

Patient Report

Sequential measurement of fecal parameters in a case of non-immunoglobulin E-mediated milk allergy

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Food allergies are divided into those mediated by allergen-specific immunoglobulin E (IgE) and those not mediated by IgE.¹ The majority of adverse food reactions in infants are to cow's milk proteins and most are not IgE-mediated.¹ The immune mechanisms of these non-IgE reactions involve T-cell responses. Direct evidence supporting this has been presented in the recent literature.² We analyzed in detail the sequential changes of stool parameters in a patient with non-IgE mediated cow's milk allergy to determine the role of cellular immunity and inflammatory cytokines in the pathogenesis.

Case report

A female newborn weighing 2536 g was delivered vaginally at 34 weeks gestation. Her father had had atopic dermatitis, allergic rhinitis and asthma. Her mother had had allergic rhinitis. She was initially fed with breast milk combined with peptide-based formula. At 7 days of age, failure to thrive, vomiting and bloody stool appeared. We performed blood examinations and bacterial cultures due to suspected bacterial infection and milk allergy. Peripheral leukocyte count was 13 300/ μ L with 8% eosinophils and serum C-reactive protein was 0.2 mg/dL. Specific IgE against cow's milk, α -lactalbumin, β -lactoglobulin and casein were negative. Fecal bacterial culture was not remarkable, and stools did not contain eosinophils. Oral feeding was discontinued and the patient received i.v. fluid supplementation. The intestinal symptoms resolved without antibiotics and breast milk alone was started again at 10 days of age. Her weight increased progressively during the breastfed period (Fig. 1), but the weight gain ceased without any intestinal symptoms after peptide-based formula was added. At 24 days of age peripheral leuko-

cyte count increased to 36 300/ μ L with 44% eosinophils but specific IgE against cow's milk remained negative. We suspected non-IgE mediated cow's milk allergy from the clinical history and eliminated cow's milk from her feeding. Her weight started to increase again after this elimination.

At 36 days of age, we performed oral challenge with cow's milk formula after informed consent had been obtained from the parents, in order to make the diagnosis of milk allergy. She was given cow's milk formula consisting of 0.37–0.6 g protein per kilogram bodyweight. After the third feeding (8 h after the initial challenge) the infant vomited a small amount of milk. Bloody stool appeared after the sixth feeding (17 h after the initial feeding) and the challenge was discontinued. The patient remained stable during the challenges, and bloody stool disappeared quickly. Fecal eosinophils remained negative. There were no skin lesions during or after the challenge test.

After the cow's milk challenge the patient was fed with breast milk alone. Because we were concerned about the shortage of breast milk, oral challenge with hydrolyzed casein-based formula was performed at 45 days of age to check the safety of feeding with hydrolyzed casein-based formula. During the 7 days of the oral challenge there were no intestinal symptoms and her weight continued to increase. The patient did not take any cow's milk proteins after the challenge test.

Materials and methods

Stool samples were collected once a day at day 26, 38–42, 45–52, 54, 75, 255 and 375. They were suspended 1:2 (w/v) in phosphate-buffered saline (pH 7.2). After vigorous vortex-mixing for 30 min, they were centrifuged for 10 min at 20 000 g and supernatant was collected and used for measurement of tumor necrosis factor- α (TNF- α), immunoglobulin A (IgA) and eosinophil-derived neurotoxin (EDN). The commercial enzyme immunoassay kits were used for TNF- α (ELISA Development Kit, Human TNF- α ; Techne, Minneapolis, MN, USA)

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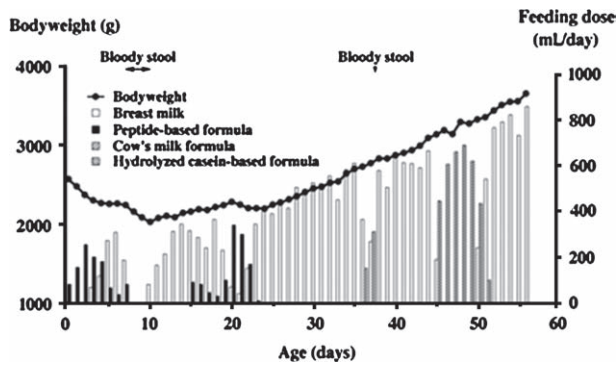


Fig. 1 Relationship between bodyweight gain and nature of feeding.

and EDN (EDN ELISA Kit; MBL, Nagoya, Japan). IgA values were measured using a sandwich enzyme-linked immunosorbent assay (ELISA) using goat anti-human IgA (BioSource International, Camarillo, CA, USA) and alkaline phosphatase-conjugated goat anti-human IgA (BioSource International). Solid parts of the samples were used to estimate the fecal fat content by the acid steatocrit method.³ Control fecal samples were obtained from age-matched healthy infants.

Statistical analysis

The Mann–Whitney *U*-test was used to analyze the significance of the differences between the two groups. Differences were considered to be significant for $P < 0.05$.

Results

Figure 2 shows the time course of fecal TNF- α , IgA, EDN and acid steatocrit. We classified these data into three groups, days 26, 39–42, 45, 52, 54, 75 (group A), days 38, 46–51 (group B) and days 255, 375 (group C). We defined group B as during the challenge tests or within 24 h of challenge tests. Group B was the acute or stimulating phase. Cow's milk formula or hydrolyzed casein-based formula might have been present in the patient's intestine during the group B period. Group A was the subacute or chronic phase. The data were compared among different groups (Fig. 3).

Fecal TNF- α concentrations were significantly elevated before the challenges as compared to control group. Unexpectedly, TNF- α concentrations were undetectable during the hydrolyzed casein-based formula challenge test except for day 46, immediately after the hydrolyzed casein-based formula challenge. TNF- α concentrations increased again after the challenges and remained high for a prolonged period. It became undetectable on days 255 and 375.

Fecal IgA concentrations were significantly lower in group B than in group A. Fecal EDN concentrations were signifi-

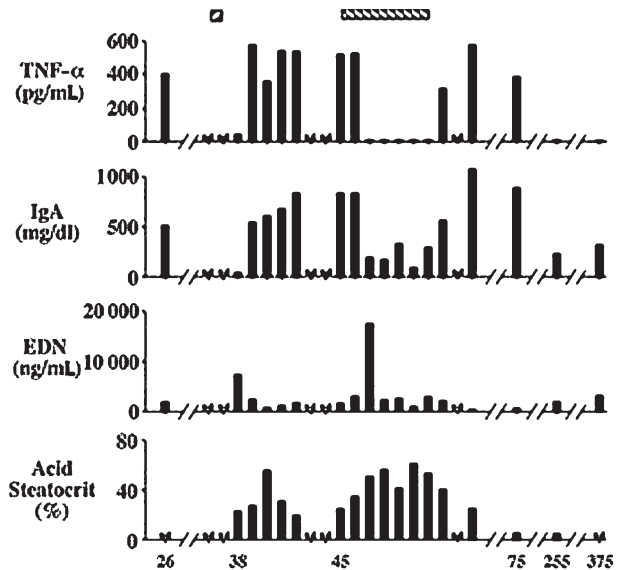


Fig. 2 Time course of the levels of fecal tumor necrosis factor- α (TNF- α), immunoglobulin A (IgA), eosinophil-derived neurotoxin (EDN) and acid steatocrit. n.d., no data. Duration of feeding with (▨) cow's milk formula or (▧) casein-based formula.

cantly higher in group B than in group A. Acid steatocrits in group A and B were significantly higher than controls.

Discussion

Diagnosis of milk allergy is made by a positive response to the oral challenge. Presence of allergen-specific IgE supports the diagnosis. In the present patient, cow's milk allergy was not suspected initially because serum-specific IgE for cow's milk, α -lactalbumin, β -lactoglobulin or casein was negative.

High concentrations of fecal TNF- α have been reported in children with inflammatory bowel disease, atopic eczema and cow's milk allergy manifested by intestinal symptoms.^{4–6} Accelerated production of TNF- α is responsible for local inflammation, structural damage and increased permeability of the intestinal mucosa to proteins and electrolytes, further rendering intestinal mucosa sensitive to food allergens.^{7–9} However, most of these studies were performed only for a short period and the clinical significance of sequential measurement of stool TNF- α in patients with food allergy is not clear. In addition, we measured fecal IgA, EDN and fat content simultaneously to evaluate the mechanisms and process of intestinal events in this patient. The levels of fecal TNF- α remained increased for a long period after the oral challenges, suggesting that the induced inflammation results in long-lasting functional disturbance of the intestine. It is particularly noteworthy that a high level of fecal TNF- α persisted for 1 month after the oral challenge, when the clinical symptoms had long

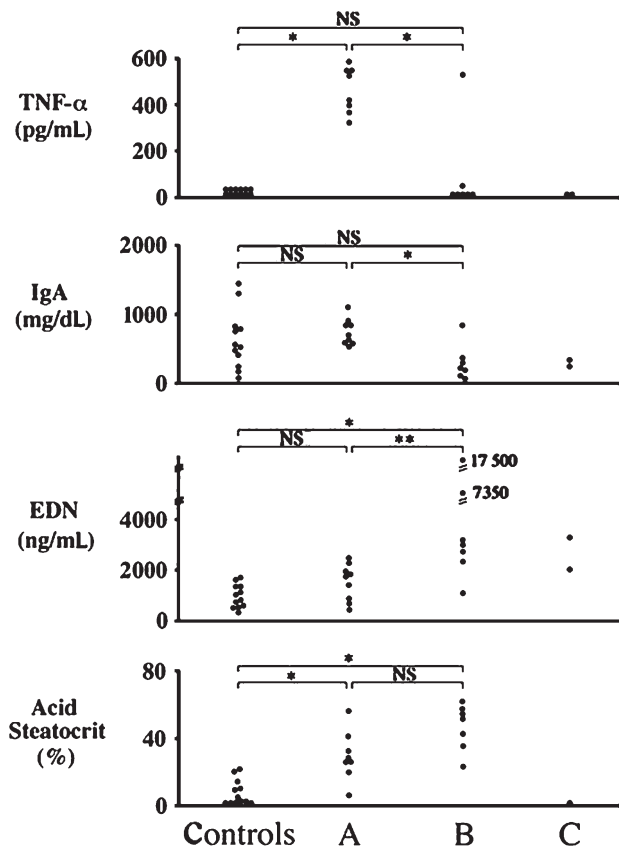


Fig. 3 Comparison of fecal tumor necrosis factor- α (TNF- α), immunoglobulin A (IgA), eosinophil-derived neurotoxin (EDN) and acid steatocrit levels among three different groups of samples. Age-matched control data are shown at the left end of the figure. Group A, days 26, 39–42, 45, 52, 54 and 75; group B, days 38 and 46–51; group C, days 255 and 375. * $P < 0.01$; ** $P < 0.05$; NS, not significant.

resolved. We do not know at present why there was transient reduction of fecal TNF- α levels during the challenge test. One explanation may be that induction of proteolytic enzymes after antigen challenges resulted in the rapid degradation of the cytokines in the stool samples.¹⁰ Supporting this hypothesis, fecal IgA levels decreased significantly during the challenge test, similar to TNF- α . In addition to the proteolytic cleavage, IgA immune complex formation may also explain the sudden decrease of IgA within stool samples. However, we need to examine many more cases to further elucidate the mechanism of the paradoxical decrease of TNF- α and IgA.

The patient presented with markedly increased eosinophil counts in the peripheral blood, and fecal EDN increased during the oral challenge test. These results suggest that eosinophils played some role during the acute phase and in the subsequent development of the chronic inflammation in this patient.^{11–13}

Fecal fat content was significantly increased during the acute or chronic phase compared with normal controls. These

results indicate that allergic intestinal reactions induced long-lasting fat malabsorption in this patient.

Measurements of fecal parameters are not quantitative analyses as compared with blood examinations. But sequential measurement of fecal TNF- α , combined with other parameters of the intestinal inflammation, may provide a sensitive and non-invasive method to evaluate non-IgE-mediated milk allergy. It also opens a way to elucidate the complex nature of food-induced intestinal diseases, and helps to diagnose cases with minimal symptoms.

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