

Markedly high eosinophilia and an elevated serum IL-5 level in an infant with cow milk allergy

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Background: Interleukin-5 (IL-5) promotes the production and function of eosinophils, and an increase in the serum soluble CD23 (sCD23) level is suggestive of enhanced type-2 helper T-cell activity. The secretion of a large amount of the proinflammatory cytokine, tumor necrosis factor alpha (TNF- α), has been reported to alter the intestinal barrier capacity.

Objective: To determine whether or not distinct profiles of cytokine production were involved in the marked peripheral eosinophilia of as high as 20,000/mm³ and the gastrointestinal symptoms seen in an infant with cow milk allergy.

Methods: The levels of IL-5, sCD23, and TNF- α in serum and the culture supernatants of mononuclear cells were compared with those in infants with anaphylaxis to cow milk and nonallergic infants.

Results: Interleukin-5 was detected in the serum (19 pg/mL) but became undetectable after 2 weeks on a milk-free diet together with clinical remission. A kinetic decrease in the serum sCD23 level was also observed during the administration of a milk-free diet with improvement of the eosinophilia in 2 months. The TNF- α produced in vitro after stimulation with cow milk protein was not different from in controls.

Conclusion: It seems likely that the allergic inflammation due to cow milk can induce marked eosinophilia with an associated increase in IL-5 production.

Ann Allergy Asthma Immunol 1999;82:253–256.

INTRODUCTION

A cow milk-based formula has been used successfully as the primary source of nutrition for many infants. On the other hand, a broad spectrum of symptoms and signs has been attributed to the ingestion of cow milk protein. Although the subject of cow milk allergy has suffered from confusion due to lack of reliable single diagnostic test, it has been defined as an adverse reaction to cow milk protein that is mediated by immunologic mechanisms.¹ Most reactions are localized to the gastrointestinal tract, but cow milk allergy may also cause systemic symptoms such as anaphylaxis and local reactions in the skin and respiratory tract. Furthermore, several well-defined but uncommon syndromes, in-

cluding Heiner's syndrome² and eosinophilic gastroenteritis,^{3,4} have been associated with cow milk allergy.

In the present report, we describe an unusual case of cow milk allergy with marked high peripheral blood eosinophilia and gastrointestinal symptoms. Interleukin-5, preferentially produced by type 2 helper T cells, appears to promote the production and function of eosinophils. In addition, peripheral blood mononuclear cells from infants with cow milk allergy and intestinal symptoms have been shown to secrete large amounts of the proinflammatory cytokine, TNF- α , which altered the intestinal barrier capacity. We considered it of significance to determine whether or not distinct profiles of cytokine production were related to the gastrointestinal damage and marked eosinophilia seen in this case.

CASE REPORT

A 9-month-old male infant was referred to us for evaluation of intermit-

tent vomiting, gastrointestinal occult bleeding, and peripheral blood eosinophilia of as high as 20,000/mm³. The infant had been fully bottle-fed from birth with a cow milk formula which he tolerated well, and had been thriving well.

He was first seen elsewhere at age 2 months because of a fever and a generalized skin rash, both of which disappeared within 2 days, and was evaluated by means of laboratory tests, the results including white blood cell counts of 8,400 to 8,700/mm³ with 0% to 7% eosinophils. At age 8 months he experienced a fever and a generalized skin rash again, both of which remained for 3 days, but no laboratory evaluation was performed at the time. With the exception of the 2 episodes described above, he had not been given any medication. During the 10 days prior to the authors' examination, he experienced intermittent vomiting once for a couple of days and multiple laboratory examinations revealed occult blood in the stools and peripheral blood leukocytosis of 37,400 to 38,400/mm³ with eosinophilia of 43.4% to 50.7% (differential counts). The peripheral blood eosinophils were well matured and morphologically normal. Serum albumin (3.9 g/dL), the hematocrit (39%), and hemoglobin (13 g/dL) were within the normal ranges. At this time, he did not exhibit diarrhea, fever, pruritus, skin lesions, or a respiratory disorder. He has no sisters or brothers, and there was no family history of allergic diseases.

The serum level of total IgE (161 IU/mL) was elevated compared with the normal range (6 to 71 IU/mL) in nonatopic age-matched controls. Serum-specific IgE was titrated by means of a fluoroenzyme immunoassay using a CAP-RAST kit (Pharmacia), and

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Received for publication June 16, 1998.

Accepted for publication October 21, 1998.

Table 1. Skin Prick, Skin Patch and CAP-RAST Results for Cow Milk Proteins During the Administration of a Milk-Free Diet

	Milk-Free Diet, wk					
	0	1	10	14	18	25
Skin prick test						
Skimmed milk	+	n.d.*	+	+	n.d.	—
Whey hydrolysates	n.d.	n.d.	—	—	n.d.	n.d.
Casein hydrolysates	n.d.	n.d.	—	—	n.d.	n.d.
Skin patch test						
Skimmed milk	n.d.	n.d.	n.d.	—	n.d.	—
CAP-RAST (UA/mL)						
Cow milk	n.d.	5.4	n.d.	n.d.	0.4	n.d.
α -Lactalbumin	n.d.	4.2	n.d.	n.d.	<0.4	n.d.
β -Lactoglobulin	n.d.	7.9	n.d.	n.d.	<0.4	n.d.
Casein	n.d.	2.9	n.d.	n.d.	<0.4	n.d.
Total IgE (IU/mL)	n.d.	161	n.d.	n.d.	111	n.d.

* n.d. = not done.

more than 0.4 unit allergen per milliliter (UA/mL) was defined as a positive result in this study. The skin prick test results were considered positive if the wheal diameter exceeded that of the saline control by at least 3 mm. As shown in Table 1, the skin prick test and CAP-RAST for cow milk were positive. The skin prick test results for casein hydrolysates and whey hydrolysates were negative, and he was found to be anergic with the skin patch test for cow milk protein. The skin prick test results for hen eggs, soybeans, wheat protein, cat fur, feather allergens, and house dust/mites were all negative. The serum concentrations of IgG (441 mg/dL), IgA (36 mg/dL), and IgM (57 mg/dL) were all within the normal ranges. Repeated stool examinations for parasites were negative, and serum immunodiagnostic tests for 12 kinds of major parasites in Japan were negative (kindly performed by Dr. Y. Nawa, Miyazaki Medical College). No eosinophils were found in the feces.

He was switched to a soy-protein formula. Once he had started the milk-free diet, the vomiting ceased. The eosinophilia improved dramatically and the guaiac reaction in the stools became negative within 4 weeks, as shown in Figure 1. The kinetics of the IgE responses to cow milk during the administration of the milk-free diet are summarized in Table 1. The CAP-

RAST results for individual cow milk proteins became negative at age 1 years 2 months. Since he had lost the sensitivity to cow milk, as judged with the skin prick test, at 9 months after the switch to the milk-free diet at age 1 year 6 months, he started to drink homogenized milk, which he tolerated well, and so the milk-free diet was suspended. Although the skin prick test result for cow milk was negative at age 2 years 6 months, the total serum level of IgE (129 IU/mL) and the CAP-RAST results for cow milk (0.47 to 1.15 UA/mL) were slightly elevated again, and the white blood cell counts ranged from 9,400 to 10,800 with 8% to 10% eosinophils, reflecting mild eosinophilia. Until the authors' final check at age 3 years, neither vomiting, gastrointestinal bleeding, nor a positive guaiac reaction in the stools was observed.

METHODS

Interleukin-5, TNF- α and Soluble CD23 (sCD23) Determination

Heparinized venous blood was obtained from the present case at age 2 years 6 months, and 3 patients (aged 2 to 4 years old) with cow milk anaphylaxis, eg, systemic hives, angioedema, wheezing, or hypotension following the ingestion of cow milk. The total IgE levels ranged from 86 to 5,330 IU/mL with CAP-RAST results as to cow milk of 10.6 to 33.8 UA/mL.

Blood was also drawn from 3 nonallergic children, aged 3 to 5 years old, with negative skin prick test results for cow milk and house dust/mites. Before this study, informed consent was obtained from all the parents. Mononuclear cells were collected using a sodium metrizoate-Ficoll solution, washed 3 times, and then adjusted to 1×10^6 cells/mL with RPMI 1640 culture medium containing 10% heat-inactivated fetal calf serum in round bottom Falcon tubes (No. 2054; Beckton Dickinson, Lincoln Park, NJ). The cells were cultured at 37°C under 5% CO₂ for 2 days without or with 20 μ g/mL of phytohemagglutinin-P (PHA) plus 2 ng/mL of phorbol myristate acetate (PMA), 200 μ g/mL of bovine serum albumin (BSA), or 200 μ g/mL of whole casein (WCA). Phytohemagglutinin, PMA, BSA, and WCA were obtained from Sigma, St. Louis, MO. The levels of IL-5, TNF- α , and sCD23 in sera and culture supernatants were determined with commercially available ELISA kits according to the manufacturers' instructions. The kits for IL-5 and TNF- α were obtained from R & D Systems, Minneapolis, MN, and the ELISA kit for sCD23 was purchased from Nichiray, Tokyo, Japan. The results are expressed as means $\pm$ 1 standard error (SE). Student's unpaired *t* test was used to compare the means of the different groups.

RESULTS

Interleukin-5 (18.9 pg/mL) was detected in the serum at 1 week after introduction of the milk-free diet, and became undetectable after 2 weeks with the milk-free diet (Table 2). The serum levels of IL-5

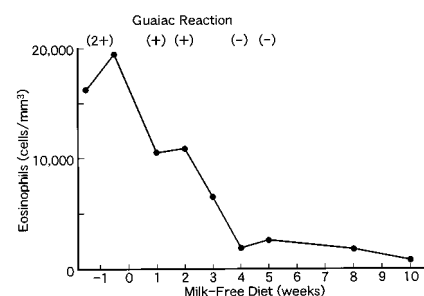


Figure 1. Kinetics of the eosinophil counts in peripheral blood and the guaiac reaction in stools.

were under the limit of sensitivity in all the patients with cow milk anaphylaxis and all the nonallergic controls. In addition, the serum levels of TNF- α were under the limit of sensitivity in all the samples examined in this study. The serum levels of sCD23 were increased in the present case when compared with those in the nonallergic controls, and kinetic decreases were observed during the administration of the milk-free diet. The amounts of IL-5 and TNF- α in the culture supernatants in the present case were within the ranges of those in the patients with cow milk anaphylaxis and the nonallergic controls (Fig 2).

DISCUSSION

There was a direct correlation of presence of cytokines with clinical symptomatology and eosinophilia and IgE responses. With cow milk elimination, there was a fall in these laboratory findings with clinical improvement that suggests their role in the pathogenesis of the disorder. Diagnostic criteria for cow milk allergy have been proposed in the literature, and a double-blind, placebo-controlled, food challenge (DBPCFC) has been claimed by some authors to be the only way (gold standard) of confirming the diagnosis of a food allergy.^{5,6} This case had been fully bottle fed at the time of his first visit, and his clinical symptoms improved within 4 weeks after the elimination of cow milk products. The controlled administration of cow milk or a placebo for full nutrition for several days will be difficult to accomplish.⁶ The peripheral blood eosinophilia in this case had disappeared at 2 months after the elimination of cow milk; therefore, one or two trial feedings of cow milk will not be sufficient to induce the disorder. Since he had lost the sensitivity to cow milk, as judged with the skin prick test, at 9 months after the switch to the milk-free diet at age 1 year 6 months, cow milk protein was reintroduced. After that, the serum-specific IgE to cow milk and the peripheral blood eosinophilia reappeared again. This evidence highly suggests that the disorder in the present case was due to the ingestion of cow milk.

Table 2. Serum Levels of IL-5, TNF- α and sCD23

	IL-5 (pg/mL)	TNF- α (pg/mL)	sCD23 (ng/mL)
Present case			
1 week of milk-free diet	18.9	<4.0	16.1
2 weeks of milk-free diet	<4.0	<4.0	13.4
3 weeks of milk-free diet	<4.0	<4.0	10.2
1 year after restarting milk-ingestion	<4.0	<4.0	10.6
Milk anaphylaxis			
Case 1	<4.0	<4.0	8.8
Case 2	<4.0	<4.0	10.6
Case 3	<4.0	<4.0	6.4
Nonallergy			(8.6 $\pm$ 2.1)*
Case 1	<4.0	<4.0	7.8
Case 2	<4.0	<4.0	6.1
Case 3	<4.0	<4.0	3.8
			(5.9 $\pm$ 2.0)*

* mean $\pm$ 1 SE, statistically not significant ($P > .1$).

The allergic manifestations in this case seemed to have dramatically worsened at 9 months of age. Although most infants with cow milk allergy develop clinical symptoms shortly after the introduction of a milk-based formula,¹ a longer duration of bottle-feeding is required to obtain for gastrointestinal symptoms.⁷ In addition, as shown in Table 1, it took almost 25 weeks to resolve the laboratory problems in this case by elimination of cow milk. This evidence might reflect the delayed appearance of clinical manifestations in this case.

Previous reports revealed two main cow milk allergy groups according to the time of onset of reaction after milk

ingestion, ie, immediate onset and delayed onset.^{6,8} Children with immediate onset usually have cutaneous manifestations such as positive skin prick test results and raised IgE values, and are atopic; the reaction is provoked by only small amounts of milk. Children with delayed onset usually have gastrointestinal manifestations, negative skin prick test results, and normal IgE values, and are nonatopic; large amounts of milk are needed to provoke the reaction. It has long been a puzzling finding that most infants with gastrointestinal symptoms caused by cow milk allergy have a predominantly non-IgE-dependent disease of which the effectors remain uncharacterized. It

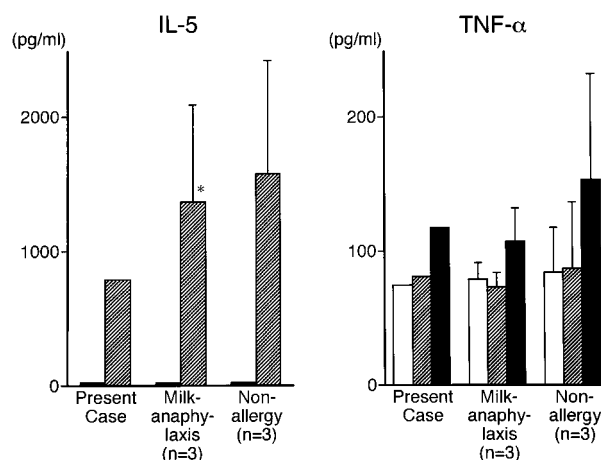


Figure 2. Interleukin-5 and TNF- α produced by mononuclear cells in vitro. A million cells were cultured for 2 days without (■) or with PHA plus PMA (▨) for the IL-5 assay. The cells were also cultured without (□) or with BSA (▨), and WCA (■) for the TNF- α assay. *mean $\pm$ 1 SE.

was reported recently that peripheral blood mononuclear cells from cow milk allergy cases with intestinal symptoms secrete greater amounts of TNF- α , which altered the intestinal functions.⁹ The in vitro TNF- α release in the present case, however, was within the ranges in the patients with cow milk anaphylaxis and the nonallergic controls. Since the improvement of his gastrointestinal disorder and marked eosinophilia was closely associated with the disappearance of immediate type skin sensitivity and decreased serum levels of specific IgE to cow milk, it is suggested that an IgE-mediated pathogenesis was involved for the disorder in the present case.

Determination of the cause of eosinophilia may be one of the most difficult endeavors in clinical medicine, however, the combination of eosinophilia with prominent gastrointestinal symptoms limits the diagnosis to a few conditions that include eosinophilic gastroenteritis. Eosinophilic gastroenteritis with predominant mucosal involvement produces gastrointestinal bleeding. Some authors have mentioned in the literature that development of the symptoms and signs of eosinophilic gastroenteritis are associated with IgE-mediated cow milk allergy.^{3,4}

To our knowledge, marked peripheral eosinophilia of up to 20,000/mm³ has never previously been reported for a patient with eosinophilic gastroenteritis. The extraordinary high eosinophilia in the present case was associ-

ated with increases in the sCD23 and eosinophilogenic cytokine IL-5 levels in the serum. The production of sCD23 is upregulated by IL-4, which induces the production of the IgE isotype by B cells.¹⁰ Since IL-4 and IL-5 are preferentially produced by type 2 helper T cells, increases in sCD23 and IL-5 in the serum are suggestive of enhanced type-2 helper T-cell activity. Although most cases with delayed onset reactions with cow milk allergy and gastrointestinal manifestations are non-atopic, a broad spectrum has been reported for cow milk allergy including patients with elevated IgE antibodies to cow milk and slow onset of clinical reactions after cow milk ingestion.^{1,8,11} Alternatively, it is probable that the clinical and laboratory problems in the present case may be due to IgE-mediated allergic inflammation as a consequence of an enhanced type-2 helper T-cell reaction to cow milk.

REFERENCES

- Host A. Cow's milk protein allergy and intolerance in infancy. *Pediatr Allergy Immunol* 1994;5(Suppl 5):5-36.
- Heiner DC, Sears JW, Kniker WT. Multiple precipitins to cow's milk in chronic respiratory disease. A syndrome including poor growth, gastrointestinal symptoms, evidence of allergy, iron-deficiency anemia, and pulmonary hemosiderosis. *Am J Dis Child* 1962;103:634-654.
- Caldwell JH, Mekhjian HS, Hurtubise PE, Beman FM. Eosinophilic gastroenteritis with obstruction. Immunological studies of seven patients. *Gastroenterology* 1978;74:825-829.
- Cello JP. Eosinophilic gastroenteritis. A complex disease entity. *Am J Med* 1979;67:1097-1104.
- Bahna SL, Gandhi MD. Milk hypersensitivity II. Practical aspects of diagnosis, treatment and prevention. *Allergy* 1983;50:295-301.
- Hill DJ, Hosking CS. Some limitations of double-blind, placebo-controlled food challenges in young children. *J Allergy Clin Immunol* 1991;87:136-137.
- Goldman AS, Anderson JR DW, Sellers WA, et al. Milk allergy I. Oral challenge with milk and isolated milk proteins in allergic children. *Pediatrics* 1963;32:425-443.
- Hill DJ, Firer MA, Shelton MJ, Hosking CS. Manifestations of milk allergy in infancy: Clinical and immunological findings. *J Pediatr* 1986;109:270-276.
- Benlounes N, Christophe D, Candalh C, et al. The threshold for immune cell reactivity to milk antigens decreases in cow's milk allergy with intestinal symptoms. *J Allergy Clin Immunol* 1996;98:781-789.
- Gordon J. CD23 and B cell activation. *Clin Exp Allergy* 1992;22:199-204.
- Fallstrom SP, Ahlstedt S, Hanson LA. Specific-antibodies in infants with gastrointestinal intolerance to cow's milk protein. *Int Arch Allergy Appl Immunol* 1978;56:97-105.

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