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

Noriaki Aoi, MD,[a,b] Tokuko Masuda, BS,[a] Daisuke Murakami, MD,[a] Toshiki Yajima, MD, PhD,[a] Hiroyuki Mizubuchi, MS,[a] Hisakata Yamada, MD, PhD,[a] Hideyuki Kawauchi, MD, PhD,[b] and Yasunobu Yoshikai, MD, PhD[a]

Fukuoka and Izumo, Japan

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 2006;117:1359-66.)

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 γδ 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 β1.[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 clones[19] and promote the survival of B cells,[20] mast cells,[21] and eosinophils.[22] Thus, the roles of endogenous IL-15 in allergic responses remain elusive.

From [a]the Division of Host Defense, Medical Institute of Bioregulation, Kyushu University, Fukuoka; and [b]the Department of Otorhinolaryngology, Shimane University, Izumo.

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Reprint requests: Yasunobu Yoshikai, MD, PhD, Division of Host Defense, Research Center of Prevention of Infectious Disease, Medical Institute of Bioregulation, Kyushu University, Fukuoka 812-8582, Japan. E-mail: yoshikai@bioreg.kyushu-u.ac.jp.

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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

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 $I\text{-}A^b$ (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 μg ovalbumin mixed with 100 μL complete Freund's adjuvant (CFA) on day 0. Seven days later (day 7), the mice were intraperitoneally injected with 100 μg ovalbumin mixed with 100 μL IFA. Nonsensitized mice were injected with PBS mixed with 100 μL CFA on day 0 followed by intraperitoneal injection with PBS mixed with 100 μL IFA 7 days later. In some experiments, mice were intraperitoneally sensitized with 100 μg keyhole limpet hemocyanin (KLH, Wako, Osaka) mixed with 100 μL CFA on day 0 followed by intraperitoneal injection with 100 μg KLH mixed with 100 μL 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 μL ovalbumin dissolved in PBS (10 mg/mL) into the bilateral nostril. Some ovalbumin-sensitized mice were intranasally challenged with 10 μL PBS as controls.

Evaluation of nasal signs

Before the last rechallenge 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, IgG_1, and IgG_{2a}

Levels of ovalbumin-specific IgE, IgG_1, 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 μL ovalbumin (100 μg/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; IgG_1, 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), IgG_1, 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 μL/well of *o*-phenylendiamine 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% CO_2. 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 μg/mL) for 48 hours at 37°C in 5% CO_2. 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 μg/mL) for 48 hours at 37°C in 5% CO_2. 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 horus 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 after the first sensitization, and T cells were enriched by though nylon-wool columns to >80%. Nylon-wool column-passed T cells