Original Article

Serum C-reactive protein in food protein-induced enterocolitis syndrome versus food protein-induced proctocolitis in Japan

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Abstract

Background: Some infants with food protein-induced enterocolitis syndrome (FPIES) have increased serum C-reactive protein (CRP) and fever in Japan. The aim of this study was therefore to clarify and compare the incidence of this in patients with FPIES versus patients with food protein-induced proctocolitis (FPIP).

Methods: One hundred and sixteen infants with non-IgE-mediated gastrointestinal food allergies were enrolled in this study and classified into three phenotypes: FPIES presenting with vomiting and/or diarrhea (n = 47); FPIP with bloody stool alone (n = 19); and the mixed phenotype (MP), bloody stool with vomiting and/or diarrhea (n = 50).

Results: Serum CRP was increased in 55.3% of the FPIES group, similar to that in the MP group (54.0%), and significantly higher than in the FPIP group (15.8%; \(P < 0.01\)). Fever was observed in 29.8% of the FPIES group, significantly higher than in the MP group (8.0%; \(P < 0.01\)) and in the FPIP group (0%; \(P < 0.05\)). Patients with fever had significantly higher serum CRP than patients without fever (median, 12.8 vs <0.2 mg/dL, \(P < 0.00001\)).

Conclusions: Serum CRP was significantly higher in the FPIES group than in the FPIP group. This suggests that serum CRP is a useful marker for differentiating the pathogenesis of FPIES from FPIP. From the perspective of serum CRP, the pathology of the intestinal inflammation in MP subjects is suggested to be similar to that of FPIES.

Key words

C-reactive protein, cow’s milk, fever, food protein-induced enterocolitis syndrome, food protein-induced proctocolitis.

It is well-known that, in addition to immunoglobulin (Ig)E-mediated immediate allergic reactions, food causes gastrointestinal (GI) symptoms via cell-mediated hypersensitivity. This has been termed “non-IgE-mediated gastrointestinal food allergy” (GIA).1 GIA consists of food protein-induced enterocolitis syndrome (FPIES), which causes vomiting (V) and/or diarrhea (D), and food protein-induced proctocolitis (FPIP), which produces bloody stool alone (B). The pathology of the intestinal lesion in FPIES and FPIP is thought to be different. Although these two conditions have been studied separately in Western countries, they are usually treated as one ailment in Japan under the name of “neonatal and infantile gastrointestinal allergy” (NIGIA).2 NIGIA contains another phenotype with a mix of symptomatic features of FPIES and FPIP, namely B in addition to V and/or D.3,4

While the difference in the symptoms may reflect the difference in the pathogenesis of those phenotypes, little is known about the pathogenesis of this disease. Laboratory tests are often useful for studying the mechanism of the diseases. Powell observed an increase in the absolute neutrophil count in patients with FPIES on oral food challenge test (OFC).5 This may provide insight into the nature of the inflammation that causes FPIES and is consistent with a study that reported an increase in the production of tumor necrosis factor (TNF)-\(\alpha\), a representative pro-inflammatory cytokine.6 Serum C-reactive protein (CRP), however, which is also a good indicator of inflammation caused by pro-inflammatory cytokines, was not examined. In Japan, many studies report on increased serum CRP 7,8 and fever.9 The combination of fever and highly increased serum CRP has been referred to as the sepsis-like phenotype of FPIES.9 Given that fever and increased serum CRP can be reproduced on OFC in patients with FPIES,10 it is suggested that this is caused by FPIES itself, but not by complications such as infectious disorders.

Thus far, increased serum CRP and fever in FPIES patients has not been studied well. The aim of the present study was therefore to clarify and compare the incidence of increased CRP and fever in infants with FPIES and with other phenotypes.

Methods

Subjects

One hundred and sixteen infants with NIGIA who were referred between 1 January 2001 and 31 May 2015, were enrolled in this study (Table 1). The causative food was cow’s milk (CM) in all cases. NIGIA was diagnosed according to the following criteria, corresponding to those of the Japanese Guidelines for Food Allergy and Clinical Immunology, Shizuoka Children’s Hospital, Urushiya 860, Aoi-ku, Shizuoka City, Shizuoka 420-0953, Japan. Email: mitsuaki-kimura@i.shizuoka-pho.jp

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Table 1  Subject profile

|                     | Median (range) or n (%) |
|---------------------|-------------------------|
| n                   | 116                     |
| M : F               | 62:54                   |
| Age at onset        | 8 days (0 days–6 months) |
| Symptoms            |                         |
| V                   | 64 (55.2)               |
| D                   | 53 (45.7)               |
| B                   | 69 (59.5)               |
| Fever               | 18 (15.5)               |
| Systemic antibiotics| 22 (19.0)               |
| CM-sIgE (kU/L)      | <0.35 (<0.35–18.9)      |
| Undetectable CM-sIgE| 97 (83.6)               |
| WBC (/mL)           | 14 260 (6500–64 540)    |
| CRP (mg/dL)         | <0.2 (<0.2–28.2)        |
| Increased CRP       | 56 (48.3)               |
| (≥0.2 mg/dL)        |                         |
| ALST positive       | 98 (84.5)               |

ALST, allergen-specific lymphocyte stimulation test; B, bloody stool; CM, cow’s milk; CRP, C-reactive protein; D, diarrhea; sIgE, specific IgE antibody; V, vomiting; WBC, white blood cells.

Allergy 2014: (i) development of GI symptoms without symptoms typically observed in IgE-mediated immediate allergic diseases after ingestion of the causative food; (ii) disappearance of symptoms after discontinuation of the causative food; (iii) reproduction of symptoms on OFC with CM formula; and (iv) exclusion of other diseases such as infections, surgical problems, and so on. The reproduction of GI symptoms on accidental re-exposure to CM formula was considered to be equivalent to a positive response at planned OFC.

The original diagnostic criteria for FPIES were proposed by Powell,11 modified by Sicherer et al.,12 and then further modified by Caubet et al. in a recent large-scale study.13 The criteria used in this study are consistent with those of Caubet et al.

Subjects were divided into three groups according to GI symptoms: FPIES with V and/or D; FPIP with B alone; and mixed phenotype (MP), B as well as V and/or D.

Data were collected from the medical records. Informed consent was obtained from the parents before the study, which was approved by the ethics committee of Shizuoka Children’s Hospital.

Oral food challenge

Planned OFC was performed using CM formula at the volume usually ingested by the patient. From 2008, planned OFC was performed daily according to a stepwise incremental protocol with an initial volume of 1 mL/kg, which then increased to the volume usually ingested by the patient in order to reduce the incidence of severe reactions.10

Laboratory tests

Serum total IgE and CM-specific IgE (sIgE) were measured using the ImmunoCAP system (Thermo Fisher Scientific, Tokyo, Japan). Allergen-specific lymphocyte stimulation test (ALST) was performed with flow cytometry or radioisotopes.14 A positive result depended on the upper limit of the normal range set by each method.

Statistical analysis

Mann–Whitney U-test was used to estimate the significance of differences, while the significance of the incidence was estimated using Fisher’s exact test. The accuracy for discriminating subjects with fever was analyzed using receiver operating characteristics (ROC) curve and expressed as the area under the curve (AUC). All analyses were performed using STATA 13 (Light Stone, Tokyo, Japan).

Results

Subject profile

One hundred and sixteen patients were enrolled in this study (Table 1). There were no significant differences in the sex ratio. Median age at onset was 8 days. CM-sIgE was negative in 97 patients (83.6%). Fever and increased CRP at onset were seen in 18 (15.5%) and in 56 subjects (48.3%), respectively. ALST was positive in 98 (84.5%). With regard to disease type, 47, 19, and 50 subjects were classified into FPIES, FPIP, and MP, respectively (Table 2). Age at onset was significantly earlier in patients with MP (median, 6.5 days) than in those with FPIES (median, 20 days; P < 0.001) or with FPIP (median, 21 days; P < 0.05).

CRP: FPIES vs FPIP

Serum CRP was significantly higher in the FPIES group than in the FPIP group (median, 0.41 vs <0.2 mg/dL; P < 0.01; Table 2). The proportion of patients with increased CRP (≥ 0.2 mg/dL) was also significantly higher in the FPIES group than in the FPIP group (55.3% vs 15.8%; P < 0.01).

Serum CRP in the FPIES group was highest in patients with both V and D (median, 2.12 mg/dL). This was significantly higher than serum CRP in patients with V alone, which was lowest in the FPIES group (median, <0.2 mg/dL; P < 0.02; Fig. 1).

CRP: FPIES vs MP

There were no significant differences in serum CRP between the FPIES and the MP groups (median, 0.41 vs 0.2 mg/dL) or the percentage of patients with increased CRP (≥ 0.2 mg/dL; 55.3% vs 54.0%; Table 2). The percentage of patients with considerably increased CRP (≥ 3 mg/dL), however, was significantly higher in the FPIES group than in the MP group (31.9% vs 10.0%; P < 0.05; Table 2).

CRP: FPIP vs MP

Serum CRP (median, 0.2 vs <0.2 mg/dL; P < 0.01) and the percentage patients with increased serum CRP (54.0% vs...
15.8%; \( P < 0.01 \) were significantly higher in the MP group than in the FPIP group.

### Characteristics of patients with fever

Fever developed in 18 patients, but was absent in 98 patients. Serum CRP was significantly higher in patients with fever than in those without fever (median, 12.8 vs \(<0.2 \text{ mg/dL}; P < 0.00001; \) Table 3). The prevalence of antibiotic treatment was also significantly higher in those with fever than in those without fever (100% vs 4.1%; \( P < 0.00001 \)). White blood cell (WBC) count was significantly higher in febrile patients than in afebrile patients (17 765 vs 13 800/\( l \); \( P < 0.005 \)). There were no significant differences in sex ratio, age at onset, C-Ms IgE, or ALST between febrile and afebrile subjects.

The prevalence of fever was significantly higher in the FPIES group (29.8%) than in the FPIP group (0%; \( P < 0.05 \)) or in the MP group (8.0%; \( P < 0.01 \); Table 2). There was no significant difference in the prevalence of fever between the FPIP and the MP groups.

### Serum CRP to discriminate patients with fever

Patients with fever were distinguished from those without fever by serum CRP level with high precision. The AUC of the ROC curve was 0.983 (Fig. 2). The OR was highest for serum CRP \( 3 \text{ mg/dL}: \) the sensitivity and the specificity at this level were 85.0% (17/20) and 99.0% (95/96), respectively.

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abnormal laboratory finding associated with FPIES may be a useful tool for the study of the pathogenesis of FPIES. Neutrophilia was noted as a characteristic of FPIES, while eosinophilia is often described in FPIP. In addition to these findings, an increase in serum CRP and fever in patients with NIGIA has also been demonstrated. Given that fever and an increase in serum CRP can be reproduced on OFC in patients with FPIES, it is suggested to be the phenomenon derived from FPIES itself, but not from other complicated disease such as infections. Further studies, however, are needed to clarify the exact mechanisms involved. The increase in serum CRP at OFC is closely correlated with that at onset. This suggests that serum CRP at onset may reflect the intensity of the intestinal inflammation in FPIES patients. In order to further clarify the significance of the increased serum CRP and fever, we analyzed the incidence and degree of these findings at onset in a large population of Japanese infants with FPIES and compared them for other phenotypes of the disease.

This study has demonstrated a clear increase in serum CRP level in FPIES at onset. Increased serum CRP has not yet been reported from Western countries in either early studies or recent studies including a large number of subjects. An increase in the serum CRP, however, might not be unexpected given that the increased production of a representative proinflammatory cytokine TNF-α was noted in patients with FPIES. Although the regulation of inflammation is very complex, increased TNF-α production may be caused by interferon (IFN)-γ, which is produced by type 1 T-helper cells (Th1). IFN-γ activates monocytes and induces the production of proinflammatory cytokines such as TNF-α, IL-6 and IL-1β. These cytokines have been shown to play a critical role in inducing neutrophilia, as well as increasing serum CRP. Neutrophilia is often accompanied by an increase in serum CRP in patients with various inflammatory disorders.

We also reported the development of fever in patients with FPIES with considerably increased CRP (≥3 mg/dL), which could be reproduced on OFC. In this study, we reconfirmed a close correlation between fever and serum CRP level. Fever was eventually limited to patients with FPIES with considerably increased CRP (≥3 mg/dL), which has been called the sepsis-like phenotype of FPIES in Japan. Clinically, this provides an important suggestion about the management of FPIES in the acute phase. If physicians see neonates or young infants with fever and markedly increased serum CRP, they must assume severe systemic bacterial infection such as sepsis or meningitis. This can sometimes lead to serious outcomes such as death or severe sequelae. Multiple examinations including invasive procedures such as spinal tap, and systemic antibiotic treatment are needed for the management of those infants.

All patients with the sepsis-like phenotype of FPIES received systemic antibiotics in this study. This should be mandatory as an empiric countermeasure for severe systemic bacterial infection in neonates and young infants with fever and high serum CRP. But, as indicated by the present results, physicians must be mindful of the fact that some patients with FPIES could show similar findings. If systemic antibiotic

| Table 3 | Patient characteristics vs presence of fever |
|---------|------------------------------------------|
|         | Fever (n) | Without fever (n) | P       |
| n       |           | Median (range)    | Median (range)    |       |
| M : F   | 18        | 98                | NS               |
| CRP (mg/dL) | 12:6   | 50:48             | <0.00001         |
| Considerably increased CRP (≥3 mg/dL) | 17 (94.4) | 3 (3.1) | <0.00001 |
| WBC (µL) | 17 765 (6500–65 540) | 13 800 (6500–32 900) | <0.005 |
| Age at onset | 7.5 days (1 day–2 months) | 8 days (0 days–6 months) | NS |
| Systemic antibiotics administered | 18 (100) | 4 (4.1) | <0.00001 |
| CM-sIgE | <0.35 (<0.35–8.33) | <0.35 (0.35–18.9) | NS |
| ALST positive | 18 (100) | 80 (81.6) | NS |

ALST, allergen-specific lymphocyte stimulation test; CM, cow’s milk; CRP, C-reactive protein; sIgE, specific IgE antibody; WBC, white blood cells.

Fig. 2 Receiver operating characteristic (ROC) curves for serum C-reactive protein (CRP) and white blood cell (WBC) count to distinguish patients with fever. Area under the curve: CRP, 0.983; WBC count, 0.717. FPF, false-positive fraction; TPF, true-positive fraction.

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treatment is ineffective, FPIES should then be considered as a possible cause. Judgment could easily be made given that symptoms and abnormal laboratory findings would improve soon after discontinuation of CM formula and be completely resolved within a few days in the case of FPIES. FPIP is also included in GIA, as is FPIES. In addition to the difference in the symptoms, eosinophilia is noted specifically in patients with FPIP, suggesting that a Th2 cytokine such as IL-5 participates in the development of FPIP. Given that Th2-skewed immune response is thought to be incompatible with Th1-skewed immune response, in which pro-inflammatory cytokines play a pivotal role, it is expected that serum CRP does not increase in patients with FPIP. In the present study, serum CRP was directly compared between the FPIES and FPIP groups, and was found to be significantly lower in the FPIP group compared with the FPIES group. No patients with FPIP had considerably increased CRP (≥3 mg/dL). Correspondingly, no patients with FPIP had fever. Thus, the supposition of the difference in the pathogenesis between FPIES an FPIP is again supported by the present results.

In Japan, approximately one-third of patients with NIGIA are classified into the MP group. According to symptoms, FPIES and FPIP seem to coexist in patients with MP. The immunopathology assumed for FPIES and FPIP, however, is thought to be incompatible. It is important for the improved understanding of the disease to clarify the major mechanism in patients with MP. The present MP serum CRP level was similar to that of the FPIES group, but not to that of the FPIP group. Fever was also observed in patients with MP, although the incidence was lower than that in the FPIES group. This suggests that pro-inflammatory cytokines play an important role in the development of the intestinal inflammation in MP, in a similar way to that observed in FPIES. It remains to be clarified how FPIP-like symptoms, namely B, develop in those patients. Given that age at onset was lower in the MP group than in the other disease types, this relative immaturity of the immune system might facilitate the transient coexistence of Th1- and Th2-skewed immunopathology, which, ordinarily, are incompatible.

There is no clear definition about whether patients with both B and D should be separated from patients with FPIP, who have B alone. In this study, symptoms of FPIP were limited to B alone. Patients with both B and D were classified as having MP. This is reasonable because patients with both B and D had increased serum CRP, similar to those with D only. This is a sharp contrast to the FPIP group. This suggests that the pathogenesis of patients with both B and D more closely resembles that of FPIES as opposed to that of FPIP. Thus, from the perspective of CRP level, patients with both B and D should be categorized into the MP group separately from those patients with B alone, who belong to the FPIP group.

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Disclosure

The authors declare no conflict of interest.

Author contributions

K.M. designed the study and wrote the manuscript; S.M., M.H., and M.T. collected data; S.S. critically reviewed the manuscript. All authors read and approved the final manuscript.

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