

Existence of Antigen-Specific Immunoglobulin E Is Not Sufficient for Allergic Nasal Eosinophil Infiltration in Mice

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Key Words

Allergic rhinitis · Eosinophils · Immunoglobulin E · Sneeze

Abstract

Background: Immunoglobulin E (IgE) is important for the development of allergic rhinitis (AR), though the contribution of IgE to the infiltration of eosinophils in the nasal mucosa has not been fully elucidated. In this study, antigen-induced sneezing and nasal eosinophil accumulation were comparatively investigated in anti-ovalbumin (OVA)-IgE transgenic (Tg) and wild-type (WT) mice. **Methods:** Tg and OVA-immunized WT mice were intranasally challenged with OVA. Antigen-specific serum IgE level, sneezing and infiltration of eosinophil into the nasal cavity were then examined. **Results:** The level of serum OVA-specific IgE in Tg mice was significantly higher than that in antigen-immunized WT mice. Compared to saline challenge, intranasal challenge with OVA significantly induced sneezing in both Tg and immunized WT mice. However, antigen-induced nasal eosinophil infiltration was observed in immunized WT mice but not in Tg mice. **Conclusions:** IgE-mediated responses might not play a crucial role in antigen-induced eosinophil infiltration in AR.

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Introduction

Allergic rhinitis (AR) is characterized by antigen-induced nasal symptoms, such as sneezing, rhinorrhea and nasal blockage. It has been reported that immunoglobulin E (IgE) plays an important role in these nasal responses [1, 2]. Following the capture of antigens by IgE, mast cells degranulate and release chemical mediators including histamine. Histamine induces sneezing and rhinorrhea by stimulating sensory nerve terminals in the nasal mucosa. Moreover, several chemical mediators accelerate plasma leakage from blood vessels and cause nasal blockage [1, 2].

Many kinds of inflammatory cells other than mast cells are also observed in the nasal mucosa of AR patients. It has been reported that eosinophils, in particular, contribute to the nasal symptoms by releasing chemical mediators such as cysteinyl leukotrienes [3]. However, the role of IgE/mast cell-mediated responses in the accumulation of inflammatory cells including eosinophils into the nasal mucosa has not been fully elucidated. It could be, at least in part, due to the fact that both IgE/mast cell- and Th2 cell-mediated responses are developed in allergic inflammation models using antigen-immunized mice. There-

fore, by employing the Th2 cell adoptive transfer system and mast cell-deficient mice, we have demonstrated that antigen-specific Th2 cells play a central role in antigen-induced infiltration of eosinophils into the lungs, whereas the IgE/mast cell-dependent pathway is implicated only supplementarily [4–6]. Furthermore, to directly investigate the role of IgE in allergic diseases, we recently established transgenic mice in which a large amount of ovalbumin (OVA)-specific IgE was constitutively produced [7]. This transgenic mouse is useful for analyzing antigen-induced responses solely dependent on IgE, especially in comparison to the responses occurred in antigen-immunized mice in which antigen-specific Th2 cells as well as IgE are developed.

In this study, in order to clarify the role of IgE in the pathogenesis of AR, antigen-induced nasal responses including eosinophil infiltration in transgenic and antigen-immunized WT mice were comparatively investigated.

Materials and Methods

Animals

Anti-OVA IgE transgenic mice of BALB/c background (Tg) were established as described previously [7]. Six-week-old female BALB/c mice (WT) were purchased from Japan SLC (Shizuoka, Japan). The experimental protocols were approved by the Animal Use and Care Committee of the Tokyo Metropolitan Institute of Medical Science.

Antigen-Induced Sneezing in Mice

WT mice were sensitized by intraperitoneal injection of 20 µg OVA (Sigma-Aldrich, St. Louis, Mo., USA) emulsified with 2.25 mg alum (Thermo Fisher Scientific, Waltham, Mass., USA) on days 0, 7, 14 and 21 of the experiment. The mice were challenged 4 times by daily intranasal injection of 600 µg OVA dissolved in 20 µl saline or saline alone (control) on days 35–38. The same challenge was performed on age-matched Tg mice. Immediately following the last challenge, the number of sneezes was counted for 5 min.

Nasal Lavage

Six hours after the last challenge, nasal lavage (NAL) was performed by introducing 150 µl PBS into the nasal cavity. The number of cells recovered in the NAL fluid (NALF) was counted by a hemocytometer. Then, differential eosinophil counts based on morphological criteria were performed by counting at least 200 cells on a cytocentrifuged preparation after staining with Diff-Quick (Sysmex Corporation, Hyogo, Japan).

ELISA

Peripheral blood of immunized WT mice and age-matched Tg mice was collected on day 38 and the level of serum antigen-specific IgE was evaluated by ELISA, with slight modifications, as described previously [8]. Briefly, serially diluted sera were

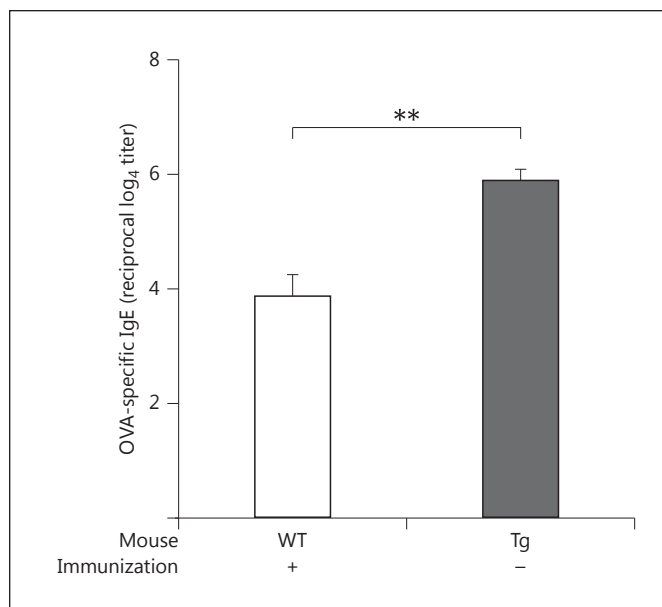


Fig. 1. Serum antigen-specific IgE levels in Tg mice and OVA-immunized WT mice. The level of serum OVA-specific IgE was measured by ELISA. Data are expressed as mean $\pm$ SEM of 4–6 animals. * $p < 0.05$ (Student's *t* test).

added to wells of an OVA-coated 96-well microtiter plate. OVA-specific IgE was detected with HRP-conjugated monoclonal anti-mouse IgE antibody (clone LO-ME-2, AbD Serotech, Oxford, UK). The last serum dilution yielding an OD₄₅₀ value of 0.1 over the background was decided as the end-point titer for the sample.

Statistical Analysis

The results were presented as arithmetic mean $\pm$ SEM. Statistical analysis was performed by Student's *t* test. $p < 0.05$ was considered to indicate statistical significance.

Results and Discussion

In order to elucidate the role of IgE in AR, we first investigated serum antigen-specific IgE levels in Tg and OVA-immunized WT mice. Considering that not only antigen-specific but also total IgE was not detectable in naïve WT mice (data not shown), sera of both mice contained a large amount of OVA-reactive IgE (fig. 1). Furthermore, the serum IgE level in Tg mice was significantly higher than that in immunized WT mice.

We next examined antigen-induced sneezing in Tg and immunized WT mice. In comparison with saline challenge, intranasal challenge with OVA significantly induced an increase in the number of sneezes in antigen-

immunized WT mice (table 1). Essentially, the same response was observed in Tg mice. Notably, the antigen-induced sneezing response in Tg mice was a little weaker than that in immunized WT mice, even though this was not statistically significant (table 1).

Antigen-induced infiltration of eosinophils in the nasal cavity was examined next. In OVA-challenged WT mice, a large number of eosinophils were recovered in NALF, in comparison with saline-challenged control mice (table 2). However, antigen-induced nasal eosinophil accumulation was hardly observed in Tg mice.

These findings are consistent with previous reports by Oshiba et al. [9] and Hamelmann et al. [10] demonstrating that antigen-induced eosinophil accumulation in the lungs was much weaker in antigen-specific IgE-transferred mice than in antigen-immunized mice. However, in recent clinical studies, eosinophil infiltration in patients with AR as well as bronchial asthma was downregulated by treatment with omalizumab, a humanized anti-IgE monoclonal antibody [11–13]. Although the reason for this apparent contradiction is unclear, apart from the IgE/mast cell-dependent pathway, Th2 cells are crucially implicated in the accumulation of eosinophils. Thus, we and many other investigators demonstrated that antigen-induced airway eosinophilic inflammation accompanied by bronchial hyperresponsiveness was indeed observed in mice transferred with antigen-specific Th2 cells [5, 6, 14–16]. It was suggested in the reports of Oshiba et al. [9] and Hamelmann et al. [10] that the weak accumulation of eosinophils in antigen-specific IgE-transferred mice was caused by the lack of activated Th2 cells. In this regard, IgE/mast cells have the ability to augment Th2 cell-mediated responses. Activated mast cells synthesize and release several cytokines responsible for the activation and migration of Th2 cells [17]. Consistently, IgE-dependent activation of mast cells potentiated Th2 cell-mediated eosinophil accumulation [18–20]. Taking these findings together, IgE might participate in the recruitment of eosinophils in the nasal cavity of antigen-immunized mice, by promoting Th2 cell-mediated responses, though the existence of antigen-specific IgE was not sufficient for the massive eosinophil infiltration.

On the other hand, it is known that murine mast cells derived from their precursor in bone marrow are matured at local tissues under the influence of various growth and differentiation factors, such as IL-3, IL-4, IL-6 and IL-9, produced, at least in part, by T cells [21]. In this study, a relatively large number of sneezes were observed upon antigen challenge in immunized WT mice com-

Table 1. Role of IgE in antigen-induced sneezing response

Challenge	Sneezes/5 min	
	WT	Tg
Saline	1.3±0.3	2.0±0.6
OVA	22.2±5.2*	11.3±4.9*

Tg and antigen-immunized WT mice were challenged with OVA or saline. Immediately after the challenge, the number of sneezes was counted for 5 min. Data are expressed as mean ± SEM of 4–6 mice.

* $p < 0.05$, compared with saline-challenged WT mice (Student's *t* test).

Table 2. Role of IgE in antigen-induced eosinophil accumulation in nasal cavity

Challenge	Eosinophils /NALF	
	WT	Tg
Saline	5±4	50±33
OVA	760±210*	62±28 [#]

Tg and antigen-immunized WT mice were challenged with OVA or saline. Six hours after the last challenge, the number of eosinophils in NALF was determined. Data are expressed as mean ± SEM of 4–6 mice.

* $p < 0.05$, compared with saline-challenged WT mice.

[#] $p < 0.01$, compared with OVA-challenged WT mice (Student's *t* test).

pared to Tg mice, even though the serum OVA-specific IgE level was significantly higher in Tg mice. This may be because, in the nasal mucosa of Tg mice, mast cells were insufficiently differentiated or migrated due to lack of Th2 cell activation. Further investigations are required to elucidate the cooperation between IgE and Th2 cells in allergic nasal eosinophilic inflammation, including the differentiation and infiltration of mast cells in the nasal mucosa.

Conclusively, nasal antigen challenge elicited sneezing in both Tg and immunized WT mice. However, nasal eosinophil accumulation was significantly induced in immunized WT mice but not in Tg mice. Antigen-specific IgE may play a supplemental role in antigen-induced nasal eosinophil infiltration in AR.

Acknowledgements

This work was supported by the research grant 'Agri-Health Translational Research Project' from the Ministry of Agriculture, Forestry, and Fisheries of Japan to T.H. and the JSPS KAKENHI grant (No. 23791952) to T.N.

Disclosure Statement

The authors declare that no financial conflict of interest exists in relation to the content of the article.

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