

Interleukin-17A-induced inflammation does not influence the development of nasal polyps in murine model

Sung-Lyong Hong, MD^{1,2,3}, Yu-Lian Zhang, PhD⁴, Sang-Wook Kim, MD, PhD⁵, Dae Woo Kim, MD, PhD^{4,6}, Sang-Heon Cho, MD, PhD^{3,7}, Yoon-Seok Chang, MD, PhD⁸, Chul Hee Lee, MD, PhD^{4,9} and Chae-Seo Rhee, MD, PhD^{3,9,10,11}

Background: Nasal polyposis associated with chronic rhinosinusitis (CRS) is a chronic inflammatory disease that is characterized by infiltration of many inflammatory cells. Meanwhile, interleukin (IL)-17A is a well-known proinflammatory cytokine that induces both eosinophilic and neutrophilic inflammation. We investigated the role of IL-17A in the development of nasal polyps in the CRS murine model.

Methods: Eosinophilic CRS with nasal polyps was induced by using ovalbumin (OVA) and *Staphylococcus aureus* enterotoxin B (SEB) in wild-type BALB/c and IL-17A knockout (KO) mice. Histopathologic changes of the sinonasal cavity were evaluated using hematoxylin and eosin, Periodic acid-Schiff, Sirius red, Masson's trichrome, and immunohistochemistry. The levels of total and OVA-specific immunoglobulin Es (IgEs) in sera were measured using enzyme-linked immunosorbent assay. The expression levels of IL-4, IL-5, and interferon- γ (IFN- γ) in the nasal mucosa were assessed by quantitative real-time polymerase chain reaction.

Results: Under the IL-17A deficiency, total and OVA-specific IgEs in sera were reduced significantly. Infiltration

of both eosinophils and neutrophils into the nasal mucosa, subepithelial fibrosis, and goblet cell count also decreased significantly in IL-17A KO mice treated with both OVA and SEB compared with those in the wild-type counterpart. However, there were no significant differences in the number of polypoid lesions among groups. Meanwhile, IL-4 increased and IFN- γ decreased in the nasal mucosa in IL-17A KO mice treated with both OVA and SEB.

Conclusion: This study suggests that even though IL-17A plays an important role in both nasal inflammation and remodeling, it does not influence the development of nasal polypoid lesions. © 2015 ARS-AAOA, LLC.

Key Words:

airway remodeling; inflammation; interleukin-17A; nasal polyps; *Staphylococcal enterotoxin B*

How to Cite this Article:

Hong SL, Zhang YL, Kim SW, et al. Interleukin-17A-induced inflammation does not influence the development of nasal polyps in murine model. *Int Forum Allergy Rhinol.* 2015;5:363-370.

¹Department of Otorhinolaryngology-Head and Neck Surgery, Pusan National University Hospital, Busan, South Korea; ²Medical Research Institute, Pusan National University Hospital, Busan, South Korea; ³Graduate School of Immunology, Seoul National University College of Medicine, Seoul, South Korea; ⁴Department of Otorhinolaryngology, Seoul National University College of Medicine, Seoul, South Korea; ⁵Department of Otorhinolaryngology, Gyeongsang National University, Jinju, South Korea; ⁶Department of Otorhinolaryngology, Seoul National University Boramae Medical Center, Seoul, South Korea; ⁷Department of Internal Medicine, Seoul National University Hospital, Seoul, South Korea; ⁸Department of Internal Medicine, Seoul National University Bundang Hospital, Gyeonggi, South Korea; ⁹Department of Otorhinolaryngology, Seoul National University Bundang Hospital, Gyeonggi, South Korea; ¹⁰Research Center for Sensory Organs, Seoul National University Medical Research Center, Seoul, South Korea; ¹¹Institute of Allergy and Clinical Immunology, Seoul National University Medical Research Center, Seoul, South Korea

Chronic rhinosinusitis with nasal polyp (CRSwNP) is a chronic inflammatory disorder characterized by infiltration of many kinds of inflammatory cells into the sinonasal mucosa.¹ Although its etiology and pathogenesis have been studied for a long time, they are not yet fully elucidated. However, its association with *Staphylococcus*

Correspondence to: Chae-Seo Rhee, MD, PhD, Department of Otorhinolaryngology, Seoul National University Bundang Hospital, 82, Gumi-ro 173, Beon-gil, Bundang-gu, Seongnam-si Gyeonggi-do, 463-707, South Korea; e-mail: csrhee@snu.ac.kr

Funding sources for the study: Seoul National University Bundang Hospital Research Fund 2011 (grant no. 12-2011-0047). Potential conflict of interest: None provided.

Received: 29 December 2014; Revised: 27 January 2015; Accepted: 3 February 2015

DOI: 10.1002/alr.21515

View this article online at wileyonlinelibrary.com.

aureus enterotoxin B (SEB) has been fairly well established. SEB is one of the exotoxins released from *Staphylococcus aureus* in the lateral wall of the nose and may act as superantigens and upregulate the V_{β} region of lymphocytes in CRSwNP.²⁻⁴ It can induce both release of cytokines and recruitment of many inflammatory cells in the sinonasal mucosa.^{2,5,6}

A recent study reported that SEB increased interleukin (IL)-17A expression in human nasal epithelial cells and in epithelial cells of SEB-instilled mice and concluded that SEB may be involved in pathogenesis of nasal polyps by enhancing IL-17A expression in epithelial cells in nasal polyps.⁷ IL-17A, the key cytokine of T helper (Th) 17 cells, is a well-known proinflammatory cytokine that induces Th1-type neutrophilic inflammation as well as Th2-type eosinophilic inflammation.⁸⁻¹⁰ It is known that IL-17A's expression increases in patients with allergic airway disease.¹¹ Moreover, several recent human studies have reported that IL-17A may be associated with CRSwNP.¹²⁻¹⁴

Considering the ability of IL-17A to induce both eosinophilic and neutrophilic inflammation and its possible association with CRSwNP, we hypothesized that it may play an important role in the pathogenesis of CRSwNP. Recently, authors established a murine model of SEB-induced eosinophilic CRSwNP.¹⁵ Based on this murine model, the role of IL-17A in the development of CRSwNP was investigated in this study.

Materials and methods

Experimental animals

Four-week-old female BALB/c (wild-type [WT]) mice and IL-17A-deficient (knockout [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, Japan). IL-17A-KO mice had been bred under specific-pathogen-free conditions. The BALB/c mice were obtained from Korea Orient Co. (Gyeonggi, South Korea). Each mouse weighed 20 to 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 were approved by Institutional Animal Care and Use Committee of Seoul National University Hospital.

Experimental protocols

Mice were categorized into 8 groups; 4 control groups (groups A, B, C, and D) and 4 experimental groups (groups E, F, G, and H). WT mice were used in the control groups, and IL-17A KO mice were used in the experimental groups. Eosinophilic inflammation of the nasal and sinus mucosa was induced according to a previously established protocol (Table 1).¹⁵

Briefly, mice in groups C, D, G, and H were sensitized intraperitoneally with 25 μ g of ovalbumin (OVA, grade V; Sigma, St. Louis, MO) + 2 mg of aluminum hydroxide gel (Alum) dissolved in 300 μ L of phosphate-buffered saline (PBS) on days 0 and 5. From days 12 to 19, a daily intranasal challenge with 3% OVA diluted in 40 μ L of PBS was administered. Thereafter, prolonged continuous inflammation was maintained by subsequent nasal exposure to 3% OVA 3 times weekly for 12 consecutive weeks. Meanwhile, mice in groups A, B, E, and F received intraperitoneal injection and nasal instillation of the same amount of PBS instead of 3% OVA.

Additionally, mice in groups B, D, F, and H were challenged with 5 ng of SEB dissolved in 20 μ L of PBS weekly from weeks 5 through 12, 30 minutes after nasal instillation of PBS (in groups B and F) or 3% OVA (in groups D and H). Mice in groups A, C, E, and G were challenged with 20 μ L of PBS instead of SEB.

Twenty-four hours after the final nasal challenge, mice were euthanized and decapitated. Serum and nasal mucosa were collected. Each group contains 15 mice, respectively. Ten mice were used for histological analyses and 5 mice were used for quantitative real-time polymerase chain reaction (PCR) for nasal mucosa.

Histological analyses

The heads of mice were fixed in 10% formalin overnight at 4°C with slow shaking, followed by decalcification and embedding 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.

To characterize inflammatory changes, 4 stains were performed; hematoxylin and eosin (H&E) stain for overall inflammation, Sirius red stain for eosinophils, Masson's trichrome stain for collagen, and Periodic acid-Schiff (PAS) for goblet cells. The numbers of polypoid lesions were counted in high-power fields (original magnification $\times 400$). A polypoid lesion was defined as distinct mucosal bulges with eosinophilic infiltration. The numbers of eosinophils, lymphocytes, and goblet cells were counted in 4 high power fields (original magnification $\times 400$) in each mouse. The maximal thicknesses of the subepithelial layers (from the perichondrium of the septal cartilage to the bottom of the epithelium) were measured at the inferior end of the nasal septum using an image analysis system. To quantify neutrophil infiltration, after antigen retrieval per manufacturer protocol, sections were stained with the NIMP R14 rat anti-mouse neutrophil antibody (Abcam, Cambridge, MA) and subsequently with anti-rat immunoglobulin G (IgG) (Dako, Copenhagen, Denmark), followed by detection with the DAB kit (Vector Laboratories, Burlingame, CA). Then, tissue was counterstained with Mayer's Hematoxylin and mounted. The numbers of neutrophils were also counted in 4 high-power fields (original magnification $\times 400$) in each mouse.

TABLE 1. Experimental protocol for the induction of CRSwNP in mice

		General sensitization (days 0 and 5)	Challenge (days 12–19)	Challenge (days 20–103)	Provocation (days 48–103)
Group A	WT	PBS	PBS	PBS	PBS
Group B	WT	PBS	PBS	PBS	SEB
Group C	WT	OVA + Alum	OVA	OVA	PBS
Group D	WT	OVA + Alum	OVA	OVA	SEB
Group E	IL-17A KO	PBS	PBS	PBS	PBS
Group F	IL-17A KO	PBS	PBS	PBS	SEB
Group G	IL-17A KO	OVA + Alum	OVA	OVA	PBS
Group H	IL-17A KO	OVA + Alum	OVA	OVA	SEB
		25 μ g of OVA + 2 mg of Alum dissolved in 300 μ L of PBS	3% OVA diluted in 40 μ L of PBS	3% OVA diluted in 40 μ L of PBS	5 ng of SEB dissolved in 20 μ L of PBS
		Intraperitoneal injection	Intranasal instillation, daily	Intranasal instillation, 3 times a week (for 12 weeks)	Intranasal instillation, 3 times a week (for 8 weeks)

CRSwNP = chronic rhinosinusitis with nasal polyps; IL-17A = interleukin 17A; KO = knockout; OVA = ovalbumin; Alum = aluminum hydroxide gel; PBS = phosphate-buffered saline; SEB = *Staphylococcal aureus* enterotoxin B; WT = wild-type.

Measurement of serum total IgE and OVA-specific IgE

Serum samples were collected 24 hours after the last intranasal challenge. To measure serum total IgE levels, 96-well plates were coated overnight with anti-mouse IgE capture monoclonal antibody (BD Pharmingen, San Jose, CA) at 4°C. The plate was washed 3 times with washing buffer (PBS containing 0.05% Tween-20) and blocked with 300- μ L of 3% bovine serum albumin (BSA) per well for 1 hour 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 hours at 4°C. After washing 3 times, bound IgE was detected using rat anti-mouse IgE-horseradish peroxidase (HRP) (Southern Biotech, Birmingham, AL) at a 3000-fold dilution with 2% BSA. Optical density (OD) at 450 nm was measured using a microplate reader.

Amounts of OVA-specific IgE were determined by enzyme-linked immunosorbent assay (ELISA) using 96-well plates. Plates were incubated overnight at 4°C with 100 μ g/mL OVA in coating buffer (0.05 M carbonate-bicarbonate), washed 3 times with washing buffer (PBS containing 0.05% Tween-20), blocked with 3% BSA in PBS for 1 hour at room temperature (RT) and incubated with 50- μ L of serum sample (16-fold dilution with 0.1% BSA/PBS) for 2 hours at RT. After washing, 100- μ L of biotin-conjugated rat anti-mouse IgE monoclonal antibody was added to each well and incubated for 1 hour at RT. The plates were then incubated with 100- μ L streptavidin-HRP (BD Pharmingen) for 30 minutes at RT. After washing, 100- μ L of 3,3',5,5'-tetramethylbenzidine (TMB) (KPL, Gaithersburg, MD) substrate solution was added to each

well and incubated for 30 minutes at RT; the reaction was then terminated by adding 1N HCl. OD at 450 nm was measured using a microplate reader.

Measurement of cytokines in the sinonasal mucosa

Cells were washed in ice-cold PBS and lysed in Trizol Reagent (Invitrogen, Carlsbad, CA). Complementary DNA (cDNA) was synthesized using Superscript[®] reverse transcriptase (Invitrogen, Camarillo, CA) and oligonucleotide (dT) primers (Fermentas, Burlington, ON). The sequences of the specific PCR primers used to amplify IL-4 (Mm00445258_g1), IL-5 (Mm00439646_m1), IFN- γ (Mm99999071_m1), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Mm03302249_g1) are given in Table 1. The reaction was performed using an ABI PRISM7000 sequence detection system (Applied Biosystems, Foster City, CA). The average gene transcript levels were then normalized to that of GAPDH. PCR cycling conditions were as follows: denaturation at 94°C and annealing at 58°C, followed by extension at 72°C (25 cycles for GAPDH, and 30 cycles for IL-4, IL-5, and IFN- γ). PCR products were separated on 1.5% agarose gels and stained with GelRed Nucleic Acid Stain (Koma Biotech, Seoul, South Korea).

Statistical analyses

The results are expressed as means $\pm$ standard deviation (SD). Statistical analysis was performed using SPSS 18.0 (SPSS Inc., Chicago, IL) and the Mann-Whitney *U* test was used for evaluation of differences in mean values. A value of *p* < 0.05 was considered statistically significant.

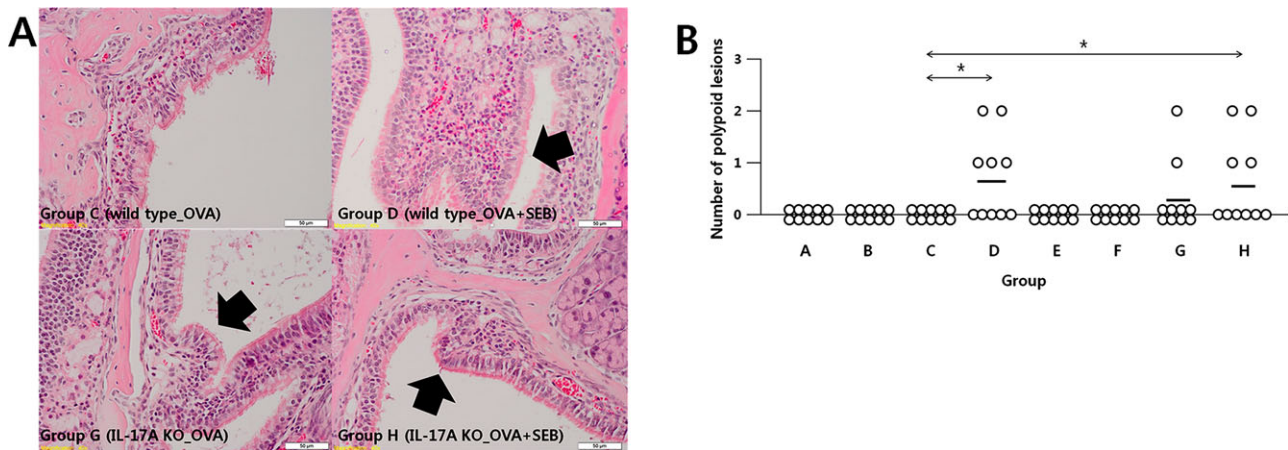


FIGURE 1. Comparison of nasal polypoid lesion counts among groups. (A) No polyp-like lesions were observed in groups A, B, C, E, or F. (B) There were 7 lesions in 5 of 10 mice in group D, 3 lesions in 2 of 10 mice in group G, and 6 lesions in 5 of 10 mice in group H. However, differences among the 3 groups were not statistically significant (hematoxylin and eosin stain, $\times 400$, $*p < 0.05$). IL-17A = interleukin 17A; KO = knockout; OVA = ovalbumin; SEB = *Staphylococcus aureus* enterotoxin B.

Results

Histology

Nasal polypoid lesion

Nasal polypoid lesions were detected in the junction olfactory and respiratory epithelia. No polyp-like lesion was observed in groups A, B, C, E, or F (Fig. 1). Seven lesions were detected in 5 of 10 mice in group D. Similarly, 3 lesions were evident in 2 of 10 mice in group G. Six lesions were detected in 5 of 10 mice in group H. Differences among the 3 groups were not statistically significant.

Inflammatory cells

Mice in groups A, B, E, and F showed no definite eosinophilic infiltration. The numbers of infiltrated eosinophils in groups C or D were statistically higher than those in groups G or H, respectively ($p = 0.012$ and $p = 0.021$, respectively; Fig. 2A). Meanwhile, there were no significant differences in the numbers of eosinophil infiltration either between groups C and D or between groups G and H, respectively.

Mice in groups A and E showed minimal neutrophilic infiltration. There were significant differences in the number of neutrophilic infiltration between groups B and F, between groups C and G, and between groups D and H, respectively ($p = 0.008$, $p = 0.008$, and $p = 0.032$, respectively; Fig. 2B). Meanwhile, there was a difference in the number of neutrophilic infiltration between groups C and D, but not between groups G and H.

Mice in groups A, B, E, and F showed no definite lymphocytic infiltration. The numbers of infiltrated lymphocytes in group D were statistically higher than those in group H ($p = 0.002$).

In total, groups C and D showed more infiltrate of inflammatory cells in the nasal tissue and cavities than groups G and H, respectively ($p = 0.009$ and $p = 0.004$; Fig. 2C).

Subepithelial fibrosis

The subepithelial layers in groups C or D were thicker than that in groups A or B, respectively (both $p = 0.004$). Group H showed thinner subepithelial layers than group D ($p = 0.023$). However, thicknesses in groups C and G were not statistically different (Fig. 3A).

Goblet cell hyperplasia

The numbers of goblet cells in groups E, F, G, and H (groups with IL-17A deficiency) were smaller than those in their wild-type counterparts (groups A, B, C, and D). However, only the difference in the numbers of the cells between groups D and H was statistically significant ($p = 0.045$; Fig. 3B).

Total and OVA-specific IgE

In general, serum total and OVA-specific IgE levels were higher in OVA-treated groups (groups C, D, G, and H) as compared with those in the negative control groups (groups A, B, E, and F) (Fig. 4). Moreover, those levels were slightly higher in WT groups (groups C and D) than in IL-17A-deficient groups (groups G and H) (both $p = 0.009$). However, there were no significant differences in those levels either between groups C and D or between groups G and H.

Messenger RNA expression of cytokines in the sinonasal mucosa

The real-time PCR was used to determine the messenger RNA (mRNA) expression of key cytokines in the murine sinonasal mucosa (Fig. 5). Group H showed the highest IL-4 expression, which was significantly lower in groups C, D, and G and rarely detected in other groups ($p < 0.05$) (Fig. 5A). Meanwhile group D showed highest IFN- γ expression, which was significantly lower in groups C, G, and H ($p < 0.05$; Fig. 5C). However, there was no significant

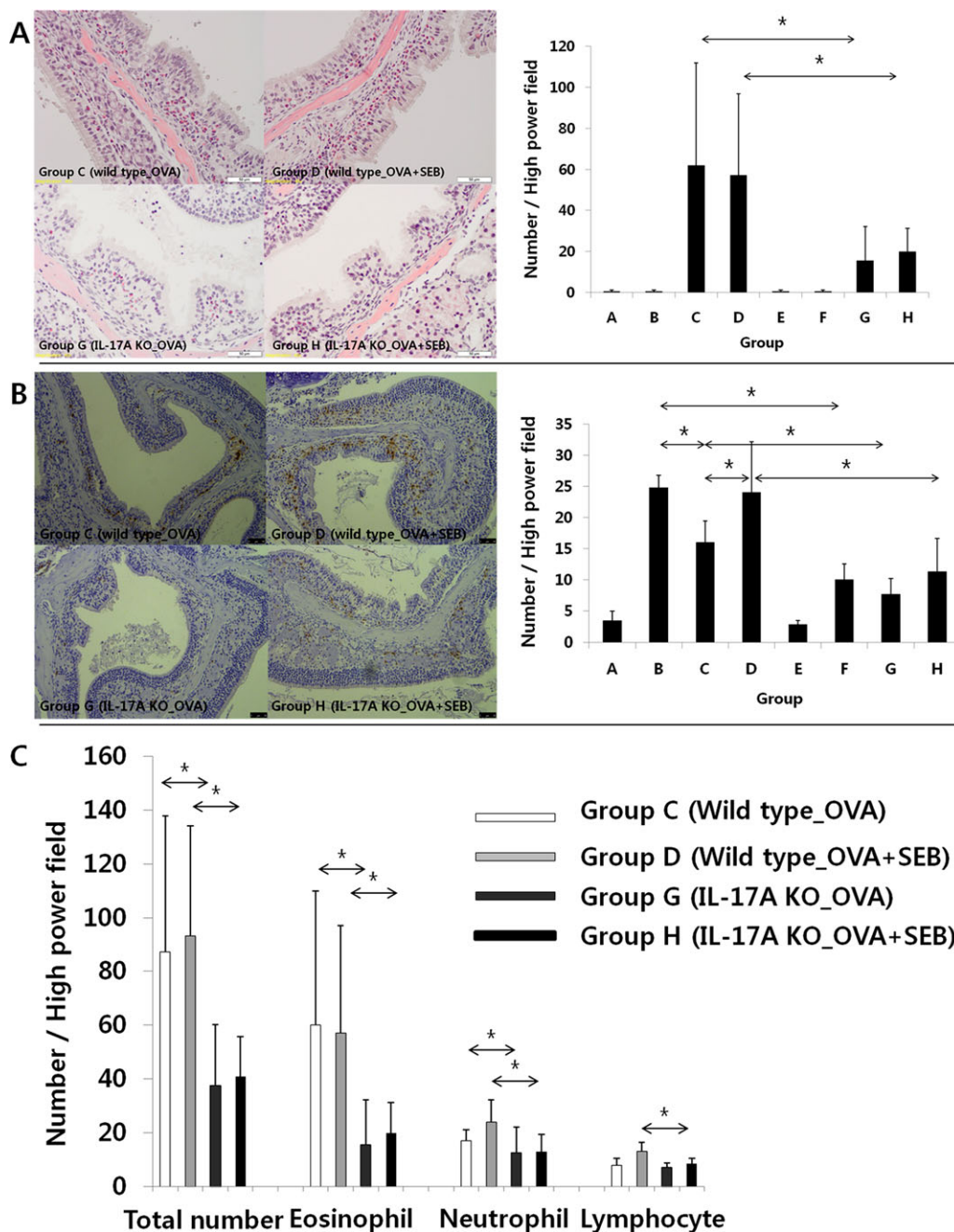


FIGURE 2. Comparison of infiltrated inflammatory cells. (A) The numbers of infiltrated eosinophils in groups C or D were statistically higher than those in groups G or H, respectively. There were no significant differences in the numbers of eosinophil infiltration either between group C and D or between group G and H, respectively (Sirius red stain, $\times 400$, $*p < 0.05$). (B) Meanwhile, the number of infiltrated neutrophils was highest in groups B and D. There was a significant difference in the number of neutrophil infiltration between groups C and D, but not between groups G and H. There were significant differences in the number of eosinophil infiltration between groups B and F, between groups C and G, and between groups D and H (immunohistochemical stain for neutrophil, $\times 200$, $*p < 0.05$). (C) In total, groups C and D showed more infiltrate of inflammatory cells in the nasal tissue and cavities than groups G and H, respectively ($*p < 0.05$). IL-17A = interleukin 17A; KO = knockout; OVA = ovalbumin; SEB = *Staphylococcus aureus* enterotoxin B.

difference in the expression level of IL-5 among groups C, D, G, and H, except between groups C and D (Fig. 5B).

Discussion

Authors have already reported that nasal exposure to SEB can induce nasal polypoid lesions in an OVA-induced CRS

murine model.¹⁵ The combination of an OVA-induced allergic response and low-dose SEB induced a significantly higher number of polypoid lesions showing more severe inflammatory cells and higher levels of eotaxin and IL-5 than SEB or OVA alone, which may explain the mechanism of nasal polyp formation. Meanwhile, IL-17A, the key cytokine of Th17 cells, mediates tissue inflammation by induction of chemokines such as CXCL-1, 2, and 8 and

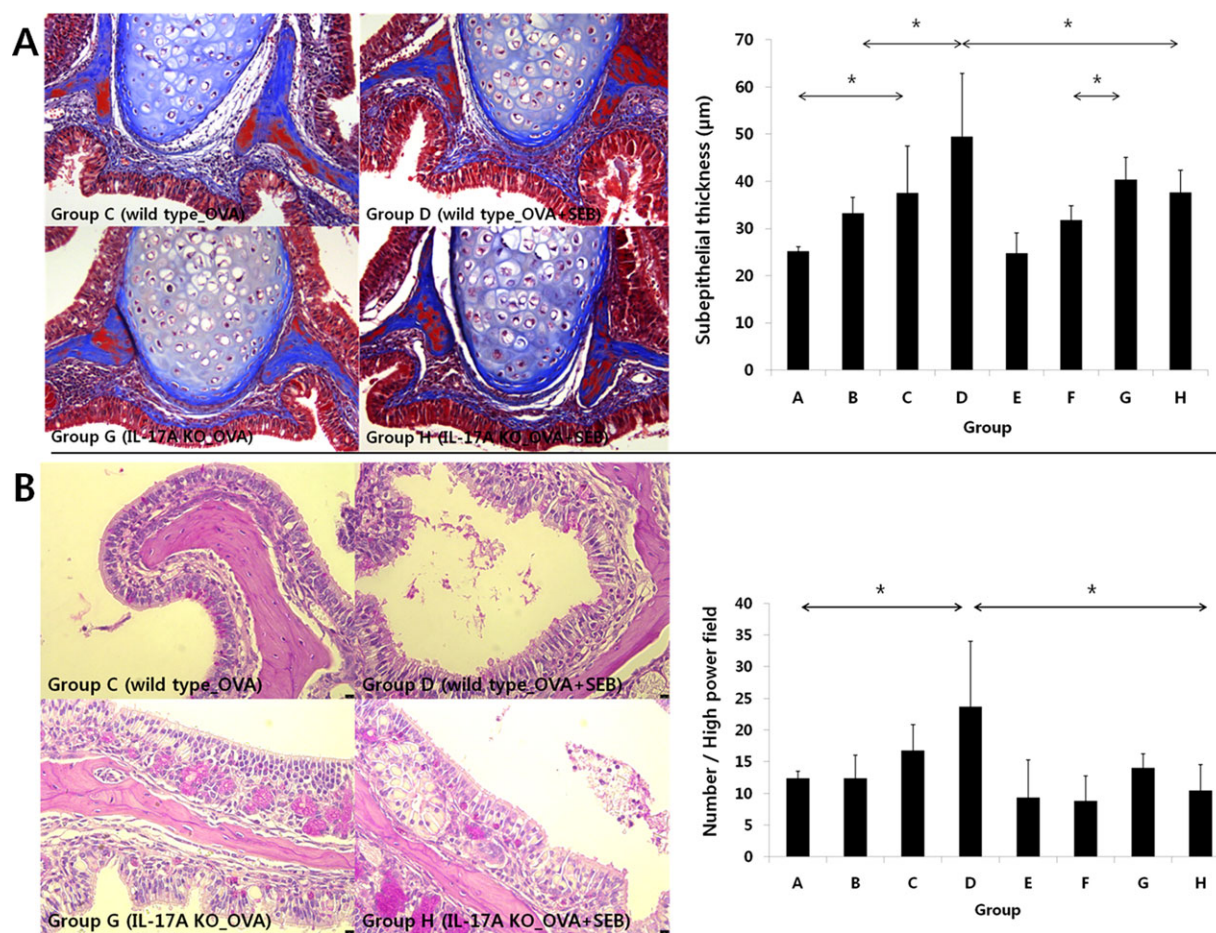


FIGURE 3. Comparison of subepithelial fibrosis and goblet cell hyperplasia among groups. (A) The subepithelial layers in groups C or D were thicker than that in groups A or B, respectively. Group H showed thinner subepithelial layers than group D did. However, thicknesses in groups C and G were not statistically different (Masson's trichrome stain, $\times 200$, $*p < 0.05$). (B) Meanwhile, the numbers of goblet cells in groups E, F, G, and H (groups with IL-17A deficiency) were smaller than those in their wild-type counterparts (groups A, B, C, and D). However, only the difference in the numbers of the cells between groups D and H was statistically significant (Periodic acid-Schiff stain, $\times 400$, $*p < 0.05$). IL-17A = interleukin 17A; KO = knockout; OVA = ovalbumin; SEB = *Staphylococcus aureus* enterotoxin B.

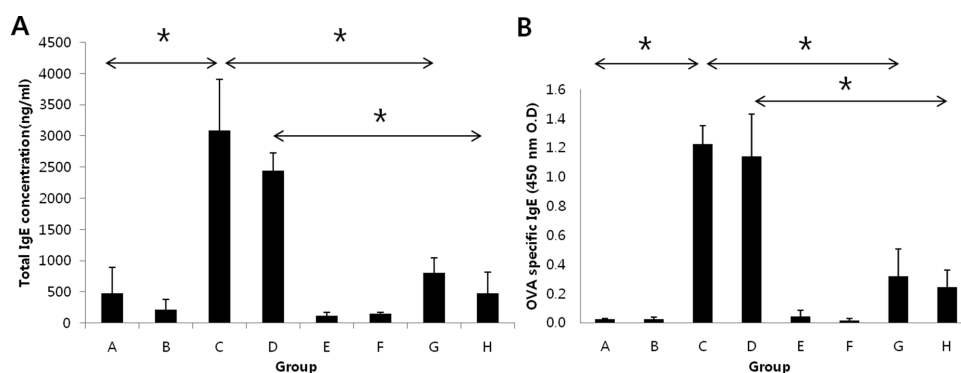


FIGURE 4. Serum total (A) and ovalbumin-specific IgE (B) levels measured by enzyme-linked immunosorbent assay. These levels were slightly higher in wild-type groups (groups C and D) than in IL-17A deficient groups (groups G and H). ($*p < 0.05$). IgE = immunoglobulin E; OD = optical density; OVA = ovalbumin.

proinflammatory cytokines such as tumor necrosis factor α (TNF- α), IL-1 β , and IL-6 in the structural cells and potentiates allergic inflammation by regulating innate immunity.¹⁶ IL-17A is known to induce Th1-type neutrophilic inflammation as well as Th2-type eosinophilic inflammation.¹⁰

Therefore, it may play an important role in the formation of nasal polypoid lesions, which is usually caused by chronic inflammatory condition in the sinonasal cavity and tried to investigate the effects of IL-17A deficiency on the formation of them in mice.

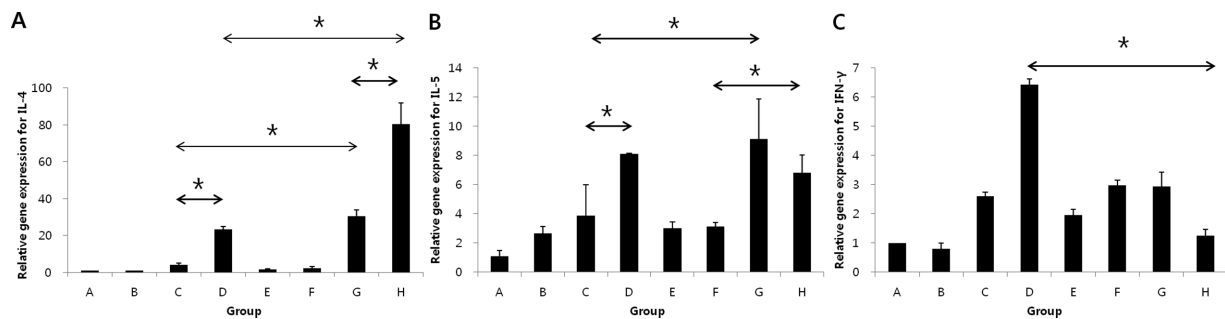


FIGURE 5. The mRNA expression of cytokines in the sinonasal mucosa measured by real-time PCR. (A) Group H showed the highest IL-4 expression, which was significantly lower in groups C, D, and G. (C) Meanwhile, group D showed the highest IFN- γ expression, which was significantly lower in groups C, G, and H. (B) However, there was no significant difference in the expression level of IL-5 among groups C, D, G, and H, except between groups C and D ($*p < 0.05$). IFN- γ = interferon γ ; IL = interleukin; mRNA = messenger RNA; PCR = polymerase chain reaction.

It has been reported that IL-17A deficiency may play an important role in nasal allergic inflammation through decrease of not only Th2 immune response but also general inflammation.¹⁷ In this experiment, as expected, it led to a significant reduction of both total and OVA-specific IgEs in sera in the long-term exposure to OVA and SEB. Both eosinophilic and neutrophilic infiltration into the nasal mucosa also decreased significantly under this condition (group H) as compared with its counterpart condition (group D). In other words, IL-17A deficiency decreased both systemic inflammation (Th1 and Th2 responses) and infiltration of inflammatory cells (eosinophils, neutrophils, and lymphocytes) into the nasal mucosa.

Moreover, it decreased the degree of both subepithelial fibrosis and goblet cell hyperplasia. They are particularly prominent in patients with asthma accompanying tissue eosinophilia.¹⁸ However, they were also reported by studies that dealt with a CRS murine model.^{19,20} These phenomena happen as a remodeling process in progression of the CRSwNP. Remodeling is generally considered to be a consequence of persistent inflammation.²¹ It includes extracellular matrix protein deposition, basement membrane thickening, goblet cell hyperplasia, subepithelial edema, and reduced vessels and glands.¹³ In this study, the long-term exposure to OVA and SEB in the murine model resulted in the subepithelial fibrosis and goblet cell hyperplasia, which decreased in the IL-17A deficiency condition. It means that IL-17A may be an important mediator of both inflammation and remodeling in CRSwNP.

However, there was no significant difference in the number of polypoid lesions between groups D and H, and real-time PCR revealed that IL-4 increased and IFN- γ decreased in the nasal mucosa under the IL-17A-deficient condition. In other words, formation of nasal polypoid lesions did not decrease under the IL-17A-deficient condition and IL-17A deficiency increased the local Th2 response and decreased the local Th1 response. Even though IL-17A can induce both Th1 and Th2 inflammation, its main role in the local inflammation of this murine model may be the induction of Th1 response but not Th2 response. Moreover, in the condition in which Th1 response decreased because of the absence of IL-17A, Th2 response could relatively in-

crease with the continuous stimuli of OVA and SEB to the nasal mucosa. This result can be partially explained by the previous report that the majority of T-cell clones which can produce IL-17A produces IL-17A alone (Th17) or IL-17A plus IFN- γ (Th17/Th1) and a smaller portion can produce both IL-17A and IL-4 (Th17/Th2).²² It suggests that IL-17A has a greater influence on Th1 inflammation than on Th2 inflammation.

It has been reported that epicutaneous exposure to SEB enhanced allergic lung inflammation via an IL-17A-dependent mechanism.²³ In their study, epicutaneous sensitization of mice with a combination of OVA and SEB significantly enhanced OVA-induced airway hyperresponsiveness and granulocytic lung inflammation than OVA alone, and when IL-17A was deleted genetically, the effect of SEB on augmenting lung inflammation and airway hyperresponsiveness markedly diminished. However, in this model in which OVA and SEB were challenged intranasally, IL-17A deficiency reduced overall inflammation except the Th2 response in the local tissue represented by IL-4. Generally, IL-17A is thought to potentiate allergic airway inflammation. However, it seems that IL-17A potentiated the Th1 response more than the Th2 response. Moreover, absence of IL-17A was insufficient to significantly reduce the formation of nasal polypoid lesions. Thus, it may be inferred that suppression of nasal inflammation by IL-17A deficiency does not have a significant effect on the formation of nasal polypoid lesions.

Previous studies have suggested that mucosal disruption may be essential for the formation of polyp in the sinonasal mucosa, and intensive inflammation induced by long-term intranasal challenge of OVA and SEB was thought to induce disruption of the nasal mucosa and formation of nasal polypoid lesions.^{15,24–26} However, our results suggest that local inflammation induced by IL-17A may not play an important role in their formation, and another mechanism may be associated with both the alteration of underlying mediators and the formation of nasal polypoid lesions.

SEB is known to induce a local increase in both IL-5 and IgE against SEB and finally result in eosinophilic inflammation.^{27,28} Several recent human studies have reported that local IgE level and key markers of local class

switching recombination to IgE are increased in CRSwNP, and these may be associated with the pathogenesis of CRSwNP.^{29,30} Therefore, in the eosinophilic CRSwNP murine model, chronic provocation of SEB may induce a local increase in IgE against SEB as well as overall inflammation, both of which may influence the development of nasal polypoid lesions. In IL-17A deficiency, overall inflammation decreased, but local IL-4 increased. IL-4 is the principal cytokine responsible for B-cell immunoglobulin class switching to the IgE phenotype.³¹ Thus, local increase in IL-4 may induce a local increase in IgE through the class switching recombination. In this condition, the local increase in IgE may cancel out the effect of reduced inflammation, and that may be the reason why decreased inflammation induced by IL-17A deficiency did not influence the development of nasal polypoid lesions.

Conclusion

IL-17A deficiency can downregulate overall inflammation in an experimentally induced CRSwNP murine model. However, instead, local Th2 response increased and formation of nasal polypoid lesion did not decrease in IL-17A deficiency conditions. Even though IL-17A plays an important role in inducing Th1-type neutrophilic inflammation as well as Th2-type eosinophilic inflammation, its absence put the sinonasal mucosa into a relatively Th2-response-dominant condition and did not reduce formation of nasal polypoid lesion. This study suggests that reduction of local inflammation induced by IL-17A deficiency may not have a significant effect on the development of nasal polyps. ④

References

- Kim JW, Hong SL, Kim YK, et al. Histological and immunological features of non-eosinophilic nasal polyps. *Otolaryngol Head Neck Surg.* 2007;137:925–930.
- Bernstein JM, Ballou M, Schlievert PM, Rich G, Allen C, Dryja D. A superantigen hypothesis for the pathogenesis of chronic hyperplastic sinusitis with massive nasal polyposis. *Am J Rhinol.* 2003;17:321–326.
- Seiberling KA, Grammer L, Kern RC. Chronic rhinosinusitis and superantigens. *Otolaryngol Clin North Am.* 2005;38:1215–1236.
- Seiberling KA, Conley DB, Tripathi A, et al. Superantigens and chronic rhinosinusitis: detection of staphylococcal exotoxins in nasal polyps. *Laryngoscope.* 2005;115:1580–1585.
- Bachert C, Zhang M, van Zele T, Gevaert P, Patou J, van Cauwenberge P. Staphylococcus aureus enterotoxins as immune stimulants in chronic rhinosinusitis. *Clin Allergy Immunol.* 2007;20:163–175.
- Bernstein JM, Allen C, Rich G, et al. Further observations on the role of *Staphylococcus aureus* exotoxins and IgE in the pathogenesis of nasal polyposis. *Laryngoscope.* 2011;121:647–655.
- Jin J, Yoon YH, Kwak SY, et al. Staphylococcal enterotoxin B induced expression of IL-17A in nasal epithelial cells and its association with pathogenesis of nasal polyposis. *Eur Arch Otorhinolaryngol.* 2014;271:525–534.
- Schnyder-Candrian S, Togbe D, Coullin I, et al. Interleukin-17 is a negative regulator of established allergic asthma. *J Exp Med.* 2006;203:2715–2725.
- 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.
- Iwakura Y, Nakae S, Saijo S, Ishigame H. The roles of IL-17A in inflammatory immune responses and host defense against pathogens. *Immunol Rev.* 2008;226:57–79.
- 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.
- Jiang XD, Li GY, Li L, Dong Z, Zhu DD. The characterization of IL-17A expression in patients with chronic rhinosinusitis with nasal polyps. *Am J Rhinol Allergy.* 2011;25:e171–e175.
- Wang H, Bai J, Ding M, et al. Interleukin-17A contributes to the expression of serum amyloid A in chronic rhinosinusitis with nasal polyps. *Eur Arch Otorhinolaryngol.* 2013;270:1867–1872.
- Hu XD, Bao YY, Zhou SH, et al. Interleukin-17A expression in patients with chronic rhinosinusitis and its relationship with clinical features. *J Int Med Res.* 2013;41:777–784.
- Kim DW, Khalmuratova R, Hur DG, et al. *Staphylococcus aureus* enterotoxin B contributes to induction of nasal polypoid lesions in an allergic rhinosinusitis murine model. *Am J Rhinol Allergy.* 2011;25:e255–e261.
- Wang YH, Liu YJ. The IL-17 cytokine family and their role in allergic inflammation. *Curr Opin Immunol.* 2008;20:697–702.
- Quan SH, Zhang YL, Han DH, et al. Contribution of interleukin 17A to the development and regulation of allergic inflammation in a murine allergic rhinitis model. *Ann Allergy Asthma Immunol.* 2012;108:342–350.
- Berry M, Morgan A, Shaw DE, et al. Pathological features and inhaled corticosteroid response of eosinophilic and non-eosinophilic asthma. *Thorax.* 2007;62:1043–1049.
- Wang H, Lu X, Cao PP, et al. Histological and immunological observations of bacterial and allergic chronic rhinosinusitis in the mouse. *Am J Rhinol.* 2008;22:343–348.
- Lim YS, Won TB, Shim WS, et al. Induction of airway remodelling of nasal mucosa by repetitive allergen challenge in a murine model of allergic rhinitis. *Ann Allergy Asthma Immunol.* 2007;98:22–31.
- Meng J, Zhou P, Liu Y, et al. The development of nasal polyp disease involves early nasal mucosal inflammation and remodelling. *PLoS One.* 2013;8:e82373.
- Cosmi L, Maggi E, Santarlasci V, et al. Identification of a novel subset of human circulating memory CD4(+) T cells that produce both IL-17A and IL-4. *J Allergy Clin Immunol.* 2010;125:222–230.e4.
- Yu J, Oh MH, Park JU, et al. Epicutaneous exposure to staphylococcal superantigen enterotoxin B enhances allergic lung inflammation via an IL-17A dependent mechanism. *PLoS One.* 2012;7:e39032.
- Norlander T, Fukami M, Westrin KM, et al. Formation of mucosal polyps in the nasal and maxillary sinus cavities by infection. *Otolaryngol Head Neck Surg.* 1993;109:522–529.
- Norlander T, Westrin KM, Fukami M, et al. Experimentally induced polyps in the sinus mucosa: a structural analysis of the initial stages. *Laryngoscope.* 1996;106:196–203.
- Norlander T, Brönnegård M, Stiern P. The relationship of nasal polyps, infection, and inflammation. *Am J Rhinol.* 1999;13:349–355.
- Van Zele T, Gevaert P, Watelet JB, et al. *Staphylococcus aureus* colonization and IgE antibody formation to enterotoxins is increased in nasal polyposis. *J Allergy Clin Immunol.* 2004;114:981–983.
- Patou J, Gevaert P, Van Zele T, Holtappels G, van Cauwenberge P, Bachert C. *Staphylococcus aureus* enterotoxin B, protein A, and lipoteichoic acid stimulations in nasal polyps. *J Allergy Clin Immunol.* 2008;121:110–115.
- Gevaert P, Nouri-Aria KT, Wu H, et al. Local receptor revision and class switching to IgE in chronic rhinosinusitis with nasal polyps. *Allergy.* 2013;68:55–63.
- Baba S, Kondo K, Toma-Hirano M, et al. Local increase in IgE and class switch recombination to IgE in nasal polyps in chronic rhinosinusitis. *Clin Exp Allergy.* 2014;44:701–712.
- Yea SS, Yang YI, Park SK, et al. Interleukin-4 C-590T polymorphism is associated with protection against nasal polyps in a Korean population. *Am J Rhinol.* 2006;20:550–553.