

Allergy to nondairy proteins in mother's milk as assessed by intestinal permeability tests

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The intestinal permeability test is a noninvasive method which, when done during a food provocation procedure, can detect the deleterious effect of food on the intestinal mucosa in allergic children. We report on a 1-month-old breast-fed boy with a history of regurgitation, diarrhea, difficult feeding, and malaise suggesting food allergy. Intestinal permeability tests were done with the mother's milk and showed breast-milk-induced alterations of intestinal permeability. No improvement occurred in the child's clinical symptoms or in the results of the intestinal permeability test when the mother withdrew dairy products from her diet. Disappearance of the child's symptoms and normalization of intestinal permeability during provocation with the milk were obtained after elimination of egg and pork from the mother's diet. This observation suggests that dietary proteins different from cow's milk antigens may be transferred to breast milk and induce adverse reactions in hypersensitive infants.

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Intestinal permeability tests (IPTs) analyze the urinary elimination of orally absorbed nonmetabolizable markers of various molecular size. IPTs allow detection of the modifications of intestinal permeability which result from alterations in the intestinal mucosa barrier in various pediatric intestinal conditions such as celiac disease and inflammatory bowel disease (1, 17, 18). IPTs have been shown to be a good tool for the diagnosis of food allergy when studied in basal conditions and after ingestion of the offending food (2, 6). We recently showed that in children with cow's milk allergy (CMA) who do not tolerate the minute amounts of cow's milk proteins contained in their mother's milk (10, 13, 14, 21), ingestion of mother's milk resulted in measurable alterations of intestinal permeability (3).

We present a child with CMA who reacted clinically to ingestion of his mother's milk even after elimination of dairy products from the mother's diet. In this child, symptoms disappeared after elimination of pork and egg from the mother's diet, in good correlation with the normalization of IPT results.

Material and methods

Intestinal permeability studies

IPTs were done according to the method previously described (6). Briefly, after an overnight fast of at

least 6 h, the subject was given a mixture of a 10% mannitol aqueous solution and a 65% lactulose aqueous solution (1001 mosm/l) at a dosage of 0.1 g/kg of body weight for each sugar. During the following 5 h, all urine was collected, merthiolate being added as a preservative. After recording the volume, aliquots were frozen at -20°C until analysis. During the fasting IPT, the solution of markers was given alone, and the subject was allowed only water during the third, fourth and fifth hours of urine collection. On the next day, a provocation IPT was done in similar conditions, the solution of markers being given mixed with 100 ml of breast milk or of the formula to be tested.

Urine was desalted and dried, and trimethylsilyl derivatives of the sugars were prepared. Analysis was performed by gas-liquid chromatography (6). The column temperature was 160°C for mannitol and 240°C for lactulose. Methylglucose and turanose were used as internal standards for mannitol and lactulose, respectively. Results were expressed as the proportion of oral mannitol and lactulose excreted (percent mannitol urinary clearance and percent lactulose urinary clearance) and as the ratio of these values (L/M ratio, percentage). For the provocation IPT, the L/M ratio was expressed by comparison with that of the fasting IPT: provocation L/M ratio/fasting L/M ratio (P/F). A provocation

IPT was considered to be abnormal if P/F was more than 1.1; i.e., the mean $\pm$ 2 SD of P/F in a previously described control population of nonallergic children tested with various foods (6).

Case report

A 1-month-old boy weighing 3.4 kg was admitted for the diagnosis of regurgitations, diarrhea, difficult feeding, and malaise with bradycardia and hypotonia after feeding. He was breast-fed because of a previous diagnosis of CMA in an elder sister.

A 24-h esophageal pH-metric recording showed a gastroesophageal reflux, but clinical symptoms persisted despite appropriate treatment (upright positioning, feed thickening, cisapride, and sodium alginate). A fasting IPT revealed a normal L/M ratio, 3.69%, while provocation with the mother's breast milk induced a dramatic increase of the L/M ratio, 16.52% (P/F: 4.47) (Fig. 1). A provocation IPT with a hypoallergenic formula (Alfaré®, Nestlé) was normal, L/M ratio: 2.39% (P/F: 0.64). The diagnosis of CMA was suggested, and the mother was asked to eliminate dairy products from her own diet. No improvement was observed either in the child's symp-

toms or in the results of the second provocation IPT with breast milk, the L/M ratio remaining still high, 12.11% (P/F: 3.28). A thorough clinical history led to the finding of a high egg and pork content in the mother's diet, and the mother was asked to eliminate also eggs and pork from her own diet. A third provocation IPT with the mother's breast milk revealed a nearly normal L/M ratio, 4.34% (P/F: 1.17) while the child's symptoms disappeared.

The mother then decided to stop breast-feeding. Weaning was done on a strict elimination diet of the implicated foods. At 10 months of age, the child remaining on the same elimination diet, a skin prick test was positive for cow's milk protein (8.5-mm wheal), eggs (17-mm), pork (8.5-mm), and fish (8.5-mm), as compared with a 0-mm wheal for the negative control and a 17-mm wheal for the positive one. At 17 months of age, under the same regimen, blood testing revealed RAST positive for cow's milk (0.63 kU/l) and eggs (0.69 kU/l), but negative for pork (<0.35 kU/l). Provocation with pork triggered the onset of atopic dermatitis lesions after a 2-day delay. After the infant followed a diet containing pork for 1 month, pork withdrawal resulted in the rapid disappearance of these lesions. At 22 months of age, a new provocation with pork was attempted, but it resulted in malaise and hypotonia 1 h after feeding. At the same age, provocation with egg was still avoided because of extensive reactions involving the whole limb during the corresponding skin prick test.

Discussion

The IPT used in this study, which has been extensively studied in the diagnosis of children's CMA (1, 2, 6), is based on the principle of differential absorption. Mannitol and lactulose differ in their molecular size, mannitol having a radius of 0.4 nm and lactulose one of 0.52 nm, and their absorption is thought to be along different pathways. Mannitol passes through the abundant transcellular routes of aqueous pores in the cell membrane, while lactulose passes through the fewer intercellular junctional complexes and extrusion zones at the villous tips (17, 18). In tests of intestinal permeability during provocation with food in healthy children, the L/M ratio slightly decreases, as compared with the fasting test, resulting in a mean P/F value of 0.85 ± 0.15 . This slight decrease is thought to be due to a decrease of intraluminal osmolarity (9, 16, 18, 22) resulting from the dilution of food by the markers mixture. In contrast, in hypersensitive subjects (2), ingestion of the offending food induces a rise in the lactulose crossing the intestinal mucosa, as compared with the fasting test, with a corresponding increase of lactulose urinary excretion and of the

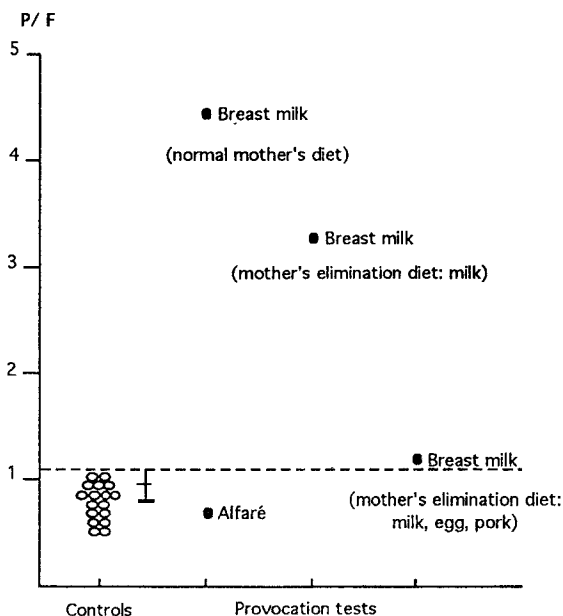


Fig 1 Provocation intestinal permeability tests done in 17 healthy children with various foods (controls) and in child with active cow's milk allergy, tested with Alfaré® and with mother's breast milk, mother receiving normal diet, cow's milk protein elimination diet, or cow's milk protein, egg, and pork elimination diet. Results were expressed as P/F, P being the lactulose/mannitol urinary ratio (L/M ratio) of the provocation IPT and F the L/M ratio of the fasting IPT in same child. Provocation IPT was considered to be positive for P/F > 1.10; i.e., mean $\pm$ 2 SD of P/F in controls.

L/M ratio. In children with CMA, during provocation with dairy products, the increase of the L/M ratio results in a P/F higher than 1.10, i.e., mean $\pm$ 2 SD of controls (6). When tested with protein hydrolysates, children with CMA exhibit a normal P/F value (7), thus demonstrating that these formulas do not alter intestinal permeability in milk-reactive children.

However, in some allergic children, hypoallergenic feeds may reputedly trigger adverse effects, as with protein hydrolysates (4, 5, 8, 19), in which the role of persisting immunologically intact milk epitopes has been suggested, and breast milk (10, 13, 14, 21), for which considerable evidence indicates the transfer of intact maternal dietary proteins. The presence of β -lactoglobulin in breast milk, suggested decades ago, has been demonstrated recently in several studies (11, 12, 20). The role of cow's milk antigens, transferred to the infant digestive tract via mother's milk, has been demonstrated in breast-fed babies with colitis (13) and verified in a previous study on intestinal permeability (3). In these cases, elimination of dairy products from the mother's diet resulted both in an improvement of symptoms and in a normalization of provocation IPTs (3).

In the present study, such intestinal permeability measurements, as well as the clinical history, indicate that dairy proteins might not account for all the clinical consequences of allergy to mother's milk. The normalization of IPT that results only after elimination of egg and pork in the mother's diet suggests that these foods cause both the clinical symptoms and the breast-milk-induced rise of the L/M ratio before the corresponding elimination diet. This hypothesis is further supported by the clinical follow-up, showing positive skin prick tests for cow's milk protein, egg, and pork at 10 months of age, despite a weaning done on a diet strictly eliminating these foods. Moreover, the two provocation procedures with pork done at an age when infant food allergy usually wanes led first to atopic dermatitis, and then to general symptoms such as malaise and hypotonia in the older infant. It is likely that, as recently demonstrated for β -lactoglobulin (11, 12, 15, 20), other dietary proteins, such as those of pork or egg in the present case, may be transferred via the blood from the mother's intestine to breast milk, where they may induce adverse reactions in hypersensitive children.

In conclusion, this is, to our knowledge, the first verification of allergic reactions to nondairy proteins transmitted via mother's milk. In the difficult diagnosis of this condition, the usefulness of the non-invasive IPT may be underlined.

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