

ORIGINAL ARTICLE

Vascular endothelial growth factor in allergen-induced nasal inflammation

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Summary

Background Increased vessel number and permeability are important features of the nasal mucosa in allergic rhinitis (AR), and are mediated in part by the cytokine vascular endothelial growth factor (VEGF). Eosinophils are the major effector cells in the nasal secretions of patients with AR during the responses to allergen challenges. To evaluate the involvement of VEGF in nasal allergic inflammation, we monitored the levels of VEGF, eosinophil cationic protein (ECP), and specific antibodies in the nasal lavage fluids (NLFs) of patients with AR in response to *Dermatophagoides pteronyssinus* (*Dpt*).

Methods Sixty-three subjects with sensitization to *Dpt* were enrolled: 29 patients with AR (group I) who showed positive responses in a nasal provocation test (NPT) with *Dpt*; and 34 asymptomatic controls (group II) who showed sensitization to *Dpt* but negative NPT results. NLF samples were collected at baseline, 10, 30, and 60 min, and at 3, 6, and 24 h during the NPT. The ECP levels in the NLF samples were measured using the ImmunoCAP system. VEGF and *Dpt*-specific IgE, IgA, and IgG in the NLF samples were detected by ELISA.

Results The eosinophil counts and ECP levels in the samples were significantly increased in group I, but not in group II, during the early and late responses. Although the baseline VEGF level was not significantly different between groups I and II, increased VEGF production was noted in group I after the NPT, especially during the early response. The level of *Dpt*-specific IgA was significantly increased in group I during the NPT. A relationship was found between the levels of VEGF and ECP or *Dpt*-specific IgA in the NLF samples collected at 10 min and at 3–6 h ($P < 0.05$, respectively).

Conclusion Nasal VEGF secretion in response to allergen exposure may augment eosinophilic inflammation in the nasal mucosa of patients with AR.

Keywords allergic rhinitis, eosinophil, nasal provocation, vascular endothelial growth factor (VEGF)

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Introduction

Allergic rhinitis (AR) is clinically defined as a symptomatic disease of the nose induced by IgE-mediated allergic inflammation after allergen exposure. Although allergic inflammation is mediated by the complex interaction of inflammatory cells (such as eosinophils), cytokines, chemokines, and neuropeptides [1, 2], numerous studies have shown that eosinophils are the major effector cells in the nasal secretions of AR patients during allergic inflammation [3–5], and that the local production of allergen-specific IgE, IgA, and IgG in the nasal secretions is involved in the pathogenesis of AR [2, 6, 7]. Furthermore, nasal vasodilation

and increased vascular permeability are important features of AR, although the underlying mechanisms are unknown. Although these effects have been thought to result from the IgE-dependent release of histamine from mast cells [8], the levels of histamine in the nasal fluids of patients with AR vary considerably, and may even be decreased [8, 9]. This suggests the involvement of vasoactive mediators other than histamine.

Vascular endothelial growth factor (VEGF) is a potent inducer of endothelial cell growth and angiogenesis and is responsible for increased capillary permeability [10]. Many inflammatory and structural cells, such as macrophages, neutrophils, eosinophils, epithelial cells, and fibroblasts, are

important sources of VEGF in inflamed tissues [11]. VEGF causes vasodilation and increases vascular permeability by stimulating the endothelial production of such vasoactive mediators as nitric oxide and prostacyclin, which allow plasma proteins to leak into the extravascular space, leading to oedema and a profound alteration of the extracellular matrix [10, 12, 13]. Recently, it has been reported that VEGF modulates immune cell function by inhibiting dendritic cell activity and T-cell development and by stimulating eosinophil chemotaxis [12, 14]. In addition, it has been shown that the levels of VEGF are increased in the nasal mucosa of patients with AR [15]. These observations suggest that VEGF plays an important role in nasal mucosal inflammation during AR. However, to our knowledge, few studies have investigated the role of VEGF in nasal mucosal inflammation in patients with AR.

In the present study, we measured the levels of VEGF in nasal lavage fluids (NLFs) to determine whether VEGF is involved in nasal allergic inflammation, and we assessed the association of VEGF with eosinophil activation and the local production of specific antibodies.

Methods

Study subjects

Sixty-three subjects who were sensitized to *Dermatophagoides pteronyssinus* (*Dpt*) were enrolled. Sensitization was determined based on the results of a skin prick test using *Dpt* allergen (Bencard, Bradford, UK); those subjects with an A/H ratio (mean weal diameter of the allergen to histamine) >2 were considered to be sensitized. All subjects were administered a nasal provocation test (NPT) with *Dpt* allergen and monitored for nasal symptoms (e.g. rhinorrhoea, nasal itching, nasal obstruction, and sneezing). Twenty-nine positive responders (i.e. those with an increase of more than three points in the total nasal symptom score) were classified as AR subjects (group I). Thirty-four negative responders (no response or a total nasal symptom score <3) were classified as asymptomatic controls (group II). None of the subjects had received antihistamine therapy, leukotriene receptor antagonist, or topical steroids for 4 weeks before the study. The subjects with asthma, chronic sinusitis, septal deviation, nasal polyps, or a history of immunotherapy were excluded from this study. All of them were non-smokers. The study was approved by the Institutional Review of Board of Ajou Medical Center, Suwon, Korea. Informed consent was obtained from all subjects.

The NPT

The subjects were first left at room temperature for 30 min in the morning to minimize the effects of daily-life stimuli. They were then given a saline challenge to

exclude nasal hyperreactivity. An NPT was performed using the *Dpt* allergen according to previously described methods [16]. An 8-mm filter paper disc (punched from a Shandon filter card; Pittsburgh, PA, USA) impregnated with the allergen solution (5000 BU/ μ L *Dpt*, prepared by Allergopharma, Reinbek, Germany) was applied to the anterior tip of the inferior turbinate on the wider side of the nose for 10 min or until the appearance of allergic symptoms. Nasal symptom scores were determined at baseline, and at 10, 30 min and 1, 3, 6, and 24 h post-provocation. Rhinorrhoea, nasal itching, and nasal obstruction symptoms were rated on an arbitrary scale from 0 to 3 (0 = no symptom, 1 = mild symptom, which was present but was of a short duration and not annoying or troublesome, 2 = moderate symptom, which was frequently troublesome but did not interfere with either normal daily activity or sleep, and 3 = severe symptom, which was troublesome and interfered with normal daily activity or sleep). Sneezes were counted and classified as follows: no sneezing, 0; one to four sneezes, 1; five to nine sneezes, 2; and >10 sneezes, 3. Positive response was defined as an increase of more than three points of the total nasal symptom scores that was derived from sum of sneezing, itching, rhinorrhoea, and nasal obstruction symptom scores during the provocation test.

Nasal lavage and sample processing

Nasal lavage was performed bilaterally before allergen provocation and at 10, 30 min, and 1, 3, 6, and 24 h after the NPT. Subjects were in a sitting position with extension of their neck backward to 30° from horizontal. And then, 5 mL of saline was instilled into one nostril using a syringe. NLF was recovered after 10 s by anterior flexion of the head with pushing out of the solution into a polypropylene tube. This process was again performed at the counter-lateral nostril. To limit posterior loss, subjects closed the posterior nasopharynx using the soft palate, holding their breath during the period of nasal lavage retention with the mouth slightly open. Measurement of recovered fluids showed an approximately 80% recovery with a range of 65–90%. The nasal secretions were immediately placed on ice to increase cell viability. The samples were centrifuged at 300 g for 10 min. The supernatants were stored at -80°C until use, while the cell pellets were used to prepare slides, which were subjected to May-Grunwald and Giemsa staining for the analysis of cellular morphology.

Measurement of inflammatory markers in the NLF samples

The number of eosinophils and other inflammatory cells were counted at two different sites at $\times 1000$. The percentage of each inflammatory cell was calculated by the number of cells per 100 cells on a slide. The levels of

eosinophil cationic protein (ECP) in the NLF samples were measured by fluoroimmunoassay using the Pharmacia CAP system (Pharmacia-Upjohn, Uppsala, Sweden). To correct the dilutional effect of samples by repeated nasal lavage, the ECP level was calculated relative to the amount of total protein, which was measured by the Bradford method using the sample supernatant. The level of VEGF in each NLF sample was measured using a commercially available ELISA kit (R&D Systems, Abingdon, UK) according to the manufacturer's instructions. The inter- and intra-assay variation of this ELISA kit are under 8.5% and 6.5%, respectively. The minimum detectable level of VEGF is 5.0 pg/mL.

Measurement of total immunoglobulin E and allergen-specific immunoglobulins (immunoglobulin E, immunoglobulin A, and immunoglobulin G)

The total IgE levels in the NLF samples were measured by fluoroimmunoassay using the Pharmacia CAP system (Pharmacia-Upjohn). The levels of allergen-specific immunoglobulins (IgE, IgG, and IgA) in the serum and NLF samples were determined by ELISA, as described previously [16].

To measure the level of *Dpt*-specific IgE, microtitre plates (Costar, Corning, NY, UK) were coated with 100 µL of *Dpt* extract (1 µL/well) and incubated overnight at 4 °C. After three washes with phosphate-buffered saline (PBS)-0.05% Tween, each well was blocked with 200 µL of 10% fetal bovine serum albumin-PBS for 1 h at room temperature, and then washed again three times. The NLF and serum specimens were pipetted into their respective wells (50 µL/well), and the plates were incubated for 1 h at room temperature. The plates were then rewashed. Next, 100 µL of 1 : 1000 (v/v) biotin-labelled goat anti-human IgE antibody (Sigma, St. Louis, MO, USA) was pipetted into each well and incubated for 1 h at room temperature. After a buffer wash, 100 µL of 1 : 1000 (v/v) streptavidin peroxidase (Sigma) was pipetted into each well and incubated for 30 min. After a final buffer wash, 100 µL of tetramethylene benzidine (TMB) in 0.05 M phosphate-citrate buffer (pH 5.0) and 10 µL of 30% H₂O₂ in phosphate-citrate buffer were added to each well, and the plate was incubated for 10 min at room temperature. The reaction was stopped by the addition of 100 µL of 2 N sulphuric acid, and the absorbance was read at 450 nm using an automated plate reader. The results are expressed in terms of the optical density (OD).

The procedure for detecting allergen-specific IgA was as described above for allergen-specific IgE, except that 50 µL of a 1 : 10 dilution of each NLF sample was incubated with 100 µL of 1 : 1000 (v/v) biotin-labelled goat anti-human IgA (Sigma). The procedure used to detect allergen-specific IgG was similar to that used for allergen-specific IgE, except that 100 µL of 1 : 5000 (v/v) alkaline phosphatase-labelled goat anti-human IgG (Sigma) was

used as the primary antibody, and binding was detected by the addition of 100 µL of phosphatase substrate to each well, followed by 20 min of incubation at room temperature. The reaction was stopped by the addition of 100 µL of 1 N NaOH, and the absorbance was read at 405 nm.

Statistical analyses

Values are presented as mean ± standard deviation (SD), unless otherwise specified. The *t*-test and the χ^2 test were used for comparison between the groups. Wilcoxon's test was also used to compare the results obtained according to the elapsed time after the nasal provocation within the group. Pearson's correlations were used to assess the relationships between VEGF and ECP, and each specific immunoglobulin. All analyses were carried out using SPSS 13.0 (SPSS Inc., Chicago, IL, USA). A *P*-value of <0.05 was considered statistically significant.

Results

Characteristics of the study subjects

The subjects were classified into two groups: AR subjects (group I, *n* = 29) with a positive NPT result; and asymptomatic sensitized controls (group II, *n* = 34) with a negative NPT result. The clinical characteristics of groups I and II are summarized in Table 1. The mean weal diameter for *Dpt* was 10.26 ± 4.23 mm in group I and 7.61 ± 4.35 mm in group II, but there was no significant difference between them (*P* = 0.68). Serum *Dpt*-specific IgE levels tended to higher in group I, without statistical significance also (*P* = 0.25). No significant differences in age, sex, or the co-morbid condition of atopic eczema were noted between the two groups (*P* > 0.05, respectively).

Inflammatory cell counts and the eosinophil cationic protein/protein ratios in nasal lavage fluids samples during the nasal provocation test

The eosinophil counts and ECP/protein ratios of the NLF samples were higher in group I, with significant differences

Table 1. Clinical characteristics of the subjects in groups I and II

	Group I (<i>n</i> = 29)	Group II (<i>n</i> = 34)	<i>P</i> -value
Sex (M/F)	22/7	21/13	0.15
Age (years)*	25.70 ± 4.17	26.18 ± 4.01	0.73
Skin reactivity to <i>Dpt</i> (mm)*	10.26 ± 4.23	7.61 ± 4.35	0.68
Serum-specific IgE to <i>Dpt</i> *	1030.61 ± 774.14	597.37 ± 891.83	0.25
Co-morbid condition			
Atopic eczema [<i>n</i> (%)]	8 (27%)	7 (20%)	0.17

Dpt, *Dermatophagoides pteronyssinus*; skin reactivity to *Dpt* was presented as mean weal diameter (mm) and the level of serum-specific IgE to *Dpt* was presented as optical density × 1000.

*Data expressed as the mean ± SD (standard deviation).

Table 2. Changes in the total symptom scores and various markers of inflammation in nasal lavage fluids collected from the subjects in groups I and II during the nasal provocation test with *Dpt* allergen

Group	Elapsed time after the nasal provocation test						
	Baseline	10 min	30 min	1 h	3 h	6 h	24 h
Total nasal symptom scores							
I	1.03±1.72*	4.66±3.37*†	3.93±2.37*†	2.83±1.91*†	2.71±1.79*†	1.76±1.85*	1.61±2.10*
II	0.37±0.85	0.62±0.78	0.47±0.82	0.43±0.73	0.30±0.60	0.43±0.49	0.18±0.50
Eosinophil counts (%)							
I	17.61±25.31*	24.33±30.32*	25.50±29.37*†	28.12±30.10*†	24.78±27.0*	20.73±25.0*	19.70±25.92*
II	0.86±1.71	3.04±5.28	3.20±5.16	2.42±4.15	2.58±4.42	2.33±3.33	1.86±4.67
ECP/protein ratio							
I	4.08±4.36*	7.47±11.09*	7.60±13.17*†	NA	4.79±5.00*	4.99±6.89*	7.31±9.42*
II	1.27±1.67	0.97±1.77	1.19±2.35	NA	1.64±3.17	1.14±1.98	0.76±1.06
Protein (µg/mL)							
I	97.04±79.99	160.95±135.31*	117.64±83.39*	120.58±145.48*	83.60±70.27	86.87±60.06*	101.64±89.48
II	63.35±39.72	62.89±59.41	59.26±47.73	52.23±41.29	56.15±58.31	48.48±43.27	61.96±56.33
SIgA- <i>Dpt</i> (OD*1000)							
I	1034±634	1196±645*†	1150±601*	1097±585*	1138±746*	1194±674*†	1152±642
II	960±575	679±400	751±390	795±449	795±502	793±439	903±533

SIgA-*Dpt*, specific IgA to *Dpt* (*Dermatophagoides pteronyssinus*); total nasal symptom scores presented as sum of sneezing, itching, rhinorrhoea, and nasal obstruction symptom scores; the results are expressed as the mean±SD.

* $P < 0.05$ (group I vs. group II).

† $P < 0.05$ (baseline vs. level at elapsed time after challenge).

ECP, eosinophil cationic protein; NA, not available.

between the groups before nasal provocation ($P < 0.05$, respectively). After provocation, both values were significantly increased in group I ($P < 0.05$, respectively), whereas no significant changes were detected in group II although the eosinophil counts tended to be increased. In group I, the eosinophil count peaked at 1 h and persisted until 24 h, whereas the ECP/protein ratio was significantly increased at 10 min and 30 min, and then increased again at 3 h post-NPT (Table 2). No significant differences in any of the other inflammatory cell counts (e.g. neutrophils and lymphocytes) were detected.

Changes in the levels of vascular endothelial growth factor during the nasal provocation test

There were no significant differences in the levels of VEGF between groups I and II at baseline ($P = 0.233$). After the NPT, the levels of VEGF increased remarkably at 10 min after nasal provocation and then decreased in group I ($P = 0.05$, respectively). The VEGF level tended to be higher than that of group II during late responses, but no statistical significance was noted compared with the baseline level within group I. In group II, there were no significant changes in the levels of VEGF during the NPT (Fig. 1).

Total and specific immunoglobulins in the nasal provocation test samples

We measured the levels of total IgE and specific antibodies in the NLF samples after nasal provocation with the *Dpt*

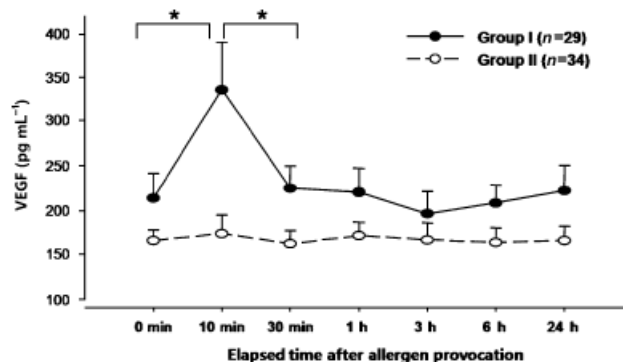


Fig. 1. Changes in vascular endothelial growth factor (VEGF) level in nasal lavage fluid during nasal provocation test with *Dermatophagoides pteronyssinus* allergen. Solid circles, group I; open circles, group II. The results are expressed as the mean±SE. The levels of VEGF are significantly increased during the early response at 10 min ($P < 0.05$) and tended to be higher during late response without statistical significance (after 3 h of nasal provocation) in group I, but not in group II, * $P < 0.05$.

allergen. There were no significant differences in the total IgE levels between groups I and II, either before or after the NPT. Furthermore, the levels of *Dpt*-specific IgE and IgG did not differ significantly between the two groups during the NPT, although the specific IgE level tended to be higher in group I after the test. In group I, the level of *Dpt*-specific IgA was significantly increased immediately and 3 h after the NPT (Table 2). Dual peaks for *Dpt*-specific IgA were identified at 10 min and 6 h after the NPT, with significant differences being noted between the two groups ($P < 0.05$, respectively).

Table 3. Correlations between the VEGF levels, ECP/protein ratios, and specific IgA levels in the nasal lavage fluids of group I during the nasal provocation test

	Elapsed time after the nasal provocation test					
	baseline	10 min	30 min	3 h	6 h	24 h
VEGF and ECP/protein ratio	$r = 0.422^*$	$r = 0.670^\dagger$	$r = 0.228$	$r = 0.798^\dagger$	$r = 0.559^*$	$r = 0.129$
<i>Dpt</i> -specific IgA and ECP/protein ratio	$r = 0.394$	$r = 0.631^\dagger$	$r = 0.400^*$	$r = 0.376^*$	$r = 0.459^*$	$r = 0.543^*$
VEGF and <i>Dpt</i> -specific IgA	$r = 0.856^\dagger$	$r = 0.932^\dagger$	$r = 0.546^*$	$r = 0.800^\dagger$	$r = 0.625^\dagger$	$r = 0.602^\dagger$

* $P < 0.05$,

$^\dagger P < 0.001$. The correlations were evaluated at the indicated time-points after nasal provocation. Significant correlations between the VEGF levels and the ECP/protein ratios, as well as the levels of *Dpt*-specific IgA were observed during the early and late responses in group I.

ECP, eosinophil cationic protein; r , Pearson's coefficient; *Dpt*, *Dermatophagoides pteronyssinus*; VEGF, vascular endothelial growth factor.

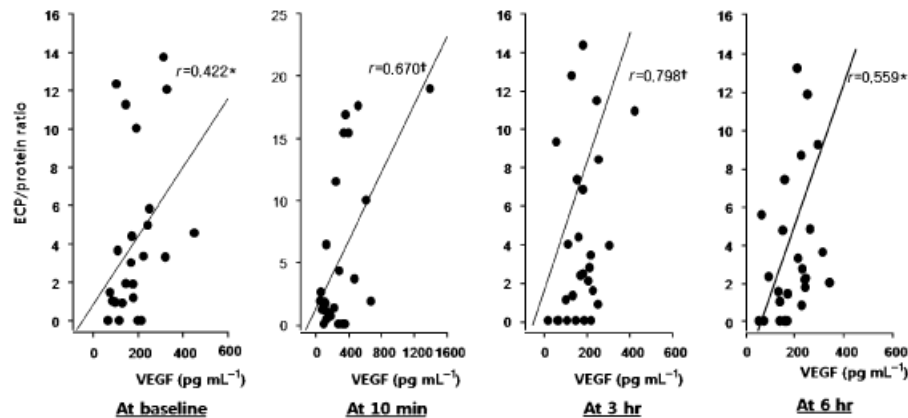


Fig. 2. Correlation between the vascular endothelial growth factor (VEGF) levels and eosinophil cationic protein (ECP)/protein ratio during the nasal provocation test in group I subjects. * $P < 0.05$, $^\dagger P < 0.001$.

Correlations between the levels of vascular endothelial growth factor, eosinophil cationic protein, and specific immunoglobulin A

We evaluated the association of VEGF with the ECP/protein ratio, and with the level of locally produced specific antibodies (Table 3). A significant correlation was found between the VEGF level and the ECP/protein ratio in the NLF samples collected at 10 min, and 3, and 6 h after the NPT with *Dpt* allergen (Fig. 2, $r = 0.670$, $P < 0.001$; $r = 0.798$, $P < 0.001$; and $r = 0.559$, $P = 0.001$, respectively). Furthermore, there were significant correlations between the levels of VEGF and *Dpt*-specific IgA at 10, 30 min, and 3, 6, and 24 h after the NPT ($r = 0.932$, $P < 0.001$; $r = 0.546$, $P = 0.001$; $r = 0.800$, $P < 0.001$; and $r = 0.625$, $P < 0.001$, respectively, data not shown). No significant correlations were found between the levels of VEGF and total IgE or other specific immunoglobulins (e.g. IgE or IgG) ($P > 0.05$, respectively).

Discussion

Nasal allergic inflammation is associated with the release of mediators from eosinophils, mast cells, and other

inflammatory cells in the nasal mucosa [17]. In the present study, we investigated the active nasal allergic inflammatory process through a serial analysis of NLF samples after nasal provocation with house dust mite allergen, which is the most prevalent allergen in the country. We detected an increase in the eosinophil counts and the levels of ECP in NLF samples collected from subjects with AR during the early and late responses to allergen exposure with the development of symptoms, which confirms that eosinophils and eosinophil-derived mediators play key roles during early and late nasal allergic inflammation [16, 18]. A previous biopsy study of seasonal AR has demonstrated that, even during the off-season, the number of nasal tissue eosinophils may be elevated compared with healthy, nonatopic individuals [19]. We also found that the baseline eosinophil counts and ECP levels were higher in the inflamed nasal tissues of AR patients than in those of asymptomatic sensitized controls. This suggests the presence of early nasal allergic inflammation without symptoms in AR patients.

VEGF is postulated to act as an angiogenic agent that is capable of altering subepithelial vascularity. Numerous *in vitro* and *in vivo* studies have demonstrated the increased expression of VEGF in asthmatic airways [13, 20–25].

VEGF increases bronchial vascularization, vasodilation, and permeability, which may result in mucosal oedema, thereby contributing to airway inner wall thickening and consequent airflow limitations [13, 26, 27]. However, in AR, the amount of available data regarding the role of VEGF is limited. A few studies have documented the increased expression of VEGF in the nasal mucosa of patients with AR using biopsies, along with a subsequent increase in nasal vascular permeability and congestion [19, 28, 29]. Nevertheless, the present study is the first to monitor changes in the levels of VEGF in the NLFs of AR subjects after allergen exposure. We found that the levels of VEGF were significantly increased immediately after allergen exposure in AR subjects, but not in asymptomatic sensitized controls. Furthermore, the dynamic pattern of the VEGF levels mirrored the total nasal symptom scores although no direct correlations were found. These findings suggest that VEGF plays important roles in the development of symptoms and nasal allergic inflammation.

It is known that eosinophils are the main effector cells of allergic inflammation and one of the important sources of VEGF with other inflammatory and structural cells in inflamed tissue [11]. Therefore, we assessed the correlation between VEGF and the ECP/protein ratio in NLF samples from subjects with AR at serial time-points during NPTs. This is the first study to assess the correlation between locally produced VEGF and eosinophil activation in the nasal mucosa after nasal provocation with *Dpt* allergen, even though several asthma studies have shown a correlation between the levels of VEGF and the numbers of eosinophils and levels of ECP [25]. We found that the levels of VEGF were significantly correlated with the ECP/protein ratio at the baseline level, which were increased further during early response in AR subjects. These findings suggest that VEGF is involved in eosinophil activation during allergic nasal inflammation, especially during the early response. It was reported that VEGF is a potent chemoattractant for leucocytes in experimental asthma [30], and that eosinophil infiltration can be reduced by the administration of anti-VEGF receptor antibodies in a murine model of toluene diisocyanate-induced asthma [31]. In addition, an *in vitro* study has demonstrated that VEGF receptor (VEGFR)-1 and VEGFR-2 are expressed on human peripheral blood eosinophils, and that VEGF induces eosinophil migration and ECP release, mainly through VEGFR-1 [14]. In the present study, the level of VEGF peaked much more rapidly than the eosinophil count or the level of ECP. Thus, the significant increase in VEGF in the NLF samples during the early response after NPT may be related to eosinophil migration and activation, as well as to increased vascular permeability and vasodilation. Additional investigations are needed to elucidate how VEGF contributes to the activation of eosinophils following allergen exposure.

Many studies have shown the mucosal and systemic production of specific immunoglobulins (IgE, IgG, and

IgA) in patients with asthma and/or AR. Although IgE is known to be a critical participant in AR, recent studies have identified specific IgA as a contributor to allergic inflammation. Clinical studies have shown a relationship between secretory allergen-specific IgA and eosinophil activation *in vivo* and *in vitro* [7, 14, 32–34]. In the present study, we detected allergen-specific IgE, IgG, and IgA in NLF during allergen provocation. Although there was no significant increase in the allergen-specific IgE and IgG, specific IgA levels were significantly increased during the early and late responses in parallel with the ECP level. These findings suggested that locally produced allergen-specific IgA might be involved in eosinophil activation during nasal allergic inflammation. Furthermore, our results demonstrate a close correlation between the levels of VEGF and allergen-specific IgA, which may be derived from significant correlations between ECP and allergen-specific IgA, or ECP and VEGF, indicating indirect involvement of VEGF in eosinophilic inflammation *via* specific IgA.

In conclusion, our results suggest that VEGF and allergen-specific IgA augment eosinophilic inflammation, especially during the early response to allergen exposure in the nasal mucosa of AR patients. Additional studies are needed to evaluate the exact role of VEGF in the pathogenesis of AR.

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