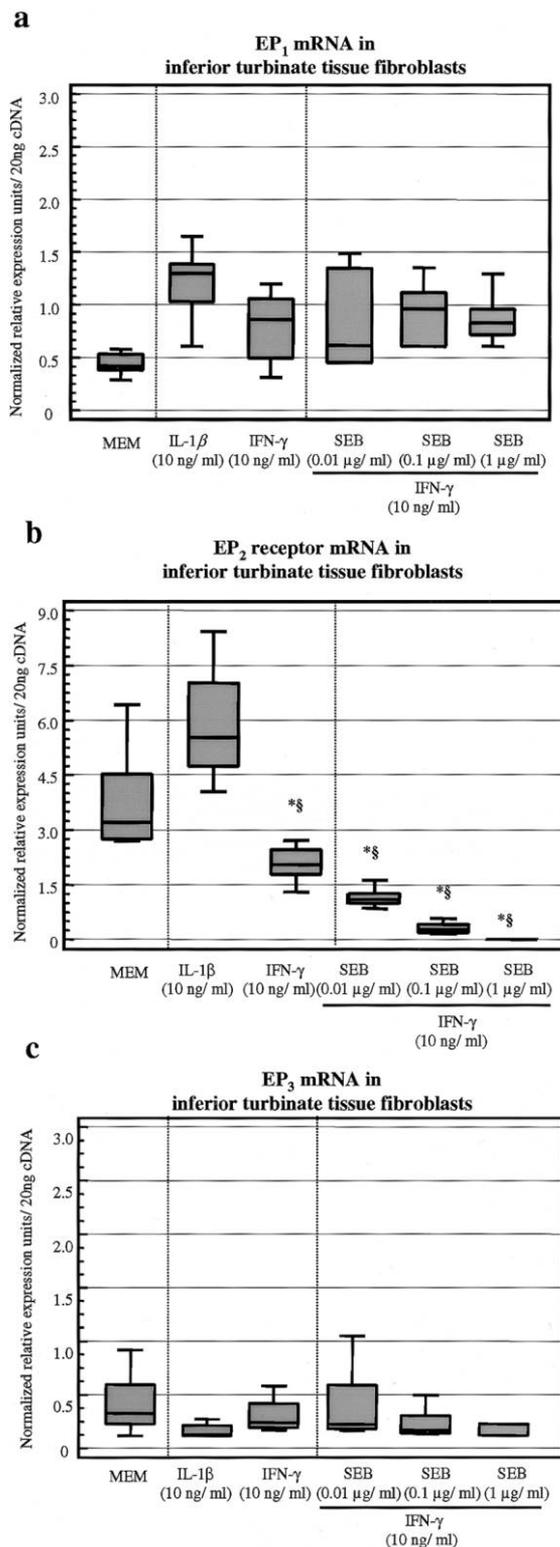
a

EP₁ mRNA in inferior turbinate tissue fibroblasts

b

EP₂ receptor mRNA in inferior turbinate tissue fibroblasts

c

EP₃ mRNA in inferior turbinate tissue fibroblasts

Figure 3. mRNA levels of prostanoid E (EP) receptors EP_1 *(a)*, EP_2 *(b)*, and EP_3 *(c)* in nasal tissue fibroblasts after stimulation with interleukin (IL)–1β (10 ng/mL), interferon (IFN)–γ (10 ng/mL), or *Staphylococcus aureus* enterotoxin B (SEB; 0.01, 0.1, or 1 μg/mL). *Significant difference compared with cells incubated with minimum essential medium (MEM); §significant difference compared with cells stimulated with IFN-γ (10 ng/mL) ($P <$ .05, Mann-Whitney U test for independent samples).

thesis and activation of cytosolic phospholipase A_2 (cPLA_2) protein in a human bronchial epithelial cell line [15]. However, it seems that nasal fibroblasts may react differently to the cytokine, because COX-2 and PGE_2 were significantly down-regulated compared with nonstimulated cells. Additionally, staphylococcal enterotoxins have been demonstrated to induce PGE_2 production and COX-2 and cPLA_2 expression in IFN-γ–treated fibroblastlike synoviocytes [10]. However, in nasal fibroblasts, SEB acted as an agonist of the cytokine by accentuating the blockage of the COX pathway.

There have been no previous studies showing the effect of superantigens on PGE_2 release in nasal fibroblasts. However, Clement et al. have demonstrated highly focalized intracellular reservoirs of *S. aureus* (including the nasal epithelium, glandular tissue, and myofibroblasts) in endonasal mucosa from patients with recurrent *S. aureus* rhinosinusitis [16]. These findings support our thinking that the presence of SEB in nasal tissue may exacerbate airway inflammation by down-regulating the production of anti-inflammatory mediators, such as PGE_2.

Moreover, staphylococcal superantigens may act as potent leukocyte and neutrophil chemoattractants [17]. In support of these findings, we have found that SEB significantly induced fibroblast migration. This effect seems to be mediated by the binding of MHC-II, because no effect was observed in the presence of the enterotoxin or IFN-γ alone. IFN-γ has been reported to increase the expression of both intercellular adhesion molecule 1 and vascular cell adhesion molecule in human lung fibroblasts [18], important regulators of cell adhesion and migration processes. Furthermore, it has been shown that SEB can induce T cell adhesion to endothelial cells (pretreated with IFN-γ) in an MHC-II–dependent mechanism [19], which may confirm the findings of this study. However, although it was not our current objective, it may be useful to evaluate the expression of cell migration–related molecules to learn more about the mechanisms modulating this process.

SEB blocked proliferation in the cell culture when it was applied in the absence of IFN-γ, and this effect may be restored by the cytokine. Although the effect of SEB on cell proliferation is unknown, the superantigen may block this process by cross-linking receptors other than MHC-II or simply by altering cell membrane permeability or activating molecules that regulate this cell function. Furthermore, PGE_2 has been reported as an important inhibitor of fibroblast proliferation and migration [20, 21]. However, analyzing our results, we suggest that the effect of SEB on fibroblast proliferation is not linked to PGE_2 regulation. On the contrary, the migration process seems to be influenced by the decrease in eicosanoid levels after SEB exposure. However, this can be confirmed only by performing experiments in the presence of PGE_2 and MHC-II antagonists, which should be the aim of further studies.
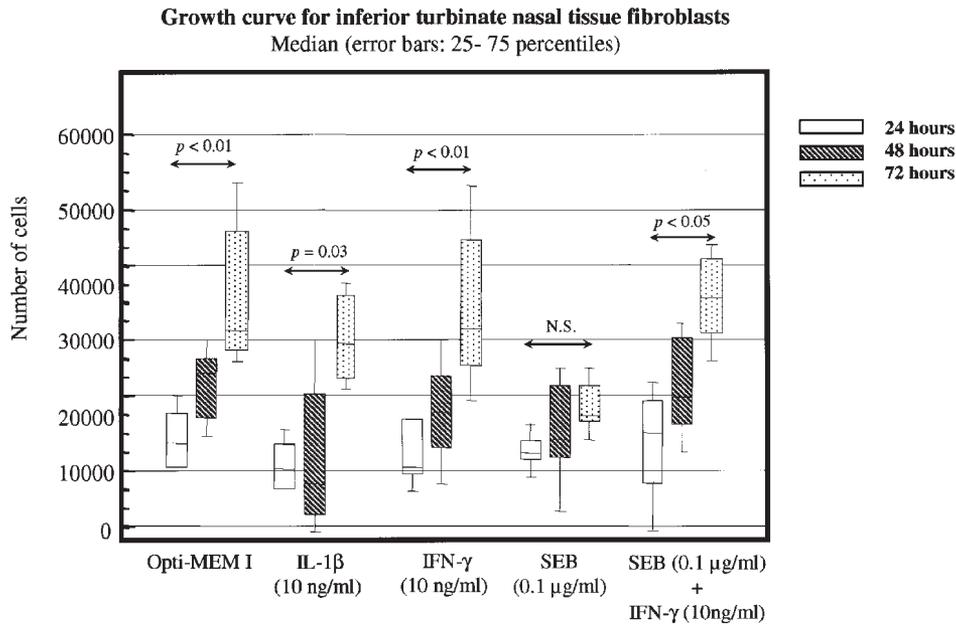
Growth curve for inferior turbinate nasal tissue fibroblasts
Median (error bars: 25- 75 percentiles)

Figure 4. Growth curve for nasal tissue fibroblasts showing the number of proliferated cells after incubation with interleukin (IL)–1β (10 ng/mL), interferon (IFN)–γ (10 ng/mL), or *Staphylococcus aureus* enterotoxin B (SEB; 0.1 μg/mL) for 24, 48, and 72 h. *P* values were determined by the Mann-Whitney *U* test for independent samples. N.S., not significant.

Finally, confocal microscopy showed the presence of SEB within cells with and without MHC-II receptors. These findings suggest that the enterotoxin may enter the cell via alternate mechanisms or receptors in cells that lack MHC-II or T cell receptors. Studies have shown that SEB may cross the epithelium by facilitated transcytosis [22], and the existence of alternative binding site proteins has been also reported in monkey kidney fibroblasts [23]. Our results indicate that the effect of SEB on human nasal fibroblast metabolism may depend on how the molecules enter the cell and may or may not depend on the classic superantigen-binding mechanism.
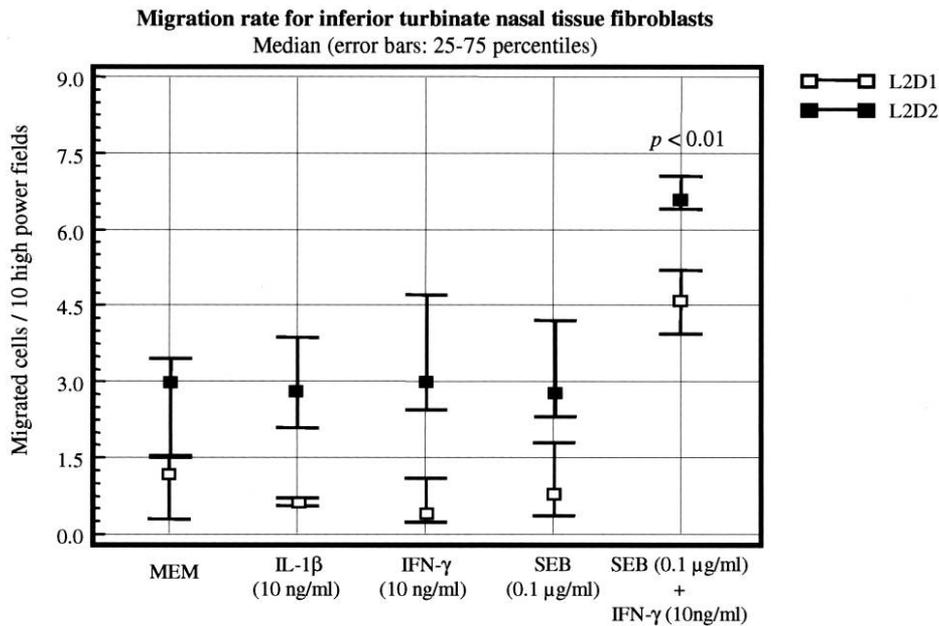
Migration rate for inferior turbinate nasal tissue fibroblasts
Median (error bars: 25-75 percentiles)

Figure 5. Migration test for nasal tissue fibroblasts after stimulation with interleukin (IL)–1β (10 ng/mL), interferon (IFN)–γ (10 ng/mL), or *Staphylococcus aureus* enterotoxin B (SEB; 0.1 μg/mL). Graph shows the number of cells migrating 240 μm from the wound site after 24 h (L2D1) and 48 h (L2D2). MEM, minimum essential medium.
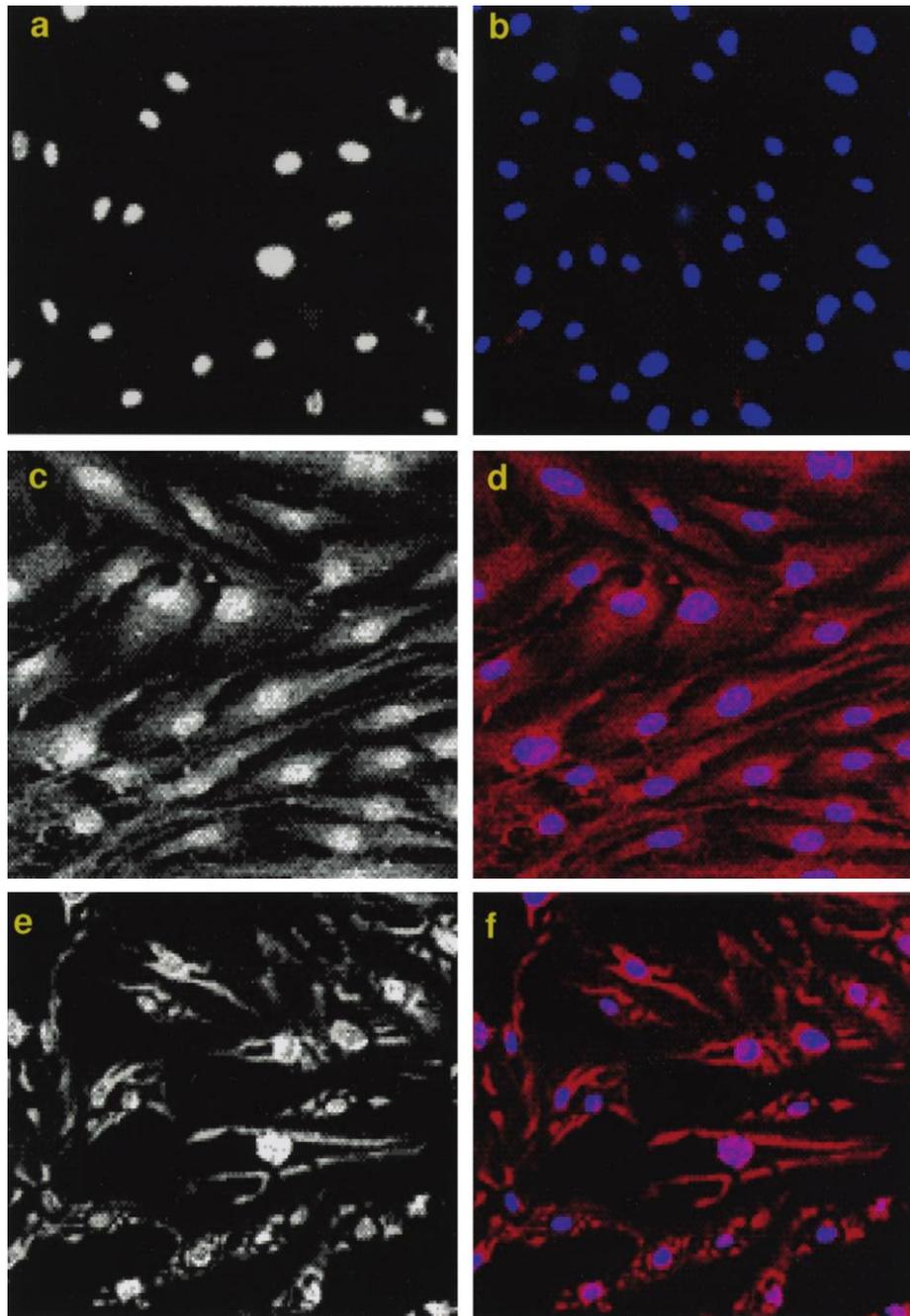
Figure 6. Confocal microscopic images (original magnification, ×40) of nasal tissue fibroblasts incubated with minimum essential medium (*a* and *b*), stimulated with *Staphylococcus aureus* enterotoxin B (SEB; 0.1 μg/mL) for 24 h (*c* and *d*), or preincubated with interferon-γ (10 ng/mL) and stimulated with SEB (0.1 μg/mL) for 24 h (*e* and *f*). Images show that SEB is internalized in the nasal fibroblasts and can be localized in the cytoplasm and in the nucleus of the cell.

In conclusion, SEB may down-regulate COX-2 gene expression and PGE_2 production in human nasal tissue fibroblasts, and this action seems to involve EP_2 but not EP_1, EP_3, or EP_4 receptors. Additionally, this enterotoxin may affect important basic cell functions, such as growth and migration. Determining the mechanism behind this action was not the objective of this study, but it seems that mechanisms other than the conventional binding via MHC-II allow the enterotoxin to enter the cell and influence its metabolism. The present study will be followed by experiments using PGE_2 and MHC-II inhibitors, which can provide more information on the regulatory mechanisms acting on these cells. Triggering of the arachidonic acid metabolism is one of the cellular events regulating the pro- and anti-inflammatory immune response in diseases. The findings of this study may have major implications for our understanding of the role played by bacterial superanti-

gens in regulating the inflammatory and remodeling mechanisms of upper airway diseases and, hence, help to elucidate their pathophysiology.

References

1. Buckley CD, Pilling D, Lord JM, Akbar AN, Scheel-Toellner D, Salmon M. Fibroblasts regulate the switch from acute resolving to chronic persistent inflammation. Trends Immunol **2001**; 22:199–204.
2. Zhang Y, Cao HJ, Graf B, Meekins H, Smith TJ, Phipps RP. CD40 engagement up-regulates cyclooxygenase-2 expression and prostaglandin E2 production in human lung fibroblasts. J Immunol **1998**; 160:1053–7.
3. Yellin MJ, Winikoff S, Fortune SM, et al. Ligation of CD40 on fibroblasts induces CD54 (ICAM-1) and CD106 (VCAM-1) up-regulation and IL-6 production and proliferation. J Leukoc Biol **1995**; 58:209–16.
4. Sacco O, Silvestri M, Sabatini F, Sale R, Defilippi AC, Rossi GA. Epithelial cells and fibroblasts: structural repair and remodelling in the airways. Paediatr Respir Rev **2004**; 5 (Suppl A):S35–40.
5. Van Zele T, Gevaert P, Watelet JB, et al. *Staphylococcus aureus* colonization and IgE antibody formation to enterotoxins is increased in nasal polyposis. J Allergy Clin Immunol **2004**; 114:981–3.
6. Vancheri C, Mastruzzo C, Sortino MA, Crimi N. The lung as a privileged site for the beneficial actions of PGE2. Trends Immunol **2004**; 25:40–6.
7. Perez-Novo CA, Watelet JB, Claeys C, van Cauwenberge P, Bachert C. Prostaglandin, leukotriene, and lipoxin balance in chronic rhinosinusitis with and without nasal polyposis. J Allergy Clin Immunol **2005**; 115:1189–96.
8. Perez-Novo CA, Kowalski ML, Kuna P, et al. Aspirin sensitivity and IgE antibodies to *Staphylococcus aureus* enterotoxins in nasal polyposis: studies on the relationship. Int Arch Allergy Immunol **2004**; 133:255–60.
9. Perez-Novo CA, Claeys C, Van Zele T, Holtapples G, van Cauwenberge P, Bachert C. Eicosanoid metabolism and eosinophilic inflammation in nasal polyp patients with immune response to *Staphylococcus aureus* enterotoxins. Am J Rhinol **2006**; 20:456–60.
10. Mehindate K, al Daccak R, Dayer JM, et al. Superantigen-induced collagenase gene expression in human IFN-gamma-treated fibroblast-like synoviocytes involves prostaglandin E2: evidence for a role of cyclooxygenase-2 and cytosolic phospholipase A_2. J Immunol **1995**; 155:3570–7.
11. Migita K, Eguchi K, Kawabe Y, et al. Superantigen-induced stromelysin production from rheumatoid synovial cells. Biochem Biophys Res Commun **1997**; 231:222–6.
12. Kraft M, Filsinger S, Kramer KL, Kabelitz D, Hansch GM, Schoels M. Synovial fibroblasts as target cells for staphylococcal enterotoxin-induced T-cell cytotoxicity. Immunology **1998**; 93:20–5.
13. Smith RS, Smith TJ, Blieden TM, Phipps RP. Fibroblasts as sentinel cells: synthesis of chemokines and regulation of inflammation. Am J Pathol **1997**; 151:317–22.
14. Hendricks A, Leibold W, Kaever V, Schuberth HJ. Prostaglandin E2 is variably induced by bacterial superantigens in bovine mononuclear cells and has a regulatory role for the T cell proliferative response. Immunobiology **2000**; 201:493–505.
15. Wu T, Levine SJ, Lawrence MG, Logun C, Angus CW, Shelhamer JH. Interferon-gamma induces the synthesis and activation of cytosolic phospholipase A_2. J Clin Invest **1994**; 93:571–7.
16. Clement S, Vaudaux P, Francois P, et al. Evidence of an intracellular reservoir in the nasal mucosa of patients with recurrent *Staphylococcus aureus* rhinosinusitis. J Infect Dis **2005**; 192:1023–8.
17. Miller EJ, Nagao S, Carr FK, Noble JM, Cohen AB. Interleukin-8 (IL-8) is a major neutrophil chemotaxin from human alveolar macrophages stimulated with staphylococcal enterotoxin A (SEA). Inflamm Res **1996**; 45:386–92.
18. Spoelstra FM, Postma DS, Hovenga H, Noordhoek JA, Kauffman HF. Interferon-gamma and interleukin-4 differentially regulate ICAM-1 and VCAM-1 expression on human lung fibroblasts. Eur Respir J **1999**; 14:759–66.
19. Kita M, Eguchi K, Kawabe Y, Tsukada T, et al. Staphylococcal enterotoxin B-specific adhesion of murine splenic T cells to a human endothelial cell line. Immunology **1996**; 88:441–6.
20. Saltzman LE, Moss J, Berg RA, Hom B, Crystal RG. Modulation of collagen production by fibroblasts: effects of chronic exposure to agonists that increase intracellular cyclic AMP. Biochem J **1982**; 204:25–30.
21. Kohyama T, Ertl RF, Valenti V, et al. Prostaglandin E_2 inhibits fibroblast chemotaxis. Am J Physiol Lung Cell Mol Physiol **2001**; 281:L1257–63.
22. Hamad AR, Marrack P, Kappler JW. Transcytosis of staphylococcal superantigen toxins. J Exp Med **1997**; 185:1447–54.
23. Rogers TJ, Guan L, Zhang L. Characterization of an alternative superantigen binding site expressed on a renal fibroblast cell line. Int Immunol **1995**; 7:1721–7.