

IgE Response to Staphylococcal Enterotoxins in Adenoid Tissues from Atopic Children

Seung-Youp Shin, MD; Gil-Soon Choi, MD; Kun-Hee Lee, MD, PhD; Sung-Wan Kim, MD, PhD;
Joong-Saeng Cho, MD, PhD; Hae-Sim Park, MD, PhD

Objectives/Hypothesis: To evaluate local production of staphylococcal superantigen (SAG)-specific IgE in adenoid tissue and to compare its prevalence with that in the tonsil and serum, as well as its relationship with markers of allergic inflammation within adenoid tissue.

Study design: Prospective randomized study.

Methods: We recruited 18 atopic children who had rhinitis symptoms and were sensitized to more than one common aeroallergen, and 22 nonatopic children undergoing adenotonsillectomy. Immunoassays were performed using adenoid tissue homogenate to quantify the levels of three staphylococcal SAG-specific IgE (SEA, SEB, and TSST-1), total IgE, eosinophil cationic protein (ECP), mast cell tryptase, and soluble CD23.

Results: Three kinds of SAG-specific IgE were detected in the adenoid and tonsil tissues of atopic patients, but not in those of nonatopic patients. In atopic children, the prevalence of SEA-, SEB-, and TSST-1-specific IgE in adenoid tissues (61.1%, 27.8%, 33.3%, respectively) were higher than those in tonsil tissues (38.9%, 5.6%, 11.1%, respectively) and in sera (11.1%, 27.8%, 16.7%, respectively). Subjects with high SEA levels showed significantly higher serum and adenoid total IgE, with higher eosinophilia. Significant correlations were noted between SAG-specific IgE levels and tryptase levels in adenoid tissue.

Conclusions: Local specific IgE response to staphylococcal SAGs, especially SEA may contribute to ongoing allergic inflammation in adenoid tissue from atopic children.

Key Words: Staphylococcal superantigen, adenoid, atopy, eosinophil, mast cell.

Laryngoscope, 119:171–175, 2009

INTRODUCTION

The adenoids are believed to serve as a reservoir for pathogens responsible for several upper airway diseases. The predominant aerobic bacteria in the core tissue are α -hemolytic and γ -hemolytic streptococci, *Haemophilus influenzae*, and *Staphylococcus aureus*, which are also known as etiologic pathogens in otitis media, rhinosinusitis, and adenotonsillitis.^{1,2} Recently, there has been an increased interest in the potential role of adenoid tissue in atopic diseases. A few studies have shown that adenoids from atopic children have cellular and cytokine profiles that differ from those seen in nonatopic children, and that eosinophils could be involved in adenoid inflammation.^{3,4}

S. aureus is a human pathogen responsible for several infectious upper airway diseases. According to recent studies, staphylococcal enterotoxins (SEs) potentially play a role in the pathophysiology of atopic diseases. SEs are a large protein family composed of SEA to SEQ, along with toxic shock syndrome toxin-1 (TSST-1), all of which may act as superantigens (SAGs). These SAGs have the capacity to activate large populations (5%–20%) of T cells through cross-linking MHC class II molecules on antigen-presenting cells (APCs) with a variable V β region on T cell receptors, thus bypassing common antigen presentation mechanisms.⁵ In addition to classic SAG effects, SAGs may also act as conventional allergens, inciting specific IgE responses in the serum. The presence of this SAG-specific IgE in serum is associated with severe atopic dermatitis (AD) and airway diseases such as asthma, rhinitis, and nasal polyps;^{6–9} the conventional allergic reaction is believed to augment local SAG-mediated inflammation. Both murine models and human studies have demonstrated Th2-type cytokine secretion and polyclonal IgE synthesis in response to SAG stimulation.^{10,11} However, to the best of our knowledge, little effort has been expanded to detect local SAG-mediated inflammation in the respiratory mucosa of children.

From the Department of Otolaryngology (S.-Y.S., K.-H.L., S.-W.K., J.-S.J.), Kyunghee University Hospital and Kyunghee University College of Medicine, Seoul, Korea; and Department of Allergy and Rheumatology (G.-S.C., H.-S.P.), Ajou University Hospital and Ajou University School of Medicine, Suwon, Korea.

Editor's Note: This Manuscript was accepted for publication July 15, 2008.

This study was supported by a grant from the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (A030001 and A050571).

Send correspondence to Hae-Sim Park, MD, PhD, Department of Allergy and Rheumatology, Ajou University, School of Medicine, Wonchondong San-5, Paldalgu, Suwon 442-749, Korea. E-mail: hspark@ajou.ac.kr

DOI: 10.1002/lary.20046

We hypothesized that locally produced SAg-specific IgE might be detected in adenoid tissue and might be involved in allergic inflammation. To confirm local production of SAg-specific IgE, we classified the study subjects into two groups according to atopy status. To evaluate the role of SAg-specific IgE in allergic inflammation in adenoid tissue, we measured SEA-, SEB-, and TSST-1-specific IgE levels in adenoid tissue homogenates from the two groups, comparing the prevalence of the specific immunoglobulins with those seen in the tonsils and serum. We measured the following parameters: serum total IgE; peripheral blood total eosinophil count (TEC); and total IgE, ECP, tryptase, and soluble CD23 (sCD23) levels in adenoid tissue homogenate. We also observed the relationship between SEA-, SEB-, and TSST-1-specific IgE and inflammatory markers within adenoid tissue.

MATERIALS AND METHODS

Subjects

Forty children participated in this study: 26 boys and 14 girls (mean age 7.9 years, range 2.8–15 years). All subjects underwent adenotonsillectomy between June and July 2007 at the Ears, Nose, and Throat (ENT) Department of the Kyunghee University Hospital in Seoul, Korea. Parental informed consent was obtained, and the study was approved by the local ethics committee. All patients were referred to the ENT department because of rhinitis symptoms, such as nasal obstruction or rhinorrhea, secondary to allergic rhinitis, recurrent infection, or adenoid hypertrophy. The parents or foster parents of the children were asked to complete an extensive questionnaire concerning symptoms and signs of each child's disease(s), medications, and family history with respect to allergy.

Before surgery, all children underwent a routine ENT examination. Blood samples obtained from patients during the operation were used for the multiple allergosorbent chemiluminescence assay (MAST-CLA) (Hitachi Chemical Diagnostics Inc., CA, USA), to determine serum total IgE concentration, and to count eosinophils. For the MAST-CLA, antigens attached to threads were sequentially incubated with patient serum, enzyme-absorbed anti-IgE, and a photoresponse reagent. The chemical fluorescence generated by each was developed on Polaroid film and scored according to a 5-point system, from 0 to 4. All reactions of grade 2 or higher were scored as positive.

Atopy was defined as a combination of rhinitis symptoms, a familial history of allergic disease, a sufficiently high total IgE for age (>10 IU/year of age), and a score of 2+ on the MAST-CLA. Patients were excluded if antihistamine-containing medications were used intraoperatively or within the preceding week, or if immunosuppressive agents, including steroids, were used intraoperatively or within the preceding six weeks.

Preparation of Adenoid and Tonsil Tissue Homogenate. Each patient's adenoids were removed with a curette, and tonsils were removed using mono-polar electrocautery. Partial pieces of the adenoids and tonsils (0.8 gm) were washed, thawed, and dispersed in 1 ml of LIPA buffer (Sigma, St. Louis, MO) with a protease inhibitor (Roche, Indianapolis, IN). The fragments were harvested by centrifugation, and the supernatants were retained and stored at -70°C before use. In order to correct for the dilution factor between individual tissues, all parameters measured in the adenoid and tonsil tissue homogenate are presented as ratios to albumin level in adenoids and tonsils, as described previously.¹² Albumin levels in adenoid and tonsil

tissue homogenate were measured using a QuantiChrom BCP Albumin Assay Kit (BioAssay Systems, Hayward, USA).

Immunoassays. The levels of SEA-, SEB-, and TSST-1-specific and total IgE were measured using the ImmunoCAP system (Pharmacia, Uppsala, Sweden), with a confidence range of 0.35–100 kIU_A/L. The adenoid and tonsil tissue homogenates were not diluted for the IgE assays. ECP (diluted 1:5) and tryptase (diluted 1:20) levels in adenoid tissue homogenates were also quantified using the ImmunoCAP system. The minimal detection limits for ECP and tryptase were 2 ng/mL and 4 ng/mL, respectively. ECP and tryptase concentrations were corrected for albumin content and expressed as nanograms per milligram of albumin. Levels of sCD23 (Bender MedSystems, Vienna, Austria) were quantified using enzyme immunoassays.

Statistical Analyses

Comparison of ECP, tryptase, and sCD23 levels, as well as SEA-, SEB-, and TSST-1-specific IgE levels between atopic and nonatopic patients, was performed through a Mann-Whitney U test, using SPSS version 12.0 (Chicago, IL, USA). Pearson correlations were applied to define the relationships among SAg-specific IgE and inflammatory marker levels. *P* values of 0.05 or below were regarded as statistically significant.

RESULTS

Clinical Characteristics of Study Subjects

Eighteen patients (45%, 13 boys and 5 girls) were found to have atopy. Twenty-two nonatopic patients (55%) served as a control group. The characteristics of the study subjects are shown in Table I. There were no

TABLE I.
Clinical Data for Study Subjects and Detected Enterotoxins.

	Atopic Patients (n = 18)	Nonatopic Patients (n = 22)	<i>P</i>
Gender (M/F)	13/5	13/9	NS
Age* (years)	8.2 ± 3.5	7.7 ± 3.5	NS
Enterotoxin detected in adenoid tissue (positive/negative)			
SEA	11/7	0/22	.001
SEB	5/13	0/22	.013
TSST-1	6/12	0/22	.005
Enterotoxin detected in tonsil tissue (positive/negative)			
SEA	7/11	0/22	.002
SEB	1/17	0/22	NS
TSST-1	2/16	0/22	NS
Enterotoxin detected in serum (positive/negative)			
SEA	2/16	0/22	NS
SEB	5/13	0/22	.005
TSST-1	3/15	5/17	NS
Serum total IgE† (IU/mL)	359.4 (328)	53.7 (53)	.001
Total eosinophil count† (/mm ³) in peripheral blood	280.6 (135)	172.4 (125)	.021

*Values are expressed as mean ± SD.

†Total IgE and eosinophil counts are expressed as median (range). Statistical comparisons between atopic and nonatopic values were performed using cross tab analysis or Mann-Whitney U test.

MAST-CLA = multiple allergosorbent chemiluminescence assay; NS = not significant.

TABLE II.
Comparison of Median Inflammatory Marker Levels in Adenoid Tissues of Atopic and Nonatopic Patients.

	Atopic Patients	Nonatopic Patients	P
ECP (ng/mL)	380.4 ± 266.3	272.9 ± 420.4	.02
Tryptase (ng/mL)	145.9 ± 169.1	87.0 ± 52.8	.42
sCD23 (U/mL)	1667.7 ± 1361.7	861.5 ± 411.8	.048
Total IgE (kU/mL)	37.0 ± 27.4	5.4 ± 4.4	.001

r = Pearson correlation coefficient; ECP = eosinophil cationic protein; sCD23 = soluble CD23.

All parameters are depicted as a ratio to the albumin level.

significant differences in gender ratio or age between the two groups. Atopic patients were most commonly reactive to house dust mites (HDM, 88.9%), with 28% of the atopic patients reactive to animal (dog and cat) dander. Serum total IgE levels and peripheral blood eosinophil counts were significantly higher in atopic patients than in nonatopic patients ($P < .01$, $P = .021$, respectively).

Comparison of SAg-specific and Total IgE Levels Between Atopic and Nonatopic Patients. On adenoid tissue homogenate analysis, 11 children were positive for SEA-specific IgE, 5 children were positive for SEB-specific IgE, and 6 children were positive for TSST-1-specific IgE. All of these patients were atopic. On tonsil tissue homogenate analysis, 7 children were positive for SEA-specific IgE, 1 child was positive for SEB-specific IgE, and 2 children were positive for TSST-1-specific IgE, and all were atopic. Two children positive for SEB-specific IgE and 7 children positive for TSST-1-specific IgE in sera were negative for SAg-specific IgE in both adenoid and tonsil tissues homogenates (Table I). On serum analysis using the ImmunoCAP system, 2 children were positive for SEA-specific IgE, and 5 children were positive for SEB-specific IgE. All SEA- and SEB-specific IgE children were atopic. Eight children

were positive for TSST-1-specific IgE, 3 of which were atopic, and 5 of which were nonatopic (Table I). Serum total IgE levels and total IgE levels in adenoid tissue homogenates for the two groups are shown in Tables I and II. Total IgE levels in serum and adenoid tissue homogenates were significantly higher in atopic patients than in nonatopic patients ($P < .01$, $P < .01$, respectively).

Inflammatory Markers in Adenoid Tissue. A comparison of ECP, tryptase, and sCD23 levels in adenoid tissue homogenates between atopic and nonatopic patients is shown in Table II. The ECP and sCD23 levels in adenoid tissue were significantly higher in atopic patients than in nonatopic patients ($P = .026$, $P = .048$, respectively), while no statistically significant difference was noted between the two groups with regard to tryptase level ($P = .422$; Table II). The levels of SEA-, SEB-, and TSST-1-specific IgE in adenoid tissues correlated significantly with the levels of adenoid tryptase ($r = .673$, $r = .572$, $r = .738$, respectively; Fig. 1), levels of adenoid total IgE ($P < .01$, $P < .01$, $P < .01$, respectively), and the eosinophil count in peripheral blood ($r = .38$, $r = .37$, $r = .30$, respectively). In addition, the levels of SEA-specific IgE in adenoid tissues correlated significantly with the levels of serum total IgE ($r = .46$), but correlated poorly with those of SEB- and TSST-1-specific IgE ($r = .25$, $r = .29$, respectively).

Stratification for Staphylococcal Enterotoxin

A Status. All 40 children were stratified for the presence or absence of detectable SEA-specific IgE in adenoid tissues, irrespective of atopy status (Table III). All children positive for SEA-specific IgE (SEA positive, $n = 11$) and 7 children negative for SEA-specific IgE (SEA negative, $n = 29$) were atopic. SEA-positive children did not differ in gender ratio or age. However, the SEA-positive children had striking increases in total IgE in adenoid tissue and sera ($P < .01$, $P < .01$, respectively), and in peripheral blood TEC, ($P = .016$) compared with SEA-negative children. SEA-positive children also showed significantly increased levels of sCD23

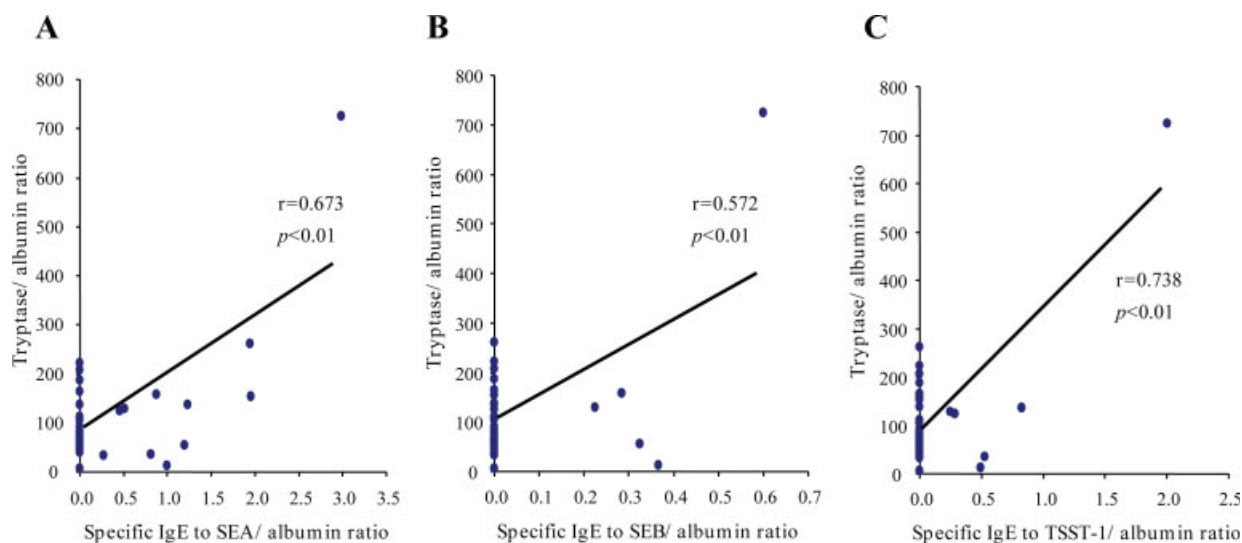


Fig. 1. Correlation between SEA-, SEB-, and TSST-1-specific IgE levels and tryptase level in adenoid tissues of atopic ($n = 18$) and nonatopic subjects ($n = 22$).

TABLE III.
Stratification of Study Subjects (n = 40) Based on the Presence or Absence of SEA-specific IgE in Adenoid Tissue.

	SEA-positive (n = 11)	SEA-negative (n = 29)	P
Gender (M/F)	7/4	19/10	NS
Age (years)	8.1 ± 3.9	7.8 ± 3.3	NS
Atopy (positive/negative)*	11/0	7/22	0.001
Chronic rhinosinusitis (positive/negative)	5/6	11/18	NS
Serum total IgE (IU/L)*	476.4 ± 354.8	83.1 ± 106.2	.001
Total eosinophil count (/mm ³) in peripheral blood*	311.8 ± 134.5	183.7 ± 124.5	.016
Adenoid total IgE (kU/L)*	50.6 ± 26.5	7.9 ± 7.4	.001
Tryptase (ng/mL)*	165.7 ± 199.0	90.3 ± 55.4	.45
ECP (ng/mL)*	397.6 ± 317.1	292.3 ± 375.0	.19
sCD23 (U/mL)*	1626.5 ± 850.9	1070.3 ± 1046.2	.047

*Values are expressed as mean ± SD. Statistical comparisons between the groups were performed using cross tab analysis or Mann-Whitney U test. NS = not significant; SEA = staphylococcal enterotoxin A; ECP = eosinophil cationic protein; sCD23 = soluble CD23.

in adenoid tissue ($P = .047$), but no differences in ECP or tryptase in adenoid tissue.

DISCUSSION

This study confirmed, for the first time, the presence of SAg-specific IgE and determined its local effect in children. This study also confirmed augmented total IgE levels in the sera and tissue homogenates, and peripheral blood eosinophilia, in atopic children positive for SAg-specific IgE. The prevalences of SEA-, SEB-, and TSST-1-specific IgE in adenoid tissues from atopic children were 61.1%, 27.8%, and 33.3%, respectively. Prevalences were 38.9%, 5.6%, and 11.1%, respectively, in tonsil tissues and 11.1%, 27.8%, and 16.7%, respectively, in sera (Table I). From these results, the following might be deduced. First, SEA was the most prevalent positive SAg in respiratory tissues from atopic children, a finding that correlates with other studies concerning respiratory mucosa. All subjects positive for SEB- and TSST-1-specific IgE were also positive for SEA-specific IgE in adenoid and tonsil tissues. Second, there was a higher prevalence of SAg positivity in the adenoid, compared to the tonsil and serum. This might be explained by the fact that the adenoid, along with the nasal mucosa, is the site of first contact for respiratory pathogens and allergens. Last, none of the nonatopic patients was determined to have SAg-specific IgE in their tissues. Interestingly, however, two atopic children had detectable SEB-specific IgE in their sera, and five nonatopic children had TSST-1-specific IgE in their sera, and none of these patients had detectable SAg-specific IgE in adenoid or tonsil tissues. This might be explained by a route of pathogen entry other than the respiratory tract.

Another important finding of our study was that the total IgE levels in adenoid and tonsil tissues, as well as in sera, were markedly higher in atopic patients than in nonatopic patients (Tables I, II). This may merely reflect increased local production of specific IgE in adenoid and tonsil tissues, as supported by the close correlation between total and specific IgE levels in adenoid

and tonsil tissues. Stratification for the presence or absence of detectable SEA-specific IgE suggested that this correlation with total IgE was largely independent of atopic or nonatopic status (Table III). In previous in vitro studies, SEA was shown to interact with helper T cells in an antigen-like manner, resulting in the activation of B cells manifesting preferential production of antigen-specific antibodies.¹³ SAgS also enhance the Th2 response by augmenting isotype switching and synthesis of IgE by B cells, potentially resulting in a local polyclonal IgE response to SAgS themselves and to other foreign bodies.¹⁴ In this study, the levels of sCD23, as the marker of Th2 cell activation, were significantly higher in atopic patients than in nonatopic patients (Table II). Moreover, the levels of sCD23 in SEA-positive patients were significantly higher than in SEA-negative patients (Table III). Therefore, we found that SEA-specific IgE might play a role in increasing the levels of sCD23 (the marker of Th2 cell activation) in adenoid tissue.

Proinflammatory cells such as mast cells, eosinophils, macrophages, and epithelial cells play a key role in the pathogenesis of inflammatory diseases. It is thought that SAgS affect proinflammatory cells directly and indirectly through T cell activation. SAgS can induce Th2 cell-mediated immune responses in the skin,¹⁵ and they also selectively stimulate IL-5 production from peripheral blood mononuclear cells.¹¹ IL-5 is produced by activated Th2 cells, mast cells,¹⁶ and eosinophils. Compared with normal tissue, IL-5 levels are elevated in nasal polyps, in correlation with the eosinophil marker ECP. In our previous study, the levels of SEA- and SEB-specific IgE correlated significantly with the levels of ECP and IL-5 in nasal polyp tissue from patients with asthma.¹⁷ In our current study, the level of mast cell tryptase correlated significantly with SEA-, SEB-, and TSST-1-specific IgE levels (Fig. 1). A possible explanatory mechanism is that specific IgE bound to its high affinity receptor, FcεRI, on mast cells may bind with staphylococcal enterotoxins, leading directly to mast cell degranulation and release of tryptase. The level of ECP

in the adenoid was significantly higher in atopic patients than in nonatopic patients, but its level did not correlate with that of SAg-specific IgE. This might be explained by the atopy status and rhinosinusitis status of our subjects, which may influence factors that activate eosinophils in local tissues. Although SAg-specific IgE did not lead to direct activation of eosinophils in adenoid tissue, the IgE level correlated significantly with the TEC in the peripheral blood, and the level of TEC in SEA-positive patients was significantly higher than that in SEA-negative patients (Table III). Therefore, we found that SAg-specific IgE in atopic patients might contribute to an augmented atopic response by direct or indirect activation of mast cells and eosinophils. To evaluate the clinical significance of these findings, further therapeutic investigation with antibiotics to reduce colony count of *Staphylococcal aureus* may be beneficial in patients with high SAg-specific IgE.

CONCLUSIONS

We confirmed local production of SAg-specific IgE in adenoid tissues from atopic children indicating that the adenoid could be involved in allergic inflammation occurring in the upper airway which is more prominent in atopic children.

BIBLIOGRAPHY

1. Brook I, Shah K, Jackson W. Microbiology of healthy and diseased adenoids. *Laryngoscope* 2000;110(6):994-999.
2. Kania RE, Lamers GE, Vonk MJ, et al. Characterization of mucosal biofilms on human adenoid tissues. *Laryngoscope* 2008;118(1):128-134.
3. Fokkens WJ, Vinke JG, De Jong SS, Bogaert DP, Kleinjan A, Eichhorn E. Differences in cellular infiltrates in the adenoid of allergic children compared with age- and gender-matched controls. *Clin Exp Allergy* 1998;28:187-195.
4. Nguyen LH, Manoukian JJ, Tewfik TL, et al. Evidence of allergic inflammation in the middle ear and nasopharynx in atopic children with otitis media with effusion. *J Otolaryngol* 2004;33(6):345-351.
5. Bernal A, Proft T, Fraser JD, et al. Superantigens in human disease. *J Clin Immunol* 1999;19:149-157.
6. Strange P, Skov L, Lisby S, Nielsen PL, Baadsgaard O. Staphylococcal enterotoxin B applied on intact normal and intact atopic skin induces dermatitis. *Arch Dermatol* 1996;132:27-33.
7. Herrmann T, Baschieri S, Lees RK, et al. In vivo responses of CD4+ and CD8+ cells to bacterial superantigens. *Eur J Immunol* 1992;22:1935-1938.
8. Proft T, Fraser JD. Bacterial superantigens. *Clin Exp Immunol* 2003;133:299-306.
9. Conley DB, Tripathi A, Ditto AM, et al. Chronic sinusitis with nasal polyps: Staphylococcal exotoxin IgE and cellular inflammation. *Am J Rhinol* 2004;18(5):273-278.
10. Laouini D, Kawamoto S, Yalcindag A, et al. Epicutaneous sensitization with superantigen induces allergic skin inflammation. *J Allergy Clin Immunol* 2003;112:981-987.
11. Heaton T, Mallon D, Venaille T, Holt P. Staphylococcal enterotoxin induced IL-5 stimulation as a cofactor in the pathogenesis of atopic disease: the hygiene hypothesis in reverse? *Allergy* 2003;58:252-256.
12. Suh KS, Park HS, Nahm DH, Kim YK, Lee YM, Park K. Role of IgG, IgA, and IgE antibodies in nasal polyp tissue: their relationships with eosinophilic infiltration and degranulation. *J Korean Med Sci* 2002;17(3):375-380.
13. Domiati-Saad R, Attrep JF, Brezinschek HP, et al. Staphylococcal enterotoxin D functions as a human B cell superantigen by rescuing VH4-expressing B cells from apoptosis. *J Immunol* 1996;156(10):3608-3620.
14. Bachert C, Gevaert P, Holtappels G, et al. Total and specific IgE in nasal polyps is related to local eosinophilic inflammation. *J Allergy Clin Immunol* 2001;107(4):607-614.
15. Cho SH, Strickland I, Tomkinson A, Fehring AP, Gelfand EW, Leung DYM. Preferential binding of Staphylococcus aureus to skin sites of Th2-mediated inflammation in a murine model. *J Invest Dermatol* 2001;116:658-663.
16. Bradding P, Roberts JA, Britten KM, et al. Interleukin-4, -5, and -6 and tumor necrosis factor-alpha in normal and asthmatic airways: evidence for the human mast cell as a source of these cytokines. *Am J Respir Cell Mol Biol* 1994;10(5):471-480.
17. Suh YJ, Yoon SH, Sampson AP, et al. Specific immunoglobulin E for staphylococcal enterotoxins in nasal polyps from patients with aspirin-intolerant asthma. *Clin Exp Allergy* 2004;34(8):1270-1275.