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의학과 면역학과정

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CCL7, CCR3 and IL-17 in a murine model of allergic rhinitis

by
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ABSTRACT

CCL7, CCR3 and IL-17 in a murine model of allergic rhinitis

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Background: The proinflammatory cytokine IL-17, produced by Th17 cells, plays a critical role in neutrophilic airway inflammation. Recent reports suggest that IL-17 is associated with eosinophil infiltration into nasal mucosa in a murine model of allergic rhinitis. The chemokine receptor CCR3, which is found on the surface of eosinophils, is involved in allergic diseases such as asthma, atopic dermatitis and allergic rhinitis. CCL7, a ligand of CCR3, induces chemotaxis of monocytes, eosinophils, basophils, NK cells and T lymphocytes.

Objectives: In this study we investigated whether IL-17 deficiency suppresses allergic inflammation in a murine allergic rhinitis model. We also determined the relationship between IL-17 and the CCL7/CCR3 pathway of eosinophil infiltration.

Methods: IL-17A-deficient and wild-type (WT) BALB/c mice were sensitized and challenged with ovalbumin to induce allergic rhinitis. Parameters of allergic responses including the nasal symptom score, serum immunoglobulin levels, and eosinophil infiltration and cytokine production in nasal mucosa were analyzed. The mRNA and protein levels of CCL7 and CCR3 in nasal tissue and serum in the two groups were compared. The chemotactic response to CCL7 in bone marrow-derived eosinophils (bmEos) from WT and IL-17 knockout (KO) mice was measured in the presence or absence of anti-IL-17 antibody.

Results: In the allergic rhinitis model, IL-17 deficiency can significantly decrease nasal symptoms, OVA-specific IgE in serum, and eosinophil infiltration and cytokine production in the nasal mucosa. The CCL7 concentration in nasal lavage fluid and serum of IL-17 KO mice was lower than that in the control group. Compared to WT mice, CCL7 mRNA level was suppressed and CCR3 mRNA and protein levels were decreased in the nasal mucosa of IL-17 KO mice. In the absence of IL-17 stimulation during differentiation, the bmEos from WT mice showed a decreased chemotactic response to

CCL7. The bmEos from IL-17 KO mice showed a significantly decreased chemotactic response to 500 ng/ml CCL7. In addition, among leukocytes only eosinophils showed decreased CCR3 expression in IL-17 KO-OVA mice.

Conclusions: Suppression of nasal inflammation by IL-17 deficiency in allergic rhinitis is partly responsible for the regulation of CCL7 secretion and eosinophil infiltration, which may be regulated by the CCL7/CCR3 pathway.

Key words: IL-17; CCR3; CCL7; eosinophil; allergic rhinitis

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LIST OF ABBREVIATION

OVA	Ovalbumin
IL-17	Interleukin 17
CCL7	Chemokine (C-C motif) ligand 7
CCR3	C-C chemokine receptor type 3
IL-17 KO-OVA	IL-17A deficient mice sensitized and challenged with OVA
WT-OVA	Wild type mice sensitized and challenged with OVA
bmEos	Bone marrow derived eosinophils

INTRODUCTION

Allergic rhinitis, the most commonly encountered chronic allergic disease, is dramatically increasing in prevalence in industrialized countries and is associated with important medical and socioeconomic problems (1). Allergic rhinitis is considered to be a typical Th2 cytokine-dominant disease that shows both influx of a large number of eosinophils and mast cells in the nasal mucosa and increased IgE production (2, 3).

Th17 cells, a subset of T helper cells, produce interleukin (IL)-17 and are developmentally distinct from Th1 and Th2 cells (4). Th17 cells are potent inducers of tissue inflammation and have been associated with the pathogenesis of many immune-mediated diseases, including psoriasis, rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease, and allergic disorders such as asthma (2, 5). IL-17 is a T cell-derived proinflammatory cytokine originally named cytotoxic T lymphocyte-associated serine esterase-8 (CTLA-8) (6). Members of the IL-17 family include IL-17A, IL-17B, IL-17C, IL-17D, IL-17E and IL-17F (7). IL-17 stimulates fibroblasts to produce IL-6, IL-8, IL-11, and growth related oncogene-α (GRO-α), which might contribute to the amplification, regulation, and/or perpetuation of local airway inflammation and remodeling (8). IL-17 may constitute a link between the activation of certain T-lymphocytes and mobilization of neutrophils in rat airways *in vivo* and in human bronchial epithelial cells *in vitro* (9). Sputum IL-17A and IL-8 mRNA levels are

significantly elevated in asthma patients, and Th17 cell infiltration in the asthmatic airway links T-cell activity with neutrophilic inflammation in asthma (10). However, few studies investigated the involvement of IL-17 in eosinophil infiltration at the site of inflammation. Th17 cells significantly enhance Th2-cell–mediated eosinophilic airway inflammation (11). Localization of IL-17A expression coincided predominantly with eosinophils and $CD4^+$ lymphocytes, but not with neutrophils, in the subepithelial layers. IL-17A also plays an important role in eosinophil accumulation in nasal polyps (12). The mechanism underlying Th17 cell-mediated enhancement of eosinophil recruitment into the airway is unknown.

Chemokine receptor CCR3 plays a well-known role in the development of allergic diseases such as asthma, atopic dermatitis, and allergic rhinitis (13). CCR3 is expressed on eosinophils, basophils, mast cells, neutrophils and endothelial cells. Among these, the highest level of CCR3 is found on eosinophils; it is the dominant cell-surface receptor with around 50,000 receptors per cell (14). CCR3 is responsible for the chemotactic responses of eosinophils to eotaxin, RANTES (regulated upon activation, normal T-cell expressed and secreted) and chemokine (C-C motif) ligand 7 (CCL7) (14).

CCL7, previously called monocyte chemotactic protein-3 (MCP-3), is a small cytokine known as a chemokine; the DNA sequence is that of MARC (mast cell activation-related chemokine)/FIC (fibroblast inducible cytokine) (15, 16). The CCL7 gene is located on chromosome 17q11.2-12, clustered with the other CC chemokines

including CCL2/MCP-1, CCL3/MIP-1α, CCL4/MIP-1β, CCL5/RANTES, and CCL11/eotaxin (17, 18). CCL7 protein is secreted by various cells, including monocytes, macrophages, mast cells, fibroblasts, endothelial cells and epithelial cells. CCL7 binds the main receptors CCR1, CCR2 and CCR3 on chemotactic monocytes, eosinophils, basophils, NK cells, T lymphocytes and neutrophils. CCL7 mRNA expression was shown to be significantly increased in the bronchial mucosa of both atopic and non-atopic asthmatic subjects. Moreover, secreted protein levels were significantly increased in the bronchoalveolar lavage fluid of asthmatic subjects and were associated with increased accumulation of bronchial mucosal eosinophils (19). Allergic rhinitis is associated with increased expression of CCL7/MCP-3 and CCL13/MCP-4, which may be closely related to the influx of inflammatory cells and may thus contribute to the pathogenesis of allergic rhinitis (20).

One study reported on the relationship between IL-17 and CCL7. In brain tissue, the expression of CCL7 was inhibited considerably after IL-17 blockade; IL-17 transgene overexpression caused inflammation in the lungs and the expression of CCL7 was significantly increased and IL-17-treated MLE12 cells (a mouse lung epithelial cell line) showed a similar gene expression profile (21). In addition, treatment of mouse embryonic fibroblasts (MEFs) with IL-17 induced the upregulation of CCL7 mRNA expression (21). CCL7 expression was significantly reduced in the central nervous system of IL-17 knockout (KO) compared with wild-type (WT) mice. Ovalbumin -treated IL-17 KO mice had fewer bronchoalveolar lavage fluid cells and eosinophils in a chronic asthma model

(21). Opposite results were observed in a dextran sodium sulfate (DSS)-induced colitis mouse model in which CCL7 mRNA expression was elevated in DSS-treated IL-17-deficient mice compared with DSS-treated WT mice (22). However, the relationship between CCL7 and IL-17 in allergic rhinitis was not investigated.

IL-17A is associated with inflammatory lung disorders by triggering an accumulation of neutrophils. Results from our previous study suggested that IL-17A contributes to the development of allergic eosinophilic inflammation in an allergic rhinitis model (23). However, the relationship between IL-17A and eosinophils in the upper airway remains uncertain.

The aim of this study was to investigate the regulation of eosinophil inflammation by IL-17A and to demonstrate the involvement of the CCR3/CCL7 pathway using an allergic rhinitis murine model.

MATERIALS AND METHODS

Reagents and antibodies

OVA (grade V) was obtained from Sigma (St Louis, MO), bovine serum albumin (BSA) was purchased from USB (Cleveland, OH) and aluminum hydroxide (Alum) was provided by Pierce (Rockford, IL). Anti-CCR3 antibody (clone Y31) was obtained from Abcam (Cambridge, UK), murine CCL7 protein and CCL7 enzyme-linked immunosorbent assay (ELISA) development kits were purchased from PeproTech (Rocky Hill, NJ) and the CCL7 instant ELISA kit was obtained from eBiosience (Vienna, Austria). Biotin-conjugated rat anti-mouse IgE (clone R35-72), IgG2a (clone R19-15), IgG1 (clone A85-1), purified mouse IgE standard (clone C38-2) and streptavidin HRP were purchased from BD Bioscience (San Jose, CA).

Animals

Four-week-old female BALB/c (WT) mice and IL-17A-deficient (KO) mice were used in all experiments. IL-17A-KO mice with a BALB/c background were purchased from Professor Yoichiro Iwakura (Center for Experimental Medicine, Institute of Medical Science University of Tokyo) (3). IL-17A-KO mice had been bred under specific-pathogen-free conditions. The BALB/c mice were obtained from Korea Orient Co. (Gyeonggi, Korea). Each mouse weighed 20–30 g and all animal experiments followed the principles for laboratory animal research, as outlined in the Animal Welfare Act and Department of Health, Education, and Welfare guidelines for the experimental use of

animals (National Institutes of Health), and was approved by our Institutional Animal Care and Use committee.

Experimental schedule

The mice were divided into four groups. [1] The negative control group (WT-PBS) was sensitized and challenged with phosphate-buffered saline (PBS), [2] the positive control group (WT-OVA) was sensitized and challenged with OVA, [3] the IL-17 KO-PBS group used IL-17A-KO mice for sensitization and challenge with PBS, and [4] the IL-17 KO-OVA group was sensitized and challenged with OVA using IL-17A deficient mice (Fig. 1). The allergen sensitization and intranasal challenge schedule is summarized in Figure 1. Briefly, WT-OVA and IL-17 KO-OVA groups were sensitized by intraperitoneal injection of 25-μg OVA mixed with 2-mg Alum on days 0, 7, and 14; intranasal challenge was carried out using 100-μg OVA on 7 consecutive days from day 21 to day 27. The WT-PBS and IL-17 KO-PBS groups were intraperitoneally injected and intranasally challenged with PBS on the same schedule.

Evaluation of nasal symptoms

On day 27, after the groups were administered a 100-μg OVA intranasal challenge, the frequencies of sneezing and nose-scratching were counted for 15 min to evaluate early allergic responses (evaluated by an investigator blind to the experimental condition of the mouse).

Histological analysis

The experimental mice were sacrificed 24 h after the final OVA challenge. The heads of mice were fixed in 10% formalin overnight at 4℃ with slow shaking, followed by decalcification and embedding in paraffin blocks. Paraffin-embedded tissue samples were cut into 4-μm-thick sections, deparaffinized in xylene, and dehydrated through a graded series of ethanol solutions.

Sirius red eosinophil staining

The sections were stained with Sirius red at room temperature for 2 h and rinsed with tap water for 10 min. The nuclei were stained with a progressive alum hematoxylin for 5 min and rinsed with tap water. After dehydration with increasing concentrations of ethanol, the sections were mounted. Eosinophils were counted under a microscope (400 × magnifications) in the submucosal area of the entire nasal septum by a pathologist blinded to the tissue section information.

Serum OVA-specific Ig levels

Serum samples were collected 24 h after the last nasal OVA challenge, and the amounts of OVA-specific IgE, IgG1 and IgG2a were determined by ELISA using 96-well plates. Plates were incubated overnight at 4℃ with 100 μg/ml OVA in coating buffer (0.05 M carbonate-bicarbonate), washed three times with washing buffer (PBS containing 0.05% Tween-20), blocked with 3% bovine serum albumin (BSA) in PBS for 1 h at room temperature (RT) and incubated with 50-μl of serum sample (16-fold dilution with 0.1%

BSA/PBS) for 2 h at RT. After washing, 100-μl of biotin-conjugated rat anti-mouse IgE monoclonal antibody was added to each well and incubated for 1 h at RT. The plates were then incubated with 100-μl streptavidin-horseradish peroxidase (HRP) (BD Pharmingen, San Jose, CA) for 30 min at RT. After washing, 100-μl of 3,3´,5,5´-tetramethylbenzidine (TMB) (KPL, Clopper road, Gaithersburg) substrate solution was added to each well and incubated for 30 min at room temperature; the reaction was then terminated by adding 1N HCl. Optical density (OD) at 450 nm was measured using a microplate reader. OVA-specific IgG1 and IgG2a levels were determined using similar methods.

Serum total IgE level

To measure IgE levels, 96-well plates were coated overnight with anti-mouse IgE capture monoclonal antibody (BD Pharmingen) at 4℃. The plate was washed three times with washing buffer (PBS containing 0.05% Tween-20) and blocked with 300-μl of 3% BSA per well for 1 h at room temperature. Serum samples (32-fold dilution with 0.1% BSA/PBS) and standard solution (BD Pharmingen) were added, and the plate was incubated for 3 h at 4℃. After washing three times, bound IgE was detected using rat anti-mouse IgE-HRP (Southern Biotech, Birmingham, AL) at a 3,000-fold dilution with 2% BSA. Optical density at 450 nm was measured using a microplate reader.

Measurement of cytokines in spleen cell culture

The spleens were removed aseptically 24 h after the final intranasal challenge. Dispersed single-cell suspensions were plated on 24-well tissue-culture plates at a final

concentration of 5×10^6 cells/well in RPMI-1640 containing 10% fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 μg/ml streptomycin. The cells were stimulated with OVA for 72 h. The supernatants were collected and stored at -70℃ until required. Cytokines (IL-4, IL-5, IFN-γ, IL-10) were assayed using ELISA kits (R&D systems, Minneapolis, MN).

Real time RT-PCR in the nasal mucosa

Total RNA was prepared from nasal mucosa with TriZol reagent (Invitrogen, Carlsbad, CA). Complementary DNA (cDNA) was synthesized using Superscript® reverse transcriptase (Invitrogen, Camarillo, CA) and oligo (dT) primers (Fermentas, Burlington, ON). To analyze IL-4 (Mm00445258_g1), IL-5 (Mm00439646_m1), IL-10 (Mm00439616_m1), IFN-γ (Mm99999071_m1), CCL7 (Mm00443113_m1), CCR3 (Mm01216172-m1) and GAPDH (Mm03302249_g1), primers and probe were purchased from Applied Biosystems (Foster City, CA). The reaction was performed using an ABI PRISM7000 sequence detection system (Applied Biosystems). The average gene transcript levels were then normalized to that of GAPDH. The reverse-transcribed cDNA was amplified by PCR for analysis of CCL11/eotaxin1, CCL24/eotaxin2, CCL26/eotaxin3, CCL5/RANTES, CCL8/MCP2, CCR1, CCR2 (24) and GAPDH. The sequences of the specific PCR primers are shown in Table 1. PCR cycling conditions were as follows: denaturation at 94℃ and annealing at 55℃ (CCL24/eotaxin2, CCL8/MCP2), 56℃ (GAPDH, CCR2, CCL26/eotaxin3), 57℃ (eotaxin1, CCR1), or 58℃ (RANTES), followed by extension at 72℃ (25 cycles for CCL8, CCL24, CCL26;

30 cycles for CCL5, CCL11). PCR products were separated on 1.5% agarose gels and stained with GelRed Nucleic Acid Stain (Koma Biotech, Seoul, Korea).

CCL7 secretion in nasal lavage fluid and serum

After sacrifice, a pipette tip was inserted into the nasopharynx through the tracheal opening. Nasal lavage fluid (NLF) was obtained from the upper airway by rinsing twice with 200 μl of PBS; supernatants were frozen at -70℃ until analysis. NLF and serum concentrations of CCL7 were measured using CCL7 Instant ELISA kits and CCL7 development ELISA kits.

Western blot for CCR3 in the nasal mucosa

Protein was obtained from the nasal mucosa using lysis buffer (0.5% Triton X-100, 150 mM NaCl, 15 mM Tris (pH 7.4) 1 mM $CaCl_2$, 1 mM $MgCl_2$). Protein concentrations were determined using BCA protein assay reagent (Thermo Fisher Scientific, Waltham, MA). Samples (50-μg protein per lane) were separated on 8% to 16% Tris–glycine mini gels (NOVEX, San Diego, CA, USA) and transferred onto PVDF membranes (Amersham Biosciences, Piscataway, NJ, USA). CCR3 and β-actin were immunoblotted with a primary rabbit monoclonal anti-CCR3 antibody and anti-β actin antibody (Cell signaling, Danvers, MA), respectively. The membrane was then immunoblotted with a secondary anti-rabbit IgG-HRP. The blots were visualized using SuperSignal West Pico Chemiluminescent Substrate (Pierce, Rockford. IL). Quantification of Western blots was performed using the TINA 2.0 software.

Bone marrow-derived eosinophils

Bone marrow-derived eosinophils (bmEos) were generated as described elsewhere (24). Briefly, bone marrow was flushed from the femurs and tibias of 4-week-old naïve BALB/c and IL-17 KO mice. Single-cell suspensions were cultured at 1×10^6 cells/ml in RPMI 1640 containing 20% FBS (GIBCO, Grand Island, N.Y.), 100 units/ml penicillin and 100 μg/ml streptomycin, 2 mM glutamine, 25 mM HEPES, 1 x MEM nonessential amino acids, 1 mM sodium pyruvate (Invitrogen, Grand Island, N.Y.), 50 μM 2-mercaptoethanol (Amresco, solon, Ohio), 100 ng/ml stem cell factor (SCF), and 100 ng/ml FLT3-ligand (FLT3-L) (Peprotech, Rocky Hill, NJ) from days 0 to 4. On day 4, the medium containing SCF and FLT3-L was replaced with medium containing 10 ng/ml recombinant mouse IL-5 (Peprotech) only. On day 8, the cells were moved to new flasks and maintained in fresh medium supplemented with recombinant murine IL-5. Every other day, from this point forward, one-half of the media was replaced with fresh media containing rmIL-5. The cells from BALB/c mice were cultured with or without anti-IL-17 mAb (5 μg/ml) for 12 days.

Flow cytometry analysis of bmEos expressing CCR3

The bmEos cells were divided into two groups. In the first group, cells from BALB/c mice cultured with or without anti-IL-17 mAb (5 μg/ml) were compared. In the second group, cells from BALB/c and IL-17 KO mice were compared. Cells were incubated with either PE-conjugated rat anti-mouse Siglec-f (BD Pharmingen) or APC-conjugated anti-mouse CCR3 (R&D systems, Minneapolis, MN) for 45 min at 4℃. After staining, the

cells were fixed in 1% paraformaldehyde and analyzed by flow cytometry. Data were acquired with a FACS Calibur flow cytometer (BD Biosciences) and analyzed using Cell Quest Pro ver. 5.2.1 (BD Biosciences). Siglec-f and CCR3-positive cells were identified by comparison with the PE-conjugated IgG2a, κ isotype and APC-conjugated IgG2a isotype controls.

Chemotaxis assay

The chemotaxis assay was performed in Transwell plates (Corning, Tewksbury, MA) with a 5.0-μm pore size polycarbonate membrane. Recombinant murine CCL7 (Peprotech) protein was diluted in medium containing recombinant murine; 600-μl of the medium containing various CCL7 concentrations IL-5 (0, 100 and 500 ng/ml) were placed in the lower wells and 100-μl of bmEos (5×10^6 cells/ml) were placed in the upper chambers. Cells were incubated at 37℃ for 3 hours to permit migration across the membrane in response to the chemotactic agent. After 3 h, the Transwells were removed, and the number of cells in the bottom chamber for 1 min was counted using a FACSCalibur flow cytometer and CellQuest software.

Flow cytometric assessment of blood and splenocytes expressing CCR3

BALB/c WT and IL-17 KO mice were sensitized and exposed to OVA. Whole blood was collected in tubes containing heparin. Spleen cells were mechanically disaggregated into a suspension of single cells, and 2×10^5 splenocytes were stained with FITC-anti-CD4 and APC-anti-CCR3. Additionally, 50-μl of packed whole blood was

stained with FITC-anti-CD4, PE-anti-siglec-f and APC-anti-CCR3 for 45 min at 4℃. For analysis of eosinophils in the blood, the cells were examined in terms of their expression of Siglec-F^+CCR3^+ cells gated on leukocytes, excluding lymphocytes. Data were acquired with a FACSCalibur flow cytometer and analyzed with Cell Quest Pro ver. 5.2.1.

Statistical analysis

The results are expressed as means ± SD. Statistical analysis was performed using the SPSS 18.0 software and the Mann-Whitney U-test was used for evaluation of differences in mean values. A value of $P < 0.05$ was considered to indicate statistical significance.

RESULTS

Symptom score

The frequencies of nasal sneezing and rubbing scores in the positive control group (WT-OVA) were 10.45 ± 8.46 and 7.05 ± 4.05, respectively (Fig. 2). The nasal sneezing and rubbing symptom scores in the IL-17 KO-OVA group were significantly reduced compared to those in the WT-OVA group [2.45 ± 2.14 (P = 0.0002) and 3.65 ± 2.37 (P = 0.0018), respectively].

Eosinophil infiltration in the nasal mucosa

Eosinophils in nasal mucosal tissue were detected by Sirius red staining. Heavy eosinophil infiltration was observed in the submucosa of the WT-OVA group. The mean number of eosinophils in the WT-OVA group was 160.00 ± 16.87, which was significantly different from that in the IL-17A-KO OVA group (96.00 ± 31.75, P = 0.012; Fig. 3).

Serum total IgE and ovalbumin-specific immunoglobulin

The total IgE level in the IL-17 KO-OVA group was significantly decreased compared to that in the WT-OVA group (652.34 ± 389.60 ng/ml *vs.* 1772.77 ± 1045.96 ng/ml, P < 0.001; Fig. 4A). The OVA-specific IgE level was significantly reduced in the IL-17 KO-OVA group compared to that in the WT-OVA group (0.51 ± 0.35 *vs.* 1.01 ± 0.37, P < 0.001; Fig. 4B). The OVA-specific IgG2a level in the IL-17 KO-OVA group was also significantly reduced compared to that in the WT-OVA group (0.65 ± 0.54 *vs.* 1.20 ± 1.05,

P = 0.04; Fig. 4D). In terms of the OVA-specific IgG1 levels, the IL-17 KO-OVA and WT-OVA groups were not significantly different (Fig. 4C).

Cytokine levels in spleen cell cultures

The levels of several cytokines in supernatants obtained from spleen cell cultures with or without OVA for 72 h were measured (Fig. 5). The levels of IL-4 and IL-10 production were significantly decreased in the IL-17 KO-OVA group compared to that in the WT-OVA group (IL-4: 322.07 ± 13.91 pg/ml *vs*. 430.94 ± 3.104 pg/ml, *P* = 0.008; IL-10: 1262.11 ± 68.19 *vs*. 1628.61 ± 26.23 *P* = 0.02). The level of IFN-γ production in the IL-17 KO OVA group showed a decreasing trend, which did not reach statistical significance, as compared with that in the WT-OVA group (606.75 ± 56.34 pg/ml *vs*. 707.06 ± 38.89 pg/ml, *P* = 0.174). The level of IL-5 production was significantly increased in the IL-17 KO OVA group compared to that in the WT-OVA group (1177.37 ± 9.86 pg/ml *vs*. 961.35 ± 21.51 pg/ml, *P* = 0.005).

Cytokine mRNA expression levels in nasal mucosa

The mRNA expression of IL-5 in the nasal mucosa was significantly decreased in the IL-17 KO-OVA group compared to that in the WT-OVA group**.** The expression levels of IL-4, IFN-γ and IL-10 mRNA were not significantly different between the IL-17 KO-OVA and WT-OVA groups. Interestingly, the baseline levels of IFN-γ and IL-10 mRNA expression were significantly increased in the IL-17 KO group (Fig. 6).

CCL7 and CCR3 mRNA expression in the nasal mucosa

We measured the mRNA expression of several chemokines in the nasal mucosa using RT-PCR. A variety of chemokines are involved in eosinophil chemotaxis, including eotaxin-1 (CCL11), eotaxin-2 (CCL24), eotaxin-3 (CCL26), CCL7 (MCP3), CCL8 (MCP2) and RANTES (CCL5). Of these, the level of CCL7 does not seem to increase with allergen challenge in the IL-17-deficient allergic rhinitis murine model. CCL7 signals through three different G-protein-coupled receptors: CCR1, CCR2 and CCR3. Of these chemokine receptors, the mRNA expression of only CCR3 was decreased in the IL-17 KO-OVA group (Fig. 7A and B). Real time-PCR gene expression quantification revealed that CCL7 (P = 0.008) and CCR3 (P = 0.011) mRNA levels were significantly reduced in the IL-17 KO-OVA group (Fig. 7C and D).

CCL7 protein secretion in nasal lavage and serum

CCL7 protein level in the nasal lavage fluid was significantly decreased in the IL-17 KO-OVA group (7.16 ± 3.31 pg/ml) compared to that in the WT-OVA group (14.60 ± 6.40 pg/ml; P = 0.004; Fig. 8A). The CCL7 serum protein level (103.77 ± 46.84 pg/ml) in IL-17-deficienct mice was significantly lower than that in the WT mice (171.66 ± 52.50 pg/ml) (P = 0.005; Fig. 8B). However, OVA challenge did not induce any significant difference in CCL7 production in either the WT or KO mice (Fig. 8B).

CCR3 protein expression in the nasal mucosa

The expression of CCR3 protein was measured in the nasal mucosa by immunoblotting. The CCR3 protein level decreased in the OVA-treated IL-17-deficient group (Fig. 9).

CCR3 expression on bmEos

No differences in CCR3 expression levels were found on bmEos cultured in the presence or absence of anti-IL-17 mAb (77.33% *vs*. 77.26%; Fig. 10A). Additionally, the expression levels of CCR3 on bmEos were similar in WT BALB/c and IL-17 KO mice (79.16% *vs*. 77.63%; Fig. 10B). These data indicate that IL-17 does not affect CCR3 expression during differentiation of eosinophils from bone marrow.

Chemotactic response of bmEos

The Transwell migration assay demonstrated that bmEos have chemotactic responses to CCL7 at concentrations of 100 and 500 ng/ml. When eosinophils were cultured in the presence of anti-IL-17 mAb (5 μg/ml), decreased eosinophil chemotactic responses to CCL7 were observed compared to those in the absence of anti-IL-17 mAb (CCL7 100 ng/ml: 130.8 ± 11.0% *vs*. 146.3 ± 3.7%; CCL7 500 ng/ml: 144.7 ± 10.9% *vs*. 165.4 ± 15.1%; Fig. 11A). We compared the eosinophil chemotactic response to CCL7 in WT and IL-17 KO mice (Fig. 11B). The CCL7-induced chemotaxis of eosinophils was significantly reduced in the IL-17 KO mice compared to that in the WT mice (CCL7 100

ng/ml: 131.0 ± 23.1% *vs*. 150.4 ± 8.8%, $P = 0.232$; CCL7 500 ng/ml: 134.2 ± 18.6% *vs*. 190.5 ± 25.0%, $P = 0.018$).

CCR3 expression on cells from the blood and spleen

We performed FACS analysis to detect CCR3 expression. Blood and spleen cells from the WT-OVA and IL-17 KO-OVA mice were stained for $CD4^+CCR3^+$ and siglec-f^+CCR3^+. The gating was set for lymphocytes in the experiments. No $CCR3^+$ cells were detected among the $CD4^+$ lymphocytes in both groups. In the other leukocyte-gating region of blood cells, CCR3 expression was lower in IL-17 KO-OVA mice than in WT-OVA mice (19.19% *vs*. 25.34%, respectively). Interestingly, only CCR3 expression on siglec-f^+ eosinophils was decreased in the IL-17 KO-OVA mice compared to the WT-OVA mice (16.46% *vs*. 22.38%, respectively; Figs. 12-14).

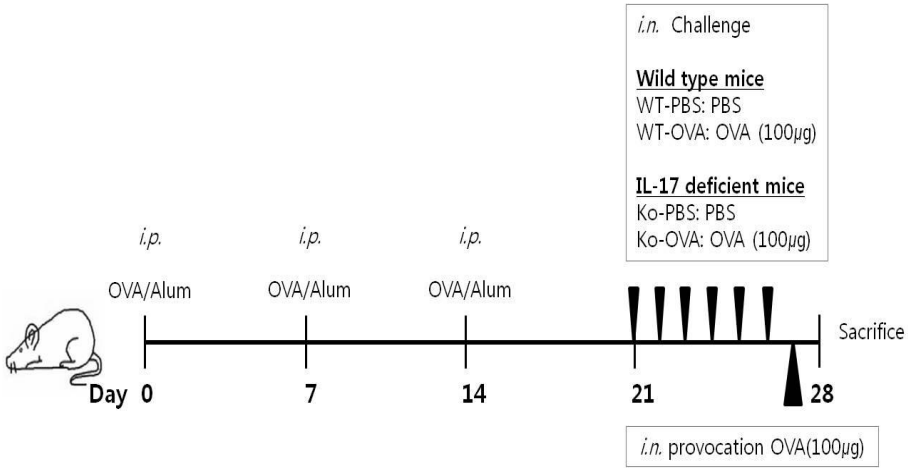


Figure 1. Experimental protocol. Mice were sensitized by intraperitoneal injection of ovalbumin mixed with aluminum hydroxide on days 0, 7, and 14. Intranasal challenge was performed by daily OVA intranasal challenge from day 21 to day 27. The wild-type (WT) PBS and interleukin (IL)-17A-deficient PBS groups were sensitized with phosphate-buffered saline (PBS) and then challenged with PBS instead of OVA. The mice were sacrificed 24 h after the final OVA challenge.

Table 1: Sequence of primers for RT-PCR

Primer	Sequence	Size
CCL11/ Eotaxin1	5'-TCC ACA GCG CTT CTA TTC CT-3'	327 bp
	5'-GCA GTT CTT AGG CTC TGG GTT-3'	
CCL24/ Eotaxin2	5'- TCA TCT TGC TGC ACG TCC TTT-3'	269 bp
	5'- TAA ACC TCG GTG CTA TTG CCA-3'	
CCL26/ Eotaxin3	5'- TTG TTC TCC TGG CCA TCT TC-3'	202 bp
	5'- GGC TGG ACA CAG AAT TGC TT-3'	
CCL8/ MCP-2	5'-ACG CTA GCC TTC ACT CCA AAA -3'	367 bp
	5'- TTC CAG CTT TGG CTG TCT CTT-3'	
CCL7/ MCP-3	5'- ATG GAA GTC TGT GCT GAA G-3'	322 bp
	5'- ACA TGA GGT CTC CAG AGC TTT-3'	
CCL5/ RANTES	5'-AGT CGA TCT CCC ACA GCC TCT-3'	337 bp
	5'- CAG GGT CAG AAT CAA GAA ACC -3'	
CCR1	5'-TTT AAG GCC CAG TGG GAG TT-3'	314 bp
	5'-CTC TGC TCA CAC TGA TTG GTG AAT-3'	
CCR2	5'-GGC ATG AGG CTG TCA G-3'	300 bp
	5'-ATT GTC CAT GTT GTC ATA GAT-3'	
CCR3	5'-TTG ATC CTC ATA AAG TAC AGG AAGC-3'	330 bp
	5'-CAA TGC TGC CAG TCC TGC AA-3'	
GAPDH	5'-ACC ACA GTC CAT GCC ATC AC-3'	451 bp
	5'-TCC ACC ACC CTG TTG CTG TA-3'	

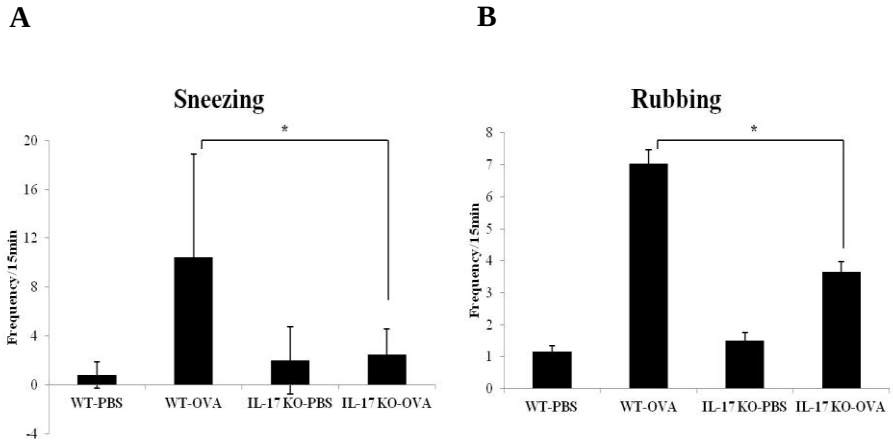
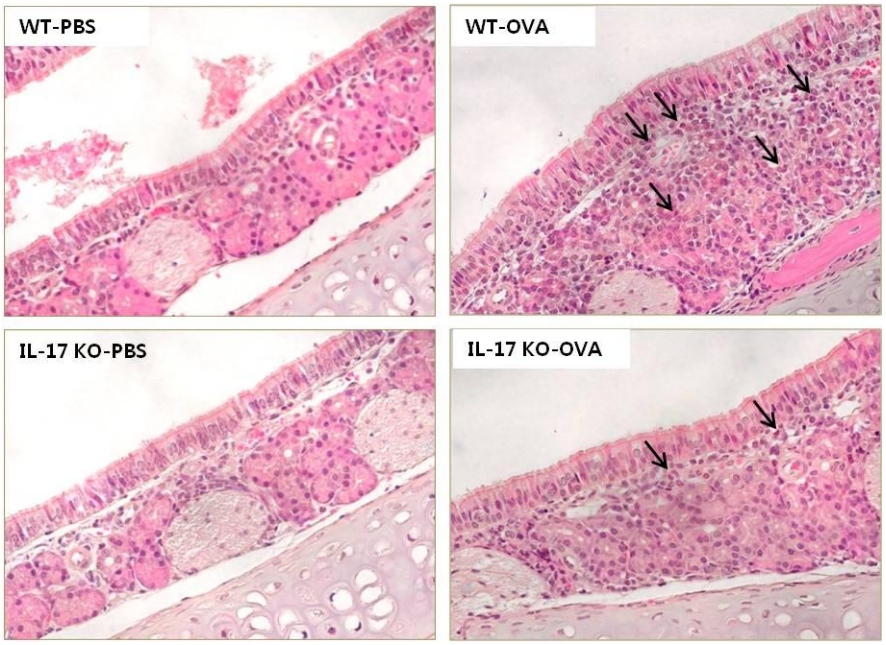
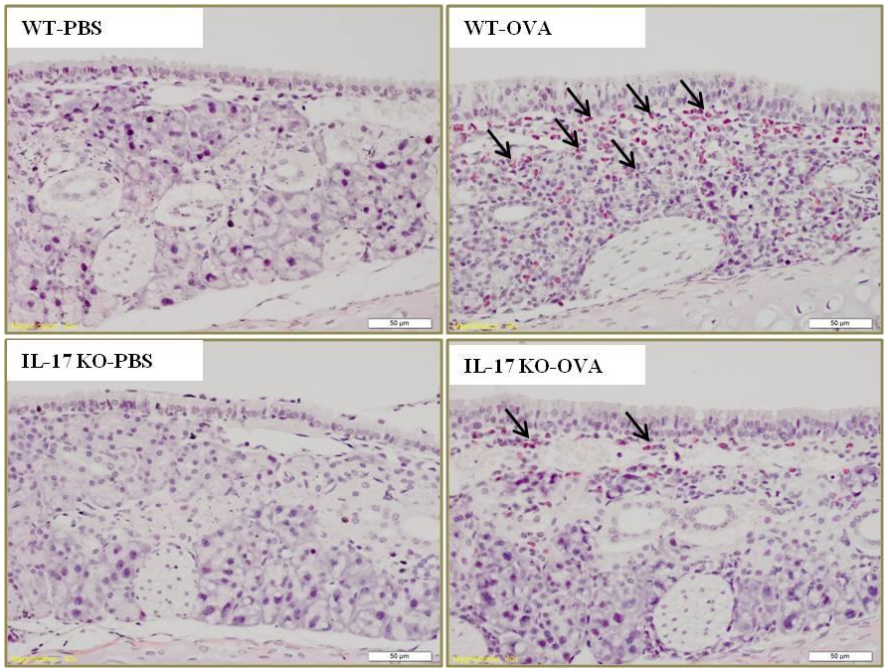


Figure 2. Nasal allergic symptom score. The numbers of (A) sneezing and (B) rubbing events for 15 min after OVA provocation were counted. In the IL-17 KO-OVA group, the nasal sneezing and rubbing symptom scores were significantly reduced compared to the WT-OVA group. Results are means ± SD, n = 20. WT-PBS, WT mice sensitized and challenged with PBS; WT-OVA, WT mice sensitized and challenged with OVA; IL-17 KO-PBS, IL-17-deficient mice sensitized and challenged with PBS; IL-17 KO-OVA, IL-17-deficient mice sensitized and challenged with OVA. **P* < 0.001 *vs.* the WT-OVA group.

A


WT-PBS
WT-OVA
IL-17 KO-PBS
IL-17 KO-OVA

B


WT-PBS
WT-OVA
IL-17 KO-PBS
IL-17 KO-OVA

C

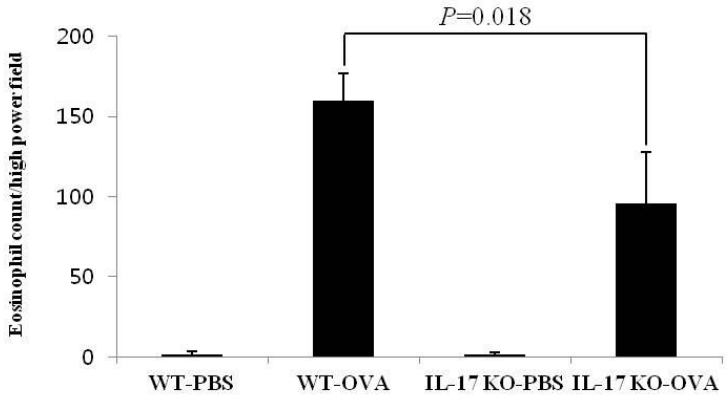


Figure 3. Eosinophil infiltration in the nasal mucosa. Histological findings of the nasal mucosa in each group (× 400 magnification) with (A) hematoxylin and eosin (H & E) stain, and (B) Sirius stain. The sections from the WT-OVA group show aggressive infiltrating eosinophils (arrows). (C) The eosinophil count was significantly reduced (P = 0.018) in the IL-17 KO-OVA group compared to the WT-OVA group. Results are means ± SD. n = 4–5.

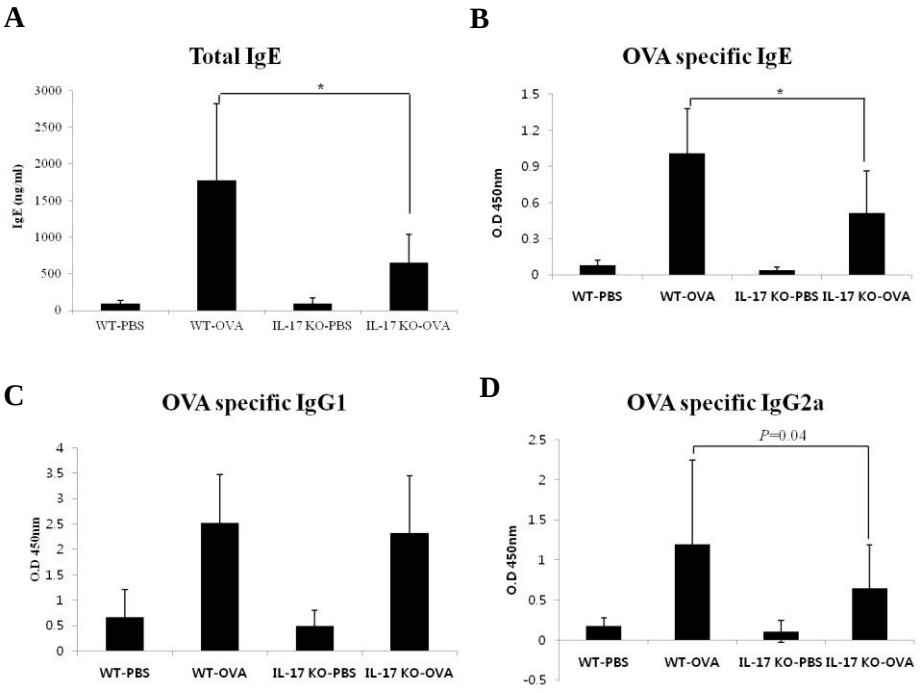


Figure 4. Serum immunoglobulin levels. (A) total IgE, (B) OVA-specific IgE, (C) OVA-specific IgG1 and (D) OVA-specific IgG2a. Serum total IgE, OVA-specific IgE, and OVA-specific IgG2a levels were significantly reduced in the IL-17 KO-OVA group compared to the WT-OVA group. OVA-specific IgG1 levels in the IL-17 KO-OVA and WT-OVA groups were not significantly different. Results are means ± SD. n = 20. *P < 0.001 *vs.* the WT-OVA group.

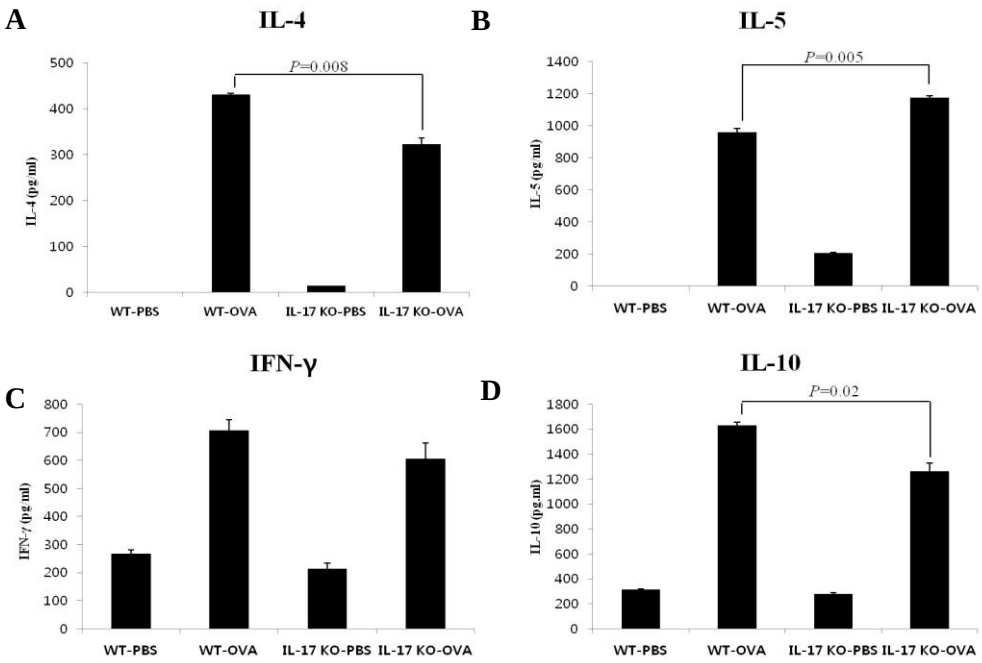


Figure 5. Cytokine levels in spleen cell culture. (A) IL-4, (B) IL-5, (C) IFN-γ and (D) IL-10. IL-4 and IL-10 levels were significantly decreased (P = 0.008, P = 0.02, respectively) in the IL-17 KO-OVA group compared to the WT-OVA group. The IL-5 level was significantly increased (P = 0.008) in the IL-17–KO OVA group compared to the WT-OVA group. The IFN-γ level in the IL-17 KO OVA group showed a decreasing tendency, which did not reach statistical significance, compared with that of the WT-OVA group. Results are means ± SD.

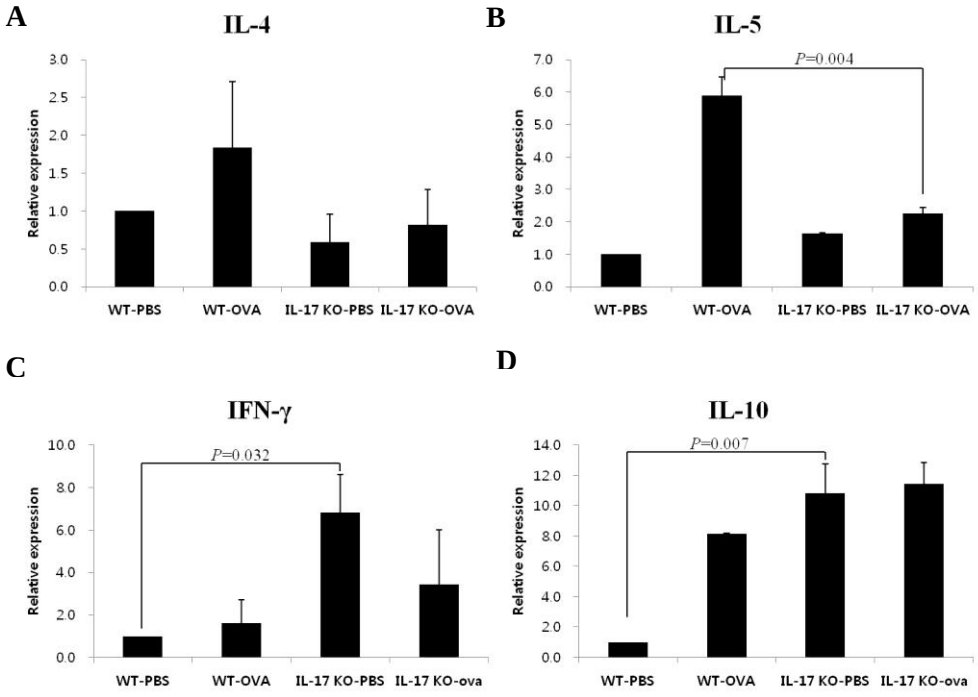
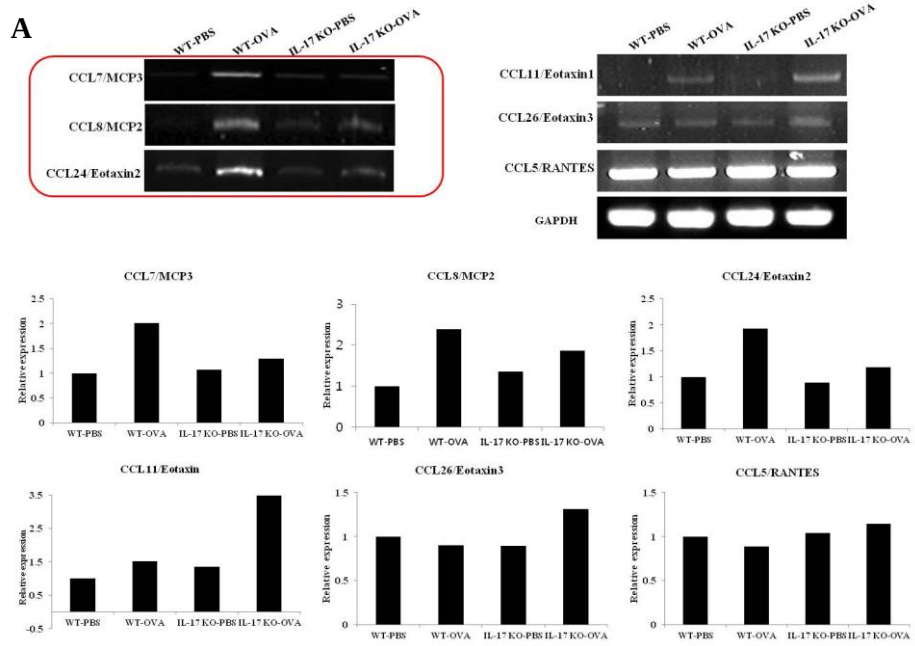


Figure 6. Cytokine mRNA levels in the nasal mucosa. (A) IL-4, (B) IL-5, (C) IFN-γ and (D) IL-10. The IL-5 mRNA level was significantly decreased (P = 0.004) in the nasal mucosa of the IL-17 KO-OVA group compared to that of the WT-OVA group**.** With regard to IL-4, IFN-γ and IL-10 mRNA levels, the IL-17 KO-OVA and WT-OVA groups were not significantly different. The baseline IFN-γ and IL-10 mRNA levels were significantly increased (P = 0.032, P = 0.007, respectively) in the IL-17 KO group. Data were normalized *vs*. GAPDH and fold-induction is relative to the WT-PBS group. Results are means ± SD, n = 5.


A
WT-PBS
WT-OVA
IL-17 KO-PBS
IL-17 KO-OVA
CCL7/MCP3
CCL8/MCP2
CCL24/Eotaxin2
CCL11/Eotaxin1
CCL26/Eotaxin3
CCL5/RANTES
GAPDH
CCL7/MCP3
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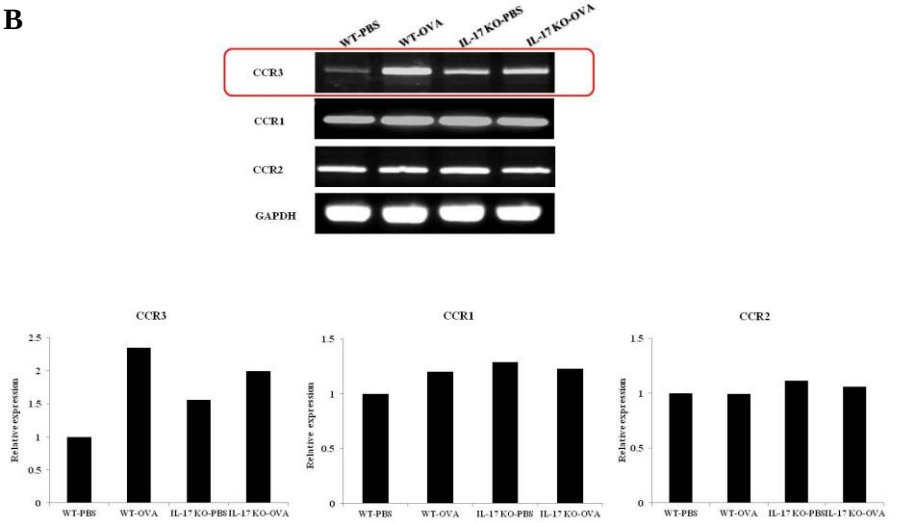
CCL26/Eotaxin3
CCL5/RANTES
Relative expression


B
WT-PBS
WT-OVA
IL-17 KO-PBS
IL-17 KO-OVA
CCR3
CCR1
CCR2
GAPDH
CCR3
CCR1
CCR2
Relative expression

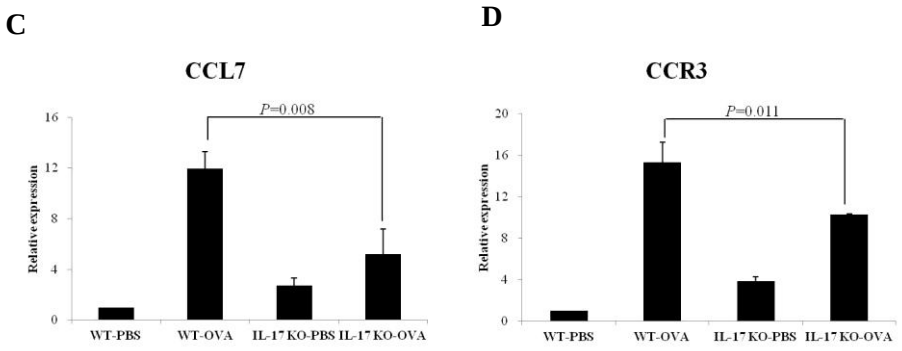


Figure 7. Chemokine and chemokine receptor mRNA levels in the nasal mucosa. (A) Real-time polymerase chain reaction (RT-PCR) for several chemokines. A variety of chemokines are associated with eosinophil chemotaxis, including eotaxin-1 (CCL11), eotaxin-2 (CCL24), eotaxin-3 (CCL26), CCL7 (MCP3), CCL8 (MCP2) and RANTES (CCL5). Of these, CCL7 did not increase upon allergen challenge in the IL-17-deficient allergic rhinitis murine model. (B) RT-PCR for CCL7 receptors. CCL7 signals through three G-protein-coupled receptors: CCR1, CCR2 and CCR3. Among these, mRNA level of only CCR3 was decreased in the IL-17 KO-OVA group. (C) CCL7 and (D) CCR3 mRNA levels were quantified by real time PCR. both CCL7 and CCR3 mRNA levels were significantly reduced (P = 0.008, P = 0.011, respectively) in the IL-17 KO-OVA group. Data were normalized to GAPDH and fold-induction is relative to the WT-PBS group. Results are means ± SD, n = 5.

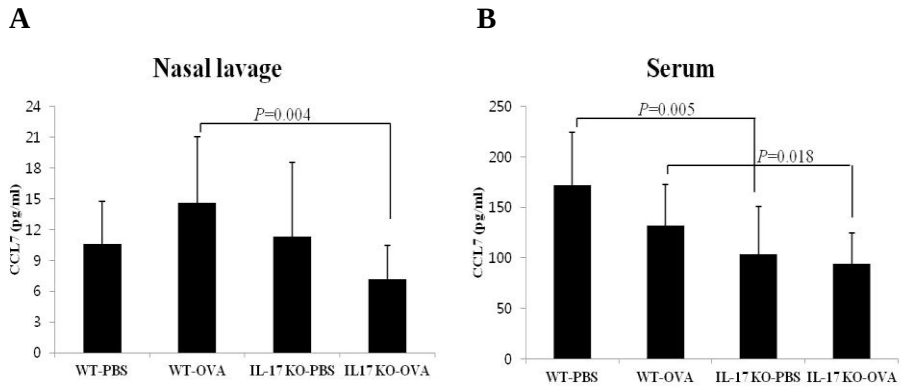


Figure 8. CCL7 protein levels in nasal lavage and serum. (A) The CCL7 protein level in nasal lavage fluid was significantly decreased in the IL-17 KO-OVA group. (B) The CCL7 serum protein level in IL-17-deficient mice was significantly lower than that in WT mice. However, OVA challenge resulted in no significant difference in CCL7 level in WT or KO mice. Results are means ± SD, n = 10–13.

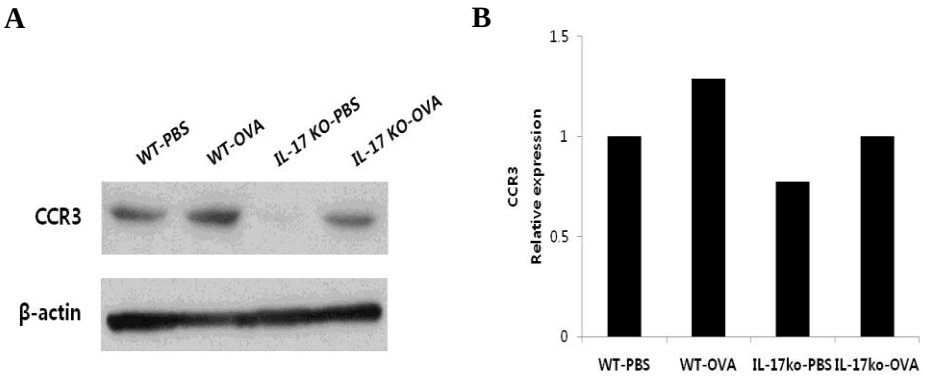


Figure 9. CCR3 protein levels in the nasal mucosa. (A, B) CCR3 protein levels were decreased in the IL-17 KO-OVA group compared to the WT-OVA group. Expression of CCR3 was normalized to β-actin and fold-induction is relative to the WT-PBS group.

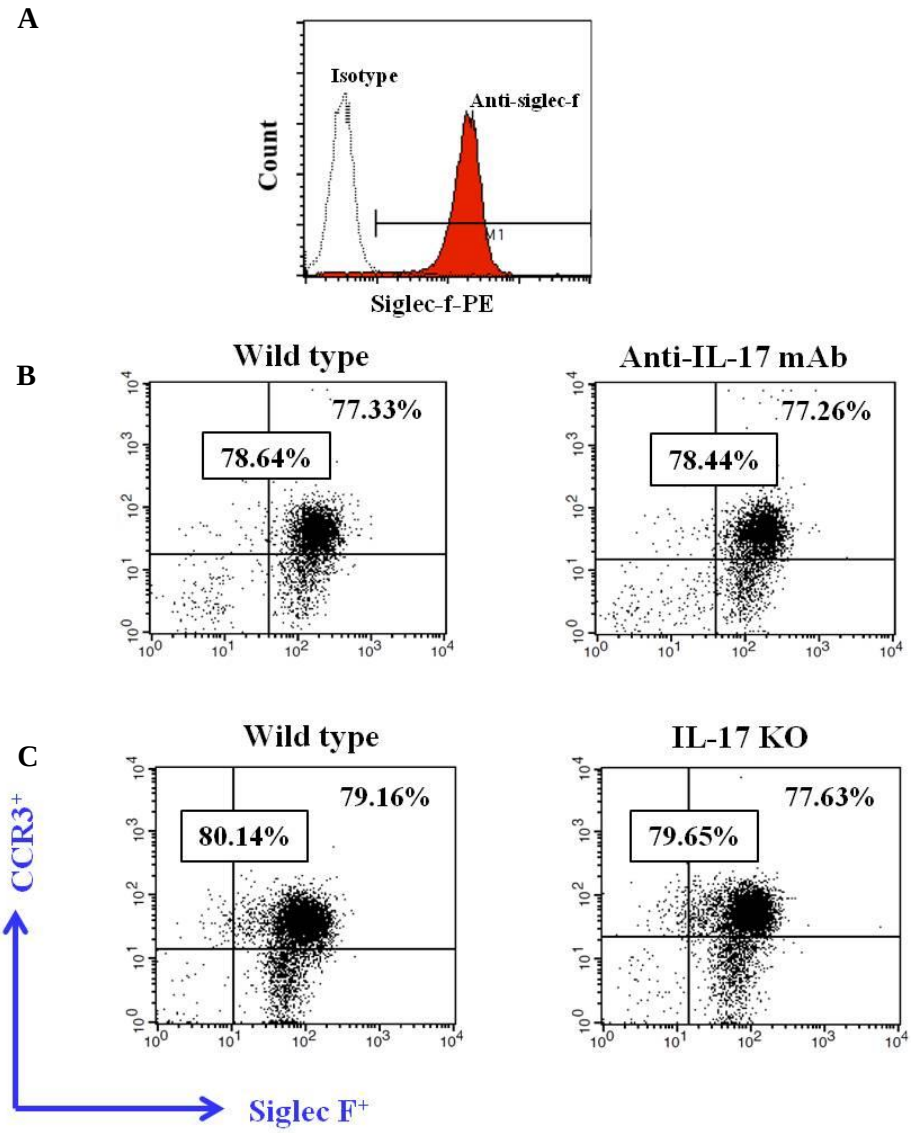


Figure 10. CCR3 expression on bone-marrow-derived eosinophils (bmEos) from naïve mice. (A) Flow cytometry of Siglec-f expression in bmEos culture. (B) Flow cytometry detected no difference in CCR3 expression on bmEos cultured in the presence or absence of anti-IL-17 mAb. (C) Flow cytometry detected similar CCR3 expression levels on bmEos in WT BALB/c and IL-17 KO mice.

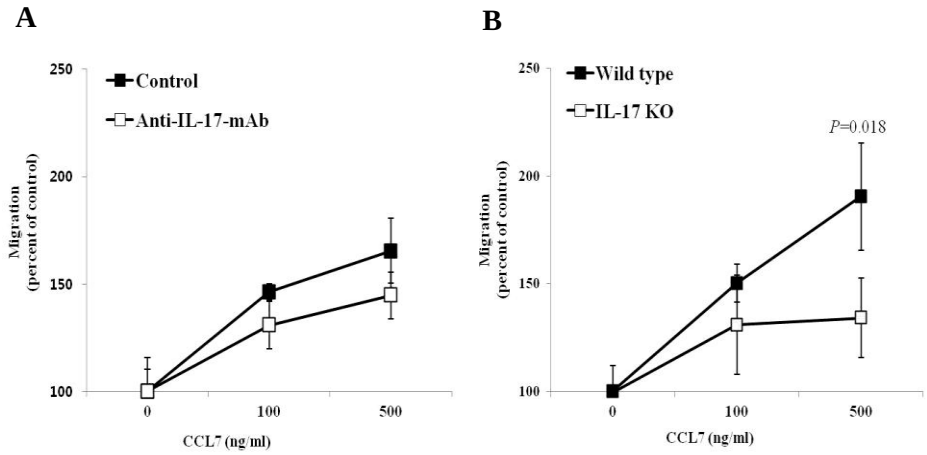


Figure 11. bmEos in chemotactic migration assays. (A) Eosinophils were cultured in the absence or presence of anti-IL-17 mAb (5 μg/ml) in whole cultures. The lower chamber contained chemotaxis medium with mouse CCL7. The bmEos from day 12 cultures were used to measure chemotaxis in the presence of a mouse CCL7 gradient, determined as a percentage of background migrating cells (control: no CCL7). The cells that migrated into the lower chamber were assessed after 3 h. The anti-IL-17-mAb-treated bmEos had a lower migration ability than cells incubated in normal medium with two CCL7 concentrations (CCL7 100 ng/ml: 146.3 ± 3.7% *vs*. 130.8 ± 11.0%; 500 ng/ml: 165.4 ± 15.1% *vs*. 144.7 ± 10.9%). (B) A comparison of the chemotactic responses of bmEos derived from WT and IL-17 KO mice. The bmEos from IL-17 KO mice had a lower migration ability than those from WT mice (CCL7 100 ng/ml: 150.4 ± 8.8% *vs*. 131.0 ± 23.1%; 500 ng/ml: 190.5 ± 25.0% *vs*. 134.2 ± 18.6%, $P = 0.018$). Results are shown as the means ± SD, $*P < 0.05$ *vs*. the WT group treated with 500 ng/ml CCL7.

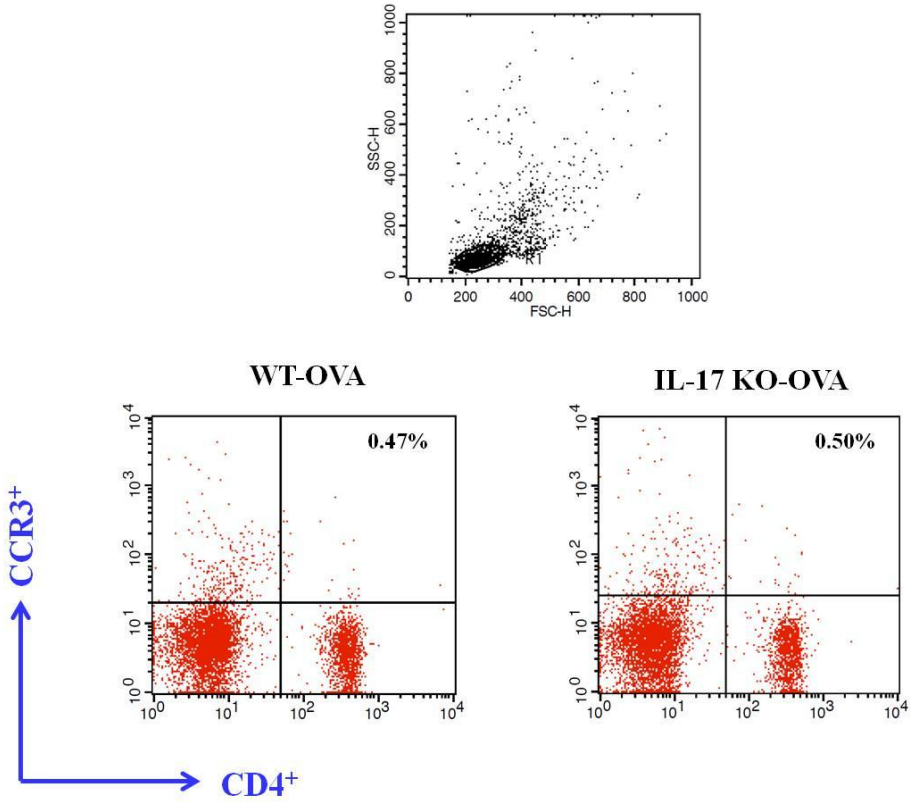


Figure 12. Flow cytometric evaluation of CCR3 expression on $CD4^+$ lymphocytes in the spleen. The spleen cells from WT-OVA and IL-17 KO-OVA mice were stained for $CD4^+CCR3^+$. Gating was set for lymphocytes. There was no difference in the $CCR3^+$ cells among the $CD4^+$ lymphocytes in both groups (0.47% *vs.* 0.50%). WT-OVA, WT mice sensitized and challenged with OVA; IL-17 KO-OVA, IL-17-deficient mice sensitized and challenged with OVA.

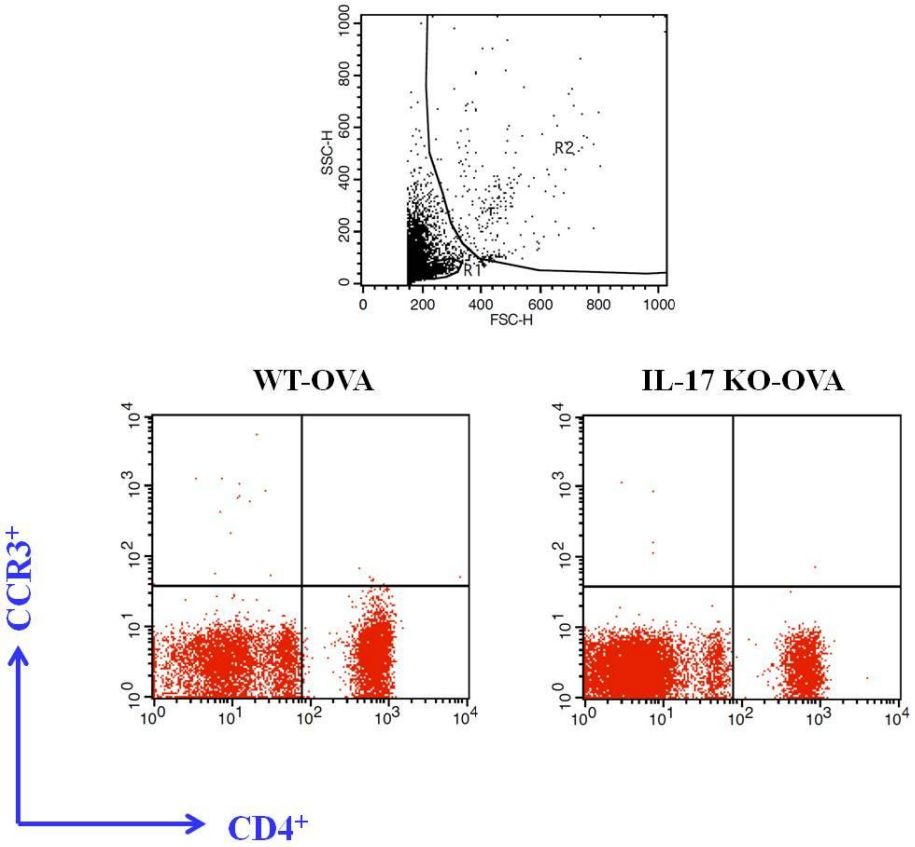


Figure 13. Flow cytometric evaluation of CCR3 expression on $CD4^+$ lymphocytes in blood. Blood from WT-OVA and IL-17 KO-OVA mice was stained for $CD4^+CCR3^+$. Gating was set for lymphocytes. No $CCR3^+$ cells were detected among the $CD4^+$ lymphocytes from both groups. WT-OVA, WT mice sensitized and challenged with OVA; IL-17 KO-OVA, IL-17 deficient mice sensitized and challenged with OVA.

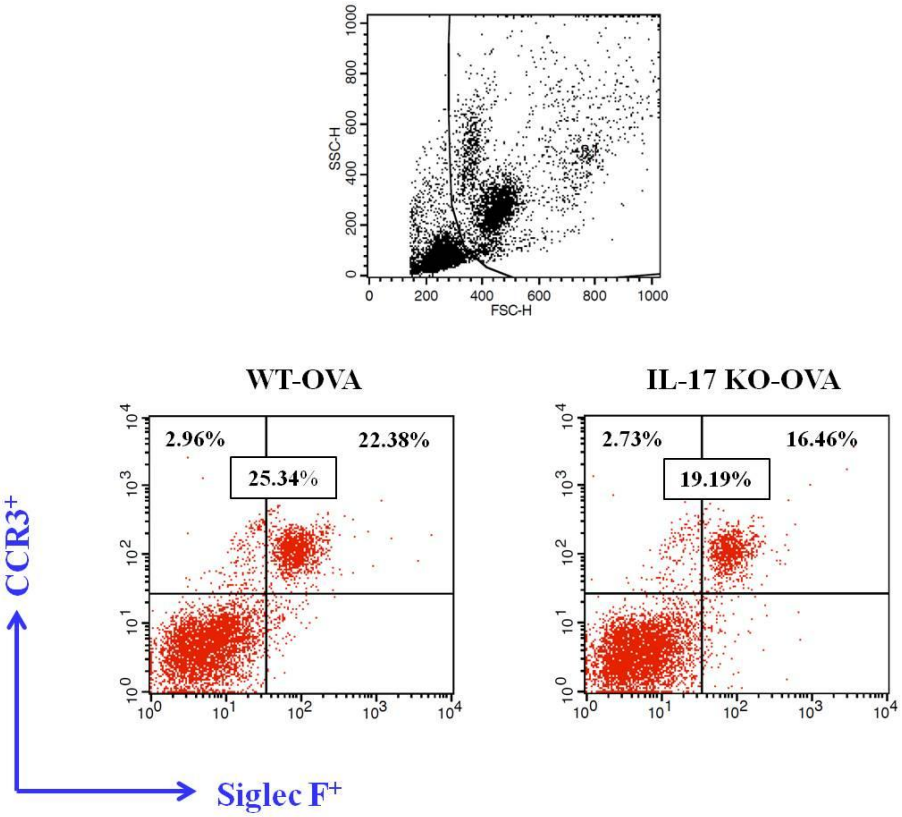


Figure 14. Flow cytometric analysis of CCR3 expression on other leukocytes, except blood lymphocytes. Blood from WT-OVA and IL-17 KO-OVA mice was stained for siglec-f^{+}CCR3^{+}. In the other leukocyte-gating region of blood, CCR3 expression was lower in IL-17 KO-OVA mice compared to in WT-OVA mice. Interestingly, only CCR3 expression on siglec-f^{+} eosinophils was decreased in the IL-17 KO-OVA mice compared to WT-OVA mice (22.38% *vs.* 16.46%).

DISCUSSION

Allergic rhinitis is an allergic inflammation of the nasal airways characterized by the accumulation of eosinophils and mast cells in the nasal mucosa. Allergen triggers a Th2-predominant immune response leading to antigen-specific IgE production. Subsequently, IgE binds to mast cells to release chemical mediators that cause early allergic reactions such as sneezing, itching and rhinorrhea. The late-phase response involves increased recruitment and activation of inflammatory cells, predominantly eosinophils (1, 25, 26).

Since the discovery of Th1 and Th2 type cells in mice (27), the Th1/Th2 balance has been considered to be important for the immune system. Th1 cells produce IFN-γ and regulate cellular immunity, whereas Th2 cells produce IL-4, IL-5, and IL-13, which induce the production of IgE and allergic responses. It is well known that antigen-induced allergic airway inflammation is mediated, in part, by Th2 cells and their cytokines. Th17 cells are a novel subset expressing IL-17, and have been shown to regulate tissue inflammatory responses including allergic airway inflammation (28). Previous studies have revealed that IL-17A deficiency suppresses allergic cellular and humoral responses in diseases such as contact hypersensitivity, delayed-type hypersensitivity, and the airway hypersensitivity response in experimental IL-17A-deficient mice (3). In addition, IL-17A deficiency attenuates allergic inflammation in a murine allergic rhinitis model (23). However, the exact mechanism of IL-17A-induced suppression of eosinophil inflammation remains unknown.

Th2-dominant chronic inflammation of the lower airway with eosinophils is widely accepted as a fundamental characteristic of asthma. Recent studies indicate that IL-17 induces neutrophilia rather than eosinophilia in the airway, providing new insight into the pathogenesis of “non-Th2-type” asthma. For example, IL-17A levels are elevated in the sputum and blood samples from asthmatics (8, 10, 29, 30) and IL-17 can stimulate release of IL-8 by bronchial fibroblast, epithelial cell and smooth muscle cell activation (31, 32). IL-17A enhances IL-6, IL-8 and GRO-α secretion by human bronchial fibroblasts (8), β-defensin-2, ICAM-1, IL-8, CXCL1, CCL20, G-CSF, MUC5B and MUC5AC expression/production by human bronchial epithelial cells (33-39), and IL-6 and IL-8 from human airway smooth muscle cells (31, 40). IL-8 and GRO-α are known to be chemoattractants for neutrophils, and IL-6 is a neutrophil-activating cytokine. These reports indicate that IL-17 is predominantly involved in neutrophil inflammation in the airway.

In the upper airway, the cellular sources for IL-17A are reported to be T lymphocytes, neutrophils, eosinophils, plasma cells and serous glands (41, 42). Recent studies using a murine asthma model have shown that IL-17A induces recruitment not only of neutrophils, but also of eosinophils into the airway (11, 28). Saitoh and colleagues clearly showed that IL-17A was predominantly expressed in both CD4-positive lymphocytes and eosinophils, but not in neutrophils. In addition, nasal polyps are characterized by a considerable number of IL-17A-positive cells that are strongly correlated with the number of eosinophils (12). The correlation between IL-17A-positive cells and eosinophils can be

explained simply by the fact that eosinophils are the major source of IL-17A (12). A primary function of IL-17A may be to induce the recruitment of eosinophils, which is enhanced by a positive feedback loop of IL-17A production by eosinophils (12).

IL-17 is known to induce allergen-specific Th2 cell activation, eosinophil accumulation, and serum IgE production (3, 28). In accordance with other studies, our results confirmed that the IL-17-deficient allergic rhinitis murine model shows decreased IgE production from serum and IL-4 expression by both spleen and nasal tissue compared to those from WT mice. Thus, IL-17 deficiency may play an important role in the development of allergic rhinitis by reducing Th2 cytokines in the response. On the other hand, we observed different systemic and local responses with respect to IFN-γ and IL-10 in the IL-17–KO mice. Basal IFN-γ and IL-10 levels in the nasal mucosa were significantly increased in the IL-17 KO mice compared to those in WT mice. These observations may be a result of negative regulation by IL-17A of cytokine production by Th1 and regulatory T cells. However, further study is needed to elucidate the exact mechanism.

The initial event in allergic diseases is the interaction between dendritic cells (DC) and T cells, leading to the generation of Th2 cells and IL-5, an important cytokine for induction of eosinophil recruitment (43). Upon allergen challenge, naïve $CD4^+$ T cells differentiate into the prevailing Th2 effector phenotype, and these cells predominantly secrete IL-4, IL-5, and IL-13 promoting the recruitment of eosinophils, mast cells and

lymphocytes, and hyperplasia of smooth muscle and airway hyperresponsiveness, which are frequently associated with an increased serum IgE concentration (44). IL-5 is indispensable for long-term eosinophilia, as it is the major factor inducing eosinophil differentiation from lineage-committed precursors. Furthermore, IL-5 extends the lifespan of infiltrating eosinophils and further activates or enhances their effector capabilities (45). Released primarily by Th17 cells, IL-17A cytokine is critical in an array of inflammatory diseases. A previous report demonstrated that eosinophils are a potential source of IL-17 within asthmatic airways, suggesting that IL-17 might have the potential to amplify inflammatory responses (8). In a model of chronic eosinophilia, IL-17–KO mice did not recruit an important number of eosinophils after allergen challenge (46). Results obtained using our allergic rhinitis model were consistent with that previous report: the numbers of infiltrating eosinophils and IL-5 mRNA expression in nasal mucosa were significantly decreased in the IL-17 KO-OVA group compared to in the WT-OVA group. However, in spleen cell culture supernatants, IL-5 secretion was higher in the IL-17 KO-OVA mice than in the WT-OVA mice. We speculate that IL-5 expression may differ between systemic and local responses.

In the present study, CCL7 and CCR3 mRNA levels were significantly increased in the nasal mucosa of mice with allergic rhinitis. This result is consistent with previous studies that showed over 90% upregulation of CCL7 and CCR3 gene expression in the nasal mucosa of patients with allergic rhinitis (47). Several reports have addressed the relationship between IL-17A and CCL7 using mouse embryonic fibroblasts and MLE12

cells (a mouse lung epithelial cell line), brain and central nervous system tissue and chronic asthma models. In these models, upregulation and downregulation of CCL7 expression were observed in the presence or absence of IL-17A, respectively (21, 22). In this study, significantly reduced CCL7 mRNA and protein levels were found in the nasal lavage fluid from the IL-17 KO-OVA group compared to the WT-OVA group. In addition, the serum CCL7 level was significantly reduced in the IL-17-deficient mice. These results are in agreement with those of previous studies. Interestingly, CCL7 secretion was decreased by OVA treatment of both WT and IL-17-deficient mice compared to PBS-treated mice. Polentarutti and colleagues reported that IL-4, IL-10 and IL-13 suppress CCL7 expression in monocytes (48). Presumably, our allergic rhinitis model with OVA results in increased IL-4 and IL-10 expression in blood, which can lead to reduced CCL7 expression.

CCR3 is expressed on eosinophils, basophils, mast cells, neutrophils, Th2 cells and endothelial cells; of these, eosinophils have the highest level (19, 49, 50). Siglec-f has emerged as a reliable distinguishing marker of eosinophils in mice (51). In the present study, we compared CCR3 expression on blood and spleen cells using the allergic rhinitis model with WT and IL-17–KO mice. No difference in CCR3 expression between CD4-positive lymphocytes and siglec-f negative cells was detected, whereas CCR3 expression was decreased on siglec-f positive eosinophils. We suggest that IL-17A deficiency reduces CCR3 expression on blood eosinophils.

CCR3 on eosinophils is responsible for chemotaxis induced by eotaxin, RANTES and CCL7 (14, 52). In this study, the mRNA and protein levels of CCR3 in the nasal mucosa were suppressed in IL-17–deficient mice. CCL7 showed an expression profile similar to that of CCR3, and eosinophil numbers were also decreased in the nasal mucosa of IL-17-deficient mice. CCR3 expression on bmEos from IL-17 KO mice did not differ from that in naïve mice, suggesting that IL-17 does not affect the development of CCR3. In addition, the eosinophil chemotactic response of bmEos toward CCL7 demonstrated that IL-17 deficiency results in a decreased CCL7chemotactic activity. These results suggest that IL-17A regulates eosinophil inflammation through the CCL7/CCR3 pathway in murine allergic rhinitis models.

In summary, the nasal symptom scores, serum IgE production and the numbers of infiltrating eosinophils in the nasal mucosa were significantly decreased in OVA-sensitized IL-17A-deficient mice compared with WT mice. IL-17A deficiency resulted not only in significantly decreased levels of IL-5, but also of CCL7 and CCR3 in the nasal mucosa. In addition, IL-17 deficiency may suppress the chemotactic response of eosinophils toward CCL7. These results suggest that IL-17A plays a role in the development of allergic rhinitis by regulating the levels of Th2 cytokines, chemokines and chemokine receptors on eosinophils. In conclusion, regulation of allergic inflammation by IL-17 is attributable at least in part to the CCL7/CCR3 pathway.

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국문 초록

연구배경: IL-17 은 Th17 세포에서 분비되는 전염증사이토카인으로 호중구성 기도염증에 관련이 있다고 알려져 있다. 이와 더불어 최근에는 알레르기비염의 호산구 침윤과도 연관되어 있다고 보고되었다. 호산구 표면에 있는 CCR3 는 케모카인 수용체로서 특히, 천식, 아토피 피부염 및 알레르기 비염과 같은 알레르기 질환에서 현저하게 많이 발현한다. CCL7 은 CCR3 의 ligand 로서 단핵구, 호산구, 호염구, NK 세포, T 세포 및 호중구 등의 면역세포에 대한 화학주성 역할을 한다.

연구목적: 알레르기 비염 모델에서 IL-17 이 결핍되면 CCL7 의 생성이 억제되는지 여부를 조사하고, IL-17 에 의한 호산구의 침윤에 CCR3/CCL7 의 경로가 관여하는지를 알아보고자 하였다.

연구방법: IL-17 Knock-out (KO) 생쥐와 wild type BALB/c (대조군)를 난알부민으로 감작시켜서 알레르기비염 모델을 제작한 후 비염증상, 코점막 내 호산구 침윤 정도와 사이토카인 발현 정도, 혈청난알부민, 특이 IgE, IgG1, IgG2a 를 측정하였다. 또한 CCL7 과 CCR3 의 유전자 및 단백질 발현의 차이를 비교하였다. Wild type 과 IL-17 KO 생쥐의 골수에서 분화시킨 호산구를 이용하여 호산구에 대한 CCL7 의 화학주성능을 비교하였다.

연구결과: 알레르기비염 모델에서 IL-17A 가 결핍되면 비염 증상이 완화되고, 점막하 호산구 침윤과 혈청난알부민 특이 IgE 가 유의하게 감소하였고, 코점막에서 IL-5 mRNA 발현이 유의하게 감소하였다. IL-17A KO 생쥐의 혈청과 비관류액에서 CCL7 의 발현이 대조군에 비해 의미있게 감소하였고, 비점막에서 CCL7 mRNA 의 발현도 감소하였다. IL-17A KO 생쥐의 비점막에서 CCR3 의 mRNA 와 단백질의 발현도 대조군에 비해 감소되었다. IL-17A 항체는 호산구에 대한 CCL7 의 화학주성능을 저하시킬 뿐만 아니라 IL-17A 유전자 결핍 생쥐에서 유도시킨 호산구는 500 ng/ml CCL7 에 대해 화학주성능이 유의하게 감소하였다. IL-17A 가 결핍되면 알레르기비염 모델에서 CCR3 를 발현하는 호산구의 수가 감소하는 경향을 보였다.

결론: IL-17 은 알레르기 염증반응에서 CCL7 의 분비를 조절하며, 비점막에서 IL-17 에 의한 호산구의 침윤에는 CCR3/CCL7 경로가 적어도 부분적으로 관여할 것으로 생각된다.

중심단어 : IL-17, CCR3, CCL7, 호산구, 알레르기 비염

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