

Japanese Cedar Pollen–Specific IgA in Nasal Secretions and Nasal Allergy Symptoms

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Abstract

Objective: IgA-dependent degranulation of eosinophils and positive correlation between IgA and eosinophil cytotoxic protein levels in nasal secretions have been reported. However, the association between IgA and allergic reactions remains unclear. Therefore, we evaluated the changes in Japanese cedar–specific IgA levels and allergy symptoms after Japanese cedar pollen scattering in symptomatic and asymptomatic individuals sensitized to Japanese cedar pollen.

Methods: Nasal secretion and serum samples were collected from 31 participants (21 symptomatic and 10 asymptomatic participants) in January (preseason) and March (peak season). Japanese cedar–specific IgA or IgE levels were measured using ELISA with diamond-like carbon-coated chips.

Results: The ratio of Japanese cedar pollen–specific IgA to total IgA (rIgA) in the nasal secretions of symptomatic participants increased significantly in March compared with that in January ($P < .01$); however, the ratio of specific IgE to total IgE (rIgE) in nasal secretions did not. rIgA in nasal secretions among asymptomatic participants also did not increase during pollen season. rIgA in nasal secretions was significantly correlated with nasal allergic symptoms ($r = 0.82$; $P < .0001$) with no significant correlation between rIgE and symptoms.

Conclusions: To our knowledge, this is the first study to show an association between nasal symptoms and rIgA in nasal secretions, suggesting that rIgA is useful as an antigen-specific biomarker for allergic rhinitis or pollinosis. Furthermore, rIgA values in nasal secretions do not increase in asymptomatic participants sensitized to Japanese cedar during the pollen season.

Keywords

allergic rhinitis, pollinosis, IgA, IgE, symptom

Introduction

IgE has been focused on because of its role in mediating type 1 allergy, namely, allergic rhinitis. IgA has been also highlighted as a promoting factor of allergic rhinitis, although its role with respect to allergic rhinitis remains unclear. IgA is mainly found as a monomer in serum but can also be found in mucous secretions as a dimer linked to a secretory component (known as secretory IgA). Abu-Ghazaleh et al¹ showed that secretory IgA was a strong trigger for eosinophil degranulation, whereas IgE was a weak trigger. Eosinophils and their products play a crucial role in allergic inflammation of mucosal tissues. Eosinophil cationic protein (ECP) is one of the main proteins released by activated eosinophils. Several studies have shown that IgA is positively correlated with nasal fluid ECP concentrations.^{2–4} Additionally, pollen-specific IgA in nasal fluid increases during the pollen season.^{5,6} Considering this, IgA secreted in nasal mucosa may be associated with allergic

reactions and symptoms via IgA-dependent eosinophil cytotoxicity.

Many studies have suggested that eosinophils or ECP could be markers of allergic responses and symptoms.^{7–11} However, whether ECP could be a marker of allergen-specific responses and symptoms remains controversial because many allergens induce ECP in individuals sensitized to multiple allergens. Therefore, markers that can be used to monitor allergen-specific allergic responses and their expected symptoms are needed.

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Many people experience allergic rhinitis worldwide. Japanese cedar is one of the main allergens that cause severe allergic rhinitis in Japan.¹² Some people are sensitized to Japanese cedar but do not exhibit symptoms during the pollen season; therefore, it is important to investigate allergic responses in both asymptomatic and symptomatic individuals. In this study, we examined Japanese cedar-specific IgE and IgA in serum and nasal secretions of both symptomatic and asymptomatic individuals sensitized to Japanese cedar.

Methods

Participants and sample collection

We included individuals with sensitization to Japanese cedar, which was determined according to the presence of Japanese cedar pollen-specific IgE in serum using the CAP system (Pharmacia & Upjohn, Uppsala, Sweden). Exclusion criteria included the existence of any nasal disease, such as sinusitis, nasal tumor, and nasal polyp; specific immunotherapy; age <20 or >60 years; pregnancy; any other diseases except allergic rhinitis or pollinosis; or refusing to provide consent. After providing a thorough explanation of the objectives and methods of this study, written consent to participate was obtained from all included participants. Patient anonymity was preserved using methods approved by the Ethics Committee. As the Japanese cedar pollen season starts in February and peaks in March throughout the study region, evaluation was performed in the middle of January (preseason) and the middle of March (peak season). Nasal secretions and serum samples were collected from all participants. Nasal secretions were collected from the nasal cavity using the gelfoam (Spongel) absorption technique. Spongel was placed in the nasal cavity for 15 minutes, and nasal specimens were eluted with 1 ml saline. After centrifugation to exclude insoluble particles, the supernatant was collected and preserved in a freezer until measurement. Participants were not allowed to use therapeutic treatments such as nasal and oral corticosteroids for 4 weeks before sample collection. The present study involved a clinical trial with an interventional approach, and the trial was pre-registered in a public registry. This study was approved by the Nagoya City University ethical committee (15-1227).

Symptom score

A questionnaire was administered to all participants to assess symptoms. Symptom severity was evaluated using the Japanese guidelines for allergic rhinitis.¹³ The frequency of sneezing was classified as none, 1 to 5, 6 to 10, 11 to 20, and >21 times per day, with scores of 0 to 4, respectively. The frequency of nose blowing, which represents the degree of nasal secretion, was classified as none, 1 to 5, 6 to 10, 11 to 20, and >21 times per day, with scores of 0 to 4, respectively. Nasal obstruction was classified as none, a feeling of

obstruction but mouth breathing was not required, obstruction that sometimes required mouth breathing, obstruction that required mouth breathing most of the day, and obstruction requiring mouth breathing for the entire day; these were also scored 0 to 4, respectively. The symptoms score was evaluated as the sum of the scores for sneezing, nose blowing, and nasal obstruction. The mean of symptom scores on the previous 1 day and 2 days before sample collection was calculated and used as the symptom score.

Antigen-specific IgA and IgE in serum and nasal secretions

Antigen-specific IgA and IgE in the serum and nasal secretions of participants were measured using ELISA with diamond-like carbon-coated chips, which is 4.3 to 142 times more sensitive than a conventional ELISA assay.¹⁴ Diamond-like carbon-coated chips have high antigen immobilization capacity via carboxylated arms on the surface of a diamond-like carbon chip.¹⁴ This high immobilization capacity allows for superior sensitivity. The ELISA procedure has been described elsewhere.¹⁴ For this study, individual chips were incubated with 20 μ L of 1:10 diluted serum or 1:1 eluted nasal secretion incubated with primary antibody for 60 minutes at 37°C. Chips were then washed twice and dried using centrifugation. HiLyte Fluor TM 555-labeled secondary antibody against human IgE (Millipore, Bedford, Massachusetts, USA) or human IgA (Zymed Laboratories Inc, South San Francisco, California, USA) was applied and incubated for 1 hour. The chips were then washed twice and dried. The fluorescent intensity of each spotted antigen was measured by scanning, and the concentration of antigen-specific immunoglobulin was determined. The ratio of Japanese cedar pollen-specific IgA (IU/mL) to total IgA (μ g/mL) (rIgA) and the ratio of specific Japanese cedar pollen IgE (IU/mL) to total IgE (ng/mL) (rIgE) for nasal secretions and serum were also calculated, as previously reported.⁵

ECP in nasal secretions

Quantities of ECP in nasal secretions were measured using sandwich ELISA, with an ECP ELISA kit (Aviscera Bioscience Inc., Santa Clara, California, USA). Nasal secretions were added to plates coated with purified antibody against ECP. Plates were then incubated with an ECP detection antibody. Standard curves were also generated to determine ECP concentrations.

Statistical analysis

Data are expressed as mean $\pm$ standard error of the mean. A statistical comparison of the results between symptomatic and asymptomatic participants was performed using the

Table 1. Levels of Antibodies in Subjects Who Are Sensitized With Japanese Cedar Pollen.

	Patients With Symptom in March		Patients Without symptom in March	
	January	March	January	March
Nasal secretion				
Specific IgA (IU/mL)	3.9 ± 1.1	12.3 ± 3.9**	3.1 ± 0.9	2.8 ± 1.0††
Total IgA (μg/mL)	142.2 ± 15.0	156.4 ± 16.7	113.6 ± 15.5	119.3 ± 6.9
rIgA	0.030 ± 0.010	0.104 ± 0.023**	0.032 ± 0.011	0.025 ± 0.009†
Specific IgE (IU/mL)	10.5 ± 8.3	9.7 ± 7.8	5.0 ± 2.9	7.8 ± 3.9
Total IgE (ng/mL)	42.3 ± 14.0	35.9 ± 12.1	44.8 ± 13.2	43.3 ± 11.4
rIgE	0.13 ± 0.05	0.11 ± 0.03	0.06 ± 0.02	0.10 ± 0.03
Sera				
Specific IgA (IU/mL)	27.3 ± 4.6	28.4 ± 3.8	22.1 ± 5.2	25.9 ± 6.5
Total IgA (μg/mL)	1925 ± 121	2071 ± 132	1947 ± 174	2128 ± 366
rIgA in sera	0.016 ± 0.003	0.015 ± 0.003	0.014 ± 0.005	0.019 ± 0.006
Specific IgE (IU/mL)	11.8 ± 4.1	14.4 ± 4.4*	10.7 ± 3.6	11.2 ± 4.1
Total IgE (ng/mL)	107.6 ± 14.9	133.5 ± 22.2	113.7 ± 29.1	105.1 ± 23.7
rIgE in sera	0.085 ± 0.015	0.092 ± 0.014	0.074 ± 0.011	0.087 ± 0.012

Abbreviations: rIgA, the ratio of Japanese cedar pollen-specific IgA to total IgA (μg/mL); rIgE, the ratio of Japanese cedar pollen-specific IgE to total IgE (ng/mL).

* $P < .05$ versus patients with symptom in January. ** $P < .01$ versus patients with symptom in January. † $P < .05$ versus patients with symptom in March.

†† $P < .01$ versus patients with symptom in March.

chi-square test for sex and Mann-Whitney test for age and antibody levels. The Wilcoxon signed-rank test was also used for comparison of the results before and during the pollen season. Correlations between nasal secretions and serum as well as those between symptoms and antibody levels were estimated using Pearson's product-moment correlation coefficient with a 2-tailed P value. A P value $< .05$ was considered significant.

Results

Patient characteristics

Thirty-one participants were included in this study. Participants were classified into 2 groups: symptomatic and asymptomatic participants in March. There were 21 symptomatic participants during Japanese cedar pollen season (13 women and 8 men, mean age 40.1 ± 1.8 years, range, 24-53 years) and 10 asymptomatic participants (6 women and 4 men, mean age 41.6 ± 3.7 years, range, 25-58 years). No participants had a past history of asthma or atopic dermatitis. There were no significant differences in age or sex between the 2 groups.

Pollen distribution

Japanese cedar pollen was monitored with a Durham sampler. The pollen sampler was placed on the roof of our institute from January 1 to March 31 in 2016. No Japanese cedar pollen scattering was seen in January. Pollen distribution started in February, and the highest daily pollen count occurred in March.

Changes in Japanese cedar pollen-specific IgA and IgE

Among participants sensitized to Japanese cedar pollen, we examined changes in nasal secretion and serum antibody levels in symptomatic and asymptomatic participants and compared the pre-pollen season (January) with the pollen season (March). As shown in Table 1, among symptomatic participants, specific IgA in nasal secretions was significantly higher among samples collected in March than those collected in January. However, there was no significant difference in specific IgA values of nasal secretions among asymptomatic participants between January and March. rIgA values in nasal secretions of symptomatic participants were also significantly higher in March than in January, whereas there was no significant difference in rIgA in nasal secretions of asymptomatic participants between samples collected in January and March. Additionally, in nasal secretions, there were significant differences in specific IgA and rIgA in March between symptomatic and asymptomatic participants. There was no significant difference between serum rIgA collected in January and that collected in March from symptomatic and asymptomatic participants.

The rIgE values in nasal secretions and serum samples collected in either January or March were also evaluated (Table 1). In both symptomatic and asymptomatic participants, there was no significant difference between rIgE in nasal secretions collected in January and March. In addition, rIgE in serum collected in January did not differ significantly from that in serum collected in March among symptomatic and asymptomatic participants.

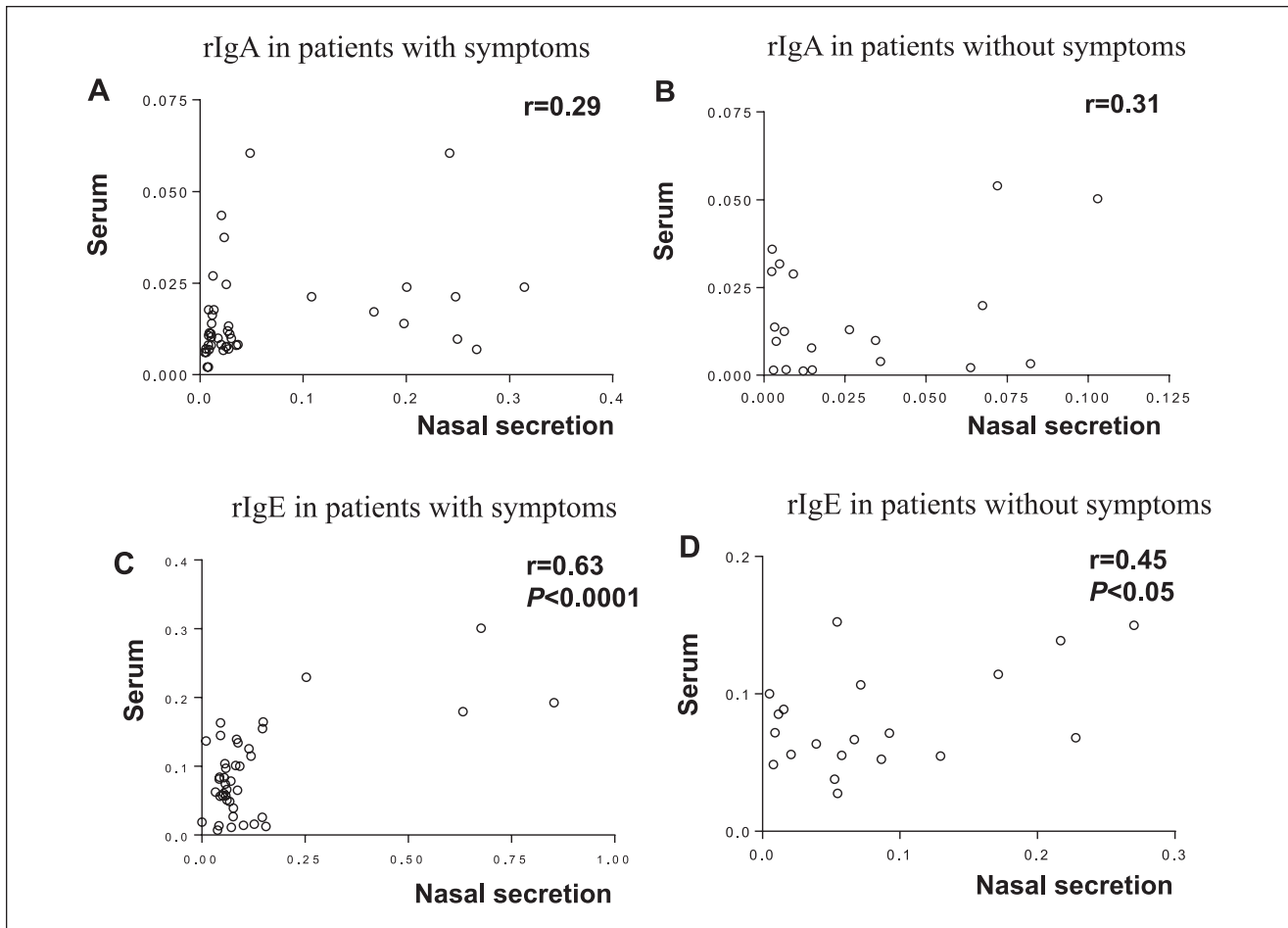


Figure 1. Correlations between antibody levels in nasal secretions and those in serum samples of symptomatic and asymptomatic participants collected in January and March. Correlations between the ratio of Japanese cedar pollen-specific IgA to total IgA (rIgA) in nasal secretions and serum among (A) symptomatic participants and (B) asymptomatic ones. Correlations between the ratio of Japanese cedar pollen-specific IgE to total IgE (rIgE) in nasal secretions and that in serum of (C) symptomatic participants and (D) asymptomatic ones.

Correlation between antibody levels in nasal secretions and serum samples

Next, we assessed whether there was a correlation between Japanese cedar pollen-specific antibody levels in nasal secretions and serum samples collected in January and March. There was no significant correlation between rIgA values for nasal secretions and those for serum among symptomatic and asymptomatic participants (Figures 1A, 1B). However, there was a significant correlation between rIgE in nasal secretions and serum of symptomatic participants (Figure 1C). There was also a significant correlation between rIgE in nasal secretions and serum of asymptomatic participants, although the correlation coefficient was lower among asymptomatic participants than symptomatic participants (Figure 1D).

Correlation between levels of Japanese cedar pollen-specific immunoglobulins and allergic nasal symptoms

We examined the correlation between levels of Japanese cedar-specific antibodies and nasal symptoms in symptomatic participants using only samples collected in March. As shown in Figure 2A, a significant correlation between symptom score and specific IgA in nasal secretions was observed. Figure 2B shows no significant correlation between symptom score and specific IgE in nasal secretions. A significant correlation between symptom score and rIgA in nasal secretions can be seen in Figure 3C. Additionally, there were no significant correlations between symptom scores and rIgE in nasal secretions (Figure 2D), rIgA in serum (Figure 2E), and rIgE in serum (Figure 2F).

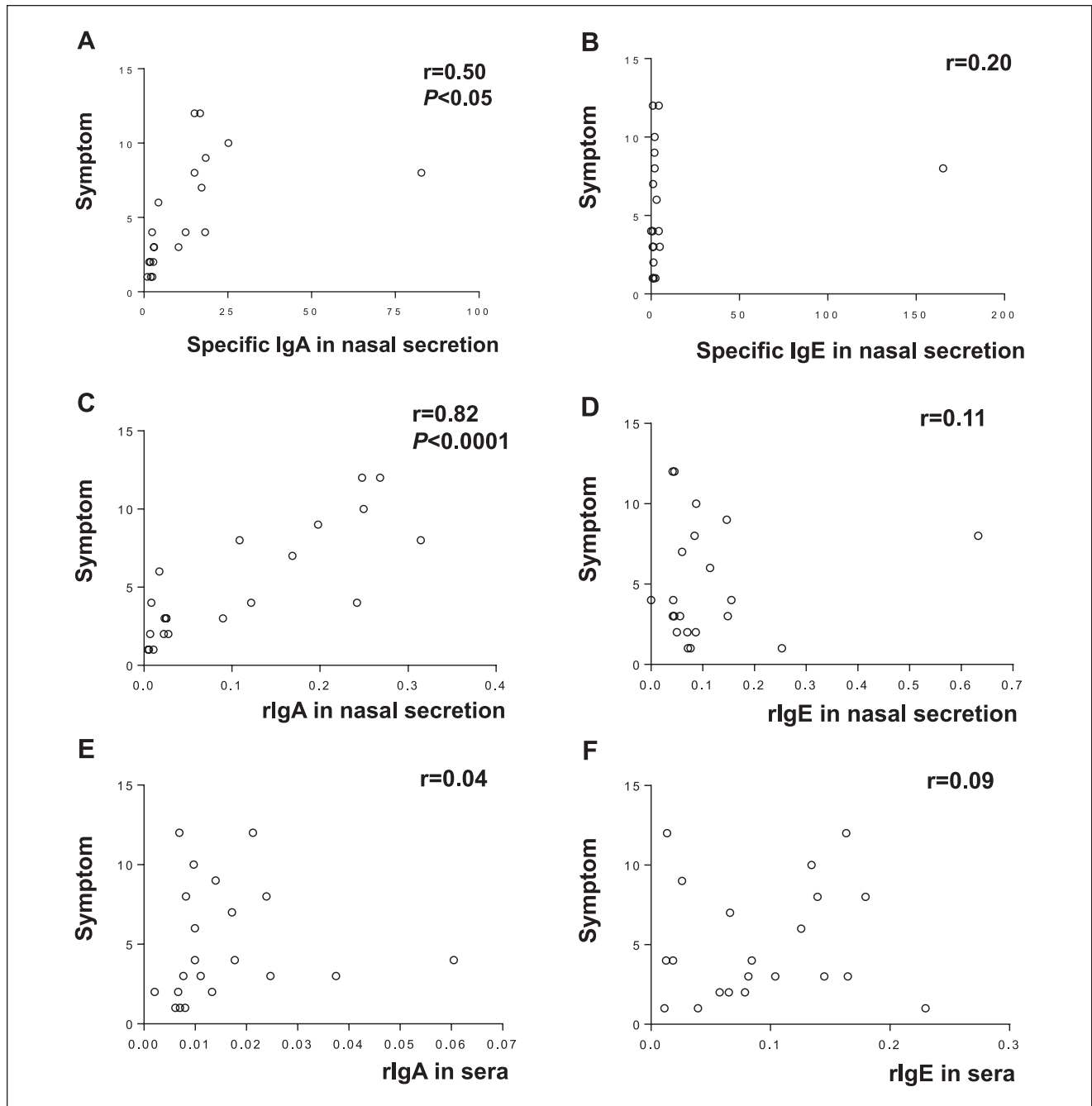


Figure 2. Correlations between symptom score and antibody levels in nasal secretions and serum samples collected in March. Correlation of (A) symptom score and Japanese cedar pollen-specific IgA and (B) those of symptoms and Japanese cedar pollen-specific IgE in nasal secretions. Correlations of (C) symptom score and ratio of Japanese cedar pollen-specific IgA to total IgA (rIgA) in nasal secretions, (D) symptoms and ratio of Japanese cedar pollen-specific IgE to total IgE (rIgE) in nasal secretions, (E) symptoms and rIgA in serum, and (F) symptoms and rIgE in serum.

ECP in nasal secretions

We examined ECP levels in nasal secretions among symptomatic participants using only samples collected in March. The ECP level in nasal secretions was 428.5 ± 73.7 ng/mL.

As shown in Figure 3A, there was a significant correlation between symptom score and ECP in nasal secretions. There was also a significant correlation between rIgA and ECP in nasal secretions (Figure 3B).

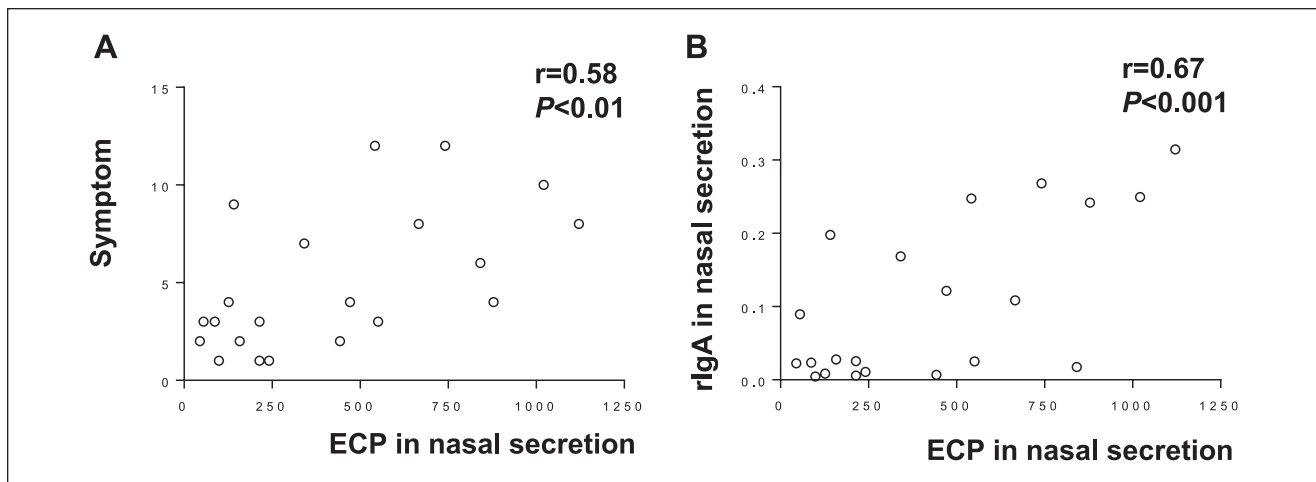


Figure 3. Nasal secretion eosinophilic cationic protein (ECP) in March among symptomatic participants. (A) Correlation of symptom score and level of ECP in nasal secretions. (B) Correlation of the ratio of Japanese cedar pollen-specific IgA to total IgA (rIgA) and ECP in nasal secretions.

Discussion

This study showed that cedar-specific IgA antibody in the nasal secretions of our symptomatic individuals was significantly increased during the pollen season, although cedar-specific IgE in nasal secretions did not change significantly. Keen et al⁵ reported that birch-specific IgA levels in nasal secretions increases ($P = .047$) during pollen season in participants with pollinosis. Reed et al⁶ also found that ragweed-specific IgA levels in nasal lavage specimens increased by the end of pollen season but IgE levels did not. Mite-specific IgA levels in lavage fluid significantly increased after a nasal provocation test.² These results of previous reports are consistent with our results.

One question that remains is the function of antigen-specific IgA in nasal secretions. It is well known that IgA secreted in the nasal mucosa binds inhaled pathogens (eg, influenza virus) and neutralizes the pathogen to prevent pathogen penetration of mucosal tissue.¹⁵ In this context, IgA may prevent antigen penetration of mucosal tissues by neutralizing antibody or acting as a competitive antibody in binding to the antigen, thereby preventing IgE from binding to the antigen. However, in allergic responses, there is no clear evidence to prove the aforementioned hypothesis. However, Abu-Ghazaleh et al.¹ found that IgA, not IgE, induced degranulation of eosinophil-derived neurotoxin, and secretory IgA was the most potent stimulus. Tomassini et al¹⁶ also found that ECP was released when eosinophils were incubated with anti-IgA monoclonal antibody; ECP could not be detected after adding anti-IgE monoclonal antibody. Additionally, Motegi et al¹⁷ demonstrated that compared with serum IgA, secretory IgA could induce greater degranulation and superoxide production by activating human eosinophils. Considering this, IgA in nasal secretions

may play an important role in regulating eosinophil functions in vivo in diseases associated with mucosal eosinophilia as well as various allergic diseases.

In the present study, specific IgA or IgE was measured using diamond-like carbon-coated chips, whose high immobilization capacity allows for high sensitivity in detection.¹⁴ Consequently, we showed a significant association between cedar-specific IgA and nasal allergy symptoms, suggesting that specific IgA in nasal secretions could serve as a biomarker for allergic rhinitis. The mechanism leading to the increase in IgA levels is unknown; however, there are several possibilities. IL-5 is a crucial type II cytokine and is an upstream mediator of allergic reaction. IL-5 is important not only for eosinophil differentiation,¹⁸ activation,¹⁹ and chemotaxis²⁰ but also for the production of IgA.²¹ Because IL-5 orchestrates both eosinophil recruitment and IgA production simultaneously, secretory IgA produced in the nasal mucosa could serve as a biomarker for eosinophil inflammation. Terada et al.²² also found that the number of eosinophils, amount of ECP, and amount of secretory IgA in nasal lavage fluid increased significantly after nasal application of IL-5 in participants with Japanese cedar pollinosis, demonstrating that IL-5 can induce both eosinophil recruitment and IgA production. Furthermore, IgA can enhance eosinophil inflammation through IgA-dependent eosinophil degranulation, as mentioned previously. Terada et al²² confirmed this enhancement with additional secretory IgA administration. Nasal administration of secretory IgA after the application of IL-5 induced significantly more ECP release to nasal lavage fluid than with administration of IL-5 alone.

Sakaida et al²³ reported that there was no significant correlation between IgE in nasal secretions and serum of

asymptomatic participants, although they found a significant correlation between IgE in nasal secretions and serum of symptomatic participants. In the present study, we also showed that there was a significant correlation between rIgE in nasal secretions and serum samples of both symptomatic and asymptomatic participants, although the correlation coefficient in symptomatic participants was higher than that in asymptomatic ones. This suggests that cedar-specific IgE in nasal secretions comes from serum. In contrast, there was no significant correlation between rIgA levels in nasal secretions and serum among symptomatic and asymptomatic participants, suggesting that specific IgA in nasal secretions is generated in the nose.

In this study, participants who were not sensitized to cedar pollen were not included because allergen-specific IgA has not been demonstrated in nasal secretions of healthy controls.²⁴ Therefore, we compared antibody levels between symptomatic and asymptomatic participants. Consequently, we found that specific IgA in nasal secretions was not increased in asymptomatic participants sensitized to cedar after pollen distribution and that the level of specific IgA in the nasal secretions of symptomatic participants was significantly higher than that of asymptomatic participants during the pollen season. Allergen-specific IgA in nasal secretions may serve as an antigen-specific biomarker to distinguish between symptomatic and asymptomatic participants.

To our knowledge, there have been no reports showing a significant correlation between symptoms and IgA in nasal secretions. Nickelson et al²⁵ demonstrated that there was no correlation between IgA in nasal discharge and symptoms during the pollen season. Platts-Mills et al²⁶ also reported that IgA in nasal discharge did not correlate with seasonal allergic symptoms in people with hay fever. The level of specific antibody is not homogeneous in nasal secretions, although we found a significant correlation between symptoms and specific IgA in nasal secretions in this study. Because an uncertain amount of nasal secretion is absorbed during elution with saline in the present study, the concentration of specific immunoglobulins in nasal secretions cannot be compared directly. To investigate IgA against influenza in nasal secretions, rIgA has been used.^{27,28} Keen et al⁵ showed that the ratio of Bet v 1-specific IgA to total IgA was increased in children with allergy. Moreover, the total IgA level in nasal washes from patients with allergy was not significantly different from that in nasal washes from normal individuals.^{24,29} Therefore, in this study, we used the ratio of Japanese cedar pollen-specific antibodies to total antibodies, as used in Keen et al's⁵ study. Consequently, a significant increase in rIgA was seen in symptomatic participants but not asymptomatic participants. And we found a significant and stronger correlation between nasal symptoms and rIgA in the nasal secretions of symptomatic participants ($r = 0.82$) compared with nasal

symptoms and specific IgA in nasal secretions ($r = 0.50$). Furthermore, the correlation coefficient ($r = 0.82$) between nasal symptoms and rIgA in the nasal secretions was higher than that ($r = 0.58$) between nasal symptoms and ECP in the nasal secretions, although there was a significant correlation between rIgA and ECP in nasal secretion. Additionally, rIgA may be an antigen-specific biomarker, whereas ECP is an antigen-nonspecific biomarker. Considering these results, rIgA in nasal secretions may be used as an antigen-specific biomarker for eosinophilic inflammation and allergic symptoms. rIgA also may be used to differentiate between participants who are only sensitized to an antigen and those who are likely to exhibit symptoms.

The limitations of this study are that the study sample was small, samples must be collected from nasal secretions, and the differences between symptomatic and asymptomatic participants were only observed postexposure. Further studies are necessary to develop IgA in nasal secretions as a useful antigen-specific biomarker to distinguish between individuals who will be symptomatic and those who will not. Additionally, our results provide some insight into the mechanisms of allergic symptom onset, thus contributing to the development of therapies for allergic rhinitis.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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