

Nasal exposure to Staphylococcal enterotoxin enhances the development of allergic rhinitis in mice

M. Okano*, H. Hattori*[‡], T. Yoshino[†], Y. Sugata*, M. Yamamoto*, T. Fujiwara*, A. A. Satoskar[‡], A. R. Satoskar[‡] and K. Nishizaki*

Departments of *Otolaryngology-Head & Neck Surgery and [†]Pathology, Okayama University Graduate School of Medicine and Dentistry, Okayama, Japan and [‡]Department of Microbiology, The Ohio State University, Columbus, OH, USA

Summary

Background Staphylococcal enterotoxins (SEs) appear to play a role in the pathogenesis of allergic disease. However, little is known whether the nasal exposure to SE affects the development of allergic rhinitis (AR).

Objective We sought to determine the *in vivo* effect of nasal exposure to SE on the development of AR using mouse model.

Methods BALB/c mice were intranasally sensitized with *Schistosoma mansoni* egg antigen (SmEA) in the presence or absence of staphylococcal enterotoxin B (SEB). Control mice were intranasally sensitized with either SEB or SmEA alone. The production of antigen-specific antibodies including IgE, nasal eosinophilia and cytokines by nasal mononuclear cells was compared among mice that had or had not received SEB treatment.

Results Nasal exposure to SEB enhanced the development of AR in SmEA-sensitized mice, as manifested by SmEA-specific IgE production, nasal eosinophilia, and IL-4 and IL-5 production by nasal mononuclear cells after Ag challenge. This treatment also elicited IFN- γ production by SmEA-primed cells. In addition, these mice produced SEB-specific IgE whereas mice treated with SEB without SmEA sensitization did not produce SEB-specific IgE or demonstrate nasal eosinophilia.

Conclusion These results suggest that the nasal exposure to SEB enhances susceptibility to AR although the exposure to SE solely does not induce AR.

Keywords allergy, enterotoxin, IgE, mouse, rhinitis

Submitted 2 April 2004; revised 5 September 2004; accepted 28 October 2004

Introduction

Superantigenic exotoxins produced by *Staphylococcus aureus*, including the staphylococcal enterotoxins (SEs), exfoliative toxins, and toxic shock syndrome toxin (TSST)-1, trigger an excessive immune response and appear to be involved in the pathogenesis of allergic disease, especially atopic dermatitis (AD) [1–4]. They elicit T cell activation, which leads to the production of IL-4 and IL-5 [5, 6]. Direct application of these toxins to the respiratory mucosa and to lesions of the skin induces an inflammatory response *in vivo* in both humans and mice [7–9]. In addition, *S. aureus*-derived toxins act as allergens [10–13]. Histamine release from basophils has been observed in patients with AD sensitized to SE upon exposure to SE toxins, however, histamine release has not been observed in un-sensitized AD patients or in normal controls upon exposure [13].

The mechanism by which *S. aureus* and/or *S. aureus* toxins contribute to the development of allergic rhinitis (AR)

remains unclear despite the fact that *S. aureus* is often found in the nasal mucosa and is one of the pathogens of acute infectious rhinosinusitis [14, 15]. Recently, Shiomori et al. have reported higher rates of nasal carriage of *S. aureus* and toxin-producing *S. aureus* among patients with perennial AR, compared with control subjects. In addition, peripheral blood mononuclear cells (PBMC) from these patients produced higher levels of IL-4 and IL-5, and decreased levels of IFN- γ response to the toxins [6]. Another group reported that TSST-1 enhanced pollen-specific IgE production by PBMC from patients with pollinosis during the pollen season [16]. More recently, we have reported an increased prevalence of sensitization to SEs among patients with AR, compared with healthy controls. Also, patients sensitized to SEs had higher levels of total serum IgE and exhibited poly-sensitization to other inhaled allergens, when compared with un-sensitized patients [17]. These findings prompted us to investigate the possibility that intranasal exposure to SEs may affect the allergic responses such as the production of allergen-specific IgE and nasal eosinophilia, in cases of AR.

We have recently developed a murine model of AR using *Schistosoma mansoni* egg antigen (SmEA) as allergen [18]. Intranasal sensitization with SmEA induces allergic hallmarks such as the production of Ag-specific IgE and local

Correspondence: Mitsuhiro Okano, Department of Otolaryngology-Head & Neck Surgery, Okayama University Graduate School of Medicine and Dentistry, 2-5-1 Shikata-cho Okayama-shi, Okayama 700-8558, Japan.
E-mail: mokano@cc.okayama-u.ac.jp

eosinophils in the absence of any adjuvants, thus we believe that this model is suitable to investigate the initiation of AR. In the present study, we investigated the role of nasal exposure to SEB, one of the commonest exotoxins found in the nasal cavity [6], in the development of AR *in vivo*. The results presented here may argue against our current understanding of the 'hygiene hypothesis' [19].

Materials and methods

Animals and antigens

Female BALB/c mice aged 7–10 weeks old were purchased from Charles River Japan (Yokohama, Japan). The mice were maintained in SPF condition at Okayama University Medical School in accordance with the guidelines set forth by the Okayama University Medical Area Research Committee. And all experimental protocols and procedures in the present study were approved by institutional animal care and use Committee. SmEA was prepared as previously described [18]. SEB was purchased from Toxin Technology Inc. (Sarasota, FL, USA). Ovalbumin (OVA: grade V) was purchased from Sigma (St Louis, MO, USA).

Sensitization of mice

Mice ($n = 6$ per group) were intranasally sensitized with SmEA in the absence of adjuvants to archive the initiation of AR as described previously with slight modification [18]. In brief, low (0.1 µg) or high (10 µg) amount of SmEA mixed with either 5 or 500 ng of SEB in 20 µL phosphate-buffered saline (PBS) was instilled into nostrils once a week for 3 weeks. One week after the third sensitization, the same treatment was challenged intranasally for 7 consecutive days. As control, mice were received either the same amount of SmEA or PBS alone. In another set of experiment, mice were applied intranasally with 500 ng of SEB alone in a same manner as described above (Fig. 1a). Peripheral blood was collected from the tail vein prior to sensitization (day 0), 6 days after the third sensitization (day 20) and/or 12 h after the final challenge (day 28), then centrifuged at 200g, and the specific Ab content in the serum was tested.

In order to investigate the effect of exposure to SEB on nasal challenge, BALB/c mice ($n = 6$ per group) were sensitized intraperitoneally with 50 µg OVA adsorbed to 1 mg alum (Kyowa Kagaku, Kagawa, Japan) in a total volume of 200 µL on day 0, 7, and 14. Two weeks after the third intraperitoneal sensitization, mice were challenged intranasally with 10 µg OVA in the presence or absence of 5 or 500 ng of SEB in 20 µL PBS for 7 consecutive days. Twelve hours after the final nasal challenge, blood was taken (Fig. 1b).

Antibody determination

The levels of Ag-specific Ab including IgE, IgG1, and IgG2a were measured by ELISA as previously described [20]. The levels of SEB-specific IgE Ab were also measured by captured ELISA using biotinylated SEB (Toxin Technology Inc.) as a detection reagent [20]. Titres for specific IgE were estimated as mean optical density (OD) at 450 nm of 1:4 diluted sera.

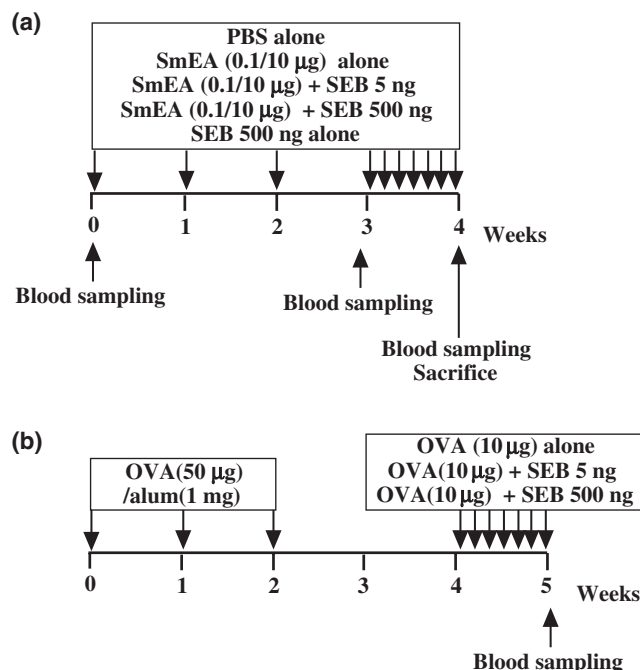


Fig. 1. Experimental design used to investigate the effect of nasal exposure with staphylococcal enterotoxin B (SEB) on the initiation (a) and nasal challenge (b) of allergic rhinitis in mice. SmEA, *Schistosoma mansoni* egg antigen.

Titres for specific IgG1 and IgG2a are estimated as mean OD at 450 nm of 1:100 diluted sera.

Histological examination

Histological examination was performed as previously described [20]. Briefly, 12 h after the final nasal challenge mice were killed by cervical dislocation. Their heads were removed, fixed in 10% formalin and decalcified with 2.5% EDTA-2Na solution. Coronal nasal section were stained with Luna solution and the number of eosinophils in the nasal mucosa was counted microscopically under a high power field (10 × 40).

In vitro culture of nasal mononuclear cells and cytokine determination

Twelve hours following the final nasal challenge, mice were sacrificed, and heads were removed. Nasal mononuclear cells were isolated as described previously [20]. In brief, skin-peeled nasal components were cut into small pieces, and incubated with RPMI 1640 (Sigma) containing 4 mg/mL collagenase (Boehringer-Mannheim, Indianapolis, IN, USA) for 30 min at 37 °C. After the incubation, a single cell suspension obtained by passing 70 µm cell strainer (BD Falcon, Bedford, MA) was placed on top of 40% and 75% Percoll density gradient solutions (Pharmacia, Uppsala, Sweden). After the centrifugation at 200g for 15 min, nasal mononuclear cells were recovered from interface between the 40% and 75% Percoll solutions. Then IL-4, IL-5, and IFN-γ production by SmEA-stimulated and unstimulated cells were measured [20]. Detection limit for IL-4, IL-5, and IFN-γ in this system was 0.1 U/mL, 40 pg/mL, and 0.1 IU/mL, respectively.

Statistical analysis

Student's unpaired *t*-test was used to determine the statistical significance of the values obtained. $P < 0.05$ was considered statistically significant. Data are expressed as the mean standard error of mean (SEM) for each subject group.

Results

Nasal exposure to staphylococcal enterotoxin enhances the development of allergic rhinitis

Following repeated intranasal sensitization with 0.1 µg of SmEA, which has been shown to induce mild sensitization [21], BALB/c mice produced detectable amounts of SmEA-specific IgE and IgG1, but not IgG2a (Figs 2a–c). An increased level of total serum IgE was also observed (Fig. 2d). Simultaneous application of 5 ng of SEB with SmEA led to a significant increase in the production of SEA-specific IgG1. This treatment also enhanced, albeit not significantly, the production of SmEA-specific IgE. On the other hand, application of

500 ng of SEB with SmEA significantly enhanced both SmEA-specific IgG1 and IgE production (Figs 2b, c). This treatment also elicited an increase in total serum IgE (Fig. 2d). In addition, both concentrations of SEB increased the production of SmEA-specific IgG2a, however, these increases in production were not statistically significant (Fig. 2c).

Similar results were obtained with regard to the development of local eosinophilia, as determined by histological examination. Mice sensitized with 0.1 µg of SmEA showed subtle eosinophil infiltration, and the average number $\pm$ SEM of eosinophils per field (10×40) of the nasal septum was 5.33 ± 1.31 (Fig. 3a). In mice treated with 5 ng of SEB, a slight increase in eosinophil infiltration was observed, compared with control mice, but this increase was not significant (Fig. 3B). Eosinophil infiltration was significantly greater in mice sensitized with SmEA mixed with 500 ng of SEB, compared with mice sensitized with SEA alone (Fig. 3c). The average number $\pm$ SEM of eosinophils per field was 8.83 ± 1.25 and 35.50 ± 6.80 in mice treated with 5 and 500 ng of SEB, respectively.

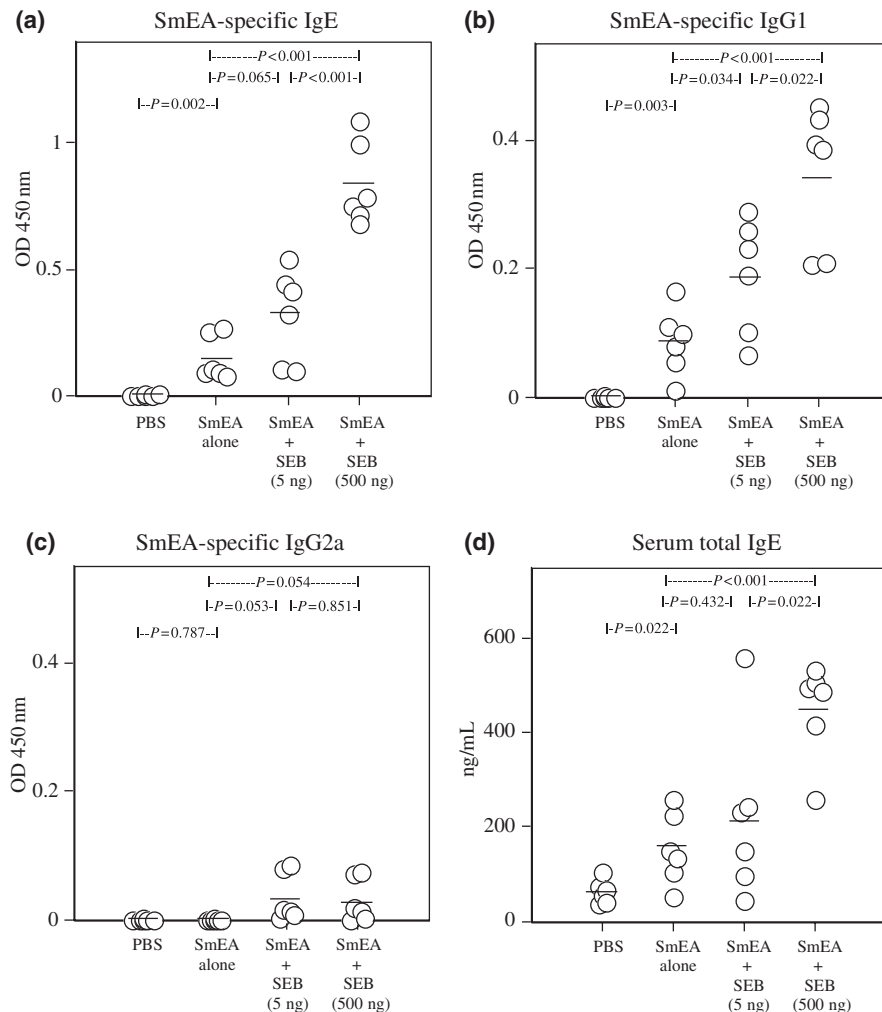


Fig. 2. Effect of nasal exposure with staphylococcal enterotoxin B (SEB) on antibody production following intranasal sensitization with 0.1 µg of *Schistosoma mansoni* egg antigen (SmEA). BALB/c mice ($n = 6$) were intranasally sensitized with 0.1 µg of SmEA or phosphate-buffered saline (PBS). Five or 500 ng of SEB or PBS, which served as a control, were applied intranasally at the same time, as shown in Fig. 1. Blood was sampled from the tail 12 h after the final nasal challenge. Titres of SmEA-specific IgE (a), IgG1 (b), IgG2a (c), and serum total IgE (d) were determined by ELISA. Results show (a) the mean optical density (OD) at 450 nm $\pm$ SEM of six serum samples from each group at a 1 : 4 dilution, and (b, c) the mean OD at 450 nm $\pm$ SEM of six serum samples from each group at a 1 : 100 dilution. Data are representative of three separate experiments.

Cytokine production from nasal mononuclear cells after challenge with SmEA was then examined. The cells were isolated by enzyme extraction and stimulated with SmEA for 72 h, after which cytokine production within the culture supernatant was assessed. SmEA-stimulated nasal mono-

nuclear cells from control mice produced detectable amounts of IL-4 and IL-5, but not IFN- γ (Figs 4a–c). Nasal application of 500 ng of SEB with each repeated intranasal application of

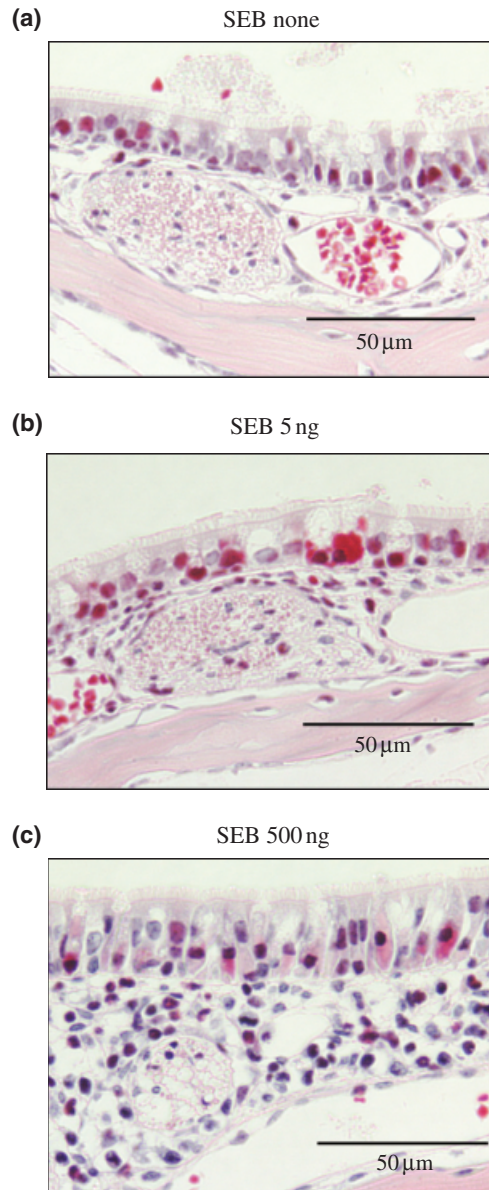
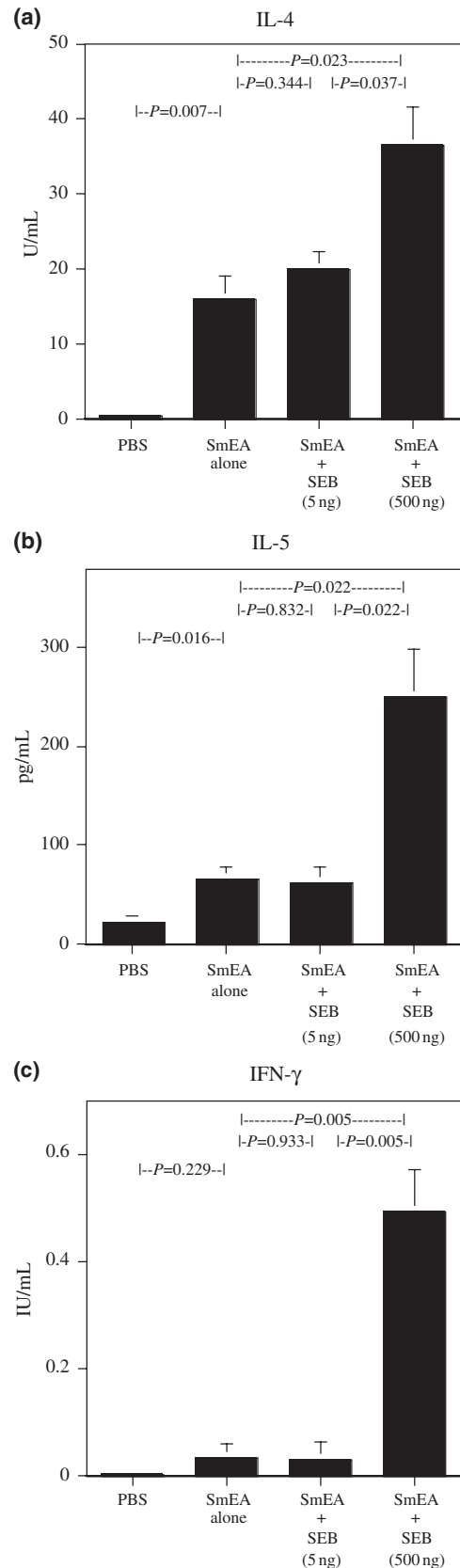


Fig. 3. Effect of nasal exposure with staphylococcal enterotoxin B (SEB) on the induction of nasal eosinophilia. BALB/c mice ($n = 6$) were intranasally sensitized with *Schistosoma mansoni* egg antigen (SmEA). Phosphate-buffered saline (a) 5 ng of SEB (b), or 500 ng of SEB (c) were applied intranasally at the same time of sensitization. Twelve hours after the final nasal challenge with SmEA, mice were sacrificed. Nasal sections were fixed, decalcified, and Luna staining was performed to detect eosinophils in the nasal mucosa.

Fig. 4. Production of IL-4 (a), IL-5 (b) and IFN- γ (c) by nasal lymphocytes from BALB/c mice intranasally exposed with staphylococcal enterotoxin B (SEB) or phosphate-buffered saline (PBS) during sensitization and subsequent challenge with *Schistosoma mansoni* egg antigen (SmEA). Nasal lymphocytes were isolated and cultured *in vitro* for 72 h with SmEA. Cytokines were measured by ELISA. Results show the mean $\pm$ SEM of three separate experiments.



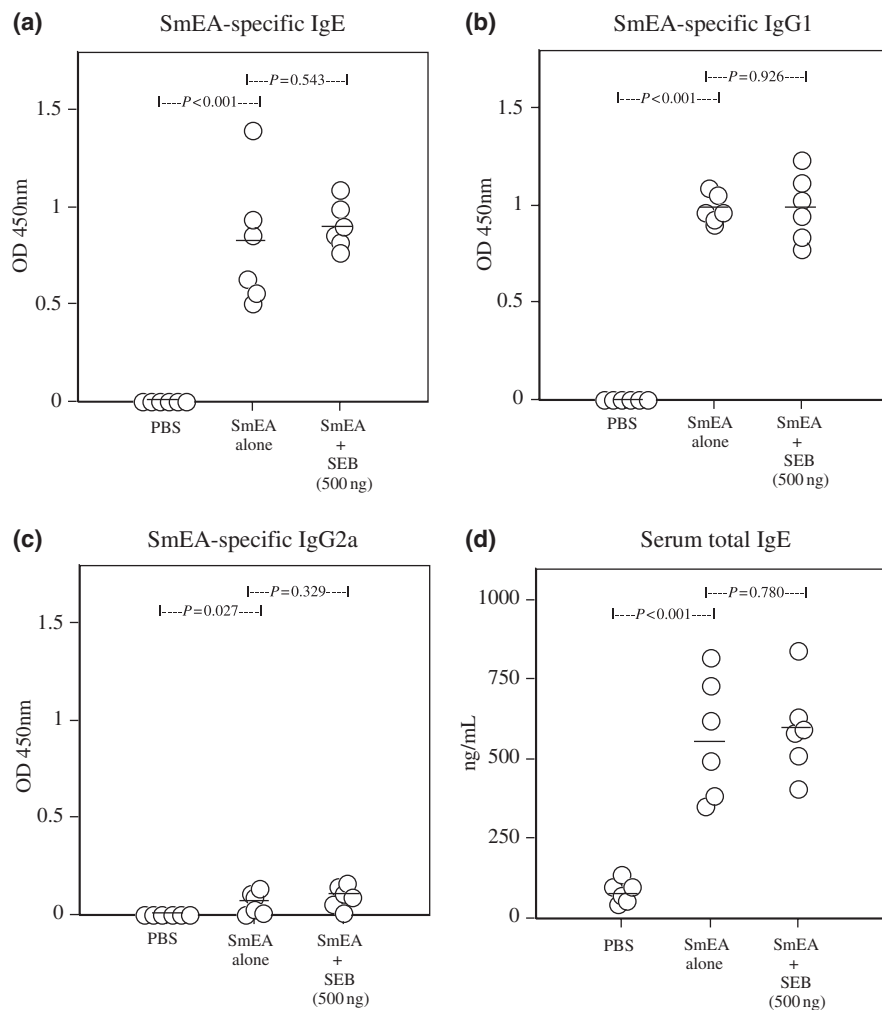


Fig. 5. Effect of nasal exposure with staphylococcal enterotoxin B (SEB) on antibody production following intranasal sensitization with 10 µg of *Schistosoma mansoni* egg antigen (SmEA). BALB/c mice ($n = 6$) were intranasally sensitized with 10 µg of SmEA or phosphate-buffered saline (PBS). Five hundred nanograms of SEB or PBS, which served as a control, were applied intranasally at the same time, as shown in Fig. 1. Blood was sampled from the tail 12 h after the final nasal challenge. Titres of SEA-specific IgE (a), IgG1 (b), IgG2a (c), and serum total IgE (d) were determined by ELISA. Results show (a) the mean optical density (OD) at 450 nm $\pm$ SEM of six serum samples from each group at a 1 : 4 dilution, and (b, c) the mean OD at 450 nm $\pm$ SEM of six serum samples from each group at a 1 : 100 dilution. Data are representative of two separate experiments.

SmEA led to an increase in IL-4 and IL-5 production (Figs 4a and b). In addition, nasal mononuclear cells also produced significantly greater levels of IFN- γ in response to SmEA, than those from mice sensitized with SmEA alone (Fig. 4c). On the other hand, nasal application of 5 ng of SEB did not significantly affect cytokine production.

Effect of nasal exposure to staphylococcal enterotoxin on the development of allergic rhinitis depends on the dose of allergen introduced

BALB/c mice were intranasally sensitized with 10 µg of SmEA, which has been shown to induce full sensitization [21]. BALB/c mice produced greater amounts of SmEA-specific IgE and IgG1, along with increased levels of total serum IgE, compared with mice treated with 0.1 µg of SmEA (Figs 2 and 5). Nasal exposure to 500 ng of SEB did not alter the production of SmEA-specific Abs or total serum IgE (Fig. 5). In addition, this treatment did not modify the degree of eosinophilia noted following repeated nasal sensitization with 10 µg of SmEA. The average num-

ber $\pm$ SEM of eosinophils per field (10×40) of the nasal septum was 80.67 ± 11.63 and 59.50 ± 6.70 in mice that did or did not receive intranasal exposure to 500 ng of SEB, respectively ($P = 0.146$).

Nasal exposure to staphylococcal enterotoxin alone does not induce the pathological features of allergic rhinitis

We sought to determine whether repeated nasal exposure to SEB alone induces the production of IgE or results in nasal eosinophilia. Levels of total serum IgE did not differ significantly among mice exposed to 500 ng of SEB alone or PBS alone throughout the experiment (Fig. 6). In addition, eosinophilia was not observed within the nasal mucosa of mice treated with 500 ng of SEB alone.

Staphylococcal enterotoxin-specific immunoglobulin E was detected only in the presence of allergen

Mice sensitized with 0.1 µg of SmEA together with 500 ng of SEB produced significant levels of SEB-specific IgE after

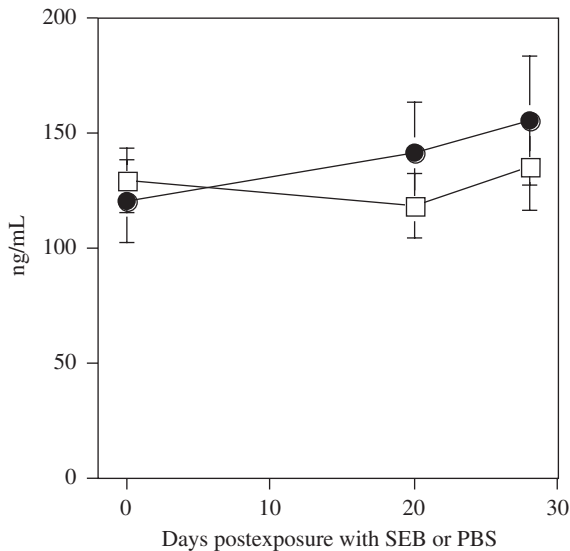


Fig. 6. Effect of nasal exposure with staphylococcal enterotoxin B (SEB) alone on the amount of serum total IgE. BALB/c mice ($n = 6$) were repeatedly applied with 500 ng of SEB (closed circle) or phosphate-buffered saline (PBS) (open square) intranasally as shown in Fig. 1. Blood was sampled prior to (day 0), 6 days after the third (day 20), and 12 h after 10th (day 28) exposure with SEB. Results show the mean amounts $\pm$ SEM of six serum samples from each group. Data are representative of two separate experiments.

nasal challenge. Mice sensitized with SmEA together with 5 ng of SEB also produced SEB-specific IgE, however, a significant increase in the level of SEB-specific IgE over that of control mice was not observed. Mice treated with 500 ng of SEB alone did not display significant SEB-specific IgE production (Fig. 7).

Effect of nasal exposure to staphylococcal enterotoxin on nasal challenge in presensitized mice

BALB/c mice presensitized with OVA were intranasally challenged with OVA in the presence or absence of 5 or 500 ng of SEB. Mice exposed to 500 ng of SEB during the nasal challenge produced significantly higher amount of OVA-specific IgE but not IgG1 or IgG2a as compared with other groups (Fig. 8).

Discussion

In the present study, we analyzed the immunological effects of nasal exposure to a *S. aureus*-derived enterotoxin, specifically with regard to the development of AR in mice. Mice sensitized with 0.1 μ g of SmEA together with 500 ng of SEB produced significantly greater amounts of SmEA-specific IgE and IgG1, as well as total serum IgE, compared with mice sensitized with SmEA alone (Fig. 2). Eosinophil infiltration of the nasal mucosa was also markedly increased in mice treated with 500 ng of SEB, compared with control mice (Fig. 3). These results suggest that nasal exposure to SEB enhances the development of AR.

There are several mechanisms by which exposure to an enterotoxin might augment the development of AR. First, SEB might enhance the immune response following presenta-

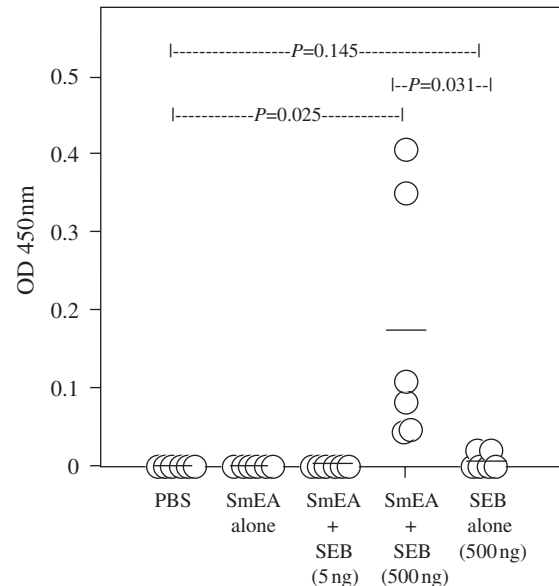


Fig. 7. Effect of nasal exposure with staphylococcal enterotoxin B (SEB) on SEB-specific IgE production. BALB/c mice ($n = 6$) were repeatedly received the intranasal application with phosphate-buffered saline (PBS), 0.1 μ g of *Schistosoma mansoni* egg antigen (SmEA) alone, 0.1 μ g of SmEA together with 5 ng of SEB, 0.1 μ g of SmEA together with 500 ng of SEB or 500 ng of SEB alone as shown in Fig. 1. Blood was sampled from the tail 12 h after the final nasal challenge. Titres of SEB-specific IgE were determined by ELISA. Results show the mean optical density at 450 nm $\pm$ SEM of six serum samples from each group at a 1 : 4 dilution. Data are representative of two separate experiments.

tion of an allergen-specific antigen. Conventional allergens, such as SmEA, are ingested by antigen-presenting cells (APCs) such as macrophages and dendritic cells, after which they are digested into small peptides, which then bind to the groove in major histocompatibility complex (MHC) class II molecules. After this, T cells display allergen-specific activation via binding between the T cell receptor (TCR) and peptide/MHC class II molecules [22]. Superantigens, such as SEB, stimulate T cells in the absence of these conventional proceeding and presentation, since superantigen can bind directly to MHC class II β chain and the TCR-V β element [23]. Thus, direct binding of SEB to an MHC class II molecule loaded with SEA-derived peptides might enhance the antigenicity of an allergen. Second, activation of immune cells, such as T cells and dendritic cells, by superantigens leads to the synthesis of a variety of pro-inflammatory cytokines, including IL-1 and TNF- α [24, 25]. In addition, SEB has been shown to induce high levels of CC chemokines, such as macrophage inflammatory protein (MIP)-1 α , MIP-1 β , and monocyte chemoattractant protein-1 (MCP-1), which are involved in the recruitment and activation of eosinophils [26, 27]. A micro-environment in which these pro-inflammatory cytokines and chemokines are present might set the stage for the development of AR. In fact, mice deficient in TNF- α have displayed a significant reduction in antigen-specific IgE production, nasal symptoms, T helper 2 (Th2)-type cytokine production, and nasal eosinophilia, in a murine model of AR [28].

Interestingly, such an adjuvant effect of SEB is not found when mice were sensitized with 10 μ g of SmEA, with which dose the pathological features of AR were fully induced (Fig. 5). In addition, nasal exposure with low dose (5 ng) of SEB

only augmented SmEA-specific IgG1 production but not IgE production or nasal eosinophilia (Figs 2 and 3). In addition, nasal exposure to a low dose of SEB (5 ng) only augmented

SmEA-specific IgG1 production, but not IgE production or nasal eosinophilia (Figs 2 and 3). These results suggest that both SEB and the SmEA allergen have dose-dependent effects on the development of AR.

Nasal mononuclear cells from SmEA-sensitized mice exposed to 500 ng of SEB, produced not only IL-4 and IL-5, but also IFN- γ , in response to recall stimulation with SmEA *in vitro* (Fig. 4). This result suggests that nasal exposure to SEB does not induce a strict Th2-skewed response. The finding of a slight, but not significant, increase in the production of SmEA-specific IgG2a upon exposure of mice to 500 ng of SEB (Fig. 2c) supports this assertion. As described above, superantigens induce the production of various pro-inflammatory cytokines and chemokines, some of which preferentially induce T helper type 1 (Th1) responses [29]. In addition, Saloga et al. [30] have revealed a shift in the immune response of mice to OVA from Th2-associated Ab production of OVA-specific IgE and IgG1 to Th1-associated Ab production of IgG2a, IgG2b and IgG3, following intradermal administration of SEB. Since SmEA is a potent Th2-inducer because of its carbohydrate content [31], it is possible that the presence of both allergen and exotoxin might facilitate both Th1 and Th2 responses.

Recent reports revealed that IgE to SE is related with local eosinophilic inflammation in nasal polyps and the severity of asthma [32, 33]. And we have observed an increased prevalence of SE-specific IgE among patients with AR, compared with healthy controls. In addition, patients sensitized with SEs significantly demonstrated increased levels of total serum IgE and polyvalent sensitization [17]. In the present study, SEB-specific IgE production, and increased levels of total serum IgE, were seen in mice exposed to SEB combined with SmEA (Figs 2 and 7). The present results are consistent with clinical observations, and suggest that nasal exposure to SEB may be involved in polyvalent sensitization.

However, intranasal application of SEB alone did not induce significant production of SEB-specific IgE or eosinophilia within the nasal mucosa (Figs 6 and 7). Herz et al. [9] have reported induction of pulmonary eosinophilia, and detection of Th2-dominant cytokines by bronchoalveolar lavage, as well as tracheal hypersensitivity, following intranasal administration of SEB. Although they did not focus on IgE production or nasal inflammation, their results are not consistent with our own. This may be because of different responses of the upper and lower airways to SEB. In fact, upper and lower airways are known to display different responses to bacterial products, such as lipopolysaccharide [34]. Our results suggest that SEB does not act as an allergen on its own, and therefore does not result in SEB-specific IgE production and nasal eosinophilia. However, simultaneous application with SmEA may enhance the antigenicity of the SEB and lead to the production of SEB-specific IgE. This

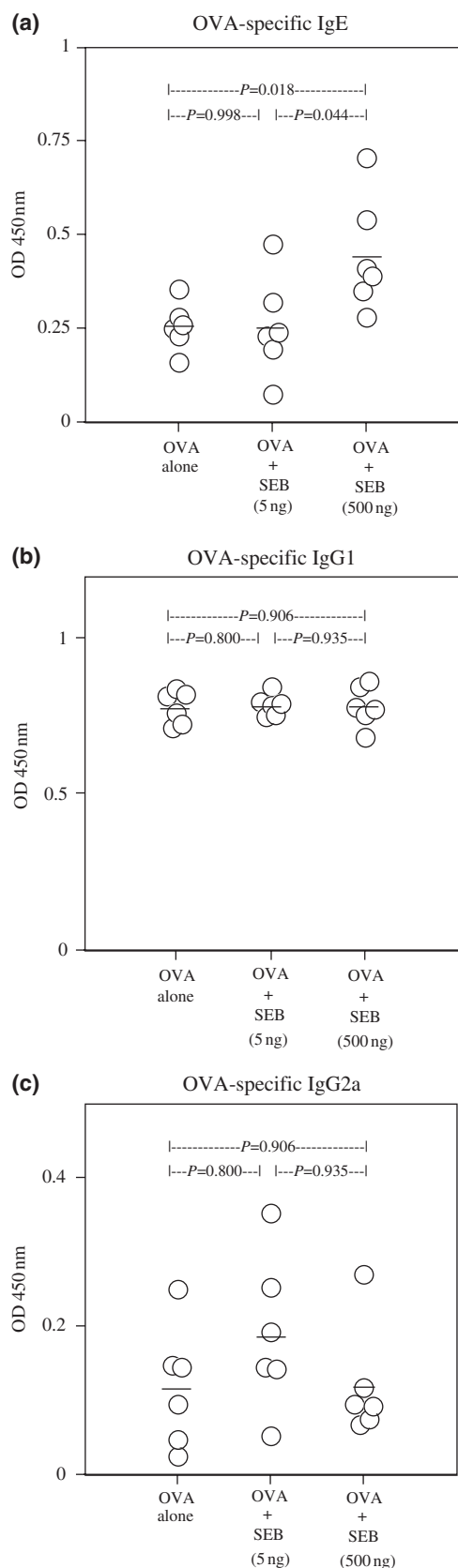


Fig. 8. Effect of nasal exposure with staphylococcal enterotoxin B (SEB) on nasal challenge. BALB/c mice ($n = 6$) were presensitized with ovalbumin (OVA)/Alum, then intranasally challenged with OVA in the presence or absence of 5 ng or 500 ng of SEB, as shown in Fig. 1. Blood was sampled from the tail 12 h after the final nasal challenge. Titres of OVA-specific IgE (a), IgG1 (b), and IgG2a (c) were determined by ELISA. Results show (a) the mean optical density (OD) at 450 nm $\pm$ SEM of six serum samples from each group at a 1:4 dilution, and (b, c) the mean OD at 450 nm $\pm$ SEM of six serum samples from each group at a 1:100 dilution. Data are representative of two separate experiments.

may be because of the adjuvant activity of SmEA since it contains immunomodulatory carbohydrate that leads to the induction of Th2 responses.

In conclusion, we demonstrated that nasal exposure to SEB enhances sensitization and allergic inflammation in a murine model of AR. The present results may argue against our current understanding of the 'hygiene hypothesis' [19]. In fact, recent report suggested that a difference in responses to Gram-negative vs. Gram-positive bacteria may affect the initiation of allergic diseases [35].

Acknowledgements

The authors thank Dr Donald A. Harn for providing SmEA. They also thank Yuko Okano for editorial assistance. This work was supported in part by the grants from Ministry of Education, Culture, Sports, Science and Technology, Japan (no. 14704043) and Research on Allergic disease and Immunology of Ministry of Health, Labor and Welfare (no. 14210301).

References

- White J, Herman A, Pullen AM, Kubo R, Kappler JW, Marriack P. The V β -specific superantigen staphylococcal enterotoxin B; stimulation of mature T cells and clonal deletion in neonatal mice. *Cell* 1989; 56:27–35.
- Kappler J, Kotzin B, Herron L et al. V beta-specific stimulation of human T cells by staphylococcal toxins. *Science* 1989; 244:811–3.
- Mcfadden JP, Noble WC, Camp RDR. Superantigen exotoxin-secreting potential of staphylococci isolated from atopic eczematous skin. *Br J Dermatol* 1993; 128:631–2.
- Akiyama H, Toi Y, Kanzaki H, Tada J, Arata J. Prevalence and producers of enterotoxins and toxic shock syndrome toxin-1 among *Staphylococcus aureus* strain isolated from atopic dermatitis lesions. *Arch Dermatol Res* 1996; 288:418–20.
- Neuber K, Steinrucke K, Ring J. Staphylococcal enterotoxin B affect in vitro IgE synthesis, interferon-gamma, interleukin-4, and interleukin-5 production in atopic eczema. *Int Arch Allergy Immunol* 1995; 107:179–82.
- 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; 105:449–54.
- Strange P, Skov L, Lisby S, Nielsen PL, Baadsgaard O. Staphylococcal enterotoxin B applied on intact normal and intact atopic skin induces dermatitis. *Arch Dermatol* 1996; 132:27–33.
- Saloga J, Leung DY, Reardon C, Giorno RC, Born W, Gelfand EW. Cutaneous exposure to the superantigen staphylococcal enterotoxin B elicits a T-cell-dependent inflammatory response. *J Invest Dermatol* 1996; 106:982–8.
- Herz U, Ruckert R, Wollenhaupt K et al. Airway exposure to bacterial superantigen (SEB) induces lymphocyte-dependent airway inflammation associated with increased airway responsiveness – a model for non-allergic asthma. *Eur J Immunol* 1999; 29: 1021–31.
- Bunikowski R, Mielke M, Skarabis H et al. Prevalence and role of serum IgE antibodies to the *Staphylococcus aureus*-derived superantigens SEA and SEB in children with atopic dermatitis. *J Allergy Clin Immunol* 1999; 103:119–24.
- Nomura I, Tanaka K, Tomita H et al. Evaluation of the staphylococcal enterotoxins and their specific IgE in childhood atopic dermatitis. *J Allergy Clin Immunol* 1999; 104:441–6.
- Tada J, Toi Y, Akiyama H et al. Presence of specific IgE antibodies to staphylococcal enterotoxin in patients with atopic dermatitis. *Eur J Dermatol* 1996; 6:552–4.
- Leung DY, Harbeck R, Bina P et al. Presence of IgE antibodies to staphylococcal exotoxins on the skin of patients with atopic dermatitis. *J Clin Invest* 1993; 92:1374–80.
- 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.
- Suzuki K, Nishiyama Y, Sugiyama K, Miyamoto N, Baba S. Recent trends in clinical isolates from paranasal sinusitis. *Acta Otolaryngol* 1996; 525 (Suppl.):51–5.
- Hofer MF, Harbeck RJ, Schlievert PM, Leung DY. Staphylococcal toxins augment specific IgE responses by atopic patients exposed to allergen. *J Invest Dermatol* 1999; 112:171–6.
- Okano M, Takishita T, Yamamoto T et al. Presence and characterization of sensitization to staphylococcal enterotoxins in patients with allergic rhinitis. *Am J Rhinol* 2001; 15:417–21.
- Watanabe T, Okano M, Hattori H et al. Roles of Fc γ RIIB in nasal eosinophilia and IgE production in murine allergic rhinitis. *Am J Respir Crit Care Med* 2004; 169:105–12.
- Braun-Fahrlander C. Environmental exposure to endotoxin and other microbial products and the decreased risk of childhood atopy: evaluating developments since April 2002. *Curr Opin Allergy Clin Immunol* 2003; 3:325–9.
- Okano M, Azuma M, Yoshino T et al. Differential role of CD80 and CD86 molecules in the induction and the effector phases of allergic rhinitis in mice. *Am J Respir Crit Care Med* 2001; 164:1501–7.
- Okano M, Nishizaki K, Abe M et al. Strain-dependent induction of allergic rhinitis without adjuvant in mice. *Allergy* 1999; 54:593–601.
- Okano M, Nagano T, Nakada M et al. Epitope analysis of HLA-DR-restricted helper T-cell responses to Der p II, a major allergen molecule of *Dermatophagoides pteronyssinus*. *Allergy* 1996; 51: 29–35.
- Irwin MJ, Hudson KR, Ames KT, Fraser JD, Gascoigne NR. T-cell receptor β -chain binding to enterotoxin superantigens. *Immunol Rev* 1993; 131:61–78.
- Brand JM, Kirchner H, Neustock P, Kruse A. Induction of cytokine in human whole blood cultures by a mitogen derived from *Mycoplasma arthritidis* and by staphylococcal enterotoxin B. *Immunobiology* 1992; 186:246–53.
- Matsuyama S, Koide Y, Yoshida TO. HLA class II molecule-mediated signal transduction mechanism responsible for the expression of interleukine-1 beta and tumor necrosis factor-alpha genes induced by a staphylococcal superantigen. *Eur J Immunol* 1993; 23:3194–204.
- Krakauer T. Induction of CC chemokines in human peripheral blood mononuclear cells by staphylococcal exotoxins and its prevention by pentoxifylline. *J Leukocyte Biol* 1999; 66:158–64.
- Dahinden CA, Geiser T, Brunner T et al. Monocyte chemoattractant protein 3 is a most effective basophil and eosinophil-activating chemokine. *J Exp Med* 1994; 179:751–6.
- Iwasaki M, Saito K, Takemura M et al. TNF- α contributes to the development of allergic rhinitis in mice. *J Allergy Clin Immunol* 2003; 112:134–40.
- Stuyt RJ, Netea MG, Kim SH et al. Differential roles of interleukin-18 (IL-18) and IL12 for induction of gamma interferon by staphylococcal cell wall components and superantigens. *Infect Immun* 2001; 69:5025–30.
- Saloga J, Lack G, Bradley K et al. Inhibition of the development of immediate hypersensitivity by staphylococcal enterotoxin B. *Eur J Immunol* 1994; 24:3140–7.
- Okano M, Satoskar AR, Nisizaki K, Harn DA. Induction of Th2 responses and IgE is largely due to carbohydrates functioning as adjuvants on *Schistosoma mansoni* egg antigens. *J Immunol* 1999; 163:6712–7.

- 32 Bachert C, Gevaert P, Holtappels G, Johansson SGO, van Cauwenberge P. Total and specific IgE in nasal polyps is related to local eosinophilic inflammation. *J Allergy Clin Immunol* 2001; 107:607–14.
- 33 Bachert C, Gevaert P, Howarth P, Holtappels G, van Cauwenberge P, Johansson SGO. IgE to *Staphylococcal aureus* enterotoxin in serum is related to severity of asthma. *J Allergy Clin Immunol* 2003; 111:1131–2.
- 34 Nonaka M, Pawankar R, Saji F, Yagi T. Distinct expression of RANTES and GM-CSF by lipopolysaccharide in human nasal fibroblasts but not in other airway fibroblasts. *Int Arch Allergy Immunol* 1999; 119:314–21.
- 35 Heaton T, Mallon D, Venaille T, Holt P. Staphylococcal enterotoxin induced IL-5 stimulation as a cofactor in the pathogenesis of atopic diseases: the hygiene hypothesis in reverse? *Allergy* 2003; 58:252–6.