Allergen endotoxins induce T-cell–dependent and non–IgE-mediated nasal hypersensitivity in mice

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Background: Allergen-mediated cross-linking of IgE on mast cells/basophils is a well-recognized trigger for type 1 allergic diseases such as allergic rhinitis (AR). However, allergens may not be the sole trigger for AR, and several allergic-like reactions are induced by non–IgE-mediated mechanisms.

Objective: We sought to describe a novel non–IgE-mediated, endotoxin-triggered nasal type-1-hypersensitivity-like reaction in mice.

Methods: To investigate whether endotoxin affects sneezing responses, mice were intraperitoneally immunized with ovalbumin (OVA), then nasally challenged with endotoxin-free or endotoxin-containing OVA. To investigate the role of T cells and mechanisms of the endotoxin-induced response, mice were adoptively transferred with *in vitro*–differentiated OVA-specific T_H2 cells, then nasally challenged with endotoxin-free or endotoxin-containing OVA.

Results: Endotoxin-containing, but not endotoxin-free, OVA elicited sneezing responses in mice independent from IgE-mediated signaling. OVA-specific T_H2 cell adoptive transfer to mice demonstrated that local activation of antigen-specific T_H2 cells was required for the response. The Toll-like receptor 4-myeloid differentiation factor 88 signaling pathway was indispensable for endotoxin-containing OVA-elicited rhinitis. In addition, LPS directly triggered sneezing responses in OVA-specific T_H2-transferred and nasally endotoxin-free OVA–primed mice. Although antihistamines suppressed sneezing responses, mast-cell/basophil-depleted mice had normal sneezing responses to endotoxin-containing OVA. Clodronate treatment abrogated endotoxin-containing OVA-elicited rhinitis, suggesting the involvement of monocytes/macrophages in this response.

Conclusions: Antigen-specific nasal activation of $CD4^+$ T cells followed by endotoxin exposure induces mast cell/basophil-independent histamine release in the nose that elicits sneezing responses. Thus, environmental or nasal residential bacteria may exacerbate AR symptoms. In addition, this novel phenomenon might explain currently unknown mechanisms in allergic(-like) disorders. (J Allergy Clin Immunol 2016;■■■:■■■-■■■.)

Key words: *Allergic rhinitis, $CD4^+$ T cell, endotoxin, histamine, hypersensitivity, IgE, monocyte/macrophage, nonallergic rhinitis, LPS*

Type 1 hypersensitivity reactions are a major pathological response in allergic diseases such as allergic rhinitis (AR), allergic asthma, and allergic anaphylaxis.[1] Classical type 1 hypersensitivity reactions are mediated by mast cells and basophils armed with antigen-specific IgE.[1,2] Cross-linking of IgE on mast cells/basophils by cognate antigens induces degranulation and the release of chemical mediators including histamine, which elicits bronchoconstriction and vasodilation and increases vascular permeability.[2] Although the IgE-mast-cell–mediated pathway is closely linked to allergic disorders, several type-1-hypersensitivity-like symptoms are induced by non–IgE-mediated mechanisms.[3-5]

The early-phase responses of AR (eg, sneezing) are mainly induced by allergen-mediated IgE cross-linking on mast cells.[6] However, the allergen-IgE pathway may not be the sole trigger, because some patients with AR do not respond to anti-IgE therapy.[7] Currently, non–IgE-mediated trigger(s) of AR symptoms are entirely unknown. AR diagnosis is based on rhinitis symptoms: sneezing, nasal discharge, and nasal clotting, and the presence of systemic or local (nasal) allergen-specific IgE.[8-10] Patients with rhinitis without allergen-specific IgE are diagnosed as suffering from nonallergic rhinitis (NAR) and, in most cases, triggers of the symptoms are unclear.[11] Although NAR is not considered an allergy, some patients with NAR show nasal infiltration of inflammatory cells including neutrophils, eosinophils, and mast cells that suggests local activation of innate and/or adaptive immunity.[12-15] In addition, histamine, a major mediator of type 1 allergic reactions, is involved in the disease symptoms, because antihistamines are often effective in patients with NAR.[11] Thus, the nasal symptoms of NAR with inflammation might be an immune-mediated hypersensitivity reaction. Currently, the underlying pathological mechanisms in these non–IgE-mediated allergic reactions have been rarely studied. Although IgE cross-linking is the classical signal for

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Abbreviations used
AR: Allergic rhinitis
DT: Diphtheria toxin
Ec-OVA: Endotoxin-containing OVA
Ef-OVA: Endotoxin-free OVA
ILC2: Group 2 innate lymphoid cell
i.n.: Intranasally
i.p.: Intraperitoneally
MyD88: Myeloid differentiation factor 88
NAR: Nonallergic rhinitis
OVA: Ovalbumin
TLR4: Toll-like receptor 4
WT: Wild type

mast cell/basophil degranulation,[1,2] many signals induce IgE-independent mast cell degranulation.[4,16] In addition, many cell types other than mast cells/basophils can produce and release histamine by non–IgE-mediated mechanisms.[17-22] These observations prompted us to investigate the causes and mechanisms of non–IgE-mediated type-1-hypersensitivity-like reactions.

LPS, also known as endotoxin, is a component of the gram-negative bacterial cell wall and is a common contaminant in several allergens including household dust[23-25] and animal dander,[26,27] as well as ambient air pollutants.[28] Low-dose LPS is a well-known T_H2-inducing adjuvant in airway application models.[29,30] Furthermore, several allergens contain ligands for Toll-like receptor 4 (TLR4), the LPS receptor, which mediates their allergenicity.[31-33] Even though mast cells also express TLR4, and LPS stimulation of mast cells induces cytokine production, LPS does not elicit mast cell degranulation.[34] Thus, even though LPS can participate in some allergic sensitizations, it is not considered a trigger of effector-phase responses.

In this study, we show the presence of a hitherto unknown non–IgE-mediated nasal allergy-like response in mice. Nasal application of endotoxin-containing ovalbumin (OVA) (Ec-OVA) triggers sneezing, a type-1-hypersensitivity-like reaction, in mice, which is IgE- and mast-cell–independent, but dependent on $CD4^+$ T cells, histamine, and monocytes/macrophages. We propose that the local activation of antigen-specific $CD4^+$ T cells followed by endotoxin exposure can elicit histamine release in the nose that triggers a type-1-hypersensitivity-like reaction. This reaction might contribute to classical and non–IgE-mediated allergic disorders of which the pathological mechanisms are not fully understood.

METHODS

More detailed methods can be found in this article's Methods section in the Online Repository at www.jacionline.org.

Mice

BALB/c wild-type (WT) mice were purchased from Charles River Laboratories Japan (Yokohama, Japan). BALB/c-background DO11.10$^+$[35] and myeloid differentiation factor 88–deficient (*Myd88*$^{-/-}$)[36] mice were maintained at the animal facilities of Hyogo College of Medicine. BALB/c-background *Fcer1a*$^{-/-}$ and *Rag2*$^{-/-}$ mice were purchased from Jackson Laboratories (Bar Harbor, Me). BALB/c-background *Tlr4*$^{-/-}$ mice were purchased from OrientalBioService (Kyoto, Japan). BALB/c-background Mas-TRECK mice were provided by Dr Masato Kubo (Division of Molecular Pathology, Research Institute for Biological Sciences, Tokyo University of Science, Tokyo, Japan). These mice were maintained under specific pathogen-free conditions. All mouse experiments were performed with the approval of, and in accordance with, the guidelines of the Institutional Animal Care Committee of Hyogo College of Medicine (No. A11-235, No. 14-007, and No. 28028).

Removal of endotoxin from OVA

Endotoxin-free ovalbumin (Ef-OVA) was generated from commercially available Ec-OVA. Overall, 1 mL of OVA solution (20 mg/mL) was mixed with 10 μL of Triton X-114 by vortexing. Samples were placed on ice for 5 minutes, then incubated at 37°C for 5 minutes. After centrifugation (9000*g* for 7 seconds), the upper aqueous phases were collected. The procedure was repeated 3 times. Residual detergent in the aqueous phase was removed by Pierce Detergent Removal Spin Columns (Pierce Biotechnology, Rockford, Ill). The endotoxin levels in Ec-OVA and Ef-OVA were determined by Pierce LAL Chromogenic Endotoxin Quantitation Kit (Pierce Biotechnology). Endotoxin levels in OVAs used in this study were 100 to 207 EU/mg (Ec-OVA) and 1.74 EU/mg (Ef-OVA), where 1 EU is equivalent to 0.1 ng of LPS from *Escherichia coli* E111:B4.

Mouse models

For the OVA immunization model, mice were injected intraperitoneally (i.p.) with a mixture of Ef-OVA (30 μg/200 μL/mouse) and alum (1 mg/200 μL/mouse) in PBS on days −18, −11, and −4. Five days after the final immunization (day 1), mice were intranasally (i.n.) administered 20 μL of PBS, Ec-OVA, or Ef-OVA (1 mg protein/dose in PBS) for 4 consecutive days. For cell-transfer models, mice were injected intravenously with naive or *in vitro*–differentiated OVA-specific T_H2 cells from DO11.10$^+$CD4$^+$ T cells (4-6 × 10^6 cells) on day −1. Two days after the transfer (day 1), mice were i.n. administered 20 μL of PBS, Ec-OVA, or Ef-OVA (1 mg protein/dose in PBS) for 4 consecutive days. Memory T_H2 cells were generated in mice transferred with DO11.10$^+$ T_H2 cells, and these were then left untreated for 6 weeks. In some experiments, mice were i.n. administered 20 μL of LPS (0.5, 5, or 50 ng/mL in PBS). To deplete mast cells and basophils, Mas-TRECK and control WT mice were injected i.p. with diphtheria toxin (DT) (250 ng/200 μL/mouse) on days −6, −5, −4, −3, −2, 1, and 3. Monocyte/macrophage depletion was achieved by intraperitoneal injection of clodronate liposome (100 μL/mouse) on day 3, 6 hours after the nasal challenge. For antihistamine treatment, mice were injected i.p. with diphenhydramine (1 or 2 mg/200 μL/mouse) or fexofenadine (5 mg/200 μL/mouse) in PBS 90 minutes before nasal challenge with OVA. Immediately after each nasal challenge, the frequency of sneezing was counted for 10 minutes. The mice were sacrificed 24 hours after the final nasal challenge, and noses were dissected for analyzing infiltrating inflammatory cells.

Statistics

Two-tailed Student *t* test and 1-way ANOVA followed by Tukey test were used to determine the statistical significance between 2 groups and among more than 2 groups, respectively. Two-way ANOVA followed by Bonferroni test was used for analyzing statistics of time-course experiments. *P* values of less than .05 were considered statistically significant.

RESULTS

Ec-OVA elicits IgE-independent rhinitis

We investigated whether AR-like symptoms can be elicited by non–IgE-mediated mechanisms. WT and *Fcer1a*$^{-/-}$ mice, deficient for FcεRI and thus deficient for IgE-mediated allergic reactions,[9,37] were i.p. immunized with Ef-OVA 3 times (days −18, −11, and −4), then i.n. challenged with 1 mg of Ef-OVA (endotoxin level, 1.74 EU/mg) or Ec-OVA (endotoxin level,