

Correlation between specific IgA and eosinophil numbers in the lavage fluid of patients with perennial allergic rhinitis

Jeong-Hoon Oh, M.D., Ph.D.,* Gyu-Young Hur, M.D.,# Young-Min Ye, M.D.,# Jeong Eun Kim, M.D.,# Keehyun Park, M.D., Ph.D.,§ and Hae-Sim Park, M.D., Ph.D.†

ABSTRACT

The mechanisms of eosinophil activation in perennial allergic rhinitis (AR) are not fully understood but may be mediated by a series of inflammatory mediators, including locally produced specific antibodies. The aim of this study was to evaluate the role of locally produced allergen-specific antibodies in the nasal lavage fluid during the recruitment of inflammatory cells in AR. Thirteen patients with AR and a positive nasal provocation test (NPT) with Dermatophagoides pteronyssinus and 10 asymptomatic subjects, sensitized to D. pteronyssinus but who had a negative NPT were enrolled. Nasal lavage fluids were collected before and at 10, 30, and 60 minutes and 3, 6, and 24 hours after the NPT. Inflammatory cells were counted, and eosinophil cationic protein (ECP) and specific immunoglobulin E (IgE), IgG, and IgA antibodies were measured by enzyme-linked immunosorbent assay. After NPT, six subjects showed early responses and seven subjects showed dual responses. The eosinophil count and ECP level in lavage fluid were significantly increased after NPT in patients with positive NPTs, and these values were significantly higher than in the negative group. The specific IgA level in lavage fluid was increased after NPT in the positive group, and significant differences were noted for both the early and the late positive responses. Significant correlations were found between specific IgA and ECP levels in lavage fluid during early and late responses. Eosinophil recruitment occurred during both early and late responses after allergen exposure in perennial AR. Locally produced allergen-specific IgA (but not IgE or IgG) may be involved in eosinophil activation in both early and late responses.

(Allergy Asthma Proc 29:152–160, 2008; doi: 10.2500/aap.2008.29.3069)

Key words: Antigen challenge, ECP, eosinophil recruitment, IgA, IgE, IgG, late phase, nasal provocation, perennial allergic rhinitis, specific antibody

Allergic rhinitis (AR) is characterized by an immunoglobulin E (IgE)–dependent release of mediators from infiltrating mast and inflammatory cells, including eosinophils.^{1,2} Challenge with a relevant allergen in patients with AR provokes an immediate response in which mast cells and IgE play important roles.^{3,4} A dual response may be observed at 3–24 hours after allergen provocation,⁵ and nasal hyperresponsiveness after allergen provocation has been associated with eosinophilia of the nasal airway and blood.^{6–8}

Allergen-specific IgE, IgA, and IgG have been shown in nasal secretions from patients with seasonal AR who are sensitive to grass and ragweed pollens, raising the

possibility of using allergen-specific antibodies as local markers of inflammation.⁹ The provocation test data and clinical symptoms of rhinitis suggest a much better correlation with local levels of specific IgE than with serum concentrations.¹⁰ However, based on a number of previous reports, the magnitude of the increase in specific antibodies after allergen stimulation can vary. The role of IgE as a key molecule in allergic inflammation has been established in numerous studies. Although many studies have implicated IgG or IgA as a contributing factor in the allergic inflammation of asthmatic airways,¹¹ the roles of IgA and IgG in AR are not fully understood.

Currently, there is no universally accepted nasal provocation model for the house-dust mite (HDM), and because of the continuous exposure to HDM allergens, it is difficult to establish a clear, symptom-free baseline in perennial rhinitis patients.¹² To the best of our knowledge, only a few reports have established allergen-specific immunoglobulins in nasal lavage fluid from patients with perennial AR.

The aim of this study was to quantify the relationship between locally produced specific antibodies and eosinophil activation in perennial AR. Therefore, we observed changes in nasal symptoms and inflammatory cell counts, including eosinophils, after allergen

From the *Department of Otolaryngology–Head and Neck Surgery, College of Medicine, The Catholic University of Korea, Seoul, Korea, and Departments of #Allergy and Rheumatology and §Otolaryngology, Ajou University School of Medicine, Suwon, Korea

Supported by a Grant A050571 from the Korea Health 21 R & D Project, Ministry of Health and Welfare, R.O.K.

This study was approved by the Ethics Committee of the Medical Faculty of Ajou University, and written informed consent was obtained from each subject

Address correspondence and reprint requests to Hae-Sim Park, M.D., Department of Allergy and Rheumatology, Ajou University School of Medicine, San-5, Wonchondong, Yongsong-gu, Suwon 442-712, Korea

E-mail address: hspark@ajou.ac.kr

Copyright © 2008, OceanSide Publications, Inc., U.S.A.

provocation in AR patients sensitive to HDM. We also measured allergen-specific immunoglobulins present in the nasal lavage fluid of AR patients at serial time points after allergen provocation and examined the correlation of immunoglobulin levels with inflammatory cell counts and eosinophil cationic protein (ECP).

MATERIALS AND METHODS

Subjects

Thirteen patients who were diagnosed with AR and had a positive response to a nasal provocation test (NPT) with the HDM (*Dermatophagoides pteronyssinus*), as indicated by an increase of more than three points in the nasal symptom score on provocation, were enrolled as the positive group. For a control, 10 asymptomatic volunteers who were sensitized to the same HDM but had no NPT response were enrolled as the negative group. AR was diagnosed based on clinical history, skin-prick tests, and a positive response on an NPT with HDM (*D. pteronyssinus*) allergen. None of the subjects exhibited acute nasal symptoms or had taken any medication, including nasal or systemic corticosteroids, during the previous 4 weeks. Subjects with a history of immunotherapy, septal deviation, chronic sinusitis, or nasal polyps were excluded from this study.

Nasal Allergen Provocation Testing

All subjects underwent an NPT with the relevant allergen (lyophilized, purified *D. pteronyssinus* extract diluted in saline solution, prepared by Allergopharma, Reinbek, Germany). After adapting at room temperature for 30 minutes in the morning to minimize the effects of daily life stimuli, the subjects' clinical symptom scores were recorded, and their basal nasal functions were measured by physical examination and acoustic rhinometry. As a placebo for detecting non-specific responses, saline-soaked filter-paper disks were applied 1 hour before the NPT, and the presence of nonspecific hyperresponsiveness was evaluated. Testing was performed by applying an 8-mm filter-paper disk (punched from Shandon filter cards; Shandon, Pittsburgh, PA) impregnated with 50 μ L of HDM (*D. pteronyssinus*) allergen solution (5000 BU/ μ L) to the anterior tip of the inferior turbinate on the wider side of the nose for 10 minutes or until the appearance of allergic symptoms.

Evaluation of Symptom Score and Acoustic Rhinometry

The subjects were asked to record the severity of rhinorrhea, itching, sneezing, and nasal obstruction before nasal lavage. These symptoms were rated on an arbitrary scale from 0 to 3 (none = 0, mild = 1, moderate = 2, and intense = 3). Sneezes were counted and

scored as no sneeze, 0; 1–4 sneezes, 1; 5–9 sneezes, 2; and >10 sneezes, 3. Simultaneously, acoustic rhinometry (SRE2100; RhinoMetrics, Lynge, Denmark) was performed with a rhinometer probe fitted with an anatomic nose adapter. Each test consisted of three consecutive measurements on each side of the nasal cavity. Two measurements were recorded, the minimal cross-sectional area (MCA) and the distance to the MCA.

Nasal Lavage and Inflammatory Cells

Nasal lavage samples were obtained before the NPT and at 10, 30, and 60 minutes and 3, 6, and 24 hours after provocation. Five milliliters of saline solution at 37°C were instilled into the nostril with a syringe. The subject's head was tilted back at a 45° angle to close the nasopharynx with the soft palate. After 10 seconds, the subject was asked to flex the neck and expel the solution into a polypropylene tube. Immediately after harvest, the nasal secretions were placed on ice. Cellular elements were immediately separated from the liquid phase by centrifugation at $300 \times g$ for 10 minutes. The cell-free supernatants were stored at -80°C until further processing. The cell pellet was used to prepare slides, which were stained using the May-Grunwald and Giemsa procedures to morphologically assess the cells in the fluid. The percentages of eosinophils and other inflammatory cells were calculated as the number of cells per 100 cells on a slide.

Measurement of Allergen-Specific IgE

Serum-specific IgE levels and changes in specific IgE levels in nasal lavage fluids were determined by enzyme-linked immunosorbent assay (ELISA). Briefly, microtiter plates (Costar, Corning, NY) were coated with 100 μ L of *D. pteronyssinus* extract (1 μ g/well) and incubated overnight at 4°C. Each well was then washed three times with 0.05% Tween in phosphate-buffered saline (PBS-T), and the remaining binding sites were blocked by incubation with 200 μ L of 10% fetal bovine serum albumin for 1 hour at room temperature. Each well was washed three times and then incubated with 50 μ L of nasal lavage fluid (undiluted) for 1 hour at room temperature. After washing the wells three times with PBS-T, 100 μ L of 1:1000 (v/v) biotin-labeled goat anti-human IgE antibody (Vector, Burlingame, CA) was added to each well, and the plate was incubated for 1 hour at room temperature. The wells were then washed three times with PBS-T, incubated with 1:1000 (v/v) streptavidin peroxidase (Sigma, St. Louis, MO) for 30 minutes, and washed again. Next, 100 μ L of tetramethylene benzidine (TMB) in 0.05 mol/L of phosphate-citrate buffer, pH 5.0, and 10 μ L of 30% H_2O_2 in phosphate-citrate buffer were added to each well, and the plate was incubated for 10 minutes at room temperature. The reaction was

Table 1 The clinical characteristics of the AR subjects with a positive NPT and the subjects with a negative NPT

	Positive (<i>n</i> = 13)	Negative (<i>n</i> = 10)	<i>p</i> Value
Number of men/women	11/2	8/2	NS
Age (yr)	28.85 ± 4.72	28.60 ± 4.65	NS
Skin reactivity to HDM (A/H)	4.5 ± 1.17	3.10 ± 1.85	0.05
Serum-specific IgE	1103.17 ± 761.01	548.78 ± 846.88	0.03
Serum-specific IgA	1687.75 ± 367.30	1317.22 ± 502.39	0.056

*The skin reactivity and serum-specific IgE level were significantly higher in the positive group (*p* = 0.05 and *p* = 0.03, respectively).*
A/H = ratio of mean diameter of a wheal formed by the allergen to that formed by histamine.

stopped by adding 100 μ L of 2 N of sulfuric acid, and the absorbance was read at 450 nm using an automated plate reader.

Measurement of Allergen-Specific IgG

Serum-specific IgG levels and changes in specific IgG levels in nasal lavage fluids were determined by ELISA. The procedure was the same as described previously for allergen-specific IgE except that the primary antibody was 100 μ L of 1:5000 (v/v) alkaline phosphatase-labeled goat anti-human IgG antibody (Sigma) per well, which was detected by adding 100 μ L of phosphatase substrate to each well and incubating for 20 minutes at room temperature. The reaction was stopped by adding 100 μ L of 1 N of NaOH, and the absorbance was read at 405 nm using an automated plate reader.

Measurement of Allergen-Specific IgA

Serum-specific IgA and changes in specific IgA levels in nasal lavage fluids were determined by ELISA. The procedure was the same as described previously for allergen-specific IgE except that 50 μ L of nasal lavage fluid (1:10 dilution) was incubated in each well and the primary antibody was 100 μ L of 1:1000 (v/v) biotin-labeled goat anti-human IgA (Vector). Antibody detection was the same as for IgE described previously.

Measurement of Total IgE and ECP

ECP and total IgE in nasal lavage fluid were measured by fluoroimmunoassay using the Pharmacia CAP system (Pharmacia-Upjohn, Uppsala, Sweden).

Statistical Analysis

For statistical analysis, a Mann-Whitney *U* test was performed using SPSS version 10.0 (Chicago, IL). Pearson's correlation coefficients were applied to define the relationships between immunoglobulins and ECP. Re-

sults with values of *p* < 0.05 were considered significant.

RESULTS

Clinical Characteristics of the Study Subjects

The clinical characteristics of the AR subjects with a positive NPT (*n* = 13) and the subjects with a negative NPT (*n* = 10) are shown in Table 1. The skin reactivity and serum-specific IgE level were significantly higher in the positive group (*p* = 0.05 and *p* = 0.03, respectively). No significant differences in total IgE, specific IgG, or specific IgA levels in serum were noted between the two groups (*p* > 0.05, respectively).

Among those with a positive NPT, we defined early (*n* = 7) and dual (*n* = 6) responders according to a further increase in the eosinophil count at 3–24 hours after the early response. No significant differences were noted between the two responder groups with respect to skin reactivity, serum-specific IgE or IgA levels, or symptom scores, including nasal obstruction, as shown in Table 2.

Nasal Symptom Scores and Acoustic Rhinometry

There was no significant difference in the symptom score before the NPT between the positive and negative groups. After provocation, the sneezing score was significantly increased in the positive group at 10 minutes (*p* = 0.02) and 30 minutes (*p* = 0.03). The rhinorrhea score was increased also in the positive group at 10 and 30 minutes (*p* = 0.04 and *p* = 0.01, respectively). The itching score was not significantly different between the two groups. The symptom score for nasal obstruction in the positive group was increased at 1 and 3 hours after provocation, although these results were not statistically significant (*p* = 0.83 and *p* = 0.72, respectively).

Acoustic rhinometry did not show a significant difference in minimal cross-sectional area (MCA) at base-

Table 2 Clinical characteristics of the early and dual responders

	Dual (n = 7)	Early (n = 6)	p Value
Number of men/women	5/2	6/0	NS
Age (yr)	26.86 ± 3.72	31.71 ± 4.79	0.053
Skin reactivity to HDM (A/H)	4.86 ± 1.12	4.33 ± 1.21	NS
Serum-specific IgE	1079.00 ± 733.12	1250.86 ± 848.24	NS
Serum-specific IgA	1753.67 ± 468.30	1653.71 ± 251.00	NS

No significant differences were noted between the two responder groups with respect to skin reactivity, serum-specific IgE or IgA levels, or symptom scores, including nasal obstruction ($p > 0.05$, respectively).

A/H = ratio of mean diameter of a wheal formed by the allergen to that formed by histamine.

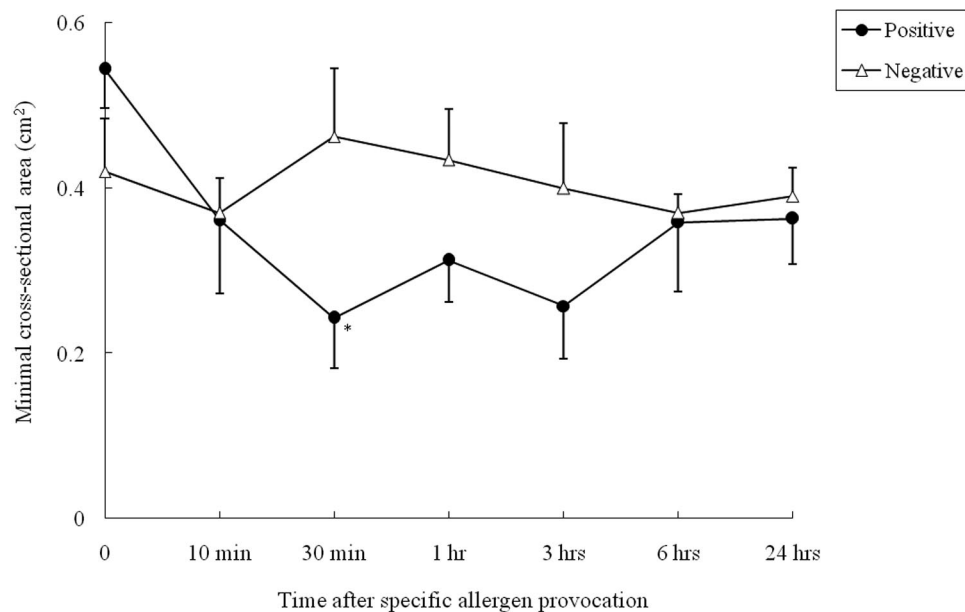


Figure 1. Changes in the MCA (cm²) in acoustic rhinometry of the positive and negative groups. The results are expressed as means ± SEM. A significant difference was noted between the groups at 30 minutes (* $p < 0.05$).

line between the two groups. After the NPT, MCA was markedly reduced in the positive group at 30 minutes ($p = 0.02$), 1 hour ($p = 0.10$), and 3 hours ($p = 0.10$). However, only the difference at 30 minutes was significant (Fig. 1). The distance to MCA tended to be reduced in the positive group at 10 minutes ($p = 0.10$).

Inflammatory Cell Counts and ECP Levels in Nasal Lavage Fluids

The eosinophil count at baseline was significantly higher in the positive group when compared with the negative group ($p < 0.001$). The eosinophil count was significantly increased in the positive group at 30 minutes and 1 and 3 hours after the NPT (* $p < 0.05$, respectively). Furthermore, these increases persisted for up to 24 hours (Fig. 2). Significant differences were noted between the two groups for all lavage samples collected before and after the NPT (* $p < 0.05$, respec-

tively). When comparing early and dual responders, the eosinophil counts at baseline and during late responses (at 1, 3, 6, and 24 hours after the NPT) were significantly higher in dual responders than in early responders ($p < 0.05$, respectively; Fig. 3). No significant differences in the other inflammatory cell counts (*i.e.*, neutrophils and lymphocytes) were noted between the positive and negative groups. The level of ECP in the nasal lavage fluid was significantly higher in the positive group than in the negative group, both before and after the NPT; significant differences were found between the two groups for all samples (Fig. 4).

Total and Specific Immunoglobulins in Nasal Lavage Fluid

There were no significant differences in the total IgE level between the positive and negative groups, either before or after the NPT. The specific IgE level tended to

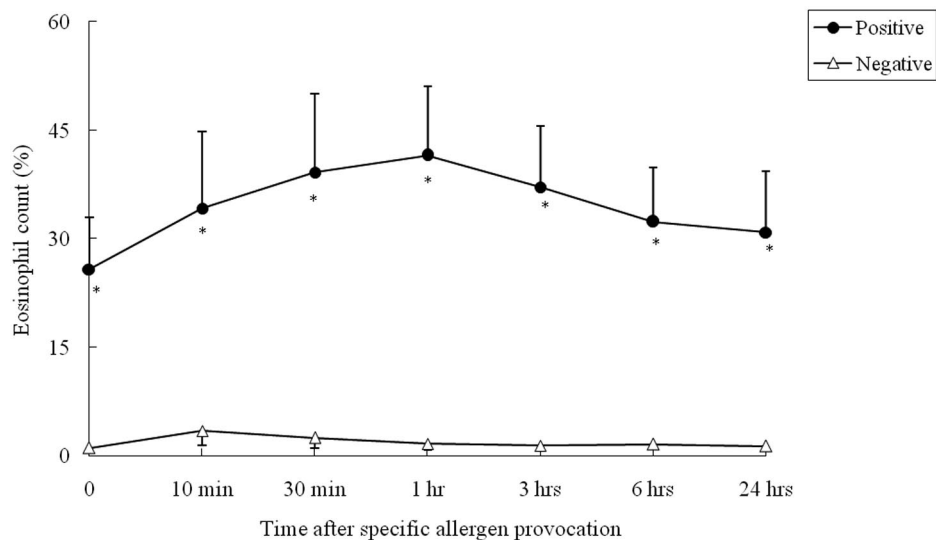


Figure 2. Changes in the eosinophil count in the nasal lavage fluid of the positive and negative groups. The results are expressed as means $\pm$ SEM. Significant differences were noted between the two groups for all lavage samples collected before and after nasal provocation (* $p < 0.05$, respectively).

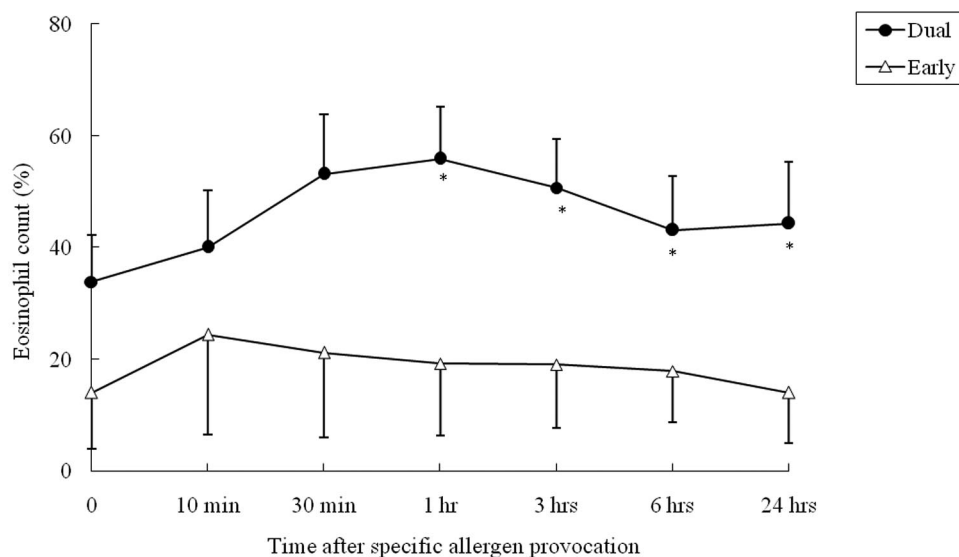


Figure 3. Changes in the eosinophil count in nasal lavage fluid of early and dual responders. The results are expressed as means $\pm$ SEM. Significant differences were noted at baseline level and during the late responses (at 1, 3, 6, and 24 hours after the NPT; * $p < 0.05$, respectively).

be higher in the positive group, but the difference was not significant (Fig. 5). There was no significant difference in the specific IgG level between the two groups after the NPT (Fig. 6). Furthermore, the specific IgA level was not significantly different between the groups before the NPT. In the positive group, the specific IgA level was increased for both the early and the late responses after provocation, and significant differences were found at 10 minutes and 3 hours (Fig. 7). The specific IgA level during the late response (6 hours after provocation) was significantly higher in the dual responders than in the early responders ($p = 0.022$).

Correlation between ECP and Specific Antibodies

We evaluated the relationship between ECP and locally produced specific antibodies. A significant correlation between the specific IgA and ECP levels was observed at baseline and at 10 minutes and 6 and 24 hours after nasal provocation ($r = 0.58$, $p = 0.004$; $r = 0.81$, $p < 0.0001$; $r = 0.46$, $p < 0.023$; and $r = 0.61$, $p = 0.02$, respectively). No significant correlation was found between the ECP level and the total IgE, specific IgE, or specific IgG level ($p > 0.05$, respectively).

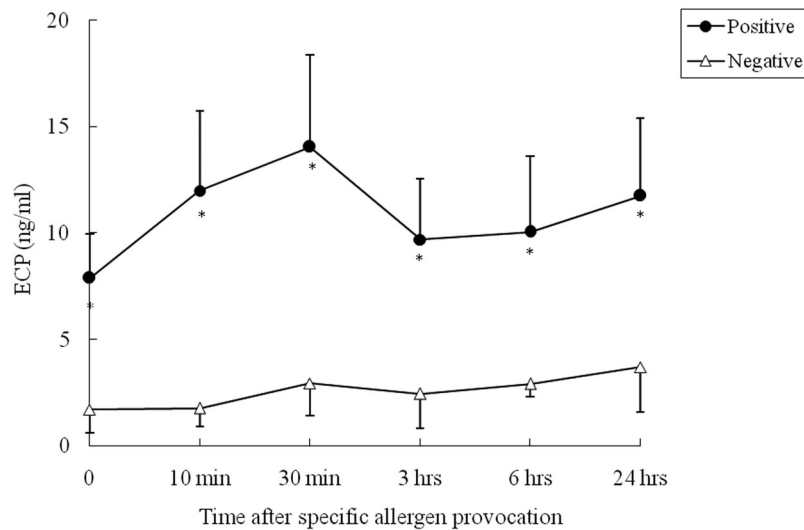


Figure 4. Changes in the ECP level in the nasal lavage fluid of the positive and negative groups. The results are expressed as means $\pm$ SEM. Significant differences were noted between the groups (* $p < 0.05$, respectively).

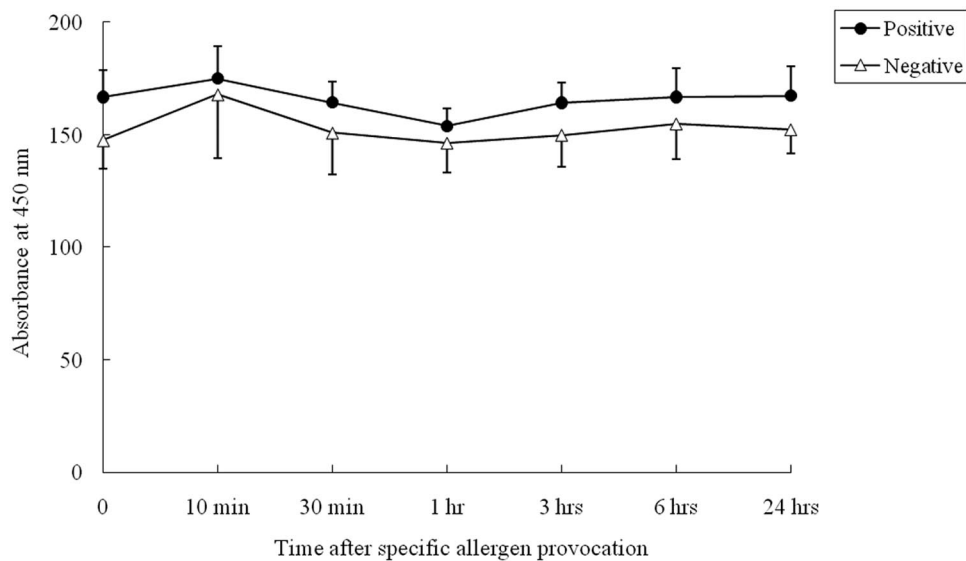


Figure 5. Changes in the D. pteronyssinus-specific IgE level in nasal lavage fluid of the positive and negative groups. The results are expressed as means $\pm$ SEM. No significant differences were found ($p > 0.05$, respectively).

DISCUSSION

The mucosal inflammation characteristic of AR is associated with an accumulation of inflammatory cells and mediators in the nasal mucosal membrane.¹³ Eosinophils and eosinophil-derived mediators are the predominant factors in the chronic allergic inflammatory process and are characteristic of the late-phase response.^{2,14,15} The number of accumulated eosinophils and/or the level of toxic granule proteins in the nasal airway correlate with the magnitude of the late-phase response and nasal hyperresponsiveness in AR patients.^{6–8} In this study, the baseline eosinophil count was significantly higher in AR patients with positive NPTs than in the negative subjects. The increased eo-

sinophil count at baseline could be partially attributable to a continuous environmental exposure to HDM allergens. A study of seasonal AR with pollen showed significant differences in the eosinophil count and ECP level between the rhinitis and control groups during the pollen season.¹⁶ In this study, subjects with a positive NPT displayed stronger reactivity on a skin-prick test, a higher serum IgE level, and an elevated eosinophil count in nasal lavage fluid at baseline. These findings may be useful for predicting whether sensitized subjects are likely to develop AR symptoms with allergen exposure. Because all subjects were asymptomatic on entering the study, we can conclude that the inflammatory components (e.g., eosinophils) found in the

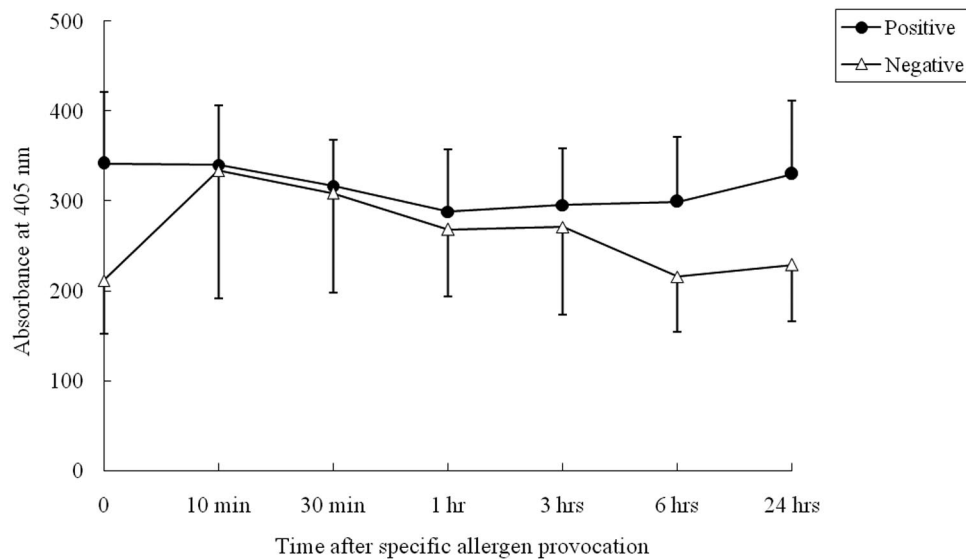


Figure 6. Changes in the *D. pteronyssinus*-specific IgG level in nasal lavage fluid of the positive and negative groups. The results are expressed as means $\pm$ SEM. No significant differences were found ($p > 0.05$, respectively).

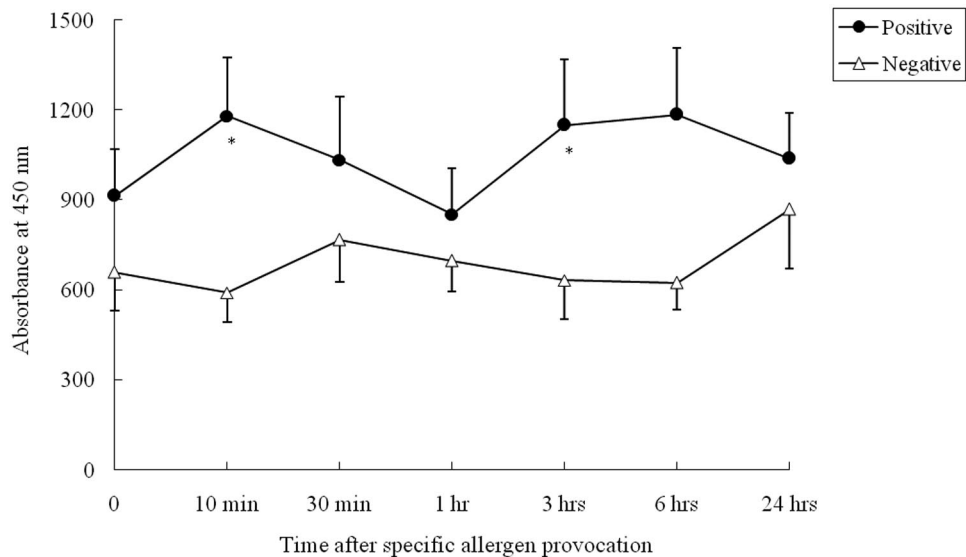


Figure 7. Changes in the *D. pteronyssinus*-specific IgA level in nasal lavage fluid of the positive and negative groups. The results are expressed as means $\pm$ SEM. Significant differences were noted at 10 minutes and 3 hours after the NPT (* $p < 0.05$, respectively).

nasal lavage fluid were the result of an active allergic inflammatory process.

Previous studies in patients with perennial AR have indicated that an NPT with HDMs can induce immediate symptoms and plasma exudation without late reoccurrence of nasal symptoms. This response can continue for up to 8 hours after provocation.¹⁷ In that study, allergen provocation increased the ECP level as early as 1 hour after provocation, and the level remained augmented for up to 8 hours. Consistent with those previously published results, we also found an immediate increase in the eosinophil count and ECP level after provocation. Moreover, we observed a further increase in the eosinophil count during the late

response in 7 of 13 patients with perennial AR; this is comparable with a previous finding that dual responders showed a significantly higher symptom score during the early response and higher ECP release during the late response.¹⁸ In addition, the eosinophil count at baseline was significantly higher in dual responders than in early responders, and it was further increased during the late response. Similar findings were noted for the ECP level. These results confirm that the more eosinophils present in the epithelium at steady state in perennial AR, the higher will be the response to nasal provocation. In this study, the number of eosinophils was increased in both the early and the late responses after allergen provocation in perennial AR. Collec-

tively, these results suggest that eosinophils are the major effector cells determining AR symptoms in the development of the late response as well as the early response in perennial AR patients with continuous stimulation by allergens.

Immunoglobulins (IgE, IgA, and IgG) are potent stimuli for eosinophil degranulation, resulting in the release of cytotoxic proteins such as ECP and eosinophil peroxidase (EPO), and eosinophil-derived neurotoxin in an *in vitro* model system.¹⁹ In a previous study showing a significant correlation between ECP and *D. pteronyssinus*-specific IgE in nasal polyp tissue homogenate, the authors concluded that an elevated IgE concentration in nasal secretions of nonallergic nasal polyps may implicate IgE-dependent but nonspecific degranulation of effector cells such as eosinophils.²⁰ Peebles *et al.* detected a positive correlation between the baseline levels of specific IgE and IgA in bronchoalveolar lavage fluids and the ragweed-induced change in ECP in patients with asthma.²¹ However, in the present study, we did not observe a correlation between ECP and specific IgE after nasal provocation. The reason for this apparent inconsistency may be that the subjects in this study were sensitized to HDM allergen.

Numerous studies have indicated that mucosal and systemic production of specific IgA occurs in patients with asthma and/or AR. Clinical studies have shown a positive correlation between allergen-specific IgA and eosinophil granule proteins.^{22,23} In some studies, allergen-specific IgA has been reported to increase during the pollen season,²² but other studies have reported no increase.⁸ Keen *et al.* observed that specific IgA to Bet v 1, the major birch pollen allergen, increased relative to total IgA during the pollen season, although this did not occur after a single nasal allergen provocation in children with seasonal AR.¹⁶ They proposed that the lack of an increase after provocation may be because of insufficient time for IgA production between the provocation and nasal lavage, given that immunoglobulin production is not instantaneous in the nasal mucosa. In the present study, we detected allergen-specific IgE, IgG, and IgA antibodies in nasal lavage fluid, with the specific IgA level being significantly higher during early and late responses after the NPT in the positive group. A close correlation was found between the ECP level and the level of specific IgA, but not IgG or IgE, suggesting a possible involvement of specific IgA in eosinophilic inflammation rather than in nonspecific protection. Additional investigations are needed to elucidate the involvement of specific IgA in eosinophil activation after allergen provocation.

CONCLUSIONS

Eosinophils were abundantly recruited in both the early and the late responses after allergen exposure in perennial AR. The close correlation between the increased levels of locally produced specific IgA and ECP during the early and late responses suggest a role for specific IgA in the pathogenic mechanism of allergic inflammation in nasal mucosa.

REFERENCES

1. Nouri-Aria KT, O'Brien F, Noble W, et al. Cytokine expression during allergen-induced late nasal responses: IL-4 and IL-5 mRNA is expressed early (at 6 h) predominantly by eosinophils. *Clin Exp Allergy* 30:1709–1716, 2000.
2. Broide DH. The pathophysiology of allergic rhinoconjunctivitis. *Allergy Asthma Proc* 28:398–403, 2007.
3. Miadonna A, Milazzo N, Gibelli S, et al. Nasal response to a single antigen challenge in patients with allergic rhinitis—Inflammatory cell recruitment persists up to 48 hours. *Clin Exp Allergy* 29:941–949, 1999.
4. Naclerio RM, Meier HL, Kagey-Sobotka A, et al. Mediator release after nasal airway challenge with allergen. *Am Rev Respir Dis* 128:597–602, 1983.
5. Pelikan Z. Late and delayed responses of the nasal mucosa to allergen challenge. *Ann Allergy* 41:37–47, 1978.
6. Yamasaki M, Mizutani N, Sasaki K, et al. No involvement of interleukin-5 or eosinophils in experimental allergic rhinitis in guinea pigs. *Eur J Pharmacol* 439:159–169, 2002.
7. Pastorello EA, Riario-Sforza GG, Incorvaia C, et al. Comparison of rhinomanometry, symptom score, and inflammatory cell counts in assessing the nasal late-phase reaction to allergen challenge. *J Allergy Clin Immunol* 93:85–92, 1994.
8. de Graaf-in't Veld C, Garrelds IM, Koenders S, et al. Relationship between nasal hyperreactivity, mediators, and eosinophils in patients with perennial allergic rhinitis and controls. *Clin Exp Allergy* 26:903–908, 1996.
9. Benson M, Reinholdt J, and Cardell LO. Allergen-reactive antibodies are found in nasal fluids from patients with birch pollen-induced intermittent allergic rhinitis, but not in healthy controls. *Allergy* 58:386–392, 2003.
10. Yoshida T, Usui A, Kusumi T, et al. A quantitative analysis of cedar pollen-specific immunoglobulins in nasal lavage supported the local production of specific IgE, not of specific IgG. *Microbiol Immunol* 49:529–534, 2005.
11. Monteiro RC, Hostoffer RW, Cooper MD, et al. Definition of immunoglobulin A receptors on eosinophils and their enhanced expression in allergic individuals. *J Clin Invest* 92:1681–1685, 1993.
12. Oldenbeuving NB, KleinJan A, Mulder PG, et al. Evaluation of an intranasal house dust mite provocation model as a tool in clinical research. *Allergy* 60:751–759, 2005.
13. Amin K, Rinne J, Haahtela T, et al. Inflammatory cell and epithelial characteristics of perennial allergic and nonallergic rhinitis with a symptom history of 1 to 3 years' duration. *J Allergy Clin Immunol* 107:249–257, 2001.
14. Bentley AM, Jacobson MR, Cumberworth V, et al. Immunohistology of the nasal mucosa in seasonal allergic rhinitis: Increases in activated eosinophils and epithelial mast cells. *J Allergy Clin Immunol* 89:877–883, 1992.
15. Gelfand EW. Inflammatory mediators in allergic rhinitis. *J Allergy Clin Immunol* 114(suppl):S135–S138, 2004.
16. Keen C, Johansson S, Reinholdt J, et al. Bet v 1-specific IgA increases during the pollen season but not after a single allergen challenge in children with birch pollen-induced intermittent allergic rhinitis. *Pediatr Allergy Immunol* 16:209–216, 2005.

17. Andersson M, Rimmer J, Salome C, et al. Dual symptomatic and exudative nasal responses are not characteristics of perennial allergic rhinitis. *Acta Otolaryngol* 121:407–413, 2001.
18. de Graaf-in't Veld C, Garrelds IM, van Toorenenbergen AW, et al. Nasal responsiveness to allergen and histamine in patients with perennial rhinitis with and without a late phase response. *Thorax* 52:143–148, 1997.
19. Tomassini M, Tsicopoulos A, Tai PC, et al. Release of granule proteins by eosinophils from allergic and nonallergic patients with eosinophilia on immunoglobulin-dependent activation. *J Allergy Clin Immunol* 88:365–375, 1991.
20. Suh KS, Park HS, Nahm DH, et al. Role of IgG, IgA, and IgE antibodies in nasal polyp tissue: Their relationships with eosinophilic infiltration and degranulation. *J Korean Med Sci* 17:375–380, 2002.
21. Peebles RS Jr, Liu MC, Adkinson NF Jr, et al. Ragweed-specific antibodies in bronchoalveolar lavage fluids and serum before and after segmental lung challenge: IgE and IgA associated with eosinophil degranulation. *J Allergy Clin Immunol* 101:265–273, 1998.
22. Reed CE, Bubak M, Dunnette S, et al. Ragweed-specific IgA in nasal lavage fluid of ragweed-sensitive allergic rhinitis patients: Increase during the pollen season. *Int Arch Allergy Appl Immunol* 94:275–277, 1991.
23. Benson M, Svensson PA, Carlsson B, et al. DNA microarrays to study gene expression in allergic airways. *Clin Exp Allergy* 32:301–308, 2002. □

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.