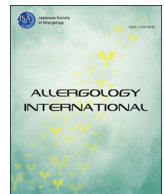




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Original article

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

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ARTICLE INFO

Article history:

Received 21 April 2016

Received in revised form

8 July 2016

Accepted 1 August 2016

Available online xxx

Keywords:

Eosinophilia

Food protein-induced enterocolitis syndrome

Food protein-induced proctocolitis

Infants

Interleukin-5

Abbreviation:

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 ($r_s = 0.60$, $p < 0.01$).

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.

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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 to 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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Peer review under responsibility of Japanese Society of Allergy.

<http://dx.doi.org/10.1016/j.alit.2016.08.003>

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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 2014¹⁸: 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.

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- μ g/mL streptomycin). Cells were cultured for 6 days after adding 100- μ g/mL α -casein (Wako Pharmaceutical, Tokyo, Japan). Lipopolysaccharide concentration was less than 100 pg/mL in the medium containing 100- μ g/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 cytometry⁷ 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).

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 ($\geq 5\%$)	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.

Results

Subject profiles

Among 113 patients with GIFA, 62 and 51 were male and female patients, respectively (Table 1). The median age at onset was 8 days, ranging from 0 day to 6 months. V, D, and B were seen in 61 (54.0%), 53 (46.9%) and 68 (60.2%) subjects, respectively. CM-sIgE and eosinophilia were seen in 19 (16.8%) and 84 (74.3%) subjects, respectively.

Eosinophilia in patients with FPIES

Among 113 GIFA patients, 94 were classified as FPIES, whereas 19 as FPIP (Table 2). Eo was increased not only in patients with FPIP (median 9.0%), but also in patients with FPIES (7.5%), and no significant differences were seen between them.

In patients with FPIES, Eo in patients with B(+)-FPIES was significantly higher than that in patients with B(–)-FPIES (10.8% vs. 6.0%, $p < 0.005$).

Among concurrent symptom groups, Eo was highest in the V–D–B group (12.9%), which was significantly higher than that in the D group (3.2%, $p < 0.005$) (Fig. 1). Eo in the V–B group (10.6%) was the second highest and significantly higher than that in the D group ($p < 0.005$). A significant difference in Eo was found between the V and D groups (7.0% vs. 3.2%, $p < 0.02$).

Correlation of eosinophilia with symptoms

The incidence of GI symptom was found to be varied with the degree of eosinophilia (Fig. 2). The incidences of V and B were increased with the degree of eosinophilia and highest in patients with marked eosinophilia. Correlations between the degree of eosinophilia and incidence of V (Cramer's $V = 0.31$, $p < 0.005$) as well as that of B (Cramer's $V = 0.34$, $p < 0.0005$) were statistically significant. In contrast, the incidence of D was not influenced by the degree of eosinophilia.

Onset age-dependent changes in Eo in patients with FPIES

Eo showed a remarkable change based on the age of onset in patients with FPIES (Fig. 3). Eo was significantly higher in the early-onset group (≤ 10 days old) (median 4.5 days) than that in the late-

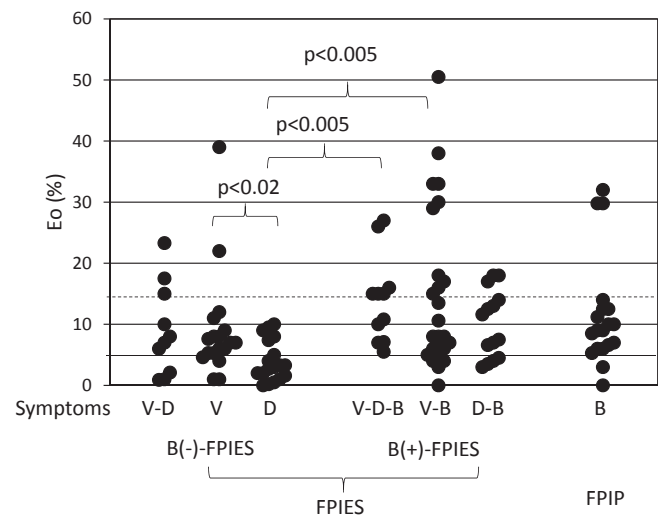


Fig. 1. Percentage of eosinophils in each symptom group. The percentage of peripheral blood eosinophils based on each symptom group is shown. Eo, percentage of peripheral blood eosinophils; FPIES, food protein-induced enterocolitis syndrome; B(+)- and B(–)-FPIES, patients with FPIES with and without bloody stool; FPIP, food protein-induced proctocolitis; V, vomiting; D, diarrhea; B, bloody stool.

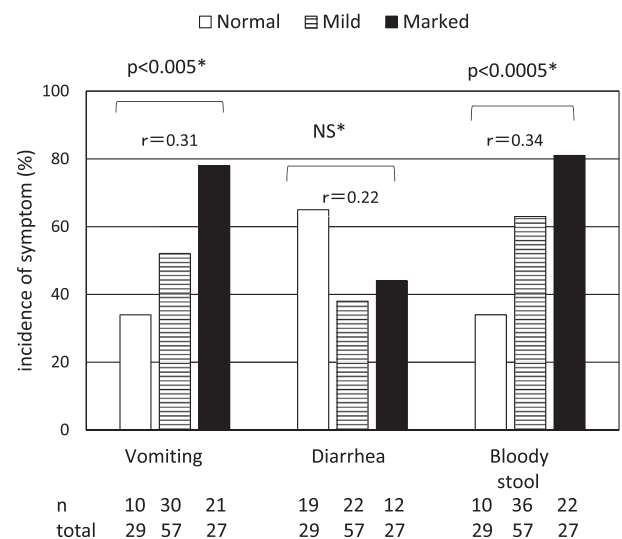


Fig. 2. Correlation of the incidence of symptom with the degree of eosinophilia. The incidence of each symptom based on the degree of eosinophilia is shown. The significance of the correlation was tested by Cochran–Armitage trend test. Normal, within normal limits (Eo < 5%); Mild, mild eosinophilia (5% ≤ Eo < 15%); Marked, marked eosinophilia (Eo ≥ 15%); n, the number of subjects with each symptom; total, total number of subjects belonging to each degree of eosinophilia.

Table 2
Eosinophilia in patients with FPIES.

Categories	Group	N	Eo
GIFA	FPIES	94	7.5 (0.0–50.5)%
	FPIP	19	9.0 (0.0–32.0)%
FPIES subgroup	B(–)-FPIES	45	6.0 (0.0–39.0)%
	B(+)-FPIES	49	10.8 (0.0–50.5)*%
Concurrent symptom group	V–D	10	7.5 (0.9–23.3)%
	V	17	7.0 (1.0–39.0)**%
	D	18	3.2 (0.0–10.0)%
	V–D–B	11	12.9 (5.5–27.0)***%
	V–B	24	10.6 (0.0–50.5)***%
	D–B	14	9.6 (3.0–18.0)%

The percentage of peripheral blood eosinophils is shown in each subject group classified by symptomatic characteristics. Data on the percentage of peripheral blood eosinophils are shown as median and range. The incidence of eosinophilia is shown as percentage.

N, number of subjects; Eo, percentage of peripheral blood eosinophil; GIFA, gastrointestinal food allergy; FPIES, food protein-induced enterocolitis syndrome; FPIP, food protein-induced proctocolitis; B(+)- and B(–)-FPIES, patients with FPIES with and without bloody stool; V, vomiting; D, diarrhea; B, bloody stool.

* Significantly higher than B(–)-FPIES ($p < 0.005$).

** Significantly higher than D ($p < 0.02$).

*** Significantly higher than D ($p < 0.005$).

onset group (> 10 days old) (median 1 month) (9.8% vs. 5.4%, $p < 0.005$) (Table 3). The incidence of marked eosinophilia in the early-onset group (35.7%) was significantly higher than that in the late-onset group (10.5%, $p < 0.01$).

Onset age-dependent changes of symptom in FPIES patients

The incidence of V was as high as 76.8% in the early-onset group of FPIES (Table 3). It decreased significantly in the late-onset group (47.4%, $p < 0.02$). The incidence of B also showed onset age-dependent changes and significantly decreased from 67.9% in the early-onset group to 28.9% in the late-onset group ($p < 0.0005$). In contrast, the incidence of D significantly increased from 42.9% in the early-onset group to 76.3% in the late-onset group ($p < 0.001$).

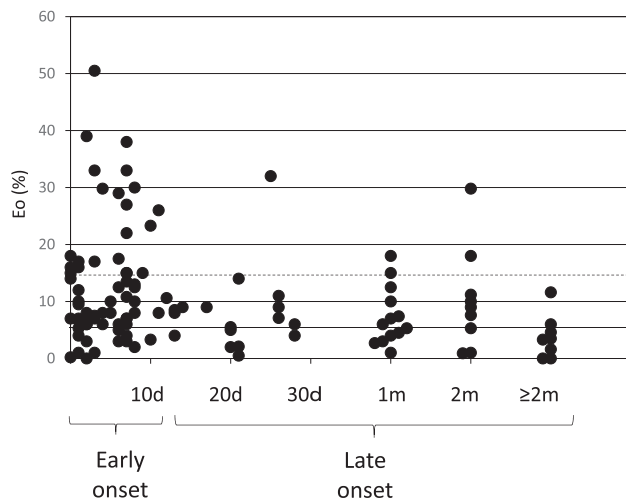


Fig. 3. Relationship of eosinophilia with age of onset. The percentage of peripheral blood eosinophils based on the age of onset is shown. Eo, percentage of peripheral blood eosinophils; d, day; m, month.

Patients with V–B were unique to the early-onset FPIES

In the early-onset FPIES group, the proportion of each concurrent symptom group ranged from 7.1% to 39.3%, among which the V–B group was the largest (Table 3). However, the proportion of the V–B group drastically decreased to 5.3% in patients with late-onset

FPIES ($p < 0.0005$). In contrast, the percentage of the D group was significantly increased from 7.1% in patients with early-onset to 36.8% in patients with late-onset ($p < 0.001$).

Cytokine production

Cytokine production was examined in 32 subjects. IL-5 level was significantly higher in the early-onset group (≤ 10 days) than that in the late-onset group (> 10 days old) (67.7 vs. 12.5 pg/mL, $p < 0.01$) (Table 4). There was a significant positive correlation between IL-5 level and Eo in patients with early-onset FPIES alone ($r_s = 0.60$, $p < 0.01$) (Fig. 4A). In this patient group, IL-5 level also showed significant positive correlations with IFN- γ level ($r_s = 0.69$, $p < 0.02$) (Fig. 4C).

In contrast, IL-5 level did not show a significant correlation with either Eo or IFN- γ level in the late-onset group (Fig. 4B, D).

Discussion

Although cell-mediated hypersensitivity is assumed to be a major allergological mechanism of FPIES, much remains to be elucidated about its pathophysiology. Powell showed an increase in the number of neutrophils during OFC.¹¹ Recently, we also demonstrated an increase in the serum C-reactive protein (CRP) level at OFC.²⁰ These findings suggest the possible role of pro-inflammatory cytokines in the pathogenesis of FPIES.⁸ In contrast, eosinophilia has seldom been noted in patients with FPIES in western countries.^{1,2,11} However, eosinophilia is not rare in patients with FPIES in Japan.^{16,17,25} In this study, we reconfirmed an

Table 3
Differences between early and late-onset FPIES.

	Number	Early onset 56	Late onset 38	Difference
Eosinophils	Eo	9.8% (0.0–50.5)	5.4% (0.0–26.0)	$p < 0.005$
	Marked eosinophilia	35.7%	10.5%	$p < 0.01$
FPIES subgroup	B(–)-FPIES	32.1%	71.1%	$p < 0.0005$
	B(+)-FPIES	67.9%	28.9%	$p < 0.0005$
Incidence of each symptom	V	76.8%	47.4%	$p < 0.02$
	D	42.9%	76.3%	$p < 0.001$
	B	67.9%	28.9%	$p < 0.0005$
Incidence of concurrent symptom group	V–D	7.1%	15.8%	NS
	V	17.9%	18.4%	NS
	D	7.1%	36.8%	$p < 0.001$
	V–D–B	12.5%	10.5%	NS
	V–B	39.3%	5.3%	$p < 0.0005$
	D–B	16.1%	13.2%	NS

The percentage of peripheral blood eosinophils and the incidence of each symptom or symptom group in the early-onset (≤ 10 days) FPIES are shown in parallel with those in the late-onset FPIES (> 10 days). Data on the percentage of peripheral blood eosinophils are shown as median and range, and other data are shown as percentage. B(+)- and B(–)-FPIES, patients with FPIES with and without bloody stool; Eo, percentage of peripheral blood eosinophil; V, vomiting; D, diarrhea; B, bloody stool.

Table 4
Cytokine production.

	Control	Whole FPIES	D-1	FPIES	D-2
				Early onset	Late onset
Number	10	32		18	14
Age at exam	3 m (0 m–7 m)	2 m (0 m–9 m)	NS	1 m (14 d–8 m)	3 m (1 m–9 m)
Time lag		1 m (12 d–9 m)		1 m (12 d–7 m)	1.5 m (14 d–5 m)
Eo (%) onset (/μl)		8.0 (1.0–50.5)		9.0 (2.0–50.5)	6.0 (1.0–20.0)
IL-5 (pg/ml)	5.4 (<3–49.3) (8)	38.3 (<3–533) (32)	$p < 0.05$	67.7 (<1.0–533) (18)	12.5 (<1.0–58.0) (14)
IFN- γ (pg/ml)	6.3 (<2.0–21.0) (10)	45.3 (<2.0–550) (19)	$p < 0.01$	45.3 (7.2–247) (11)	77.0 (1.1–550) (8)
IL-4 (fg/ml)	<200 (<200) (10)	<200 (<200–685) (19)	NS	<200 (<200–671) (11)	<200 (<200–685) (8)

The level of cytokine in the culture supernatant is compared between control subjects and patients with FPIES and between the early- (≤ 10 days) and late-onset FPIES (> 10 days). Data on age, time lag, eosinophils, and cytokine levels are shown as median and range. The number of subjects is shown at the bottom of the cytokine data. FPIES, food protein-induced enterocolitis syndrome; D-1, difference between control subjects and patients with FPIES; D-2, difference between patients with early- (≤ 10 days) and late-onset FPIES (> 10 days), NS, not significant; exam, examination; time lag, time lag between onset and examination; Eo, percentage of peripheral blood eosinophils.

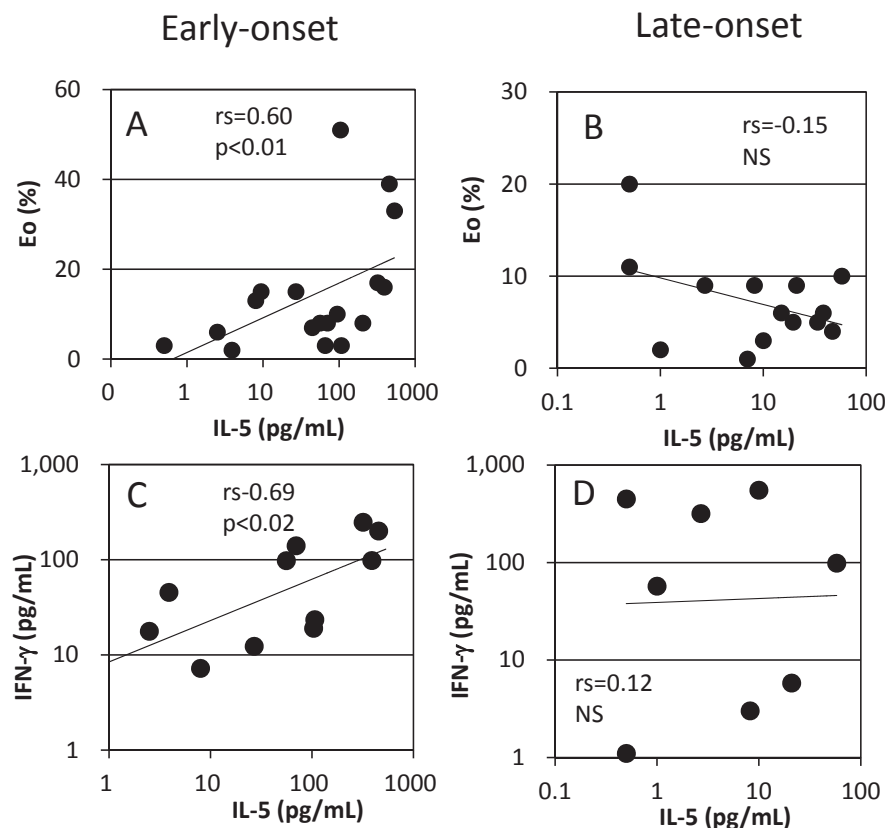


Fig. 4. Correlation of IL-5 levels with Eo and IFN-γ levels. Correlations of IL-5 level with the percentage of peripheral blood eosinophils (A, B) and IFN-γ level (C, D) are shown. Eo, percentage of peripheral blood eosinophils.

apparent increase in Eo in most patients with FPIES. Moreover, this study first demonstrated a distinctive difference in the severity of eosinophilia between the early and late-onset FPIES patients.

Eosinophils are well-known effector cells in allergic inflammation,¹⁴ wherein Th2 cytokine such as IL-5 plays a critical role.²⁶ Eosinophilia in the peripheral blood is often linked to tissue eosinophilia.²⁷ However, many eosinophils reside in the gut in a normal condition, in contrast to the bronchial tissue.²⁸ Thus, the pathological significance of eosinophilia in the gut should be considered carefully.²⁹ Eosinophils are shown to play some beneficial roles in maintaining homeostasis of the intestine.³⁰ For example, eosinophils are presumed to contribute to protection against various infectious microorganisms such as helminths, bacteria, or viruses. Eosinophils might also help in maturation of the GI system in the fetus and newborn infants, as they do in the maturation of the mammary gland.³¹

Homing of eosinophils into the gut is initiated before birth.³² Although the number of intestinal eosinophils at birth is smaller than that at adulthood, it rapidly increases after birth and reaches to the adult level within a few weeks. Investigators have reported that eosinophils in the peripheral blood correspondingly increase after birth even in healthy neonates and reach the peak level at about 2 weeks after birth.^{33,34} Although this neonatal eosinophilia persists for a few months, the severity is usually mild.

These characteristics of physiological eosinophilia seen in young infants are similar to those of eosinophilia seen in patients with late-onset FPIES in this study. Thus, eosinophilia seen in late-onset FPIES may not be a specific finding to food allergy but a physiological phenomenon. However, the fact that most infants with FPIES develop GI symptoms within a few months after birth, which

corresponds to the period of physiological eosinophilia in young infants, indicates that the immaturity of the gut characterized by mild eosinophilia may underlie the development of infantile FPIES. Further investigations are needed on the relationship between eosinophilia in young infants and the development of FPIES.

Conspicuous eosinophilia in patients with early-onset FPIES; however, it should be distinguished from mild eosinophilia seen in patients with late-onset FPIES because of its severity. To our knowledge, such definite eosinophilia has never been reported previously. It is widely accepted that IL-5, a representative Th2 cytokine, is essential for an increase in eosinophil count.²⁶ In order to confirm the role of IL-5, cytokine profile was analyzed in this study. As a result, IL-5 level in patients with early-onset FPIES was shown to be significantly higher than that in patients with late-onset FPIES (Table 4). Because a significant positive correlation was seen between IL-5 levels and Eo in patients with early-onset FPIES (Fig. 4A), IL-5 is suggested to play a critical role in the induction of marked eosinophilia in those patients.

In this study, the age of cytokine measurement in the early-onset FPIES group is significantly younger than that in the late-onset group (Table 4). However, IL-5 production did not show any clear correlation with the age of examination (data not shown). Indeed, although IL-5 level was measured after 1 month of age in 11 patients in the early-onset group (median, 2 months, range 1 month–8 months), their IL-5 level (median, 56.0 pg/mL, range 3.9–455 pg/mL) was significantly higher than that in the late-onset group ($p < 0.02$) despite the age of examination not being different between the groups. Thus, remarkable increases in IL-5 levels in patients with early-onset FPIES cannot be attributed to the age of examination, but rather to the age of onset.

IFN- γ is a representative Th1 cytokine. In the mature acquired immune system, Th1 and Th2 cells oppose each other, so that Th1 and Th2 cytokines cannot be positively correlated.³⁵ However, IL-5 level showed a significant positive correlation with IFN- γ level in patients with early-onset FPIES. This may be explained by the immaturity of T cells in those subjects. It is shown that T cells are immature at birth, thus producing both Th1 and Th2 cytokines.³⁶ Th2 cytokine levels decrease and Th1 cytokines become dominant after birth.³⁷ The disturbance of the shift to Th1 dominant immunity is presumed to be the cause of atopic disorders.³⁸

The shift to Th1 dominance is shown to be initiated as early as a week after birth.³⁹ In this study, cytokine production in patients with early-onset FPIES was examined at approximately 1–2 months of age. Because a remarkable increase in IL-5 level and a clear positive correlation with IFN- γ level can be seen at the time of examination, the immaturity of the immune system in patients with early-onset FPIES seems robust and persists at least a few months after birth. Thus, such a delay in the shift to the Th1 dominant immunity might be an essential defect in patients with early-onset FPIES.

It is widely accepted that eosinophilia is associated with the development of B.^{1,15} In this study, we first demonstrated that the incidence of V also shows a significant positive correlation with the degree of eosinophilia. As a result, approximately 40% of patients in the early-onset FPIES group show V and B simultaneously. So far, the rationale for increased incidence of V in patients with marked eosinophilia remains unknown. However, if the eosinophil counts increased in the upper GI tract in patients with marked eosinophilia, an increased sensitivity of the upper GI tract against the invasion of allergic food might be explained.

It is also shown that many patients showed B and eosinophilia in the early-onset group, which resembles to patients with FPIP. Thus, B in the early onset FPIES might share some common pathophysiology with that in FPIP. However, the age of onset in early-onset FPIES is significantly earlier than that in FPIP (4.5 days (0–10 days) vs. 21 days (1 day–4 months), $p < 0.001$). Although the cause of such a remarkable difference in the onset age is so far unknown, some additional factors may participate in the development of B in the early-onset FPIES.

In contrast to V and B, any positive correlation was not shown between eosinophilia and the incidence of D. It is also interesting that Eo is the lowest in patients with D alone, which is significantly lower than that in patients with V alone (Table 2). The incidence of D is higher in the late-onset FPIES group than in the early-onset FPIES group, which is opposite to the incidence of V or B. These findings may indicate that, although both V and D are representative symptoms of FPIES, the pathophysiology of D is not the same as that of V in young infants. To elucidate the specific mechanism and pathology for the development of D as well as V may be crucial for comprehensive understanding of FPIES.

Acknowledgements

This study was partly supported by grants from the Shizuoka Prefectural Hospital Organization.

Conflict of interest

The authors have no conflict of interest to declare.

Author's contributions

MK designed the study and wrote the manuscript. MS, HM, and TM contributed to data collection. SS provided critique for revision.

All authors read and approved the final manuscript.

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