

# Relationship of nasal carriage of *Staphylococcus aureus* to pathogenesis of perennial allergic rhinitis

Teruo Shiomori, MD,<sup>a</sup> Shin-ichi Yoshida, MD, PhD,<sup>b</sup> Hiroshi Miyamoto, MD, PhD,<sup>c</sup>  
and Kazumi Makishima, MD, PhD<sup>a</sup> Kitakyushu and Fukuoka, Japan

**Background:** Several studies have previously shown some factors that modify the pathogenesis of perennial allergic rhinitis (PAR). However, the association between bacterial colonization and PAR has not been well understood.

**Objective:** We sought to study the association between superantigen-producing *Staphylococcus aureus* and PAR.

**Methods:** *S aureus* colonization in the nasal cavity and its superantigen production were studied in 65 patients with PAR and 45 nonallergic control subjects. The nasal symptom scores of the patients were evaluated. Furthermore, we examined the response to staphylococcal enterotoxin B or toxic shock syndrome toxin 1 of peripheral blood lymphocytes from both patients and control subjects by measuring proliferative responses and production of cytokines (IFN- $\gamma$ , IL-4, and IL-5). **Results:** The rate of nasal carriage of *S aureus* in the patients (44%) was significantly higher than that of the control subjects (20%,  $P < .01$ ). Moreover, the rate of nasal carriage of superantigen-producing *S aureus* in the patients (22%) was significantly higher than that of the control subjects (6.7%,  $P < .05$ ). The nasal symptom scores of the *S aureus*-positive patients were significantly higher than those of the *S aureus*-negative patients ( $P < .05$ ), although there was no significant association between symptom scores and superantigen production. Peripheral blood lymphocytes from the patients showed significantly higher proliferative responses and were more likely to produce  $T_{H2}$  type cytokines in response to superantigens ( $P < .01$ ), but the responses were not different among the patients with *S aureus* carriage and superantigen production.

**Conclusion:** This study suggests that PAR leads to a higher carriage rate of *S aureus*, and nasal carriage of *S aureus* may aggravate PAR. (J Allergy Clin Immunol 2000;105:449-54.)

**Key words:** Perennial allergic rhinitis, *Staphylococcus aureus* colonization, superantigen,  $T_{H2}$  type cytokine

### Abbreviations used

|         |                                      |
|---------|--------------------------------------|
| PAR:    | Perennial allergic rhinitis          |
| PBL:    | Peripheral blood lymphocyte          |
| RPLA:   | Reversed passive latex agglutination |
| SE:     | Staphylococcal enterotoxin           |
| SI:     | Stimulation index                    |
| TN:     | Total nitrogen                       |
| TSST-1: | Toxic shock syndrome toxin 1         |

Allergic rhinitis is an inflammation of the nasal mucosa that is characterized by the infiltration of inflammatory cells and the release of inflammatory mediators. Although the pathophysiologic mechanism of allergic rhinitis is not well understood, there has recently been much interest in the role of cytokines in allergic inflammatory reactions.<sup>1-3</sup> Numerous in vitro studies have suggested that  $T_{H2}$  type cytokines play an important role in the pathogenesis of allergic inflammatory diseases.<sup>1,3,4</sup> IL-4 is required for the production of IgE by inducing an isotype switch in B cells, and this function of IL-4 is inhibited by IFN- $\gamma$ .<sup>5</sup> IL-5 stimulates the growth, differentiation, and survival of eosinophils.<sup>6</sup>

Factors that modify the pathogenesis of allergic rhinitis have been reported to be environmental factors (temperature, desiccation, and air pollution),<sup>7</sup> sex hormones,<sup>8</sup> stress, and alcohol. Although for other atopic disorders, including bronchial asthma and atopic dermatitis, it has been reported that bacteria could play a role in the pathogenesis of allergic inflammatory diseases,<sup>9-12</sup> there are few reports regarding the association between bacterial colonization and allergic rhinitis.<sup>13,14</sup> To our knowledge, no data can be found in the literature concerning nasal bacterial flora, such as *Staphylococcus aureus*. *S aureus* is a representative constituent of bacterial flora in the nasal cavity and a common pathogen. It produces several exotoxins with superantigenic properties, which have been shown to induce inflammatory reactions.<sup>15</sup>

In this study, to gain insight into bacterial association, we examined the nasal carriage of *S aureus* and its superantigen production. Furthermore, we investigated the in vitro proliferative response and the production of IFN- $\gamma$ , IL-4, and IL-5 in response to stimulation with staphylococcal enterotoxin (SE) B and toxic shock syndrome

From the Departments of Otorhinolaryngology<sup>a</sup> and Microbiology,<sup>c</sup> University of Occupational and Environmental Health, School of Medicine, Kitakyushu, Japan; and the Department of Bacteriology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.<sup>b</sup>

Received for publication July 19, 1999; revised Oct 6, 1999; accepted for publication Oct 29, 1999.

Reprint requests: Teruo Shiomori, MD, Department of Otorhinolaryngology, University of Occupational and Environmental Health, School of Medicine, Yahatanishi-Ku, Kitakyushu, 807-8555, Japan.

Copyright © 2000 by Mosby, Inc.

0091-6749/2000 \$12.00 + 0 1/104256

doi: 10.1067/mai.2000.104256

toxin 1 (TSST-1) in peripheral blood lymphocytes (PBLs) from patients with perennial allergic rhinitis (PAR) and nonallergic control subjects.

## METHODS

### Patients

Forty-five nonallergic healthy volunteers (control group) and 65 patients with PAR (allergic group) caused by house dust mites (*Dermatophagoides pteronyssinus* or *Dermatophagoides farinae*) were enrolled in this study. The mean age of the subjects was 27.6 years (range, 6–68 years; 8 children and 37 adults) in the control group and 25.4 years (range, 8–66 years; 10 children and 55 adults) in the allergic group. Peripheral venous blood was collected from 34 patients with PAR (24 with nasal carriage of *S aureus* [positive group] and 10 without nasal carriage of *S. aureus* [negative group]) and 15 nonallergic control subjects.

All subjects in the allergic group satisfied the following criteria: (1) well-documented history of PAR for at least a year; (2) no history of asthma, atopic dermatitis, eczema, and urticaria; (3) no seasonal deterioration during pollen season; (4) no chronic sinusitis of the paranasal sinus and no previous sinus surgery, as determined by history and radiography; (5) no nasal polyposis; (6) positive intracutaneous skin test response (wheal diameter, >5 mm) to house dust mite (concentration 1:1000 wt/vol, total nitrogen [TN] value of 0.66 µg/mL) glycerinated extracts (Torii Co Ltd, Tokyo, Japan) but negative skin test responses to other major allergens in Japan: Japanese cedar (concentration 1:1000 wt/vol, TN value 0.62 µg/mL), orchard grass (concentration 1:1000 wt/vol, TN value 3.426 µg/mL), and short ragweed (concentration 1:1000 wt/vol, TN value 3.35 µg/mL) glycerinated extracts (Torii Co Ltd; a volume of 0.02 mL of each antigen was used for intracutaneous skin tests); (7) eosinophilia (>5% of cells) in nasal smears; (8) no medical treatment, including antibiotics and topical steroids, for nasal symptoms during the 4 weeks before enrollment; (9) no infection, including upper respiratory infection, during the 4 weeks before enrollment; and (10) no previous immunotherapy.

The healthy volunteers in the control group had negative skin test responses and had no history of allergic rhinitis, asthma, atopic dermatitis, eczema, and urticaria.

Informed consent was obtained from all adult patients participating in this study and from the parents of all children in this study. This study was approved by an institutional review board.

### Study for nasal carriage of *S aureus*

Nasal culturing was performed on the first day of the collection of daily symptom cards. Bacterial samples of the subjects were taken as previously described<sup>16</sup> by using sterile cotton swabs moistened with sterilized saline solution and rotated with gentle pressure around each nostril, including the inferior turbinate. The samples were inoculated on Salt Egg Yolk Agar plates (Nissui Pharmaceutical Co, Ltd, Tokyo, Japan). The plates were incubated at 37°C for 48 hours. After the incubation, identification of *S aureus* was done with the standard microbiological method, including Gram staining, growth on salt egg yolk agar, and agglutination by protein A and clumping factor.<sup>17</sup>

### Detection of superantigenic staphylococcal exotoxins

Three *S aureus* strains were examined in each allergic patient and each control subject. After preliminary growth on the agar plates, staphylococcal cultures were transferred to brain heart infusion broth and cultured aerobically at 37°C for 20 hours by shaking.<sup>18–20</sup> The culture supernatant was centrifuged at 900g for 20

minutes and sterilized by filtration (pore size, 0.45 µm). The reversed passive latex agglutination (RPLA) for detection of staphylococcal toxin, enterotoxins, and TSST-1 was carried out by using SET-RPLA and TST-RPLA kits (Denka Seiken Co, Ltd, Tokyo, Japan), as previously described.<sup>19–21</sup> Briefly, latex particles coated with specific rabbit IgG against staphylococcal enterotoxins (SEA, SEB, SEC, and SED) and TSST-1 were added to the culture supernatants diluted from 2 to 256 times in 96-well V-type bottomed plates left for 16 hours at room temperature and then inspected for agglutination. The sensitivity of the kits was approximately 2 ng/mL staphylococcal enterotoxins or TSST-1.

### Clinical evaluation

The nasal symptom scores (maximum of 9) were evaluated according to the criteria of Okuda et al<sup>22</sup> by using daily symptom cards. In brief, 53 patients were asked to complete daily symptom cards during the 14 days before the assessment of nasal symptom scores. Some patients (12/65) were not evaluated by symptom score because we did not ask them detailed questions about their condition, and they did not arrange return visits. The daily symptom card included the number of sneezing attacks, the number of nose blows, and the degree of nasal obstruction. A careful survey of the daily card graded the 3 nasal symptoms (sneeze, rhinorrhea, and obstruction) on a scale of 0–3 (0, none; 1, mild; 2, moderate; and 3, severe) according to severity. The average daily scores of the sum total of the 3 scores were used as nasal symptom scores.

### Cell culture and stimulation by superantigens

PBLs were separated from heparinized venous blood by means of density gradient centrifugation with a Lymphoprep Tube (NYCOMED; Pharma AS, Oslo, Norway) and washed 3 times in sterile PBS. PBLs were cultured at a concentration of  $1 \times 10^6$ /mL in RPMI-1640 medium (Daigo) supplemented with heat-inactivated 10% FBS (56°C for 30 minutes; Gibco Laboratories, Grand Island, NY), 2 mmol/L L-glutamine, and 50 µg/mL kanamycin sulfate. The cultures were incubated in both 24-well and 96-well, flat-bottomed, culture plates (FALCON; Becton Dickinson, Franklin Lakes, NJ) at 37°C in a humidified atmosphere with 5% CO<sub>2</sub>. The cultures were then stimulated with SEB or TSST-1 (Toxin Technology, Inc, Sarasota, Fla) at concentrations ranging from 1 to 0.0001 µg/mL or left unstimulated for both proliferation assay and cytokine analysis. The supernatants were collected after 48 hours of culture and stored at –20°C until assayed.

### PBL proliferation assay

PBLs ( $2 \times 10^5$  per 0.2 mL of culture medium) were added in triplicate to 96-well, flat-bottomed, culture plates. After 72 hours of SEB or TSST-1 stimulation, thymidine incorporation was assessed by pulsing with 1 µCi of tritiated thymidine (Amersham Plc, Buckinghamshire, UK) per cell during the last 16 hours of culture. Cells were harvested onto glass-fiber filter paper, and radioactivity was measured by using a liquid scintillation counter (LSC-3100; Aloka Co, Tokyo, Japan). The stimulation index (SI) was calculated by the ratio of tritiated thymidine incorporation (cpm) of the mean of the stimulated/unstimulated sample.

### Cytokine production

The amounts of IFN-γ, IL-4, and IL-5 in the cell culture supernatants from 24-well, flat-bottomed, culture plates were measured by commercially available sandwich ELISA kits (Cytoscreen; Biosource International, Camarillo, Calif), with a lower limit of sensitivity of 4 pg/mL (IFN-γ), 3 pg/mL (IL-4), and 4 pg/mL (IL-5). The acquired optical density at 450 nm was adjusted to picograms per milliliter by using our standard curve. All samples were run in duplicate.

**TABLE I.** Nasal carriage rate of *S aureus* and symptom scores

|                                                    | Allergic patients (n = 65) |                         | Control subjects (n = 45) |          |
|----------------------------------------------------|----------------------------|-------------------------|---------------------------|----------|
|                                                    | Positive                   | Negative                | Positive                  | Negative |
| Nasal carriage of <i>S aureus</i>                  | 29 (45%)*                  | 36                      | 9 (20%)                   | 36       |
| Carriage of superantigen-producing <i>S aureus</i> | 14 (22%)*                  | —                       | 3 (6.7%)                  | —        |
| Symptom score                                      | 5.88 ± 1.80<br>(n = 24)    | 4.76 ± 1.77<br>(n = 29) |                           |          |

\*Significant difference versus control.

## Statistical analysis

Data are expressed as the means ± SEM. Statistical significance was determined by using the Mann-Whitney *U* test, the  $\chi^2$  test, or the 1-way ANOVA test. Significant differences were accepted when *P* values were less than .05.

## RESULTS

### The carriage rate of *S aureus* in nasal cavity

*S aureus* was isolated from nose swabs in 29 (45%) of 65 patients with PAR and 9 (20%) of 45 control subjects (Table I). The rate of patients with PAR was found to be significantly higher than that of the control subjects, as determined by using the  $\chi^2$  test ( $P < .01$ ).

### Superantigenic exotoxins produced by *S aureus*

A  $\chi^2$  test showed that the rate of nasal carriage of *S aureus*-producing superantigenic exotoxins of patients with PAR was 21.5% (14/65), which was significantly higher than the 6.7% (3/45) found in the control subjects ( $P < .05$ , Table I). About half of the patients (14/29) were colonized by *S aureus* strains producing one or more exotoxins, whereas 33% (3/9) of *S aureus* from the control subjects produced detectable exotoxins. All of the culture supernatants were agglutinated after dilution up to 256 times. The rates of culture supernatants containing the individual superantigenic exotoxins were 13% for SEA, 54% for SEB, 13% for SEC, and 20% for TSST-1 in patients with PAR. The rate of SEB was the highest among the enterotoxins. Therefore the ensuing experiments were performed by using SEB and TSST-1 as representative *S aureus* toxins.

### Clinical evaluation

The nasal symptom scores of the 53 patients studied ranged from 2 to 9, with a mean of 5.26. The nasal symptom scores were significantly higher in the *S aureus*-positive groups (n = 24; mean ± SD, 5.88 ± 1.80) compared with the *S aureus*-negative groups (n = 29; 4.76 ± 1.77;  $P = .01$ ; Table I). The nasal symptom scores of the nasal carriers of *S aureus*-producing SEB (n = 8), nasal carriers of *S aureus*-producing superantigenic exotoxins other than SEB (n = 6), and nasal carriers of *S aureus* not producing superantigenic exotoxins (n = 10) were 5.86 ± 1.13, 5.83 ± 2.32, and 5.90 ± 1.97, respectively. There was no significant difference between the nasal symptom scores of the 3 groups.

## Proliferative responses of PBLs

The effects of SEB and TSST-1 on the proliferation of PBLs from patients with PAR (n = 34) and control subjects (n = 15) were examined (Fig 1, A). The proliferation of PBLs from both patients and control subjects increased in a dose-dependent fashion. When stimulated with 1  $\mu$ g/mL SEB and TSST-1, the SI of PBLs of patients with allergic rhinitis (SI: SEB, 97.7 ± 25.9; TSST-1, 91.3 ± 26.5) displayed a significantly greater proliferation than that of the normal control subjects (SI: SEB, 55.3 ± 3.9; TSST-1, 52.3 ± 4.2;  $P < .01$ ). However, there was no significant difference in tritiated thymidine incorporation between the *S aureus*-positive and *S aureus*-negative allergic groups at any toxin concentration (data not shown).

### IFN- $\gamma$ production from PBLs after SEB and TSST-1 stimulation

As shown in Fig 1, B, SEB and TSST-1 induced a dose-dependent increase in IFN- $\gamma$  production by PBLs obtained from normal control subjects over the range of concentrations tested. Also, after stimulation with SEB and TSST-1 at 1  $\mu$ g/mL, 29.1 ± 9.0 and 29.3 ± 7.8 ng/mL IFN- $\gamma$ , respectively, was measured in the allergic group. PBLs from patients with PAR produced significantly less IFN- $\gamma$  than that from the control subjects in response to SEB and TSST-1 ( $P < .01$ ). However, there was no significant difference in IFN- $\gamma$  production between the *S aureus*-positive and *S aureus*-negative allergic groups at any toxin concentration (data not shown).

### IL-4 and IL-5 production from PBLs after SEB and TSST-1 stimulation

As shown in Figs 1, C and D, SEB and TSST-1 induced a dose-dependent increase in IL-4 and IL-5 production by PBLs from normal control subjects over the range of concentrations tested. At 0.01, 0.1, and 1  $\mu$ g/mL SEB and TSST-1, PBLs from patients with PAR produced significantly higher levels of IL-4 than that from control subjects ( $P < .01$ ). Similar results were obtained in the case of IL-5 (Fig 1, D). However, there were no significant differences in IL-4 and IL-5 production between the *S aureus*-positive and *S aureus*-negative allergic groups at any toxin concentration (data not shown).

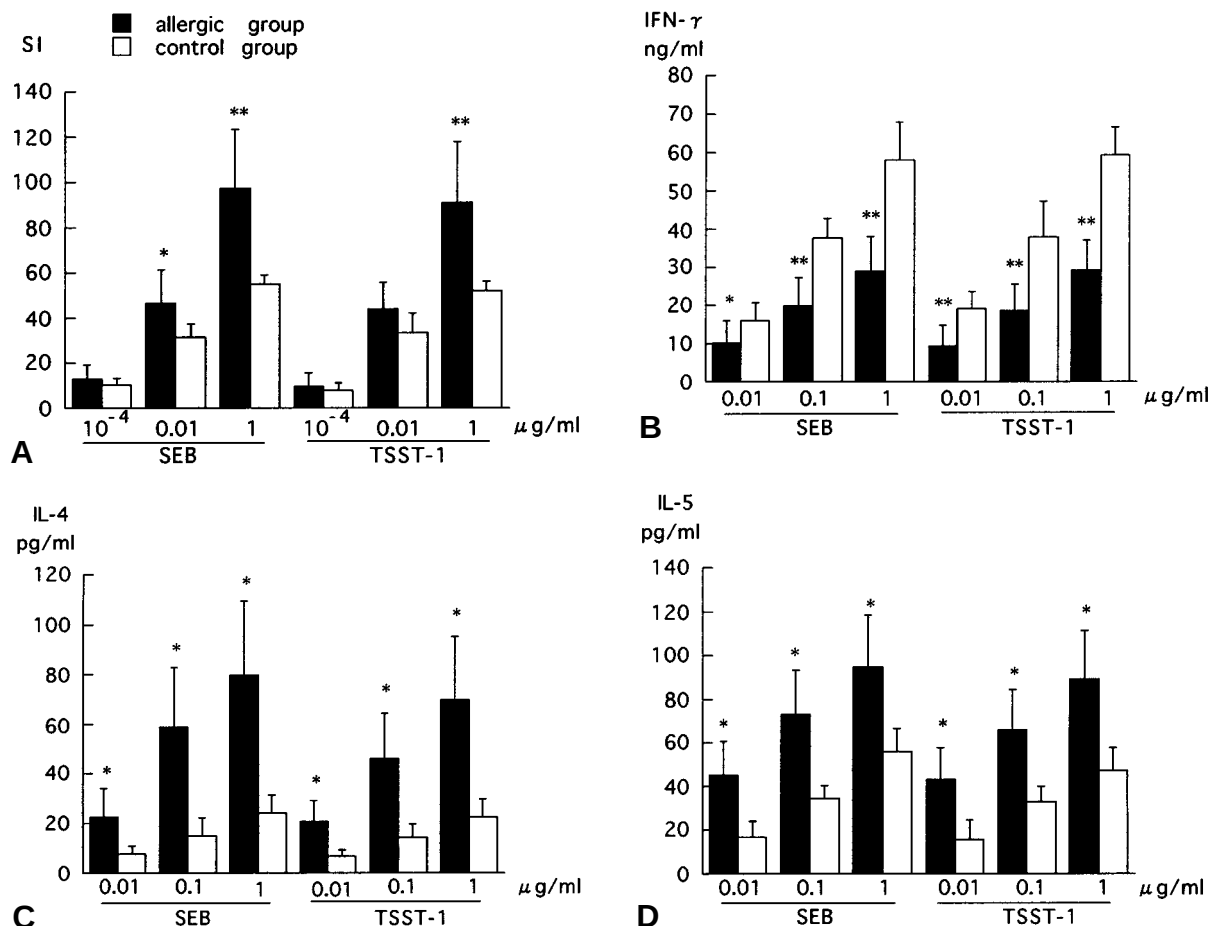


**FIG 1.** Effects of SEB and TSST-1 on tritiated thymidine incorporation of PBLs (**A**), IFN- $\gamma$  production (**B**), IL-4 production (**C**), and IL-5 production (**D**) from PBLs from allergic patients ( $n = 34$ ) and normal control subjects ( $n = 15$ ). Statistically significant differences are indicated, as determined by the Mann-Whitney  $U$  test. Paired values of allergic patients and control subjects are compared at each SEB and TSST-1 concentration. **A**, Lymphocyte proliferation. SI was calculated by the ratio of tritiated thymidine incorporation (cpm) of mean stimulated/unstimulated samples. All samples were done in triplicate (\* $P < .05$ , \*\* $P < .01$ ). **B**, IFN- $\gamma$  production (\* $P < .05$ , \*\* $P < .01$ ). **C**, IL-4 production (\* $P < .01$ ). **D**, IL-5 production (\* $P < .01$ ).

### Cytokine production by PBLs from allergic patients after SEB and TSST-1 stimulation for the nasal carriage of *S aureus*-producing SEB

Fig 2 shows the productions of IFN- $\gamma$  (Fig 2, A), IL-4 (Fig 2, B), and IL-5 (Fig 2, C) by PBLs from 34 patients with PAR and 15 control subjects. The allergic patients were divided into 4 groups as follows: nasal carriers of *S aureus*-producing SEB ( $n = 8$ ); nasal carriers of *S aureus*-producing superantigenic exotoxins other than SEB ( $n = 6$ ); nasal carriers of *S aureus* not producing superantigenic exotoxins ( $n = 10$ ); and negative nasal carriers of *S aureus* ( $n = 10$ ). PBLs from all subjects in all groups showed cytokine (IFN- $\gamma$ , IL-4, and IL-5) production to SEB and TSST-1, but not all cytokine levels were elevated. However, the differences in the cytokine productions were significant between the allergic and control groups as mentioned above.

### DISCUSSION

Although several studies have previously shown some factors that modify the pathogenesis of allergic rhinitis,<sup>7,8</sup> the association between bacterial colonization and allergic rhinitis has not been well documented.<sup>13,14</sup> For other atopic disorders, including bronchial asthma and atopic dermatitis, there have been many reports on the association with bacteria.<sup>10-12,23,24</sup>

In this study we focused on a representative bacterial flora of the nasal cavity and showed the existence of a higher carriage rate of *S aureus* (45%) in patients with PAR in comparison with control subjects (20%). Furthermore, half of the allergic patients were colonized by *S aureus*, producing identifiable superantigenic exotoxins, enterotoxins, and TSST-1. The colonization of *S aureus* in the nasal cavity varies according to the population studied. Previous studies have reported that the mean carriage rate in the general population is 37.2%, with a range of 19.0% to 55.1%.<sup>25</sup> There are many factors that may influ-

ence the rate of *S aureus* nasal carriage. The adherence of the bacteria is mediated by receptors on the cell wall for fibronectin<sup>26</sup> and laminin<sup>27</sup> present on the mucosal epithelial cells. The allergic nasal mucosa may provide better nutrition and less host defense. The increased *S aureus* carriage rate in PAR may be partly due to increased hand-to-nose contact caused by blowing, picking, or rubbing. However, the reason for the higher carriage rates in patients with PAR is unclear in detail.

The nasal symptom scores were significantly higher in the *S aureus*-positive groups compared with the *S aureus*-negative groups ( $P = .0103$ ). This observation suggests that the nasal carriage of *S aureus* in PAR may have an influence on the pathogenesis of allergic rhinitis or may give rise to infectious rhinitis symptoms. However, there was no significant difference between the nasal symptom scores of the nasal carriage of *S aureus*-producing superantigenic exotoxins group and the nasal carriage rate of the group with *S aureus* not producing superantigenic exotoxins. It has been reported that SEB and TSST-1 lead to a marked release of cytokines.<sup>15</sup> However, our results suggested that production of superantigens from *S aureus* colonizing in the nasal cavity does not influence the symptoms or otherwise that *S aureus* in the nasal cavity may not produce superantigenic exotoxins in situ. This suggests that other staphylococcal products may also be involved in PAR. Further studies are also needed to confirm whether *S aureus* produces superantigens in vivo in patients with PAR. Nevertheless, our finding of enhanced proliferation to SEB and TSST-1 of PBLs from patients with PAR supports a potential role for SEB and TSST-1 in the pathogenesis of allergic rhinitis. It has been known for many years that T cells from patients with allergic disorders respond with a reduced tritiated thymidine incorporation to mitogens (eg, PHA or concanavalin A).<sup>28</sup> In contrast to these findings, stimulation of PBLs with the potent T-cell stimuli SEB and TSST-1 resulted in a significantly increased T-cell proliferation in patients with PAR compared with cells from control subjects. We found that PBLs from patients with PAR produced lower amounts of IFN- $\gamma$  and larger amounts of IL-4 and IL-5 after stimulation with SEB and TSST-1.  $T_H2$  type cytokines, IL-4 and IL-5, are known to induce the IgE switching activity of B cells and eosinophilia.<sup>1-3</sup> These findings suggested that staphylococcal superantigens activated T cells and produced  $T_H2$  type cytokines in PBLs of patients with allergic rhinitis and therefore could promote local inflammation contributing to the lesions of allergic rhinitis. Our findings were similar to those reported regarding atopic dermatitis.<sup>29-31</sup>

As shown in Fig 2, we divided allergic patients into 4 groups according to superantigen production and examined cytokine productions from PBLs from 34 patients with PAR and 15 normal control subjects. PBLs from all subjects in all groups showed elevated cytokine production to SEB and TSST-1, with no significant differences between the groups. When TSST-1 was added to PBLs of allergic patients with nasal carriage of *S aureus*-produc-

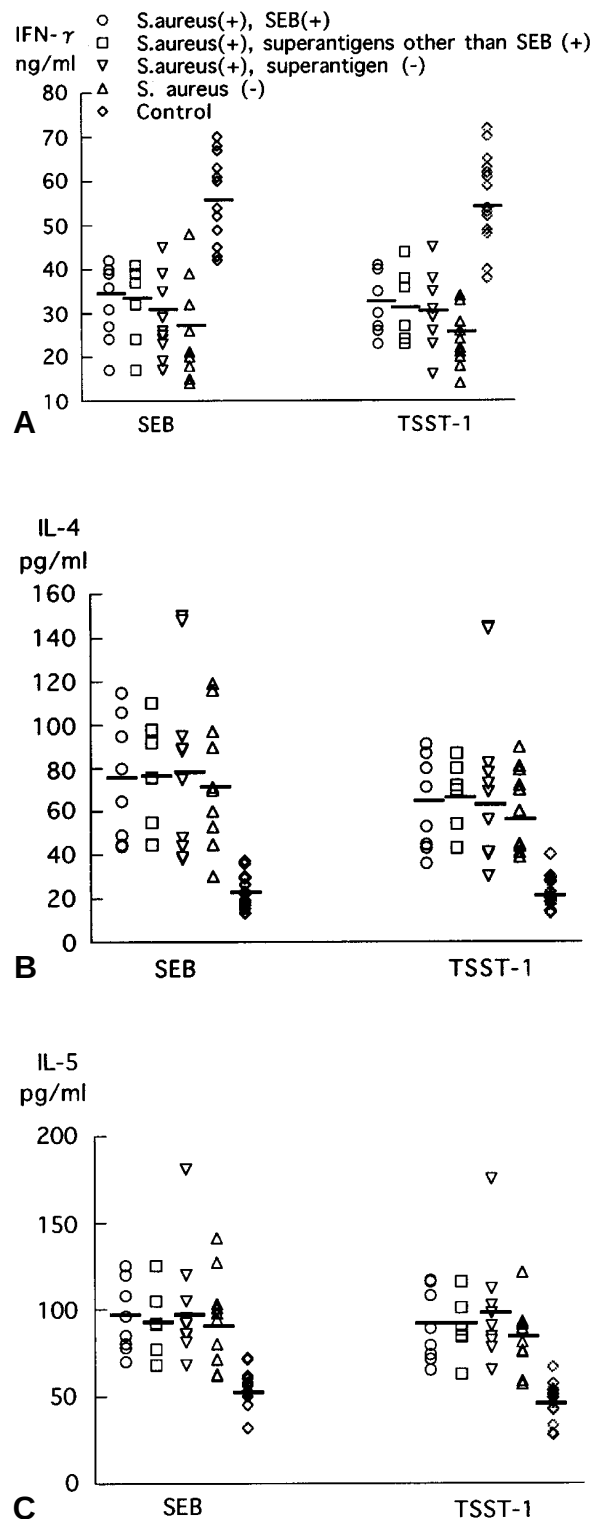


**FIG 2.** IFN- $\gamma$  (A), IL-4 (B), and IL-5 (C) production from PBLs from allergic patients nasal carriage of *S aureus*-producing SEB (n = 8), nasal carriage rate of *S aureus*-producing superantigenic exotoxins other than SEB (n = 6), nasal carriage rate of *S aureus* not producing superantigenic exotoxins (n = 10), negative nasal carriage rate of *S aureus* (n = 10), and control subjects (n = 15). For comparison of paired values, the 1-way ANOVA test was used.

ing SEB, TSST-1 did cause the production of more IL-4 and IL-5 in SEB producers than control subjects, but it was not different from SEB nonproducers. In this respect the results regarding response of PBLs are consistent with the grade of the nasal symptom.

Superantigen-induced upregulation of IL-4 and IL-5 may be important in the pathogenesis of PAR. The resultant T-cell proliferation and production of T<sub>H</sub>2 type predominant cytokines promotes local inflammation contributing to the lesions of PAR. Furthermore, diminished IFN- $\gamma$  production against staphylococcal superantigen may lead to an inappropriate elimination of *S aureus* that persists in the nasal cavity of patients with PAR. Therefore the nasal carriage rate of *S aureus* and superantigen production could aggravate the pathogenesis of PAR. Further studies are needed to determine whether antimicrobial treatment may have a role in PAR.

In conclusion, we have demonstrated that the rate of nasal carriage of *S aureus* of patients with PAR was significantly higher than that of control subjects, the nasal symptom scores were significantly higher in the *S aureus*-positive groups compared with the *S aureus*-negative groups, and PBLs from allergic patients were significantly more likely to produce T<sub>H</sub>2 type cytokines, IL-4 and IL-5, in response to both SEB and TSST-1. Therefore PAR leads to a higher carriage rate of *S aureus*, and the nasal carriage rate of *S aureus* may play a role in the pathogenesis and promote local inflammation of PAR. In addition to superantigens, *S aureus* can express protein A and other toxins, which induce epithelial cell and immune activations.<sup>32</sup> In the future, additional analyses should also examine the potential role of these factors as well because *S aureus*, but not necessarily superantigen production, correlated with severity of nasal symptoms.

## REFERENCES

- Imada M, Estelle F, Simons R, Jay FT, Hayglass KT. Allergen-stimulated interleukin-4 and interferon-gamma production in primary culture: responses of subjects with allergic rhinitis and normal controls. *Immunology* 1995;85:373-80.
- Lee CH, Rhee CS, Oh SH, Min YG, Lee MS. Increase in expression of IL-4 and IL-5 mRNA in the nasal mucosa of patients with perennial allergic rhinitis during natural allergen exposure. *Ann Otol Rhinol Laryngol* 1997;106:215-9.
- Saito H, Asakura K, Ogasawara H, Watanabe M, Kataura A. Topical antigen provocation increases the number of immunoreactive IL-4-, IL-5- and IL-6-positive cells in the nasal mucosa of patients with perennial allergic rhinitis. *Int Arch Allergy Immunol* 1997;114:81-5.
- Terada N, Konno A, Terada Y, Fukuda S, Yamashita T, Abe T, et al. IL-4 upregulates Fc epsilon RI alpha-chain messenger RNA in eosinophils. *J Allergy Clin Immunol* 1995;96:1161-9.
- Pene J, Rousset F, Briere F, Chretien I, Paliard X, Banchereau J, et al. IgE production by normal human B cells induced by alloreactive T cell clones is mediated by IL-4 and suppressed by IFN-gamma. *J Immunol* 1988;141:1218-24.
- Coffman RL, Seymour BW, Hudak S, Jackson J, Rennick D. Antibody to interleukin-5 inhibits helminth-induced eosinophilia in mice. *Science* 1989;245:308-10.
- Peterson B, Saxon A. Global increases in allergic respiratory disease: the possible role of diesel exhaust particles. *Ann Allergy Asthma Immunol* 1996;77:263-8.
- Sorri M, Hartikainen Sorri AL, Karja J. Rhinitis during pregnancy. *Rhinology* 1980;18:83-6.
- Konig B, Neuber K, Konig W. Responsiveness of peripheral blood mononuclear cells from normal and atopic donors to microbial superantigens. *Int Arch Allergy Immunol* 1995;106:124-33.
- Hahn DL, Dodge RW, Golubjatnikov R. Association of *Chlamydia pneumoniae* (strain TWAR) infection with wheezing, asthmatic bronchitis, and adult-onset asthma. *JAMA* 1991;266:225-30.
- Shirakawa T, Enomoto T, Shimazu S, Hopkin JM. The inverse association between tuberculin responses and atopic disorder. *Science* 1997;275:77-9.
- Falanga V, Campbell DE, Leyden JJ, Douglas SD. Nasal carriage of *Staphylococcus aureus* and antistaphylococcal immunoglobulin E antibodies in atopic dermatitis. *J Clin Microbiol* 1985;22:452-4.
- Till S, Durham S, Dickason R, Huston D, Bungre J, Walker S, et al. IL-13 production by allergen-stimulated T cells is increased in allergic disease and associated with IL-5 but not IFN-gamma expression. *Immunology* 1997;91:53-7.
- Shinogi J, Majima Y, Takeuchi K, Harada T, Sakakura Y. Quantitative cytology of nasal secretions with perennial allergic rhinitis in children: comparison of noninfected and infected conditions. *Laryngoscope* 1998;108:703-5.
- Marrack P, Kappler J. The staphylococcal enterotoxins and their relatives. *Science* 1990;248:705-11.
- Wilson SZ, Martin RR, Putman M. In vivo effects of josamycin, erythromycin, and placebo therapy on nasal carriage of *Staphylococcus aureus*. *Antimicrob Agents Chemother* 1977;11:407-10.
- Branger C, Fournier JM, Loulergue J, Bouvet A, Goullet P, Boutonnier A, et al. Epidemiology of *Staphylococcus aureus* in patients with cystic fibrosis. *Epidemiol Infect* 1994;112:489-500.
- Leung DY, Harbeck R, Bina P, Reiser RF, Yang E, Norris DA, et al. Presence of IgE antibodies to staphylococcal exotoxins on the skin of patients with atopic dermatitis. Evidence for a new group of allergens. *J Clin Invest* 1993;92:1374-80.
- Wieneke AA. The detection of enterotoxin and toxic shock syndrome toxin-1 production by strains of *Staphylococcus aureus* with commercial RPLA kits. *Int J Food Microbiol* 1988;7:25-30.
- Takeuchi S, Ishiguro K, Ikegami M, Kaidoh T, Hayakawa Y. Production of toxic shock syndrome toxin by *Staphylococcus aureus* isolated from mastitic cow's milk and farm bulk milk. *Vet Microbiol* 1998;59:251-8.
- McFadden JP, Noble WC, Camp RD. Superantigenic exotoxin-secreting potential of staphylococci isolated from atopic eczematous skin. *Br J Dermatol* 1993;128:631-2.
- Okuda M, Sakaguchi K, Ohtsuka H. Intranasal beclomethasone: mode of action in nasal allergy. *Ann Allergy* 1983;50:116-20.
- Hanifin JM, Homburger HA. Staphylococcal colonization, infection, and atopic dermatitis-association not etiology. *J Allergy Clin Immunol* 1986;78:563-6.
- Motala C, Potter PC, Weinberg EG, Malherbe D, Hughes J. Anti-*Staphylococcus aureus*-specific IgE in atopic dermatitis. *J Allergy Clin Immunol* 1986;78:583-9.
- Kluytmans J, van Belkum A, Verbrugh H. Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. *Clin Microbiol Rev* 1997;10:505-20.
- Bibel DJ, Aly R, Shinefield HR, Maibach HI. The *Staphylococcus aureus* receptor for fibronectin. *J Invest Dermatol* 1983;80:494-6.
- Lopes JD, dos Reis M, Brentani RR. Presence of laminin receptors in *Staphylococcus aureus*. *Science* 1985;229:275-7.
- Elliott ST, Hanifin JM. Lymphocyte response to phytohemagglutinin in atopic dermatitis. *Arch Dermatol* 1979;115:1424-6.
- Hofer MF, Lester MR, Schlievert PM, Leung DY. Upregulation of IgE synthesis by staphylococcal toxic shock syndrome toxin-1 in peripheral blood mononuclear cells from patients with atopic dermatitis. *Clin Exp Allergy* 1995;25:1218-27.
- Lester MR, Hofer MF, Renz H, Trumble AE, Gelfand EW, Leung DY. Modulatory effects of staphylococcal superantigen TSST-1 on IgE synthesis in atopic dermatitis. *Clin Immunol Immunopathol* 1995;77:332-8.
- Campbell DE, Kemp AS. Proliferation and production of interferon-gamma (IFN-gamma) and IL-4 in response to *Staphylococcus aureus* and staphylococcal superantigen in childhood atopic dermatitis. *Clin Exp Immunol* 1997;107:392-7.
- Ezpechuk YV, Leung DY, Middleton MH, Bina P, Reiser R, Norris DA. Staphylococcal toxins and protein A differentially induce cytotoxicity and release of tumor necrosis factor-alpha from human keratinocytes. *J Invest Dermatol* 1996;107:603-9.