알레르기내과학 박사학위 논문

The local production of allergen-specific antibodies in nasal secretion from allergic rhinitis patients sensitive to *D.farinae*: Relationships with inflammatory mediators

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집먼지 진드기에 의한 알레르기 비염 환자의 비점막에서 생성되는 국소 항체 및 염증 조절 매개체들과의 연관성

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이 논문을 의학 박사학위 논문으로 제출함.

2017 년 8월

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April, 28th, 2017

Abstract

Background and Purpose: Allergic rhinitis (AR) is a common and increasing allergic disease, in which *Dermatophagoides* (*D.) farinae* is the most common causative allergen. The aims of this study are to compare locally produced antibodies to *D. farinae* in nasal mucosa between nasal provocation test (NPT)-positive and NPT-negative groups on NPT for *D. farinae*, to evaluate their correlation with the levels of inflammatory mediators, and to determine adaptive and innate immune responses in nasal mucosa.

Subjects and Methods: Sixty AR patients underwent NPT for *D. farinae*. The sinus packs were placed in both nasal cavities of the patients for 5 minutes to obtain nasal secretion after NPT. The levels of total IgE, specific IgE to *D. farinae*, ECP, and tryptase were measured by using ImmunoCAP. Specific IgE, IgA to *D. farina*, and secretory IgA antibodies were measured by using ELISA. IL-8, VEGF, IL-25, and IL-33 levels were also measured by using ELISA

Results: The high levels of total IgE, specific IgE, specific IgA, and secretory IgA, as well as inflammatory mediators, such as ECP, IL-8, VEGF and tryptase, were detected in nasal secretion, although the differences were not statistically significant between the NPT-positive and NPT-negative groups. The levels of total IgE, specific IgE to *D. farinae*, specific IgE, IgA to *D. farina*, and secretory IgA correlated with ECP (r=0.757, 0.436, 761, 0.737 and 0.692, respectively; all

p<0.001). IL-33 and IL-25 were also highly detected, and IL-25 significantly correlated with IL-8 (r=0.625, p<0.001). Compared to the ELISA method, the ImmunoCAP system was more sensitive in the detection of specific IgE to *D. farinae*. Differences between right and left nasal secretions had no statistical significance.

Conclusion: These findings confirmed the presence of locally produced specific antibodies to *D. farinae* in nasal secretion obtained from *D. farinae*-sensitive AR patients in which close correlations were noted between the antibodies and nasal inflammatory responses. It is suggested that both innate and adaptive immune responses may be involved in nasal inflammation of AR patients.

Keywords*: Allergic rhinitis, nasal mucosa, local specific antibody*

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Abbreviations

AR: allergic rhinitis

BA: bronchial asthma;

D.: *Dermatophagoides*

ELISA: enzyme-linked immunosorbent assay

ECP: eosinophil cataionic protein

FBS: fetal bovine serum

IL: interleukin

NPT: nasal provocation test

PBS: phosphate buffered saline

SPT: skin prick test;

VEGF: vascular endothelial growth factor

I. Introduction

Allergic rhinitis (AR) is a chronic inflammatory condition in the mucosa of the nasal cavity and paranasal sinus characterized by rhinorrhea, sneezing, itching, and nasal congestion (Bousquet et al., 2008). The prevalence of AR is increasing, emerging as a global problem that affects approximately 10% to 30% of adult populations. AR not only interferes with the quality of life but also attributes to risk factors for asthma and rhinosinusitis, that early diagnosis and intervention in AR is essential in preventing asthma and further sensitization to other allergens (Greiner et al., 2012).

The most common causative inhalant allergens responsible for AR are the house dust mites, *Dermatophagoides (D.) pteronyssinus* and *D. farinae* (Bousquet et al., 2008; Platts-Mills et al., 2001). Exposure to house dust mites induces specific antibody production and nasal inflammation through various inflammatory cells, including mast cells, eosinophils, and structural cells, such as nasal epithelial cells (Ferrera et al., 2000; Rydell-Tormanen K et al., 2008; Shin et al., 2009).

The innate immune response has been identified to augment inflammation in nasal airway mucosa (Gregory et al., 2011). IL-33 and IL-25 produced by airway epithelial cells are important Th2-augmenting cytokines to affect eosinophilic homoestasis and airway inflammation (Reynolds et al., 2010; Cayrol et al., 2014). These 2 cytokines are involved in the development of allergic disease as a link

between innate and adaptive airway mucosal immunity (Saenz et al., 2008; Kariyawasam et al., 2013).

Nasal secretion is the first line defense mechanism that contains cytokines and biologic factors, such as protein, cells, and mediators to protect airway epithelium from inoculation with allergens and pathogens like bacteria and virus, and to regulate immune response in nasal inflammation (Jankowski et al., 2000). The most important point in the collection of nasal secretion is the method of collection and storage which require careful and coordinated efforts. Various methods, such as blowing of nose, suction, absorbent cotton wool samplers, and nasal lavage fluids, are applied to collect nasal secretion in order to investigate upper airway mucosa (Riechelmann et al., 2003; Lu et al., 2010). However, there has been no consensus on a standardized method for the collection of nasal secretion.

In this study, we applied a simple, non-invasive method using a cotton-ball sinus pack to collect nasal secretion in nasal provocation test (NPT)-positive and NPT-negative groups on NPT in AR patients in order to confirm the presence of locally produced specific antibodies to *D. farinae* and also evaluated their correlation with inflammatory mediators in nasal secretion between the 2 groups. Moreover, we determined the link between innate and adaptive immune responses in nasal inflammation of AR.

II. Materials and Methods

A. MATERIALS

1. Study subjects

Sixty patients who were diagnosed with AR according to ARIA classification (Rottem et al., 2010) and underwent NPT were enrolled in the study. The study subjects were divided into 2 groups according to the NPT results: the NPT-positive (n=39) and NPT-negative (n=21) groups. None of the patients received any antihistamine, leukotriene receptor antagonist or topical steroid prior to NPT. The results of skin prick test, levels of serum total and specific IgE to *D. farinae* and *D. pteronyssinus*, and eosinophilic cationic protein (ECP) levels were obtained. The study was approved by the Institutional Review Board of Ajou Medical Center, Suwon, Korea. Informed consent was obtained from each study subject.

B. Methods

1. Nasal provocation test

NPT with *D. farinae* was performed according to previously described methods(Oh et al., 2008; Kim et al., 2011). Subjects were first placed at room temperature for 30 minutes to minimize the effects of daily-life stimuli. They

underwent saline challenge tests to exclude nasal hyper reactivity. NPT was performed by applying an 8-mm filter paper disk impregnated with an allergen solution (5000 BU/μL, *D. farinae*; prepared by Allergopharma, Reinbek, Germany) to the anterior tip of inferior turbinate on the wider side of the nose for 10 minutes or until the allergic symptoms appear. Nasal symptoms for rhinorrhea, nasal itching, nasal obstruction, and sneezing on a VAS scale from 0 to 10 (subjective symptoms reported by patients) were determined at baseline and at 30 minutes after NPT. A positive response to NPT with *D. farinae* was defined as a 3 point or more increase in the total nasal symptom score, which is the sum of rhinorrhea, nasal itching, nasal obstruction, and sneezing during the test.

2. Sample collection and processing

A cotton ball sinus packs were placed on the middle turbinate for 5 minutes after NPT. After 3 ml of normal saline was added to the pack and incubated at 4℃ for 2 hours, the pack was put in the shaft of a syringe (Kovax-Syringe 10 ml; Korea Vaccine Co. LTD, Ansan, Kyeonggi-do, Korea). The syringe with the pack and no piston was placed in a Falcon tube. The Falcon tube was centrifuged at 1,500 *g* for 10 minutes at 4℃ to recover all fluid. Aliquots of 500 μl were prepared and stored at -70 ℃ for analysis. The experimental steps were modified from the method suggested by Ghent University Hospital (Watelet et al., 2004) and

adjusted according to the circumstances.

3. Measurements of local antibodies, inflammatory mediators, and inflammatory markers in the nasal secretion samples

The levels of total IgE and specific IgE to *D. farinae* and *D. pteronyssinus* in the nasal secretion were measured by using ImmunoCAP® (Thermo Fisher Scientific, Uppsala, Sweden).

The levels of specific IgE to *D. farinae*, IgA to *D. farina*, and secretory IgA in the nasal secretion were measured by using enzyme-linked immunosorbent assay (ELISA) as previously described (Ferrara et al., 2000; Oh et al., 2008). In brief, 96-well microtiter plates (Corning, Corning, NY, USA) were coated with *D. fariane* (2 μg/well, homemade antigen) (Jeong et al., 2012) and incubated overnight at 4 ℃. After washing, the plates were blocked with 10% fetal bovine serum (FBS) in phosphate buffered saline (PBS) and incubated for 2 hours. 50 μl of nasal secretion were incubated for 2 hours and biotin-labeled goat anti-human IgE antibody (Vector Lab, Burlingame, CA, USA) at 1:1000, dilution biotinylated anti-human IgA (Sigma Co., St. Louis, MO, USA) at 1:2000 dilution and monoclonal anti-secretory IgA (Sigma Co.) at 1:2000 dilution were added and incubated for 1 hour to detect specific IgE to *D. farina*, specific IgA to *D. farina*, and secretory IgA. After washing, the plates were incubated with streptavidine-

peroxidate (Sigma Co.) at 1:1000 dilution for 30 minutes, anti-goat IgG-AP (Sigma Co.) at 1:10000 dilution and anti-mouse IgG-AP (Sigma Co.) at 1:10000 dilution for 1 hour at room temperature. After washing, *o*-phenylene diamine was added as a substrate solute and the optical densities were measured by using an ELISA reader **(Bio-Tek Instruments, Winooski, VT, USA)** at 405 nm.

ECP and tryptase were measured by using ImmunoCAP® (Thermo Fisher Scientific). The level of interleukin (IL)-8 and vascular endothelial growth factor (VEGF) in the nasal secretion samples were measured by using ELISA kits (Endogen, Woburn, MA, USA and R&D Systems, Inc, Minneapolis, MN, USA, respectively). To evaluate the role of innate immune responses, IL-25 and IL-33 levels in the nasal secretion samples were measured by using ELISA kits (R&D systems).

4. Calculation of concentrations and statistical analysis

After 3 ml of normal saline were added to each sample, concentrations of local antibodies and inflammatory mediators were recalculated using protein concentration for each measurement to correct the dilution effect of the samples. All the values were shown as the protein ratio.

5. Statistical analysis

All analyses were carried out by using SPSS 20.0 (SPSS Inc., Chicago, IL, USA).

The values are presented as mean ± SD. The levels of antibodies in the nasal mucosa were compared by using Student's *t* test between the NPT-positive and NPT-negative groups. Pearson's correlation coefficients were applied to examine the correlation between local antibodies and inflammatory mediators. A *p* value of <0.05 was considered statistically significant.

III. Results

A. Characteristics of the study subjects

There were no significant differences in age or sex between the NPT-positive and NPT-negative groups. Twelve of the 39 patients in the NPT-positive group and 3 of the 18 patients in the NPT-negative group had bronchial asthma. The total nasal symptom scores before and after NPT were 2.89 ± 2.37 and 4.98 ± 2.03, respectively, in the NPT-positive group with significant difference (p<0.001) and 1.7 ± 1.83 and 1.88 ± 2.07, respectively, in the NPT-negative group without significant difference (p<0.001). The serum specific IgE level to *D. farinae* was significantly higher in the NPT-positive group than in the NPT-negative group. The clinical characteristics of the patients are summarized in Table 1.

Table 1. Clinical characteristics of the subjects

	NPT-positive group (n=39)	NPT-negative group (n=21)	*p value*
Age (years)	30.92±10.94	36.67±12.78	0.073
Sex (male/female)	15/24	13/8	0.107
Skin reactivity to *D. farinae*	8.26±7.46	7.95±7.03	0.878
AR vs AR with BA	27/12	18/3	0.218
Serum total IgE*	370.18±412.33	478.00±1095.68	0.585
Serum specific IgE to *D. fariane**	29.79±37.27	2.63±8.03	<0.001
Total nasal symptom score before NPT	2.89±2.37	1.7±1.83	0.035
Total nasal symptom score after NPT	4.98±2.03	1.88±2.07	<0.001

All data are expressed as mean±SD.

*Measured by ImmunoCAP method. D: *Dermatophagoides*, AR: allergic rhinitis, BA: bronchial asthma, VEGF: vascular endothelial growth factor, NPT: nasal provocation test

B. Comparison of local antibody levels in nasal secretion between the NPT-positive and NPT-negative groups

Fig. 1 shows comparison of total IgE, specific IgE to *D. farinae*, specific IgA to *D. farina*, and secretory IgA; between the NPT-positive and NPT-negative groups, although some AR patients had high levels of local antibodies in nasal secretions, there were no significant differences in the variables between the NPT-positive and NPT-negative groups (p=0.656, 0.285, 0.988, 0.699, and 0.507, respectively).

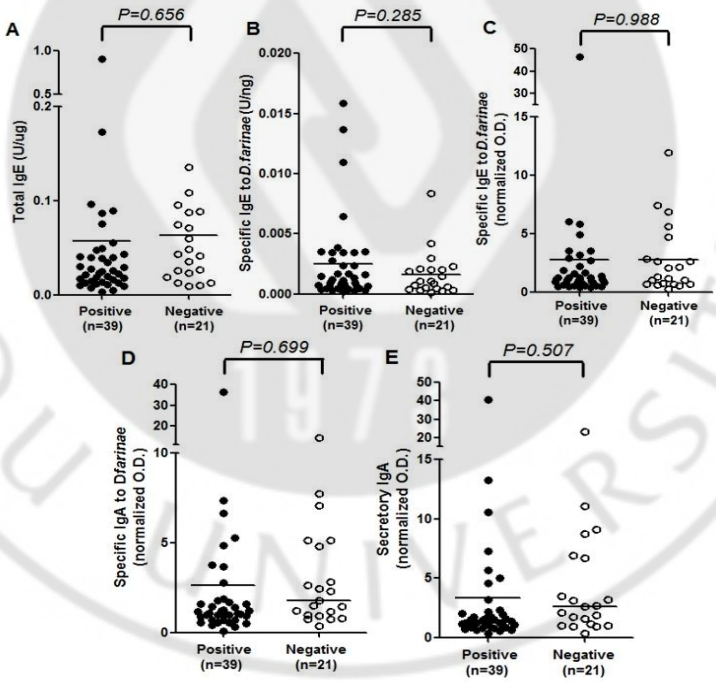

Fig. 1. Detection of local specific antibodies in nasal secretion of the NPT-positive and NPT-negative groups. Total IgE (A) specific IgE to *D.farinae* (B) were measured by using ImmunoCAP. Specific IgE *to D.farinae* (c), Specific IgA *to D. farina* (D), and E. secretory IgA (E) were measured by using ELISA method. All values were adjusted by the protein ratio.

C. Comparison of inflammatory markers in nasal secretion between the NPT-positive and NPT-negative groups

Fig. 2 shows comparison of the levels of ECP, tryptase, IL-8, and VEGF between the NPT-positive and NPT-negative groups. Some AR patients had high levels of ECP, tryptase, IL-8, and VEGF. There were no significant differences in ECP, tryptase, or VEGF levels between the NPT-positive and NPT-negative groups (*P*=0.508, 0.490, and 0.972, respectively), while the IL-8 level was significantly higher in the NPT-negative group than in the NPT-positive group (*p*=0.02)

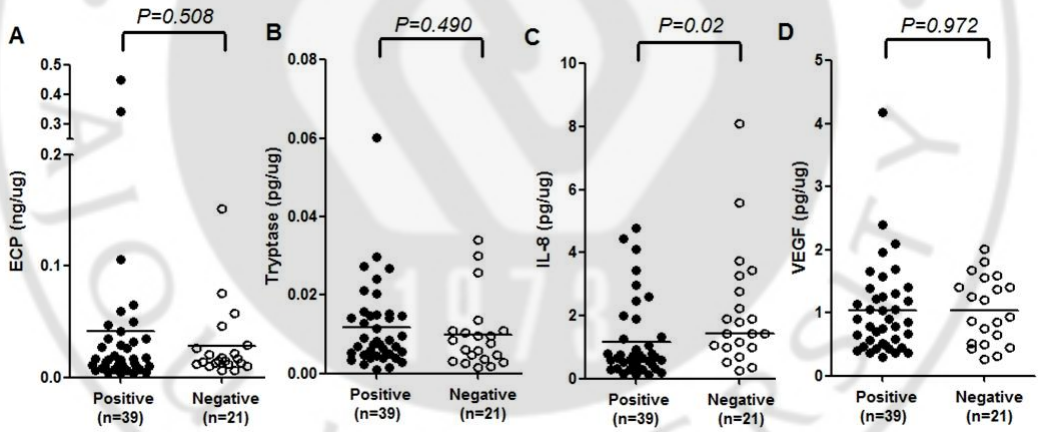

Fig.2. Detection of inflammatory mediators in nasal secretion in the the NPT-positive and NPT-negative groups. ECP (A) and tryptase (B) were measured by using ImmunoCAP. IL-8 (C) and. VEGF (D) measured by using ELISA method. All values were adjusted by the protein ratio.

D. Comparison of IL-33 and IL-25 levels in nasal secretion between the NPT-positive and NPT-negative groups

Fig. 3 shows comparison of IL-33 and IL-25 between the NPT-positive and NPT-negative groups. Some AR patients had high levels of IL-33 and IL-25 but did not show significant difference (p=0.163 and 0.392, respectively)

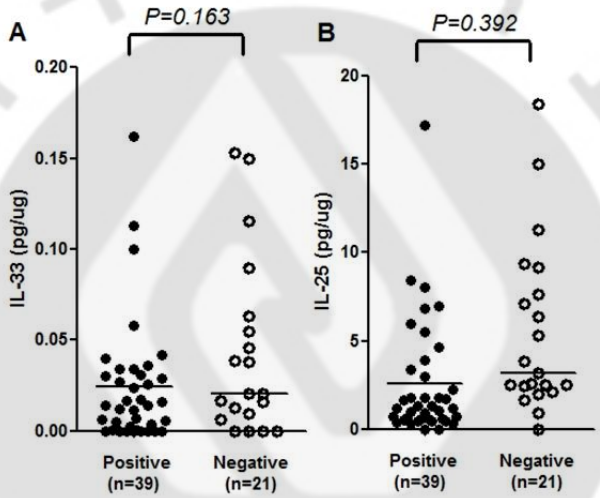

Fig.3. Detection of IL-33 and IL-25 in nasal secretion of the NPT-positive and NPT-negative groups. IL-33 (A) and IL-25 (B) measured by using ELISA method. All values were adjusted by the protein ratio.

E. Correlation between local specific antibodies to D. farinae and inflammatory markers in nasal secretion

ECP correlated with total IgE, specific IgE to *D.farinae*, specific, IgA to *D. farina*, and secretory IgA (r=0.757, 0.463, 0.761, 0.737 and 0.692, respectively) with

statistical significance (p<0.001) (Fig. 4). However, there was no significant correlation between tryptase and local antibodies (r=0.179, 0.208, 0.219, 0.169, and 0.170, respectively; all p>0.05) (Fig. 5). IL-8 and VEGF showed a significant correlation with local specific antibody levels and statistically significant (r=0.283, 0.239, 0.306, 0.299, 0.295 and r=0.593, 0.387, 0.618, 0.612, and 0.566, respectively: p<0.05) (Figs. 6 and 7).

A significant correlation was noted between ECP and VEGF (r=0.520, p<0.001), but not between tryptase, IL-8 and VEGF.

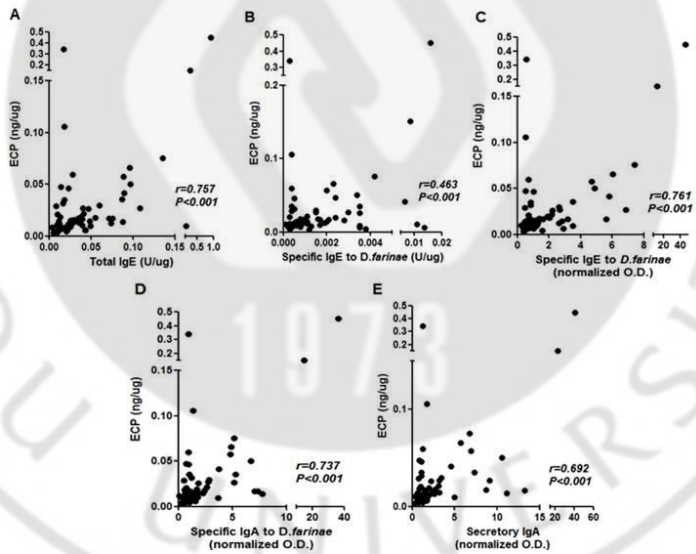

Fig. 4. Correlation of ECP levels with local specific antibodies in nasal secretion. A. Total IgE (A) and specific IgE to *D. farinae* (B) were by using ImmunoCAP. Specific IgE to *D. farinae* (C), specific IgA to *D. farinae* (D), and secretory IgA measured by using ELISA. All values were adjusted by the protein ratio. Pearson's rank correlation coefficients were applied to the test.

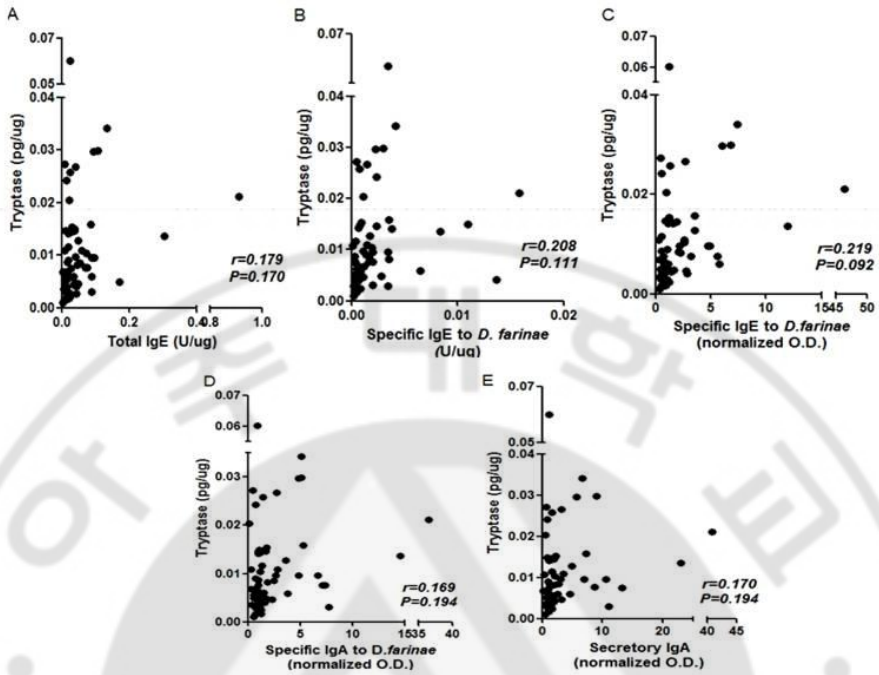

Fig. 5. Correlation of tryptase levels with local specific antibodies in nasal secretion.. Total IgE (A) and specific IgE to D. *farinae* (B) were measured by ImmunoCAP. Specific IgE to *D. farina* (C) specific IgA to *D. farina* (D), and secretory IgA (E) were by using ELISA. All values were adjusted by the protein ratio. Pearson's rank correlation coefficients were applied to the test.

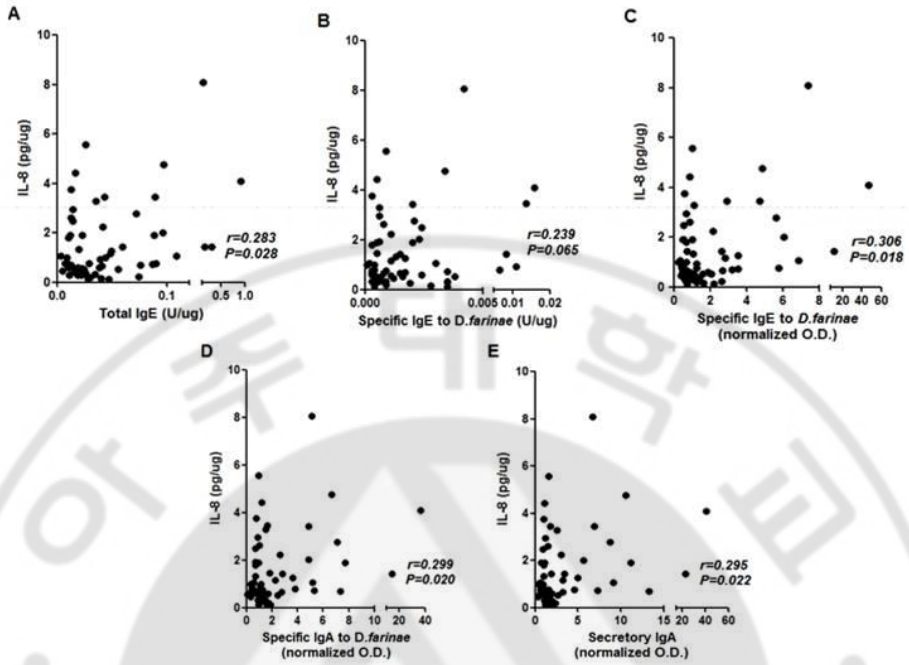

Fig. 6. Correlation of IL-8 levels with local specific antibodies in nasal secretion. IL-8 levels were measured by using ELISA. Total IgE (A) and specific IgE to *D. farinae* (B) were measured by using ImmunoCAP. Specific IgE to *D. farinae* (C), D. specific IgA to *D.farinae* (D), and secretory IgA (E) were measured by ELISA. All values were adjusted by the protein ratio Pearson's rank correlation coefficients were applied to the test.

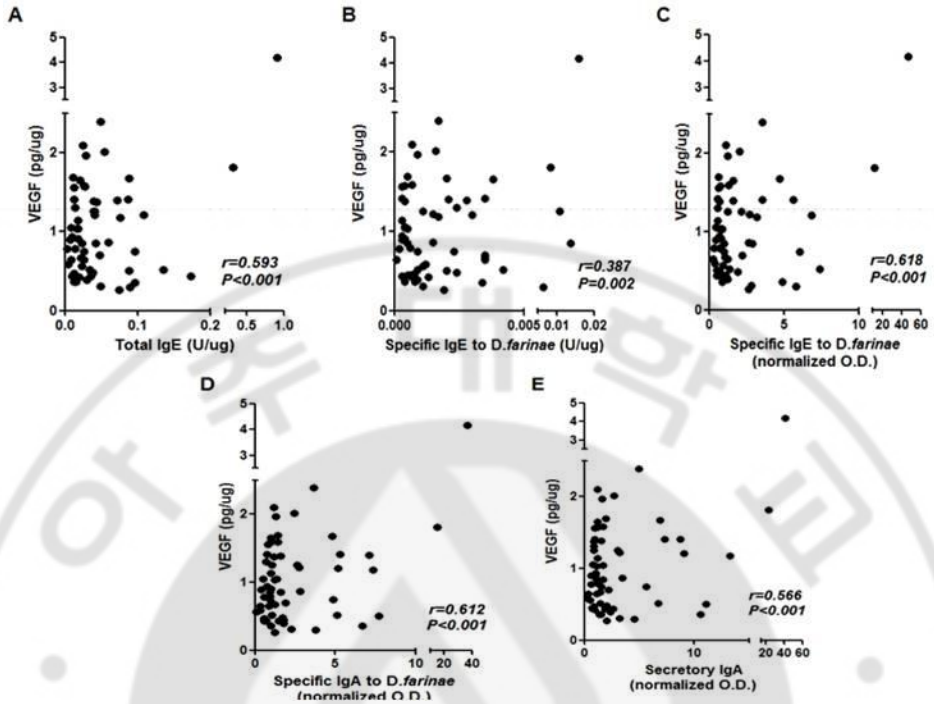

Fig. 7. Correlation of VEGF levels with local specific antibodies in nasal secretion. VEGF levels were measured by using ELISA. Total IgE (A) and specific IgE to D. *farinae* (B) were measured by using ImmunoCAP. C. Specific IgE to *D. farinae* (C), specific IgA to *D. farinae* (D), and E. secretory IgA were measured by ELISA. All values were adjusted by the protein ratio. Pearson's rank correlation coefficients were applied to the test.

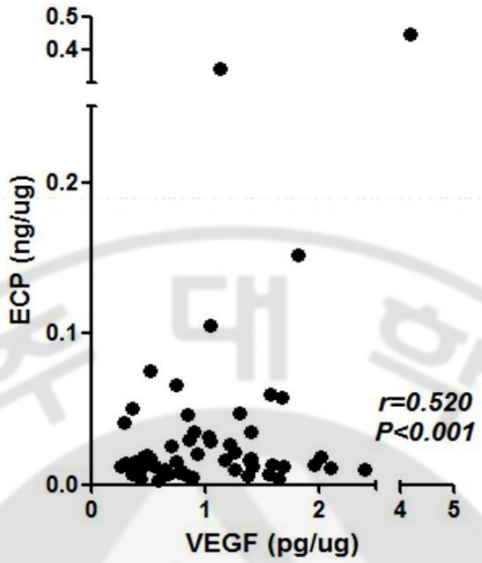

Fig. 8. Correlation of ECP with VEGF in nasal secretion. All values were adjusted by the protein ratio Pearson's rank correlation coefficients were applied to the test.

F. Correlation of local specific antibodies and inflammatory mediators with IL-25 and IL-33

The correlation between local specific antibodies and inflammatory mediators with IL-25 and IL-33 was evaluated (Table 2). No significant correlation noted except between IL-25 and IL-8 (r=0.625, p<0.001).

Table 2. Correlation of local specific antibodies *to D. farina* and inflammatory mediator with IL-25 and IL-33

	IL-33*		IL-25	
	r	*p*-value	*r*	*p* value
Total IgE*	-0.171	0.191	0.079	0.546
Specific IgE to *D.farinae**	-0.162	0.216	0.218	0.095
Specific IgE to *D.farinae*†	-0.0186	0.164	0.141	0.283
Specific IgA to *D.farinae*†	-0.0186	0.156	0.086	0.516
Secretory IgA†	-0.199	0.127	0.072	0.583
Specific IgG to *D.farinae*†	-0.186	0.154	0.141	0.283
ECP*	0.017	0.900	0.025	0.850
Tryptase*	-0.173	0.187	0.021	0.873
IL-8†	-0.039	0.768	0.625	<0.001
VEGF†	0.025	0.851	0.084	0.523
IL-25†	-0.012	0.926		

All values were adjusted by the protein ratio.

*Measured by using ImmunoCAP method.

†Measured by using ELISA method.

Pearson's rank correlation coefficients were applied to the test. All values were adjusted by the protein ratio.

D=Dermatophagoides; ECP=eosinophil cationic protein*;* IL=interleukin*;* VEGF=vascular endothelial growth factor

IV. Discussion

IgE plays a key role in type I hypersensitivity that manifests various allergic diseases, such as AR, allergic asthma, and food allergy (De Schryver et al., 2015). IgA is an antibody produced mostly in the mucosal lining and plays a critical role in mucosal immunity (Fagarasan et al., 2003). Secretory IgA, a dimeric form of IgA found in mucous secretion such as nasal secretions and saliva, is associated with allergic diseases (Corthésy. 2013). Although allergen-specific IgA is known to be involved in allergen tolerance, few studies on physiologic functions of specific IgA has been conducted (Vazquez-Ortiz et al., 2013). In this study, high specific IgA and secretory IgA levels were noted in nasal secretion in the NPT-positive and NPT-negative groups. A study in chronic rhinitis patients with nasal polyp showed subepithelial accumulation of secretory IgA, suggesting that it may be involved in Th2-related eosinophilic inflammation (Hupin et al., 2013). Many studies have revealed the role of IgA in innate immunity, suggesting that natural helper cells can help B cells to produce IgA or the IgA-based immune complex against certain antigens (Jankowski et al., 2000; Wijburg et al., 2006; Koyasu et al., 2011). In our study, locally produced specific IgE and IgA were detected in nasal secretions in both the NPT-positive and NPT-negative groups of AR patients. A significant correlation was noted between specific IgE and IgA (specific and secretory), and between ECP and specific IgE/IgA. These findings suggest that

locally produced specific and secretory IgA, as well as specific IgE, may play a role in the nasal inflammation of AR patients.

Eosinophilic inflammation plays a key role in the pathogenesis of AR; therefore, the ECP level in nasal secretion may be used to monitor the activity of eosinophilic inflammation and the efficacy of anti-inflammatory treatment (Bystrom et al., 2012; Kariya et al., 2015). In this study, we confirmed a higher ECP level in the nasal secretion of AR patients. However, a significant correlation was found between ECP and local specific IgE/IgA, between various inflammatory mediators, such as IL-8 and VEGF levels. These findings suggest that ECP is a key mediator of nasal inflammation in AR patients in that specific antibodies, including IgE and IgA, could be involved in eosinophilic inflammation in AR patients.

Mast cells contain cytokines, chemokines, and growth factors as well as mediators, such as histamine and tryptase. They participate in transition from innate to adaptive immunity (Galli et al., 2011). Tryptase is the most common mediator contained in mast cells, and has been used as a marker for mast cell activation. Numerous studies have proven the function of mast cells in type I hypersensitivity reaction, alteration of vascular permeability, and tissue swelling associated with IgE (Wedemeyer et al., 2000; Williams et al., 2000). In this study, high tryptase levels were noted in nasal secretion of AR patients in both the NPT-

positive and NPT-negative groups, but there was no significant correlation between the tryptase levels and local antibodies/other inflammatory mediators. Mast cell plays an important role in innate immunity as a central component against bacterial infections and inflammation in different organs, such as the airway or intestine, as well as in immune responses associated with Th2 cells and IgE (Galli et al., 1999). Tryptase shows no correlation with local antibodies; however, mechanisms other than local may be involved in the activation of mast cells.

Epithelial cells are positioned on the first line of exposure to allergens and pathogens and regulate innate and adaptive immune systems. By interactions with cells and production of functional molecules IL-8 produced by epithelial cells, macrophages, and smooth muscle cells is released to the nasal fluid and recruits other inflammatory cells, such as eosinophils and neutrophils (Ohkubo et al., 1998). Other study (Zuyderdyun et al., 2006) have reported that IL-8 is released by airway smooth muscle cells in response to airway inflammation, and Simpson (Simpson et al., 2007) have suggested that airway disease is an innate immune response associated with neutrophilic inflammation by elevation of IL-8. In this study, high IL-8 levels were noted in nasal secretion of AR patients. Moreover, the IL-8 level was significantly higher in NPT-negative group than in NPT-positive group. A significant correlation was noted between specific antibodies

and various inflammatory mediators, such as ECP, tryptase, and VEGF. IL-25, a cytokine released from activated epithelial cells, increases in nasal secretion after exposure to house dust mites and correlates with the IL-8 level. These findings suggest that activated epithelial cells may be involved in nasal inflammation of AR patients via innate and adaptive immune responses.

VEGF is a potent signal protein to induce vascular angiogenesis, permeability, remodeling, and wound protection/healing, which is derived from macrophages, neutrophils, eosinophils, epithelial cells and fibroblasts. Recent studies have demonstrated that VEGF regulates immune responses by inhibiting T-cell development and dendritic-cell activity and by stimulating eosinophil chemotaxis(Ferrara et al., 2000; Dvorak et al., 1995). Choi (Choi et al., 2009) have reported EGF-induced eosinophilic inflammation in the nasal lavage fluid in patients with AR sensitized to house dust mites. In this study, high VEGF levels were noted in AR patients in both the NPT-positive NPT-native groups, with a significant correlation with local specific antibodies, such as specific IgE, specific IgA, and secretory IgA. These findings suggest that the vascular factor may be another key component for nasal inflammation of AR regardless of positivity to NPT, which can be a target for anti-inflammatory treatment.

IL-25 and IL-33 both produced by sinonasal epithelial cells are reported to have critical roles in promoting Th2-mediated inflammation (De Schryver et al., 2015).

Previous studies have shown a role of IL-25 in augmenting allergic inflammation by epithelial cell hyperplasia, secretion of mucus, airway hyperresponsiveness, and production of specific eosinophilic chemokines and Th2 cytokines (Fort et al., 2001; Hurst et al., 2002). In this study, the innate immunity-related cytokines, IL-25 and IL-33, were detected in nasal secretion of AR, which correlated strongly with IL-8, while no significant correlation was found between IL-33 and inflammatory mediators/local specific antibodies. These findings suggest that innate immune responses induced by nasal epithelial cells may augment nasal inflammation of AR.

NPT is an effective method for the diagnosis of AR in patients without definitive history or clinical manifestation. Although NPT is time-consuming and has a potential for anaphylactic reactions, it has been widely used to confirm causative allergens in occupational asthma or in aspirin exacerbated respiratory disease. Nasal secretion is a heterogeneous fluid composed of protein, various cells, plasma exudates, and mucus. Since considerable inter-individual variations exist in terms of amount, composition, biological activity, and cellular components, nasal secretion is difficult to obtain and needs techniques to collect sufficient amounts (Lu et al., 2010). In our study, a non-invasive and inexpensive method using a cotton ball sinus pack was introduced, and both ELISA and ImmunoCAP methods were applied to detect specific IgE to *D. farinae* in order to validate

consistency of the method and showed good results. It allows collection of sufficient amounts of nasal secretion, need no special techniques or instruments, and does not cause discomfort to patients. Our method was conducted to all 60 patients, and the specific antibodies and inflammatory mediators were detected, which suggests that this method may be steady and highly reproducible.

NPT provokes rhinorrhea, which allowed collection of sufficient amounts of nasal secretion. Since nasal secretion is insufficient before NPT or in normal controls, nasal secretion samples are difficult to collect. Nasal secretion is easy to collect after NPT, which allow investigators to further elucidate mechanisms underlying nasal inflammation and therapeutic outcomes of pharmacologic treatment and allergen immunotherapy.

V. CONCLUSION

In conclusion, we confirmed the presence of local specific antibodies and inflammatory mediators in the nasal secretion of AR patients by using a simple, non-invasive method of collecting nasal secretion. Specific IgE and IgA antibodies may be involved in nasal eosinophilic inflammation and innate immune responses via IL-25 and can be involved in nasal inflammation via epithelial-cell activation.

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국문 초록

서론 : 알레르기 비염은 점차 증가하고 있는 흔한 질환으로 북미형집먼지 진드기 (*Dermatophagoides farinae*)는 가장 흔히 알려지 원인 항원이다. 본 연구의 목적은 비강 유발검사 후 비즙을 채취하고 비강유발검사 양성 및 음성군으로 나누어 국소 항체의 다양성을 확인하고 염증 매개 물질과의 연관성 그리고 비점막의 선천 면역과 획득 면역을 확인해 보고자 하였다.

대상 및 방법 : 집먼지 진드기에 감작된 60명의 환자를 대상으로 북미형 집먼지 진드기를 이용하여 비강 유발검사를 시행하였다. 이후 환자의 부비강에 5분강 비강 패킹을 시행하여 비즙을 채취하였다. 비강유발검사 양성군 39명과 음성군 21명에 대해 ImmunoCAP 방법으로 IgE, 미형 집먼지 진드기에 대한 특이 IgE, ECP, 및 tryptase를 측정하였고 ELISA 방법을 통해 특이 IgE, 특이 IgA와 분비 IgA 항체 및 IL-8, VEGF, IL-25, IL-33을 측정하였고 양군간에 비교, 분석을 시행하였다.

결과 : 총 IgE 및 특이 IgE, 특이 IGA 및 분비 IgA 뿐만 아니라 ECP, IL-8, VEGF와 trypase 등의 염증 매개 물질 또한 높은 농도로 측정이 되었으나 유발검사 양성군과 음성군 상의 통계적인 의의는 없었다. 총

IgE, 특이 IgE, 특이 IgE, 분비 IgA는 ECP와 연관성을 보여주었고 (각각 r=0.757, 0.436, 0.761, 0.737, 0.692, 모두 p<0.001), IL-33과 IL-25도 높은 도로 측정이 되었으며 특히 IL-25와 IL-8의 연관성을 확인하였다. (r=0.625, p<0.001). 또한 북미형 집먼지 진드기 검출 방법으로는 ELISA와 비교하였을 때 ImmunoCAP 방법이 민감도가 더 좋은 것을 확인하였다.

결론 : 이 연구를 통해 북미형집먼지 진드기에 감작이 된 비염 환자의 비점막에 존재하는 국소항체의 존재와 염증 매개 물질 사이의 연관성 및 비염증 반응에 관여하는 것을 확인하였다. 또한 비염 환자의 비염증 반응에 선천 및 획득 면역 모두 관여하는 것도 확인할 수 있었다.

Keyworlds: Allergic rhinitis, nasal mucosa, local specific antibody