

IL-15 prevents allergic rhinitis through reactivation of antigen-specific CD8⁺ cells

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Background: Allergic rhinitis is one of the most common allergic inflammatory diseases characterized by a predominant T_H2 response with antigen-specific IgE synthesis. IL-15 plays important roles in activation and maintenance of memory CD8⁺T cells capable of producing IFN- γ , which regulates T_H2 responses.

Objective: To investigate the roles of endogenous IL-15 in allergic inflammation, we examined allergic rhinitis in IL-15 knockout (KO) mice sensitized with ovalbumin followed by intranasal rechallenge with ovalbumin.

Methods: IL-15KO mice were sensitized intraperitoneally with ovalbumin/complete Freund's adjuvant on day 0 and ovalbumin/IFA on day 7, and then were intranasally challenged with ovalbumin on days 21, 22, 23, 24, and 25. Nasal symptoms and histologic changes were examined. IgE production and T_H2 responses were measured by ELISA. Purified CD8⁺T cells or recombinant IL-15 were administered into ovalbumin-sensitized mice.

Results: The levels of IgE production and T_H2 responses in IL-15KO mice were comparable to those in control mice after ovalbumin sensitization. However, sneezing, infiltration of eosinophils into the nasal mucosa, and T_H2 cytokine production were aggravated in ovalbumin-sensitized IL-15KO mice after intranasal challenge with ovalbumin. Adoptive transfer of CD8⁺T cells from ovalbumin-sensitized mice suppressed the T_H2 responses in mice but not in IL-15KO mice. Administration of IL-15 with ovalbumin significantly prevented the development of allergic rhinitis in ovalbumin-sensitized mice.

Conclusion: We demonstrate with IL-15KO mice that endogenous IL-15 plays an important role in suppression of

allergic rhinitis at effector phase. Intranasal administration of IL-15 is useful as a therapeutic approach to control allergic rhinitis.

Clinical implications: Intranasal administration of recombinant IL-15 might become new immunotherapy for allergic rhinitis. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: IL-15, allergic rhinitis, T_H2 cells, CD8⁺T cells

Allergic rhinitis is one of the most common allergic inflammatory diseases, affecting between 10% to 25% of the world's population, and its prevalence has increased over the last decade.¹ Allergic rhinitis is characterized by a predominant T_H2 response, antigen-specific IgE synthesis and infiltration of eosinophils into the nasal mucosa.² The allergic responses are largely divided into 2 phases: systemic response in the induction phase and allergic inflammation in the effector phase.³⁻⁶ T_H2 cells and IgE specific for the allergen are generated in secondary lymphoid tissues at the induction phase after sensitization. The effector phase is characterized by sneezing, pruritus and rhinorrhea, and nasal congestion with infiltration of the nasal mucosa with inflammatory cells, including basophils, eosinophils, neutrophils, newly synthesized mast cells, and mononuclear cells, which occur at mucosa after challenge with the allergen.

IL-15 was initially identified by its ability to stimulate proliferation of T-cell clones, an activity similar to that of IL-2.^{7,8} However, in contrast with IL-2, which is produced only by activated T cells, IL-15 is produced by many types of cells, including monocytes, macrophages,⁹ dendritic cells,¹⁰ keratinocytes,¹¹ and epithelial cells.¹² Studies using IL-15 knockout (KO) mice have revealed that IL-15 plays important roles in the development, proliferation, and maintenance of natural killer (NK) cells,^{9,13} T-cell receptor $\gamma\delta$ intestinal intraepithelial lymphocytes,¹⁴ and memory phenotype CD8⁺T cells.^{15,16} IL-15 has been reported to promote IFN- γ production from murine T_H1 clones via upregulation of IL-12 receptor β 1.¹⁷ We previously found that IL-15 transgenic mice contained an increased number of memory CD8⁺T cells in a naive state and showed an impaired T_H2 responses in a murine model of asthma via activation of CD8⁺T cells.¹⁸ On the other hand, IL-15 has been reported to induce IL-5 production by allergen-specific T_H2 clones¹⁹ and promote the

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Abbreviations used

CFA: Complete Freund's adjuvant
 KLH: Keyhole limpet hemocyanin
 KO: Knockout
 LN: Lymph node
 NK: Natural killer
 WT: Wild-type

survival of B cells,²⁰ mast cells,²¹ and eosinophils.²² Thus, the roles of endogenous IL-15 in allergic responses remain elusive.

In this study, to elucidate the role of endogenous IL-15 in allergy, we analyzed allergic responses at the induction and effector phases in mouse models for allergic rhinitis using IL-15KO mice. We found that IL-15 attenuated the allergic symptoms at the effector phase by activation of antigen-specific CD8⁺ T cells. On the basis of this finding, we have proposed a therapeutic application of IL-15 for allergic rhinitis.

METHODS

Mice

IL-15KO mice were purchased from Taconic (Germantown, NY). Age-matched and sex-matched C57BL/6 wild-type (WT) mice were purchased from Charles River Japan Inc (Hino, Japan) and used as control mice. These mice were bred in specific pathogen-free conditions in our institute. All mice were used at 6 to 10 weeks of age. The animal experiments were approved by our institutional review committee according to a notice of the Prime Minister's Office of Japan (#6 of March 27, 1980) for the care and use of laboratory animals.

Antibodies and reagents

Fluorescein isothiocyanate-conjugated anti-mouse IgE (23G3), phycoerythrin-conjugated anti-CD117 (2B8), biotin-conjugated antimouse/human CD45R (RA3-6B2), biotin-conjugated antimouse NK1.1 (PK136), and purified antimouse CD11b (M1/70) were purchased from eBioscience (San Diego, Calif). Biotin-conjugated antimouse CD11c (HL3) and biotin-conjugated antimouse I-A^b (25-9-3) were purchased from Becton Dickinson (Franklin Lakes, NJ). MicroBeads conjugated to monoclonal rat antimouse CD4 (L3T4) antibodies (GK1.5), MicroBeads conjugated to streptavidin, and MicroBeads conjugated to goat antirat IgG were purchased from Miltenyi Biotec GmbH (Bergisch Gladbach, Germany). Ovalbumin (grade V) was purchased from Sigma (St Louis, Mo). CFA and IFA were purchased from Rockland Immunochemicals, Inc (Gilbertsville, Pa).

Sensitization and challenge

IL-15KO mice and WT mice were intraperitoneally sensitized with 100 μ g ovalbumin mixed with 100 μ L complete Freund's adjuvant (CFA) on day 0. Seven days later (day 7), the mice were intraperitoneally injected with 100 μ g ovalbumin mixed with 100 μ L IFA. Nonsensitized mice were injected with PBS mixed with 100 μ L CFA on day 0 followed by intraperitoneal injection with PBS mixed with 100 μ L IFA 7 days later. In some experiments, mice were intraperitoneally sensitized with 100 μ g keyhole limpet hemocyanin (KLH, Wako, Osaka) mixed with 100 μ L CFA on day 0 followed by intraperitoneal injection with 100 μ g KLH mixed with 100 μ L IFA

on day 7. On days 21, 22, 23, 24, and 25 after the first sensitization, ovalbumin-sensitized IL-15KO and WT mice were intranasally challenged with 10 μ L ovalbumin dissolved in PBS (10 mg/mL) into the bilateral nostril. Some ovalbumin-sensitized mice were intranasally challenged with 10 μ L PBS as controls.

Evaluation of nasal signs

Before the last challenge with ovalbumin or PBS, the mice were placed into an observation cage (1 animal/cage) for about 10 minutes for acclimatization. After the challenge with ovalbumin or PBS, the mice were placed into the observation cage again, and the numbers of sneezes were counted for 10 minutes by the method of Sugimoto et al.²³

Measurement of ovalbumin-specific IgE, IgG₁, and IgG_{2a}

Levels of ovalbumin-specific IgE, IgG₁, and IgG_{2a} on day 21 were measured by ELISA according to the method of Ishimitsu et al.¹⁸ Briefly, sample wells of an ELISA plate were coated with 100 μ L ovalbumin (100 μ g/mL) overnight at 37°C, washed with borate-buffered saline containing 0.05% Tween 20, and then blocked with 1% BSA in borate-buffered saline for 30 minutes. The plates were incubated with diluted samples (samples for IgE diluted 1:100; IgG₁, 1:100000; and IgG_{2a}, 1:100) for 90 minutes at room temperature. After further washing, the plates were incubated with peroxidase-conjugated antimouse IgE (Nordic Immunology, Minneapolis, Minn), IgG₁, or IgG_{2a} (Zymed Laboratories, Inc, San Francisco, Calif) for 90 minutes at room temperature. After further washing, the plates were incubated for 20 minutes at room temperature with 100 μ L/well of *o*-phenylenediamine solution, and the reactions were stopped with sulfuric acid. Then the plates were read for OD at 492 nm.

Measurement of ovalbumin-specific cytokine production by CD4⁺ T cells from the spleen and cervical lymph nodes

On day 21 after the first sensitization with ovalbumin or PBS, spleen cells from IL-15KO and WT mice were incubated on nylon-wool columns for 60 minutes at 37°C in 5% CO₂. After elution, CD4⁺ T cells and CD8⁺ T cells were positively purified to >95% using magnetic adsorbent sorting (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). Purified CD4⁺ T cells (1×10^6) from the spleen were cultured with mitomycin C-treated naive splenocytes (1×10^6) and ovalbumin (200 μ g/mL) for 48 hours at 37°C in 5% CO₂. In some experiments, 12 hours after the last nasal challenge with ovalbumin or PBS, purified CD4⁺ T cells (5×10^5) from cervical lymph nodes (LNs) were cultured with mitomycin C-treated naive splenocytes (5×10^5) and ovalbumin (100 μ g/mL) for 48 hours at 37°C in 5% CO₂. The concentrations of IL-4, IL-5, IL-13, and IFN- γ in the supernatants were analyzed by ELISA using a Cytokine Development Kit (GT, Minneapolis, Minn).

Histologic examination

Mice were killed 12 hours after the last nasal challenge with ovalbumin or PBS. The heads were removed and fixed in 10% formaldehyde solution for 24 hours at room temperature. After fixation, the heads were decalcified in 5% formic acid for 36 hours at room temperature and neutralized in 5% sodium sulfate solution for 12 hours at room temperature. Coronal nasal sections were then stained with hematoxylin and eosin, and the number of eosinophils in each side of the posterior edge of the nasal septum was counted microscopically in a high power field.

Transfer assays

Spleen cells of WT mice that had been sensitized with ovalbumin/ CFA and ovalbumin/IFA at a 7-day interval were obtained 21 days

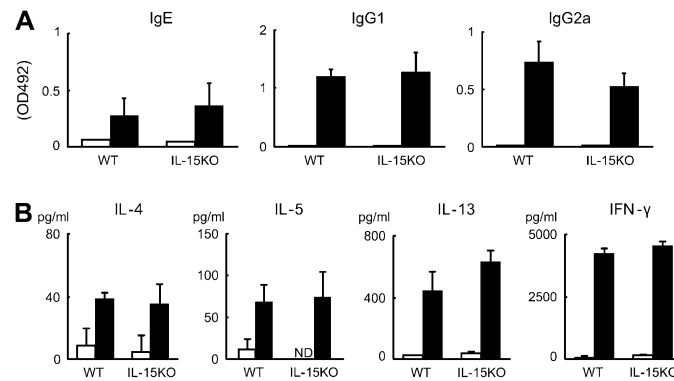


FIG 1. Ovalbumin-specific immunoglobulin production and T_H2 response in IL-15KO and WT mice on day 21 after ovalbumin sensitization. **A**, Serum levels of ovalbumin-specific immunoglobulins in IL-15KO mice and WT mice that had been sensitized with ovalbumin/CFA followed by ovalbumin/IFA (■) or PBS/CFA followed by PBS/IFA as controls (□). **B**, Cytokine production by $CD4^+$ T cells from the spleens of IL-15KO and WT mice that had been sensitized with ovalbumin/CFA followed by ovalbumin/IFA in the presence (■) or absence (□) of ovalbumin. The data are representative of 3 independent experiments. Pooled cells from 3 to 4 mice of each group were used in each experiment. Results are shown as the means of triplicate determinations $\pm$ SDs.

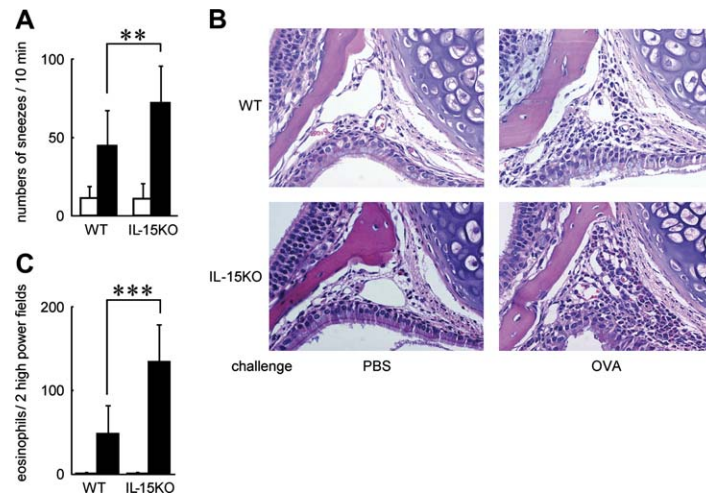


FIG 2. Allergic symptoms and eosinophil infiltration into the nasal mucosa after nasal challenge with ovalbumin (OVA) or PBS in OVA-sensitized IL-15KO and WT mice. **A**, The numbers of sneezes after challenge with ovalbumin (■) or PBS (□). **B**, Coronal nasal sections stained with hematoxylin and eosin. **C**, The number of eosinophils infiltrating into the nasal mucosa after the last nasal challenge with OVA (■) or PBS (□). Data were obtained from 3 independent experiments, and results are geometric means $\pm$ SDs. In each experiment, 5 to 8 mice were used. ** $P < .01$; *** $P < .001$.

after the first sensitization, and T cells were enriched by though nylon-wool columns to $>80\%$. Nylon-wool column-passed T cells (2×10^7) were transferred intravenously to naive IL-15KO and WT mice. In some experiments, spleen cells of WT mice that had been sensitized with ovalbumin/CFA and ovalbumin/IFA at a 7-day interval or with KLH/CFA and KLH/IFA at a 7-day interval were obtained 21 days after the first sensitization and passed through nylon-wool columns. $CD8^+$ T cells (1×10^7) were negatively purified by using MACS by depletion of the cells expressing CD4, CD11b, CD11c, NK1.1, CD45R, or I-A^b to $>90\%$ and were adoptively transferred into IL-15KO and WT mice that had been sensitized with ovalbumin/CFA and ovalbumin/IFA at a 7-day interval 21 days previously. Some ovalbumin-sensitized IL-15KO and WT mice were intravenously injected with $CD8^+$ T cells (1×10^7) from WT mice that had been intraperitoneally sensitized with KLH/CFA and KLH/IFA

at a 7-day interval as controls. Just after the transfer, these recipient mice were intranasally challenged with 10 μ L ovalbumin or PBS into the bilateral nostrils for 5 days. Mice were killed 12 hours after the last intranasal challenge, and then analysis of cytokine production by $CD4^+$ T cells from cervical LNs and histologic examinations of nasal mucosa were performed as described.

Intranasal administration of exogenous IL-15

Wild-type mice were intraperitoneally sensitized with ovalbumin/CFA and ovalbumin/IFA at a 7-day interval. On days 21, 22, 23, 24, and 25 after the first sensitization, ovalbumin-sensitized WT mice were intranasally challenged with 10 μ L ovalbumin dissolved in PBS (10 mg/mL) containing recombinant murine IL-15 (10 μ g/mL) into the bilateral nostrils. Some ovalbumin-sensitized WT mice were intranasally challenged with 10 μ L ovalbumin or PBS for 5 days as

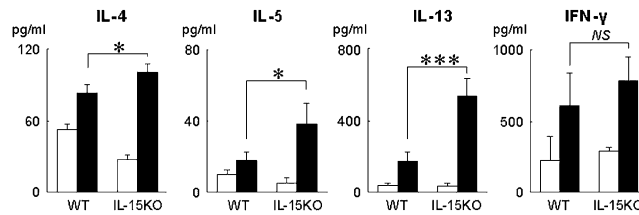


FIG 3. Cytokine production by CD4⁺ T cells from cervical LNs after nasal challenge with ovalbumin or PBS in ovalbumin-sensitized IL-15KO and WT mice in the presence (■) or absence (□) of ovalbumin. The data are representative of 3 independent experiments. Pooled cells from 3 to 5 mice of each group were used in each experiment. Results are shown as the means of triplicate determinations $\pm$ SDs. * $P < .05$; *** $P < .001$; NS, nonsignificant difference.

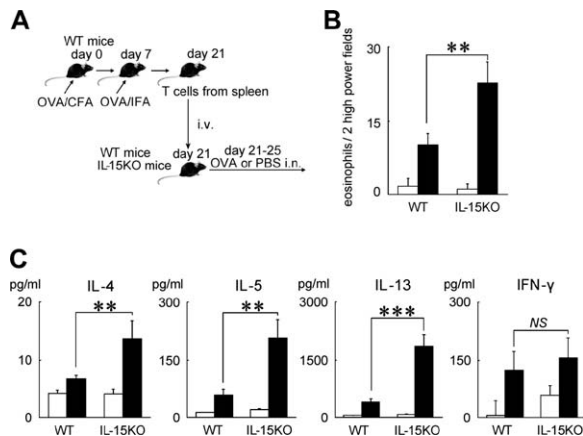


FIG 4. Effect of adoptive transfer of ovalbumin (OVA)-sensitized T cells at the effector phase of allergic rhinitis. **A**, Immunization protocol. T cells from spleens of OVA-sensitized WT mice were transferred into naive IL-15KO and WT mice, which were subsequently challenged with OVA or PBS. **B**, The number of eosinophils infiltrating into the nasal mucosa after the last nasal challenge with OVA (■) or PBS (□). Data were obtained from 3 independent experiments, and results are geometric means $\pm$ SDs. In each experiment, 3 to 5 mice were used. **C**, Cytokine production by CD4⁺ T cells from cervical LNs of the recipient mice in the presence (■) or absence (□) of ovalbumin. The data are representative of 3 independent experiments. Pooled cells from 3 to 5 mice of each group were used in each experiment. Results are shown as the means of triplicate determinations $\pm$ SDs. ** $P < .01$; *** $P < .001$; NS, nonsignificant difference.

controls. Counting of the number of sneezes and histologic examinations of nasal mucosa were performed as described.

Statistical analysis

The statistical significance of the data was determined by Student *t* test. A value of $P < .05$ was taken as significant.

RESULTS

IL-15KO mice showed aggravated allergic inflammation at the effector phase of allergic rhinitis

To determine the role of IL-15 in T_H2 response with IgE production at the induction phase after immunization, IL-15KO and WT mice were sensitized with ovalbumin/CFA and then with ovalbumin/IFA 7 days later. ovalbumin-

specific IgE, IgG₁, and IgG_{2a} in the serum from ovalbumin-sensitized IL-15KO and WT mice were assessed on day 21 after the first sensitization. There was no significant difference between ovalbumin-specific IgE, IgG₁, and IgG_{2a} levels in ovalbumin-sensitized IL-15KO and those in WT mice (Fig 1, A).

We next examined cytokine production by CD4⁺ T cells from the spleen of ovalbumin-sensitized IL-15KO and WT mice on day 21. There was no significant difference in IL-4, IL-5, IL-13, or IFN- γ production in response to ovalbumin by CD4⁺ T cells from the spleens of ovalbumin-sensitized IL-15KO mice and WT mice (Fig 1, B). Thus, IL-15KO mice showed IgE production and T_H2 response comparable to those in WT mice at the induction phase.

We next assessed the role of IL-15 at the effector phase in an allergic rhinitis model of mice. Ovalbumin-sensitized IL-15KO and WT mice were intranasally challenged with 10 μ L ovalbumin into the bilateral nostrils on days 21, 22, 23, 24, and 25. The number of sneezes by each mouse over a 10-minute period after the last nasal challenge with ovalbumin or PBS was counted. The number of sneezes was significantly greater in IL-15KO mice than in WT mice ($P < .01$; Fig 2, A). Thus, IL-15KO mice showed aggravated symptoms at the effector phase of allergic rhinitis.

We counted the number of eosinophils infiltrating into the nasal mucosa microscopically 12 hours after the last nasal challenge with ovalbumin or PBS. The infiltration of eosinophils into the nasal mucosa was aggravated in IL-15KO mice compared with that in WT mice ($P < .001$; Fig 2, B and C).

We next examined cytokine production by CD4⁺ T cells from cervical LNs at effector phase. CD4⁺ T cells from cervical LNs of IL-15KO mice produced large amounts of IL-4, IL-5, and IL-13 in response to ovalbumin compared with the amounts produced by CD4⁺ T cells from cervical LNs of WT mice ($P < .05$; Fig 3). Thus, IL-15KO mice showed aggravated T_H2 responses at effector phase of allergic rhinitis.

IL-15-dependent CD8⁺ T cells suppressed T_H2 response at the effector phase of allergic rhinitis

To determine which cells are responsible for inhibition of T_H2 response at the effector phase of allergic rhinitis, we first transferred 2×10^7 nylon wool columns-passed

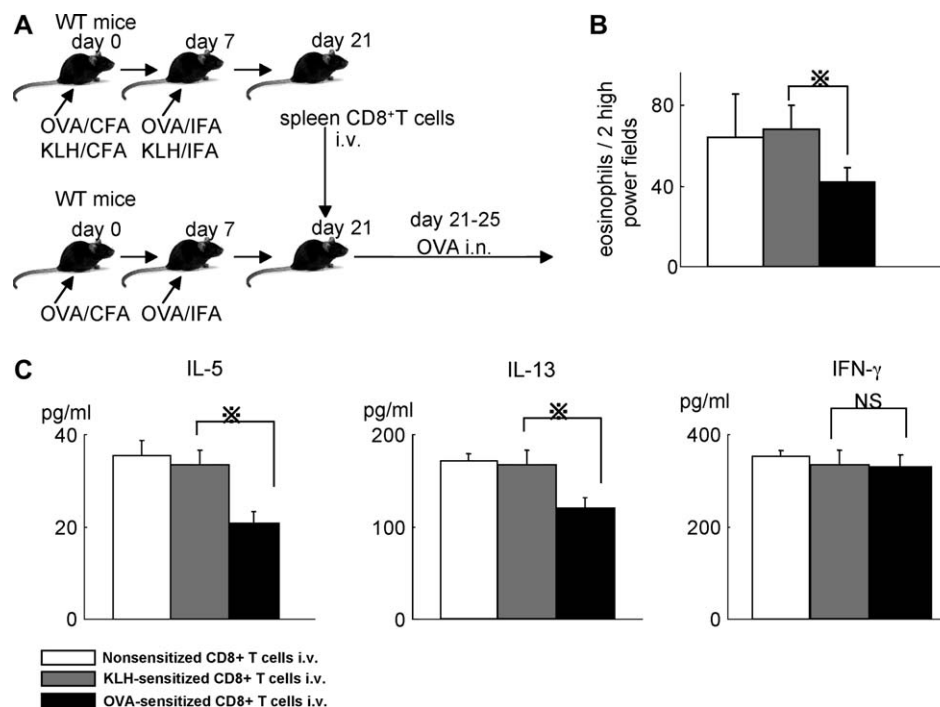


FIG 5. Effect of adoptive transfer of CD8⁺ T cells at the effector phase on allergic rhinitis in WT mice. **A**, Immunization protocol. CD8⁺ T cells from spleens of naive, ovalbumin (OVA)-sensitized, or KLH-sensitized WT mice into OVA-sensitized WT mice, which were subsequently challenged with OVA or PBS. **B**, The number of eosinophils infiltrating into the nasal mucosa. Data were obtained from 3 independent experiments, and results are geometric means $\pm$ SDs. In each experiment, 3 to 5 mice were used. **C**, Cytokine production by CD4⁺ T cells (5×10^5) from cervical LNs of the recipient mice in response to OVA. The data are representative of 3 independent experiments. Pooled cells from 3 to 4 mice of each group were used in each experiment. Results are shown as the means of triplicate determinations $\pm$ SDs. * $P < .05$; NS, nonsignificant difference. i.n., Intranasally.

spleen cells from ovalbumin-sensitized WT mice into naive IL-15KO or WT mice and then intranasally challenged ovalbumin. Transfer of nylon-wool-passed spleen cells induced eosinophil infiltration with T_H2 response after intranasal challenge with ovalbumin (Fig 4, A). The degree of eosinophil infiltration and T_H2 response were significantly aggravated in naive IL-15KO mice compared with naive WT mice ($P < .01$; Fig 4, B and C). These results suggested that regulatory T cells existed in spleen of ovalbumin-sensitized WT mice and suppressed T_H2 response in IL-15-dependent manner at the effector phase of allergic rhinitis.

IL-15 has been reported to stimulate memory CD8⁺ T cells to expand and show increased IFN- γ production and cytotoxic activities.²⁴⁻²⁷ We next transferred CD8⁺ T cells from ovalbumin-sensitized or KLH-sensitized WT mice into ovalbumin-sensitized WT mice, which were then intranasally challenged with ovalbumin. As shown in Fig 5, B and C, transfer of CD8⁺ T cells from ovalbumin-sensitized WT mice but not from naive or KLH-sensitized WT mice suppressed infiltration of eosinophils into the nasal mucosa and T_H2 cytokine production by CD4⁺ T cells from cervical LNs of ovalbumin-sensitized and challenged WT mice ($P < .05$). These results suggested that ovalbumin-specific CD8⁺ T cells had a

regulatory function in T_H2 response at the effector phase of allergic rhinitis.

We next transferred the CD8⁺ T cells from ovalbumin-sensitized mice into ovalbumin-sensitized IL-15KO mice and examined T_H2 responses after intranasal challenge with ovalbumin (Fig 6, A). When 1×10^7 CD8⁺ T cells were transferred into ovalbumin-sensitized WT mice, infiltration of eosinophils into the nasal mucosa and T_H2 cytokine production by CD4⁺ T cells of cervical LNs were significantly suppressed ($P < .05$; Fig 6, B and C). On the other hand, the T_H2 responses were not affected after transfer with the CD8⁺ T cells in ovalbumin-sensitized IL-15KO mice (Fig 6, B and C). These results suggested that ovalbumin-specific CD8⁺ T cells suppressed the T_H2 response at the effector phase of allergic rhinitis and that IL-15 was indispensable for activation of these CD8⁺ T cells for suppressive function.

Exogenous IL-15 prevents allergic rhinitis in ovalbumin-sensitized WT mice

Our results revealed that endogenous IL-15 attenuated allergic responses at the effector phase of allergic rhinitis. These results raise the possibility that IL-15 is applicable to therapeutic use for controlling allergic rhinitis. To test

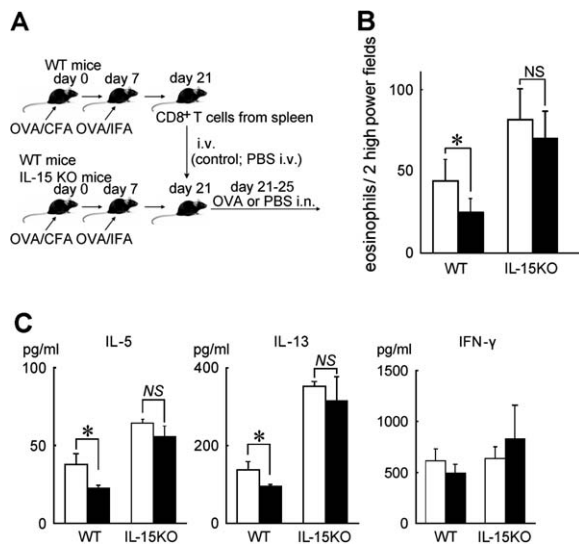


FIG 6. Effect of adoptive transfer of ovalbumin (OVA)-sensitized CD8⁺ T cells at the effector phase on allergic rhinitis in IL-15KO mice. **A**, Immunization protocol. CD8⁺ T cells from spleens of OVA-sensitized WT mice were transferred into OVA-sensitized IL-15KO and WT mice, which were subsequently challenged with OVA. **B**, The number of eosinophils infiltrating into the nasal mucosa. Data were obtained from 3 independent experiments, and results are geometric means $\pm$ SDs. **C**, Cytokine production by CD4⁺ T cells from cervical LNs of the recipient mice in the presence of OVA. The data are representative of 3 independent experiments. Pooled cells from 3 to 4 mice of each group were used in each experiment. Results are shown as the means of triplicate determinations $\pm$ SDs. * P < .05; NS, nonsignificant difference. i.v., Intravenously.

this, we examined the effects of exogenous IL-15 on allergic rhinitis at the effector phase. After nasal challenge of ovalbumin together with IL-15 for 5 days, the number of sneezes, histologic change of nasal mucosa, and cytokine production by CD4⁺ T cells from cervical LNs were examined. The number of sneezes was reduced by addition of IL-15 at the effector phase compared with PBS (P < .05; Fig 7, A). The number of eosinophils infiltrating into the nasal mucosa was also reduced by addition of IL-15 at the effector phase (Fig 7, B and C). Thus, administration of IL-15 at effector phase was able to prevent allergic rhinitis.

DISCUSSION

In this study, we examined the role of endogenous IL-15 in allergic rhinitis using IL-15KO mice. T_H2 response with antigen-specific IgE production was normally induced in IL-15KO mice after sensitization with ovalbumin. However, allergic rhinitis as assessed by symptoms, eosinophil infiltration into the nasal mucosa, and T_H2 responses in regional LN was aggravated in ovalbumin-sensitized IL-15KO mice after intranasal challenge with ovalbumin. Ovalbumin-specific CD8⁺ T cells showed a suppressive effect on T_H2 response at the effector phase of allergic rhinitis only in the presence of IL-15. Thus,

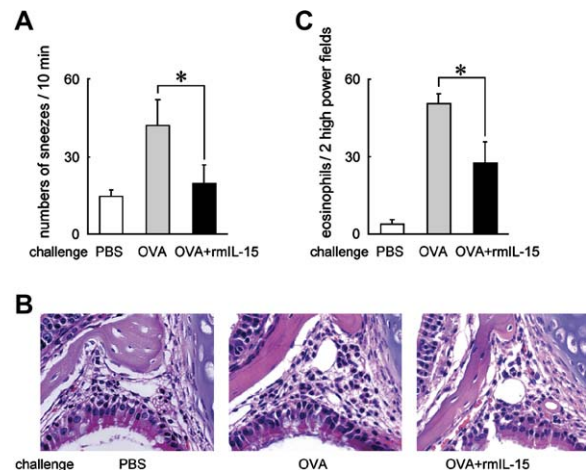


FIG 7. Effect of exogenous IL-15 administration at the effector phase on allergic rhinitis. Ovalbumin (OVA)-sensitized WT mice were intranasally rechallenge with OVA together with or without recombinant murine (rm) IL-15. **A**, The number of sneezes. **B**, Coronal nasal sections stained with hematoxylin and eosin. **C**, The number of eosinophils infiltrating into the nasal mucosa. Data were obtained from 3 independent experiments, and results are geometric means $\pm$ SDs. * P < .05.

these results suggest that endogenous IL-15 plays a regulatory role in allergic rhinitis via suppression of T_H2 response at the effector phase. It is notable that *in vivo* addition of exogenous IL-15 at the effector phase significantly ameliorated allergic rhinitis, providing an insight into a therapeutic approach using IL-15 to control allergic diseases.

There are several lines of evidence that IL-15 affects CD4⁺ T_H1 or T_H2 responses at the induction phase after primary immunization. IL-15 has been reported to induce IL-12 receptor β 1 directly on mouse T cells at the transcriptional level via activation of signal transducer and activator of transcription 5.¹⁷ We have reported that overexpression of IL-15 induced CD4⁺ T_H1 response after *Listeria monocytogenes* infection via bystander activation of memory CD8⁺ T cells producing IFN- γ .²⁸ We have also reported that the CD4⁺ T_H2 response was significantly reduced in IL-15 transgenic mice sensitized with ovalbumin.¹⁸ On the other hand, IL-15 has been shown to induce IL-5 production of human T_H2 clones¹⁹ and to induce proliferation of activated B cells *in vitro*, corresponding to the enhancement of IgE and IgG.²⁰ However, in the current study, we showed that IgE production and CD4⁺ T_H2 response normally occurs in IL-15KO mice at the induction phase after sensitization with ovalbumin. In contrast with overexpression of IL-15, endogenous IL-15 may not be mandatory in induction of systemic T_H2 response after ovalbumin immunization.

The effector phase in allergic rhinitis is characterized by sneezing, pruritus and rhinorrhea, and nasal congestion with infiltration of the nasal mucosa with inflammatory cells, including basophils, eosinophils, neutrophils, newly synthesized mast cells, and mononuclear cells, which are mainly mediated by secondary T_H2 responses by memory

CD4⁺ T cells.^{5,6} We found that ovalbumin-specific CD8⁺ T cells are responsible for suppression of the T_H2 response in regional LNs at the effector phase of allergic rhinitis. Notably, transfer of CD8⁺ T cells from ovalbumin-sensitized WT mice could not control infiltration of eosinophils into the nasal mucosa and the T_H2 response in regional LNs at the effector phase in IL-15KO mice, suggesting that the regulatory function in the CD8⁺ T cells is totally dependent on IL-15 at the effector phase. IL-15-dependent CD8⁺ T cells may be mainly responsible for suppression of allergic rhinitis at the effector phase.

T_H1 cells, which secrete IL-2, IFN- γ , and TNF- α for the induction of cell-mediated immunity, inhibit the proliferation of T_H2 cells, which secrete IL-4 for IgE production, whereas T_H2 cells shut down IFN- γ production by T_H1 cells.^{29,30} Thus, T_H1 and T_H2 cells are mutually regulated. We previously reported that the CD4⁺ T_H2 response was significantly reduced in IL-15 transgenic mice sensitized with ovalbumin, whereas the T_H1 response capable of inducing IFN- γ production in response to ovalbumin was not increased in sensitized IL-15 transgenic mice.¹⁸ Instead, CD8⁺ T cells from ovalbumin-sensitized IL-15 transgenic mice produced an appreciable amount of IFN- γ in response to ovalbumin and depletion of CD8⁺ T cells aggravated allergic asthma, indicating that CD8⁺ T cells were responsible for impaired T_H2 response with IgE production. Therefore, it is possible that IL-15-dependent CD8⁺ T cells suppress the secondary T_H2 response at the effector phase of allergic rhinitis via production of IFN- γ . Recently, Miyahara et al³¹ have reported that effector CD8⁺ T cells producing IL-13 are responsible for airway hyperresponsiveness in mice immunized with ovalbumin/alum. These CD8⁺ T cells appeared to be activated in an IL-15-independent manner. On the other hand, IL-15-dependent central memory CD8⁺ T cells showed no suppressive effect on airway hyperresponsiveness.³² Ruckert et al³³ have recently reported that treatment with soluble IL-15 receptor α both at induction and effector phases prevents allergen-specific T_H2 cell response and allergic inflammation at the effector phase *in vivo*.³³ The discrepancy may be explained by the differences in mouse strains and adjuvants used for sensitization. They used T_H2-prone BALB/c mice immunized with ovalbumin/alum, which develop preferentially a T_H2 response, whereas we used T_H1-prone C57BL/6 mice immunized with ovalbumin/CFA, which develop both T_H1/T_C1 and T_H2 responses.^{34,35} We speculate that IL-15-dependent CD8⁺ T cells capable of producing IFN- γ are preferentially generated in C57BL/6 mice sensitized with ovalbumin/CFA but not in BALB/c mice sensitized with ovalbumin/alum. We have recently reported that IL-15 plays an important role in early activation of memory CD8⁺ T cells for cytotoxicity after re-exposure to antigen.²⁷ Thus, it is also possible that memory CD8⁺ T cells kill antigen-presenting cells such as dendritic cells for T_H2 response and suppress the afterresponse in allergic rhinitis. There are several lines of evidence that regulatory CD8⁺ T cells downregulate immune responses via production of

suppressive cytokines such as IL-10 and TGF- β and via inductions of cytotoxic T lymphocyte-associated antigen expression,³⁶ raising another possibility that regulatory CD8⁺ T cells producing suppressive cytokines are responsible for the suppression. However, we did not detect such cytokines in the CD8⁺ T cells in response to ovalbumin. Further experiments are needed to clarify the mechanism whereby the CD8⁺ T cells suppress the allergic rhinitis at the effector phase.

It is notable that administration of exogenous IL-15 together with allergen prevented allergic rhinitis in mice. Our results obtained by using IL-15KO mice revealed that antigen-specific CD8⁺ T cells downregulated T_H2 response at the effector phase of allergic rhinitis in the presence of endogenous IL-15. These results provide an insight into a therapeutic approach to control allergic rhinitis by IL-15. In fact, we showed that administration of exogenous IL-15 together with allergen prevented allergic rhinitis in mice. We have recently reported that exogenous IL-15 is able to activate memory CD8⁺ T cells at a very early stage after reinfection, conferring robust protection against *L. monocytogenes*.²⁷ Exogenous IL-15 may expand and activate memory CD8⁺ T cells to downregulate the T_H2 response at the effector phase of allergic rhinitis. IL-15 may be useful for treatment of allergic rhinitis.

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