

Development of a mouse model of eosinophilic chronic rhinosinusitis with nasal polyp by nasal instillation of an *Aspergillus* protease and ovalbumin

Ho Chan Kim¹ · Ji Youn Lim² · Seoyeon Kim² · Ji Heui Kim² · Yong Ju Jang² 

Received: 16 May 2017 / Accepted: 12 August 2017
© Springer-Verlag GmbH Germany 2017

Abstract One subtype of chronic rhinosinusitis with nasal polyps (CRSwNP) is characterized by the development of a T-helper type 2 (Th2) response and eosinophilic infiltration. Here, we aimed to establish an eosinophilic CRSwNP murine model, which would be essential to understand the underlying pathogenesis and establish a treatment strategy. C57BL/6 mice were challenged intranasally with a mixture of an *Aspergillus oryzae*-derived protease (AP) and ovalbumin (OVA) for 6, 8, or 12 consecutive weeks (12 mice/group); control mice received the same volume of phosphate-buffered saline for 12 weeks ($n = 12$). Sinonasal samples were evaluated histologically, and interleukin (IL)-4, IL-5, IL-13, eotaxin, keratinocyte chemoattractant, and macrophage inflammatory protein-2 mRNA levels in sinonasal mucosa were measured by real-time PCR. Protein levels of Th2 cytokines, INF- γ , IL-17A, and chemokines in nasal lavage fluid, and total serum IgE were measured by ELISA. Greater eosinophil infiltration in the subepithelial layer was observed in the challenged groups, compared with the control group. Polypoid mucosal lesions were predominantly observed in the 12-week group, which also exhibited mucosal thickening on micro-CT scans. The IL-4, IL-5, and IL-13 mRNA and protein levels were elevated in the

sinonasal mucosa and nasal lavage fluid. INF- γ and IL-17A were undetectable or not elevated relative to the control group levels. In contrast, eotaxin levels were particularly elevated in the sinonasal mucosa and nasal lavage fluid in the 12-week group. In conclusion, intranasal AP and OVA exposure successfully induced Th2-specific CRSwNP in a murine model.

Keywords Administration · Intranasal · Endopeptidases · Mice · Nasal polyps · Ovalbumin · Sinusitis

Introduction

Chronic rhinosinusitis with nasal polyps (CRSwNP) is a chronic inflammatory disease of the sinonasal cavity mucosa; this disease has a multifactorial etiology and is often associated with other respiratory diseases such as asthma, aspirin hypersensitivity, idiopathic bronchiectasis, and cystic fibrosis [1, 2]. Nasal polyps (NP) can be classified as eosinophilic or non-eosinophilic polyps, based on the dominant inflammatory cell types. Among the various CRSwNP types, eosinophilic CRSwNP, which is characterized by a marked increase in the expression of T-helper type 2 (Th2) cytokines and eosinophilic infiltration, is among the most common and clinically significant, and it is difficult to treat and is prone to recurrence [3, 4]. The establishment of an appropriate murine model of eosinophilic CRSwNP may be a critically important step toward improving treatment. Several animal models of chronic rhinosinusitis (CRS) are currently available. However, researchers lack an easily inducible and appropriate animal model that accurately represents the human counterpart of eosinophilic CRS, especially with regard to successful NP development.

✉ Ji Heui Kim
jhkim0217@amc.seoul.kr

✉ Yong Ju Jang
jangyj@amc.seoul.kr

¹ Department of Otolaryngology-Head and Neck Surgery, Eulji Medical Center, Eulji University School of Medicine, Seoul, Republic of Korea

² Department of Otorhinolaryngology-Head and Neck Surgery, Asan Medical Center, University of Ulsan College of Medicine, 88 Olympic-ro 43-gil, Songpa-gu, Seoul 05505, Republic of Korea

In a previous study, we developed a murine model of eosinophilic CRS without nasal polyps via intranasal challenge with an *Aspergillus oryzae* protease (AP) plus ovalbumin (OVA) (AP + OVA) for 5 weeks without intraperitoneal OVA sensitization [5]. In the present study, we tested and confirmed the hypothesis that extended exposure to AP + OVA may induce eosinophilic CRSwNP by developing a Th2-specific eosinophilic CRSwNP murine model induced by prolonged intranasal treatment with AP + OVA.

Materials and methods

Eosinophilic CRSwNP murine model

C57BL/6 mice (5 weeks old) were purchased from Orient Bio Inc. (Sunngam, Korea). The mouse intranasal challenge was performed as previously described [5], with slight modifications. Briefly, a mixture of 2 U AP (Sigma-Aldrich, St. Louis, MO, USA) and 75 µg OVA (grade V; Sigma-Aldrich) was diluted in sterile phosphate-buffered saline (PBS) to a total volume of 20 µl and was intranasally instilled into each mouse 3 times per week for a total of 6, 8, or 12 weeks (12 mice per group). Control mice were administered 20-µl PBS but were otherwise treated identically (12 mice per group). After nasal instillation, the mouse head was maintained in a downward position to prevent reagent influx into the lungs. Histological and nasal lavage fluid analyses involved seven mice per group, whereas sinonasal tissue mRNA levels were measured in five mice per group. All experiments were approved by the Institutional Animal Care and Use Committee of the Asan Institute for Life Science (2015-12-012) and conducted according to international guidelines on animal experimentation.

Histological analysis

At the end of the experiment, the mice were decapitated and the skulls were fixed in 10% neutral-buffered formalin for 1 day, decalcified, and embedded in paraffin. The embedded tissue was cut into serial 4-µm-thick coronal sections and stained with hematoxylin and eosin (H&E) to detect NP lesions, Sirius red to detect eosinophils, and an anti-mouse neutrophil monoclonal antibody (1:200, NIMR-R14, ab2557, Abcam, Cambridge, UK) to detect neutrophils in the subepithelial layer. NP lesions were defined as distinct elevations of the mucosa with eosinophil infiltration and microcavity formation [6]. The true maxillary sinus and ethmoid labyrinths were also identified. The numbers of NP lesions and infiltrating eosinophils were counted in two coronal sections using a high-power field (HPF; 400× magnification), and the average value from four different areas was used for between-group comparisons. This analysis was performed

independently by two examiners blinded to the group assignments. When the values from the two examiners differed by 10%, a consensus was reached via a simultaneous review of the slides under a multihead microscope. On the same two coronal sections, the maximal subepithelial thickness at the transition zone between the olfactory and respiratory epithelia of the ethmoid labyrinths was measured using an image analysis system (cellSens Standard software version 1.7; Olympus, Tokyo, Japan) [7].

Micro-CT evaluation

One day before sacrifice, the skull of each mouse in the 12-week group was subjected to micro-CT scanning (Sky-scan 1176; Bruker-microCT, Kontich, Belgium) with an 18-µm resolution protocol. Each mouse was sedated with an intraperitoneal injection of Zoletil (15 mg/kg) and Rumpun (5 mg/kg). For the sinusitis evaluation, the same coronal plane showing the true maxillary sinuses and ethmoid labyrinths was selected for each mouse.

Analysis of mRNA and protein levels of cytokines and chemokines in the sinonasal mucosa and nasal lavage fluid

Total mRNA from the sinonasal mucosa was prepared using a RNeasy Mini Kit (Qiagen, Venlo, The Netherlands), and the levels of interleukin (IL)-4, IL-5, IL-13, eotaxin/chemokine (C–C motif) ligand 11 (CCL11), keratinocyte chemoattractant/chemokine (C–X–C motif) ligand 1 (KC/CXCL1), and macrophage inflammatory protein-2/chemokine (C–X–C motif) ligand 2 (MIP-2/CXCL2) mRNA were determined. Primers were purchased from Bioneer (Daejeon, Korea). cDNA was synthesized using the M-MuLV reverse transcriptase (RT-&GOTM kit, MP Biomedicals, Santa Ana, CA, USA) and specific primers for IL-4 (forward: 5'-GGTCTCAACCCCCAGCTAGT-3'; reverse: 5'-GCCGATGATCTCTCTCAAGTGAT-3'), IL-5 (forward: 5'-GGGCTTCCTGCTCCTATCTA-3'; reverse: 5'-CAGTCATGGCACAGTCTGAT-3'), IL-13 (forward: 5'-CCTGGCTCTTGCTTGCTT-3'; reverse: 5'-GGTCTTGTGTGATGTTGCTCA-3'), eotaxin/CCL11 (forward: 5'-CTGCTTGATTCCTTCTCTTCCTAA-3'; reverse: 5'-GGAACCTACATGAAGCCAA GTCCTT-3'), KC (forward: 5'-TGGCTGGGATTACCTCA A-3'; reverse: 5'-GAGTGTGGCTATGACTTCGGTTT-3'), and MIP-2 (forward: 5'-ACCAACCACCAGGCTACAG-3'; reverse: 5'-GCGTCACACTCAAGCTCT-3'). The reactions were performed using a LightCycler 480 real-time PCR system (Roche, Pleasanton, CA, USA).

Nasal lavage fluid was collected as previously described [5]. Briefly, after a partial tracheal resection was performed under anesthesia, a 24-gauge catheter was inserted into the tracheal opening in the direction of the posterior choanae.

Thereafter, 400 μ l of PBS was gently introduced into the nasal cavity, and the fluid from the nostril was collected and centrifuged. The levels of IL-4, IL-13, interferon (IFN)- γ , IL-17A, KC/CXCL1, and MIP-2/CXCL2 in the nasal lavage fluid were measured using a multiplex immunoassay (ProcartaPlex[®] Multiplex Immunoassays, eBioscience, Vienna, Austria) according to the manufacturer's protocols. The concentrations of IL-5 and eotaxin/CCL11 were measured using the DuoSet Mouse ELISA Kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

Analysis of serum total IgE levels

The serum levels of total IgE were measured using the BD OptEIA[™] Mouse IgE ELISA Set (BD Pharmingen, San Diego, CA, USA) according to the manufacturer's instructions.

Statistical analysis

Between-group comparisons were performed using a non-parametric Mann–Whitney *U* test. Statistical analyses were performed using SPSS statistical software (version 16.0; SPSS, Chicago, IL, USA). A *p* value of <0.05 was considered statistically significant.

Results

Histological analysis

Polypoid lesions were observed in 6 of the 7 mice (85.7%) in the 12-week group (Fig. 1a, b). Only 3 of the 7 mice (42.9%) in the 8-week group had polypoid lesions, whereas none of the mice in the 6-week group had polyps. Additionally, no polypoid lesions were observed in the control group.

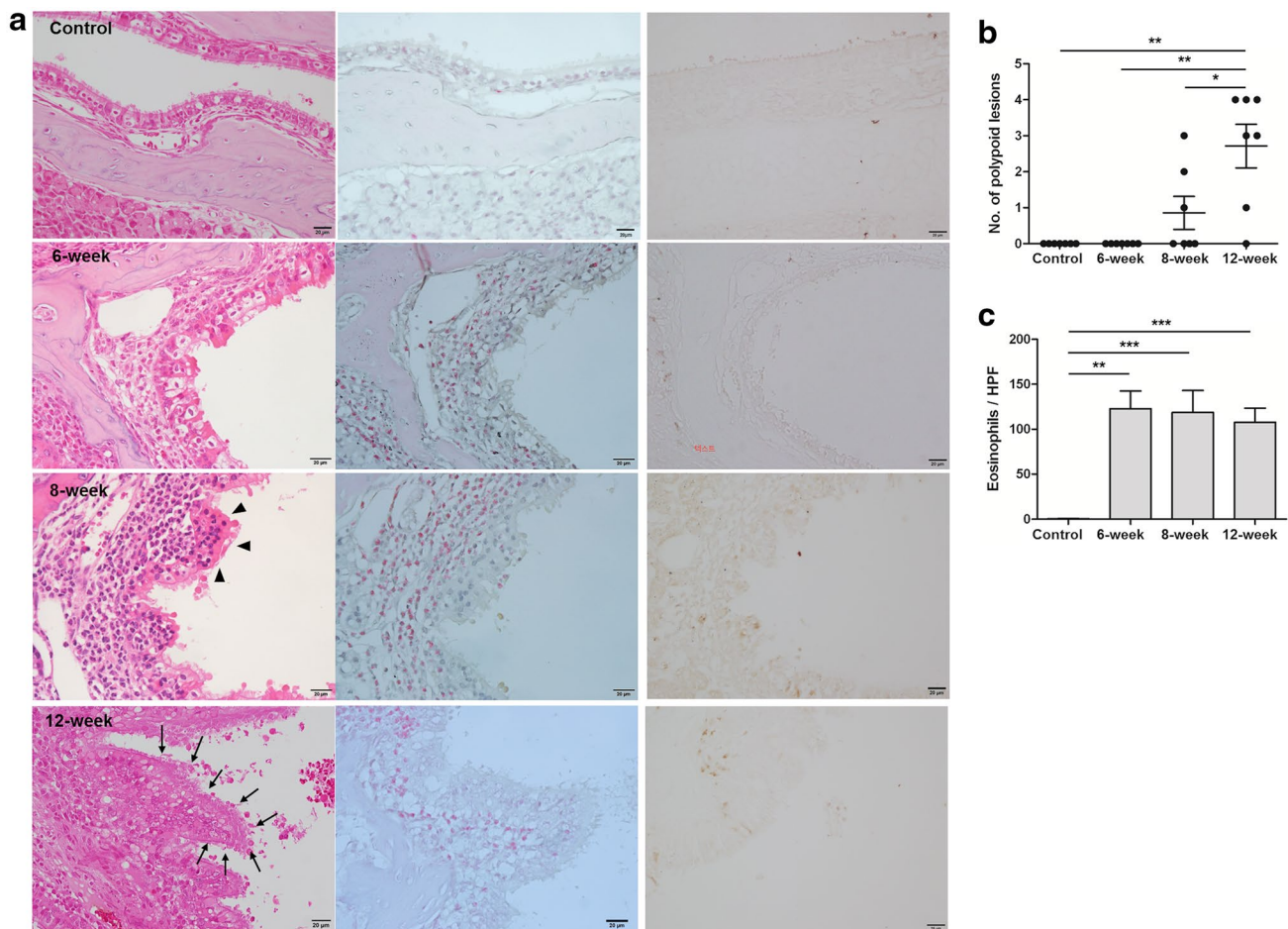


Fig. 1 Histologic findings of mice challenged with *Aspergillus* protease and ovalbumin for 6-, 8-, and 12 weeks. **a** Mucosal bulges (arrowheads) were detected in the 8- and 12-week groups, which also developed polypoid lesions (arrows). Hematoxylin–eosin stain (left column), sirius red stain (middle column), and neutrophil-specific

immunohistochemistry (brown, right column) (×400 magnification). **b** In the 12-week group, polypoid lesions were found in 6 out of 7 mice (85.7%). **c** Eosinophil counts in the 6-, 8-, and 12-week groups were significantly elevated, compared to the control group. **p* < 0.05; ***p* < 0.01; ****p* < 0.001

Significantly greater numbers of infiltrating eosinophils were observed in the 6-week (127.3 ± 47.9 cells/HPF), 8-week (118.5 ± 69.3 cells/HPF), and 12-week (105.4 ± 45.0 cells/HPF) groups, compared with the control group (0.4 ± 0.9 cells/HPF) ($p = 0.003$, $p = 0.002$, and $p = 0.003$, respectively); however, the eosinophil counts did not differ significantly among the 6-, 8-, and 12-week groups (Fig. 1a, c). Eosinophilic exudates and Charcot–Leyden crystal-like materials were also observed in the 6-, 8-, and 12-week groups. Thus, the AP + OVA intranasal challenge likely induces polyp formation between 8 and 12 weeks of exposure. Furthermore, we observed no prominent increase in infiltrating neutrophils in the lamina propria of AP + OVA challenged mice (Fig. 1a). The maximal subepithelial thickness was significantly greater in the 6- (84.5 ± 20.9 μ m), 8- (95.0 ± 21.8 μ m), and 12-week (102.7 ± 20.8 μ m) groups relative to the control group (42.0 ± 21.7 μ m) ($p = 0.010$,

$p = 0.002$, and $p = 0.003$, respectively). Although the subepithelium thickness tended to increase over time, the differences observed between the 6-, 8-, and 12-week groups were not statistically significant (Fig. 2).

Micro-CT evaluation

Coronal CT scan images of the 12-week group appeared consistent with the histology findings. The CT findings from the 12-week group included diffuse mucosal thickening of the nasal septum, ethmoid labyrinths, and true maxillary sinuses. These mice also exhibited an uneven mucosal surface, whereas control mice exhibited a smooth and thin mucosal lining (Fig. 3).

Th1/2/17 cytokines and chemokines in sinonasal mucosa and in nasal lavage fluid

The AP + OVA-challenged groups exhibited elevated levels of IL-4, IL-5, IL-13, and eotaxin/CCL11 mRNA in the sinonasal mucosa (Fig. 4a). Notably, the IL-5 mRNA levels in the 6-, 8-, and 12-week groups were significantly higher than those in the control group ($p = 0.021$, $p = 0.021$, and $p = 0.021$, respectively). The eotaxin/CCL11 mRNA level was also significantly elevated in the 12-week group relative to the control, 6-, and 8-week groups ($p = 0.016$, $p = 0.029$, and $p = 0.029$, respectively). In contrast, the mRNA levels of KC/CXCL1 and MIP-2/CXCL2 did not differ significantly among the experimental groups (AP + OVA challenged and control) (Fig. 4b).

The IL-4, IL-5, and IL-13 protein levels in the nasal lavage fluid were significantly elevated in the three AP + OVA-challenged groups. In particular, the IL-4 levels were significantly higher in the 12-week group than in the 6- and 8-week

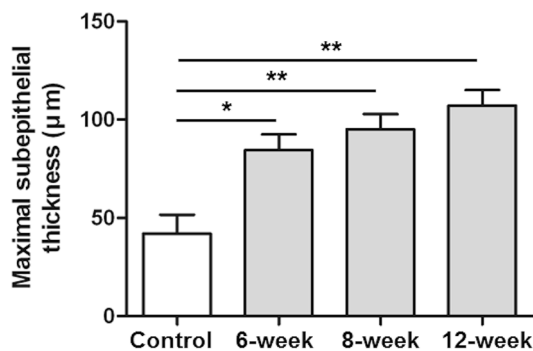


Fig. 2 Subepithelial thicknesses in experimental mice challenged with *Aspergillus* protease and ovalbumin for 6-, 8-, and 12 weeks. The maximal subepithelial tissue thicknesses were greater in the 6-, 8-, and 12-week groups than in the control group. ** $p < 0.01$; *** $p < 0.001$

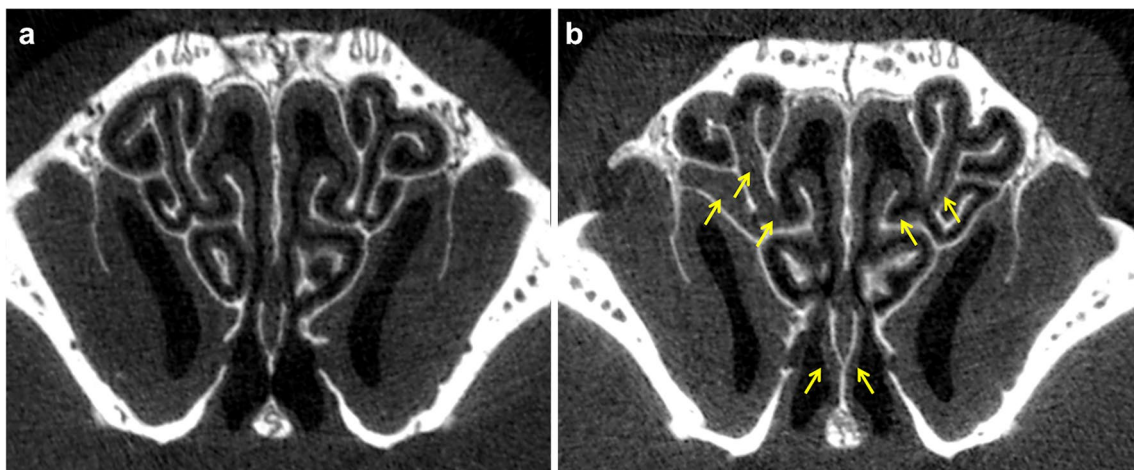


Fig. 3 Micro-CT scan images. The control group (a) exhibits smooth and thin mucosa, whereas the 12-week group (b) exhibits uneven and diffusely swollen mucosa (arrow)

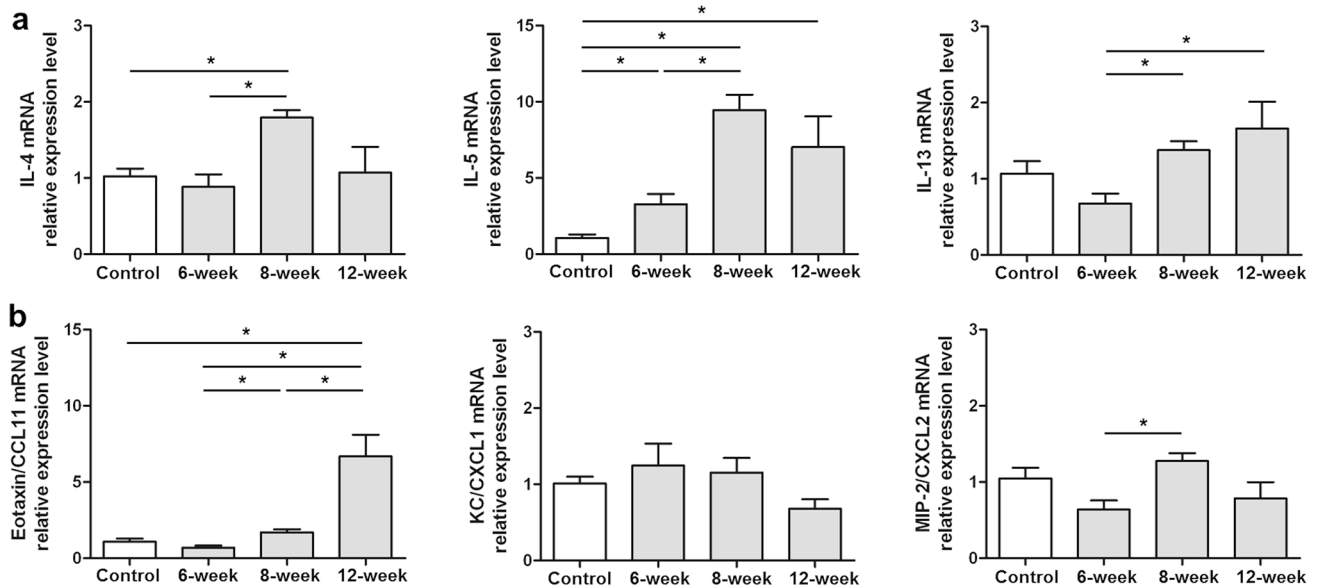


Fig. 4 mRNA levels of T-helper type 2 (Th2) cytokines and chemokines in the sinonasal mucosa of mice challenged with *Aspergillus* protease and ovalbumin for 6-, 8-, and 12 weeks. The mRNA

levels of IL-4, IL-5, IL-13, and eotaxin/CCL11 were significantly elevated in the AP + OVA-challenged groups relative to controls. * $p < .05$

groups ($p < 0.001$ and $p = 0.03$, respectively) (Fig. 5a). In contrast, the IFN- γ and IL-17A levels remained undetectable or did not differ among the study groups (Fig. 5b). The eotaxin/CCL11 protein levels were significantly higher in the 6-, 8-, and 12-week groups than in the control group ($p = 0.001$, $p < 0.001$, and $p < 0.001$, respectively). Furthermore, the eotaxin/CCL11 levels were significantly higher in the 12-week group than in the 6- and 8-week groups ($p = 0.03$ and $p = 0.001$, respectively) (Fig. 5c). The protein levels of KC/CXCL1 and MIP-2/CXCL2 were elevated in all three AP + OVA challenged groups than in the control group, whereas the MIP-2/CXCL2 levels were elevated only in the 12-week group relative to the 6- and 8-week groups ($p = 0.008$ and $p = 0.15$, respectively) (Fig. 5c).

Total serum IgE levels

The total serum IgE levels were higher in AP + OVA-challenged mice than in control mice (30.7 ± 13.3 ng/ml). However, there were no significant differences in the total serum IgE levels among the 6- (324.31 ± 175.5 ng/ml), 8- (491.1 ± 387.8 ng/ml), and 12-week (415.6 ± 146.5 ng/ml) groups (Fig. 6).

Discussion

In the present study, we successfully developed a murine model of eosinophilic CRSwNP via the nasal instillation of AP and OVA without systemic sensitization. This model

showed histological changes typical of human eosinophilic CRSwNP, including polypoid lesions, eosinophil infiltration, epithelial thickening, and subepithelial fibrosis. The levels of IL-4, IL-5, IL-13, and eotaxin/CCL11, but not IFN- γ and IL-17A, were elevated in both the sinonasal mucosa and nasal lavage fluid. In humans, eosinophilic polyp is characterized by edematous stroma containing numerous eosinophils and a thickened basement membrane [8]. Despite interspecies differences, previous researchers defined the features of eosinophilic NP in mice as mucosal bulges with eosinophilic infiltration and microcavities, similar to human NP [6, 8]. In the current study, NP lesions were successfully established and exhibited eosinophilic infiltration with mucosal bulging, microcavities, and a thickened subepithelial layer. Micro-CT scans of mice in the 12-week group revealed diffuse thickening and irregular surfaces in the sinonasal mucosa, but did not provide detailed evidence for mucosal bulging, compared with the histologic findings.

A few attempts have been made to develop an animal model of NP associated with CRS. In one previous study, *Staphylococcus aureus* enterotoxin B (SEB) was used as an adjuvant to induce NPs in an allergic CRS murine model [6]. In that study, a mixture of SEB and OVA was administered through the mouse nostril to trigger an allergic reaction and successfully establish an NP lesion. However, that model required systemic sensitization with OVA, followed by daily intranasal instillation with OVA from day 12 to day 17, a thrice-weekly intranasal challenge with OVA from day 21 to day 102, and a once-weekly SEB challenge day 49 through day 102 after OVA instillation [6, 9, 10]. Lindsay et al. [11],

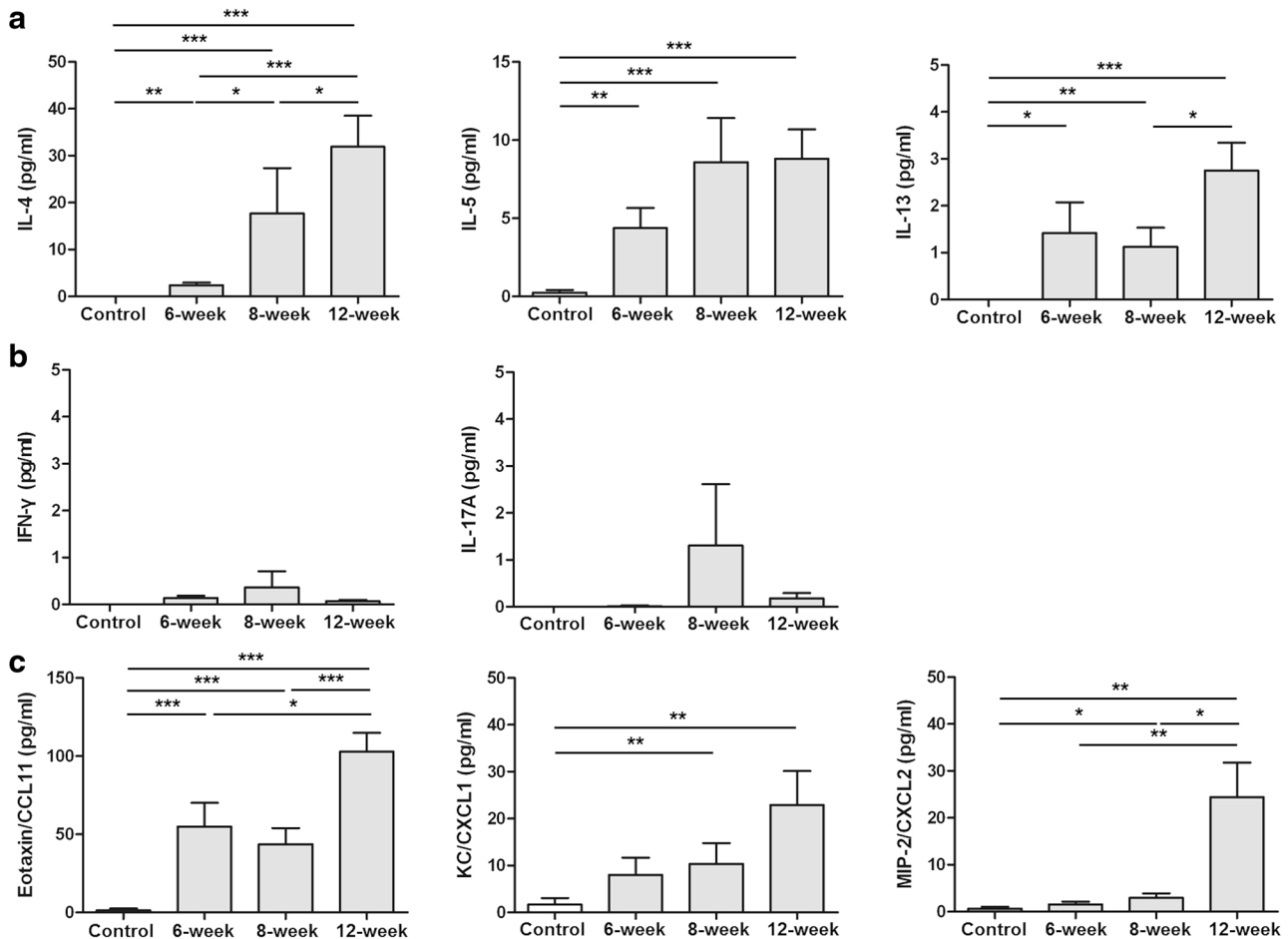


Fig. 5 Protein levels of T-helper type 1 (Th1)/Th2/Th17 cytokines and chemokines in the nasal lavage fluid of mice challenged with *Aspergillus* protease (AP) and ovalbumin for 6-, 8-, and 12 weeks.

IL-4, IL-5, and IL-13 protein levels were significantly elevated in the 3 AP + OVA-challenged groups relative to controls. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

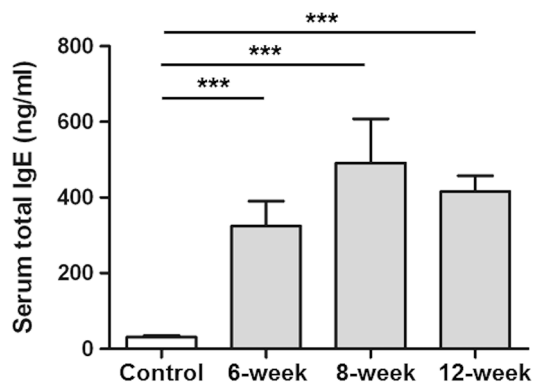


Fig. 6 Total serum IgE levels in mice challenged with *Aspergillus* protease (AP) for 6-, 8-, and 12 weeks. The total serum IgE levels were greater in AP + OVA-challenged mice than in control mice. *** $p < 0.001$

also developed a murine model of eosinophilic CRS without NP using an *A. fumigatus* extract for both the sensitization (intraperitoneal) and challenge (intranasal) material. In contrast, the experimental protocol used in the current study was relatively simple and did not require systemic sensitization. The entire protocol required a thrice-weekly AP + OVA intranasal instillation for 12 consecutive weeks. Although we did not observe an advantage with respect to the duration of polyp development, our protocol could minimize the risk of investigator errors during a long experimental period, and may increase the reproducibility of eosinophilic CRSwNP mouse generation.

Fungi are well-known inhaled antigens and have been strongly linked to human allergic illnesses. Several studies have assessed the allergenicity of *A. fumigatus* in the airways. This species was found to readily induce allergic lung inflammation when administered only topically, without the need for additional adjuvants [12, 13]. According to previous reports, protease activity is the main mechanism by which

airway hyper-responsiveness to fungal extracts is induced [14, 15]. In a study by Kheradmand et al. the inhalation of protease-active *A. oryzae*, but not its protease-inactive counterpart, elicited nearly equivalent airway hyper-responsiveness in the presence or absence of OVA, suggesting that the protease is a key inducer of allergic inflammation in the airway mucosa [14]. In our previous study, we noted that topical nasal challenges with AP + OVA over 5 consecutive weeks could induce more severe allergic inflammation in mice, compared with OVA alone [5]. In the present study, prolonged intranasal exposure to AP + OVA over 12 weeks induced the development of Th2-specific eosinophilic CRSwNP in mice. *Aspergillus*-derived proteases may contribute to NP development by inducing allergic inflammation, as well as by causing damage to the epithelial layer and thus enhancing presentation to the immune system (Th2 response) [16]. Because eosinophilic CRSwNP can be classified as an allergic airway disease, the majority of CRS mouse model studies have adopted and modified allergic airway disease animal models to generate eosinophilic CRSwNP mouse models [6, 9, 10, 17].

In the present study, we assessed the time course of intranasal challenge with AP + OVA. All mice but one (85.7%) developed polyps after a 12-week challenge. Subepithelial eosinophil infiltration was enhanced after a 6-week challenge, consistent with the results of a previous study [5] wherein an eosinophilic CRS murine model was developed via intranasal challenge with AP + OVA within 5 consecutive weeks and was sustained for 12 weeks. Furthermore, in the present study, the mRNA levels of IL-5 and eotaxin/CCL11 in the sinonasal mucosa were significantly higher in the 6-, 8-, and 12-week AP + OVA-challenged groups than in the control group. In the nasal lavage fluid, the levels of Th2 cytokines and eotaxin/CCL11 were also elevated in the 6-, 8-, and 12-week AP + OVA-challenged groups. Notably, the levels of eotaxin/CCL11 in sinonasal mucosa and nasal lavage fluid were significantly greater in the 12-week group than in the 6- and 8-week groups. These results suggest that the period of exposure to an eosinophilic Th2-milieu condition is among the most important factors affecting NP development. Moreover, our model may reflect a Th2-specific CRSwNP murine model that is distinct from the OVA plus SEB-induced CRSwNP murine model, which exhibits mixed Th1/Th2/Th17 features [9].

Our study had certain limitations. First, we did not clarify the discordance between the mRNA levels of the neutrophilic chemokines KC/CXCL1 and MIP-2/CXCL2 in the sinonasal mucosa and the corresponding protein levels in the nasal lavage fluid from the AP + OVA challenged groups; specifically, the protein levels of these chemokines were increased in the nasal lavage fluid, although the mRNA levels were not elevated in the sinonasal mucosa. However, as an immunohistochemistry analysis with an

anti-neutrophil-antibody did not reveal increased neutrophil infiltration in the subepithelial layer, the mRNA levels of these chemokines in the sinonasal mucosa might be a more reliable measure than the protein levels in nasal lavage fluid. Second, the sample size in each experimental group was relatively small. However, other sinusitis animal model studies also used fewer than 10 mice/group. Additionally, we used this model in other studies, where we consistently identified polypoid tissues in a 12-week exposure model.

Conclusion

In conclusion, we developed an eosinophilic CRSwNP mouse model via the local instillation of AP and OVA. Our results identify the exposure period to a Th2 milieu consequent to the AP and OVA challenge as one of the most important factors affecting eosinophilic NP development. The elevated levels of Th2-specific cytokines and eosinophilic chemokines in the sinonasal mucosa and nasal lavage fluid indicate that our model may serve as a useful tool for studying the pathophysiology of eosinophilic CRSwNP.

Compliance with ethical standards

Funding This study was supported by Grants from the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Education, Science, and Technology (2012R1A1A2020059) and the Ministry of Science, ICT, and Future Planning (2014R1A1A3050498).

Conflict of interest The authors declare no potential conflicts of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This study was approved by the Institutional Animal Care and Use Committee of the Asan Institute for Life Science (2015-12-012).

References

1. Chaaban MR, Walsh EM, Woodworth BA (2013) Epidemiology and differential diagnosis of nasal polyps. *Am J Rhinol Allergy* 27:473–478. doi:10.2500/ajra.2013.27.3981
2. McGarry G, Melia L (2009) Nasal polyps: an update. *Br J Hosp Med (Lond)* 70:500–504. doi:10.12968/hmed.2009.70.9.43865
3. Zhang N, Van Zele T, Perez-Novoa C, Van Bruene N, Holtappels G, DeRuyck N, Van Cauwenberge P, Bachert C (2008) Different types of T-effector cells orchestrate mucosal inflammation in chronic sinus disease. *J Allergy Clin Immunol* 122:961–968. doi:10.1016/j.jaci.2008.07.008
4. Payne SC, Borish L, Steinke JW (2011) Genetics and phenotyping in chronic sinusitis. *J Allergy Clin Immunol* 128:710–720. doi:10.1016/j.jaci.2011.05.022 (quiz 721–712)
5. Kim JH, Yi JS, Gong CH, Jang YJ (2014) Development of *Aspergillus* protease with ovalbumin-induced allergic chronic

- rhinosinusitis model in the mouse. *Am J Rhinol Allergy* 28:465–470. doi:[10.2500/ajra.2014.28.4100](https://doi.org/10.2500/ajra.2014.28.4100)
6. Kim DW, Khalmuratova R, Hur DG, Jeon SY, Kim SW, Shin HW, Lee CH, Rhee CS (2011) *Staphylococcus aureus* enterotoxin B contributes to induction of nasal polypoid lesions in an allergic rhinosinusitis murine model. *Am J Rhinol Allergy* 25:e255–e261. doi:[10.2500/ajra.2011.25.3727](https://doi.org/10.2500/ajra.2011.25.3727)
 7. Kim SW, Kim JH, Jung MH, Hur DG, Lee HK, Jeon SY, Kim DW (2013) Periostin may play a protective role in the development of eosinophilic chronic rhinosinusitis with nasal polyps in a mouse model. *Laryngoscope* 123:1075–1081. doi:[10.1002/lary.23786](https://doi.org/10.1002/lary.23786)
 8. Hellquist HB (1996) Nasal polyps update. *Histopathol Allergy Asthma Proc* 17:237–242
 9. Shin HW, Kim DK, Park MH, Eun KM, Lee M, So D, Kong IG, Mo JH, Yang MS, Jin HR, Park JW, Kim DW (2015) IL-25 as a novel therapeutic target in nasal polyps of patients with chronic rhinosinusitis. *J Allergy Clin Immunol* 135(1476–1485):e1477. doi:[10.1016/j.jaci.2015.01.003](https://doi.org/10.1016/j.jaci.2015.01.003)
 10. Lee KI, Kim DW, Kim EH, Kim JH, Samivel R, Kwon JE, Ahn JC, Chung YJ, Mo JH (2014) Cigarette smoke promotes eosinophilic inflammation, airway remodeling, and nasal polyps in a murine polyp model. *Am J Rhinol Allergy* 28:208–214. doi:[10.2500/ajra.2014.28.4055](https://doi.org/10.2500/ajra.2014.28.4055)
 11. Lindsay R, Slaughter T, Britton-Webb J, Mog SR, Conran R, Tadros M, Earl N, Fox D, Roberts J, Bolger WE (2006) Development of a murine model of chronic rhinosinusitis. *Otolaryngol Head Neck Surg* 134:724–730. doi:[10.1016/j.otohns.2005.11.048](https://doi.org/10.1016/j.otohns.2005.11.048) (**discussion 731–722**)
 12. Mehlhop PD, van de Rijn M, Goldberg AB, Brewer JP, Kurup VP, Martin TR, Oettgen HC (1997) Allergen-induced bronchial hyper-reactivity and eosinophilic inflammation occur in the absence of IgE in a mouse model of asthma. *Proc Natl Acad Sci USA* 94:1344–1349
 13. Corry DB, Grunig G, Hadeiba H, Kurup VP, Warnock ML, Shepard D, Rennick DM, Locksley RM (1998) Requirements for allergen-induced airway hyperreactivity in T and B cell-deficient mice. *Mol Med* 4:344–355
 14. Kheradmand F, Kiss A, Xu J, Lee SH, Kolattukudy PE, Corry DB (2002) A protease-activated pathway underlying Th cell type 2 activation and allergic lung disease. *J Immunol* 169:5904–5911
 15. Balenga NA, Klichinsky M, Xie Z, Chan EC, Zhao M, Jude J, Laviolette M, Panettieri RA Jr, Druey KM (2015) A fungal protease allergen provokes airway hyper-responsiveness in asthma. *Nat Commun* 6:6763. doi:[10.1038/ncomms7763](https://doi.org/10.1038/ncomms7763)
 16. Kauffman HF (2003) Interaction of environmental allergens with airway epithelium as a key component of asthma. *Curr Allergy Asthma Rep*. doi:[10.1007/s11882-003-0021-x](https://doi.org/10.1007/s11882-003-0021-x)
 17. Kim SW, Kim DW, Khalmuratova R, Kim JH, Jung MH, Chang DY, Shin EC, Lee HK, Shin HW, Rhee CS, Jeon SY, Min YG (2013) Resveratrol prevents development of eosinophilic rhinosinusitis with nasal polyps in a mouse model. *Allergy* 68:862–869. doi:[10.1111/all.12132](https://doi.org/10.1111/all.12132)