

Role of IgG, IgA, and IgE Antibodies in Nasal Polyp Tissue : Their Relationships with Eosinophilic Infiltration and Degranulation

To confirm local production of IgE, and evaluate role of immunoglobulins on eosinophil activation in nasal polyp (NP) tissue, we measured IgG, IgA, secretory IgA (sIgA), total (tIgE) and specific IgE (sIgE) to *Dermatophagoides pteronyssinus* (DP) by ELISA in NP tissue homogenates from 51 subjects. They were classified according to skin reactivity to DP: group I, 15 highly atopic subjects; group II, 18 weakly atopic subjects; and group III, 18 non-atopic subjects. Eosinophil cationic protein (ECP) level was measured by CAP system. Highest level of DP-sIgE was noted in group I, followed by group II and III ($p < 0.05$). Nine (60%) of group I and 4 (22%) of group II subjects had detectable level of DP-sIgE with no significant differences in IgA, sIgA, and IgG. All of NP tissue had eosinophilic infiltration with no significant difference in activated eosinophil count or ECP level among 3 groups. A significant correlation was noted between EG2+ cell count and tIgE ($r = 0.55$, $p < 0.05$), and DP-sIgE level ($r = 0.60$, $p < 0.05$). A significant correlation was also noted between ECP and IgG ($r = 0.51$, $p < 0.05$) and DP-sIgE level ($r = 0.47$, $p < 0.05$) with no significant correlation with IgA or sIgA. These results suggest that DP-sIgE was detectable in NP tissue from weakly atopic subjects as well as highly atopic subjects. IgG and sIgE may have potential roles in eosinophil degranulation in NP tissue.

Key Words : Nasal Polyps; Immunoglobulin E; Immunoglobulin G; Immunoglobulin A; Eosinophil Cationic Protein

Kyung-Sik Suh*, Hae-Sim Park,
Dong-Ho Nahm, Yoon-Keun Kim,
Young-Mok Lee, Keehyun Park*

Department of Otolaryngology*, Allergy and
Clinical Immunology, Ajou University School of
Medicine, Suwon, Korea

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Address for correspondence

Hae-Sim Park, M.D.
Department of Allergy and Clinical Immunology,
Ajou University School of Medicine, San-5
Wonchon-dong, Paldal-gu, Suwon 442-712, Korea
Tel : +82.31-219-5196, Fax : +82.31-219-5154
E-mail : hspark@madang.ajou.ac.kr

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INTRODUCTION

Several studies have consistently demonstrated the active role of eosinophils in the development of nasal polyp (1-4). An involvement of IgG, IgA and IgE has been suggested in pathogenesis of nasal polyp (5) since these immunoglobulin receptors can be found on the surface of eosinophil (6). Regarding IgE-mediated mechanism in nasal polyp generation, there have been a few studies demonstrating local production of IgE within nasal polyp tissue (7, 8). Our previous study (9) revealed that some non-atopic subjects by skin prick test result had detectable specific IgE. However, they showed variable results which may be derived from different study subjects and atopy criteria (7-9). To the best of our knowledge, there has been little attempt to elucidate the contribution of immunoglobulins in eosinophil activation in nasal polyp tissue.

In this study, to confirm local production of *Dermatophagoides pteronyssinus* (DP)-specific IgE, we classified the study subjects into three different groups according to of skin reactivity to DP. To evaluate role of immunoglobulins on eosinophil activation we measured major immunoglobulins such as IgG, IgA, sIgA, and IgE and DP-specific IgE levels as well as eosinophil cationic protein (ECP) as eosinophil degranulation

product, in nasal polyp tissue homogenate in three different groups, and observed the relationships between ECP and immunoglobulins.

MATERIALS AND METHOD

Subject

Fifty-one subjects who had underwent nasal polypectomy participated in this study and they were classified into three groups according to skin reactivity to DP on skin prick test. Group I consisted of 15 highly atopic subjects showing >3 mm wheal response to DP and group II consisted of 18 weakly atopic subjects showing 1-3 mm wheal response to DP. Group III consisted of 18 non-atopic subjects, who showed all negative responses to all common inhalant allergens on skin prick test with serum total IgE level less than 100 IU/mL. The polyps, soon after surgical removal, were washed with normal saline to remove stagnant mucus. The middle portion of the polyp was cut off and immediately sent to a pathology laboratory to prepare the paraffin-embedded tissue block. Half of them were made into tissue homogenate. All the subjects

gave their informed consent as regulated by the institutional review board of Ajou University Hospital, Suwon, Korea.

Preparation of nasal polyp tissue, measurement of ECP and albumin level

The polyp tissues were frozen at -70°C immediately after the operation. To be prepared for the experiments, the samples were thawed and grounded in a homogenizer (POLY-TRON, Switzerland) with phosphate buffered saline (PBS, pH 7.5) including 1% Triton X 100. They were centrifuged and the separated supernatants were kept at -70°C until the measurement of ECP, albumin and immunoglobulin levels on the same day. ECP level in nasal polyp tissue homogenate was measured using CAP system (Pharmacia, Sweden) and albumin level was measured by nephelometry. All values were presented as ratio to albumin level.

Measurement of total IgG and IgA within polyp tissue homogenate

Microtiter plates were coated with 50 μL of anti-IgG or anti-IgA antibodies (Sigma, St. Louis, MO) at 2 $\mu\text{g}/\text{mL}$ concentration and kept overnight at 4°C . After the washing step, the plates were blocked with PBS with 3% bovine serum albumin and 0.05% Tween 20. They were incubated with serial dilutions of duplicate sample of tissue homogenate (50 $\mu\text{L}/\text{well}$) or immunoglobulin standard (The binding site, Birmingham, U.K.) for 2 hr at 4°C . After washings, the plates were incubated with peroxidase conjugated affinity-purified goat anti-IgG and anti-IgA antibodies (Sigma, St. Louis, MO, U.S.A.) at 1:2,500 dilution (v/v) for 1 hr at room temperature. After washing, we added a substrate solution that consisted of 0.04% w/v *o*-phenylene diamine dissolved in 24.3 mM citric acid, 51.4 mM NaH_2PO_4 (pH 5.0) and 0.03% H_2O_2 . After 15 min, the reaction was stopped by adding 2.5 N H_2O_4 . Amounts of total IgG and IgA antibodies in samples were calculated from control curves prepared by optical densities from serial dilutions of immunoglobulin standard solution using a spline fit program (SoftMax, Molecular Devices).

Measurement of total sIgA within polyp tissue homogenate

Microtiter plates were coated with 50 μL of anti-secretory piece antibodies (The binding site, Birmingham, U.K.) in 0.1 M carbonate buffer at 2 $\mu\text{g}/\text{mL}$ concentration and kept overnight at 4°C . After the washing step, the plates were blocked with PBS with 3% bovine serum albumin and 0.05% Tween 20. They were incubated with serial dilutions of duplicate sample of tissue homogenate (50 $\mu\text{L}/\text{well}$) or purified sIgA (The binding site, Birmingham, U.K.) for 2 hr at 4°C . After repeated washings, the plates were incubated with biotinylated affinity-purified goat anti-IgA antibody (Sigma, St.

Louis, MO) at 1:2,500 dilution (v/v) for 1 hr at room temperature. After another washing step, the plates were incubated with streptavidin-peroxidase (0.5 $\mu\text{g}/\text{mL}$) for 30 min at room temperature. Then we added *o*-phenylene diamine as a substrate solution. The absorbance at 490 nm was measured by microplate reader (Molecular device, CA, U.S.A.) and the amounts of sIgA were calculated as described above.

Measurement of total and specific IgE to DP in polyp tissue homogenate

The levels of total IgE in polyp tissues were measured by sandwich ELISA as described previously (7). Microtiter plates (Immulon II; Dynatech Laboratories, Inc., Chantilly, VA, U.S.A.) were coated with 50 μL of affinity-purified goat anti-IgE antibody (Vector Laboratories, Inc., Burlingame, CA, U.S.A.) at 1:500 dilution (1 $\mu\text{g}/\text{mL}$) with 0.1 M carbonate buffer, pH 9.6, for 2 hr at 4°C . After the washing step, the plates were blocked with PBS with 3% bovine serum albumin and 0.05% Tween 20. The plates were incubated with dilutions of duplicate samples of polyp tissue homogenate. After washings, the plates were incubated with biotinylated affinity-purified goat anti-IgE antibody (Vector Laboratories, Inc., Burlingame, CA) at 1:500 dilution (1 $\mu\text{g}/\text{mL}$) for 1 hr at room temperature. After another washing step, plates were incubated with streptavidin-peroxidase (0.5 $\mu\text{g}/\text{mL}$) for 30 min at room temperature. After washing, we added *o*-phenylene diamine as a substrate solute and the absorbance was measured by a microplate reader. The absolute level of total IgE in the sample was calculated from the curve derived from serial dilutions of IgE standard (DPC, LA, CA). All samples were measured simultaneously to reduce possible variations in measurements.

For the measurement of DP-specific IgE, the procedure was essentially the same as in the above method except the microtiter plates were coated with DP (2 $\mu\text{g}/\text{well}$, Allergopharma, Germany) instead of anti-IgE antibody, and a fixed sample dilution was used (1:50 dilution in blocking buffer). Positive cut-off value for specific IgE was determined as mean + 2 folds of standard deviation of the absorbance values from the group III subjects.

Staining procedures

Four-micrometer thick tissue sections were stained with each monoclonal antibody to activated eosinophils. The antibodies were applied at optimal dilutions with Tris-buffered saline, which were determined by the previous titration experiments (2, 3). After deparaffinization and hydration, sections were incubated with 10% normal goat serum to block non-specific binding. Then, they were incubated overnight with primary antibodies at 1:200 dilution (EG2, Pharmacia, Sweden) at room temperature. A streptavidin-biotin complex (Dako, Denmark) detection system with aminoethylcarbazole as chro-

mogen was used, and sections were counter-stained with Meyer's hematoxylin. For negative control, the primary antibody was omitted.

Evaluation

The sections were evaluated with conventional light microscopy ($\times 100$, $\times 200$, $\times 400$). The number of stained cells were counted and presented as cells/mm² of tissue area as reported in our previous study (2, 3). Four areas per tissue were estimated by superimposing a grid of 100 points (intersection of crosses, covering a surface area of 0.5 mm²) on the section and by using an eyepiece graticulate at a magnification of $\times 200$. The mean cell count was derived from the sum of the four fields.

Statistical analysis

ANOVA and Student t tests were applied using the SPSS version 7.0 (Chicago, IL) to evaluate the statistical differences among the data. Pearson's correlation analysis was applied to evaluate the statistical significance between two values: A *p* value of 0.05 or less was regarded as significant.

RESULTS

IgA, sIgA, and IgG levels in nasal polyp tissue

Fig. 1 shows the comparison of each immunoglobulin concentration in nasal polyp tissue homogenate presented as its ratio to albumin ($\mu\text{g}/\text{mg}/\text{mL}$). IgA/albumin ($1,215.7 \pm 905.7$

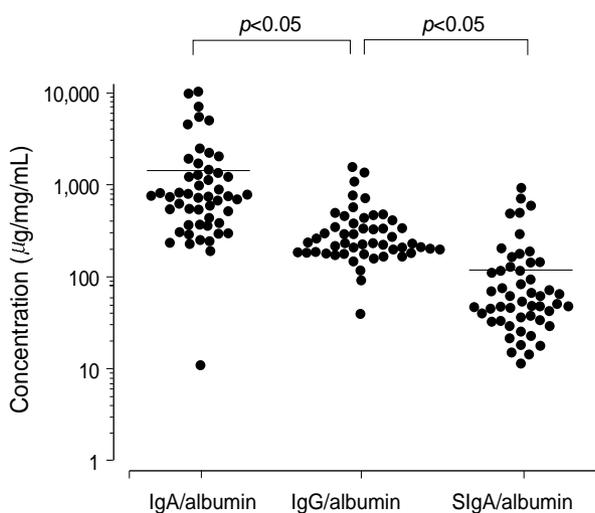


Fig. 1. The ratios of IgG, IgA, and secretory IgA (sIgA) to albumin in nasal polyp tissues from the study subjects. Significant differences are noted between IgA/albumin and IgG/albumin, and IgG/albumin and sIgA/albumin ($p < 0.05$).

$\mu\text{g}/\text{mg}/\text{mL}$) showed the highest level, followed by IgG/albumin ($335.6 \pm 273.7 \mu\text{g}/\text{mg}/\text{mL}$), and sIgA/albumin ($137.1 \pm 192.8 \mu\text{g}/\text{mg}/\text{mL}$) and a significant correlation was found between IgA and sIgA levels ($r = 0.80$, $p < 0.05$). There was no significant difference in IgA, sIgA, IgG and albumin levels among the three groups ($p > 0.05$, respectively).

Comparison of total and DP-specific IgE among the three groups

Fig. 2 shows comparison of total IgE level in both polyp tissue homogenate and sera of the three groups. Total IgE level of group I in both serum and polyp tissue was significantly higher than in group II and group III ($p < 0.05$, respectively). Moreover a significant difference was noted between group II and III ($p < 0.05$). Fig. 3 shows the comparison of DP-specific IgE level in the three study groups. Nine (60%) subjects of group I and four (22%) of group II had high specific IgE antibody. Specific IgE level of group I was significantly higher than that of group II or group III ($p < 0.05$, respectively). A significant difference was noted between group II and III ($p < 0.05$).

Relationship between ECP and three immunoglobulin levels in nasal polyp tissue

No significant correlation was found between EG2+ cell count and ECP level ($p > 0.05$, data was not shown). Although there was no significant correlation between ECP and IgA, sIgA or total IgE level, significant correlations were noted between ECP and IgG ($r = 0.51$, $p < 0.05$, Fig. 4A), and DP-

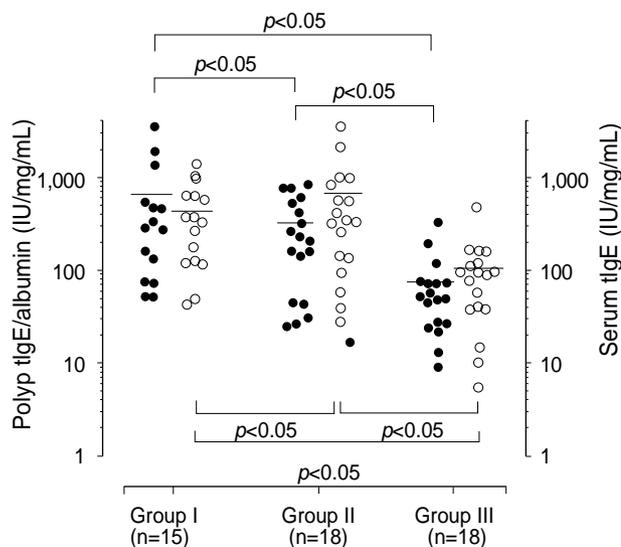


Fig. 2. Comparison of the total IgE (tIgE) levels in nasal polyp tissue (\bullet) and serum (\circ) of the three study groups. The values are presented as the ratio of the total IgE level to albumin. Horizontal bars indicate mean values. Significant differences are noted between the two groups by Student t-test ($p < 0.05$).

specific IgE ($r=0.55$, $p<0.05$, Fig. 4B) in nasal polyp tissue. When group I subjects were separated, higher correlation coefficient was noted between ECP and DP-specific IgE ($r=0.62$, $p<0.05$), and IgG ($r=0.76$, $p<0.05$) levels.

Relationship between EG2+ cell count and IgG, IgA, or IgE level

A significant correlation was found between EG2+ cell count and total IgE ($r=0.55$, $p<0.05$) and DP-specific IgE in nasal polyp ($r=0.60$, $p<0.05$ data was not shown). Poor

correlations were found with IgA ($r=-0.05$), sIgA ($r=-0.29$) and IgG ($r=-0.11$, $p>0.05$, respectively).

DISCUSSION

Studies of inflammatory cells infiltrating nasal polyps have shown that they consist of mast cells, eosinophils, lymphocytes, and neutrophils and most authors have found a consistent infiltration of stroma with eosinophils (1-3) whose numbers are reported to be higher than those of mast cells. In accordance with previous observations (10, 11), we have found that the infiltrate is, in many respects, similar to that of the bronchial mucosa of atopic asthmatics in that eosinophilia was a consistent feature. Our previous study (2) showed that EG2+ cells were abundant in both epithelial and stromal layers of patients with nasal polyp, irrespective of whether they were atopic or nonatopic. In this study, all the subjects had EG2+ cells and no significant differences existed in EG2+ cell count among the three groups. However, EG2+ cell numbers tended to be higher (2) in nasal polyps from allergic subjects than from non-allergic subjects, indicating activated eosinophils could participate in the generation of nasal polyps in both allergic and non-allergic groups.

Although the role of atopy in development of nasal polyp has been unclear, some studies (12, 13) have reported that 60-66% of patients who had undergone nasal polypectomy have had positive skin prick tests to common aeroallergens, suggesting an association (9, 14, 15) between atopy and nasal polyp. Other studies including our previous study, suggested that the IgE-mediated mechanism may be present in subpopulation of patients with nasal polyposis.

In this study, to confirm local production of IgE in nasal

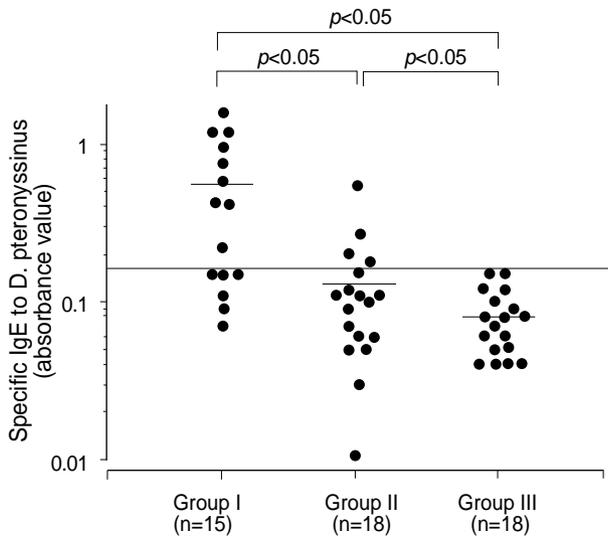


Fig. 3. Comparison of the *Dermatophagoides pteronyssinus*-specific IgE levels in nasal polyp tissue of the three study groups. Horizontal bars indicate mean values. Significant differences are noted between the two groups by student t-test ($p<0.05$).

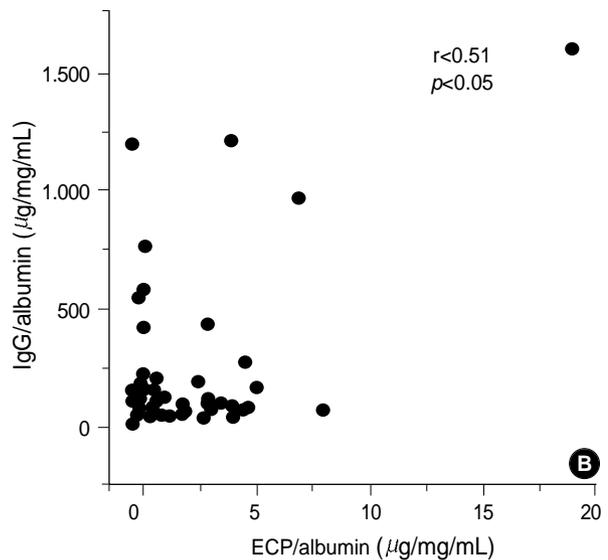
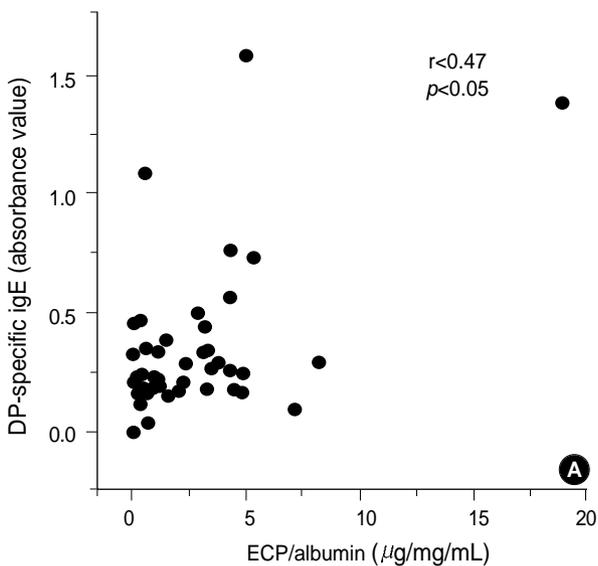


Fig. 4. Correlation between eosinophil cationic protein (ECP) level, and IgG (A) and specific IgE antibody to *Dermatophagoides pteronyssinus* (B) in nasal polyp tissues from the study subjects.

polyp tissue, we classified the study subjects into three groups according to their skin reactivity to house dust mite: highly atopic, weakly atopic and non-atopics, and compared IgG, IgA, sIgA, total and specific IgE levels to house dust mite in nasal polyp tissue homogenate. There were no significant differences in IgA, sIgA and IgG levels among the three groups. However, there was significant difference in total and specific IgE levels. Interestingly, some weakly atopic subjects had high specific IgE in nasal polyp tissue, while none of group III had specific IgE. Both total and specific IgE levels in nasal polyp tissue were significantly higher in weakly atopic than in non-atopic subjects. These findings suggest that total and specific IgE antibodies are locally produced within the nasal polyp tissue in some weakly atopic subjects as well as highly atopic subjects. Both atopics and non-atopics produce IgG, IgA, and sIgA in nasal polyp tissue. These immunoglobulins may contribute to inflammatory responses occurring in nasal polyp tissue.

Regarding the role of immunoglobulins (IgA, IgE and IgG) in eosinophil activation, they can stimulate eosinophil degranulation, resulting in the release of cytotoxic proteins such as ECP, eosinophil peroxidase and eosinophil-derived neutrophils in vitro condition (16). IgA, particularly sIgA, has been shown to mediate eosinophil degranulation in vitro (17), and increased expression of IgA receptors on eosinophils of allergic individuals has been reported (18). Recent studies have implicated IgG or IgA as a contributor of allergic inflammation of asthmatic airways (18, 19). Bronchoalveolar lavage (BAL) fluids from asthmatic subjects allergic to ragweed could lead to a degranulation of eosinophils in vitro, and the degree of degranulation correlates with the levels of antigen-specific IgA, IgG, and IgG1 found in those fluids (20). These results suggest that these immunoglobulin isotypes may mediate eosinophil degranulation in an antigen-specific fashion.

Peebles et al. (21) found significant correlations between the levels of ECP in BAL fluid after segmental lung challenge (SLC) and the levels of ragweed-specific IgA both in serum and BAL fluid after SLC, while there was no correlation between the concentrations of ragweed-specific IgA in the pre-SLC BAL fluids and ECP. However, another study revealed that there was a poor correlation between ragweed-specific IgA levels in either serum or in BAL fluid in antigen-challenged segment and total eosinophil numbers, suggesting that antigen-specific IgA is not involved in eosinophil recruitment. The present study is the first to observe the relationship between IgG, IgA, and sIgA antibodies within nasal polyp tissue and EG2+ cells or ECP as eosinophil degranulation product. This study also showed that IgA was the most abundant immunoglobulin in nasal polyp tissue compared to other immunoglobulins with a significant correlation between IgA and sIgA. Because of its abundance in nasal polyp tissue, IgA antibody may be one of major immunoglobulin isotype involved in eosinophil degranulation. However, in this study, no significant correlations were noted between ECP or EG2+

cell count and IgA or sIgA in nasal polyp tissue. Instead, significant correlations were noted between ECP and IgG, and DP-specific IgE, which were comparable to the previous study (22). Furthermore, a higher correlation coefficient was found between ECP and IgG in highly atopic subjects. These findings may be attributable to the fact that the IgA and sIgA measured in this study was total IgA and sIgA, not allergen-specific IgA, sIgA and IgG. Our results support the hypothesis that IgG antibody may be involved in the eosinophil degranulation found in nasal polyp inflammation.

Recent investigations in BAL study from allergic asthma patients have demonstrated that ragweed-specific IgE is most strongly correlated with ECP release (21) and it has been reported that IgE receptors are present on eosinophils (23, 24). However, an in vitro study revealed that IgE or anti-IgE did not induce any direct effector function such as leukotriene C4, superoxide production or any degranulation product (25). To date, the mechanism of IgE-mediated eosinophil degranulation has been unclear. This is the first study to observe the relationship between ECP and EG2+ cell count, and total and DP-specific IgE levels in nasal polyp tissue. In this study, a significant correlation was found between ECP and DP-specific IgE, and furthermore, a higher correlation coefficient was noted between ECP and specific IgE in highly atopic subjects. Moreover, a significant correlation was found between EG2+ cell count and total and DP-specific IgE levels in nasal polyp tissue. These findings suggest that IgE may be involved in eosinophil recruitment and priming as well as eosinophil degranulation in nasal polyp tissue.

In conclusion, the results from this study suggested a possible involvement of immunoglobulins such as IgG and allergen-specific IgE in degranulation and/or recruitment of eosinophils in nasal polyp tissue. Further investigations are needed to clarify the exact mechanism of IgG- or IgE-mediated eosinophil degranulation and/or recruitment occurring in nasal polyp tissue.

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