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Local IgE-mediated hypersensitivity to Alternaria in pediatric adenoid tissue☆

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ABSTRACT

Objective: Fungus may contribute to the development and exacerbation of allergic airway diseases. Several studies have demonstrated the presence of humoral immune responses to fungi, including Alternaria and Aspergillus, in patients with nasal polyposis, asthma, or rhinitis. The purpose of this study was to evaluate the role of Alternaria- and Aspergillus-specific IgE antibodies in allergic inflammation of adenoid tissue.

Methods: Thirty-nine atopic subjects who were sensitized to more than one common aeroallergen and 39 non-atopic subjects undergoing adenotonsillectomy were recruited. The Phadia ImmunoCAP was used to quantify total IgE, Alternaria- and Aspergillus-specific IgE, eosinophil cationic protein (ECP), and mast cell tryptase in adenoid tissue homogenates. Alternaria- and Aspergillus-specific IgE were detected in the adenoid tissues from some of the subjects (37.2% and 24.4%, respectively) without systemic sensitization to common airborne fungi.

Results: Both Alternaria- and Aspergillus-specific IgE were more prevalent in adenoid tissues from atopic children (48.7% and 38.5%, respectively) than in tissues from non-atopic children (25.6% and 10.3%, respectively). Subjects with high Alternaria-specific IgE level showed significantly higher serum and adenoid total IgE and adenoid ECP and tryptase than those without specific IgE. Alternaria-specific IgE levels were significantly correlated with serum and adenoid total IgE and with tryptase and ECP levels in adenoid tissue.

Conclusions: Adenoid tissues from atopic and non-atopic children displayed local IgE-mediated hypersensitivity to fungi in the absence of systemic fungal hypersensitivity. Locally-produced Alternaria-specific IgE may contribute to mast cell and eosinophil activation, especially in the presence of tissue eosinophilia.

1. Introduction

Fungi, ubiquitous in the environment, may coexist without effect in a host with normal cellular immunity [1] or may contribute to allergic airway diseases in those who are susceptible and/or heavily exposed [2–4]. Among the normal contaminants in nasal mucus, *Alternaria alternata* and *Aspergillus fulmigatus* are frequently detected [5], and patients with nasal polyposis, asthma and fungal sinusitis express humoral immune responses to such fungi [6–8].

The two chief effector cells in allergic response are the mast cells and the eosinophils. In the nasal and bronchial mucosa, allergic inflammation is characterized by tissue eosinophilia. According to a recent study, the innate direct response of eosinophils to certain fungi (e.g., Alternaria), as well as the humoral immune response, may play a prominent role in the pathophysiology and exacerbation of asthma and other eosinophil-related airway diseases [9].

In previous studies, we demonstrated the local production of total IgE and allergen-specific IgE in adenoid tissues and the relationship between local allergen-specific IgE and allergic inflammatory mediators such as tryptase and ECP in adenoid tissues [10]. However, to the best of our knowledge, few studies have evaluated local IgE-mediated fungal hypersensitivity in tissues from pediatric patients. To confirm local production of Alternaria- and Aspergillus-specific IgE, we recruited pediatric patients with and without atopy. To evaluate the roles of Alternaria- and Aspergillus-specific IgE in allergic inflammation of adenoid tissue, we measured Alternaria- and Aspergillus-specific IgE levels in adenoid tissue homogenates from the two groups. We also measured the following parameters: serum total IgE; peripheral blood total eosinophil counts (TEC); and total IgE, tryptase, and ECP levels in adenoid tissue homogenates. We also

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observed the relationships between Alternaria- and Aspergillus-specific IgE and inflammatory markers within adenoid tissues.

2. Materials and methods

2.1. Subjects

Seventy-eight children (56 boys and 22 girls, mean age 7.5 years, range 3.2–13.5) participated in this study. All patients visited the ENT department in need of surgery for recurrent infections, including pharyngotonsillitis, rhinosinusitis, adenitis, and otitis media, or for problems related to adenotonsiller hypertrophy, including upper airway obstruction, sleep disorder, abnormal dentofacial growth, and dysphagia. The parents or foster parents of the children were asked to complete an extensive questionnaire on symptoms and signs of the child's disease(s), medication, and family history with respect to allergy, rhinoconjunctivitis, asthma, eczema, smoking habits, and contact with pets. Before surgery, all children underwent a routine ENT examination. Children with congenital anomalies in the head and neck, Down syndrome, systemic diseases, or those suspected of having congenital or acquired immune deficiency were excluded from this study. Patients were also excluded if medication containing antihistamines were used intraoperatively or within the preceding week or if immunosuppressive agents, including steroids, were used intraoperatively or within the preceding six weeks.

Blood samples obtained from patients before the operation were used for multiple allergosorbent chemiluminescence assay (MAST-CLA), to determine serum total IgE concentration using a BN II assay (Dade Behring, Marburg, Germany) and to count eosinophils using the ADVIA120 Hematology System (Bayer Diagnostics, NY, USA). For MAST-CLA, 40 antigens, including house dust mite, cockroach, cat, dog, tree pollen, grass pollen, weed pollen, and common airborne fungi, attached to threads were sequentially incubated with patient serum, enzyme-linked anti-IgE, and a photoresponse reagent. The chemical fluorescence generated by each thread assay was developed on Polaroid film and scored according to a five-point system, from 0 to 4. Atopy status was defined as a total IgE higher than expected for age (>10 IU per year of age) and a score of more than 2+ on the MAST-CLA.

All subjects underwent adenotonsillectomy between May 2008 and July 2008 at the ENT Department of the Kyung Hee University Hospital in Seoul, Korea. Participation was subject to parental informed consent, and the study was approved by the local ethics committee.

2.2. Tissue collection

Adenoidectomy was performed using a curette, and tissues were stored frozen after washing. Later, the washed adenoids were thawed and dispersed in LIPA buffer (Sigma–Aldrich, St. Louis, MO, USA) containing a protease inhibitor (Roche Diagnostics, Indianapolis, IN, USA). After centrifugation, the supernatants were collected and stored at −70 °C until use. All parameters were presented relative to the albumin level in adenoid tissue homogenates to correct for differences in the dilution factors between tissue samples [10]. Albumin levels in adenoid tissue homogenates were measured using a QuantiChromTM BCP Albumin Assay Kit (BioAssay Systems, Hayward, CA, USA).

2.3. Measurement of Alternaria- and Aspergillus-specific IgE in serum and adenoid tissue homogenates

Alternaria- and Aspergillus-specific IgE were measured in serum and adenoid tissue homogenates using the ImmunoCAP system (Pharmacia, Uppsala, Sweden). An Alternaria- and/or Aspergillus-specific IgE level higher than 0.34 kUA/L was considered a positive result.

2.4. Measurement of total IgE, ECP, and tryptase in adenoid tissue homogenate

Total IgE was measured using the ImmunoCAP system (Pharmacia, Uppsala, Sweden), with the confidence range of 0.35–100 kUA/L. ECP and tryptase in adenoid homogenates diluted 1:5 and 1:20, respectively, were quantified by fluoroimmunoassay using the ImmunoCAP system (Pharmacia). The minimum detection limits for ECP and tryptase were 2 and 4 ng/mL, respectively. All parameters were corrected for albumin content and expressed as nanograms per milligram of albumin.

2.5. Statistical analyses

The levels of Alternaria- and Aspergillus-specific IgE and inflammatory markers, including ECP and tryptase, were compared between atopic and non-atopic patients and between Alternaria-specific IgE-positive and negative patients using Mann–Whitney *U*-tests with SPSS version 12.0 (Chicago, IL, USA). Pearson's correlation coefficients were applied to evaluate relationships between levels of Alternaria- and Aspergillus-specific IgE and inflammatory markers. *P*-values 0.05 or less were regarded as significant.

3. Results

3.1. Clinical characteristics of study subjects

Thirty-eight patients (50%), including 29 boys and 10 girls, were found to have atopy, meaning they had excessively high total IgE for their age (>10 IU/year of age), and a score of more than 2+ in the MAST-CLA. Thirty-eight patients (50%) were non-atopic and served as a control group.

The characteristics of the study subjects are shown in Table 1. The two groups did not differ significantly in age or sex ($P > 0.05$, respectively). Total IgE in sera and total eosinophil counts in peripheral blood of atopics were significantly higher than those of non-atopics ($P = 0.016$, $P = 0.041$, respectively).

None of 78 children had specific IgE antibody to *Alternaria alternata* and *Aspergillus fulmigatus* in serum using MAST-CLA and ImmunoCAP.

Table 1
Clinical data of the study subjects.

	Atopic ($n = 39$)	Non-atopic ($n = 39$)	*P*
Gender (M/F)	29/10	27/12	NS
Age (years)	7.9 ± 3.0	7.2 ± 2.5	NS
Cormorbid diseases (positive/negative)			
Chronic rhinosinusitis	16/23	21/18	NS
Otitis media effusion	6/33	8/31	NS
Asthma	1/37	2/38	NS
Atopic dermatitis	3/34	4/37	NS
Serum total IgE (IU/mL)	333.44 ± 543.84	99.17 ± 110.91	**0.016**
Adenoid total IgE/albumin	22.25 ± 20.58	8.80 ± 9.14	**0.012**
Total eosinophil count (/mm^3) in peripheral blood	255.49 ± 150.36	189.56 ± 125.28	**0.041**
Tryptase/albumin in adenoid tissue	2794.69 ± 2647.71	2696.72 ± 2637.38	0.87
ECP/albumin in adenoid tissue	371.36 ± 315.43	203.63 ± 140.80	**0.004**

Values are expressed as mean ± SD. The Mann–Whitney *U*-test was used to compare atopic and non-atopic values.