

ORIGINAL ARTICLE

Blocking interleukin-17 attenuates enhanced inflammation by staphylococcal enterotoxin B in murine allergic rhinitis model

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Abstract

Conclusion: The results of this study suggest that staphylococcal enterotoxin B (SEB) enhances inflammation in allergic rhinitis (AR) and interleukin (IL)-17 may play a significant role in regulation of the inflammatory process induced by SEB. **Objectives:** Exposure to SEB is associated with severe symptoms in AR patients. However, how SEB influences the inflammatory process in AR has not been elucidated so far. The aim of this study was to identify the role of SEB and IL-17 in AR. **Methods:** BALB/c and IL-17-deficient mice were divided into three groups: control, OVA, and SEB groups. The control group was challenged with phosphate-buffered saline, the AR group was challenged with ovalbumin (OVA), and the SEB group was treated with SEB before OVA challenge. Nasal symptoms, eosinophil infiltration, serum total and OVA-specific immunoglobulin E levels, and cytokine levels including IL-1 β , IL-4, IL-6, and interferon (IFN)- γ in splenocyte culture were compared between the groups. **Results:** The SEB group produced significantly greater amounts of cytokines such as IL-1 β , IL-4, IL-6, and IFN- γ than the OVA group in wild-type mice. However, these enhanced inflammatory responses in the SEB group compared with the OVA group were not observed in IL-17-deficient mice.

Keywords: Superantigens, inflammatory process, cytokines, allergic rhinitis

Introduction

Allergic rhinitis (AR) is an allergic inflammatory reaction in the nasal mucosa, which is characterized by increased expression of T helper (Th) 2 type cytokines interleukin (IL)-4 and IL-5, elevated antigen-specific IgE, and a marked inflammatory cell infiltration [1].

Staphylococcus aureus (*S. aureus*) is a gram-positive pathogen responsible for a wide range of human disease, and is often found as part of the

microflora of the upper respiratory tract [2]. It has been reported that the mean carriage rate of *S. aureus* in the nasal cavity is 37.2% in the general population, and the anterior nares are the primary reservoir of *S. aureus* [3]. In addition, another study has revealed that the rate of nasal carriage of *S. aureus* is significantly higher in patients with perennial allergic rhinitis (AR, 44%) than that of control subjects (20%). Of interest, *S. aureus*-positive groups in allergic patients revealed significantly more severe symptoms than *S. aureus*-negative groups [4]. *S. aureus*

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produces various toxins with superantigen activity, such as staphylococcal enterotoxins (SEs) and toxic shock syndrome toxin 1 [5]. Among these, in particular the SEs are known to be powerful superantigens. Superantigens have an ability to cross-link the antigen-presenting cells (APCs) and T-cell receptors. Therefore, they can activate up to 30% of the T-cell population in the body nonspecifically and cause an excess immune response [6]. Also, SEs act like a proinflammatory cytokine and activate macrophages. Activated macrophages can induce IL-1 β and IL-6, which are the most potent inducers of IL-17 [7,8].

IL-17 is the key cytokine of Th17 cells, and serum IL-17 levels are increased in patients with AR and are related to clinical symptoms, peripheral eosinophil count, and medication usage [9]. In addition, evidence for the involvement of IL-17 in the pathogenesis of AR is further provided by the finding that expression of IL-17 receptor beta is up-regulated in patients with seasonal AR [10]. IL-17 is involved in the pathogenesis of allergic disease and is known to orchestrate the neutrophilic influx into the airways and also enhance Th2 cell-mediated eosinophilic airway inflammation [11].

Although SEs and IL-17 are believed to play roles in the development of inflammation, the association between SEs and IL-17 in AR has not been clearly elucidated until now. Therefore, the aim of this study was to investigate the role of SEs and IL-17 in a mouse model of ovalbumin (OVA)-induced AR.

Material and methods

Animals

Four week-old female BALB/c mice and IL-17-deficient mice were used in all experiments. IL-17-deficient mice were purchased from Professor Yoichiro Iwakura at the Center for Experimental Medicine Institute of the Medical Science University of Tokyo. IL-17-deficient mice were on a BALB/c genetic background and were bred in specific pathogen-free conditions. Each mouse weighed 20–30 g. The studies herein followed the principles for laboratory animal research as outlined in the Animal Welfare Act and Department of Health, Education, and Welfare (National Institutes of Health) guidelines for the experimental use of animals, and these experimental protocols were approved by our institution's animal subjects committee.

Sensitization, antiangiogenic agent delivery, and allergen challenge

Forty-eight mice were divided into the following six groups: (1) the negative control group (group

A) was challenged with phosphate-buffered saline (PBS), (2) the AR group (group B) was challenged with ovalbumin (OVA; Grade V, Sigma-Aldrich, St Louis, MO, USA), (3) the staphylococcal enterotoxin B (SEB; Sigma-Aldrich) group (group C) was treated with SEB before intranasal OVA challenge, (4) the negative control group (group D) of IL-17-deficient mice, (5) the AR group (group E) of IL-17-deficient mice, and (6) the SEB group (group F) of IL-17-deficient mice.

The procedure for allergen sensitization and challenge was performed and is summarized in Figure 1. Briefly, on days 0, 7, and 14, mice were sensitized by intraperitoneal administration of 25 μ g of OVA mixed with 1 mg of aluminum hydroxide (Sigma) in 300 μ l of PBS or PBS only (groups A and D). One week after the last sensitization (day 21), mice were intranasally challenged daily with 100 μ g of OVA from day 21 to day 27 (AR group and SEB group). In the negative control groups (groups A and D), mice were intranasally challenged with PBS by the same schedule. To investigate the effect of exposure to SEB on nasal challenge, the SEB groups (groups C and F) were given intranasally 500 ng of SEB dissolved in 20 μ l of saline. At 24 h after the final OVA challenge, each mouse was killed and specimens were obtained for analysis.

Evaluation of allergic responses

Nasal symptom scores. At each sacrifice, after intranasal allergen provocation with 200 μ g of OVA, the frequency of sneezing and nose scratching was counted for 15 min to evaluate early allergic responses.

Measurement of total IgE and OVA-specific IgE. The serum levels of total IgE and OVA-specific IgE were measured by solid-phase enzyme-linked immunosorbent assay (ELISA). The serum samples were collected 24 h after the last nasal OVA challenge, and 10 μ g/ml of OVA was used to coat a microtiter plate. Bound immunoglobulin isotypes were detected with specific secondary antibody (biotin-conjugated rat anti-mouse IgE monoclonal antibody (mAb) was purchased from BD Pharmingen, San Jose, CA, USA). Total serum IgE was measured by standard ELISA using anti-mouse IgE capture mAb (BD Pharmingen) and signal from a fluorescently labeled detection antibody was measured as described for the OVA-specific IgE ELISA.

Measurement of cytokines in splenocyte cultures. The spleen was removed aseptically 24 h after the last intranasal challenge. Single cells were plated on 96-well plates at a final concentration of 5×10^6 cells/well in RPMI-1640 containing 10% fetal bovine serum (FBS) with penicillin-streptomycin. The cells

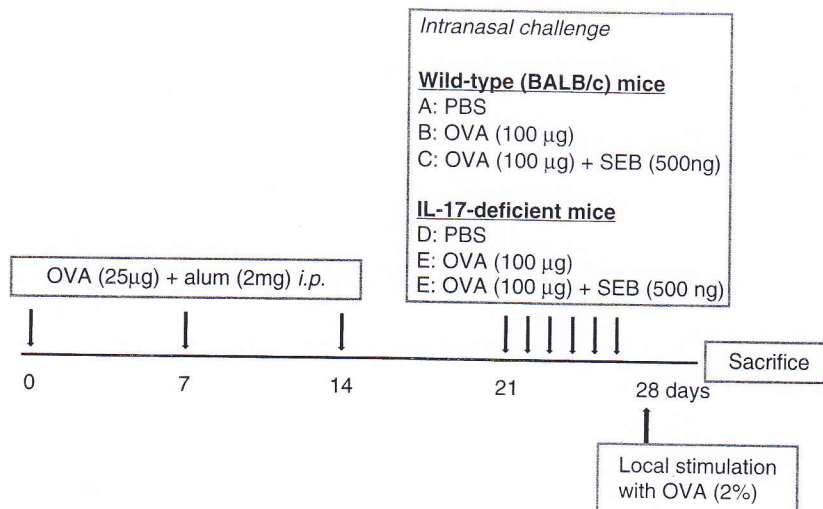


Figure 1. Experimental protocol. Mice were sensitized by intraperitoneal injection of ovalbumin (OVA) mixed with aluminum hydroxide on days 0, 7, and 14. Intranasal challenge was performed by daily OVA inhalation from day 21 to day 28. Control mice were sensitized but challenged with phosphate-buffered saline (PBS) instead of OVA. The mice were killed 24 h after the final OVA challenge. SEB, staphylococcal enterotoxin B.

were stimulated with OVA for 72 h. The supernatants were collected and stored at -70°C until needed.

The levels of IL-1 β , IL-4, IL-6, and interferon (IFN)- γ were measured in splenocyte supernatant after stimulation with OVA using commercially available ELISA kits (R&D Systems, Inc., Minneapolis, MN, USA). After measuring the optical density at 450 nm, the concentration of IL-1 β , IL-4, IL-6, and IFN- γ was determined by interpolation from a standard curve and expressed as ng/ml.

Histologic assessment

The experimental mice were sacrificed 24 h after the final OVA challenge and nasal tissues were obtained for analysis. The nose sections were stained with hematoxylin and eosin to assess inflammatory cell infiltration. Eosinophils were defined morphologically

by the presence of eosinophilic granules in the cytoplasm and two-lobed nucleus. Eosinophils were counted under a microscope ($\times 400$) in the whole nasal septal mucosa.

Statistical analysis

The results are expressed as mean $\pm$ SD. Statistical analysis was performed using PASW 18.0 software (SPSS Inc., Chicago, IL, USA) and the Kruskal-Wallis test and Tukey post hoc test were used for mean analysis.

Results

Eosinophil counts

Eosinophil counts in the nasal mucosa are shown in Figure 2a. The average number of eosinophils

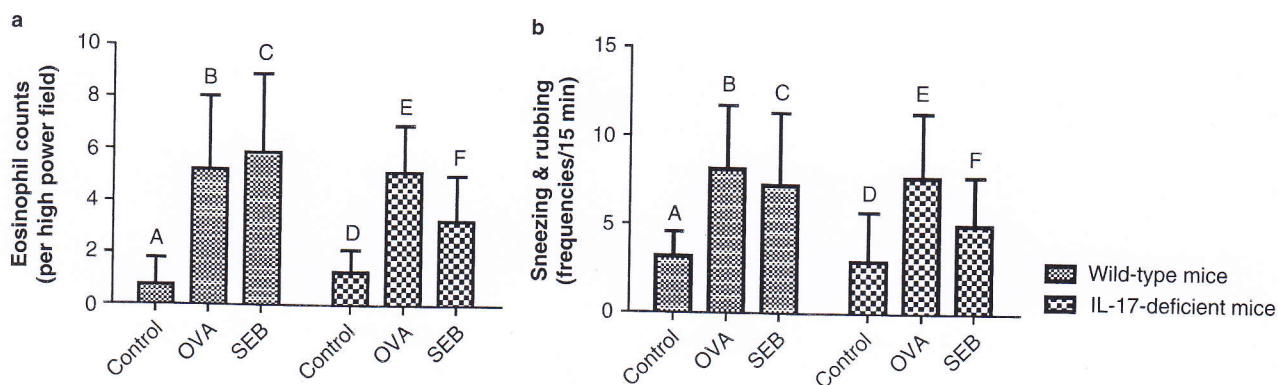


Figure 2. (a) Eosinophil counts (per high power field, original magnification $\times 400$) in the nasal mucosa and (b) nasal symptom scores following intranasal ovalbumin (OVA) challenge. Eosinophil counts in the nasal mucosa and nasal symptom scores following intranasal OVA challenge in the OVA and SEB (staphylococcal enterotoxin B) groups were similar in wild-type mice. However, in IL-17-deficient mice, the eosinophil counts and nasal symptom scores in the SEB group were lower than those in the OVA group.

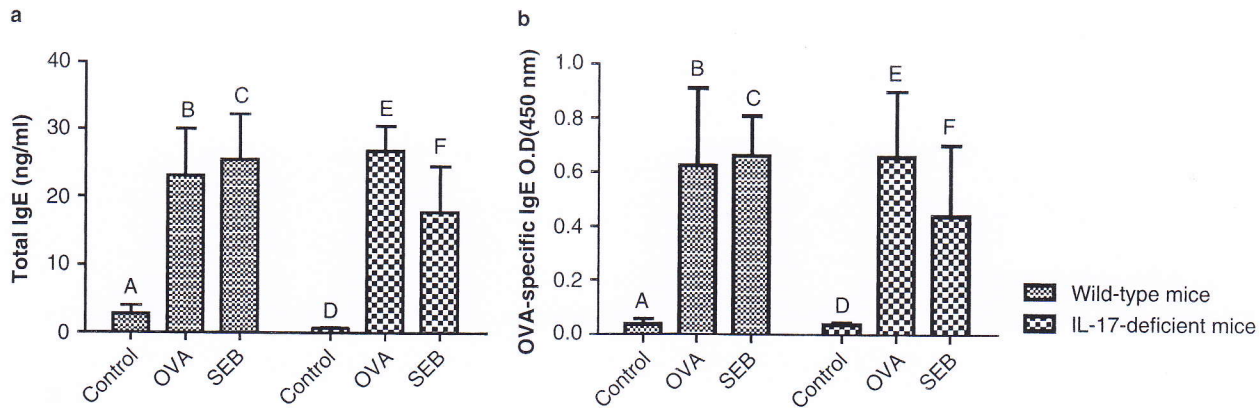


Figure 3. Serum (a) total IgE and (b) ovalbumin (OVA)-specific IgE levels. In wild-type mice, the SEB (staphylococcal enterotoxin B) group showed slightly increased serum total IgE and OVA-specific IgE levels as compared with the OVA group, even though statistical significance was not observed. However, in IL-17-deficient mice, serum total IgE and OVA-specific IgE levels in the SEB group were lower than those in the OVA group.

per high power field ($\times 400$) in the OVA and SEB groups (groups B and C, and groups E and F, respectively) were significantly higher than those in the negative control groups (groups A and D) ($p < 0.001$ and $p < 0.001$, respectively). A Tukey post hoc test revealed that there was no significant difference regarding infiltrated eosinophils between OVA and SEB groups (groups B and C) in wild-type mice. However, the eosinophil count in the SEB group (group F) was lower than that of the OVA group (group E) in IL-17-deficient mice ($p = 0.001$).

Nasal symptoms and total and specific IgE levels

The frequencies of nasal rubbing and sneezing were evaluated for 15 min after intranasal allergen challenge, and are displayed in Figure 2b. The frequencies of nasal rubbing and sneezing of groups B (OVA group) and C (SEB group) in wild-type mice were 8.1 ± 3.6 and 7.2 ± 4.1 , respectively, which were increased significantly compared with those of group A (negative control group) (3.1 ± 1.4) ($p = 0.002$). However, no significant difference was observed between groups B and C. Similarly, the symptom scores for groups E (OVA group) and F (SEB group) in IL-17-deficient mice were 7.7 ± 4.1 and 5.0 ± 2.8 , respectively, significantly different from that of group D (negative control group) ($p = 0.007$). In contrast to the result in wild-type mice, the score for group E was significantly higher than that of group F in IL-17-deficient mice.

In addition, all of the OVA-treated groups (groups B and C) in wild-type mice showed significantly higher total IgE levels than group A (negative control group) ($p < 0.001$) (Figure 3a). However, there was no significant difference in terms of total IgE levels between groups B and C. In IL-17-deficient mice,

similar results were observed in that group E and F showed significantly higher total IgE levels than group D ($p < 0.001$). A Tukey post hoc test revealed that the group F showed significantly higher total IgE levels compared with group E ($p < 0.001$). Furthermore, even though the difference in total IgE levels in groups B and E was not prominent, the total IgE level in group F was significantly lower than that in group C ($p < 0.001$).

Similarly, all the OVA-treated groups (groups B and C, and groups E and F) showed significantly higher OVA-specific IgE levels than the negative control groups (group A and D) in both wild-type and IL-17-deficient mice ($p < 0.001$ and $p < 0.001$, respectively) (Figure 3b). The OVA-specific IgE level in group F was significantly lower than that of group E by a Tukey post hoc test ($p < 0.001$). In addition, the OVA-specific IgE level in group F was significantly lower than that of group C ($p < 0.001$).

Inflammatory cytokines

The levels of IL-1 β , IL-6, and IL-4 were significantly elevated in OVA-treated groups (groups B and C, and groups E and F) as compared with the negative control groups (groups A and D) in the splenocyte cultures in both wild-type and IL-17-deficient mice ($p < 0.001$ and $p < 0.001$, respectively) (Figure 4a, b, c). Moreover, addition of SEB (group C) increased OVA-induced production of IL-1 β , IL-6, and IL-4 in splenocytes compared with the OVA-treated group (group B) in wild-type mice ($p < 0.001$). In contrast, in IL-17-deficient mice, the levels of IL-1 β , IL-6, and IL-4 in the SEB group (group F) were not higher than those in the OVA group (group E). Furthermore, IL-1 β , IL-6, and IL-4 levels in groups E and F

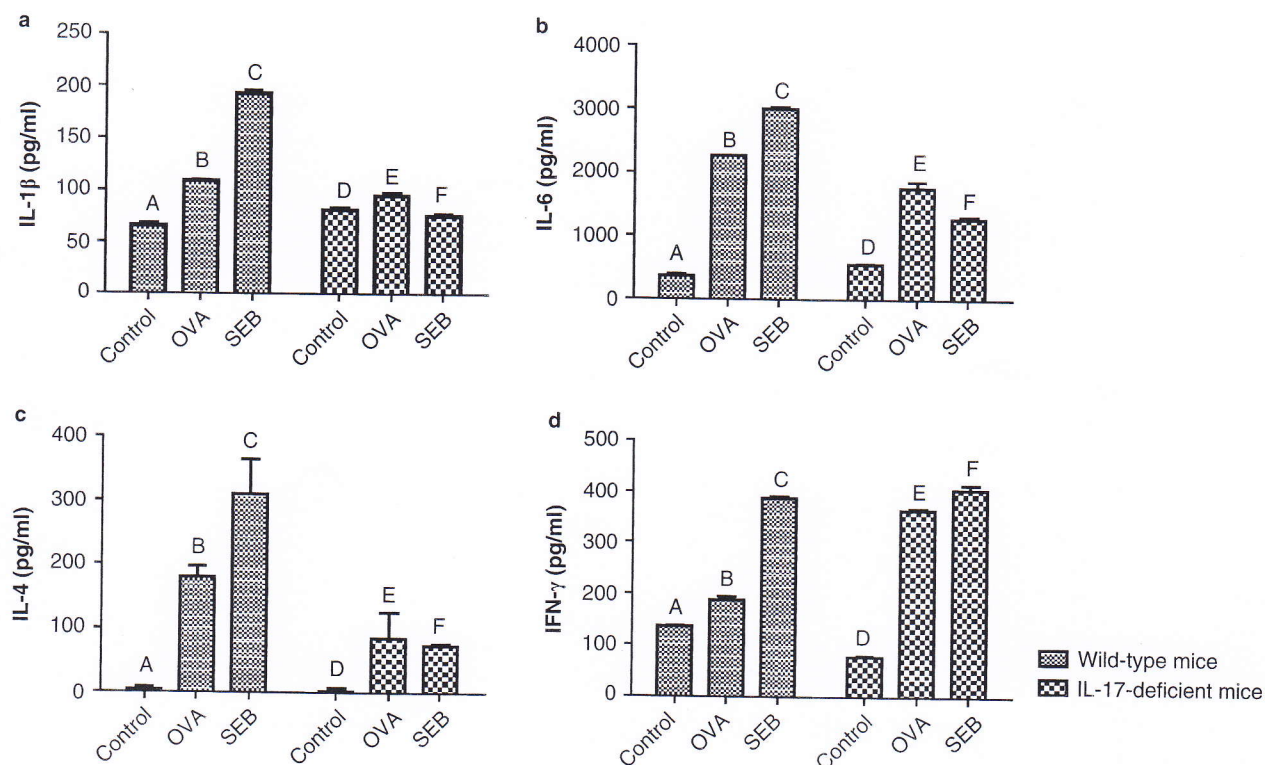


Figure 4. (a) IL-1 β , (b) IL-6, (c) IL-4, and (d) IFN- γ levels in splenocyte culture. The SEB (staphylococcal enterotoxin B) group showed increased IL-1 β , IL-6, IL-4, and IFN- γ levels as compared with the OVA (ovalbumin) group in wild-type mice ($p < 0.001$). However, these increases in cytokine levels in the SEB group compared with the OVA group were not observed in IL-17-deficient mice.

were significantly lower than those in group B and C ($p < 0.001$ and $p < 0.001$, respectively).

The level of IFN- γ in the OVA groups (groups B and E) and the SEB groups (groups C and F) were significantly higher than those in the negative control groups (groups A and C) ($p < 0.001$ and $p < 0.001$, respectively) (Figure 4d). In both wild-type and IL-17-deficient mice, a Tukey post hoc test revealed that the SEB groups (groups C and F) showed significantly higher IFN- γ level than the OVA groups (groups B and E) ($p < 0.001$ and $p < 0.001$, respectively). Additionally, group E showed a significantly increased IFN- γ level compared with group B ($p < 0.001$). However, there was no statistically significant difference regarding IFN- γ level in groups C and F.

Discussion

In the present study, we analyzed the immunologic effects of nasal exposure to SEB with regard to the development of AR in a murine model, and the role of IL-17 in this process. Mice sensitized with OVA together with SEB produced significantly greater amounts of cytokines such as IL-1 β , IL-6, IL-4, and IFN- γ . In addition, even if statistically significant differences were not observed, nasal administration of

SEB together with OVA increased eosinophil infiltration and OVA-specific and total IgE production as compared with administration of OVA only. These results suggest that nasal exposure to SEB enhances the development of AR and the inflammatory process. This is in accordance with the results of a previous study where intranasally sensitized BALB/c mice with SEB exposure developed AR more extensively than mice without SEB exposure [12].

Although SEB exposure enhances allergic inflammation, the exact mechanism regarding connection between SEB and AR has not been elucidated completely. SEB is supposed to act both as a conventional allergen and an immunomodulator and is considered to be an inducer of inflammatory response.

SEB is a well known toxin that acts as a superantigen. Superantigens, including SEB, have an ability to bind directly to the class II major histocompatibility complex (MHC) APCs and β -domain of the variable region of the T-cell receptor. This action occurs outside the conventional antigen-binding site. Therefore, they can stimulate up to 30% of the T-cell population in the body nonspecifically and cause an excessive immune response [6,13]. Activation of T cells results in the production of various proinflammatory cytokines and chemokines, some of which preferentially induce Th1 responses [14].

Moreover, it has been reported that there is a shift in the immune response of mice to OVA from Th2-associated antibody production of OVA-specific IgE and IgG1 to Th1-associated antibody production of IgG2a, IgG2b, and IgG3, following administration of SEB [15]. In the present study, mice sensitized with OVA together with SEB produced significantly greater amounts of proinflammatory cytokines, such as IL-1 β , IL-6, and both Th1 and Th2 cytokines, such as IFN- γ and IL-4. These additionally enhanced Th1 and Th2 responses following administration of SEB may be responsible for the enhanced allergic inflammatory process. Furthermore, this enhanced Th1 response was in accordance with the previous results showing that SEB does not induce a strict Th2-skewed response in a murine model of AR [12].

IL-17 can mediate tissue inflammation by the induction of chemokines and proinflammatory cytokines in the structural cells, and can potentiate allergic inflammation by regulating innate immunity [16]. IL-17 has been known to induce allergen-specific Th2 cell activation, eosinophil accumulation, and serum IgE production [17,18]. However, the IL-17/Th17 cell plays a more important role in the induction of Th1-type neutrophilic allergic inflammation than in Th2-type eosinophilic inflammation [19]. Furthermore, a recent study has reported that IL-17 secretion was enhanced by the SEB itself [20]. Therefore, it can be assumed that IL-17 plays an important role in the enhancement of allergic response in AR caused by administration of SEB by enhancing Th1 response as well as Th2 response.

We aimed to elucidate the role of IL-17 on SEB in AR using IL-17-deficient mice. Of interest, increased allergic symptoms, eosinophil infiltration, increased total IgE, and OVA-specific IgE were observed after SEB administration in wild-type mice. However, mice deficient in IL-17 displayed a significant reduction in these allergic responses caused by SEB administration. Furthermore, increased production of proinflammatory cytokines, such as IL-1 β and IL-6, and Th2 cytokine IL-4 were observed following SEB administration in wild-type mice, but in IL-17-deficient mice, increased production of those cytokines was not found even after administration of SEB. Therefore, from these results it is inferred that IL-17 has a critical role in allergic inflammatory response enhanced by SEB in AR.

However, the partially decreased response implies that mechanisms other than IL-17 could be involved in SEB-induced enhancement of allergic inflammation. One possible mechanism is that SEB might enhance the immune response of conventional allergens, such as OVA. A representative superantigen, SEB, can stimulate T cells by direct binding to

MHC class II APCs loaded with OVA-derived peptides, and this might enhance the antigenicity of the allergen, OVA.

In conclusion, exposure to SEB may enhance the allergic inflammatory responses in wild-type mice. However, these enhanced responses were not observed in IL-17-deficient mice. The results of this study demonstrate that IL-17 could play a significant role in aggravation of AR after SEB exposure.

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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