Original article

Elevation of Plasma Eotaxin Levels in Children with Food Allergy

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summary

Background: Eosinophils play an important role in allergic responses. Eotaxin is a CC chemokine that promotes the selective recruitment of eosinophils. This study was performed to investigate the significance of eotaxin in pediatric food allergies.

Methods: The study population included 35 patients with food allergy, 18 patients with atopic dermatitis but without food allergy, and 19 age-matched non-allergic controls. Eotaxin-1 and eotaxin-3 levels in plasma were assayed by enzyme-linked immunosorbent assay. Simultaneously, eosinophil counts in peripheral blood and serum immunoglobulin E (IgE) values were assessed.

Results: Plasma eotaxin-1 levels were 93.6 ± 33.4 pg/ml in patients with food allergy, 78.0 ± 31.8 pg/ml in patients with atopic dermatitis, and 60.4 ± 15.7 pg/ml in controls. Differences between the food allergy and control groups were significant ($P < 0.001$). Circulating eosinophil counts in patients with food allergy were higher than those in controls ($5.84 \pm 9.46 \times 10^9/l$ vs. $1.20 \pm 1.11 \times 10^9/l$, $P < 0.001$). Nevertheless, eotaxin-1 levels in children with food allergy were not correlated with eosinophil counts or serum IgE levels. There were no significant differences in eotaxin-3 levels between the 3 groups.

Conclusion: Plasma levels of eotaxin-1 were elevated in children with food allergy. The pathophysiological relevance of the increase in eotaxin is discussed.

Key words—food allergy; eotaxin; eosinophil; immunoglobulin E

Introduction

Food allergies (FA) are common, with up to 8% of children under 3 years of age and around 2% of adults experiencing allergic reactions to food¹⁾. Although IgE is commonly used as a clinical marker of allergy, this class of antibody does not always reflect the allergic status accurately, because allergic symptoms involve the activation of both IgE- and non-IgE-mediated pathways^{2,3)}. Eosinophils, CD4⁺ T cells, and mast cells play important roles in allergic responses⁴⁾. In particular, eosinophils include a variety of proinflammatory mediators that can contribute to allergic reactions⁵⁾ and accumulate in the foci of allergic responses. Eosinophils normally account for only a small percentage of circulating or tissue-dwelling cells, and their numbers are increased markedly in allergic diseases. Numerous mediators have been identified as eosinophil chemoattractants, including lipid mediators (platelet-activating factor, leukotrienes), bacterial products (formyl-methionyl-

leucyl-phenylalanine), and chemokines (regulated on activation, normal T cells expressed and secreted, and macrophage inflammatory protein-1). However, none of these mediators selectively promote eosinophil recruitment. Therefore, they are unlikely to be the primary mediators of the tissue eosinophilia observed in numerous hypereosinophilic disorders.

Eotaxin-1/CCL11, eotaxin-2/CCL24 and eotaxin-3/CCL26 are CC chemokines that promote the recruitment of eosinophils^{6~8)}. The eotaxins act via interaction with a specific receptor, CCR3, which is expressed mainly on eosinophils⁹⁾. In addition to eosinophils, basophils express CCR3 and migrate toward eotaxin¹⁰⁾. Although Th2 cells have also been shown to express CCR3, it is controversial¹¹⁾. Previous studies showed that plasma eotaxin levels in allergic patients with bronchial asthma and atopic dermatitis are higher than those in normal controls^{12,13)}. However, little is known about the correlations among eotaxin group levels, eosinophil counts, and IgE levels in patients with FA. In the present study, we showed that levels of eotaxin-1, but not eotaxin-3, were significantly elevated in FA, and discuss the role of eotaxins in this condition.

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Subjects and methods

Subjects

The study population consisted of 35 patients with food allergy (FA) and 18 patients with atopic dermatitis (AD) but without FA (Table). The FA group included 18 patients with and 17 patients without AD. Therefore, the AD group consisted of a total of 36 patients, including 18 with and 18 without FA. The patients with FA showed food-mediated acute allergic reactions above grade 2¹⁴ including anaphylaxis and/or urticaria. The clinical diagnoses were based on a careful history, laboratory findings (total and specific IgE), dietary elimination tests, and food challenge tests. Of the 35 FA patients, 25 underwent food elimination treatment. Specific IgE values against food antigens were scored as 3 or more in all patients. The control group consisted of 19 disease-free children without allergy. Subjects with fever, diarrhea, infectious diseases, or other chronic diseases were excluded. The differences in age were not significant among FA patients (mean $\pm$ SD: 2.5 ± 2.0 years), AD patients (2.5 ± 1.9 years), and controls (2.9 ± 1.9 years). Informed consent was obtained from caregivers of the patients and controls before enrollment in this study.

Measurements

Blood samples were harvested in tubes containing EDTA by venous puncture. Plasma specimens were separated and stored at -30°C . Hemolyzed samples were avoided. Concentrations of both eotaxin-1 and eotaxin-3 were measured by enzyme-linked immunosorbent assay (ELISA) (Quantikine; R&D Systems, Minneapolis, MN). The limits of detection were 5 pg/ml for eotaxin-1 and 0.87 pg/ml for eotaxin-3. Specific IgE levels were measured using the Phadea CAP System FEIA (formerly Pharmacia & Upjohn AB Diagnostics, Uppsala, Sweden).

Statistical analysis

Data are expressed as means $\pm$ SD. The results were analyzed using a commercially available computer software package (Stat View; Abacus Concepts Inc, Berkeley, CA). The significance of differences between two independent groups was determined using the Mann-Whitney U test. Relationships between plasma eotaxin levels and other indices were assessed based on the Pearson correlation coefficients. Differences were considered significant at $P < 0.05$.

Results

Samples were collected from 35 FA patients, 18 AD patients without FA, and 19 disease-free controls. As indicated in the Table, FA patients had significantly larger numbers of circulating eosinophils than the controls ($5.84 \pm 9.46 \times 10^9/\text{l}$ vs. $1.20 \pm 1.11 \times 10^9/\text{l}$, $P < 0.001$). Serum IgE levels in FA patients were higher than those of the controls (1007 ± 1315 U/ml vs. 16 ± 18 U/ml, $P < 0.001$). Eosinophil counts ($7.04 \pm 8.42 \times 10^9/\text{l}$, $P < 0.001$) and IgE levels (878 ± 1843 U/ml, $P = 0.005$) in AD patients without FA were higher than those of the controls. In contrast, there were no significant differences between FA and AD patients without FA in IgE levels ($P = 0.119$) or eosinophil counts ($P = 0.505$).

As shown in Fig. 1, plasma eotaxin-1 levels in FA patients (93.6 ± 33.4 pg/ml, 46.0–200.3 pg/ml, $n = 35$) were significantly higher than those of the controls (60.4 ± 15.7 pg/ml, 35.3–91.3 pg/ml) ($P < 0.001$). The difference between plasma eotaxin-3 levels in FA patients (2.0 ± 3.2 pg/ml, 0.1–18.1 pg/ml)

Fig. 1 Eotaxin-1 (A) and eotaxin-3 (B) levels in the plasma of patients with food allergy (FA) and atopic dermatitis (AD). The FA group ($n = 35$) included patients both with and without AD. The AD group ($n = 36$) included patients both with and without FA. Eotaxin-1 levels in FA and AD were higher than those of disease-free controls (A).

and the controls (1.4 ± 1.1 pg/ml, $0.1\text{--}3.4$ pg/ml) was not significant ($P=0.978$), although a small number of FA patients had high eotaxin-3 levels.

In previous study¹²⁾, AD subjects did not exclude those with FA. To obtain comparable results, first we analyzed AD subjects both with FA and without FA. Figure 1 shows that eotaxin-1 levels were significantly higher in AD patients (84.3 ± 31.1 pg/ml, $40.7\text{--}163.0$ pg/ml, $n=36$) than in controls ($P=0.003$). In contrast, eotaxin-3 levels in AD patients (2.1 ± 3.4 pg/ml, $0.1\text{--}18.1$ pg/ml) were not significantly different from those of controls ($P=0.951$). There were no significant differences in eotaxin-1 ($P=0.223$) or eotaxin-3 levels ($P=0.850$) between FA patients and AD patients.

Half of the patients with FA also had AD (Table 1). To determine whether the elevated levels of plasma eotaxin-1 in the FA patients reflect the status of AD, we then divided the FA group into subjects with and without AD, and repeated the analysis for AD subjects without FA only, as shown in Fig. 2. Eotaxin-1 levels not only in FA patients with AD (FA+/AD+, 90.7 ± 29.8 pg/ml, $n=18$) but also in FA patients without AD (FA+/AD-, 96.6 ± 37.5 pg/ml, $n=17$) were significantly higher than those of the controls ($P<0.001$). Eotaxin-3 levels were not significantly different among FA+/AD+ (2.4 ± 4.1 pg/ml), FA+/AD- (1.7 ± 2.1 pg/ml), and controls.

There was no significant difference in eotaxin-1 level between AD patients without FA (FA-/AD+, 78.0 ± 31.8 pg/ml, $n=18$) and the controls ($P=0.081$). Eotaxin-3 levels were not significantly different between FA-/AD+ (1.9 ± 2.8 pg/ml) and the controls ($P=0.616$). Neither eotaxin-1 nor eotaxin-3 levels were significantly different between FA+/AD+ and FA-/AD+ ($P=0.092$ and $P=0.704$, respectively). In addition, differences in eotaxin-1 and eotaxin-3 levels were not significant between FA subgroups with and without AD ($P=0.817$ and $P=0.255$, respectively).

The correlations among eotaxins, eosinophils, and IgE were examined. The correlations between eotaxin-1 and eotaxin-3 were not significant in FA ($r=0.018$, $P=0.918$) or the controls ($r=0.413$, $P=0.079$). In FA patients, there were no significant correlations between either eotaxin-1 ($r=-0.024$, $P=0.892$) or eotaxin-3 ($r=-0.014$, $P=0.935$) and circulating eosinophil counts. In the controls, no significant correlation was found between either eotaxin-1 ($r=-0.263$, $P=0.281$) or eotaxin-3 ($r=0.236$, $P=0.336$) and eosinophil counts. The results were similar between both eotaxin-1 ($r=-0.176$, $P=0.314$) and eotaxin-3 ($r=0.265$, $P=0.125$) and IgE in the FA patients. Consistent results were observed in the controls (eotaxin-1 and IgE, $r=-0.368$, $P=0.149$; eotaxin-3 and IgE, $r=-0.071$, $P=0.791$).

Table 1. Clinical characteristics of allergic patients and controls

	FA			AD		Controls
	Total	with AD	without AD	Total	without FA	
Subjects	35	18	17	36	18	19
Age (years)	0.4–7.8 (2.5 ± 2.0)	0.4–7.8 (2.6 ± 2.2)	0.5–7.0 (2.4 ± 1.9)	0.4–7.8 (2.5 ± 2.0)	0.4–6.1 (2.5 ± 1.9)	0.5–6.2 (2.9 ± 1.9)
Complications						
Anaphylaxis	26	10	16	10	0	0
Bronchial asthma	10	7	3	14	7	0
Atopic dermatitis	18	18	0	36	18	0
Urticaria	7	4	3	5	1	0
Food allergens						
Egg	21	13	8	13	0	0
Milk	12	8	4	8	0	0
Wheat	2	0	2	0	0	0
Nuts etc	13	6	7	6	0	0
Eosinophils ($\times 10^6/l$)	97–5771 (584 ± 941)	97–5771 (766 ± 1277)	102–1209 (392 ± 300)	49–5771 (735 ± 1067)	49–3705 (704 ± 842)	0–321 (121 ± 111)
IgE (U/ml)	30–5288 (1007 ± 1315)	30–5288 (1371 ± 1622)	39–2676 (622 ± 756)	1–7606 (1124 ± 1729)	1–7606 (878 ± 1843)	0–82 (17 ± 18)

Numbers in parentheses are means $\pm$ SD.

Abbreviations: FA, food allergy; AD, atopic dermatitis; IgE, immunoglobulin E.

Fig. 2 Eotaxin-1 (A) and eotaxin-3 (B) levels in plasma of patients with food allergy (FA) and atopic dermatitis (AD). To determine whether elevated levels of plasma eotaxin-1 in the FA patients reflect the status of AD, the FA group was divided into subjects with (FA + /AD +, $n = 17$) and without AD (FA + /AD -, $n = 18$), and compared with AD subjects only without FA (FA - /AD +, $n = 18$). Eotaxin-1 levels in FA + /AD + and FA + /AD - were significantly higher than those of the controls. Eotaxin-1 levels in FA - /AD + were not significantly different from FA + /AD + or disease-free controls.

Finally, the effects of food elimination treatment on eotaxin levels were analyzed in food allergy patients. There were no significant differences in eotaxin-1 levels in subjects with (94.0 ± 30.1 pg/ml, $n = 25$) or without food elimination treatment (92.5 ± 42.3 pg/ml, $n = 10$, $P = 0.635$) (Fig. 3). Similar results were observed for eotaxin-3 in subjects with (2.2 ± 3.7 pg/ml) and without food elimination treatment (1.7 ± 1.5 pg/ml, $P = 0.743$).

Discussion

In the present study, plasma eotaxin-1 levels were significantly elevated in patients with FA compared to the values in disease-free controls. Increases in eotaxin-1 levels have also been reported in bronchoalveolar lavage fluid¹⁵⁾ and sputum^{16,17)} from asthmatics, human milk in allergic mothers¹⁸⁾, and skin biopsy specimens from AD patients¹⁹⁾. Recently, Fulkerson et al. demonstrated that chronic allergic airway in-

Fig. 3 Eotaxin-1 (A) and eotaxin-3 (B) levels in plasma of FA patients receiving food elimination treatment. There were no significant differences in FA patients with or without food elimination treatment.

flammation was impaired in mice with defects in eosinophils, CCR3 knockout mice, and mice deficient in both eotaxin-1 and eotaxin-2²⁰⁾. They concluded that eosinophils play a central role in chronic allergic airway disease via CCR3²⁰⁾. These lines of evidence suggest a crucial role of eotaxin-1 in the pathogenesis of allergic disorders.

Previous studies showed increases in eotaxin-1 levels in AD patients^{12,21,22)}, as observed in the present study in AD patients with FA and without FA. Our FA group, which included both patients with and without AD, showed increased levels of eotaxin-1. This suggested the possibility that only the AD patients among our FA patients contributed to the elevation of eotaxin-1. To determine whether our observations were due to the AD patients, the FA subjects were divided into an AD group and a non-AD group. Eotaxin-1 levels were significantly higher in not only in FA patients with AD but also in those without AD than in controls. There was no significant difference in eotaxin-1 level between the AD and non-AD groups in FA patients. In addition, eotaxin-1 levels in AD subjects without FA were not significantly different from FA patients with AD or disease-free controls. These results indicated that eotaxin-1 levels are increased in FA patients regardless of AD.

In the present study, there were no significant differences in eotaxin-3 level among the FA group, AD group, and disease-free controls. In contrast, Berkman et al. reported that baseline eotaxin-3 mRNA expression was not increased in asthmatic patients in comparison with control subjects, but was markedly enhanced 24^h after allergen challenge in asthmatic patients²³). Kagami *et al.* suggested that serum levels of eotaxin-3 were closely correlated with disease activity of AD²⁴). Poltzer et al. showed that eotaxin-3, in contrast to eotaxin-1 and eotaxin-2, was elevated in serum samples of patients with active Churg-Strauss Syndrome, characterized by eosinophilia and granulomatous vasculitis²⁵). They further reported that eotaxins were not elevated in other eosinophilic diseases²⁵). Allergen-induced eosinophil recruitment into the airway was abolished in mice deficient in both eotaxin-1 and eotaxin-2 as well as in CCR3-deficient mice²⁰). Taken together, these observations indicate that the association between eotaxin-3 and eosinophil-induced allergic inflammation is not strong. Further studies of eotaxin-3 in FA are required.

There were no correlations between plasma eotaxins and circulating eosinophil counts in FA patients or controls. These findings may be related to the hypothesis of Rothenberg *et al.*¹⁰) that eotaxin plays an important role in the maintenance of basal levels of circulating eosinophils. Given the ability of eotaxins-1 to recruit eosinophils into the tissues and to activate the proinflammatory effector functions of the cells²⁰), eotaxin-1 may be a candidate pathogenic molecule in FA.

In the present study, food elimination treatment did not significantly affect plasma levels of eotaxin-1 or eotaxin-3. It is difficult to avoid all antigens, including minor antigens, in the clinical treatment of FA. It is possible that minor antigens could stimulate allergic inflammation, and still result in high levels of plasma eotaxin-1. To elucidate the precise role of eotaxin-1 in food elimination for FA, a strict prospective study is required.

In summary, the results of the present retrospective study suggested that levels of plasma eotaxin-1 are elevated in food allergy. Eotaxin-1 may be a useful parameter to assess this disorder.

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