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Selectively High Level of Serum Interleukin 5 in a Newborn Infant With Cow's Milk Allergy

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KEY WORDS

milk allergy, eosinophil, IL-5, neonate, cytokine profile

ABBREVIATIONS

CMA—cow's milk allergy

IgE—immunoglobulin E

Th—T helper

TNF- α —tumor necrosis factor α

IL—interleukin

WBC—white blood cell

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abstract

Cow's milk allergy (CMA) in the neonatal period is thought to include several clinical conditions, yet the pathophysiology remains unclear. We report here the case of a term newborn infant who showed hematochezia 36 hours after the first feeding with cow's milk formula. His serum immunoglobulin E levels were not elevated, although eosinophils were detected in the stool. Elimination of cow's milk formula resolved the symptoms, and from the clinical course and laboratory data the infant was diagnosed with CMA. The serum interleukin 5 (IL-5) (125 pg/mL) level in this patient was selectively elevated. However, serum levels of other T-helper 2 (Th2) cytokines (including IL-4 and IL-13), Th1 cytokines (including interferon γ), and proinflammatory cytokines (including tumor necrosis factor α) were not elevated. These findings suggest that, for this patient, IL-5 and eosinophils might have played a role in the development of neonatal CMA. Although this finding is reported from only 1 case, it highlights the need for serum IL-5 to be determined in more neonatal patients with CMA to further clarify the pathophysiology of this condition in the neonatal period. *Pediatrics* 2011;127:e231–e234

Cow's milk protein allergy is the most common food allergy in infants and young children. Cow's milk allergy (CMA) in the neonatal period has been thought to include several clinical conditions. In 1940, newborn infants were reported to show intestinal bleeding as a clinical syndrome caused by cow's milk formula.¹ Later, in a 1967 study by Gryboski² on a group of 21 children who developed gastrointestinal symptoms from cow's milk formula, the term "milk allergy" was established. Afterward, when 9 infants presented with diarrhea and vomiting after consuming a cow's milk-based formula, the disease was named food protein-induced enterocolitis (FPIES) of infancy.^{3,4} Allergic proctocolitis is often seen in infants in the neonatal period as spots or streaks of blood in the stool,⁵ and this clinical condition is also called food-induced eosinophilic proctocolitis.⁶

Food allergies can be categorized on an immunologic basis as those that are mediated by immunoglobulin E (IgE) directed toward a specific causal protein and those that are non-IgE-mediated. Food protein-induced enterocolitis and allergic proctocolitis are placed in the non-IgE-mediated allergy category, yet the pathophysiology of neonatal CMA remains unclear. Characteristics of the intestinal inflammatory response are largely determined by the cytokine response of T cells. In food allergies mediated by IgE directed toward a specific causal protein, antigen-specific cytokine production is skewed toward T-helper 2 (Th2).⁷ In contrast, in infants with non-IgE-mediated food allergy, immune reactivity is most likely mediated by the production of Th1 cytokines or tumor necrosis factor α (TNF- α).⁸

Recent advances using the bead array system have made cytokine profiling possible with only small amounts of blood taken from infants and even ne-

onates.⁹ We report here a selectively high level of serum interleukin 5 (IL-5) in a newborn infant who showed hematochezia with eosinophils in his stool caused by cow's milk formula.

CASE REPORT

The patient was a male newborn infant whose gestational age was 40 weeks and whose birth weight was 3000 g. He was born transvaginally without complications. Both his parents had allergic rhinitis against pollen occurring in their adult ages but no history of atopic disease. The patient received both cow's milk formula and breast milk initially at 12 hours of life. Approximately 36 hours after the first feeding, he showed hematochezia. His general condition was not ill, and he continued to receive feedings of both cow's milk formula and breast milk. The next day he was still showing hematochezia and was transferred to our NICU.

On admission to the NICU, the patient was clinically well, and his abdomen was soft and nondistended. His skin color was normal, and there was no exanthem. His chest-abdominal radiograph was normal. His white blood cell (WBC) count was 11 400/ μ L, and the percentage of eosinophils was 1% (114/ μ L). His hemoglobin level was 15.1 g/dL, and his hematocrit level was 44.9%. The platelet count was 3.72×10^5 / μ L, and results of a blood-coagulation profile was within the normal range. His serum C-reactive protein level was normal at 0.8 mg/L, and other serum chemistry data were within the normal ranges. Stool cultures were negative for abdominal pathogens.

Because the patient was not in acute distress, we chose to observe his clinical course without oral feeding and instead gave him fluids intravenously. We considered the possibility that he might have CMA and performed laboratory examinations to test for aller-

gies. His serum level of IgE was <3 U/mL, and his cow's milk-specific IgE level was <0.35 U/mL. Eosinophils were recognized in his loose stool. Allergen-specific lymphocyte proliferation was measured, as described previously, on the 11th day of his life.¹⁰ The response was converted into a stimulation index (SI) measured by flow cytometry. His lymphocytes did not show a significantly high response against β -lactalbumin (SI: 315) or κ -casein (SI: 70), compared with the normal ranges (SI: <450 and <150 , respectively). His lymphocytes showed a slightly higher response against α -lactalbumin (SI: 570) compared with normal (SI: <500), although this value was not necessarily considered positive. We investigated his serum cytokine profile (including 17 cytokines) at admission on day 3 of life. We found a selectively elevated level of serum IL-5 (125 pg/mL) and low levels of the other Th2 cytokines, Th1 cytokines, and proinflammatory cytokines, as outlined below.

Once oral feeding was stopped, the bloody stool gradually improved and disappeared 2 days after NICU admission. We started feeding with extensively hydrolyzed casein-based formula (MA-1 [Morinaga Milk Industry Co Ltd, Tokyo, Japan]) on the 5th day of life, and on the 10th day of life we gave mixed feeding with the extensively hydrolyzed formula and breast milk. The patient's WBC count was 11 000/ μ L with 440 eosinophils (4% of WBCs) on the 7th day of life (4 days after NICU admission), and on the 12th day of life his WBC count gradually increased to 20 000/ μ L with 1400 eosinophils (7% of WBCs). The patient was discharged from our hospital on the 14th day of life, because he no longer showed hematochezia and his eosinophil count had decreased. Exclusive breastfeeding continued at home. The mother was recommended not to eradicate cow's milk protein from dairy prod-

TABLE 1 Concentrations of Serum Cytokines, Chemokines, and Growth Factors in the Patient

	Patient	Control (N = 8), Mean $\pm$ SD ^a
Birth weight, g	3000	2736.9 $\pm$ 501.5
Gestational age, wk	40	38.8 $\pm$ 1.6
Age, d	3	3.8 $\pm$ 1.8
Proinflammatory cytokines, pg/mL		
TNF- α	4.1	9.9 $\pm$ 5.2
IL-1 β	1.0	2.0 $\pm$ 1.7
IL-6	7.7	12.2 $\pm$ 18.2
Th1 cytokines, pg/mL		
Interferon γ	6.9	8.8 $\pm$ 7.4
IL-2	<0.02	1.1 $\pm$ 1.6
IL-12	6.9	5.9 $\pm$ 5.8
Th2 cytokines, pg/mL		
IL-4	0.02	0.23 $\pm$ 0.24
IL-5	125.0	1.9 $\pm$ 0.92
IL-10	8.5	6.0 $\pm$ 7.5
IL-13	2.88	1.3 $\pm$ 1.0
Th17 cytokine, pg/mL		
IL-17	3.1	13.2 $\pm$ 17.0
Growth factors, pg/mL		
IL-7	0.24	0.34 $\pm$ 0.35
Granulocyte/macrophage colony-stimulating factor	1.33	14.4 $\pm$ 11.1
Granulocyte colony stimulating factor	21.9	14.2 $\pm$ 7.9
Chemokines, pg/mL		
IL-8	29.0	33.6 $\pm$ 39.3
Monocyte chemoattractant protein 1	87.3	395.8 $\pm$ 198.1
Macrophage inflammatory protein 1 β	381.3	251.9 $\pm$ 199.2

^a The control group included 8 term newborn patients who were admitted to the Jichi Medical University NICU with various risks. Among them, 2 had slightly low birth weight, 2 developed mild pneumomediastinum, 2 showed initial vomiting, 1 had mild asphyxia, 1 had mild transient tachypnea of the newborn, and 1 had hyperbilirubinemia caused by breastfeeding. Peripheral blood was taken from those in the control group between the second and eighth days of life (with informed consent from parents).

ucts, although she was suggested not to give cow's milk products directly to her son until he was 1 year of age. The patient received a challenge of cow's milk at the age of 1 year, but he did not show any allergic reaction against the challenge.

We measured serum cytokine levels by using the BioPlex protein array (Bio-Rad, Hercules, CA) and Luminex 100 (Mirai Bio, Alameda, CA) as described previously.^{9,11} A cytokine panel (BioPlex Human Cytokine17-Plex Panel [Bio-Rad, San Diego, CA]) was used to detect cytokines (IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-10, IL-12[p70], IL-13, IL-17, interferon γ , and TNF- α), chemokines (IL-8, monocyte chemoattractant protein 1 [MCP-1], and macrophage inflammatory protein 1 β), and growth factors (IL-7, granulocyte colony stimulating factor, and granulocyte/macrophage colony-

stimulating factor). Table 1 lists serum cytokine levels in both our patient and the controls. The most striking finding was the selectively elevated level of serum IL-5 in our patient. In contrast, serum levels of the other Th2 cytokines (IL-4, IL-13, and IL-10) were not elevated. Those of Th1 cytokines and proinflammatory cytokines, including TNF- α , were also not elevated. Regarding the chemokines, the serum level of MCP-1 was quite low.

DISCUSSION

It is estimated that up to 50% of the CMA in pediatric patients is non-IgE-mediated.⁵ Non-IgE-mediated CMA includes several clinical entities such as food protein-induced enterocolitis, allergic proctocolitis, and eosinophilic gastroenteritis with protein-losing gastroenteropathy.⁵ Food-protein chal-

lenge is required to provide a definitive diagnosis of food allergy. However, food challenge has a risk of causing allergic shock and in nearly all cases, has not been performed until 1 year of age. Our case was not diagnosed as CMA by challenge test. However, we believe that the patient had CMA, because he showed resolution of symptoms by the eradication of cow's milk formula within 48 hours. He was challenged with cow's milk at 1 year of age and did not show any reaction. This finding is compatible with allergic proctocolitis.⁵ We believe that the clinical findings of our patient are similar to those in others with food-protein eosinophilic proctocolitis, although we did not perform endoscopic investigation.⁶

Schade et al⁷ reported that antigen-specific cytokine production is Th2-skewed in infants with atopic dermatitis and CMA, who display high production levels of IL-4, IL-5, and IL-13. Ward et al⁸ investigated cytokine production in non-IgE-mediated CMA and showed that cord blood mononuclear cells isolated from a patient with fetal sensitization to cow's milk protein revealed elevated production of TNF- α with cow's milk protein as the stimuli. In our patient, however, the Th2 response without IL-5 was not enhanced, and his serum level of TNF- α was not elevated compared with the controls. Our data suggest that for our patient, IL-5 and eosinophils played a role in the development of CMA.

IL-5 promotes the production and function of eosinophils. Infiltration of eosinophils has been identified as a potential common feature of mucosal lesions induced by food allergy.^{12,13} Eosinophils develop and differentiate in the bone marrow under the influence of at least 3 cytokines (granulocyte/macrophage colony-stimulating factor, IL-3, and IL-5); IL-5 is more specific

for eosinophils. We feel that IL-5 and eosinophils may contribute to the occurrence and progression of CMA especially in the neonatal period. Sherman and Cox¹³ suggested that eosinophilic colitis could be attributed to transplacental sensitization of the colon to bovine-like antigens. Ohtsuka et al¹⁴ reported 2 neonatal cases of CMA and used the clinical entity “neonatal transient eosinophilic colitis.” CMA-antigen sensitization in utero might have contributed to the IL-5 production in our patient as well.

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CONCLUSIONS

Because the finding of elevated IL-5 has only been seen at a single point in our case, it needs to be further supported by other neonatal cases of CMA. It is also important to investigate the chronological change of the cytokine profile in the clinical course of such patients. IL-5 may be up-regulated first, and later the other Th2 cytokines may then be elevated. The difference in cytokine expression between our patient and the past reports^{7,8} may be caused by the timing of investigation. There-

fore, we suggest that the serum level of IL-5 be studied in other neonatal patients with CMA for further clarification of the pathophysiology of CMA in the neonatal period.

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