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Original article

Eosinophilia in infants with food protein-induced enterocolitis syndrome in Japan

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Abbreviations:
ALST, allergen-specific lymphocyte stimulation test; B, bloody stool; CM, cow milk; CRP, C-reactive protein; D, diarrhea; Eo, the percentage of peripheral blood eosinophils; FPIES, food protein-induced enterocolitis syndrome; FPIP, food protein-induced proctocolitis; GIFA, gastrointestinal food allergy; GI, gastrointestinal; sIgE, specific IgE antibody; V, vomiting

ABSTRACT

Background: Many Japanese infants with food protein-induced enterocolitis syndrome (FPIES) show eosinophilia, which has been thought to be a characteristic of food protein-induced proctocolitis (FPIP).
Methods: To elucidate the characteristics of eosinophilia in Japanese FPIES patients, 113 infants with non-IgE-mediated gastrointestinal food allergy due to cow's milk were enrolled and classified into FPIES (n = 94) and FPIP (n = 19).
Results: The percentage of peripheral blood eosinophils (Eo) was increased in most FPIES patients (median, 7.5%), which was comparable with that in FPIP patients (9.0%). Among FPIES patients, Eo was the highest in patients who had vomiting, bloody stool, and diarrhea simultaneously (12.9%) and lowest in patients with diarrhea alone (3.2%). Eo showed a significant positive correlation with the incidence of vomiting (Cramer's V = 0.31, $p < 0.005$) and bloody stool (Cramer's V = 0.34, $p < 0.0005$). A significant difference was found in Eo between early- (≤10 days, n = 56) and late-onset (>10 days, n = 38) FPIES (median, 9.8% vs. 5.4%; $p < 0.005$). IL-5 production by peripheral blood T cells stimulated with cow's milk protein in early-onset FPIES was significantly higher than that in late-onset FPIES (67.7 pg/mL vs. 12.5 pg/mL, $p < 0.01$), and showed a significant positive correlation with Eo (rs = 0.60, $p < 0.01$).
Conclusions: This study demonstrated two types of eosinophilia in Japanese FPIES infants: conspicuous and mild eosinophilia in early- and late-onset FPIES patients, respectively. Conspicuous eosinophilia in early-onset FPIES is suggested to be caused by abnormally high IL-5 production.

Introduction

Food protein-induced enterocolitis syndrome (FPIES) is a non-immunoglobulin (Ig) E-mediated gastrointestinal food allergy (GIFA) characterized by gastrointestinal (GI) symptoms such as vomiting (V) and/or diarrhea (D).[1,2] The pathological mechanism of the disease is intestinal inflammation,[3,4] and cell-mediated hypersensitivity is presumed to play an important role in the development of the disease.[5–7] However, further aspects related the pathogenesis and pathophysiology of FPIES remain to be elucidated. Although some investigators suggest a critical role of proinflammatory cytokines such as tumor necrosis factor-α,[8] others indicate the possible role of T Helper (Th) 2 cytokine such as interleukin (IL)-5 in the pathophysiology of the disease.[9,10]

Laboratory data reflecting inflammation characteristics may be useful for studying the nature of the inflammation in the intestine. It is well known that remarkable neutrophilia is induced during oral food challenge (OFC) in patients with FPIES.[11,12] Since neutrophilia is a good marker for proinflammatory cytokine-induced inflammation,[13] this may suggest the significance of proinflammatory cytokines in its pathophysiology. In contrast, eosinophilia, which is often related to Th2-cell-mediated allergic inflammation,[14] has often been noted in patients with food protein-induced proctocolitis syndrome (FPIP),[1,15] which is another GIFA characterized by bloody stool (B). The pathophysiology of FPIES is thought to be quite different from that of FPIP because eosinophilia has not yet been noted in patients with FPIES.

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However, we previously reported that eosinophilia is often seen in patients with FPIES in Japan.[16,17] This may indicate the complexity of FPIES pathophysiology. Further information is needed to elucidate the mechanism of inflammation in this disease. In this study, the relationship of eosinophilia with symptoms and the age of onset was studied by using a large study population of Japanese infants with FPIES.

Methods

Subjects

One-hundred thirteen infants with GIFA who were referred to our institute between January 1, 2001 and May 31, 2015 were enrolled in this study (Table 1). The causative food was cow's milk (CM) in all cases. GIFA was diagnosed based on the following criteria defined by the Japanese Guidelines for Food Allergy 2014[18]: 1) development of GI symptoms without symptoms typically observed in IgE-mediated food allergy after ingestion of causative food, 2) disappearance of symptoms after discontinuing intake of causative food, 3) reproducibility of symptoms during OFC with CM formula, and 4) exclusion of other diseases such as infections, surgical problems, and so on. The reproducibility of GI symptoms by an accidental re-exposure to CM formula was considered equivalent to a positive response during planned OFC.

The original diagnostic criteria for FPIES were proposed by Powell,[2] modified by Sicherer,[12] and then further modified by Caubet *et al.* in a recent large-scale study.[19] The criteria applied in this study are consistent to those of Caubet *et al.*

The ethics committee of Shizuoka Children's Hospital approved this study. The parents provided informed consent before participating into the study. Data were collected from the medical records.

Classification of subjects

Patients with GIFA were divided into two groups based on their GI symptoms: FPIES group included patients with V and/or D, whereas FPIP group included those with B alone. Patients with FPIES were further divided into two subgroups based on the complication of B: B(+)- and B(−)-FPIES groups were defined as patients with FPIES with and without B, respectively. Patients with FPIES were also divided into the six symptom groups based on concurrent GI symptoms: 1) V–D, patients with both V and D; 2) V, patients with V alone; 3) D, patients with D alone; 4) V–D–B, patients with simultaneous V, D, and B; 5) V–B, patients with both V and B; and 6) B–D, patients with both B and D.

Patients with FPIES were also divided into two groups based on the age of onset: early- (≤10 days) and late-onset (>10 days) groups.

Table 1
Subject profile.

	Data
Number	113
Sex (M/F)	62/51
Age at onset	8 d (0 d–6 m)
GI symptoms	
V	61 (54.0%)
D	53 (46.9%)
B	68 (60.2%)
CM-sIgE (UA/ml)	<0.35 (<0.35–18.92)
Positivity in CM-sIgE	19 (16.8%)
Eo	8.0 (0.0–50.5)%
Eosinophilia (≥5%)	84 (74.3%)
CRP (mg/dL)	<0.2 (<0.2–28.24)
Positivity in ALST	94 (83.2%)

Demographic data of subjects are shown together with clinical symptoms and laboratory data. Data are presented as the mean and range for age at onset, CM-sIgE, the percentage of peripheral blood eosinophils, and serum CRP levels. The incidence of symptoms, positive results for CM-sIgE and ALST, and eosinophilia are shown as the number of subjects and percentage.
M/F, male/female; d, day; m, month; GI, gastrointestinal; V, vomiting; D, diarrhea; B, bloody stool; CM, cow's milk; sIgE, specific IgE antibody; Eo, percentage of peripheral blood eosinophil; CRP, C-reactive protein; ALST, allergen-specific lymphocyte stimulation test.

OFC

A planned OFC was performed using CM formula at the volume usually ingested by the patient. From 2008, a planned OFC was performed according to a stepwise daily incremental protocol, with an initial volume of 1 mL/kg, which was then increased to the volume usually ingested by the patient to reduce the incidence of severe reactions.[20]

Measurement of cytokines

Thirty-two subjects who visited us from March 1, 2001 to December 31, 2006 were examined for CM-specific cytokine production. Cytokine production by peripheral blood mononuclear cells (PBMCs) was measured according to previously described methods.[21] Briefly, PBMCs were resuspended at a concentration of 1×10^6 cells/mL in RPMI 1640 medium (Sigma–Aldrich Fine Chemical, Tokyo, Japan) supplemented with 10% heat-inactivated human AB serum, 2-mM glutamine, and antibiotics (100-U/mL penicillin and 100-μg/mL streptomycin). Cells were cultured for 6 days after adding 100-μg/mL α-casein (Wako Pharmaceutical, Tokyo, Japan). Lipopolysaccharide concentration was less than 100 pg/mL in the medium containing 100-μg/mL α-casein. The supernatant was stored at −70 °C after incubation for cytokine measurement.

IL-5 level in the culture supernatant was measured by using an enzyme-linked immunosorbent assay (ELISA) kit with a sensitivity of 1.0 pg/mL (Cytoscreen human IL-5, BioSource International, Camarillo, CA, USA). Interferon (IFN)-γ and IL-4 levels were similarly measured by using ELISA kits, Cytoscreen human IFN-γ (sensitivity, 1.0 pg/mL), and IL-4 US (sensitivity, 200 fg/mL). The difference between cytokine concentration in stimulated and unstimulated samples was shown as CM-specific cytokine production.

Laboratory examinations

The upper limit of the percentage of peripheral blood eosinophils (Eo) was set at 5%,[22] which is comparable with that in the United States.[23] Eosinophilia was divided into two groups by the 15% cut-off level, which is near the 75th percentile of Eo in patients with GIFA in this study (14.0%). Marked eosinophilia was defined as Eo of higher than or equal to 15%, whereas mild eosinophilia was defined as Eo from 5% to less than 15%.

CM-specific IgE (sIgE) level was measured using the ImmunoCAP system (Thermo Fisher Scientific, Tokyo, Japan). An allergen-specific lymphocyte stimulation test (ALST) was performed by using flow cytometry[7] or radioisotopes.[24] The positivity in the test was determined based on the upper limit of the normal range set for each method.

Statistical analysis

Mann–Whitney *U*-test was used to estimate significant differences, whereas the significance of the incidence was estimated using Fisher's exact test or Cochran–Armitage trend test. The significance of the correlation was calculated by using Spearman's rank correlation coefficient. All analyses were performed using STATA 13 statistical software (Light Stone Corp., Tokyo, Japan).