

Short communication

Evidence of very delayed clinical reactions to cow's milk in cow's milk-intolerant patients

Background: In patients with cow's milk protein intolerance (CMPI), delayed clinical reactions to cow's milk (CM) ingestion may be misdiagnosed if the clinical symptoms are not "classical" and there is a long time lapse between ingestion of CM and the clinical reaction. The aim was to evaluate the clinical outcome of CMPI in a cohort of CM-intolerant children, with particular attention to the occurrence of clinical manifestations beyond 72 h after CM challenge.

Methods: Eighty-six consecutive patients (44 boys, 42 girls) with new CMPI diagnoses were enrolled; median age at diagnosis was 4 months. Patients were followed up for a mean period of 40 months. In all patients, CMPI diagnosis was made on the observation of symptoms, their disappearance after elimination diet, and their reappearance on double-blind CM challenge. At CMPI diagnosis, immunologic tests to demonstrate IgE-mediated hypersensitivity were performed. After 12 months of CM-free diet, CM tolerance was re-evaluated with a CM challenge continued at home for up to 30 days, according to a double-blind, placebo-controlled method. Patients who did not achieve CM tolerance continued a CM-free diet and subsequently underwent yearly CM challenge.

Results: The percentages of CMPI patients who became CM-tolerant after 1, 2, and 3 years of CM-free diet were 30%, 54.5%, and 70%, respectively. At the end of the follow-up period, 26/86 subjects showed persistent CMPI; these patients had a higher percentage of positivity of total serum IgE ($P < 0.05$), RAST ($P < 0.01$), and cutaneous prick tests for CM antigens ($P < 0.001$) than all the others. At CMPI diagnosis, all patients had a clinical reaction within 72 h from the beginning of the CM challenge; at the subsequent "cure" challenges, we observed patients who first reacted to CM more than 72 h after ingestion. In total, 10 out of 86 patients showed "very delayed reactions"; in these patients, the mean time between the beginning of CM challenge and the onset of a clinical symptom was 13.3 days (range 4–26 days). The number of "very late reactors" increased from the first to the third of the "cure" CM challenges, performed at yearly intervals. The "very delayed" CMPI manifestations in these subjects were constipation (five cases), wheezing (two cases), dermatitis plus constipation (two cases), and dermatitis alone (one case); in 6/10 patients, the symptoms observed at the "cure challenge" were different from those at CMPI onset.

Conclusions: Very delayed clinical reactions to reintroduction of CM in the diet can occur in CMPI patients; thus, accurate follow-up and frequent outpatient observation in patients with a long history of CMPI are probably more useful and safer than prolonged CM challenge.

**A. Carroccio, G. Montalto,
N. Custro, A. Notarbartolo**

Internal Medicine, University Hospital of Palermo,
Palermo

**F. Cavataio, D. D'Amico,
L. Alabrese, G. Iacono**

I Division of Pediatrics, Ospedale "Di Cristina",
Palermo, Italy

Key words: constipation; cow's milk challenge;
cow's milk intolerance; dermatitis; wheezing.

A. Carroccio, MD
via Coffaro 25
90124 Palermo
Italy

Accepted for publication 7 February 2000

Cow's milk protein intolerance (CMPI) is a very common clinical problem in children (1–3), and diagnosis is based exclusively on clinical observation. In fact, as there are no tests for CMPI with a high enough sensitivity or specificity, symptoms during an elimination diet and on challenge with cow's milk (CM) must be observed (4, 5). CM challenge is therefore very important for diagnosis; it is normally performed in

hospital to allow an objective evaluation of reaction by the medical staff, and because of the risk that administration of CM protein may trigger acute and severe symptoms. Once CMPI has been diagnosed, the reintroduction of CM protein is advised, but not until 9–12 months after commencement of an elimination diet (5). This challenge to ascertain cure ("cure challenge") is also normally begun in hospital and

subsequently continued at home for 1 week. However, in a recent retrospective study (6), we reported that the reaction to reintroduction of CM tends to be delayed in patients with CMPI persisting beyond 4 years of age, and this delay makes it difficult to attribute a specific symptom to CMPI.

The present prospective study was designed to evaluate the frequency of delayed reactions during the "cure CM challenge" by a double-blind, placebo-controlled (DBPC) method.

Material and methods

Patients

We enrolled 101 consecutive patients with new CMPI diagnoses admitted to the II Division of Pediatrics at the Di Cristina Hospital (Italy), between January and December 1995, but only 86 patients (44 boys, 42 girls) completed this study, because 15 were lost during follow-up (eight children moved out of the area, and seven parents refused to participate in the follow-up study). The age range at diagnosis was 15 days–32 months (median 4 months). These patients were followed up for a mean period of 40 months (range 35–47 months).

In all patients, CMPI diagnosis had been made on the observation of symptoms, their disappearance after elimination diet, and their reappearance on CM challenge.

At first visit, 66 patients presented gastroenterologic symptoms (vomiting, chronic diarrhea, gastroesophageal reflux, poor growth, and chronic constipation), seven had respiratory symptoms (rhinitis or wheezing), seven had atopic dermatitis, and six had mixed symptoms (both gastroenterologic and respiratory and/or dermatologic). At the first observation, the following laboratory examinations were performed in all patients: skin prick tests with whole milk and purified milk proteins (PRICK), as well as total serum IgE and IgE antibodies to specific milk antigens (RAST). On diagnosis, full details of family history of atopic diseases were recorded; in particular, we considered to be suffering from atopic disease those patients' parents who presented bronchial asthma, rhinitis, dermatitis, or conjunctivitis, and who had the following characteristics:

- 1) positive skin prick tests and/or RAST for food or environmental antigens (tests performed in our laboratory)
- 2) no evidence of other causes of their diseases
- 3) successful treatment with steroids and/or antihistaminic drugs.

After CMPI diagnosis, patients avoided CM and its derivatives (such as cheese and yoghurt) and took various commercially available hydrolyzed protein

milk-based formulas or soy milk-based formulas. In all cases, the "cure challenge" was performed after at least 9 months of elimination diet (median 10 months) and after patients had achieved satisfactory growth (weight/height above 25th percentile) during the period. Other CM challenges were later performed yearly, all in our hospital, and we always evaluated the results in a double-blind manner.

Informed consent was obtained from all the parents of the patients involved in the study, which was approved by the ethics committee of the hospital.

Methods

Diagnostic milk challenge. The diagnostic challenges were performed in double-blind manner after 4–6 weeks of CM-free diet, with the same procedures and precautions previously described (7, 8). They began in the hospital, with a CM formula or a placebo formula containing the same CM-free milk formula used during the previous 4–6 weeks. A computer-generated randomization scheme was used to determine the challenge sequences, so that both parents and doctors were unaware of the nature of the formula challenge. The challenge was performed beginning with an initial quantity of 5 ml and reaching the total equivalent of a full feed in 3 h. If no reaction was observed, the patients were discharged and continued the challenge at home with bottles coded A or B by a member of the hospital medical staff not involved in this study. The challenge period was 1 week for both the placebo and the CM formula; during this period, the parents recorded any clinical symptoms and described all stools passed by the patient as either loose or solid. The challenge was stopped if a clinical reaction occurred. Patients were re-examined in hospital in the case of any adverse reaction and/or at the end of the challenge period.

The CM challenge test was considered positive if there was an objective observation (vomiting, change in the quality or frequency of feces, erythema, wheezing, or infantile colic) by the medical staff. CMPI was defined as an unequivocal adverse reaction to CM challenge.

Finally, at the moment of the first challenge, 20 infants were exclusively breast-fed, and, in these cases, their mothers, after a CMP-free diet, began to receive the feeds of milk coded as A or B, always according to a double-blind procedure, but no other CM derivatives.

"Cure" CM challenge. Patients underwent CM challenge to ascertain achievement of CM tolerance at yearly intervals. These challenges were begun with the same procedure as the first, "diagnostic" challenge and were then continued at home. In this case, the patients also received several feeds of milk coded as A or B. For infants aged under 15 months,

the bottles contained a formula based on CM; for infants aged over 15 months, feeds contained commercially available whole CM; in both cases, the placebo contained the same milk the infants had received during the CM-free diet period. The challenge period lasted 4 weeks for both the placebo and the CM formula in order to detect also very delayed clinical reactions to CM. Depending on each patient's dietary habits and age, the total amount of milk given to the infants during the 4 weeks ranged from 10 to 22 l. During these 8 weeks, the parents recorded any clinical symptoms, especially those dermatologic, respiratory, and gastroenterologic. The challenge was stopped if a clinical reaction occurred, and the patients were re-examined in hospital. When there were symptoms attributable to diseases other than CMPI during challenge, the test was repeated to exclude the possibility that nonimmediate reactions might be the result of intervening infection or accidental exposure to other environmental allergens.

The end point of a positive challenge was the same as in the diagnostic CM challenge.

To ensure that the infants did not receive any other kind of milk and milk-containing foods during the study periods, parents were provided with a list including all the most common milk-containing foods to be avoided. Furthermore, the parents were able to contact us whenever necessary, and frequent telephone contacts helped to ensure diet adherence.

When there were no clinical reactions to CM challenge, the patients were left on a free diet and followed up for another 3 months. If CM challenge revealed persistent CMPI, the patients remained on CM-free diet for a further year, after which a new CM challenge was performed by the same procedure described above.

Identification of patients according to reaction time after CM challenge. In accordance with Bishop et al. (9), we classified as:

- 1) "immediate reactors" those patients with clinical manifestations (anaphylaxis, eczema, or urticaria) within 45 min after commencement of CM challenge
- 2) "intermediate reactors" patients with symptoms (vomiting or diarrhea) developing within 1–24 h
- 3) "late reactors" patients presenting symptoms (dermatologic, respiratory, or gastroenterologic) 24–72 h after the ingestion of normal volumes of CM.

Furthermore, we identified as "very delayed reactors" those patients who began to show symptoms more than 72 h after the start of the CM challenge.

Laboratory examination. A series of immunologic tests evaluated patient status at CMPI diagnosis. In

particular, serum levels of total IgE were assayed, and RAST was performed with whole CM, β -lactoglobulin, α -lactalbumin, and casein. In addition, skin prick tests were carried out with whole milk and its single proteins.

Serum levels of total IgE were assayed with a commercial kit (Phadebas IgE paper radioimmunosorbent test kit, Pharmacia Diagnostics, Uppsala, Sweden); the upper normal limit for serum IgE varied according to age-dependent cutoffs (for each age class, the cutoff was equal to the mean value plus 2 SD observed in our laboratory in healthy infants of the same age range). IgE antibodies to specific antigens were measured by Phadebas RAST (Pharmacia Diagnostics) and expressed as PRU/ml; values greater than 1.43 PRU/ml were considered positive. Skin prick tests (PRICKs) were performed with commercial kit (Lofarma Diagnostic, Milan, Italy); any wheal diameter that exceeded that of the control and was more than one-fourth the size of the histamine wheal was regarded as positive (a planimeter was used to measure the area of the papule).

Statistical analysis. Frequency analysis was performed with the chi-square test. Two-tailed *P* values <0.05 were considered statistically significant.

Results

One year after CMPI diagnosis, 26/86 patients (30%) became CM tolerant; after 2 and 3 years, 47/86 (55%) and 60/86 (70%), respectively, achieved CM tolerance. Thus, at the end of the follow-up study period, 26 children remained CM intolerant (30%). No difference in age at diagnosis or sex was observed between subjects with persistent CMPI and the other subjects.

As regards the immunologic tests performed at CMPI diagnosis, 37/86 patients had elevated serum IgE, and 40/86 had elevated specific IgE antibodies (RAST) for whole CM and CM proteins; PRICKs for milk antigens were positive in 36/86 patients. When we looked at the distribution of positivity of immunologic tests according to the achievement of CM tolerance, we found a higher frequency of positive tests in patients with persistent CMPI: in particular, a statistically significant difference was observed for the frequency of positivity of serum total IgE (21/60 cases in patients achieving CM tolerance vs 16/26 cases in persistent CMPI patients: $\chi^2=4.185$, $P<0.05$), specific IgE (RAST) (22/60 patients become CM tolerant vs 18/26 in persistent CM intolerants: $\chi^2=6.49$, $P<0.01$), and cutaneous PRICKs (18/60 cases vs 18/26 cases: $\chi^2=9.92$, $P<0.001$).

Family history of atopy in one of the first-degree-related family members was found in 12/60 (20%)

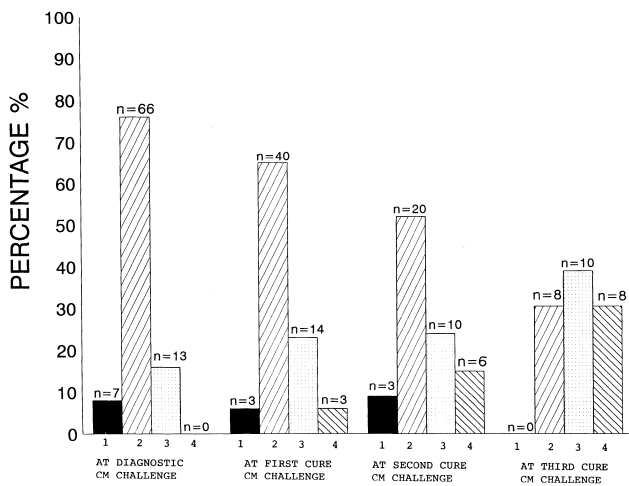


Figure 1. Percentage of subjects with immediate (1), intermediate (2), late (3), and very delayed (4) reactions to CM challenge at diagnostic CM challenge and at "cure" challenge performed after 1, 2, and 3 years of CMPI-free diet.

CMPI patients who achieved CM tolerance and in 20/26 (80%) with persistent CMPI at the end of the study ($\chi^2 = 22.8$, $P < 0.0001$).

Fig. 1 shows the time of the appearance of the CMPI manifestations in the 86 studied patients at the first diagnostic CM challenge and the subsequent yearly "cure CM challenges". As can be seen, at CMPI diagnosis, the great majority of the patients had symptoms within a few hours after ingestion of CM ("immediate or intermediate reactors"), and all had a clinical reaction within 72 h after the beginning of the challenge. At the subsequent "cure challenges", patients reacted to CM over 72 h after ingestion; the number and percentage of these "very delayed CM reactors" increased over time. In fact, we recorded three cases after 1 year of CM-free diet, six cases after 2 years, and eight cases after 3 years. In all the "very delayed CM reactors", the subsequent yearly CM challenges showed either a persistent very delayed reaction or the achievement of CM tolerance. Two of the three "very

delayed reactors" observed after 1 year of CM-free diet also remained CM intolerant after 2 years of CM-free diet, and at that time another four patients showed a similar delayed reaction. Of these six patients, five remained "very delayed reactors" and another three showed very delayed reactions at the CM challenge performed after 3 years of CM-free diet.

In total, 10 out of 86 patients we studied showed "very delayed reactions"; in these patients, the mean time between the commencement of the CM challenge and the onset of a clinical symptom was 13.3 days (range 4–26 days). The "very delayed" CMPI manifestations in these subjects were constipation (five cases), wheezing (two cases), dermatitis plus constipation (two cases), and dermatitis alone (one case). In all cases, these manifestations disappeared when the patients returned to a CM-free diet, and they were symptom-free during the subsequent year of CM-free diet. At the following "cure CM challenge", however, these symptoms reappeared. In particular, in infants presenting constipation, the symptom appeared 7–26 days after the beginning of the CM challenge. In five of these seven patients, anal erythema and edema preceded the onset of constipation; subsequently, hard stools and discomfort on defecation began. In three patients, dermatitis appeared 7–14 days after CM challenge and was characterized by widespread eruptions on the face, inner elbow, and behind the knees. Two patients reacted with wheezing 16–20 days after the beginning of the CM challenge; in these subjects, no environmental antigens as the possible cause of wheezing were identified by PRICK, whereas both PRICK and RAST for milk antigens were positive both at CMPI diagnosis and during the follow-up period. After removal of CM from the diet and β -stimulant aerosol therapy for 2–3 days, wheezing disappeared; during the following year, on CM-free diet, these two patients suffered from one and two wheezing episodes respectively, along with airway infections (presence of fever), which resolved within 3–6 days with steroids and β -stimulants. At the subsequent

Table 1. Clinical characteristics of 10 CMPI patients with very delayed clinical reactions to CM challenge

Case	Sex	Age at onset (months)	Symptoms at onset	Time reaction at diagnostic challenge	Elevated total IgE	Positive PRICK	Positive RAST	Delayed symptoms	Time of reaction at cure challenge (days)	Achieving CM tolerance
1	F	3	Vomiting	Immediate	Yes	Yes	Yes	Wheezing	20	No
2	M	4	Diarrhea	Intermediate	No	No	No	Dermatitis, constipation	7	No
3	F	5	Vomiting, GER	Intermediate	No	No	No	Constipation	8	Yes
4	M	5	Dermatitis	Intermediate	Yes	Yes	Yes	Dermatitis, constipation	14	No
5	M	5	Constipation	Late	No	No	No	Constipation	12	Yes
6	F	2	Vomiting, diarrhea	Immediate	Yes	Yes	Yes	Dermatitis	8	No
7	F	1	Wheezing	Intermediate	Yes	Yes	Yes	Wheezing	16	No
8	M	1	Vomiting, diarrhea	Intermediate	No	No	No	Constipation	26	No
9	M	2	Diarrhea	Intermediate	No	No	No	Constipation	10	No
10	F	14	Constipation	Late	No	No	No	Constipation	12	No

Time of reaction at diagnostic challenge: patients were classified as "immediate", "intermediate", and "late" reactors according to criteria quoted in Methods section. GER: gastroesophageal reflux.

yearly CM challenge, wheezing reappeared, thus confirming the relation between CM protein ingestion and the bronchial reactivity. Table 1 summarizes the clinical characteristics of these “very delayed reactor” patients; it can be seen that in 6/10 patients the symptoms observed at the “cure challenge” were different from those at the onset of CMPI; furthermore, activation of IgE-associated immunity was demonstrable in only four of these 10 patients.

Discussion

Elimination diet and subsequent DBPC challenge is the reference standard for the diagnosis of CMPI, as no single immunologic test can be considered absolutely indicative of CM intolerance (10). The diagnostic CM challenge is normally begun in hospital, whereas the “cure CM challenge” is often begun in the outpatient clinic; if no clinical reaction is observed within the first few hours, both challenges continue at home for a period of 1 week. If no clinical reaction is recorded during this week, the patients remain on a free diet, and the CMPI is considered “cured”. However, we recently reported the clinical characteristics of a small group of children with persistent CMPI beyond 4 years of age (6) and noted, over time, a more prolonged delay between CM consumption and manifestation of CMPI symptoms. In particular, in that study (6), we reported two cases with reaction to CM after 3 weeks of free diet.

The present prospective study, performed on a large sample of CMPI patients, validates and extends the conclusions of our previous study. In fact, in the present cohort of CMPI patients, we observed that a total number of 10/86 children had a clinical reaction during the “cure CM challenge” after 4 days of free diet; furthermore, the tendency for these very delayed reactions increased over time, as the number of “very late reactors” increased from the first to the third of the “cure CM challenges”, each performed at yearly intervals. In these “very delayed reactors”, we recorded a mean time lapse of 13.3 days between reintroduction of CM in the diet and the onset of clinical symptoms. This observation should have an objective weight because we protracted the DBPC challenge until 30 days, and the clinical evaluation of any new symptom or sign was performed at hospital by a physician of our staff; in this way, we should have been able to exclude overestimation of the CM-related manifestations due to parents’ observations, as previously reported (11, 12). However, it is possible that the patients noticed the difference in taste between CM and the placebo formula; thus, the influence of psychological factors could not be completely excluded. Nevertheless, all the very delayed reactions at CM challenge we observed

were objectively reproducible on repeating the CM challenge, and this would confirm the organic nature of the clinical symptoms.

The present study does not identify a distinct group of CM-intolerant patients who have a higher probability of developing delayed clinical reaction to CM; in fact, according to our clinical experience, both patients showing an IgE-mediated immediate reaction at CMPI diagnosis and patients with late – predominantly gastrointestinal – symptoms presented very delayed reactions to CM during the subsequent course of the disease. Thus, evidence of the activation of a specific immunologic mechanism, at the CMPI diagnosis, is not useful in predicting the subsequent appearance of very delayed reactions to the reintroduction of CM. This observation also raises the question of whether IgE played a role in determining CM reaction in any of the very late reactions we observed. The most probable hypothesis is that elevated IgE to CM antigens, at the time of the very delayed reactions, constituted an epiphenomenon and that the immune reaction in these cases was based on other mechanisms: in particular, it is very probable that there was a heightened T-cell reactivity to milk proteins, but, to date, this has not been demonstrated, and further studies must be performed to understand the mechanisms of these reactions. However, we can confirm that laboratory evidence of an IgE-mediated reaction is associated with a higher probability of persistent CMPI over time (13); in fact, those of our patients who did not achieve CM tolerance during the follow-up study period showed a higher frequency of positive RAST and PRICK for CM antigens than patients who became CM tolerant.

As regards the delayed clinical manifestations of CMPI, constipation was the most frequent symptom, being recorded in seven cases. According to our previous observation (14), this symptom is very often delayed after the reintroduction of CM in the diet. However, it is noteworthy that in these children constipation is associated with hard stools and often anal lesions, signs which can be objectively recorded by medical staff, like the other delayed clinical manifestations of CMPI we observed (wheezing and dermatitis). The data of this study also confirm the pathomorphism of CMPI; in fact, in 6/10 patients with very delayed reactions to CM, the symptoms observed at the final CM challenge were different from those observed at the beginning of the study. An identical changing pattern was observed in 16 out of the 26 patients with persistent CMPI at the end of the 3-year study period (data not shown).

As regards the natural history of CMPI in the cohort of patients we studied, our results confirm previous observations. In fact, after 3 years of observation, 30% of children had persistent CMPI, a result very similar to that recorded by Schrandt et al. (3) and lower than that recorded by Bishop et al. (9).

In conclusion, we must emphasize that the DBPC design of the cure challenge in this study is not useful in clinical practice because it is too cumbersome and time-consuming; all the delayed CMPI manifestations we observed can be easily recognized by physicians if they bear in mind that the symptoms we reported may be due to CMPI and may also appear after several weeks of free diet. We feel that after a standard CM challenge (1-week challenge), a careful follow-up and frequent

outpatient observation of the reintroduction of CM in the diet in patients with a long CMPI history are more useful and safer than prolonged CM challenge.

Acknowledgment

This work was supported by a 60% research grant to G.M. from MURST, for 1997.

References

1. WOOD C. How common is food allergy? *Acta Paediatr Scand* 1986;**323**(Suppl):76–83.
2. BOCK SA. Prospective appraisal of complaints of adverse reactions in children during the first 3 years of life. *Pediatrics* 1987;**79**:683–687.
3. SCHRANDER JJP, VAN DEN BOGART J, FORGET PP, SCHRANDER-STUMPEL CTRM, KUIJTEN RH, KESTER ADM. Cow's milk protein intolerance in infants under 1 year of age: a prospective epidemiological study. *Eur J Pediatr* 1993;**152**:640–644.
4. GOLDMANN AS, ANDERSON DW Jr, JELLERS WA. Milk allergy I. Oral challenge with milk and isolated milk proteins in allergic children. *Pediatrics* 1963;**32**:425–431.
5. ESPGAN Working Group for the Diagnostic Criteria for Food Allergy. Diagnostic criteria for food allergy with predominantly intestinal symptoms. *J Pediatr Gastroenterol Nutr* 1992;**14**:108–112.
6. IACONO G, CAVATAIO F, MONTALTO G, SORESI M, NOTARBARTOLO A, CARROCCIO A. Persistent cow's milk protein intolerance in infants: the changing faces of the same disease. *Clin Exp Allergy* 1998;**28**:817–823.
7. IACONO G, CARROCCIO A, CAVATAIO F, MONTALTO G, CANTARERO MD, NOTARBARTOLO A. Chronic constipation as a symptom of cow milk allergy. *J Pediatr* 1995;**126**:34–39.
8. IACONO G, CARROCCIO A, CAVATAIO F, et al. Gastroesophageal reflux and cow's milk allergy in infants: a prospective study. *J Allergy Clin Immunol* 1996;**97**:822–827.
9. BISHOP JM, HILL DJ, HOSKING CS. Natural history of cow milk allergy: clinical outcome. *J Pediatr* 1990;**116**:862–867.
10. BOCK SA, SAMPSON HA, ATKINS FM, et al. Double-blind, placebo-controlled food challenge as an office procedure: a manual. *J Allergy Clin Immunol* 1988;**82**:986–997.
11. SAMPSON HA, ALBERGO R. Comparison of results of skin tests, RAST and double blind, placebo controlled food challenges in children with atopic dermatitis. *J Allergy Clin Immunol* 1984;**74**:26–33.
12. BOCK SA, ATKINS FM. Patterns of food hypersensitivity during sixteen years of double-blind placebo controlled food challenges. *J Pediatr* 1990;**117**:561–567.
13. HILL DJ, FIRER MA, BALL G, HOSKING CS. Natural history of cow's milk allergy in children: immunological outcome over 2 years. *Clin Exp Immunol* 1993;**23**:124–131.
14. IACONO G, CAVATAIO F, MONTALTO G, et al. Intolerance of cow's milk and chronic constipation in children. *N Engl J Med* 1998;**339**:1100–1104.