

Recent Advances in Research on the Mechanisms and Regulation of Allergic Diseases

Review

T Cell-Mediated Nasal Hyperresponsiveness in Allergic Rhinitis

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Allergic rhinitis patients suffer various symptoms such as sneezing, runny nose, and nasal congestion. As disease severity and chronicity progress, nasal hyperresponsiveness (NHR) develops in those patients. During the generation of a mouse allergic rhinitis model, we discovered that immunized mice developed NHR upon repeated nasal antigen challenge. Using genetically modified mice and an originally developed T cell-transferred mouse model, we confirmed the critical role of CD4⁺ T cells after differentiation into several helper subsets in NHR. On the other hand, immunoglobulin E/mast cell-dependent responses that are critical for evoking nasal symptoms and eosinophils that accumulate in allergic inflammation sites were dispensable. A steroid, but not drugs targeting mast cell-derived mediators, was effective in alleviating NHR. The possible generation of a new means to treat allergic rhinitis by targeting T cell-derived NHR-inducing factors is suggested.

Key words eosinophil; immunoglobulin E; mast cell; nasal lavage; T cell

1. INTRODUCTION

An increasing number of people, especially those living in Western countries, develop allergic rhinitis (hay fever), particularly between the ages of 20 and 40.¹⁾ Their typical symptoms such as sneezing, runny nose, nasal congestion, and itchy eyes are induced by chemical mediators including histamine released from mast cells that are degranulated upon recognition of specific antigens through immunoglobulin E (IgE) and its receptor.²⁾ Accordingly, antiallergic agents such as antagonists and release inhibitors for chemical mediators have been used to alleviate rhinitis symptoms.³⁾ However, as disease severity and chronicity progress, inflammatory cells other than mast cells, such as eosinophils and lymphocytes, also accumulate in the nasal mucosa. In many patients with severe allergic rhinitis, the administration of antiallergic agents targeting mast cell-derived mediators is not sufficient and treatment with steroids exerting strong antiinflammatory activity is necessary.³⁾ Patients often develop hyperresponsiveness, in which more severe symptoms are induced more easily and when stimulated not only by specific antigens but also by nonspecific stimuli.⁴⁾ The detailed mechanisms of hyperresponsiveness induction have not been clarified. However, during the generation of a murine allergic rhinitis model, we found that pathophysiological responses reflecting nasal hyperresponsiveness (NHR) were induced in antigen-immunized and -challenged mice.⁵⁾ Based on the mechanisms of NHR induction suggested by our recent findings, we propose a possible new strategy for the treatment of allergic rhinitis.

2. DEVELOPMENT OF NHR IN A MOUSE MODEL OF ALLERGIC RHINITIS

To elucidate the mechanisms of and develop a new treatment for allergic rhinitis, experimental animal models were generated. Allergic inflammation can be induced in various target organs, such as the lungs, skin, eyes, and gastrointestinal tract, of immunized mice.⁶⁾ We therefore attempted to induce symptoms of allergic rhinitis in mice *via* intraperitoneal immunization and nasal challenge with cedar pollen antigen. Following antigen challenge every other day for 10 d, an obvious sneezing response was observed immediately after the last challenge. Several hours later, many inflammatory cells including eosinophils and neutrophils were recovered in nasal lavage fluid (NALF). We therefore successfully reproduced the pathophysiological responses of allergic rhinitis patients in mice.⁷⁾

It is also noteworthy that bronchial hyperresponsiveness (BHR) seen in patients with bronchial asthma is reproducible in mouse models through procedures similar to those used in creating allergic rhinitis models. Upon nasal provocation with a relatively large amount of antigen, augmentation of acetylcholine-induced bronchoconstriction and lowering of its threshold dose occurred in immunized mice.⁸⁾ Although we confirmed that the amount of antigen delivered is a critical requisite for determining the target organ of allergic inflammation,⁹⁾ whether a response similar to that seen in BHR occurs in allergic rhinitis models has not been fully investigated.

In our allergic rhinitis model, the number of sneezes in-

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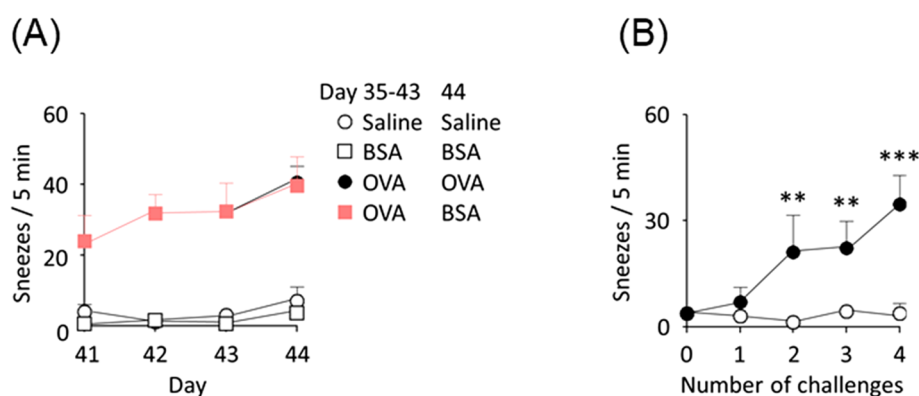


Fig. 1. Antigen-Induced NHR in Immunized Mice⁵⁾

Mice were immunized 4 times with intraperitoneal injections of OVA plus alum. Two weeks after the last immunization, mice were challenged once a day with daily intranasal injection of OVA or BSA solution, or of saline on days 35–38 and 41–43. Then, the mice were challenged with OVA, BSA, or saline on day 44. On days 41–44, the number of sneezes was counted for 5 min immediately after intranasal administration of OVA, BSA, or saline (A). The histamine-evoked sneezing response was evaluated 6 h after the 0 (day 34) to 4th (day 38) challenges with OVA (black) or saline (white) (B). Data are expressed as mean \pm standard error of the mean (S.E.M.) for 4–10 animals. ** $p < 0.01$, *** $p < 0.001$ compared with saline-challenged control mice (Mann–Whitney U -test). (Color figure can be accessed in the online version.)

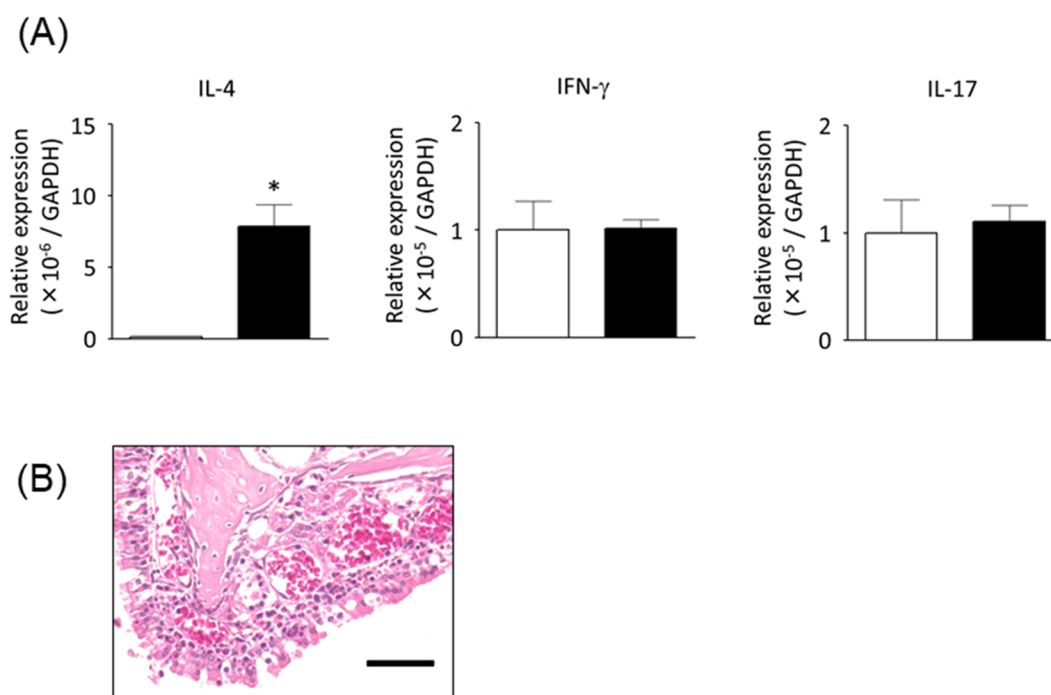


Fig. 2. Antigen-Induced Nasal Inflammation in Immunized Mice⁵⁾

OVA-immunized mice were challenged 4 times with OVA (black) or saline (white) on days 35–38. Six hours after the last challenge, the expression of IL-4, IFN- γ , and IL-17 mRNA in the nasal tissue was examined (A). Data are expressed as mean \pm S.E.M. for 4–9 animals. * $p < 0.05$ compared with saline-challenged control mice (Mann–Whitney U -test). Representative image of the lateral nose section stained with hematoxylin and eosin is shown in B. Bar = 50 μ m. (Color figure can be accessed in the online version.)

duced was correlated with the number of nasal antigen challenges. We initially considered that the augmented sneezing response simply reflected the increasing severity and chronicity of the rhinitis pathology. However, when ovalbumin (OVA)-immunized and -challenged mice were administered bovine serum albumin (BSA), a nonspecific protein, instead of OVA on the final challenge day, we were surprised that essentially the same level of sneezing response was induced as in mice receiving OVA on the final day (Fig. 1A). These findings clearly suggest that the sneezing response was not mainly caused by the antigen-specific response but reflected the augmentation of responsiveness against nonspecific stimuli, or NHR.⁵⁾

3. MECHANISMS OF ANTIGEN-INDUCED NHR DEVELOPMENT

As the severity of allergic rhinitis is known to be correlated with the degree of NHR, this parameter is clinically evaluated using nasal symptom inducers such as histamine, methacholine, and norepinephrine. For example, in the histamine challenge test, nasal symptoms are observed after the direct application of histamine or of histamine-absorbed filter paper to the inferior nasal turbinate. Then the threshold dose (sensitivity) and degree of responses (responsiveness) in inducing nasal symptoms are evaluated.¹⁰⁾

Accordingly, we evaluated the NHR observed in our model

using histamine as a symptom inducer. The sneezing response was induced by histamine administration to some degree, although it was gradually augmented in immunized mice with repeated antigen challenge (Fig. 1B). As in allergic rhinitis patients, we found that NHR can be evaluated by the histamine challenge test in the mouse model. In addition to the augmented expression of T-helper 2 (Th2) cytokines, but not of Th1 or Th17 cytokines, in the nasal tissues (Fig. 2A), derangement of epithelial cells and accumulation of inflammatory cells including eosinophils were observed in nasal mucosa specimens (Fig. 2B), suggesting that Th2-dominant allergic inflammation developed in our rhinitis model.

From the pathological examination and NALF, the accumulation of eosinophils, neutrophils, and lymphocytes in the nasal mucosa following antigen challenge was indicated. Eosinophils are recognized in various allergic diseases and are considered to be involved in their pathogenesis. Therefore, the role of eosinophils in antigen-induced NHR was determined by employing eosinophil-deficient Δ dblGATA mice.¹¹⁾ Antigen-induced nasal eosinophil accumulation completely disappeared in Δ dblGATA mice, although essentially the same level of NHR was seen in Δ dblGATA and wild-type mice (Fig. 3A). The lesser contribution of eosinophils to NHR in our model was suggested.

For the antigen-initiated sneezing response, the degranulation of mast cells stimulated through antigen-specific IgE should be required. Therefore, the contribution of mast cells and IgE to NHR was evaluated by employing mast cell-deficient WBB6F1- W/W^v mice (W/W^v)¹²⁾ and OVA-specific IgE transgenic (IgE-Tg) mice,¹³⁾ respectively. The same degree of NHR was induced in immunized W/W^v and littermate mice upon antigen challenge (Fig. 3B). Although the same serum level of antigen-specific IgE as in antigen-immunized BALB/c mice was seen in IgE-Tg mice, antigen challenge to IgE-Tg mice did not induce significant NHR (Fig. 3C). The dispensable roles of mast cells and IgE in antigen-induced NHR developed in our allergic rhinitis model were thus suggested.

Since we confirmed that $CD4^+$ T cells play an essential role in the development of BHR in a mouse model of bronchial asthma,¹⁴⁾ the contribution of $CD4^+$ T cells to NHR was investigated. Following the depletion of peripheral $CD4^+$ T cells in immunized mice by anti- $CD4$ antibody administration, antigen-induced NHR was significantly suppressed (Fig. 4A), suggesting the critical participation of $CD4^+$ T cells in NHR.

Consistent with the implication of $CD4^+$ T cells in various immune responses and diseases, we demonstrated that bronchial asthma-like airway inflammation with BHR can be reconstituted in normal mice without the assistance of IgE and mast cells by adoptive transfer of *in vitro* differentiated antigen-specific $CD4^+$ T cells.¹⁵⁾ $CD4^+$ T cells are also considered to play specific roles in various diseases after differentiation into characteristic subsets such as Th1, Th2, and Th17 cells.¹⁶⁾ Therefore, antigen-specific T cell subsets were developed from $CD4^+$ T cells of OVA-reactive T cell receptor (TCR)-expressing transgenic DO11.10 mice by antigen stimulation culture with respective cytokines and cytokine antibodies. Upon nasal OVA challenge, mice transferred with Th1, Th2, and Th17 cells developed the same degree of NHR as seen in antigen-immunized and -challenged mice (Fig. 4B), regardless of their specific phenotypes; interferon (IFN)- γ , interleukin (IL)-4, and IL-17 mRNA were preferentially expressed in the

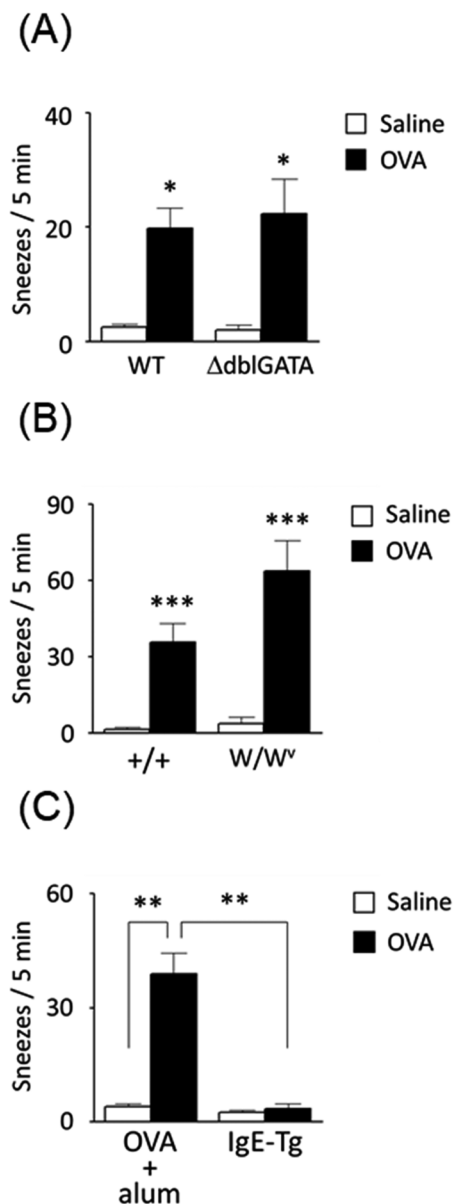


Fig. 3. Contribution of Eosinophils, Mast Cells, and IgE to Antigen-Induced NHR⁵⁾

OVA-immunized Δ dblGATA and WT mice (A) and W/W^v and $+/+$ mice (B) were challenged 7 times with OVA or saline, as shown in Fig. 1. IgE-Tg mice as well as antigen-immunized BLAB/c mice (C) were challenged 4 times with OVA or saline, as shown in Fig. 2. Six hours after the last challenge, the number of sneezes evoked by histamine was examined. Data are expressed as mean \pm S.E.M. for 4–9 animals. * p < 0.05, *** p < 0.001 (Mann–Whitney U -test compared with saline-challenged mice for A and B, Dunnett's test for C).

nasal mucosa, respectively.⁵⁾

It is recognized that $CD4^+$ T cells, especially Th2 cells, contribute to mast cell-mediated allergic symptoms through regulating immunoglobulin synthesis and IgE-class switching in B cells. However, under our experimental conditions that evaluated nasal inflammation within only 3–4 d after T cell transfer, a significant amount of antigen-specific IgE was not produced. Therefore, NHR seen in mice transferred with each T cell subset most likely developed independently of the IgE/mast cell-related pathway. The nasal accumulation of neutrophils was clearly seen in mice transferred with Th1 and Th17 cells, but not with Th2 cells, upon antigen challenge, suggesting that neutrophils did not play a major role in NHR.

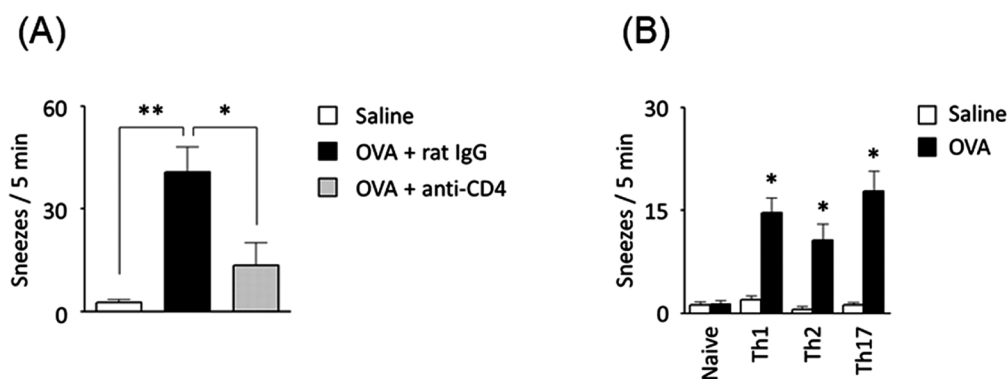


Fig. 4. Essential Contribution of CD4⁺ T Cells to Antigen-Induced NHR⁵⁾

Immunized mice were challenged 4 times with OVA or saline, as shown in Fig. 2. Anti-CD4 mAb or control rat IgG was administered twice at 9 and 6 d before the last challenge (A). Twenty-four hours after transfer of Th1, Th2, or Th17-polarized cells, mice were challenged 3 times with daily administration of OVA or saline (B). Six hours after the last challenge, the number of sneezes evoked by histamine was examined. Data are expressed as mean \pm S.E.M. for 4–8 animals. * p < 0.05, ** p < 0.01 (Dunnett's test, compared with naïve T cell-transferred and OVA-challenged mice for B).

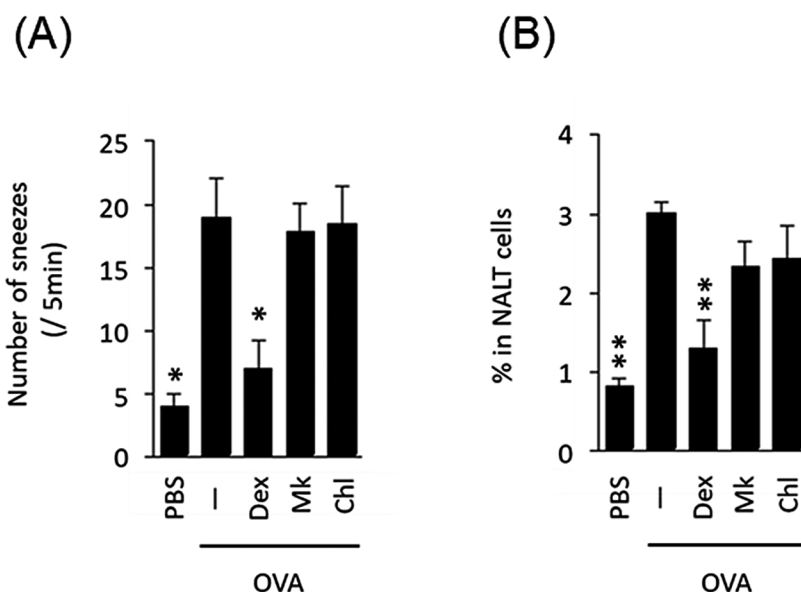


Fig. 5. Effects of Antiallergic Drugs on Th2 Cell-Mediated NHR¹⁷⁾

OVA-specific Th2 cell-transferred mice were challenged 4 times with daily administration of OVA or PBS. Dex (10 mg/kg), Mk (30 mg/kg), or Chl (100 mg/kg) was subcutaneously administered 30 min before each antigen challenge except for the last challenge. Six hours after the last challenge, NHR was evaluated by counting the number of sneezes evoked by BSA administration (A). The CD4⁺DO11.10-TCR⁺ population in the NALT was assessed by flow cytometry (B). Data are expressed as mean \pm S.E.M. of 4–6 mice. * p < 0.05 and ** p < 0.01 compared with OVA-challenged control mice (Dunnett's test).

Interestingly, obvious NHR was not observed in antigen-challenged mice transferred with naïve CD4⁺ T cells (Fig. 4B). Therefore, cytokines commonly produced by differentiated T cell subsets, but not naïve CD4⁺ T cells, *e.g.*, granulocyte macrophage colony-stimulating factor, are probably related to the development of NHR.

4. EFFECTS OF ANTIALLERGIC DRUGS ON T CELL-MEDIATED NHR

Several clinical studies indicated that patients with severe and chronic allergic rhinitis can be treated with steroids that exhibit strong T cell-suppressing activity but are resistant to drugs targeting mast cells and mast cell-derived chemical mediators.³⁾ Therefore, the effects of these drugs on NHR developed in immunized and Th2 cell-transferred mice were investigated. Consistent with the clinical observations, the administration of dexamethasone (Dex) significantly suppressed

antigen-induced NHR, as well as inflammatory cell accumulation, in both immunized and Th2 cell-transferred mice (Fig. 5A). On the other hand, these responses were refractory to chlorpheniramine (Chl), a histamine H1 receptor antagonist, and montelukast (Mk), a cysteine (Cys)-LT1 receptor antagonist. The downregulation of antigen-specific T cell accumulation in the nasal lymphoid tissue (NALT) was involved in the mechanisms by which steroids efficiently suppressed NHR¹⁷⁾ (Fig. 5B).

5. CONCLUSION

During the generation of a mouse allergic rhinitis model, we confirmed the development of NHR and partially elucidated its mechanisms. Nasal symptoms that develop in immunized animals following repeated antigen challenge, as observed in previously reported allergic rhinitis models, possibly reflect not only the IgE/mast cell activation pathway but

also antigen-induced T cell-dependent NHR. In addition to the requirement of reevaluating those previous findings, a novel strategy for the treatment of allergic rhinitis through the development of a new means to inhibit unknown NHR-inducing factors is suggested.

Conflict of Interest The authors declare no conflict of interest.

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