Specific immunoglobulin E for staphylococcal enterotoxins in nasal polyps from patients with aspirin-intolerant asthma


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Summary

Background Nasal polyps infiltrated with eosinophils are commonly found in chronic asthmatic patients, more frequently in those with aspirin-intolerant asthma (AIA) than aspirin-tolerant asthma (ATA). Some studies have suggested a contribution of superantigens derived from Staphylococcus sp to nasal polyposis and eosinophilia, but their relative importance in AIA and ATA subjects is unknown.

Objective We investigated whether local production of specific IgE to staphylococcal enterotoxins A and B (SEA and SEB) and relationships with markers of eosinophilic inflammation differ in the nasal polyps of AIA and ATA subjects.

Methods Fifteen AIA subjects with positive responses to lysine–aspirin bronchoprovocation and 15 ATA subjects underwent polypectomy. Immunoassays were used to quantify eosinophil cationic protein (ECP), IL-5, mast cell tryptase, soluble IL-2 receptors (sIL-2R), total IgE, and specific IgE for SEA and SEB.

Results ECP levels in nasal polyp homogenates were higher in AIA subjects than in ATA subjects (\(P<0.02\)), with no significant differences in tryptase, IL-5 or sIL-2R. Total IgE, and specific IgE to both SEA and SEB, were detectable in some nasal polyps from both subject groups, but median levels were markedly higher in AIA subjects than in ATA subjects (\(P=0.04, 0.01, 0.05\), respectively). Levels of specific IgE to SEA and SEB correlated significantly with levels of ECP and IL-5, but not those of tryptase or sIL-2R.

Conclusion These findings suggest that staphylococcal superantigens may drive local eosinophilic inflammation in nasal polyp tissue, and that this is exacerbated in subjects with AIA.

Keywords aspirin, asthma, eosinophil, IgE, nasal polyposis, Staphylococcus, superantigen

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Introduction

Nasal polyps represent a T cell-orchestrated eosinophilic upper airway disease of unknown pathogenesis. Nasal polyposis is often associated with asthma, and the inflammatory cellular infiltrate in nasal polyps, comprising T cells, eosinophils, plasma cells and mast cells, is similar to that in the bronchial mucosa of asthmatics, suggesting that inflammatory mechanisms of the two diseases may be related [1–4]. Eosinophilia is a consistent finding in nasal polyp tissue, and activation and enhanced survival of eosinophils may affect polyp formation and growth [5, 6].

Superantigens, predominantly derived from Staphylococcus aureus, can activate T cells, induce IgE synthesis in B cells, and directly affect the activities of inflammatory leukocytes such as eosinophils. A role of staphylococcal superantigens in atopic dermatitis has recently been recognized [7, 8] and similar mechanisms might be relevant in airway disease [9].

IgE antibodies to S. aureus enterotoxins have been described in nasal polyp tissue, and linked to local polyclonal IgE production and eosinophilic inflammation [10]. Such IgE antibodies are also present in the serum of asthmatic patients and correlate with disease severity [11]. Bilateral nasal polyps are particularly common in patients with aspirin-intolerant asthma (AIA), in whom cyclooxygenase inhibitors can trigger adverse nasal and bronchial reactions because of activation of mast cells and eosinophils. The presence of nasal polyps, asthma and aspirin-intolerance comprises the classical 'aspirin triad'. Previous studies suggested that nasal polyposis in AIA subjects was associated with more extensive eosinophilic infiltration and mucosal hypertrophy in the paranasal sinuses compared with aspirin tolerant asthma (ATA) subjects [12, 13]. IgE responses to staphylococcal enterotoxins in nasal polyps have not been compared in patients with AIA and ATA.

We hypothesized that the nasal polyps of AIA subjects may reveal greater evidence of staphylococcal superantigens driving eosinophilic inflammation than their ATA counterparts. The study revealed for the first time that compared with ATA patients, nasal polyps from subjects with AIA had...
enhanced total IgE and elevated levels of IgE specific for the staphylococcal enterotoxins A (SEA) and B (SEB). Nasal polyp levels of specific IgE for SEA and SEB correlated closely with amounts of eosinophil cationic protein (ECP) and the eosinophilopoietic cytokine IL-5, but not with mast cell tryptase or T cell-associated soluble IL-2 receptors (sIL-2R). The results suggest that IgE specific for staphylococcal superantigens might drive the enhanced eosinophilic inflammation described in nasal polyps of AIA subjects compared with their ATA counterparts. Greater susceptibility to mounting immune responses to staphylococcus superantigens may underlie the pathophysiology of nasal polyposis in patients with AIA.

Materials and methods

Subjects

Nasal polyps were obtained by polypectomy in 30 patients with bronchial asthma (15 AIA, 15 ATA) aged 27–56 years (mean ± SEM: 40.0 ± 3.4 years), who visited the Allergy Clinic of Ajou University Hospital, Suwon, Korea. The study was approved by the ethics committee of Ajou University Hospital and all subjects gave informed consent. Asthma was diagnosed according to the American Thoracic Society guidelines. Diagnosis of AIA was confirmed by lysine–aspirin bronchoprovocation tests as described [14], with a positive result defined as a 20% fall in forced expiratory volume in 1 s. ATA patients had a clinical diagnosis of asthma but negative results on lysine–aspirin bronchoprovocation. Clinical details of the AIA and ATA subjects are shown in Table 1. Five of the AIA subjects and eight of the ATA subjects had undergone polypectomy on a previous occasion. Skin prick responses to common aeroallergens (Bencard, Brentford, UK) were performed and atopy was defined as one or more positive responses (>3+ by allergen/histamine ratio). None of the study subjects had used topical steroids for at least 4 weeks prior to polypectomy. Immediately after surgical removal, polyps were washed in normal saline to remove stagnant mucus and frozen at −70 °C.

Preparation of nasal polyp homogenates

Polyps were thawed and dispersed in a homogenizer (Polytron, Cincinnati, OH, USA) with phosphate-buffered saline (pH 7.5) including 1% Triton X-100. After centrifugation, the supernatants were kept at −70 °C before assays of ECP, tryptase, IL-5, sIL-2R, and total and specific IgE antibodies. All values are presented relative to protein content as measured by the Bradford method.

Inflammatory marker assays

ECP and tryptase in nasal polyp homogenates (diluted 1:20) were quantified by fluorimunoassay using the Pharmacia CAP system (Pharmacia, Uppsala, Sweden) as previously described [3]. The minimal detection limits for ECP and tryptase were 2 and 4 ng/mL, respectively. ECP and tryptase concentrations were corrected for total protein content and expressed as nanogram per milligram of protein. Levels of IL-5 and sIL-2R were quantified by enzyme immunoassays (R&D System Inc., Minneapolis, MN, USA).

Measurement of total and specific IgE

Levels of total IgE and of specific IgE to SEA and SEB were measured by the CAP system (Pharmacia) according to the manufacturer’s instructions. Nasal polyp homogenates were diluted 1:2 for the IgE assays.

Statistical analyses

Comparisons of markers such as ECP, tryptase and the staphylococcal superantigens SEA and SEB between the AIA and ATA groups were performed by Mann–Whitney U-tests using SPSS version 10.0 (Chicago, IL, USA). Pearson’s correlations were applied to define the relationships between levels of enterotoxin-specific IgE and inflammatory markers. P-values of 0.05 or below were regarded as significant.

Results

Clinical characteristics of aspirin intolerant asthma and aspirin tolerant asthma subjects

The clinical characteristics of the AIA and ATA subject groups are shown in Table 1. There were no significant differences in gender, age or duration of symptoms between the study groups (P>0.05). The prevalence of atopy was similar in the AIA and ATA groups, but serum levels of total IgE were significantly higher in AIA subjects than in ATA subjects (P = 0.004).

Inflammatory markers and enterotoxin-specific IgE in nasal polyp tissue of aspirin intolerant asthma and aspirin tolerant asthma subjects

The levels of ECP, IL-5, tryptase and sIL-2R in nasal polyp tissue homogenates of AIA and ATA subjects, expressed as nanograms per milligram of total protein are shown in Fig. 1. Median ECP levels were markedly higher in AIA subjects than in ATA subjects (P = 0.019), while no significant differences between the groups were noted in levels of IL-5 (P = 0.76), tryptase (P = 0.89) or sIL-2R (P = 0.16).

AIA subjects had higher median levels of total IgE in nasal polyp homogenates than ATA subjects (P = 0.04). Specific IgE for the SEA and SEB were each detected in about two-
thirds of AIA nasal polyps and in about two-fifths of ATA nasal polyps; the AIA group showed significantly higher median levels of SEA (\(P = 0.01\)) and SEB (\(P = 0.05\)) than the ATA group (Fig. 2).

Pearson’s correlations were used to examine relationships between the levels of specific IgE for SEA and SEB and inflammatory markers in nasal polyp homogenates (Table 2). Levels of specific IgE to SEA showed striking correlations not only with total IgE levels in polyp tissue (\(r = 0.629, P = 0.003\)) and serum (\(r = 0.601, P = 0.001\)), but also with levels of the eosinophil-associated markers ECP (\(r = 0.56, P = 0.001\)) and IL-5 (\(r = 0.63, P = 0.0001\)) (Fig. 3). The significant overall associations of SEA-specific IgE with ECP and IL-5 were driven by particularly strong correlations within the subgroup of 15 AIA subjects (\(r = 0.56, P = 0.03\), and \(r = 0.66, P = 0.008\), respectively). Levels of SEB-specific IgE in nasal polyps showed similar, but slightly weaker, relationships with total serum IgE (\(r = 0.645, P = 0.002\)) and with ECP (\(r = 0.407, P = 0.026\)) and IL-5 (\(r = 0.588, P = 0.002\)) in nasal polyps. Neither SEA- nor SEB-specific IgE levels correlated with mast cell-derived tryptase (\(P > 0.3\)) or with the T cell activation marker sIL-2R (\(P > 0.05\)) (Table 2).

**Stratification for staphylococcal enterotoxin A status**

Irrespective of AIA and ATA status, all 30 subjects were stratified for the presence or absence of detectable SEA-specific IgE in nasal polyp tissue (Table 3). Subjects positive for SEA-specific IgE (SEA positive, \(n = 17\)) did not differ from those negative for SEA-specific IgE (SEA negative, \(n = 13\)) in gender ratio, age, atopic status, duration of symptoms or recurrence of polyposis. However, the SEA-positive
subjects had striking increases in inflammatory markers in nasal polyp tissue compared with the SEA-negative subjects; these included 30-fold higher levels of total IgE ($P = 0.0001$), 3-fold higher levels of ECP ($P = 0.008$), and 8-fold higher levels of IL-5 ($P = 0.02$). SEA-positive subjects also showed modest (2-fold) increments in nasal polyp tryptase ($P = 0.038$) and sIL-2R ($P = 0.016$) (Table 3).

**Discussion**

Nasal polyposis is a multi-factorial disease of unknown aetiology, but its frequent association with asthma and aspirin intolerance suggests common mechanisms. Recent reports of high levels of IgE in nasal polyp tissue suggest that IgE may be generated locally against one or more aeroallergens. A subgroup of nasal polyposis patients with specific IgE to staphylococcal enterotoxins has been defined [10], pointing to the possibility that bacterial superantigens may drive the non-seasonal eosinophilic inflammation that is a hallmark of nasal polyps [1–3]. It is not known whether such a mechanism is common to patients with and without aspirin intolerance.

Initially, our study compared levels of the ECP in nasal polyposis patients with AIA or ATA. Using selective and sensitive immunoassays on nasal polyp homogenates from 15 subjects in each group, this study revealed a significantly higher level of the eosinophil marker ECP in the AIA polyps, while tryptase, a mast cell marker, was not different (Fig. 1). Although eosinophils were not counted directly, this suggests the presence of a larger and/or more highly activated population of eosinophils in AIA nasal polyps compared with their ATA counterparts. Previous studies have provided only ambiguous evidence for increased eosinophil populations in AIA polyps [12, 13], although polyps from both AIA and ATA subjects contained more eosinophils and ECP than non-polyp nasal tissue [10]. Higher eosinophil counts have been reported in the bronchial biopsies of AIA subjects compared with ATA bronchial biopsies [15].

To investigate possible mechanisms for the enhanced ECP levels in AIA nasal polyps, our study next compared the nasal polyp levels of specific IgE for SEA and SEB in the AIA and ATA groups. There were markedly higher median levels of specific IgE for SEA and for SEB in AIA nasal polyps compared with ATA polyps, in which the median levels were zero (Fig. 2). There was also a tendency for a larger proportion of AIA subjects to have detectable levels of specific IgE for SEA (73% vs. 43%) and SEB (60% vs. 36%). Furthermore, when the 30 AIA and ATA subjects were analysed together, levels of IgE specific for SEA and SEB each correlated significantly with ECP levels (Table 2, Fig. 3); the relationships with SEA-specific IgE were particularly strong within the AIA group (Fig. 3). Taken together, the results suggest that elevated IgE-mediated responses to staphylococcal superantigens may directly or indirectly drive the enhanced eosinophilia in AIA nasal polyps compared with ATA polyps. Whether this is because of higher levels of exposure to staphylococcal superantigens or an increased propensity to mount a specific IgE response to these antigens in AIA subjects is unclear. The outcome is nevertheless relatively restricted to eosinophils, as AIA subjects did not show increased levels of the mast cell marker tryptase. This is consistent with the view that mast cell activation, as shown by tryptase and histamine production [10], may play only a relatively minor role in nasal polyposis.
The third important outcome of our study was that total IgE levels were markedly higher in AIA nasal polyps than in ATA polyps (Fig. 2). This may merely reflect increased local production of specific IgE in AIA polyps, as supported by the close correlation between total and specific IgE levels in polyps shown in Table 2. Alternatively, elevated total IgE in AIA polyps may be secondary to the significantly higher total IgE in the serum of these patients (Table 1). The latter finding is unexpected as the AIA and ATA subject groups had a similar prevalence of atopy as defined by skin tests.

Overall, our data are consistent with other studies in supporting the notion that staphylococcal superantigens can trigger or exacerbate allergic and inflammatory diseases of the skin [7, 8], airways [9, 16] and vasculature [17]. In the nasal airways, the presence of superantigen-producing *S. aureus* was found to be significantly higher in patients with perennial allergic rhinitis (PAR) compared with non-allergic controls [18]. PAR patients who were *S. aureus* positive experienced more nasal symptoms than their counterparts without *S. aureus* [18]. Our data are the first to reveal exaggerated IgE responses to staphylococcal enterotoxins in patients with AIA compared with ATA.

The mechanisms by which superantigens may drive eosinophilic inflammation in nasal polyp tissues remain to be elucidated. Our previous investigations revealed significant correlations between ECP levels, activated (EG2) eosinophils and levels of total IgE and house dust mite-specific IgE in nasal polyp tissue [4]. The present study revealed analogous correlations between ECP, total IgE and enterotoxin-specific IgE within nasal polyp tissue. A possible mechanism is that specific IgE bound to its cell-surface receptors on eosinophils may bind staphylococcal enterotoxins, leading directly to eosinophil degranulation and release of ECP. The presence of high-affinity IgE receptors (FcRI) on eosinophils has been suggested [19, 20], but is not universally accepted to apply in vivo. In contrast, the presence of FcRI on tissue mast cells is undisputed, but the lack of difference between the AIA and ATA groups in tryptase levels, and the lack of overall correlation between tryptase and the other markers, argue against direct activation of mast cells by staphylococcal superantigens in nasal polyps.

Other cell types may mediate the putative effect of these staphylococcal enterotoxins on eosinophil activation. When exposed to SEA in vitro, the peripheral blood lymphocytes of PAR patients proliferated to a greater extent than normal lymphocytes, and also generated significantly greater amounts of IL-4 and IL-5, in a dose-dependent manner [18]. IL-5 is a key cytokine for eosinophil differentiation, migration, activation and survival. Compared with normal tissue, IL-5 levels are elevated in nasal polyps and their concentrations correlate with the eosinophil marker ECP [21, 22]. Staphylococcal superantigens can induce T-helper type 2 mediated immune responses in the skin [23], and they selectively stimulate IL-5 production from peripheral blood mononuclear cells [24]. In the present study, significant correlations were noted overall between levels of SEA- and SEB-specific IgE and levels of IL-5 (Table 2). Stratification for the presence or absence of detectable SEA-specific IgE (Table 3) suggested that these correlations with IL-5 were largely independent of aspirin tolerance or intolerance. The higher sIL-2R levels in the SEA-specific IgE-positive group further supports the view that staphylococcal superantigens may promote eosinophilia in nasal polyps of any origin via T cell-derived IL-5. In contrast, the lack of differences in IL-5 and sIL-2R between the AIA and ATA groups suggests that additional mechanisms may be required to explain how enhanced specific IgE to staphylococcal enterotoxins can induce the further increment in ECP levels seen in AIA polyps compared with ATA polyps.

In conclusion, we have confirmed the presence of specific IgE to staphylococcal superantigens in nasal polyp tissue, and that its levels correlate with markers of eosinophil activation and recruitment. Most importantly, the study revealed that levels of enterotoxin-specific IgE are markedly elevated in the nasal polyps of subjects with AIA compared with their ATA counterparts. This finding may underlie the highly pronounced eosinophilia reported in the nasal polyps of AIA patients.

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References


