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A case of allergy to cow's milk hydrolysate

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Key words: atopy; children; cow's milk allergy; cow's milk hydrolysate; food allergy.

We here report a girl, now 3 years old, who has suffered from severe food allergy since her first year of life. She was strongly allergic to cow's milk, and had high levels of IgE antibody (AB) to casein (210 kU/l), β -lactoglobulin (43 kU/l), and α -lactalbumin (23 kU/l) at 12 months of age. In addition, at the same age, she showed positive (2–4+) skin prick reactions to both unboiled and boiled formulas (Profylac, Nutramigen, and Neocate), besides being positive in RAST to Nutramigen (0.6 kU/l). During the first 3 years, IgE Ab levels against casein and Nutramigen increased to 310 and 1.6 kU/l, respectively. Furthermore, at 3 years of age, she had positive RAST to 14 of 15 tested food allergens, being negative only to codfish. Assessment of eosinophil-related markers revealed high total eosinophil count, increased eosinophil activity, and a low ratio of interferon (IFN)- γ :IL-5, indicating enhanced IL-5 production. The food allergy was correlated to poor weight gain and increasing problems with atopic allergy in the airways.

In early childhood, food allergy, defined as an immunologically mediated adverse reaction to dietary antigens, is the major cause of allergic reactions. Cow's milk allergy is an important problem in the first 2 years of life. Several studies indicate a prevalence of cow's milk allergy of 2–3% (7). Besides posing a risk of life-threatening reactions, severe food allergy can be associated with risk of poor growth (8).

In the present case report, we describe a girl with severe food allergy from the age of 5 months to 3 years. This case illustrates a type of patient whose reactions and treatments could be informative in other cases of severe food allergy in children.

Date:

Accepted for publication 8 July 1999

To cite this article:

Nilsson C., Öman H., Halldén G., Lilja G., Lundberg M.
© Härfast B. A case of allergy to cow's milk hydrolysate.
Allergy 1999; 54, 1322–1326.

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ISSN 0105-4538

Material and methods

Subject

M.M. is a girl, now about 3 years of age, suffering from a potent and multivalent food allergy. She is an only child and was born at full term. Despite 4 months of exclusive breastfeeding, M.M. revealed acute allergic symptoms, urticaria, and vomiting after her first teaspoon of a milk-based formula (Milkotal). Her hypersensitivity to food developed gradually to include more, not to say most, of the food groups and was combined with a loss of weight. During her first year, she showed asthmatic symptoms when exposed to cow's milk. When M.M., at 10 months of age, reacted with obstructive symptoms even to casein hydrolysate (Nutramigen) and reacted with urticaria to an amino-acid hydrolysate (Neocate), a thorough allergic monitoring was initiated. M.M. was challenged on three separate occasions with Nutramigen, and each challenge resulted in obstructive symptoms. However, no double-blind challenge was performed.

Specific IgE

IgE antibodies (Ab) in serum were tested with the Pharmacia CAP System FEIA, according to the instructions of the manufacturer (Pharmacia & Upjohn Diagnostics, Uppsala, Sweden). Nutramigen ImmunoCAP from MIAB AB (Uppsala, Sweden) was obtained. A value of ≥ 0.35 kU/l (RAST class 1) was considered positive.

Skin prick tests (SPT)

SPTs were performed with pure cow's milk (3 % fat) and pure soy (Sojasemp[®], Semper AB, Stockholm, Sweden) and with the following food extracts from ALK (Copenhagen, Denmark): hen's egg white (1:100), hazelnut (1:100), wheat (1:20), peanut (1:20), codfish (1:20), and green peas (1:20). SPTs for inhalant allergens of rabbit, dog, cat, horse, *Dermatophagoides farinae*, *D. pteronyssinus*, *Alternaria*, *Cladosporium*, birch pollen, timothy, and mugwort were performed with Soluprick (10 HEP, ALK, Copenhagen, Denmark). Moreover, SPT for diluent alone without allergen was included as negative control. In addition, SPTs were performed with boiled and unboiled Nutramigen, Profylac, and Neocate.

IgE inhibition test

Serum samples were mixed and incubated with equal volumes of a serial dilution of Nutramigen (1/10–1/100 000). The mixture was analyzed for Nutramigen-specific IgE with the Pharmacia CAP System, and the inhibition ratio was calculated.

Analysis of serum eosinophil cationic protein (ECP)

Peripheral blood was treated according to the instructions of the manufacturer, and serum ECP levels were measured with the ECP CAP System FEIA (Pharmacia & Upjohn).

Preparation of blood leukocytes and flow cytometry

Intracellular analysis of eosinophils was performed as previously reported (4, 5). The eosinophils were identified by their high scatter signals, expression of CD9, and expression of the EG2-epitope on ECP, as previously described (3). Cell-surface antigens were detected by adding FITC-conjugated monoclonal antibodies (mAb) against CD9 (final concentration: 10 μ g/ml) (clone: P1/33/2) and phycoerythrin-conjugated mAb against CD11b (5 μ g/ml) (clone: 2LPM19c) (DAKO A/S, Glostrup, Denmark) before the FOG treatment. Measurements of intracellular ECP were performed with FITC-conjugated EG2 monoclonal antibodies (40 μ g/ml) (Pharmacia & Upjohn) added to FOG-treated leukocytes. The proportions of immunostained eosinophils were determined by the use of isotype control antibodies to define the cutoff for positively labeled cells, and the quantitative expression was measured as mean fluorescence intensity of the defined positive cell population. The proportion and absolute number of eosinophils were calculated.

Isolation of peripheral blood mononuclear cells (PBMC)

Venous blood was collected into heparinized vacutainer tubes. PBMC were isolated as previously described (9) and diluted to a concentration of 1×10^6 cells/ml in serum-free medium (AIM-V, GIBCO, Life Technologies).

Stimulation of PBMC

For analyses of the net production of cytokines, PBMC (0.5×10^6 cells/well) were cultured in 48-well plates (Costar) for 3 days in the presence of either phytohemagglutinin (PHA) (10 μ g/ml) (Pharmacia & Upjohn) or OKT3 mAb

(Ortho Diagnostics Systems, Rariton Inc, Johnson & Johnson, New Brunswick, NJ, USA) (1/200) in a humidified incubator at 37°C, 6% CO₂. Cell-free supernatants were harvested and assessed in ELISA for presence of cytokines.

ELISA for determination of cytokines

The cytokines IL-4, IFN- γ , and IL-5 were determined in supernatants by sandwich ELISA, according to the instructions of the manufacturers. Monoclonal antibodies to IL-4 and IFN- γ were purchased from Mabtech AB, Stockholm, Sweden. mAbs to IL-5 were purchased from Pharmingen Research Products, San Diego, CA, USA.

Results

Specific IgE

M.M. was tested for IgE Ab on three different occasions: at 12, 22, and 32 months of age (Table 1). Casein was the main allergen. In addition, high levels of IgE Ab against cereals were observed. In addition to the listed foods, M.M. displayed positive reactions to soy, hazelnut, corn, and peanut.

During the follow-up, there was an increase in IgE to peanut, but otherwise the levels were rather constant. Interestingly, the Nutramigen test was positive at both 12 months (0.6 kU/l) and 22 months (1.6 kU/l). A dose-dependent inhibition test revealed specific IgE binding to Nutramigen (Fig. 1). In addition to reacting to food allergens, M.M. was also RAST positive for all the inhalant allergens tested in this way (horse, rabbit, cat, dog, *D. pteronyssinus*, and birch pollen).

SPT

SPTs were performed at 5, 12, 22, and 32 months of age. At 5 months, M.M. had positive SPTs for cow's milk (4+), egg (3+), and soy (2+). Results of SPTs to food allergens at 12, 22, and 32 months of age are given in Table 1. At 12 months of age, a positive (3+) SPT to Nutramigen was observed for both boiled and unboiled extract. The corresponding values with Profylac were 3+ and 4+ and with Neocate 2+ and 3+, respectively. SPTs for inhalation allergens tested at 32 months of age gave the following results: birch pollen (4+), horse and cat (3+), timothy (2+), and dog and rabbit (1+). *Alternaria*, *Cladosporium*, *D. farinae*, *D. pteronyssinus*, and mugwort were negative.

Eosinophil markers

At 12 months of age, the proportion of blood eosinophils was 8.6 and the absolute number 1075/ μ l. The expression of three cellular antigens, the EG2 epitope on intracellular ECP, CD9, and CD11b, in eosinophils was determined with flow cytometry. The values were 36.6, 112.2, and 16.4 mean fluorescence intensity (MFI), respectively. The serum ECP was 13.7 μ g/ml.

Cytokine analysis

The cytokine production of PBMC from M.M. was determined after stimulation with PHA (at 12 and 18 months) and after anti-CD3 stimulation (at 18 months). In addition, PBMC from the parents were also tested for cytokine production when the child was 18 months old (Table 2).

At 12 months of age, the IFN- γ :IL-5 ratio after PHA stimulation was 6.0. At 18 months, the ratio was 2.9. None of the samples displayed detectable levels of IL-4. The parents had considerably higher IFN- γ :IL-5 ratios (Table 2). Moreover, in the PBMC from M.M. at 18 months of age, the amount of IFN- γ and IL-5 produced in the presence of PHA was much higher than that produced in the presence of anti-CD3 (Table 2).

Discussion

Sensitization to cow's milk proteins and epitopes has been found to be highly diverse (11, 15). Interestingly, T-cell

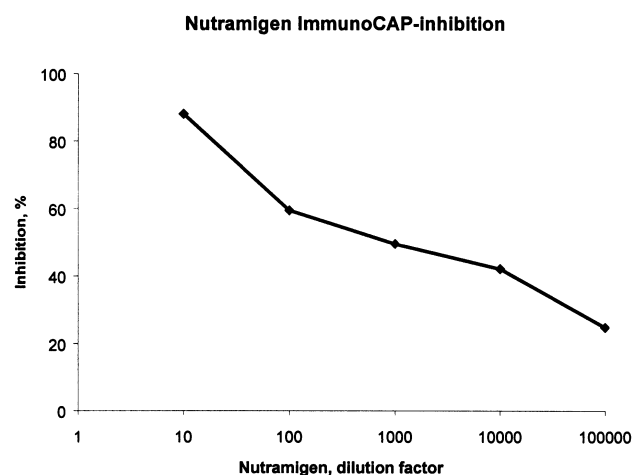


Figure 1. Ability of soluble Nutramigen to inhibit Nutramigen CAP reaction. Serum derived from M.M. at age of 12 months was preincubated with different dilutions of Nutramigen: x-axis. Inhibition of Nutramigen ImmunoCAP-reaction: y-axis.

Table 1. Summary of M.M.'s RAST and SPT to food allergens

Allergen	12 months RAST class/kU/l	SPT*	22 months RAST class/kU/l	SPT	32 months RAST class/kU/l	SPT
Cow's milk	6/>100	5+	5/82	5+	6/280	5+
Casein	6/210	ND	5/82	ND	6/310	ND
β -lactoglobulin	4/43	ND	4/35	ND	5/64	ND
α -lactalbumin	4/23	ND	4/29	ND	5/64	ND
Wheat	6/>100	4+	5/89	5+	5/85	5+
Rye	6/120	ND	5/70	ND	6/130	ND
Barley	4/47	ND	4/48	ND	5/69	ND
Oats	3/8.6	ND	4/32	ND	3/16	ND
Egg	4/34	5+	4/31	3+	4/34	5+
Codfish	0/0	0	0/0	0	0/0	0
Nutramigen	1/0.6	3+	2/1.6	ND	ND	ND

*Interpretation of SPT – 0: no skin reaction; 1+: 25% of histamine wheal; 2+: 50% of histamine wheal; 3+: equal to histamine wheal, 4+: two times histamine wheal; 5+: over two times histamine wheal.

responses after *in vitro* challenge indicate that hydrolyzed milk proteins are much less immunogenic than intact milk proteins (14). Thus, children with cow's milk allergy are frequently given extensively hydrolyzed milk-based formulas, in attempts to eliminate cow's milk allergens. Most children tolerate formulas of extensively hydrolyzed bovine casein or whey proteins, but several cases of adverse reactions to this formula have been reported, including anaphylactic shock (12). Here we show by the IgE inhibition test the presence of IgE Ab to hydrolyzed Nutramigen. In a recent publication, Hoffman & Sampson (6) reported that Nutramigen, although the purest of four formulas analyzed, still contained trace amounts of casein and whey. M.M.'s adverse reaction to Nutramigen was probably due to these impurities.

Children experiencing adverse reactions to hydrolysate have successfully been given synthetically produced amino-acid formulas (1, 6). M.M. was also given such a formula, Neocate, but displayed urticaria reactions even to this formula. In addition, M.M. had positive (2–3+) SPTs to Neocate. However, as M.M. tested this formula only once, we doubt that the urticaria reactions were due to Neocate.

In addition, we performed cytokine analysis on PBMC from M.M. after stimulation with PHA, in an attempt to determine whether anything special could be detected in the Th1:Th2 balance. In a previous study comprising asthmatic and nonasthmatic children, this ratio was found to be useful in distinguishing between the two groups of children (9). In the present study, we found that the IFN- γ :IL-5 ratio in PBMC from M.M. at 18 months of age was considerably lower than that of her parents. Although this could be an indication of a Th2-skewed response, there are no data available on normal values at this low age. This makes interpretation difficult. However, the IFN- γ :IL-5 ratio declined between 12 and 18 months from 6 to 2.9, thus indicating an enhanced Th2 skewing during this period of time. Interestingly, the concentration of both IFN- γ and IL-5 was much lower after anti-CD3 stimulation than after PHA stimulation. It is known that cord-blood cells proliferate poorly in the presence of anti-CD3 although they proliferate vigorously when challenged with PHA (12). To our knowledge, no studies on the effect of anti-CD3 on PBMC in small children have been performed. Thus, the

Table 2. Cytokine production of PBMC at 18 months of age

Donor	Stimuli	IL-4 (pg/ml)	IFN- γ (pg/ml)	IL-5 (pg/ml)	Ratio IFN- γ :IL-5
M.M.	PHA	<30	1640	571	2.9
M.M.	Anti-CD3	<30	63	<30	–
M.M.'s mother	PHA	<30	2592	114	22.7
M.M.'s mother	Anti-CD3	<30	3165	56	56.5
M.M.'s father	PHA	<30	2295	394	17.1

Cytokines determined as described in Material and methods.

low cytokine production induced by anti-CD3 could be due to the low age.

Our results indicate that M.M.'s peripheral blood eosinophils were in a state of activation since there was an increased number of eosinophils and increased expression of the activity markers EG2, CD9, and CD11b (2) (compared to reference material from healthy adult subjects analyzed in our

laboratory). Suomalainen et al. (13) found ECP levels to increase after cow's milk provocation. However, this increase was restricted to children with skin manifestations and was not found in children with gastrointestinal symptoms.

In addition to the high levels of IgE, the apparently enhanced IL-5 production and the elevated numbers and activity of eosinophils suggest an activated Th2 response.

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