IgE and Cytokines (IL-6 and IL-13) in Sinonasal Polyposis

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Abstract: Background: This study aimed to evaluate local IgE together with cytokines IL-6 and IL-13 in nasal polyposis. Twenty eight patients with sinusal polyposis were classified into allergic group and non allergic group. Serum and polyp fluid IgE, IL-6 and IL-13 were measured by ELISA. The amount of serum and polyp tissue albumin was determined. Result: The mean value of IgE in polyp fluid in allergic group is statistically significantly higher compared to that in non allergic group. Regarding cytokines assay, the mean values of both IL-6 and IL-13 in polyp fluid of allergic group are statistically significantly higher than that in non allergic group. In allergic group, the mean value in serum IgE expressed as percentage to serum albumin is statistically significantly higher than that of non allergic group. There is no significant difference regarding IL-6 concentration in sera of both groups. There is a statistically significant increase in the amount of IL-13 in sera of allergic group compared to control group. Conclusion: There is increased expression of IgE, IL-6 and IL-13 in nasal polyps extracted from patients with allergic rhinitis. There is a statistically significant increase in the amount of IgE and IL-13 in sera of allergic group compared to control group.

Key words: Sinonasal polyposis, immunology, IgE, IL-6 and IL-13, cytokines, allergic rhinitis

INTRODUCTION

Nasal polyposis is a chronic inflammatory disease of the paranasal sinuses, with a prevalence of 1-4% in the general population. Rhinosinusitis (including nasal polyps) is defined as:

- Inflammation of the nose and the paranasal sinuses characterized by two or more symptoms, one of which should be either nasal blockage/obstruction/congestion or nasal discharge (anterior/posterior nasal drip): ± facial pain/pressure; ±reduction or loss of smell and either
- Endoscopic signs of: polyps and/or, mucopurulent discharge primarily from middle meatus and/or, oedema/mucosal obstruction primarily in middle meatus and/or
- CT changes:
  - Mucosal changes within the ostiomeatal complex and/or sinuses (Fokkens et al., 2007)
  - The pathogenesis of nasal polyps is far from clear. Tos and Mogensen (1977) have suggested that the early stage of polyp formation was characterized by infiltration and edema of the nasal mucosa, rupture of the epithelium and production of granulation tissue Bachert et al. (2000) believed that formation of nasal polyps began as exudates in the nasal mucosa and that growth of nasal polyps was due to vascular stalk and vascular congestion

Predisposing factors for development of polyps of the nose and paranasal sinuses are chronic inflammation, cystic fibrosis, allergies, production of cytokines and genetic influences (Hassid et al., 1997). These conditions may lead to a proliferation of the respiratory epithelium with an edema of the underlying stroma, chronic inflammation, infiltration with lymphocytes, eosinophilic granulocytes and finally to the development of polyps in the nasal cavity and paranasal sinuses. Several cytokines have been identified that may support the development of these benign lesions (Naclerio, 1997; Mygind and Lund, 2008).

CD4 cell populations are characterized by marked variation of their cytokine secretion patterns. They have been divided into type I (Th1) cells which secrete predominantly IL-2 and IFN-γ and type II (Th2) cells that secrete IL-4, IL-5, IL-6 and IL-10 (Ishizaka et al., 1990).

IL-6, IL-9 and tumor necrosis factor-alpha act synergistically with suboptimal amounts of IL-4 (Kuna et al., 1991). Evidence for altered activity of the IL-6 pathway in chronic rhinosinusitis with nasal polyps has been shown recently (Peters et al., 2010).

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Levy et al. (1997) demonstrated that IgE production by atopic peripheral blood mononuclear cells is dependent on endogenously secreted IL-4 and IL-13, since it could be blocked by a combination of anti-IL-4 plus anti-IL-13 antibodies.

IL-13, but not IL-4, promotes the chemotaxis and prolongs the survival of eosinophils in vitro (Horie et al., 1996). IL-13 levels at baseline in rhinitis subjects did not differ from those of normal subjects and also indicated that allergen provocation of the rhinitic individuals resulted in substantial upregulation of IL-13 which could in part account for the clinical manifestations of allergen-induced late nasal response (Ghaffar et al., 1998).

In this study we aimed to evaluate cytokines IL-6 and IL-13 in nasal polyposis in allergic and non allergic groups in a trial to detect a possible relation between these cytokines and local IgE formation at the local site of allergic inflammation.

**MATERIALS AND METHODS**

This study was carried on 28 patients with nasal polyps. 14 of them were allergic to different allergens (test group) and 14 were not allergic. The diagnosis of allergic rhinitis was confirmed by clinical history, clinical examination, positive skin test for a certain inhalant allergen and elevated total serum IgE.

**Skin testing (Samter, 1971):** Prick skin testing was done with 0.1 concentrations for 6 inhalant allergens including mixed fungus, mixed pollens, house dust, smoke, hay dust and Aspergilus fumigatus. Determination of serum and polyp IgE by ELISA (Hassid et al., 1997; Levy et al., 1997).

Nasal polyps were surgically removed and put in sterile saline and transferred to laboratory as quickly as possible. Nasal polyps were minced in sterile saline using homogenizer and polyp fluid was centrifuged at 5000 rpm for 5 min and the supernatants together with patient and control sera were stored at -20°C till assay was done.

Serum and polyp fluid IgE were measured by ELISA kit (Elitech diagnostics - France). ELISA reader (Spectra III, Austria) was used.

The amount of serum and polyp tissue albumin was determined by photometric colorimetric analysis (BCG-method). The polyp tissue total IgE/albumin as well as serum total IgE/albumin ratio was determined.

**Determination of serum and polyp IL-6 and IL-13 by ELISA (Kim et al., 1998):** Nasal polyps were frozen at -20°C immediately after surgical removal. The polyps were thawed and then minced in sterile saline using homogenizer and polyp fluid was centrifuged at 5000 rpm for 5 min to remove particles and the supernatants together with patient sera were stored at -20°C till assay was done.

Serum and polyp fluid IL-6 and IL-13 were measured by ELISA kits. For IL-13 Enzyme immunoassay kit from Immunotech A Beckman couler company (Marseille-France) was used. It is a sandwich type assay using bound monoclonal anti-IL-13 antibody, a second Biotinylated monoclonal anti-IL-13 antibody and streptavidin-peroxidase conjugate. For IL-6 measurements IL-6 EASIA (Biosource, Europe S.A., Nivelles, Belgium) was used. It is a solid phase enzyme amplified sensitivity immunoassay (EASIA). For IL-6 and IL-13 assays ELISA reader (Spectra III, Austria) was used with its linearity up to 4 OD units and after construction of standard curves for both cytokines, amount in pg mL⁻¹ were calculated.

**RESULTS**

Our results in Table 1 indicated that the mean value of IgE in polyp fluid in allergic group was statistically significantly higher compared to that in non allergic. Regarding cytokines assay, the results in Table 1 show that the mean values of both IL-6 and IL-13 in polyp fluid of allergic group were statistically significantly higher than that in non allergic group.

The results in Table 2 show that in allergic group, the mean value in serum IgE expressed as percentage to serum albumin was statistically significantly higher than that of non allergic group. There is no significant difference regarding IL-6 concentration in sera of both groups. There is a statistically significant increase in the amount of IL-13 in sera of allergic group compared to nonallergic group.

**Table 1:** The expression of IgE. IL-6 and IL-13 in emulsified (homogenized) polyp tissue of allergic and non allergic (control) groups (expressed as a percentage to polyp fluid albumin)

<table>
<thead>
<tr>
<th></th>
<th>Allergic group</th>
<th>Control group</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td><strong>Mean</strong></td>
<td><strong>SD</strong></td>
<td><strong>Mean</strong></td>
<td><strong>SD</strong></td>
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<tr>
<td>IgE</td>
<td>28.6</td>
<td>0.76</td>
<td>12.90</td>
</tr>
<tr>
<td>IL-6</td>
<td>72.8</td>
<td>1.03</td>
<td>34.90</td>
</tr>
<tr>
<td>IL-13</td>
<td>23.25</td>
<td>0.22</td>
<td>14.65</td>
</tr>
</tbody>
</table>

**Table 2:** The expression of IgE. IL-6 and IL-13 in sera of allergic and non allergic groups (expressed as a percentage to serum albumin)

<table>
<thead>
<tr>
<th></th>
<th>Allergic group</th>
<th>Control group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean</strong></td>
<td><strong>SD</strong></td>
<td><strong>Mean</strong></td>
<td><strong>SD</strong></td>
</tr>
<tr>
<td>IgE</td>
<td>129.3</td>
<td>4.81</td>
<td>11.4</td>
</tr>
<tr>
<td>IL-6</td>
<td>8.32</td>
<td>0.77</td>
<td>7.90</td>
</tr>
<tr>
<td>IL-13</td>
<td>4.12</td>
<td>0.11</td>
<td>0.71</td>
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</table>
DISCUSSION

The immunohistological study of Th2 cytokine positive cells in allergic and nonallergic nasal mucosa revealed that the number of immunoreactive IL-4, IL-5 and IL-6 positive cells were significantly higher in allergic nasal mucosa than non-allergic mucosa. Furthermore, these Th2 cytokine positive cells were revealed to increase following topical antigen challenge. The main origin of IL-4 and IL-6 might be T cells and those of IL-5 might be T-cells and eosinophils (Kataura and Asakura 1996). A similar increase of Th2 cytokine mRNA positive cells was also reported by Durham et al. (1992) using in situ hybridization method.

Regarding cytokines assay, the mean values of both IL-6 and IL-13 in polyp fluid of allergic group are statistically significantly higher than that in non allergic group. The results showed that in allergic group, the mean value in serum IgE expressed as percentage to serum albumin is statistically significantly higher than that of non allergic group. There is no significant difference regarding IL-6 concentration in sera of both groups. There is a statistically significant increase in the amount of IL-13 in sera of allergic group compared to nonallergic group.

Local tissue IgE profile reflects more specifically the allergic status of patients with nasal polyps than does the systemic serum test or the presentation of allergic symptoms (Wei and Fang, 2005).

Endoscopic nasal polyposis severity directly correlates to total serum IgE levels and inclusion of anti-IgE therapy in the post polypectomy management of atopic asthmatic individuals may reduce the severity of nasal polyposis recurrence (Penn and Mikula, 2007).

Buchert et al. (1995) reported that the baseline levels of IL-1, IL-8 and TNF were significantly higher in allergic subjects than in control subjects.

IL-6 messenger RNA was expressed by a significantly greater proportion of epithelial and sub epithelial cells in chronic sinusitis in allergic and non allergic subjects than in normal controls with no difference between chronic sinusitis in allergic and non allergic patients (Ghaffar et al., 1998).

After allergen challenge in patients with nasal allergy to house dust mite, there was significantly greater levels of IL-6 and IL-8 secretion on the challenged side than on the contralateral side (Ohtuso et al., 1998). Saito et al. (1997) concluded that antigen-induced up regulation of IL-4, IL-5 and IL-6 is important in the pathogenesis of perennial allergic rhinitis as the numbers of immunoreactive IL-4, IL-5 and IL-6 positive cells were significantly higher in allergic mucosa than in non allergic mucosa.

Our results regarding increased expression of IL-13 in nasal polyposis concur that of Pawankar et al. (1997) who detected distinct IL-4 and IL-13 expression in nasal mast cells of perennial allergic rhinitis patients and a remarkable proportion of these cells expressed IL-4 (64.2%) and IL-13 (82.4%). Stimulation of nasal mast cells from perennial allergic rhinitis patients with Der III (mite antigen) induced 10-fold more IL-13 secretion than IL-4.

Ghaffar et al. (1997) indicated that IL-13 immunoreactivity was observed in nine of 10 normal controls and in all rhinitic subjects with no significant difference between the 2 groups indicating that the presence of IL-13 in the nasal mucosa may not be associated with atopy. They detected a pronounced increase in the numbers of IL-13 mRNA-positive cells 24 h after local allergen challenge of rhinitic patients suggesting that IL-13 is involved in allergen- induced late nasal response.

Rogalewska et al. (1999) measured IL-13 and IL-4 in nasal lavage fluid of 13 allergic rhinitis patients after specific antigen challenge and indicated that both cytokines were found to be present in the nasal lavage in 6 patients, the IL-13 concentration was higher than that of IL-4. Cocks et al. (1993) indicated that the availability of IL-4 and IL-13 produced in the nasal mucosa creates the possibility for isotype switching of B cells to IgE-positive B cells and proliferation and maturation of B cells to IgE-producing plasma cell.

A novel and direct evidence in vivo for increased expression of IL-4 mRNA and decreased expression for IFN-γ mRNA in the nasal mucosa of sensitized and aerosolized DBA/2J (Brown-Norway) rats together with increased expression of OVA (ovalbumin) specific IgE compared to control native rats (El-Naggar et al., 1998).

Increased levels of IgE have been identified in sinonasal tissues in allergic and nonallergic rhinitis atopic and non atopic sinonasal polyposis and allergic fungal rhinosinusitis. The ability to identify local tissue IgE in inflammatory sinonasal diseases may have significant diagnostic and therapeutic implications (Wise et al., 2009). Anti-IgE for the treatment of allergic rhinitis and eventually nasal polyposis would pave the way for anti-IgE treatment for severe nonatopic lower airway disease (Verbruggen et al., 2009).

Staphylococcal exotoxin B is able to modulate proinflammatory factors, T helper type 1/Th2 profiles and suppress T regulatory activity in cultured nasal polyps, which were rescued by blocking IL-6 activity. Therefore, IL-6 is essential for SEB induced T regulatory cell insufficiency in nasal polyps (Xu et al., 2009). IL-13 seems to be an important marker in eosinophilic CRS and plays a pivotal role in eosinophilic inflammation (Sauter et al., 2008).
CONCLUSION

IgE antibody could be locally produced from the nasal polyp tissue of non atopic as well as atopic subjects and in most cases the amount of polyp tissue IgE is more than that found in serum. IgE may have significant diagnostic and therapeutic implications. There is increased expression of IL-6 and IL-13 in nasal polyps extracted from patients with allergic rhinitis. In the future, IL-6 and IL-13 may have a role in management; follow up as well as prevention of recurrence of sinonasal polyposis.

REFERENCES


