Increased C-reactive protein and fever in Japanese infants with food protein-induced enterocolitis syndrome

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Abstract Background: Increased C-reactive protein (CRP) and fever are observed in some infants with food protein-induced enterocolitis syndrome (FPIES) in Japan, but the reproducibility of these findings has not yet been confirmed on oral food challenge (OFC).

Methods: Fourteen infants with FPIES induced by cow’s milk (CM) formula were enrolled. OFC using CM formula was performed on each infant once or repeatedly (total 18 tests), with a stepwise incremental protocol in an infection-controlled setting. CRP was measured 24 h after the last ingestion of the CM formula.

Results: Increased CRP was observed in 11 of the 18 OFC conducted (median, 2.60 mg/dL; range, 0.22–4.84 mg/dL). Fever was induced in six occasions during OFC. Serum CRP in the patients with fever increased to median 3.76 mg/dL (range, <0.7–4.84 mg/dL), which was significantly higher than that of the patients without fever (median <0.1 mg/dL; range, <0.1–2.6 mg/dL; P < 0.001). CRP during OFC significantly correlated with that at disease onset (rs = 0.62, P < 0.02). Three of the four patients with fever at disease onset also had fever during OFC.

Conclusions: Increased CRP and fever are reproducible during OFC in some infants with FPIES, suggesting that these are not accidental phenomena, but instead are associated with FPIES itself in Japanese patients.

Key words cow’s milk, C-reactive protein, fever, food protein-induced enterocolitis syndrome, oral food challenge test.

Food protein-induced enterocolitis syndrome (FPIES) is a cell-mediated allergic disorder that involves gastrointestinal (GI) symptoms such as vomiting and diarrhea. With regard to laboratory data, Powell demonstrated a definite increase in neutrophil count during oral food challenge (OFC). This is reasonable given that intestinal inflammation is the pathology of the disease, but neither an increase in C-reactive protein (CRP), a sensitive and representative marker of inflammation, nor fever, a well-known symptom of inflammatory disorders, was noted, although some patients had a septicemia-like presentation. In contrast, these findings are not rare in infants with FPIES in Japan. It is not easy, however, to conclude that these findings are relevant to FPIES because they are commonly observed in various inflammatory disorders, especially infection. Further studies to rule out the complications of other diseases are needed for these findings to be accepted as FPIES-specific observations.

The aim of this study was therefore to confirm the reproducibility of increased CRP and fever at OFC for FPIES, performed in an infection-controlled environment.

Methods

Subjects

Fourteen infants with FPIES who were referred between 1 January 2008 and 31 December 2014, were enrolled in this retrospective study (Table 1). The diagnosis of FPIES was based on the following criteria proposed by Powell: (i) development of GI symptoms after ingestion of the causative food; (ii) disappearance of symptoms after discontinuation of causative food; (iii) exclusion of other disorders that could cause GI symptoms, such as infection or surgical problems; and (iv) recurrence of GI symptoms during OFC without immunoglobulin E (IgE)-mediated allergic symptoms, such as skin rash, hives, respiratory symptoms, or anaphylaxis. Subjects with food protein-induced-proctocolitis (FPIP), who had bloody stool alone, were excluded because of the immunopathological difference between FPIP and FPIES.

Data were collected from OFC records. Informed consent was obtained from each infant’s parents prior to examination, and the study was approved by the ethics committee of Shizuoka Children’s Hospital.

Infection control during OFC

The OFC were performed in the hospital ward, where full attention was paid to the prevention of nosocomial infection. Patients with confirmed or suspected infectious disease were isolated in the special rooms for infection control. All medical and nursing staff were always encouraged to adhere to standard precautionary measures.
including hand hygiene and personal protective equipment such as masks and plastic gloves. The OFC was postponed, if the patient had contact with any individual before admission with suspected infection, for the duration of the incubation period of the disease.

**OFC**

Each patient underwent OFC for the diagnosis of FPIES or the estimation of acquisition of tolerance. OFC was performed using cow’s milk (CM) formula according to a stepwise incremental protocol to reduce the incidence of severe reaction. All patients were confirmed as having no acute or chronic inflammatory disorders prior to participation.

Patients were given standard CM formula 1 mL/kg (up to 10 mL) once a day at 10:00 am on the first day of the OFC. If no symptoms were induced by the first ingestion of CM formula, they were given 5 mL/kg (up to 50 mL) of CM formula once at 10:00 am on the next day. If the patients remained asymptomatic, they were allowed to ingest CM formula at the volume they usually ingested (up to 200 mL), once at 10:00 am on the third day.

If any GI symptoms occurred after the ingestion of CM formula, the OFC was discontinued, and the patient was kept in the inpatient ward until the symptoms completely subsided, usually for 1 day. The time of symptom occurrence was calculated as the period between the last ingestion of CM formula and the appearance of symptoms.

**Laboratory tests**

Serum CRP was measured before the OFC and 24 h after the last ingestion of CM formula. Serum total IgE and CM-specific IgE were measured using the ImmunoCAP system (Thermo Fisher Scientific, Tokyo, Japan).

**Statistical analysis**

The significance of differences was estimated using Mann–Whitney U-test, while the significance of the correlation was analyzed using Spearman rank correlation coefficient test. The significance of the incidence was estimated using Fisher’s exact test. All analyses were performed using STATA 12 (Light Stone, Tokyo, Japan).

### Results

#### Subject profile

The subjects included 14 patients (10 boys, four girls) with a median age of 10.5 days at FPIES onset (Table 1). The subjects underwent OFC at a median 6 months of age (Table 2). Three patients underwent OFC twice or more (Table 3), while the other 11 took the test once. Serum CM-IgE was <0.35 UA/mL in 80% of patients at disease onset and in 85% at OFC. Serum CRP before OFC was within the normal range in all patients (Table 2).

Vomiting and diarrhea were observed in 64.3% of patients at disease onset, and fever was observed in 28.6% of patients (Table 1). At OFC, vomiting and diarrhea were observed in 66.7% and in 55.6% of patients, respectively, and fever in 33.3% of patients (Table 2). GI symptoms developed during OFC within 2 h after ingestion of CM formula in approximately half of all tests performed (8/18), and within 4 h in more than two-thirds of all tests performed (13/18; Table 3).

#### Fever during OFC

Fever was observed in patients 1–3 at the first OFC (O-1-1, O-2, O-3-1; Table 3), and all of these patients also had fever at disease onset. Although OFC were repeated in two of three patients (patients 1, 3), fever was always observed at all follow-up OFC (O-1-2, O-3-2, O-3-3; Fig. 1a). Fever manifested at approximately 6 h after the ingestion of CM formula and subsided within 24 h.

#### CRP course during OFC

Chronologic change in serum CRP was examined in five cases (Fig. 1b). Serum CRP was within the normal range in all patients before OFC (Table 2). In contrast to the trend observed for fever induction, CRP did not increase within 6 h after ingestion of CM formula. CRP increased 24 h after ingestion of CM formula and decreased thereafter. On the basis of this observation, CRP at 24 h after the ingestion of CM formula was analyzed.

### Table 1 Subject profile at onset

| Category             | Data Mean (range) or n (%) |
|----------------------|----------------------------|
| Demographic data     | n 14                       |
| Sex (M/F)            | 10/4                       |
| Gestational age (weeks) | 37.5 (33–39)             |
| Birth weight (g)     | 2690 (2430–3094)           |
| Age at onset         | 10.5 days (1 day–6 months) |
| Laboratory data      | Total IgE (IU/mL) 5.72 (<2.0–126.0) |
|                      | CM-IgE (UA/mL) <0.35 (<0.35–1.74) |
|                      | CRP (mg/dL) 0.32 (<0.10–12.1) |
| Symptoms             | Vomiting 9/14 (64.3)       |
|                      | Diarrhea 9/14 (64.3)       |
|                      | Fever 4/14 (28.6)          |

**CM, cow’s milk; CRP, C-reactive protein; IgE, immunoglobulin E.**

### Table 2 OFC results

| Category             | Data Median (range) or n (%) |
|----------------------|-------------------------------|
| Basic data           | No. OFC 18                    |
| Age at OFC           | 6 months (8 days–20 months)   |
| Total IgE (IU/mL)    | 7.67 (<2.0–156.0)             |
| CM-IgE (UA/mL)       | <0.35 (<0.35–4.62)            |
| Induced symptoms     | Vomiting 12/18 (66.7)         |
|                      | Diarrhea 10/18 (55.6)         |
|                      | Fever 6/18 (33.3)             |
| CRP                  | Before (mg/dL) <0.10          |
|                      | After (mg/dL)                 |
|                      | Whole 0.27 (<0.10–4.84)       |
|                      | >2.0 mg/dL 6/18 (33.3)        |
|                      | 0.1–2.0 mg/dL 5/18 (27.8)     |
|                      | <0.1 mg/dL 7/18 (38.9)        |

**CM, cow milk, CRP, C-reactive protein; IgE, immunoglobulin E; OFC, oral food challenge.**
Increase in CRP

C-reactive protein increased during 11 of 18 OFC (61.1%), and was >2 mg/dL in six cases (Table 2). Serum CRP increased to median 3.76 mg/dL (range, 0.7–4.84 mg/dL) in patients with fever, which was significantly higher than in those without fever (median <0.1 mg/dL; range, <0.1–2.6 mg/dL; P < 0.001).

Reproducibility of the increase in CRP was examined in patients 1, 3, and 9, who underwent repeated OFC to estimate the acquisition of tolerance. Two patients (patients 1, 3) who had distinct increase in CRP to >2 mg/dL at first OFC, also had similar marked increase in CRP at the second OFC (Fig. 2). One patient (no. 9) who had a minor increase in CRP level to <1 mg/dL at the first OFC had a similar minor increase in the second OFC.

To further analyze the reproducibility of the increase in CRP, increased CRP during OFC were compared with that at disease onset, and a significant positive correlation was found (rs = 0.62, P < 0.02; Fig. 3).

Discussion

FPIES is considered to be a cell-mediated food allergy manifesting as GI symptoms.1–3 During the last 10 years, many new cases have been reported from various countries.11–18 Although a distinctive increase in neutrophils, an indicator of inflammation, has been
noted in patients with FPIES, neither increase in serum CRP nor fever, another well-known marker of inflammation, has been noted in any of the studies. To our knowledge, these findings were described in only one case of FPIES from Taiwan. Recently, another patient with FPIES from Korea was described as having mild fever.

In contrast, a considerable number of patients with FPIES with increased CRP and fever have been reported in Japan. Because these patients are often seriously ill, they are categorized as having the sepsis-like phenotype, but neither increased CRP nor fever are currently accepted as being associated with FPIES. In this study, we first confirmed that increased CRP and fever can be reproduced in patients with sepsis-like FPIES phenotype at OFC. This suggests that these findings are not accidental phenomena but are relevant to FPIES.

It is very important to rule out infectious diseases in the process of diagnosing FPIES, but, when patients develop increased CRP and fever, it is impossible to completely rule out infectious disease at onset, even though many tests are performed as possible. To solve this problem, we performed OFC in a setting in which the infectious diseases were strictly controlled. Patient contact with sick children was also checked before OFC. Thus, the possibility of complications due to infections would have been very rare in this study, suggesting that the increased CRP and fever reproduced in patients with FPIES are the results of the pathology of FPIES rather than of infection.

FPIES is considered a cell-mediated food allergy. Indeed, the responsiveness of T cells to CM protein stimulation is increased in patients with FPIES. Thus, from the viewpoint of the pathological features of allergy, FPIES resembles contact dermatitis, which is widely accepted as a typical cell-mediated allergic disorder. Although fever or increased serum CRP is not observed under ordinary conditions in patients with contact dermatitis, both can be caused under an extraordinary condition, wherein a causative allergen is administered systemically. Increases in serum concentration of various inflammatory cytokines such as interleukin (IL)-1β, tumor necrosis factor (TNF)-α and IL-6 have been noted.

The present results indicate that the systemic administration of antigens, which ordinarily interact on the specific organ and cause topical cell-mediated allergic reactions, can induce systemic reactions such as increased serum CRP and fever. Food protein molecules with antigenicity are known to be transferred into the bloodstream in infants. The amount of transferred antigen has been shown to be increased in patients with allergic disorders. Although intestinal permeability in FPIES patients has not yet been examined, it might be increased because of the damage to the mucosal barrier due to intestinal inflammation. Thus, the question of whether the transport of CM protein macromolecules into the body is related to the development of systemic reactions, such as increased serum CRP and fever, in patients with FPIES with a sepsis-like phenotype, is of interest.

Both increased CRP production and fever are caused by pro-inflammatory cytokines such as IL 1β, TNF-α, and IL-6. IL-6 is suggested to play a critical role in the induction of fever and in the synthesis of CRP by hepatocytes. The production of IL-6 by peripheral blood lymphocytes stimulated by CM proteins has been shown to be increased, as was that of other pro-inflammatory cytokines, in patients with FPIES.

So far, however, no information is available on serum pro-inflammatory cytokine level in patients with FPIES with sepsis-like phenotype. Precise analysis of the relationship between serum pro-inflammatory cytokine level and the development of systemic reactions, such as increased serum CRP and fever, might be useful for clarifying the mechanism of septicemia-like symptoms.

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Disclosure

The authors declare no conflicts of interest.

References

1. Sicherer SH, Sampson HA. Food allergy. J Allergy Clin Immunol. 2010; 125 (2 Suppl 2): S116–25.
2. Powell GK. Milk- and soy-induced enterocolitis of infancy. Clinical features and standardization of challenge. J Pediatr. 1978; 93: 553–60.
3. Sicherer SH, Eigenmann PA, Sampson HA. Clinical features of food protein-induced enterocolitis syndrome. J Pediatr. 1998; 133: 214–9.
4. Gryboski JD. Gastrointestinal milk allergy in infants. Pediatrics 1967; 40: 354–62.
5. Fontaine JL, Navarro J. Small intestinal biopsy in cow’s milk protein allergy in infancy. Arch. Dis. Child. 1975; 50: 357–62.
6. Kimura M Gastrointestinal cow’s milk allergy. Allergy Pract. 2008; 28: 857–60 (in Japanese).
7. Horimukai K, Dohmoto T, Mino Y, Funada H, Kanzaki S, Kimura M. Four cases of milk allergic enteritis in newborn and early infancy. Jpn. J. Pediatr. 2008; 61: 251–6 (in Japanese).
8. Kakita H, Yamamoto H, Ito M, et al. Three cases of milk protein allergy with various symptoms. Jpn. J. Soc. Prem. Newborn Med. 2009; 21: 119–24 (in Japanese).
9. Ninchoji T, Nomura T, Nozuhara M, et al. Six cases of milk allergy including three preterm infant cases. Jpn. J. Pediatr. 2010; 63: 993–8 (in Japanese).
10. Kimura M, Taguchi T, Narabayashi S, Oh S. Relationship between eosinophilia and elevated CRP levels in infants with intestinal cow’s milk allergy. J. Jpn. Pediatr. Soc. 2011; 115: 777–81 (in Japanese).

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11 Nowak-Węgrzyn A, Muraro A. Food protein-induced enterocolitis syndrome. *Curr. Opin. Allergy Clin. Immunol.* 2009; 9: 371–7.
12 Mehr S, Kakakios A, Frith K, Kemp AS. Food protein-induced enterocolitis syndrome: 16-year experience. *Pediatrics* 2009; 123: e459–64.
13 Hwang J-B, Sohn SM, Kim AS. Prospective follow-up oral food challenge in food protein-induced enterocolitis syndrome. *Arch. Dis. Child.* 2009; 94: 425–8.
14 Katz Y, Goldberg MR, Rajuan N, Cohen A, Leshno M. The prevalence and natural course of food protein-induced enterocolitis syndrome to cow’s milk: A large-scale, prospective population-based study. *J. Allergy Clin. Immunol.* 2011; 127: 647–53.
15 Kimura M, Oh S, Narabayashi S, Taguchi T. Usefulness of lymphocyte stimulation test for the diagnosis of intestinal cow’s milk allergy in infants. *Int. Arch. Allergy Immunol.* 2012; 157: 58–64.
16 Sopo SM, Giorgio V, Delle lacono I, Novembre E, Mori F, Onesimo R. A multicenter retrospective study of 66 Italian children with food protein-induced enterocolitis syndrome: Different management for different phenotypes. *Clin. Exp. Allergy* 2012; 42: 1257–65.
17 Ruffner MA, Ruymann K, Barni S, Cianferoni A, Brown-Whitehorn T, Spergel JM. Food protein-induced enterocolitis syndrome: Insights from reviews of a large referral population. *J. Allergy Clin. Immunol. Pract.* 2013; 1: 343–9.
18 Caubet JC, Simone L, Sickle L, et al. Clinical features and resolution of food protein-induced enterocolitis syndrome: 10-year experience. *J. Allergy Clin. Immunol.* 2014; 134: 382–9.
19 Marr HY, Chen WC, Lin LH. Food protein-induced enterocolitis syndrome: Report of one case. *Acta Paediatr. Taiwan.* 2001; 42: 49–52.
20 Hwang J-B. Is this symptom even a food allergy? Clinical types of food protein-induced enterocolitis syndrome. *Pediatr. Gastroenterol. Hepatol. Nutr.* 2014; 17: 74–9.
21 Shek LP, Bardina L, Castro R, Sampson HA, Beyer K. Humoral and cellular responses to cow milk proteins in patients with milk-induced IgE-mediated and non-IgE-mediated disorders. *Allergy* 2005; 60: 912–9.
22 Moller H, Ohlsson K, Kinder C, Bjorkner B, Braue M. Cytokines and acute phase reactants during flare-up of contact allergy to gold. *Am. J. Contact Dermat.* 1998; 9: 15–22.
23 Walker WA. Allergen absorption in the intestine: Implication for food allergy in infants. *J. Allergy Clin. Immunol.* 1986; 78: 1003–9.
24 Majamaa H, Isolauri E. Evaluation of the gut mucosal barrier: Evidence for increased antigen transfer in children with atopic dermatitis. *J. Allergy Clin. Immunol.* 1996; 97: 982–90.
25 Benard A, Desreuemaux P, Huglo D, Hoorelbeke A, Tonnel A-B, Wallaert B. Increased intestinal permeability in bronchial asthma. *J. Allergy Clin. Immunol.* 1996; 97: 1173–8.
26 Zetterstrom M, Sundgren-Andersson AK, Oslund P, Bartfai T. Delineation of the proinflammatory cytokine cascade in fever induction. *Ann. NY Acad. Sci.* 1998; 856: 48–52.
27 Yokota S. Interleukin 6 as a therapeutic target in systemic-onset juvenile idiopathic arthritis. *Curr. Opin. Rheumatol.* 2003; 15: 581–6.
28 Kramer F, Torzewski J, Kamenz J, et al. Interleukin-1beta stimulates acute phase response and C-reactive protein synthesis by inducing an NFkappaB- and C/EBPbeta-dependent autocrine interleukin-6 loop. *Mol. Immunol.* 2008; 45: 2678–89.
29 Morita H, Nomura I, Orihara K, et al. Antigen-specific T-cell responses in patients with non-IgE-mediated gastrointestinal food allergy are predominantly skewed to Th2. *J. Allergy Clin. Immunol.* 2013; 131: 590–2.