

Synergism of *Staphylococcus aureus* Colonization and Allergic Reaction in the Nasal Cavity in Mice

Nobusuke Hohchi^a Koichi Hashida^a Jun-ichi Ohkubo^a Tetsuro Wakasugi^a
Takanori Mori^a Khac-Hung Nguyen^a Etsushi Kuroda^b Takako Ikeno^c
Hatsumi Taniguchi^c Hideaki Suzuki^a

Departments of ^aOtorhinolaryngology, ^bImmunology and Parasitology, and ^cMicrobiology, School of Medicine, University of Occupational and Environmental Health, Kitakyushu, Japan

Key Words

Staphylococcus aureus • Nasal allergy • Ovalbumin • Synergism

Abstract

Background: The aim of this study is to investigate the reciprocal effect of *Staphylococcus aureus* colonization and allergic rhinitis in an allergy model of mice. **Methods:** BALB/c mice with intraperitoneal ovalbumin (OVA) sensitization and/or intranasal *S. aureus* inoculation were prepared. The following 4 groups were designed: an OVA-sensitized *S. aureus*-inoculated (AR-SA) group, an OVA-sensitized uninoculated (AR) group, a nonsensitized *S. aureus*-inoculated (SA) group, and a nonsensitized uninoculated (control) group. After intranasal OVA challenge, nasal lavage fluid, peripheral blood, and nasal mucosa were collected. Polymorphonuclear cells in the nasal lavage fluid were counted, serum OVA-specific IgE and IgG1 were measured by enzyme immunoassays, and *IL-4*, *IL-5*, and *IFN- γ* mRNAs in the nasal mucosa were assessed by quantitative real-time reverse transcription-PCR. The number of *S. aureus* in the nasal mucosa and lavage fluid was counted. **Results:** Both eosinophil and neutrophil counts were larger in the AR-SA group than in the other

groups. Both IgE and IgG1 levels were higher in the AR and AR-SA groups than in the SA and control groups, and the IgG1 level was higher in the AR-SA group than in the AR group. The expression of *IL-4* mRNA was higher in the AR-SA group than in the other groups, and the expression of *IL-5* mRNA was higher in the AR-SA group than in the SA group. The AR-SA group showed higher counts of *S. aureus* in the nasal mucosa than the SA group. **Conclusion:** These results indicate the mutually potentiating effect of *S. aureus* colonization and allergic rhinitis.

Copyright © 2012 S. Karger AG, Basel

Introduction

Staphylococcus aureus is one of the opportunistic pathogens in humans, and is commonly detected in the normal microflora of the skin, intestine, and upper aerodigestive tract. In the upper aerodigestive tract, the naris, nasal cavity, and pharynx are the major reservoirs of this bacterium. The nasal carriage rate of *S. aureus* in healthy people is about 80%. Persistent carriers account for a quarter of all nasal carriers, and the other three quarters are intermittent carriers [1]. Approximately 20%

of all human staphylococcal infections are considered to be autogenous [2].

Previous studies have shown that *S. aureus* colonization and staphylococcal enterotoxins (SEs) may aggravate atopic dermatitis and allergic rhinitis [3, 4]. Allergic reactions are enhanced by nasal instillation of staphylococcal enterotoxin B (SEB) in an allergic animal model [4]. In humans, peripheral blood mononuclear cells from patients with allergic rhinitis have been shown to produce higher levels of IL-4 and IL-5 in response to SEs than those from nonallergic subjects [5]. In an actual clinical setting, allergic rhinitis is often aggravated by upper respiratory infection [6, 7]. Despite the fact that *S. aureus* is an indigenous bacterium in the nasal cavity and is one of the pathogens causing acute infective rhinosinusitis, the mechanism by which *S. aureus* participates in the development of allergic rhinitis is not fully understood.

SEs have superantigenic properties, and unselectively activate inflammatory and immune processes: SEs directly bind to the major histocompatibility complex class II molecule expressed on antigen-presenting cells as well as to the V β region of the T cell receptor, and thereby couple antigen-presenting cells and T cells, leading to the activation and massive proliferation of corresponding T cells [8–12]. There are more than 10 subtypes of SEs identified to date, and each SE has a different but wide range of affinity for the V β repertoire of the T cell receptor. For instance, SEB has a high affinity for V β 3, 12, 13.2, 14, 15, 17 and 20, and thus activates a large proportion of the T cell population [13, 14]. SEs can also be target antigens of IgE-mediated immune responses. Increased levels of serum SEA- and SEB-specific IgE have been reported in patients with allergic rhinitis and bronchial asthma [9].

On the other hand, some authors have reported allergy to be one of the risk factors for bacterial colonization/infection. Allergic constitution is associated with increased bacterial carriage in the upper respiratory tract [15], and is one of the risk factors for sinus infection [16]. In fact patients with allergic rhinitis show a higher rate of *S. aureus* carriage in the nasal cavity compared to control subjects [5].

These lines of evidence suggest that allergic reaction and bacterial colonization/infection may synergize each other. Although several investigators have shown the synergistic effect of bacterial components on allergy in animal models, few of them have used the whole bacterium to enhance allergic reactions. The aim of this study is to investigate the reciprocal effect of *S. aureus* colonization and allergic rhinitis in an allergy model of mice.

Table 1. Group design

	Intraperitoneal injection	<i>S. aureus</i> inoculation	Intranasal OVA challenge
AR-SA group	OVA	+	+
AR group	OVA	–	+
SA group	saline	+	+
Control group	saline	–	+

Materials and Methods

Experimental Animals

Six- to 8-week-old male BALB/c mice were obtained from SLC Japan Inc. (Shizuoka, Japan). The animals were kept in a pathogen-free environment in a biohazard containment facility. Each group of animals was kept isolated from the other groups. The care and use of the animals were in accordance with the Guidelines for Animal Experiments of the University of Occupational and Environmental Health.

Ovalbumin Sensitization and Challenge

Ovalbumin (OVA) sensitization was carried out by the method of Sejima et al. [17] with modification. Four hundred nanograms of OVA (Sigma Chemical Co., St. Louis, Mo., USA) was dissolved in 18 ml of saline and then mixed with 2 ml of Alum Inject adjuvant solution (Pierce Biotechnology Inc., Rockford, Ill., USA). Animals were sensitized by intraperitoneal injection of 0.5 ml of the resultant solution (OVA/alum) on days 1, 5, 14 and 21. For nonsensitized animals, the same volume of saline was substituted for OVA/alum. Nasal allergic reaction was provoked by daily intranasal administration of 0.5 mg OVA/20 μ l saline from day 22 through 28 or 35 until the animal was killed.

Inoculation of *S. aureus*

The *S. aureus* used for intranasal inoculation was a clinical isolate from a patient with chronic rhinosinusitis, and was methicillin susceptible (oxacillin MIC = 0.25 μ g/ml). Toxicogenicity tests using a RIDASCREEN EIA kit (R-Biopharm GmbH, Darmstadt, Germany) and a TST-RPLA TSST-1 kit (Denka Seiken Co. Ltd., Tokyo, Japan) showed that the strain was positive for toxic shock syndrome toxin-1 and SEs A and D. *S. aureus* was grown on mannitol salt agar in humidified air containing 5% CO₂ at 37°C, and suspended in sterile 10 mM phosphate-buffered saline (PBS) at pH 7.3 immediately before inoculation at a concentration of 1.0×10^9 colony-forming units/ml. Twenty microliters of the *S. aureus* suspension was intranasally administered on day 19.

Group Design

The following 4 groups were set up (table 1): an OVA-sensitized *S. aureus*-inoculated group (AR-SA group), an OVA-sensitized uninoculated group (AR group), a nonsensitized *S. aureus*-inoculated group (SA group), and a nonsensitized uninoculated group (control group). The control group consisted of 6 mice, and the other groups consisted of 12 mice each. All animals received intranasal OVA challenge. No mouse died prior to the designated time point of sacrifice.

Sample Collection

Sample collection was performed on days 28 and 35 (the 7th and 14th day from the start of intranasal challenge) from 3 mice each in the control group and from 6 each in the other groups. Under intraperitoneal anesthesia with 120 mg/kg sodium pentobarbital (Dainippon-Sumitomo Seiyaku Co. Ltd., Osaka, Japan), the blood was collected by cardiac puncture and spun at $500 \times g$ for 15 min to obtain the sera, which was stored at -80°C until the following analyses. The animals were then killed by cervical dislocation and tracheostomized, and a 4-Fr catheter was inserted through the tracheostoma upward to the nasopharynx. The nasal cavity was gently perfused back to front with 1.1 ml of PBS via the catheter, and the lavage fluid was collected at the nares. After nasal lavage, the animals were decapitated, the skull was split in the midline to expose the nasal cavity, and the nasal mucosa was dissected under a dissecting microscope.

Histological Examination

The nasal mucosa was fixed with 4% paraformaldehyde in 0.1 M phosphate buffer at 4°C overnight, and embedded while frozen in an optimum cutting temperature compound. Then 7- μm -thick sections were prepared using a cryostat, and mounted on a glass slide. The slide was air-dried, stained with Hansel solution, and examined under a light microscope.

Cytological Examination of the Nasal Lavage Fluid

0.5 ml of the nasal lavage fluid was cytocentrifuged at $70 \times g$ for 5 min [18]. The resultant cytospin preparations were rapidly air-dried and stained with Wright-Giemsa stain to examine differential cell counts.

Measurements of OVA-Specific Immunoglobulins

Serum OVA-specific IgE was measured using a mouse OVA-specific IgE ELISA kit (DS PharmaBiomedical Co. Ltd., Osaka, Japan) according to the manufacturer's instructions [19].

Serum OVA-specific IgG1 was also measured by an enzyme immunoassay as described previously [20]. A MaxiSorp polystyrene 96-well microtiter plate (Nunc Laboratories, Roskilde, Denmark) was coated with 10 $\mu\text{g}/\text{ml}$ OVA in PBS at 4°C overnight. After blocking with 10% fetal bovine serum/PBS, serum samples were loaded and incubated at room temperature for 2 h. The plate was then washed and incubated with 200 ng/ml horseradish peroxidase-conjugated goat anti-mouse IgG1 (Bethyl Laboratories Inc., Montgomery, Tex., USA) at room temperature for 1 h. After washing, 100 μl of 1.46 mM 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) with 0.03% hydrogen peroxide was added as substrate solution. The plate was allowed to develop at room temperature for 15 min, and absorbance at 405 nm was measured.

Preparation of Total RNA

The nasal mucosa specimens were soaked in RNA stabilization reagent (Qiagen Inc., Valencia, Calif., USA) at 4°C overnight and stored at -80°C until use. Total RNA was extracted using an RNeasy Midi Kit (Qiagen) according to the manufacturer's instructions. The purity of RNA was assessed by determining the ratio of light absorption at 260 to that at 280 nm (an A_{260}/A_{280} ratio between 1.9 and 2.1 was considered acceptable). The RNA concentration was determined from A_{260} .

Quantitative Real-Time Reverse Transcription-PCR

Total RNA (2 μg) was reverse transcribed to cDNA with a High Capacity RNA-to-cDNA Kit (Applied Biosystems Inc., Foster City, Calif., USA), which uses random primers. Quantitative real-time reverse transcription-PCR analysis was performed with an Applied Biosystems StepOnePlus real-time PCR system using the TaqMan Fast Universal PCR Master Mix (Applied Biosystems) for 3 target genes (*IL-4*, *IL-5*, and *IFN- γ*) and for β -actin mRNA (*β -actin*) as a housekeeping gene according to the manufacturer's specifications. The TaqMan gene expression assays for *IL-4* (assay identification No. NM021283_m1), *IL-5* (assay identification No. NM010558_m1), *IFN- γ* (assay identification No. NM001009803_m1), and *β -actin* (assay identification No. NM007393_m1) were purchased from Applied Biosystems. One hundred nanograms per microliter of cDNA was mixed with TaqMan Universal PCR Master Mix with AmpErase (uracil N-glycosylase) and a target primer/probe set of the TaqMan gene expression assays, and subjected to PCR amplification with real-time detection. The thermal cycler conditions were as follows: holding at 95°C for 2 min, followed by two-step PCR of 40 cycles at 95°C for 1 s followed by 60°C for 20 s. All the samples were assayed in duplicate.

The measured threshold cycle (C_T) was normalized by subtracting C_T for the housekeeping gene (*β -actin*) of each sample from that for the target genes (*IL-4*, *IL-5*, and *IFN- γ*). From the obtained ΔC_T , the ratio of the target gene to the housekeeping gene was calculated as follows: target mRNA/ β -actin mRNA ratio = $2^{-\Delta C_T}$.

Count of *S. aureus*

The nasal mucosa specimens were soaked in PBS and mashed using a pestle. The nasal lavage fluid and the suspension of the mashed mucosa were diluted $\times 10$ – 100 in PBS. Aliquots of the dilutions were incubated on mannitol salt agar in humidified air containing 5% CO_2 at 37°C for 24 h, and the number of colonies of *S. aureus* was counted. We also performed bacterial culture of the lung and blood to confirm no systemic infection.

Statistics

Data values were expressed as the mean $\pm$ SEM. Statistical significance of differences was tested using the two-tailed Student t test. Differences were considered significant when $p < 0.05$.

Results

Differential Cell Counts in the Nasal Lavage Fluid

Figure 1 shows eosinophil and neutrophil counts in the nasal lavage fluid. On day 28, both eosinophil and neutrophil counts were larger in the AR-SA group than in the SA and control groups ($p = 0.001$ – 0.046), and were larger in the AR group than in the SA group ($p = 0.019$ – 0.034). On day 35, both eosinophil and neutrophil counts were larger in the AR-SA group than in the other groups ($p < 0.023$), and the eosinophil count was larger in the AR group than in the SA group ($p = 0.021$).

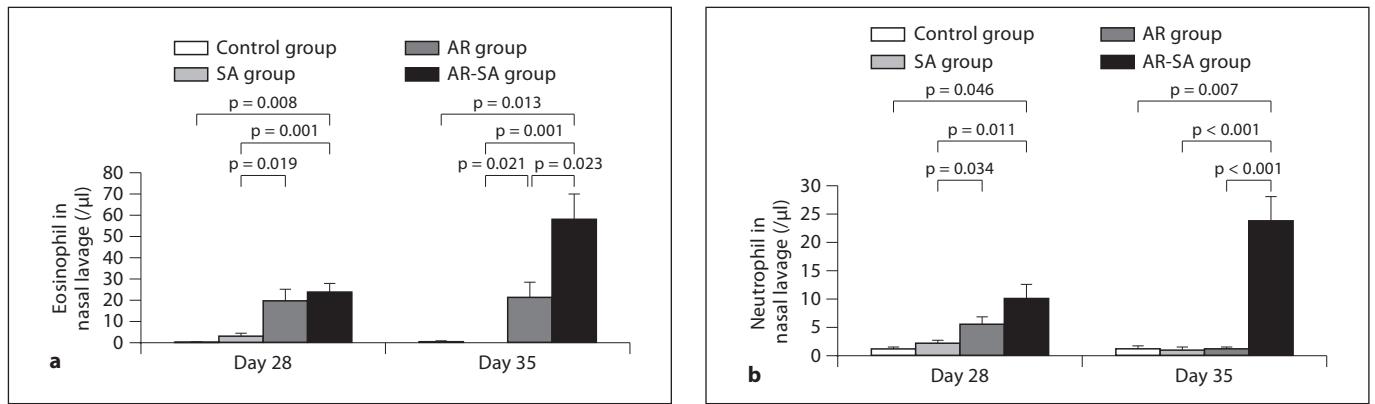


Fig. 1. Eosinophil (a) and neutrophil (b) counts in the nasal lavage fluid.

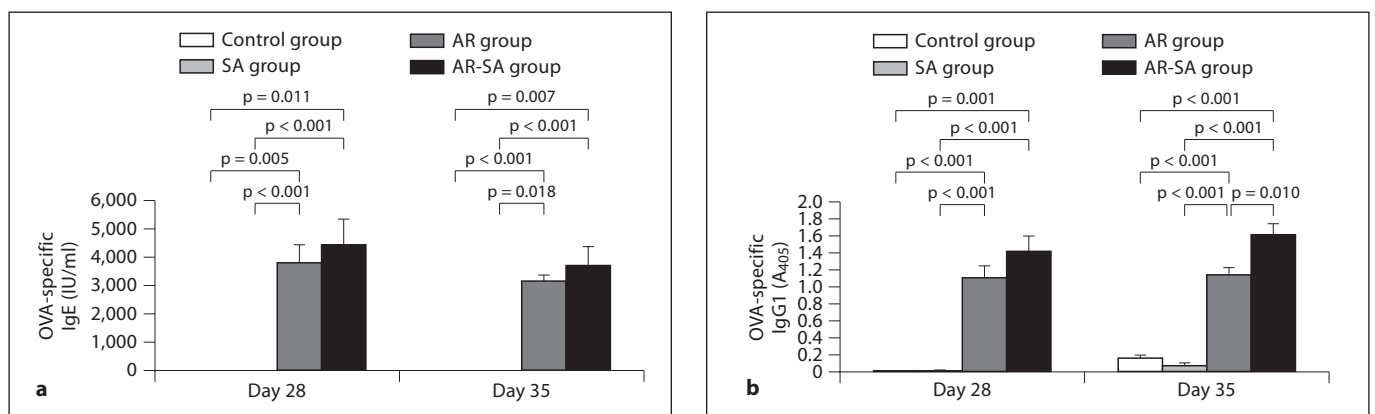


Fig. 2. OVA-specific IgE (a) and IgG1 (b) levels in the serum.

Histological Finding

No breakdown of the epithelial layer was observed at the light-microscopic level in either OVA-sensitized or nonsensitized mice.

Serum OVA-Specific Immunoglobulins

The serum OVA-specific IgE and IgG1 levels are summarized in figure 2. Both IgE and IgG1 levels were higher in the AR and AR-SA groups than in the SA and control groups on days 28 and 35 ($p < 0.018$). No significant difference was observed in the IgE level between the AR and AR-SA groups, whereas the IgG1 level was higher in the AR-SA group than in the AR group on day 35 ($p = 0.010$).

Expression of IL-4, IL-5 and IFN- γ mRNA in the Nasal Mucosa

The expressions of cytokine mRNAs in the nasal mucosa are summarized in figure 3. The expression of IL-4

mRNA was higher in the AR-SA group than in the other groups on both days ($p = 0.001$ – 0.033), and higher in the AR group than in the SA and control groups ($p < 0.018$). The expression of IL-5 mRNA was higher in the AR-SA group than in the SA group on both days ($p = 0.001$ – 0.024), and higher in the AR group than in the SA and control groups on day 28 ($p < 0.042$). On the other hand, no significant difference was observed in the expression of IFN- γ mRNA among the 4 groups.

Count of *S. aureus*

Figure 4 represents the number of colony-forming units of *S. aureus* in the nasal lavage fluid and in the nasal mucosa. The AR-SA group generally showed higher counts than the SA group, and this difference was statistically significant in the nasal mucosa on both days ($p = 0.009$ – 0.038). On the other hand, no bacteria were detected in either lung or blood. In addition, all animals

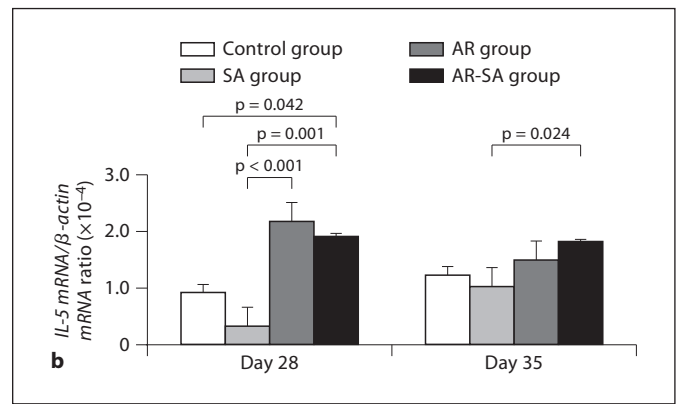
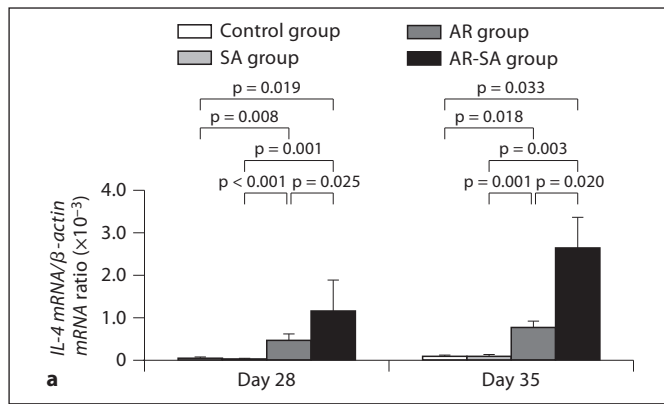


Fig. 3. Cytokine mRNA expressions in the nasal mucosa: IL-4 (a), IL-5 (b) and IFN- γ (c).

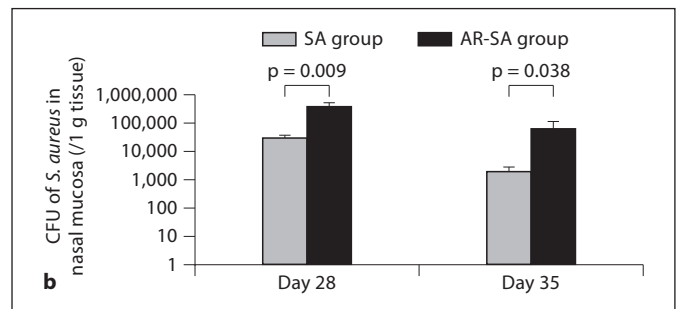
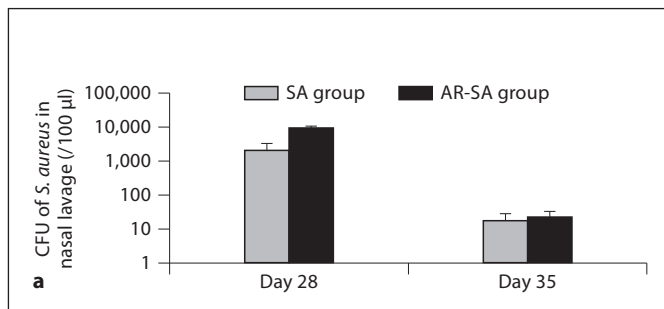
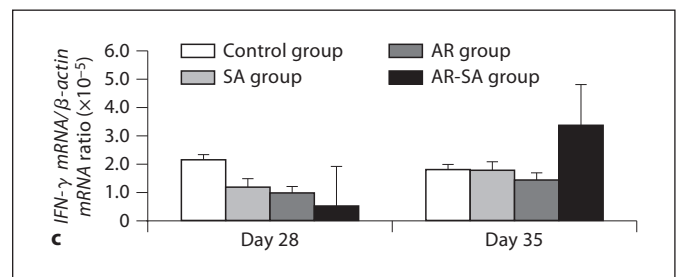


Fig. 4. CFU of *S. aureus* in the nasal lavage fluid (a) and in the nasal mucosa (b). CFU = Colony-forming units.

showed good general condition throughout the period of experiment, and none of them died prior to the designated time of sacrifice. These observations indicate that the animals did not develop systemic infections, and inoculated *S. aureus* probably stayed in the nasal cavity.

Discussion

In the present study, we demonstrated the mutually potentiating effect of *S. aureus* colonization and allergic rhinitis. *S. aureus* inoculation increased the exudation of

eosinophils and neutrophils, the specific IgG1 level in the serum, and the expression of *IL-4 mRNA*. Simultaneously, provocation of nasal allergic reaction enhanced *S. aureus* colonization.

Although there exist only a few inflammatory cells in the mucus and mucosa of normal nasal and paranasal cavities, numerous polymorphonuclear cells are locally recruited in rhinosinusitis [21–23]. Recruitment of such cells is regulated by a number of adhesion molecules and chemotactic substances. Polymorphonuclear cell-related adhesion molecules include E selectin, intercellular adhesion molecule-1, and vascular cell adhesion molecule-1

(VCAM-1), which are expressed on vascular endothelial cells [24]. Chemoattractants for polymorphonuclear cells include a variety of chemokines and chemical mediators [25]. Bacterial colonization/infection probably promotes the expression of these substances, and thereby upregulates the recruitment of inflammatory cells in allergic reaction. Particularly, SEs act as potent superantigens, which trigger a strong immune response and induce neutrophil recruitment. A large proportion of T cell population is activated by superantigens, and secretes various cytokines such as IL-2, IL-6, IFN- γ , and TNF- α [26]. These cytokines then activate other immune cells, inflammatory cells, fibroblasts, and epithelial cells [27], which release a number of chemical mediators including neutrophil chemotactic factors such as CXC chemokines and arachidonic acid metabolites.

SEs are known to induce the production of various Th1 and Th2 cytokines by lymphocytes and antigen-presenting cells [13, 28]. Okano et al. [4] have reported that nasal instillation of high-dose SEB increased IL-4, IL-5 and IFN- γ production from nasal mononuclear cells, and serum-specific IgE/IgG1 levels in *Schistosoma mansoni* egg antigen-sensitized mice. However, they also observed that low-dose SEB only increased the specific IgG1 level, and did not significantly change the production of the above cytokines or specific IgE [4]. In the present study, we used *S. aureus* instead of SEs in order to simulate the clinical situation more effectively. We observed that *S. aureus* inoculation increased the serum-specific IgG1 level and the expression of *IL-4 mRNA* but did not change IgE, *IL-5 mRNA* or *IFN- γ mRNA*. These observations suggest that the *S. aureus* inoculation used in the present study may correspond to a medium dose of SE.

IL-4 is one of the major Th2 cytokines, and is essential for regulating allergic immune responses. This cytokine is produced by mast cells, basophilic cells, and activated Th2 cells [29, 30]. IL-4 is known as an autocrine factor that promotes the proliferation and differentiation of Th2 cells themselves [31, 32]. IL-4 also induces immunoglobulin class switching from IgM to IgG1/E in activated B cells in mice, leading to sequential allergic responses [33–35]. Eosinophil infiltration is one of the distinct allergic reactions. Antigen-responding mast cells and activated Th2 cells secrete IL-5, a potent eosinophil chemotactic factor. Eotaxin, another potent chemokine for eosinophils, would also be secreted from fibroblasts and epithelial cells [36, 37] in response to the combined stimulation of IL-4 and bacterial components [38, 39]. Furthermore, IL-4 elicits the expression of VCAM-1, an eosinophil-spe-

cific adhesion molecule, on vascular endothelial cells, and thereby upregulates eosinophil infiltration [40]. Such processes involving IL-4 may underlie the synergistic effect of bacterial colonization on allergic reactions.

Some of the proinflammatory cytokines, such as IL-4, TGF- β , and IFN- γ , trigger immunoglobulin class switch recombination from IgM to other isotypes. IL-4 induces not only IgE but also IgG1 production. Moon et al. [33] have documented that low-dose IL-4 induced IgG1 production while high-dose IL-4 induced IgE production from mouse splenic B cells. In the present allergy model, the simultaneous increase of *IL-4 mRNA* and IgG1 suggests that IL-4 plays a key role in immunoglobulin class switching. Antigen-specific IgG may either suppress or enhance allergic response; activation of inhibitory Fc γ receptors on immunocompetent cells plays a significant role in maintaining immune tolerance by suppressing autoreactive B cells [41]. On the other hand, in addition to IgE, antigen-specific IgG also contributes to the pathogenesis of Th2-mediated airway inflammation via Fc γ receptor III [42]. The role of IgG in the development of allergy has been substantiated by such conflicting observations, and is still controversial.

We also found that the AR-SA group showed higher counts of *S. aureus* colonizing in the nasal mucosa than the SA group. This may be at least partially due to the impairment of the mucus blanket and mucociliary function of the nasal epithelium caused by allergic reaction. Laoukili et al. [43] reported that IL-13 inhibits the differentiation of ciliated cells, impairs ciliary beating, and stimulates the differentiation of goblet cells. In addition, more severe epithelial damage may expose subepithelial and/or submucosal extracellular matrix components such as collagen, fibrinogen, laminin, and fibronectin, to which staphylococcal adhesions are thought to bind. Such adhesion molecules on bacteria are generically referred to as 'microbial surface components recognizing adhesive matrix molecules', and are known to mediate the adherence of the bacteria to the host [44–46]. Nasal epithelial damage in allergic rhinitis is indistinguishable at the light-microscopic level [47], but has been evidenced at the ultrastructural level. Vacuolation of the epithelial cells, widening of the intercellular spaces, and breakdown of the tight junction between the epithelial cells [48–50] have been demonstrated in the nasal mucosa in patients with allergic rhinitis. In light of all these findings, nasal allergy can be considered to facilitate bacterial adhesion and colonization onto the nasal mucosa. Electron microscopy in the present allergic model remains to be performed in a future study.

Conclusions

In the present study we investigated the reciprocal effect of *S. aureus* colonization and allergic rhinitis in an OVA allergy model of mice. Nasal inoculation of *S. aureus* increased the exudation of polymorphonuclear cells, specific IgG1 in the serum, and the expression of *IL-4*

mRNA in the nasal mucosa. Provocation of nasal allergic reaction enhanced *S. aureus* colonization. These results indicate the mutually potentiating effect of *S. aureus* colonization and allergic rhinitis. Molecular and cell biological mechanisms underlying this phenomenon remain to be further investigated in future studies.

References

- Kiser KB, Cantey-Kiser JM, Lee JC: Development and characterization of a *Staphylococcus aureus* nasal colonization model in mice. *Infect Immun* 1999;67:5001–5006.
- Bachert C, van Zele T, Gevaert P, De Schrijver L, Van Cauwenberge P: Superantigens and nasal polyps. *Curr Allergy Asthma Rep* 2003;3:523–531.
- Beenken KE, Blevins JS, Smeltzer MS: Mutation of *sarA* in *Staphylococcus aureus* limits biofilm formation. *Infect Immun* 2003;71:4206–4211.
- Okano M, Hattori H, Yoshino T, Sugata Y, Yamamoto M, Fujiwara T, Satoskar AA, Satoskar AR, Nishizaki K: Nasal exposure to staphylococcal enterotoxin enhances the development of allergic rhinitis in mice. *Clin Exp Allergy* 2005;35:506–514.
- Shiomori T, Yoshida S, Miyamoto H, Makishima K: Relationship of nasal carriage of *Staphylococcus aureus* to pathogenesis of perennial allergic rhinitis. *J Allergy Clin Immunol* 2000;105:449–454.
- Ramadan HH, Fornelli R, Ortiz AO, Rodman S: Correlation of allergy and severity of sinus disease. *Am J Rhinol* 1999;13:345–347.
- Huang SW: The risk of sinusitis in children with allergic rhinitis. *Allergy Asthma Proc* 2000;21:85–88.
- Rossi RE, Monasterolo G: Prevalence of serum IgE antibodies to the *Staphylococcus aureus* enterotoxins (SAE, SEB, SEC, SED, TSST-1) in patients with persistent allergic rhinitis. *Int Arch Allergy Immunol* 2004;133:261–266.
- Lee JH, Lin YT, Yang YH, Wang LC, Chiang BL: Increased levels of serum-specific immunoglobulin E to staphylococcal enterotoxin A and B in patients with allergic rhinitis and bronchial asthma. *Int Arch Allergy Immunol* 2005;138:305–311.
- Tripathi A, Conley DB, Grammer LC, Ditto AM, Lowery MM, Seiberling KA, Yarnold PA, Zeifer B, Kern RC: Immunoglobulin E to staphylococcal and streptococcal toxins in patients with chronic sinusitis/nasal polyposis. *Laryngoscope* 2004;114:1822–1826.
- Seiberling KA, Conley DB, Tripathi A, Grammer LC, Shuh L, Haines GK 3rd, Schleimer R, Kern RC: Superantigens and chronic rhinosinusitis: detection of staphylococcal exotoxins in nasal polyps. *Laryngoscope* 2005;115:1580–1585.
- Zhang N, Gevaert P, van Zele T, Perez-Novo C, Patou J, Holtappeis G, van Cauwenberge P, Bachert C: An update on the impact of *Staphylococcus aureus* enterotoxins in chronic sinusitis with nasal polyposis. *Rhinology* 2005;43:162–168.
- Kotb M: Bacterial pyrogenic exotoxins as superantigens. *Clin Microbiol Rev* 1995;8:411–426.
- Fraser J, Arcus V, Kong P, Baker E, Proft T: Superantigens – powerful modifiers of the immune system. *Mol Med Today* 2000;6:125–132.
- García-Rodríguez JA, Fresnadillo Martínez MJ: Dynamics of nasopharyngeal colonization by potential respiratory pathogens. *J Antimicrob Chemother* 2002;50(suppl S2):59–73.
- Gwaltney JM Jr: Acute community-acquired sinusitis. *Clin Infect Dis* 1996;23:1209–1223.
- Sejima T, Madoiwa S, Minoru J, Sugo T, Okada K, Ueshima S, Matsuo O, Ishida T, Ichimura K, Sakata Y: Protection of plasminogen activator inhibitor-1-deficient mice from nasal allergy. *J Immunol* 2005;174:8135–8143.
- Ragab A, Clement P, Vincken W: Correlation between the cytology of the nasal middle meatus and BAL in chronic rhinosinusitis. *Rhinology* 2005;43:11–17.
- Takahashi H, Nakazawa M, Takahashi K, Aihara M, Minami M, Hirasawa T, Ikezawa Z: Effects of zinc deficient diet on development of atopic dermatitis-like eruptions in DS-Nh mice. *J Dermatol Sci* 2008;50:31–39.
- Ohno H, Tsunemine S, Isa Y, Shimakawa M, Yamamura H: Oral administration of *Bifidobacterium bifidum* G9–1 suppresses total and antigen specific immunoglobulin E production in mice. *Biol Pharm Bull* 2005;28:1462–1466.
- Suzuki H, Takahashi Y, Wataya H, Ikeda K, Nakabayashi S, Shimomura A, Takasaka T: Mechanism of neutrophil recruitment induced by IL-8 in chronic sinusitis. *J Allergy Clin Immunol* 1996;98:659–670.
- Demoly P, Crampette L, Mondian M, Campbell AM, Lequeux N, Enander I, Schwartz LB, Guerrier B, Michel FB, Bousquet J: Assessment of inflammation in noninfectious chronic maxillary sinusitis. *J Allergy Clin Immunol* 1994;94:95–108.
- Van Cauwenberge P, Van Hoecke H, Bachert C: Pathogenesis of chronic rhinosinusitis. *Curr Allergy Asthma Rep* 2006;6:487–494.
- Bently AM, Durham SR, Robinson DS, Menz G, Storz C, Cromwell O, Kay AB, Wardlaw AJ: Expression of endothelial and leukocyte adhesion molecules intercellular adhesion molecule-1, E-selectin, and vascular cell adhesion molecule-1 in the bronchial mucosa in steady-state and allergen-induced asthma. *J Allergy Clin Immunol* 1993;92:857–868.
- Nomiyama R, Okano M, Fujiwara T, Maeda M, Kimura Y, Kino K, Yokoyama M, Hirai H, Nagata K, Hara T, Nishizaki K, Nakamura M: CRTH2 plays an essential role in the pathophysiology of Cry j 1-induced pollinosis in mice. *J Immunol* 2008;180:5680–5688.
- Huang W, Koller LD: Superantigen activation and kinetics of cytokines in the Long-Evans rat. *Immunology* 1998;95:331–338.
- Desouza IA, Franco-Penteado CF, Camargo EA, Lima CS, Teixeira SA, Muscará MN, De Nucci G, Antunes E: Inflammatory mechanisms underlying the rat pulmonary neutrophil influx induced by airway exposure to staphylococcal enterotoxin type A. *Br J Pharmacol* 2005;146:781–791.
- Ferens WA, Goff WL, Davis WC, Fox LK, Deobald C, Hamilton MJ, Bohach GA: Induction of type 2 cytokines by a staphylococcal enterotoxin superantigen. *J Nat Toxins* 1998;7:193–213.
- Ho IC, Kaplan MH, Jackson-Grusby L, Glimcher LH, Grusby MJ: Marking IL-4-producing cells by knock-in of the IL-4 gene. *Int Immunol* 1999;11:243–247.
- Sokol CL, Medzhitov R: Role of basophils in the initiation of Th2 responses. *Curr Opin Immunol* 2010;22:73–77.
- Le Gros G, Ben-Sasson SZ, Seder R, Finkelman FD, Paul WE: Generation of interleukin 4 (IL-4)-producing cells in vivo and in vitro: IL-2 and IL-4 are required for in vitro generation of IL-4-producing cells. *J Exp Med* 1990;172:921–929.

- 32 Swain SL, Weinberg AD, English M, Huston G: IL-4 directs the development of Th2-like helper effectors. *J Immunol* 1990;145:3796–3806.
- 33 Moon HB, Severinson E, Heusser C, Johansson SG, Möller G, Persson U: Regulation of IgG1 and IgE synthesis by interleukin 4 in mouse B cells. *Scand J Immunol* 1989;30:355–361.
- 34 Vitetta ES, Ohara J, Myers CD, Layton JE, Krammer PH, Paul WE: Serological, biochemical, and functional identity of B cell-stimulatory factor 1 and B cell differentiation factor for IgG1. *J Exp Med* 1985;162:1726–1731.
- 35 Coffman RL, Ohara J, Bond MW, Carty J, Zlotnik A, Paul WE: B cell stimulatory factor-1 enhances the IgE response of lipopolysaccharide-activated B cells. *J Immunol* 1986;136:4538–4541.
- 36 Mochizuki M, Schröder J, Christophers E, Yamamoto S: IL-4 induces eotaxin in human dermal fibroblasts. *Int Arch Allergy Immunol* 1999;120(suppl 1):19–23.
- 37 Li L, Xia Y, Nguyen A, Lai YH, Feng L, Mosmann TR, Lo D: Effects of Th2 cytokines on chemokine expression in the lung: IL-13 potentially induces eotaxin expression by airway epithelial cells. *J Immunol* 1999;162:2477–2487.
- 38 Mochizuki M, Bartels J, Mallet AI, Christophers E, Schröder JM: IL-4 induces eotaxin: a possible mechanism of selective eosinophil recruitment in helminth infection and atopy. *J Immunol* 1998;160:60–68.
- 39 Nonaka M, Pawankar R, Fukumoto A, Ogihara N, Sakanushi A, Yagi T: Induction of eotaxin production by interleukin-4, interleukin-13 and lipopolysaccharide by nasal fibroblasts. *Clin Exp Allergy* 2004;34:804–811.
- 40 Schleimer RP, Sterbinsky SA, Kaiser J, Bickel CA, Klunk DA, Tomioka K, Newman W, Lusinskas FW, Gimbrone MA Jr, McIntyre BW, Bochner BS: IL-4 induces adherence of human eosinophils and basophils but not neutrophils to endothelium. Association with expression of VCAM-1. *J Immunol* 1992;148:1086–1092.
- 41 Ravetch JV, Bolland S: IgG Fc receptors. *Annu Rev Immunol* 2001;19:275–290.
- 42 Bandukwala HS, Clay BS, Tong J, Mody PD, Cannon JL, Shilling RA, Verbeek JS, Weinstock JV, Solway J, Sperling AI: Signaling through FcγRIII is required for optimal T helper type (Th)2 responses and Th2-mediated airway inflammation. *J Exp Med* 2007;204:1875–1889.
- 43 Laoukili J, Perret E, Willems T, Minty A, Parthoens E, Houcine O, Coste A, Jorissen M, Marano F, Caput D, Tournier F: L-13 alters mucociliary differentiation and ciliary beating of human respiratory epithelial cells. *J Clin Invest* 2001;108:1817–1824.
- 44 Weidenmaier C, Kokai-Kun JF, Kulauzovic E, Kohler T, Thumm G, Stoll H, Götz F, Peschel A: Differential roles of sortase-anchored surface proteins and wall teichoic acid in *Staphylococcus aureus* nasal colonization. *Int J Med Microbiol* 2008;298:505–513.
- 45 Cho SH, Strickland I, Boguniewicz M, Leung DY: Fibronectin and fibrinogen contribute to the enhanced binding of *Staphylococcus aureus* to atopic skin. *J Allergy Clin Immunol* 2001;108:269–274.
- 46 Schaffer AC, Solinga RM, Cocchiari J, Portoles M, Kiser KB, Risley A, Randall SM, Valtulina V, Speziale P, Walsh E, Foster T, Lee JC: Immunization with *Staphylococcus aureus* clumping factor B, a major determinant in nasal carriage, reduces nasal colonization in a murine model. *Infect Immun* 2006;74:2145–2153.
- 47 Kawabori S, Nakamura A, Kanai N: Tissue density and state of activation of eosinophils in the nasal mucosa of allergic and nonallergic rhinopathic patients. *Allergy* 1994;49:81–85.
- 48 Nagata H, Motosugi H, Sanai A, Suzuki H, Ohno K, Numata T, Konno A: Enhancement of submicroscopic damage of the nasal epithelium by topical allergen challenge in patients with perennial nasal allergy. *Ann Otol Rhinol Laryngol* 2001;110:236–242.
- 49 Topozada HH, Talaat MA: Human nasal epithelium and cellular elements in chronic allergic rhinitis. Electron-microscopic study. *ORL J Otorhinolaryngol Relat Spec* 1975;37:333–343.
- 50 Gershwin LJ: Effects of allergenic extracts on airway epithelium. *Curr Allergy Asthma Rep* 2007;7:357–362.