Residual Intestinal Disease After Milk Allergy in Infancy

*Jorma Kokkonen, *Sami Tikkanen, and †Erkki Savilahti

Departments of Pediatrics, University Hospital, *Oulu and †Helsinki, Finland

ABSTRACT

Background: The subsidence of cow’s milk allergy (CMA) has been a subject of controversy. In this study the authors examined whether children with this condition in infancy developed full tolerance or whether they continue to have vague gastrointestinal (GI) symptoms relating to the consumption of milk or dairy products and/or signs of mucosal lesion in the GI tract.

Methods: The authors reexamined 56 10-year-old subjects who manifested CMA before 1 year of age, and compared the results with a group of 204 randomly selected age-matched school children. Fifty-three and 90 subjects respectively attended a reexamination and were evaluated for growth, lactose tolerance, and immunoglobulin A (IgA) and IgG-class antibodies to whole cow’s milk. The subjects reporting milk-related GI symptoms were encouraged to do a 4-week blind elimination-challenge test with 1 week of low-lactose milk flour. Sixteen of the 25 children were able to complete the trial.

Results: Approximately half the study subjects (45%) reported milk-related GI symptoms, whereas the respective figure among the control subjects was 10%. Three of six study subjects and seven of 10 control subjects, although completing the challenge, responded with intestinal symptoms. The growth of the former CMA subjects was retarded compared with the control subjects, and the difference in height was most striking in those subjects still reporting milk-related GI symptoms. However, all subjects had normal hemoglobin and whole-blood folic acid levels. The CMA subjects had significantly ($P < 0.014$) lower concentrations of milk antibodies compared with the control subjects. Lactose malabsorption, defined as high counts in a hydrogen breath test and related clinical symptoms, was found in eight CMA subjects (14%) and six control subjects (3%).

Conclusions: In a certain proportion of subjects with CMA in infancy, GI intolerance seems to persist even after small-dose tolerance has been achieved. The intestinal symptoms and the increased prevalence of lactose intolerance may be caused by a disturbance of the surface epithelial cells—a state to which the authors refer as residual intestinal disease. JPGN 32:156–161, 2001. Key Words: Cow’s milk allergy—Lactose intolerance—IgA-class antibodies. © 2001 Lippincott Williams & Wilkins, Inc.
Helicobacter pylori infection and celiac disease. Those subjects reporting milk-related GI symptoms participated in a blind challenge test with low-lactose milk flour. Our findings suggest that, in a certain proportion of CMA subjects, local reactivity persists in the GI mucosa.

MATERIALS AND METHODS

Patients and Methods

The original enrollment consisted of all children in whom CMA had been diagnosed before 2 years of age in the primary care catchment area of Oulu University Hospital during the years 1986 through 1987. According to Finnish practice, public social insurance provides an allowance if a basic food has to be replaced in the diet. Because of this practice, all patients with milk allergy are registered on a national basis. Of the 2-year cohort including 108 children with milk allergy (0.9% of the total 2-year cohort), we enrolled those subjects whose diagnosis had been confirmed reliably. Sixty-five of the 75 children who fulfilled these criteria were still living in the area, and they were invited to a reexamination that was done in the outpatient clinic of the hospital. The final study group consisted of 56 children (21 girls; mean age, 10.5 ± 0.6 years; age range, 9–11 years), 53 of whom participated personally and three of whom completed a questionnaire regarding the results of the milk challenge tests and tolerance, and GI problems. All children had been on an elimination diet for milk until at least 2 years of age, after which they had been challenged for milk at first twice and later once every year. For the analysis, the study group was divided into two subgroups according to the main symptoms at the onset of CMA: mainly GI symptoms (n = 23, eight girls) and predominantly skin symptoms (n = 30, 11 girls).

A sample of control children was collected by enrolling nine school classes of fourth- and fifth-graders in one urban and one rural elementary school. Of the 245 control children, three had CMA and were included in the study group, and 204 (99 girls; mean age, 10.9 ± 0.8 years; age range, 9–11 years) completed the questionnaire. In the questionnaire, we proposed a lactose tolerance test and serum tests (described later) to be performed on the children if they reported any symptoms that they could link to the use of milk or dairy products, or if they had had recurrent abdominal pain during the last half year. Subsequently, 90 children (49 girls) participated in these studies.

Study Design

The study protocol included a retrospective evaluation of a clinical follow-up based on a detailed questionnaire completed by the parents; a clinical reexamination performed by one of us (S.T.); a lactose load test, which was performed only if the child had abdominal pain or reported complaints compatible with lactose intolerance (e.g., flatulence, diarrhea, abdominal pain); a controlled blind milk challenge among the subjects who reported GI symptoms relating to milk consumption and/or abdominal pain; and a determination of IgA- and IgG-class antibodies to whole cow’s milk and IgA-class antibodies to H. pylori and endomysium.

Diagnosis of CMA

For this study, the diagnosis of CMA was considered reliable if it was based on one of the following three criteria: 1) by IgG class antibodies to whole cow’s milk ≥1 ku/L or skin test (4 mm wheal or more) and at least one elimination-challenge test, 2) one positive elimination-challenge test performed in the hospital, or 3) two elimination-challenge tests performed at home. A positive challenge was defined as recurrence of the patient’s former complaints.

Blind, Placebo-Controlled Milk Challenge

The blind milk challenges were done at home after 2 weeks of total milk protein elimination. Rice flour was used as a carrier. The test powders contained either plain rice flour or a mixture of half rice flour and half low-fat, low-lactose milk powder (Valio Ltd, Helsinki, Finland). The flour mixtures were randomized by adding Indian saffron and vanilla sugar to prevent identification by taste or appearance. The instructions advised the subjects to start from one third of the full designated dose for the first day, followed by two thirds on the second day, and continued for five more days. The protein content of the milk powder corresponded to the use of 550 mL pasteurized skimmed milk. The subjects kept a daily diary about their skin and GI symptoms, and their intensity.

Lactose Absorption

The ability of the subjects to digest lactose was determined by measuring their end-alveolar hydrogen concentrations every half hour for 4 hours after the ingestion of 2 g/kg body weight lactose in 250 mL water and by monitoring their symptoms for the next 24 hours. The subjects were classified as having lactose malabsorption if their breath hydrogen concentrations increased by more than 20 parts per million and they reported diarrhea, flatulence, and/or abdominal pain during the follow-up. The lactose load test was performed in those who reported GI symptoms when they ingested unrestrictedly milk or dairy products, or reported clinically important GI symptoms (two or more bouts of abdominal pain per week and/or continuous loose stools or diarrhea) unrelated to the use of dairy products. The test was performed on 23 study subjects and 37 control subjects. Moreover, we checked the results of the subjects by informing on the questionnaire if a lactose load test had been performed previously. Two study subjects and one control subject had in the test a blood glucose increment of less than 1.1 mmol/L, and were considered lactose intolerant.

Determination of Cow’s Milk Antibodies

The IgA and IgG isotype antibodies to whole cow’s milk and its specific proteins of β-lactoglobulin (β-LG), bovine serum albumin (BSA), and α-casein (α-CAS) were measured using enzyme-linked immunosorbent assay. Microtiter plates (Linbro; Flow Laboratories, McLean, VA, USA) were coated with either diluted, defatted (1:500), and adapted liquid cow’s milk formula (Tutteli; Valio, Helsinki, Finland) of bovine β-LG (Sigma, St. Louis, MO, USA) at a concentration of 1 μg/mL in
carbonate buffer (pH 9.6) overnight. Diluted (1:40) for cow’s milk (1:20 for β-LG), serum was applied in triplicate to the antigen-coated plates, and in duplicate to the wells of the same microtiter plates coated with a blocking solution (1% sheep serum). The plates were incubated overnight at room temperature. After washing, 75 μL alkaline–phosphatase-conjugated monospecific swine antihuman IgG, IgA, and IgM antisera (diluted 1:200; Orion Diagnostica, Helsinki, Finland) were added, and the plates were incubated for 60 minutes at 37°C. After washing, 75 μL of p-nitro-phenylphosphate substrate (2 mg/mL in diethanolamine buffer; pH 10.0; IT Baker Chemical, Deventer, The Netherlands) was added. The reaction was stopped after 30 minutes with 75 μL 1 M NaOH.

The end product was measured at 405 nm in a semiautomatic photometer (Titrtek Multiscan; Ellab, Helsinki, Finland). The mean value of the two absorbencies for the wells coated with the blocking solution was subtracted from the mean value for the three absorbances in the antigen-coated wells. The levels of antibodies were expressed as percentages of the standard with a very high titer of whole cow’s milk and other specific antibodies.

Serum IgA-class endomysium antibodies were measured using a routine, indirect immunofluorescence method using human umbilical cord tissue as an antigen. A serum dilution of 1:5 was considered positive.

Serum IgA- and IgG-class antibodies to H. pylori were measured using an enzyme immunoassay method. The levels were expressed as relative units, which were derived from negative and high-positive standard serum pools. The values exceeding the mean ±3 standard deviations (SDs) of the control sera of the negative children were defined as positive.

Endoscopic Examinations

With the patient under general anesthesia, upper intestinal endoscopy was performed on two study subjects and one control subject to assess their severe clinical symptoms with an Olympus GIF-XQ 140 (KeyMed, Southend-on-Sea, Essex, U.K.), as is usual in our hospital. Biopsies were acquired for routine histology from the duodenum below the sphincter of Oddi, the antrum of the stomach, and the lower part of the esophagus, each at the most demonstrative site where local pathology was seen.

The data collected from the questionnaires and the clinical findings were analyzed using SPSS (version 7.5) software (SPSS Inc., Chicago, IL). Student’s t test, the χ2 test, and the Mann–Whitney U test were used to estimate the significance of the differences between the subgroups. The study protocol was accepted by the ethics committee of Oulu University Hospital.

RESULTS

Milk Tolerance and GI Symptoms

By the age of 10 years, all but four subjects had become tolerant of at least small amounts of milk without having immediate and severe symptoms. They were considered to have immediate milk intolerance, and they did not use milk protein-containing foodstuffs.

However, 24 study subjects (45%) reported having GI symptoms if they used milk and milk products freely, and were considered GI-intolerant subjects (Table 1). They all restricted their dairy product consumption. The GI and dermatitis-onset subjects equally experienced milk-related GI complaints. Because only 10% of the control subjects reported these symptoms if they used dairy products freely, the difference compared with the study group was highly significant (χ2 = 31.9, P < 0.0001). Moreover, the reported incidence of nonspecific GI symptoms was 1.5 fold in the study subjects (18%) compared with the control subjects (12%).

The age of the acquisition of small-dose tolerance was significantly higher in the GI-intolerant subjects compared with the fully tolerant ones (40.9 months vs. 27.0 months; P < 0.001). However, age at onset of CMA did not differ between the groups (4.7 months vs. 3.7 months respectively).

Milk Protein Challenge

None of the 16 subjects (six study subjects and 10 control subjects) who completed a 3-week double-blind elimination-challenge test reported marked GI symptoms during the elimination week, nor did they have symptoms during the rice flour week. Three of the six study subjects and six of the 10 control subjects who finished the test reported significant GI symptoms during the week encoded to contain milk protein. Loose, mucous stools with flatulence (n = 5) and abdominal pain with abdominal swelling (n = 4) were the main symptoms caused by low-lactose milk flour. The time since initiating the challenge ranged from 2 to 5 days.

Lactose Intolerance

On the questionnaire, 17 study subjects (30%) and 18 control subjects (9%) reported lactose intolerance (Table 2). This difference is also highly significant (P < 0.0001). However, according to a lactose load test and clinical symptoms, eight CMA subjects (14%) and six control

TABLE 1. Gastrointestinal symptoms in study subjects with CMA in infancy and control subjects at 10 years of age

<table>
<thead>
<tr>
<th></th>
<th>GI-onset (n = 25)</th>
<th>CMA (n = 56)</th>
<th>AD-onset (n = 31)</th>
<th>Controls (n = 203)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk ingestion-</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>related GI symptoms</td>
<td>12 (48%)</td>
<td>25 (45%)</td>
<td>13 (42%)</td>
<td>21 (10%)</td>
</tr>
<tr>
<td>Other GI symptoms</td>
<td>4 (16%)</td>
<td>10 (18%)</td>
<td>6 (19%)</td>
<td>25 (12%)</td>
</tr>
<tr>
<td>No GI complaints</td>
<td>9 (36%)</td>
<td>21 (37%)</td>
<td>12 (39%)</td>
<td>158 (78%)</td>
</tr>
</tbody>
</table>

* χ2 = 31.9, P < 0.0001.
subjects (3%) could definitely be judged as having clinical lactose intolerance, and the difference was highly significant ($\chi^2 = 10.9$, $P < 0.001$).

**Growth and Nutritional Status**

The mean SD in height among the study subjects at 10 years of age was $-0.21 \pm 1.2$ SD and among the control subjects was $+0.39 \pm 1.6$ SD ($P = 0.02$). The GI-intolerant subjects showed a trend to a more severe height retardation compared with the tolerant ones ($-0.5$ SD vs. $+0.1$ SD on average; $P = 0.09$). Relative weight was equal among the tolerant and intolerant subjects, and the control subjects.

The mean hemoglobin level of the patients was significantly higher than that of the control subjects (136 ± 8 g/L vs. 130 ± 7 g/L; $P < 0.0001$). The concentrations of whole-blood folic acid were similar in both groups.

**Antibodies to H. pylori and Endomysium**

Only four subjects of the 145 tested (3%), one with CMA and three control subjects, were positive for IgA-class H. pylori antibodies. All were assessed for H. pylori infection and were treated accordingly. None of the study subjects or the control subjects showed endomysium antibodies.

**Levels of Cow’s Milk Antibodies**

As demonstrated in Figure 1, the subjects with CMA in infancy had significantly ($P = 0.014$) lower concentrations of IgA-class antibodies to whole cow’s milk. The difference in the IgG-class antibodies was similar. The patients reporting milk-related GI symptoms showed the lowest titers. The GI-onset subjects had significantly higher levels ($P = 0.04$, Mann–Whitney $U$ test) of IgA-class antibodies to whole cow’s milk than the dermatitis-onset CMA subjects. Conversely, there was no significant difference in the titers of the antibodies to β-LG, BSA, and α-CAS.

Those with high IgE levels (>360 kU/L) at the time of the study showed a trend toward a lower IgA-class antibody ($P = 0.057$, Mann–Whitney $U$ test) to whole cow’s milk than the low-IgE subjects. The concentrations of IgG-class antibodies were equal.

**Endoscopic Findings**

One of the CMA children with growth retardation and prolonged diarrhea showed lymphonodular hyperplasia of the duodenal bulb and lymphoid nodules with germinal centers during histologic examination. Neither she nor any of the other three children studied with gastro-duodenoscopy showed signs of crypt hyperplasia or mononuclear inflammation of the lamina propria, such as that seen in subjects with celiac disease or with infantile cow’s milk protein intolerance. Nor did we find eosinophilic infiltration.

**DISCUSSION**

The main conclusion to be drawn from this study is that a certain proportion of the subjects with CMA in infancy seem to continue to have persistent GI intolerance even after they have developed small-dose tolerance. However, the symptoms of these subjects were mild and vague, and the response to the milk protein challenge came after a few days and with an increased dose. As a sign of this “residual intestinal disease,” the subjects experienced increased incidence of GI complaints, lactose intolerance, and growth retardation.

Reactivity could also be demonstrated by a blind, placebo-controlled milk protein challenge in a majority of the subjects who agreed to take the test. Judged from the reported symptoms, the number of milk reactors may

**TABLE 2.** Self-reported and verified lactose intolerance at 10 years of age in children with CMA and in control subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>CMA patients ($N = 56$)</th>
<th>Control subjects ($N = 204$)</th>
</tr>
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<tbody>
<tr>
<td>Self-reported lactose intolerance, n (%)</td>
<td>17 (30)$^a$</td>
<td>18 (9)</td>
</tr>
<tr>
<td>Verified lactose intolerance with breath test and clinical symptoms, n (%)</td>
<td>8 (14)$^b$</td>
<td>6 (3)</td>
</tr>
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</table>

$^a$ $\chi^2 = 17.2$, $P < 0.001$ compared with control subjects. $^b$ $\chi^2 = 10.9$, $P < 0.001$ compared with control subjects. CMA, cow’s milk allergy.
have been even higher both among the study children and among the control subjects, but those with mild complaints and those who did well with self-diminished, low-lactose dairy products could not be motivated to perform the laborious blind challenge. As far as we know, this is the first study to suggest that CMA in infancy may change over time from general and immediate reactivity to local and delayed reactivity of the GI mucosa. Another interesting conclusion is that GI intolerance to milk was also diagnosed definitely in six control subjects (3%) suggesting that this may be one major reason for GI complaints in school-age children. The same incidence has recently been reported in young adults (9).

Based on the clinical symptoms, milk intolerance at school age may no longer be IgE mediated. As discussed thoroughly by Sampson and Anderson (10) at the conclusion of a consensus meeting on adverse immunologic reactions to foods, there seems to be a wide spectrum of immunologic reactions to foods and dietary products. The non-IgE-mediated reactors are difficult to classify or diagnose by any method. In another study with respective material, we found evidence to suggest that cell-mediated immunity may be active and may cause the symptoms of children with CMA at school age (11). The clinical change seems to follow the general downregulation of IgE-mediated immunity (12).

It was highly interesting that the study subjects designated as GI intolerant according to their persistent symptoms had significantly lower average height than the tolerant ones or the control subjects. In addition, because the definite incidence of clinical lactose intolerance among the study subjects was fourfold, the results suggest that the GI-intolerant subjects have some abnormality in the surface lining (e.g., enterocytic cells). Moreover, the few endoscopic examinations performed confirmed that the subjects with persistent GI symptoms do not have villous atrophy or mononuclear infiltration of the lamina propria, which is compatible with CMA in infancy or celiac disease. Nor did the GI-intolerant patients show any laboratory signs of malabsorption, with the average hemoglobin concentration being even higher than in the control subjects. The difference in the hemoglobin level may be explained partly by the lower intake of dairy products of the former CMA subjects. In summary, the results of this study help us to understand the syndrome of “weak tummy” after CMA in infancy.

The GI-intolerant patients had confused their symptoms with those of lactose intolerance. The definite incidence of lactose malabsorption, based on a positive finding in a lactose load test and clinical symptoms during the next 24 hours, was 14% in the study subjects and 3% in the control subjects, the latter figure being considered true among the Finnish population at this age (13). Although we did not do a breath test in all subjects, we consider that we really diagnosed all true cases of the whole group because, according to the inclusion criteria, we performed the test in all subjects who reported adverse affects—from vague abdominal complaints to full-blown symptoms if dairy products were used. We also consider that the fourfold rate of lactose intolerance in former CMA subjects is true, and further suggest a secondary epithelial cell abnormality. Taken together, these results also suggest that most of the subjects who consider themselves lactose intolerant actually were milk allergic, but reacted at higher doses. A similar overestimation of self-reported lactose intolerance in adults has been reported in adults (14).

Significantly lower concentrations of IgA-class antibodies to whole cow’s milk at the age of 10 years in children with CMA in infancy is probably the result of a low milk consumption throughout infancy and later (15). Although we, unfortunately, did not measure the total IgA concentrations, we found an inverse correlation between the serum concentrations of IgE- and IgA-class antibodies to whole cows milk. The result supports the view, presented recently in the literature, that the down-regulation of IgE-mediated reactivity of infancy against food antigens is associated with a rise of IgA antibody production, and vice versa (16–18). CMA in infancy is often related to transient IgA deficiency, and according to the current findings it seems that the remaining GI-intolerant subjects have the most severe delay in the production of specific IgA-class antibodies.

In conclusion, we found evidence that CMA in infancy, even when treated properly, may persist as a local reaction on the GI mucosa in a certain group of patients. This residual intestinal disease typically seems to involve symptoms of lactose intolerance, recurrent abdominal pain, and relative growth retardation. The persistence of this GI intolerance was associated with low IgA-class antibodies against cow’s milk.

REFERENCES

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