

Contribution of interleukin 17A to the development and regulation of allergic inflammation in a murine allergic rhinitis model

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ABSTRACT

Background: Interleukin (IL) 17A, a key cytokine of T_H17 cells, is a well-known proinflammatory cytokine. Despite the important role of T_H17 cells in acute airway inflammation, the role of IL-17A in allergic rhinitis (AR) remains unclear.

Objective: To investigate the role of IL-17A in the allergic response in AR.

Methods: Wild-type BALB/c and IL-17A-deficient mice were immunized intraperitoneally and were challenged intranasally with ovalbumin. Allergic symptom scores, eosinophil infiltration, serum IgE level, and the levels of several cytokines in nasal lavage fluid and splenocyte supernatants were analyzed.

Results: IL-17A levels increased significantly more in ovalbumin-sensitized wild-type mice than in the negative control group. IL-17A-deficient mice showed a significant decrease in allergic symptoms, serum IgE levels, and eosinophil infiltration into the nasal mucosa compared with wild-type mice. IL-17A-deficient mice also showed decreased histamine and cysteinyl leukotriene release. Bone marrow-derived mast cells from IL-17A-deficient mice showed significantly lower degranulation and secretion of tumor necrosis factor α . Moreover, IL-17A deficiency attenuated the IL-5 level in nasal lavage fluid and its production in response to ovalbumin but did not increase interferon γ production and its level in nasal lavage fluid. In addition, secretion of IL-17A from spleen cells induced the expression of proinflammatory cytokine messenger RNA in macrophages. The mean level of proinflammatory cytokines, including tumor necrosis factor α and IL-17, decreased in IL-17A-deficient mice.

Conclusions: These results suggest that IL-17A may partly contribute to the development of nasal allergic inflammation in an AR animal model and regulate AR via the activation of proinflammatory cytokines and modulation of T_H2 cytokine.

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Introduction

Allergic rhinitis (AR) is an inflammatory disease of the nasal mucosa dominated by a T_H2 immune response¹ and exhibiting unbalanced T_H1/T_H2 responses. Inhalation of the allergen into the nose leads to a high level of serum IgE² and induces T_H1 to T_H2 shift; the ratio of T_H2 cytokines, such as interleukin (IL) 4, IL-5, and IL-13, to interferon γ (IFN- γ) increases. Many cell types, such as mast cells,

eosinophils, lymphocytes, macrophages, and neutrophils, contribute to allergic inflammation.^{3,4}

T_H17 cells are a subset of T-helper cells whose discovery has required the revision of accepted concepts in immunopathology and T-cell regulation.⁵ Activated T_H17 cells produce IL-17A, IL-21, and IL-22, as well as tumor necrosis factor α (TNF- α), which enhance T_H2-induced effects in allergic responses.^{6,7} IL-17A was found to induce TNF- α and IL-1 β in human blood-derived macrophages *in vitro*⁸ and to recruit and promote the survival of airway macrophages in allergic airway inflammation.⁹ High levels of IL-17A messenger RNA (mRNA) have been detected in human peripheral blood T cells, and IL-17A expression increases in patients with allergic or autoimmune disease.^{10,11} Previous studies have revealed that IL-17A deficiency suppresses allergic cellular and humoral responses in diseases such as contact hypersensitivity, delayed-

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type hypersensitivity, and airway hypersensitivity response in experimental IL-17A-deficient mice.¹² Furthermore, IL-17A may be involved in neutrophilic acute airway inflammation.¹³ It was recently demonstrated that IL-17A expression is upregulated in the sputum, bronchoalveolar lavage fluid, and blood from asthmatic patients and that eosinophils and CD4⁺ T cells are the source of IL-17A.¹⁴ Increasing evidence suggests that IL-17A plays an active role in the development of allergic reactions.¹⁵ However, the role of IL-17A in allergic inflammation of the nasal mucosa remains unclear.

To investigate the role of IL-17A in AR, we evaluated the allergic responses of ovalbumin-sensitized wild-type (WT) and IL-17A-deficient (knockout [KO]) mice. We found that IL-17A levels increased after sensitization with ovalbumin and that IL-17A deficiency attenuated nasal allergic responses. Our results suggest that IL-17A may contribute to the development of allergic inflammation in AR.

Methods

Reagents and antibodies

Ovalbumin and bovine serum albumin (BSA) were obtained from Sigma (St Louis, Missouri). Neutralizing anti-IL-17A antibody and recombinant mouse (rm) IL-17A were purchased from R&D Systems (Minneapolis, Minnesota). Isotype-matched control rat IgG2a antibody (clone R35-95) was purchased from BD Bioscience (San Jose, California). The histamine enzyme immunoassay kit was obtained from Immunotech (Villepinte, France), and the cysteinyl leukotriene immunoassay kit was purchased from BioSource (Camarillo, California). The levels of IL-5, IFN- γ , TNF- α , IL-1 β , and IL-17A were measured using enzyme-linked immunosorbent assay (ELISA) kits purchased from R&D Systems. The mast cell degranulation assay kit was purchased from Chemicon (Temecula, California).

Animals

Female BALB/c mice (WT, 4 weeks of age) were maintained under pathogen-free conditions. IL-17A-deficient mice (KO) with a BALB/c background that had been bred under pathogen-free conditions were purchased from Professor Yoichiro Iwakura (Center for Experimental Medicine, Institute of Medical Science, University of Tokyo, Tokyo, Japan).¹² All animal experiments were approved by the Institutional Animal Care Committee of the Clinical Research Institute of Seoul National University Hospital.

Experimental schedule

The mice were divided into 4 groups and sensitized by intraperitoneal injection with 25 μ g of ovalbumin complexed with 2 mg of aluminum hydroxide (alum) on days 0, 7, and 14. WT and KO mice were then subjected to an intranasal challenge with 100 μ g of ovalbumin on 7 consecutive days from day 21 to day 27.¹⁶ Control WT and KO mice were intraperitoneally injected and intranasally challenged with phosphate-buffered saline (PBS) on the same schedule. Each group had at least 15 mice.

Evaluation of nasal symptoms

On day 28, after intranasal allergen provocation with 200 μ g of ovalbumin, the frequency of sneezing and nose-scratching behaviors was determined during a 15-minute period to evaluate early allergic responses (by an investigator masked to the experimental condition of the mouse).

ELISA to detect ovalbumin specific IgE

Nunc, 96-well, flat-bottom plates were coated with 100 μ g/mL of ovalbumin in coating buffer (0.05M carbonate-bicarbonate) overnight at 4°C, washed 3 times with washing buffer (PBS contain-

ing 0.05% Tween-20), and blocked with 3% BSA in PBS for 1 hour at 37°C. Serum samples (50 μ L) were added to ovalbumin-coated plates along with serial dilutions of a high titer ovalbumin IgE standard and incubated for 2 hours at 37°C. After washing, 100 μ L of biotin-conjugated rat anti-mouse IgE monoclonal antibody (BD Pharmingen, San Jose, California) was added to each well and incubated for 1 hour at 37°C. The plates were then incubated with 100 μ L of streptavidin and horseradish peroxidase-conjugated secondary antibody (BD Pharmingen) for 30 minutes at 37°C. After washing, 100 μ L of 3,3',5,5'-tetramethylbenzidine substrate solution was added per well and incubated for 30 minutes at room temperature. Optical density was measured at 450 nm using an absorbance detector.

Measurement of total serum IgE

Nunc, 96-well, flat-bottom plates were coated overnight with anti-mouse IgE capture monoclonal antibody (BD Pharmingen) at 4°C. The plate was washed with 0.1% Tween-PBS 3 times and blocked with 300 μ L of 3% BSA per well for 1 hour at room temperature. Serum samples (32-fold dilution) and standard solution (BD Pharmingen) were added, and the plate was incubated for 3 hours at 4°C. After washing 3 times, bound IgE was detected using rat anti-mouse IgE-horseradish peroxidase (SouthernBiotech, Birmingham, Alabama) at a 3,000-fold dilution. Optical density was measured in a microplate reader at 450 nm.

Histologic analysis

Heads of mice were fixed in 10% formalin overnight at room temperature with slow shaking and then decalcified and embedded in paraffin blocks. Paraffin-embedded tissue samples were cut into 4- μ m-thick sections, deparaffinized in xylene, and dehydrated through a graded series of ethanol solutions. Hematoxylin-eosin staining and periodic acid-Schiff staining were performed. Slides were dehydrated, cleared, and mounted under a coverslip. Eosinophils were counted under a microscope ($\times 400$ magnification) in 4 fields of the nasal septal mucosa by a masked pathologist. Eosinophils were defined morphologically by the existence of eosinophilic granules in the cytoplasm and the presence of a 2-lobed nucleus.

Lung and nasal lavage fluid

After the mice were killed, a 24-gauge catheter was inserted into the nasopharynx through the tracheal opening. Nasal lavage fluid (NLF) was obtained from the upper airway with 0.5 mL of PBS. In the lower airway, the lung was lavaged with 0.5 mL of PBS. The lavage fluid was centrifuged and supernatants were frozen at -70°C until analysis. Histamine, cysteinyl leukotriene, IL-5, IFN- γ , TNF- α , and IL-1 β concentrations were measured in lung and NLF using ELISA kits. The sensitivity of the histamine enzyme immunoassay kit is 0.5 nM. The sensitivity of the cysteinyl leukotriene immunoassay kit is 26.6 pg/mL. DuoSet ELISA kits for IL-5, IFN- γ , TNF- α , IL-1 β and IL-17 were purchased from R&D Systems.

Splenocyte culture

Spleens were removed aseptically 24 hours after the final intranasal challenge. Dispersed splenocytes were plated in 96-well plates at a final concentration of 5×10^6 cells per well in RPMI-1640 containing 10% fetal bovine serum (FBS) supplemented with penicillin-streptomycin. The cells were stimulated with ovalbumin for 72 hours. The supernatants were collected and stored at -70°C until cytokine measurement using ELISA kits.

Mouse bone marrow-derived mast cell culture and degranulation assay

Bone marrow was harvested from the femurs of BALB/c mice. Bone marrow-derived mast cells (BMMCs) were obtained by culturing in Iscove modified Dulbecco medium (IMDM) supplemented

Table 1
Sequence of primers for reverse transcription–polymerase chain reaction

Primer	Sequence	Size, bp
IL-4	5'-CAACCCCGAGCTAGTTGTCA-3' 5'-AATCCCGAAAGAGTCTCTGC-3'	302
IL-13	5'-CAGCTCCTGGTCTCTCAC-3' 5'-CCACACTCCATACCATGCTG-3'	212
TNF- α	5'-ACACCGTCAGCCGATTGTC-3' 5'-CCCTGAGCCATAATCCCTT-3'	353
IL-1 β	5'-ATAACCTGCTGGTGTGTGAC-3' 5'-TGCAGACTCAAACTCCATT-3'	365
GAPDH	5'-ACCACAGTCCATGCCATCAC-3' 5'-TCCACCACCTGTGTCTGTA-3'	451

Abbreviations: IL, interleukin; TNF- α , tumor necrosis factor α .

with 10% FBS, 50 μ M β -mercaptoethanol, 2mM glutamine, 0.5 μ g/mL of amphotericin B, 100 U/mL of penicillin, 100 μ g/mL of streptomycin, and 10% WEHI-3–conditioned medium as the source of IL-3. After 4 to 5 weeks of culture, more than 95% of the nonadherent cells contained granules, as assessed by toluidine blue staining or Wright–Giemsa staining. The cells (5×10^6) were stimulated with ovalbumin for 1, 3, 6, or 24 hours, and the supernatants were assayed for TNF- α . The BMMCs were stimulated with ovalbumin at 37°C for 1 hour as control. Degranulation of mast cells was assayed using an assay kit (Chemicon) according to the manufacturer's instructions. Briefly, BMMCs (5×10^6) were treated with A23187 (a calcium ionophore, 100nM) at 37°C for 1 hour. Tryptase samples were collected by centrifugation at 700g, and the assay mixture was prepared in a 96-well microtiter plate or standard microcentrifuge tubes. The colorimetric reaction was initiated by adding the tryptase substrate, and the optical density was measured in a microplate reader at 405 nm.

Raw264.7 cell culture

Raw264.7 cells (a mouse macrophage cell line) were maintained in Dulbecco modified Eagle medium supplemented with 10% FBS, penicillin (100 U/mL), and streptomycin (100 μ g/mL) in a humidified atmosphere of 5% carbon dioxide at 37°C. The day before each experiment, cells were seeded into 6-well tissue culture plates at a density of 5 to 6×10^5 cells. After 24 hours, the cells were incubated with 50 ng/mL of rIL-17A in triplicate for 24 hours. Cells were harvested and assayed for TNF- α and IL-1 β by reverse transcription–polymerase chain reaction (RT-PCR).

Secretion assay for IL-17A

Raw 264.7 cells (2×10^6 per well) were seeded in the bottom plate with Dulbecco modified Eagle medium, and splenocytes were added onto the upper plate of the Transwell-24 system (Corning, Lowell, Massachusetts), which has 5- μ m pores. The splenocytes

were treated with anti-IL-17A monoclonal antibody (5 μ g/mL) in the presence of ovalbumin. Raw 264.7 cells were harvested after cultivating for 24 hours and assayed for TNF- α and IL-1 β .

RT-PCR analysis

Cells were washed in ice-cold PBS and lysed in Trizol Reagent (Invitrogen, Carlsbad, California). Three micrograms of prepared RNA was reverse transcribed with SuperScriptII reverse transcriptase (Invitrogen). Ten percent of the reverse-transcribed complementary DNA was amplified by PCR. The sequences of the specific PCR primers used to amplify IL-4, IL-13, TNF- α , IL-1 β , and GAPDH are given in Table 1. PCR cycling conditions were as follows: 94°C and either 56°C (IL-1 β) or 58°C (IL-4, IL-13, TNF- α , and GAPDH), followed by extension at 72°C (30 cycles for IL-4, IL-13, TNF- α , and IL-1 β ; 25 cycles for GAPDH). The PCR products were separated on 1.2% agarose gels and stained with ethidium bromide. Band intensity of PCR products was analyzed by densitometry using the Image J (<http://rsbweb.nih.gov/ij/>) program.

Statistical analysis

Results are expressed as mean (SD). Statistical analysis was performed using SPSS 12.0 software (SPSS Inc, Chicago, Illinois) and the Mann-Whitney *U* test. *P* < .05 was considered significant.

Results

IL-17A Levels in a Murine AR Model

The murine AR model was induced by sensitization and intranasal challenge with ovalbumin, in compliance with the approved experimental protocol. Control mice received PBS on the same schedule. IL-17A levels were measured by ELISA. In AR mice, IL-17A secretion increased significantly in spleen cells, lung lavage fluid, and NLF compared with control mice (Fig 1).

Nasal symptoms and released mediators

To further investigate the effect of IL-17A on the inflammation of the nasal mucosa, IL-17A-deficient mice were used in the ovalbumin-induced AR model. The frequency of nose rubbing and sneezing decreased in IL-17A-deficient mice (ovalbumin KO mice) compared with that of WT mice (ovalbumin WT mice; Fig 2A and B). Histamine and leukotriene secretion in NLF collected from ovalbumin KO mice decreased compared with ovalbumin WT mice (Fig 2C and D).

Histologic findings and eosinophils in nasal mucosa

As shown in Figure 2E, ovalbumin WT mice recruited inflammatory cells composed of eosinophils in nasal mucosa. However, IL-17A deficiency resulted in decreased eosinophil infiltration after ovalbumin challenge. The number of eosinophils in ovalbumin KO

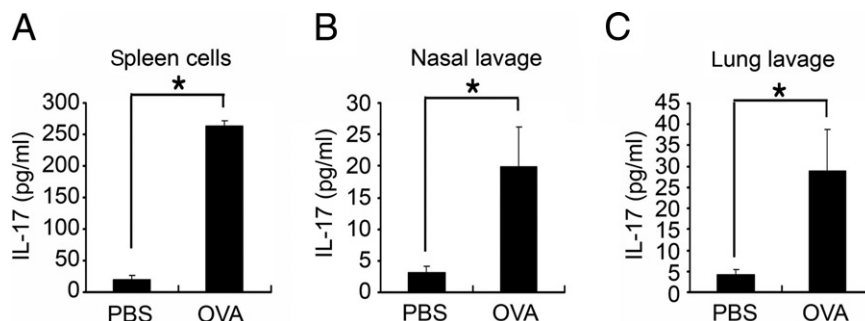


Fig. 1. Interleukin (IL) 17A levels in ovalbumin (OVA)–sensitized and –challenged mice. Spleen cells from allergic rhinitis (AR) or control mice were cultured with OVA, and culture supernatants were harvested at 72 hours. Enzyme-linked immunosorbent assay was used to quantify IL-17A production in spleen cell supernatant (A), nasal lavage fluid (B), and lung lavage fluid (C). IL-17A levels increased significantly in AR mice (OVA sensitization) compared with controls (phosphate-buffered saline [PBS] treatment). Data were expressed as the mean (SD) of 3 independent experiments for each group (*n* = 5; **P* < .05 vs the control).

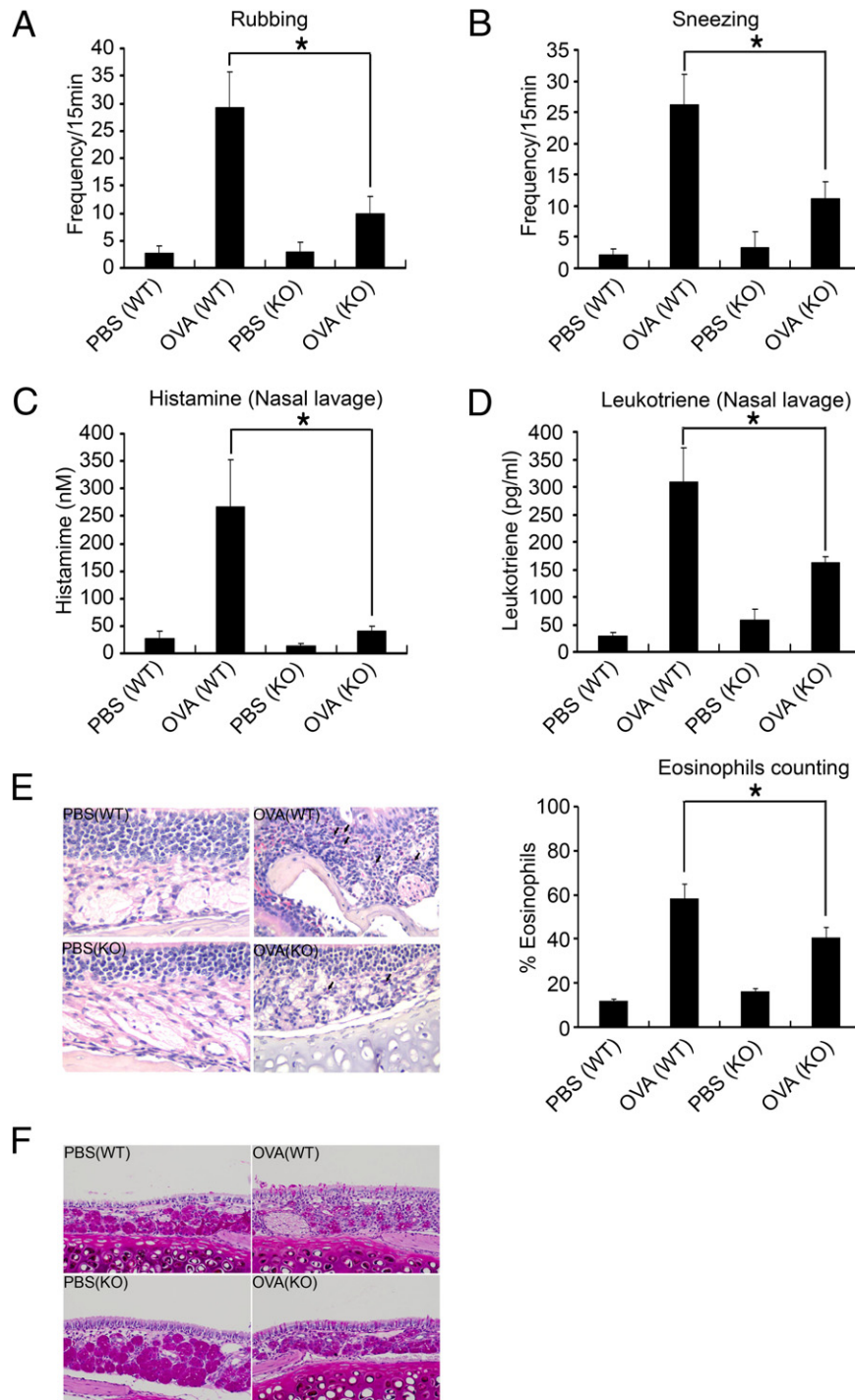


Fig. 2. Nasal symptoms and released mediators from nasal lavage fluid (NLF) in interleukin (IL) 17A-deficient mice. Wild-type (WT) BALB/c mice and IL-17A-deficient mice (knockout [KO]; BALB/c background) were sensitized and challenged with ovalbumin (OVA) or phosphate-buffered saline (PBS) (A, B). The levels of histamine (C) and total cysteinyl leukotriene (D) in NLF collected from IL-17A-deficient mice (OVA KO mice) were lower than in NLF collected from WT OVA mice. Data are expressed as the mean (SD) of 3 independent experiments for each group ($n = 5$; $*P < .05$ vs the OVA WT group). Nasal tissues were stained with hematoxylin-eosin, and the number of eosinophils that had infiltrated into the nasal septum was counted (E). The mean number of eosinophils decreased significantly in OVA KO mice compared with ovalbumin WT mice ($*P < .05$). Data are expressed as mean (SD) ($n = 5$). In contrast, periodic acid-Schiff staining showed no difference of mucous-producing goblet cells between OVA WT and OVA KO mice (F).

mice was significantly less than that in that in ovalbumin WT mice. In contrast, periodic acid-Schiff staining showed no difference of mucous-producing goblet cells (Fig 2F).

Serum total IgE and ovalbumin specific immunoglobulins

The level of total IgE in ovalbumin KO mice decreased significantly compared with ovalbumin WT mice (Fig 3A). Furthermore,

the levels of ovalbumin specific IgE, IgG2a, IgG1, and IgM decreased significantly in ovalbumin KO mice compared with the ovalbumin WT mice ($P < .05$; Fig 3B-E).

Antigen-induced T_H2 cytokine production in nasal lavage

We next investigated whether IL-17A is involved in the cellular response in the late phase of AR. To assess the effect of

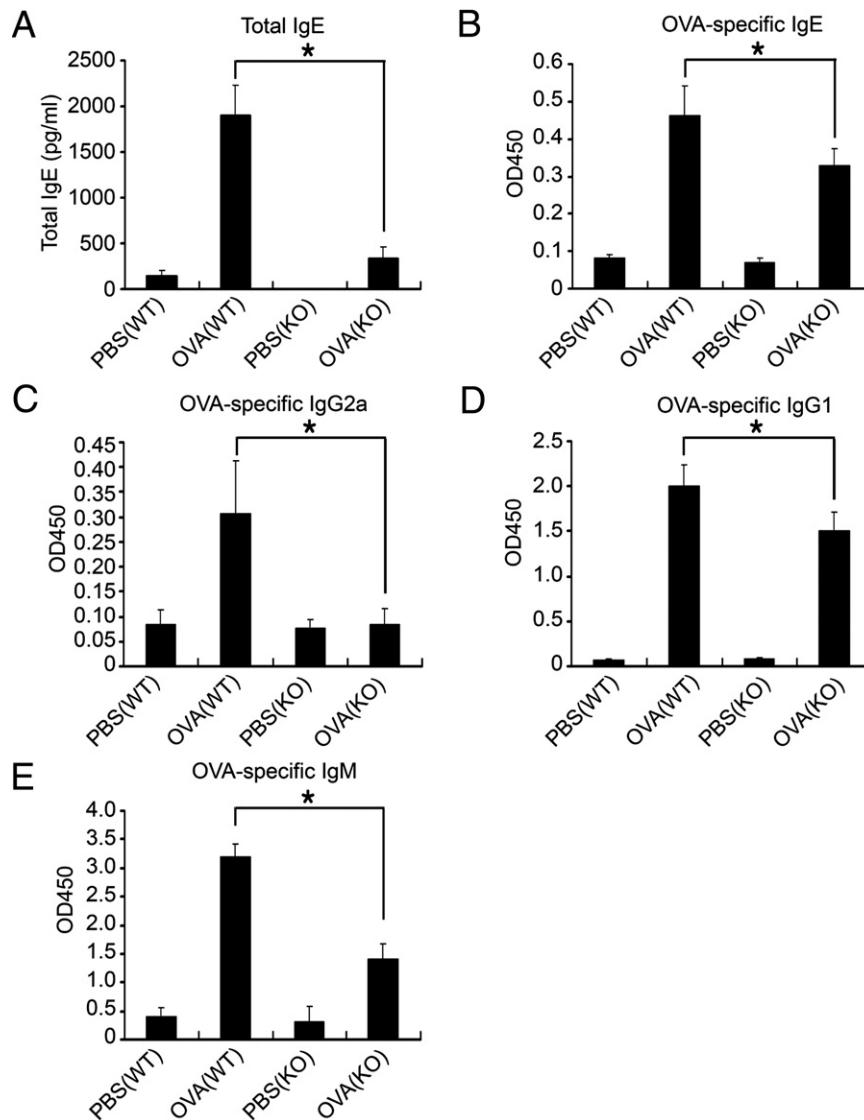


Fig. 3. Serum immunoglobulin levels in interleukin (IL) 17A-deficient mice. The levels of serum total IgE (A), ovalbumin (OVA) specific IgE (B), OVA specific IgG2a (C), OVA specific IgG1 (D), and OVA specific IgM (E) decreased in OVA knockout (KO) mice compared with OVA wild-type (WT) mice. Data are expressed as means (SD) (* $P < .05$ vs the OVA WT group). PBS indicates phosphate-buffered saline; OD, optical density.

IL-17A deficiency on the production of T-helper cytokines and inflammatory cytokines, cytokine levels were measured by ELISA. IL-17A deficiency reduced ovalbumin-induced splenic IL-4, IL-5, TNF- α , and IL-1 β compared with ovalbumin WT mice. However, ovalbumin specific IL-13 production from splenocytes was not reduced in IL-17A KO mice (Fig 4A). On average, mean IL-5, TNF- α , and IL-1 β levels in NLF collected from ovalbumin KO mice decreased by more than 50%, whereas NLF IFN- γ levels were unchanged (Fig 4B). Unfortunately, IL-4 and IL-13 were not detected in NLF. Alternatively, we examined expression of IL-4 and IL-13 mRNA in nasal tissue. The expression of IL-4 was decreased in IL-17A KO mice, but IL-17A deficiency did not affect the mRNA expression of IL-13 (Fig 4C).

Effect of IL-17A on mast cell degranulation

In allergic reactions, mast cells release characteristic granules and various mediators, such as histamine and cytokines. These chemical mediators cause the symptoms of AR in the nose. In the present study, BMMCs were cultured as described, and nonadherent cells were stained with toluidine blue (Fig 5A) or Wright-Giemsa (Fig 5B). More than 95% of the cells were identifiable as

mast cells. To examine the effect of IL-17A deficiency on mast cells, we measured TNF- α levels and mast cell degranulation in ovalbumin-stimulated BMMCs. TNF- α expression increased in ovalbumin-stimulated BMMCs. After 24 hours of ovalbumin stimulation, TNF- α levels increased in both BMMCs from WT AR mice and IL-17A KO mice (Fig 5C). However, the concentration of TNF- α in BMMCs from the ovalbumin KO group decreased significantly compared with that of the ovalbumin WT group ($P < .05$). As shown in Figure 5D, the percentage of degranulated mast cells was significantly lower in the ovalbumin KO group than in the ovalbumin WT group, indicating that IL-17A significantly enhanced the degranulation of mast cells.

Effect of IL-17A on the expression of inflammatory cytokines in macrophages

To examine the effect of IL-17A on inflammatory cytokines, we investigated IL-17A-induced mRNA expression of TNF- α and IL-1 β in macrophages. Raw264.7 cells were cultured and stimulated with rIL-17A. The expression of TNF- α and IL-1 β was increased in macrophages after IL-17A stimulation (Fig 6A).

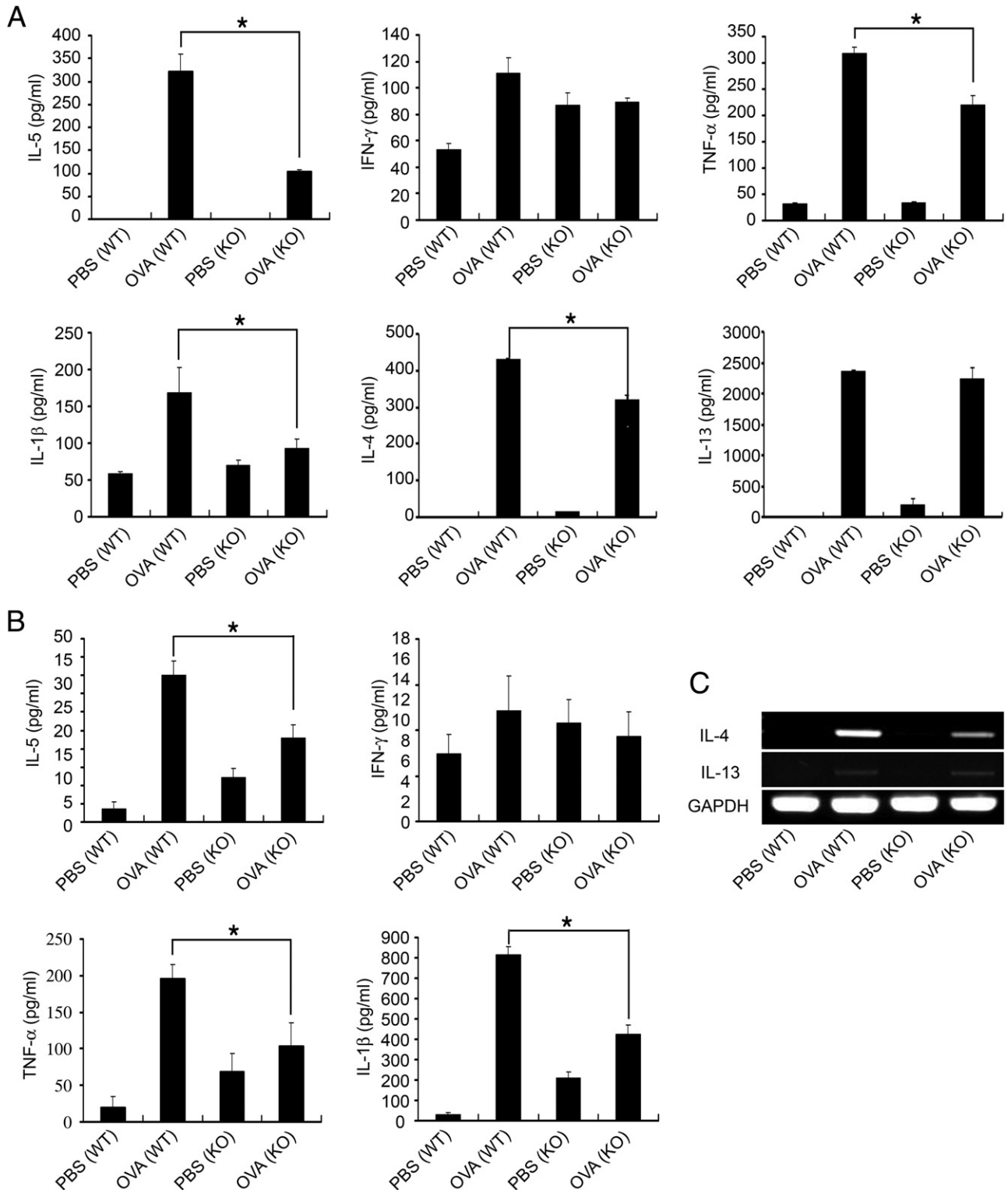


Fig. 4. Cytokine production in interleukin (IL) 17A-deficient mice. Spleen cells were cultured and stimulated with ovalbumin (OVA) for 72 hours, after which cell supernatants were prepared. (A) IL-5, interferon γ (IFN- γ), tumor necrosis α (TNF- α), IL-1 β , IL-4, and IL-13 levels were analyzed by enzyme-linked immunosorbent assay (ELISA). IL-17A deficiency reduced the secretion of IL-5, IL-4, TNF- α , and IL-1 β but not IFN- γ and IL-13. One day after the final intranasal challenge, nasal lavage fluid was collected and (B) IL-5, IFN- γ , TNF- α , and IL-1 β levels were analyzed by ELISA. IL-17A deficiency reduced the secretion of IL-5, TNF- α , and IL-1 β but not IFN- γ . Data are expressed as mean (SD) (* P < .05 vs the OVA wild-type [WT] group). (C) The expression of IL-4 and IL-13 messenger RNA in nasal tissue. KO indicates knockout.

We observed that activated T cells released IL-17A after direct stimulation by ovalbumin (Fig 6B). To confirm that IL-17A secreted from activated T cells modifies the production of inflammatory cytokines in macrophages, we established a macrophage/T-cell Transwell culture system. In Transwell experiments, ovalbumin

induced the secretion of IL-17A, which is thought to be secreted from ovalbumin-specific T cells. The addition of anti-IL-17A monoclonal antibody decreased the mRNA expression of TNF- α and IL-1 β (Fig 6C). IL-17A antibody also significantly reduced the protein level of TNF- α (Fig 6D) and IL-1 β (Fig 6E). These results indicate that

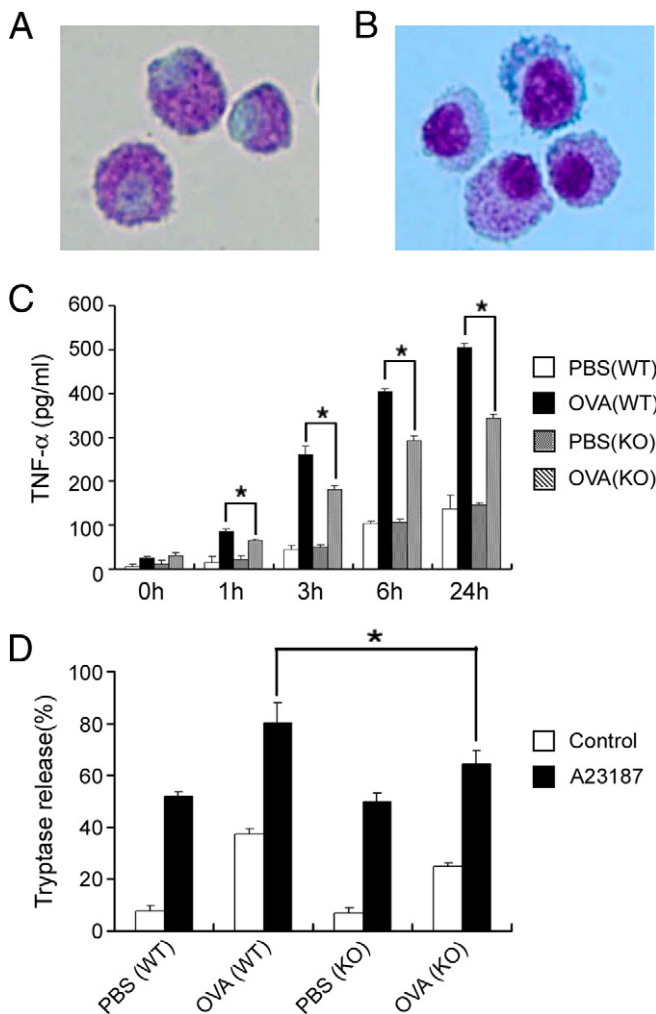


Fig. 5. Effect of interleukin (IL) 17A deficiency on bone marrow-derived mast cells (BMMCs). BMMCs were cultured in Iscove modified Dulbecco medium (IMDM) in the presence of WEHI-3–conditioned medium as a source of IL-3 for 4 to 5 weeks. Nonadherent cells were stained with toluidine blue (A) or Wright-Giemsa (B). BMMCs (5×10^6 per well) were stimulated with ovalbumin (OVA) for 1, 3, 6, or 24 hours. Tumor necrosis factor α (TNF- α) was assayed by enzyme-linked immunosorbent assay (C). BMMCs were cultured at 37°C with OVA for 1 hour as control in IMDM containing WEHI-3–conditioned medium. A mast cell degranulation assay was performed (D). The percentage of tryptase release was calculated as follows: $100 \times \text{supernatant activity} / (\text{supernatant activity} + \text{cell lysate activity})$. Data are expressed as mean (SD) (* $P < .05$ vs the OVA wild-type [WT] group) of 3 independent experiments. KO indicates knockout; PBS, phosphate-buffered saline.

activated T cells release IL-17A, which in turn promotes the release of inflammatory cytokines, including TNF- α and IL-1 β , from macrophages.

Discussion

To determine whether IL-17A is involved in the pathogenesis of AR, we investigated the effects of IL-17A deficiency on AR using IL-17A–deficient mice. The frequency of nasal rubbing and sneezing decreased significantly in IL-17A–deficient mice. Moreover, the levels of inflammatory mediators, such as histamine and leukotriene, decreased in NLF collected from IL-17A–deficient mice. In addition, IL-17A–deficient mice showed decreased numbers of eosinophils and a decrease in the ovalbumin-induced production of IL-5 and ovalbumin specific IgE compared with control mice. Thus, our experiments suggest that IL-17A deficiency plays a significant

role in the development of AR by reducing the levels of cytokines involved in the T_H2 immune response. Unexpectedly, we observed that basal IFN- γ levels were high in KO mice and not affected by ovalbumin provocation. It might be explained that IL-17A KO mice are T_H1 biased and ovalbumin induction did not affect IFN- γ level. However, further study would be needed to elucidate the exact mechanism.

Recently, IL-13 was shown to negatively regulate the secretion of IL-17A in an IL-10–dependent pathway.¹⁷ Moreover, IL-13 is a crucial mediator in the pathogenesis of allergic asthma by inducing airway hyperreactivity and mucous production.^{18,19} However, IL-17A deficiency did not decrease the levels of IL-13 from splenocyte and the mRNA expression of IL-13 from nasal tissue in our study. In addition, periodic acid–Schiff staining revealed no difference of mucous-producing goblet cells between ovalbumin WT and ovalbumin KO mice. Taken together, IL-17A may attenuate the allergic response in the IL-13–independent pathway.

Mast cells are important mediators of AR. These cells release potent inflammatory agents (eg, histamine) and cytokines (eg, TNF- α) that act on various cell types, including inflammatory cells. As a proinflammatory cytokine, TNF- α plays an essential role in the development of AR in mice by inducing antigen specific IgE and T_H2 cytokines.²⁰ In our data, IL-17A deficiency decreased the release of TNF- α from mast cells, suggesting that IL-17A may contribute to AR in mice via TNF- α –dependent mechanisms.

It has been reported that, in contrast to T_H1 and T_H2 cells, T_H17 cells mediate tissue inflammation by supporting neutrophil recruitment and survival, matrix degradation, and the induction of proinflammatory cytokines in structural cells. Similarly, IL-17A deficiency in mice led to a significant reduction in proinflammatory cytokines (TNF- α and IL-1 β) in NLF and spleen cell supernatants in our experiments. These results provide further evidence that IL-17A may play an important role in nasal allergic inflammation through decrease of not only T_H2 immune response but also general inflammation. The inflammatory response is initiated by macrophages or dendritic cells that recognize pathogens, become activated, and produce cytokines and chemokines.²¹ Proinflammatory cytokines, such as TNF- α and IL-1 β , play a crucial role in the induction of other cytokines, chemokines, and adhesion molecules that are important in various inflammatory responses. IL-17A has been shown to induce TNF- α and IL-1 β in macrophages.^{5,8,22} Recently, it has been reported that activated mast cells release mediators to stimulate alveolar macrophages to produce IL-17 in the ovalbumin-induced allergic asthma.²³ In our study, a specific antigen induced the production of IL-17A in spleen cells harvested from ovalbumin-sensitized mice. Furthermore, IL-17A induced the expression of TNF- α and IL-1 β mRNA in macrophages. To determine whether ovalbumin-induced IL-17A production stimulated the production of proinflammatory cytokines in macrophages, we established an in vitro Transwell culture system using ovalbumin-sensitized spleen cells and macrophages. The results showed that the secretion of IL-17A from spleen cells induced the expression of proinflammatory cytokine mRNA in macrophages. These results may explain the mechanism by which IL-17A can regulate inflammation in AR.

In conclusion, we demonstrated that IL-17A can upregulate the allergic response in an experimentally induced murine model of AR. To the best of our knowledge, this is the first report examining the role of IL-17A in an experimental AR animal model. The secretion of the T_H17 cytokine IL-17A was elevated in this model, and allergic responses were attenuated in IL-17A–deficient mice through the inhibition of mast cell degranulation and the modulation of T_H2 cytokine expression. In addition, proinflammatory cytokines, including TNF- α and IL-1 β , were decreased in IL-17A–deficient mice during the development of

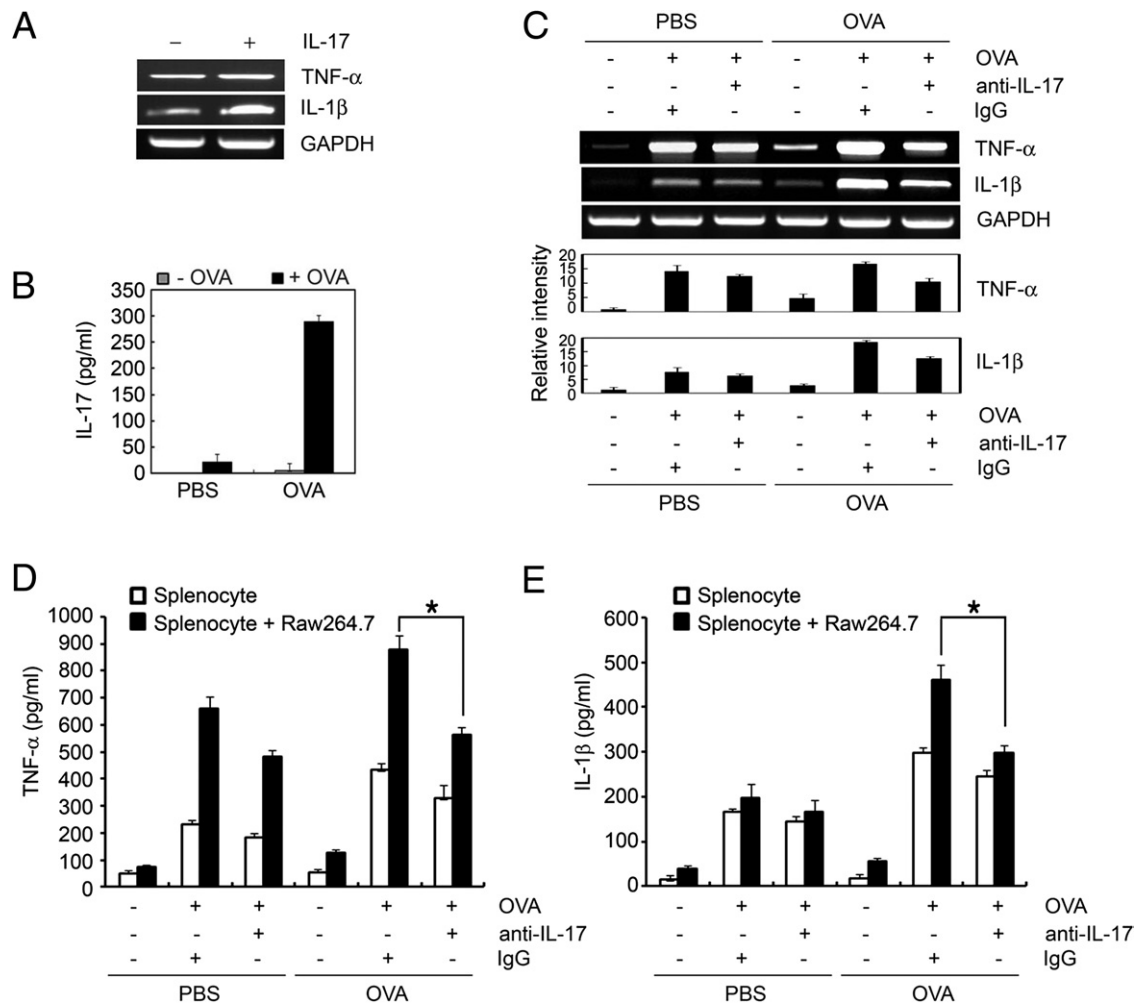


Fig. 6. Interleukin (IL) 17A regulates inflammatory cytokines induced by ovalbumin (OVA). (A) Raw264.7 cells were treated with IL-17A (50 ng/mL). After incubation for 24 hours, the messenger RNA (mRNA) levels of tumor necrosis factor α (TNF- α) and IL-1 β were examined by reverse transcription-polymerase chain reaction (RT-PCR). (B) OVA induced the secretion of IL-17A in spleen cells from OVA-sensitized mice. Spleen cells purified from OVA-sensitized or phosphate-buffered saline (PBS)-sensitized mice were added onto upper plate of the Transwell, and Raw264.7 macrophages were seeded in the bottom plate. Secretion of IL-17A by the spleen cells was detected by enzyme-linked immunosorbent assay. (C) Cells were treated with anti-IL-17A monoclonal antibody (5 μ g/mL) in the presence of OVA, and the mRNA expression of TNF- α and IL-1 β in Raw264.7 cells was quantified by RT-PCR. Bar graphs show band intensity of PCR products. (D) The protein level of TNF- α . (E) The protein level of IL-1 β . Data are expressed as mean (SD) (* P < .05) of 3 independent experiments.

AR. This study also suggests that IL-17A may regulate AR via the activation of proinflammatory cytokines and modulation of the T_H2 cytokine.

Acknowledgments

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References

- [1] Baena-Cagnani CE, Passalacqua G, Gomez M, Zernotti ME, Canonica GW. New perspectives in the treatment of allergic rhinitis and asthma in children. *Curr Opin Allergy Clin Immunol*. 2007;7:201–206.
- [2] Wilson DR, Merrett TG, Varga EM, et al. Increases in allergen-specific IgE in BAL after segmental allergen challenge in atopic asthmatics. *Am J Respir Crit Care Med*. 2002;165:22–26.
- [3] Baraniuk JN. Pathogenesis of allergic rhinitis. *J Allergy Clin Immunol*. 1997;99: S763–S772.
- [4] Seth D, Secord E, Kamat D. Allergic rhinitis. *Clin Pediatr (Phila)*. 2007;46:401–407.
- [5] Albanesi C, Cavani A, Girolomoni G. IL-17 is produced by nickel-specific T lymphocytes and regulates ICAM-1 expression and chemokine production in human keratinocytes: synergistic or antagonist effects with IFN- γ and TNF- α . *J Immunol*. 1999;162:494–502.
- [6] Artis D, Humphreys NE, Bancroft AJ, Rothwell NJ, Potten CS, Grencis RK. Tumor necrosis factor alpha is a critical component of interleukin 13-mediated protective T helper cell type 2 responses during helminth infection. *J Exp Med*. 1999;190:953–962.
- [7] Thomas PS. Tumour necrosis factor-alpha: the role of this multifunctional cytokine in asthma. *Immunol Cell Biol*. 2001;79:132–140.
- [8] Jovanovic DV, Di Battista JA, Martel-Pelletier J, et al. IL-17 stimulates the production and expression of proinflammatory cytokines, IL-beta and TNF-alpha, by human macrophages. *J Immunol*. 1998;160:3513–3521.
- [9] Sergejeva S, Ivanov S, Lotvall J, Linden A. Interleukin-17 as a recruitment and survival factor for airway macrophages in allergic airway inflammation. *Am J Respir Cell Mol Biol*. 2005;33:248–253.
- [10] Toda M, Leung DY, Molet S, et al. Polarized in vivo expression of IL-11 and IL-17 between acute and chronic skin lesions. *J Allergy Clin Immunol*. 2003;111:875–881.
- [11] Park H, Li Z, Yang XO, et al. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat Immunol*. 2005;6:1133–1141.
- [12] Nakae S, Komiyama Y, Nambu A, et al. Antigen-specific T cell sensitization is impaired in IL-17-deficient mice, causing suppression of allergic cellular and humoral responses. *Immunity*. 2002;17:375–387.
- [13] Schmidt-Weber CB, Akdis M, Akdis CA. TH17 cells in the big picture of immunology. *J Allergy Clin Immunol*. 2007;120:247–254.
- [14] Bullens DM, Truyen E, Coteur L, et al. IL-17 mRNA in sputum of asthmatic patients: linking T cell driven inflammation and granulocytic influx? *Respir Res*. 2006;7:135.

- [15] Louten J, Boniface K, de Waal Malefyt R. Development and function of TH17 cells in health and disease. *J Allergy Clin Immunol*. 2009;123:1004–1011.
- [16] Rhee CS, Libet L, Chisholm D, et al. Allergen-independent immunostimulatory sequence oligodeoxynucleotide therapy attenuates experimental allergic rhinitis. *Immunology*. 2004;113:106–113.
- [17] Newcomb DC, Boswell MG, Huckabee MM, et al. IL-13 regulates Th17 secretion of IL-17A in an IL-10-dependent manner. *J Immunol*. 2012;188:1027–1035.
- [18] Grunig G, Warnock M, Wakil AE, et al. Requirement for IL-13 independently of IL-4 in experimental asthma. *Science*. 1998;282:2261–2263.
- [19] Wills-Karp M, Luyimbazi J, Xu X, et al. Interleukin-13: central mediator of allergic asthma. *Science*. 1998;282:2258–2261.
- [20] Iwasaki M, Saito K, Takemura M, et al. TNF- α contributes to the development of allergic rhinitis in mice. *J Allergy Clin Immunol*. 2003;112:134–140.
- [21] Kaisho T, Akira S. Critical roles of Toll-like receptors in host defense. *Crit Rev Immunol*. 2000;20:393–405.
- [22] Albanesi C, Scarponi C, Cavani A, Federici M, Nasorri F, Girolomoni G. Interleukin-17 is produced by both Th1 and Th2 lymphocytes, and modulates interferon- γ - and interleukin-4-induced activation of human keratinocytes. *J Invest Dermatol*. 2000;115:81–87.
- [23] Song C, Luo L, Lei Z, et al. IL-17-producing alveolar macrophages mediate allergic lung inflammation related to asthma. *J Immunol*. 2008;181:6117–6124.