

Short communication

Allergy to cow's milk proteins in mother's milk or in hydrolyzed cow's milk infant formulas as assessed by intestinal permeability measurements

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E. Barau, C. Dupont

Hôpital Saint Vincent de Paul, Paris, France

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Edith Barau
Hôpital Saint Vincent de Paul
82 Avenue Denfert Rochereau
75674 Paris
Cedex 14
France

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An intestinal permeability test analyzing the differential urinary elimination of lactulose and mannitol orally ingested at the same dosage allowed us to establish a significant correlation between alterations of intestinal permeability and ingestion of reputedly hypoallergenic foods, breast milk, and hydrolyzed protein formulas in some infants with cow's milk allergy. In all, clinical disappearance of symptoms was observed after removal of milk from the mother's diet and/or elimination from the child's diet of any cow's-milk-based hypoallergenic formula.

Some children intolerant of milk, the most common allergen source in infancy, appear not to tolerate the minute amounts of cow's milk proteins contained in their mother's milk (7, 9, 14, 15, 24). Some of them also happen to be highly reactive to cow's milk substitutes in which the protein part has been hydrolyzed to reduce its allergenicity (1, 2, 6, 19).

An intestinal permeability test (IPT) analyzing the differential urinary elimination of lactulose and mannitol orally ingested at the same dosage allowed us to establish (4) a significant correlation between ingestion of milk and alterations of intestinal permeability in infants with cow's milk allergy (CMA) and the usual absence of modifications observed in the same patients when provocation tests are performed with a hypoallergenic formula, such as one based on a soy and beef collagen hydrolysate (5).

We demonstrate in this study that in children reacting to ingestion of their mother's milk, or of reputedly hypoallergenic hydrolyzed protein milk substitutes, the clinical adverse effects of these foods are correlated with their ability to induce alterations of intestinal permeability during provocation procedures.

Material and methods

Intestinal permeability studies

IPTs were performed according to the method previously described (4). On the morning following an overnight fast of at least 6 h and after voiding overnight urine, subjects were given a 1.001 mmol/l aqueous solution of mannitol and lactulose at a dosage of 0.1 g/kg of body weight for each sugar. During the following 5 h, all urine, including that passed at the end of the 5 h, was collected, and its lactulose and mannitol content was analyzed by gas chromatography. IPTs were repeated twice. During the fasting IPT, the solution of markers was given alone, and subjects were allowed only glucose water during the third, fourth and fifth hours of urine collection. During the following day, a provocation IPT was performed in similar conditions, the solution of markers being given mixed with 100 ml of breast milk or of the formula to be tested.

The ratio of the urinary concentrations of oral lactulose and mannitol (L/M ratio, %) was determined. For the provocation IPTs, the results were

expressed by a ratio comparing the L/M ratio of the provocation IPT (P) with the L/M ratio of the fasting IPT (F) of the same subject: P/F. A provocation IPT was considered to be abnormal if P/F was > 1.1 ; i.e., the mean + 2 SD of P/F in a previously described control population of nonallergic children tested with various foods (4).

Case reports

Case report 1

A 9-month-old girl weighing 6400 g was referred to the outpatient pediatric gastroenterology unit for the diagnosis of failure to thrive. She had been breast-fed because of a previous diagnosis of CMA in an elder brother. At age 6 months, a single attempt of feeding fish resulted in atopic dermatitis lesions, and at age 8 months, feeding a hypoallergenic formula (Pregestimil®, Mead Johnson) induced bouts of diarrhea. She was therefore maintained on a strict elimination diet, according to which, in addition to breast milk, only potatoes, wheat flour, chicken, and fruit were allowed. Prick tests were positive (wheal diameter 3 mm larger than positive control) with cow's milk, casein-based infant formulas, various dairy products, egg white, and tomato, whereas RAST (Cap System® Pharmacia) was negative. A fasting IPT revealed an abnormal L/M ratio, 5.57%, suggesting chronic small intestinal suffering, in good agreement with the persistence of loose stools. The mother was asked to eliminate dairy products from her own diet, and the fasting L/M ratio returned to normal: 2.36%. A provocation IPT was thereafter undertaken with the mother's breast milk, after a 3-d normalization of her own diet (Fig. 1). Ingestion of mother's milk was associated with a raised L/M ratio, 2.95% (P/F = 1.25). At age 14 months, progressive weaning with a hypoallergenic substitute (Alfaré®, Nestlé) resulted in the recurrence of loose stools together with abdominal distention. A provocation IPT with Alfaré gave an abnormal response, with a P/F value of 1.53. Gastrointestinal symptoms resolved after elimination from the diet of hydrolyzed-protein-based formula.

Case report 2

A 4-month-old boy on exclusive breast-feeding was referred to the outpatient pediatric gastroenterology unit for chronic urticaria caused by CMA, as evidenced by giant urticaria after first ingestion of a milk-based adapted infant formula. Blood testing revealed a positive RAST for cow's milk. Despite exclusive breast feeding and complete elimination of cow's milk proteins from the child's diet, the chronic urticarial eruption remained unchanged. A fasting IPT revealed a normal L/M ratio: 3.31%. Provoca-

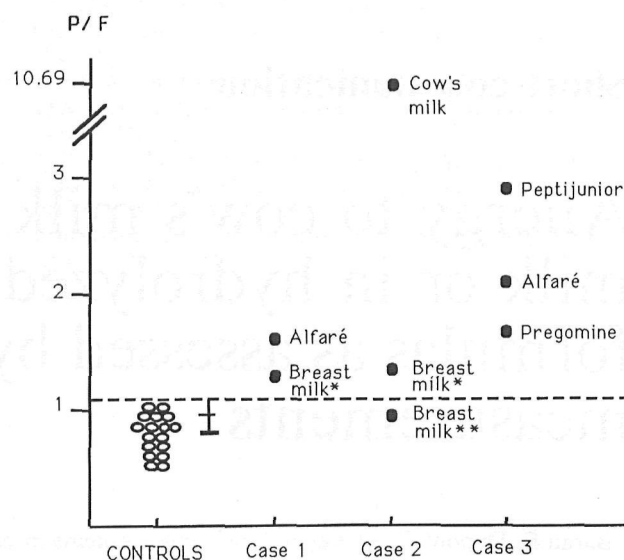


Fig. 1. Provocation intestinal permeability tests performed in 17 healthy infants with various foods (CONTROLS) and in three infants with active CMA tested with mother's breast milk (*mother on normal diet; **mother on cow's milk protein elimination diet), cow's-milk-based infant adapted formula, and various hydrolyzed-protein-based formulas. Results are expressed as P/F, P being lactulose/mannitol urinary ratio (L/M ratio) of provocation IPT, and F being L/M ratio of fasting IPT in same child. Provocation IPT was considered to be positive for P/F > 1.10 ; i.e., mean + 2 SD of P/F in controls.

tion with an adapted formula induced a dramatic increase of P/F: 10.69 (Fig. 1). A provocation IPT with the mother's milk when the mother was on normal diet was also positive: P/F = 1.28, whereas a provocation IPT performed with the mother's milk after elimination of dairy products from her own diet for 3 d yielded a P/F value in the normal range: 0.97. Urticaria disappeared completely as soon as the mother eliminated cow's milk proteins from her own diet.

Case report 3

A 3-month-old girl was referred to the pediatric gastroenterology unit for rectal bleeding associated with poor growth. Rectal bleeding had started at age 4 weeks. In the absence of any pathogens in the stools, the diagnosis of CMA was suggested and prompted a hypoallergenic diet, based on a soy and collagen hydrolysate formula (Pregomine®, Milupa), which did not lead to any significant improvement. On admission, the laboratory work-up revealed a clear decrease in the plasma hemoglobin level (7.9 g/dl) and a positive RAST for cow's milk. Iterative IPTs revealed a normal fasting L/M ratio, 3.67%, together with abnormal provocation IPTs results, the P/F value being increased when provocation was performed both with Pregomine, 1.62, and with a

wey protein hydrolysate formula (Peptijunior®, Nutricia), 2.87. The girl was therefore put on a milk- and soy-free diet in which the milk substitute was another whey protein hydrolysate (Alfaré, Nestlé). An immediate, significant improvement was observed, soon followed by relapse to difficult feeding and abdominal distention. A third provocation IPT was performed with Alfaré, with an abnormal P/F value of 2.03. All milk substitutes were dropped with adequate replacement of vitamins and minerals, leading to the disappearance of all clinical symptoms.

Discussion

When IPTs are performed in healthy children, provocation with food induces a slight decrease in the L/M ratio, as compared with the fasting test, resulting in a mean P/F value of 0.85 ± 0.15 . On the basis of these results, we defined an individual criterion of positivity for the provocation IPT: $P/F > 1.10$; mean $+ 2$ SD of controls. This upper limit confers on the provocation IPT a good specificity: in controls, P/F never exceeded a value higher than 1.1, whatever the food chosen for the provocation test (4). In infants with active CMA, cow's milk ingestion during a provocation IPT is associated with an increased permeability to lactulose, resulting in an increased L/M ratio, as compared with the fasting IPT, and a P/F value > 1.1 (4). In the same population, ingestion of hydrolyzed protein substitutes generally results in a P/F value comparable to that observed in controls (5). We present three infants with active CMA in whom reputedly hypoallergenic milk substitutes, mother's milk, and hydrolyzed protein formulas still exhibited the ability to trigger intestinal adverse reactions, demonstrated through intestinal permeability studies and necessitating either the complete elimination of cow's milk from the mother's diet or the elimination from the infant's diet of hydrolyzed protein formulas.

The presence of betalactoglobulin in breast milk, suggested decades ago, has been demonstrated recently in several studies (10, 12, 16, 22). The adverse role of cow's milk antigens transferred to the infant digestive tract via the mother's milk was shown to be evidenced in breast-fed babies by vomiting and diarrhea (8), eczema (7), colitis (14), and infantile colic (9, 24). In these observations, as in the present one, it is likely that minute amounts of cow's milk protein are sufficient to provoke intestinal mucosal reactions with various clinical manifestations. In the presence of long-lasting clinical symptoms of CMA, the reputed anallergenicity of breast milk may render an appropriate diagnosis difficult. The IPT is a noninvasive method for monitoring small intestinal mucosal suffering, with a sensitivity previously dem-

onstrated to be higher than that of intestinal biopsies (11, 21). In the present case, the mere removal of cow's milk from the mother's diet led to a complete remission of symptoms.

Modifications of cow's milk formulas to reduce their allergenicity includes the use of hydrolyzed casein (Pregestimil) and, more recently, hydrolyzed whey proteins (Alfaré, Peptijunior) or hydrolyzed soy protein and beef collagen hydrolysate (Pregomine). These hydrolysate-based formulas are generally considered to be safe and are recommended for the dietetic management of children with CMA (18). However, the sensitizing power of casein hydrolysate was demonstrated more than a decade ago in rabbits (20) and in man (13), and anaphylactic reactions to cow's milk protein hydrolysates are now thoroughly documented (1, 2, 6, 19). It has been shown by several authors (3, 17, 18, 23) that cow's milk hydrolysate formulas contain various quantities of immunologically intact milk proteins, as determined by inhibition ELISA, and that some infants show positive reactions to one or all such formulas by epicutaneous testing. Obviously, these epitopes are responsible for the reported explosively anaphylactic reactions. In case 3, intolerance of Pregomine may have stemmed either from the frequent association between soy and milk allergy, as already described (13), or from a cross-reaction between beef collagen, which is a constituent of Pregomine, and cow's milk proteins. In our observations, the absence of therapeutic effect of the replacement of cow's milk proteins by hydrolyzed protein formulas could have led to misdiagnosis of CMA. Repeated IPTs allowed a specific demonstration of the deleterious effect of protein hydrolysate formulas when used in these infants and provided a biologic basis for a replacement diet eliminating all cow's milk derivatives.

In conclusion, our data 1) confirm the alterations of intestinal permeability induced by cow's milk allergens in infants with symptoms of CMA, 2) indicate that in some cow's-milk-reactive infants reputedly hypoallergenic milk substitutes can cause clinical adverse effects correlated with intestinal hyperpermeability alterations, 3) support the validity of such a noninvasive *in vivo* biologic testing of the hypoallergenicity of milk substitutes.

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