

Short communication

Identification of a masked allergen, α -lactalbumin, in baby-food cereal flour guaranteed free of cow's milk protein

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We report on a baby food, cereal flour P (CFP), which, although guaranteed free of cow's milk protein, caused failure to thrive and diarrhea, vomiting, and coughing fits in a 22-month-old child. The purpose of this study was to identify the allergen involved. The investigation used prick tests, RAST, and the RAST inhibition method. Specific IgE was elevated to 100 kU/l for cow's milk and to 15.3 kU/l for α -lactalbumin (2.5 kU/l for casein, 0.7 kU/l for β -lactoglobulin). Antibovine IgG IgEs were associated. RAST inhibition experiments demonstrated the presence of α -lactalbumin in "food-quality" lactose used in this flour, at a dose of 1–5 μ g/g of CFP. The daily intake of α -lactalbumin was found to be less than 70 μ g. This exquisite clinical sensitization was attributed to the intestinal hyperpermeability (IH) which favors the access of milk allergen to the blood, leading to an ever-growing state of hypersensitivity. It could have been due to egg- and mustard-associated allergies as well as to giardiasis and intestinal candidosis. This work underlines the risk of masked food allergens and the need of thoroughly informative labeling.

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The diagnosis of IgE-dependent food allergy is now commonly made thanks to prick tests backed up by the detection of specific IgE by single- or double-blind, placebo-controlled oral challenges (1–3). The study of special cases and the carrying out of challenges has shown that in certain cases the reactogenic dose is extremely small (4–8). Growing interest has consequently been shown in masked allergens, which are present in such small doses in foodstuffs as to be almost undetectable. Masked allergens either are present in traditional culinary preparations, or are the result of involuntary contamination related to the technology of the foodstuffs industry (9–14). Even legally authorized preparations may contain masked allergens, as we show for a baby food, cereal flour P (CFP), guaranteed free of cow's milk proteins. If labeling does not supply any guarantee, the question of a strict checking of this labeling is raised.

Case report

A.G., 22 months old, was referred to the Department of Clinical Immunology and Allergology of the University Hospital in Nancy, in March 1993 for failure to thrive, vomiting, diarrhea, and coughing fits, relapsing several times a week. She was hypotrophic, weighing 9.5 kg (–1.5 SD) for 78 cm in height. A family history of atopy was noted. From the age of 4 months, the infant had presented mild lesions due to atopic dermatitis. She had repeated episodes of infant's diarrhea and colic. She had suffered from angioedema on several occasions after the ingestion of a sauce containing mustard. A checkup in May 1992, at the age of 11 months, revealed a high level of total IgE (600 kU/l), eosinophilia (1400/mm³), and specific IgE of class 3 relative to α -lactalbumin and gluten and of class 2 relative to β -lactoglobulin. Partial villous atrophy

was noted. The infant was given hydrolysate of collagen and soy peptides (Pregomine, Milupa, France), and a strict elimination of gluten and dairy products from the diet was tried for several months. Despite this diet, there was no improvement. In October 1992, biopsy of the mucosa of the small intestine showed normalization of the mucosa and ruled out the diagnosis of celiac disease and lactose intolerance. The diagnosis of allergy to cow's milk proteins was considered.

Material and methods

The food questionnaire which is currently used for the preliminary screening of food allergies was filled out by the parents for 1 week, noting food intake, labeling, and the times at which meals were taken and symptoms appeared.

Biologic investigations included blood cell counts, total IgE, mycologic and parasitologic examination of stools, and a test of intestinal permeability to mannitol (M) and lactulose (L) (normal values: $1.3\% < L/M < 4.1\%$) (15).

Immunoallergologic investigations were as follows. Prick tests were performed with Stallergènes Pasteur extracts (Fresnes, France) for milk, egg, peanut, soy, fish, CFP, and other foodstuffs ingested by the child. Milk and egg proteins were obtained from Sigma (St Louis, MO, USA), and tested at 1 mg/ml. The negative control was carried out with the diluent. The positive control was 9% codeine phosphate. The criterion for positivity was a wheal diameter of 3 mm (1, 16, 17). The RAST Phadebas Cap System (Kabi Pharmacia, Uppsala, Sweden) was applied for milk, egg, mustard, CFP, α -lactalbumin, β -lactoglobulin, and casein.

RAST inhibition tests were performed as described (16). Inhibition of RAST with the three milk proteins by CFP was carried out (100 mg/ml). An inhibiting mixture of 25 μ l of serum and 25 μ l of CFP (100 mg/ml) was incubated for 30 min while stirring. A control mixture was prepared with 25 μ l of serum and 25 μ l of phosphate buffer. RAST was performed twice with each mixture. The inhibition percentage corresponded to the ratio: $1 - (\text{cpm bound to inhibiting mixture} / \text{cpm bound to control mixture})$.

The inhibition of RAST to α -lactalbumin was studied with α -lactalbumin solution in phosphate-buffered saline (PBS) at doses of from 10 to 10^{-7} mg/ml (nine concentrations), and with CFP solution in PBS at concentrations of from 200 to 20 mg/ml (seven concentrations).

The inhibition of RAST to milk was consecutively performed as follows:

- With foodstuff-quality lactose (lactose 1): 96% pure (Société Sucre du Lait, 59 Sains du Nord,

France): 23, 17.25, 11.5, 9.2, 6.9, and 4.6 mg/ml. These quantities correspond to 200 to 40 mg/ml of CFP.

- With pharmaceutical quality lactose (lactose 2): 99% pure (same supplier), at the same concentrations.
- With lactose for biochemical use (lactose 3): <98% pure (Merck no. 7760, Darmstadt, Germany) at 23, 11.5, and 4.6 mg/ml. This kind of lactose is currently used in our department for breath tests, aiming at the diagnosis of lactase deficiency resulting in intolerance to lactose.

RAST to identify antbovine IgG IgEs was performed as follows. A solution of bovine IgG (Sigma no. 5506) was prepared at 5 mg/ml. After 1/50 dilution in carbonate/bicarbonate buffer (pH 9.6), 100 μ l of this solution was pipetted into each well of an Immulon III plate (Dynatech, Oslo, Norway) and incubated overnight at 4°C.

After washing, 100 μ l of serum was added and incubated for 3 h. ^{125} I-labeled anti-IgE (Pharmacia) was added, and the bound radioactivity (cpm) was determined in a gamma counter and related to the total radioactivity to establish the binding capacity.

Three negative controls were tested on a coated well: PBS, serum presenting total IgE at 600 kU/l, and serum presenting cow's milk IgE at class 4. Another negative control was prepared by testing the serum of the child on a noncoated well. A control by a reference RAST inhibition was also prepared, the solution of bovine IgG being used as an inhibitor.

All these tests were done in duplicate.

Finally, an oral challenge test with the incriminated food was not performed, as it was judged unethical. The result of the elimination was controlled through 1 year.

Results

The screening of the food questionnaire confirmed the strict dietary elimination of dairy products and wheat flour. But attention was drawn to the onset of vomiting and coughing fits after use of feeding bottles containing CFP and after the ingestion of a cake containing wheat flour and egg. This flour is guaranteed gluten-free and cow's-milk-protein-free. It contains rice, maize, tapioca, and lactose (11.5%). Feeding bottles containing hydrolysate of beef and soy proteins without CFP were well tolerated.

Total IgE rose to 5000 kU/l and eosinophilia to 2000/mm³. Stool examinations revealed giardiasis associated with candidosis (*Candida albicans*). Hyperpermeability of the intestine was shown by an L/M index raised by 8.2%.

Prick tests were positive with mustard ovomucoid and peanut, and negative with α -lactalbumin, β -lactoglobulin, casein, bovine gammaglobulins, bovine serum albumin, and wheat flour (Table 1). Specific IgE was over 100 kU/l for milk (class 6), 79 kU/l for egg, and 30 kU/l for peanut. Specific IgE for α -lactalbumin reached 15.3 kU/l (casein = 2.5 kU/l, β -lactoglobulin = 0.7 kU/l).

RAST performed to identify antiovine IgG IgEs was positive: the binding capacity of the serum was 1.8%, and the upper limit of normal values was 0.4% in controls. The RAST inhibition rate by bovine IgG was 77%, confirming the presence of antiovine IgG IgEs.

RAST to milk was inhibited by the lactose of flour P from 52% to 75% at the respective concentrations of 4.6 and 23 mg/ml. The inhibition was weaker for the Merck lactose (lactose 3). It was nonexistent for "pharmaceutical quality" lactose (lactose 2) (Fig. 1).

RAST to casein or to β -lactoglobulin was not inhibited by CFP.

RAST to α -lactalbumin was inhibited by α -lactalbumin at the respective concentrations from 10^{-7} to 10 mg/ml reaching, respectively, 14% to 88% (Fig. 2). In the same way, it was inhibited from 2% to 58% by CFP, at the respective concentrations of 20 to 200 mg/ml (Fig. 3). These results indicated that CFP contained about 1–5 μ g α -lactalbumin/g CFP.

Discussion

Allergy to milk in our patient was diagnosed in May 1992 and led to a strict elimination of dairy products. However, the child's condition was not improved, and a considerable increase in total IgE (from 600 to 5100 kU/l) and specific IgE for milk was observed. The sensitization to mustard (salad dressing) and egg could not explain the chronic symptoms since these foodstuffs were ingested only about once a week. Peanut was not consumed. On the contrary, CFP guaranteed free of cow's milk protein was used every day and seemed to induce wheezing cough and vomiting. Therefore, the presence of milk proteins as masked allergens in this flour was suspected, the more so as specific IgE to milk had risen from class 3 to 6, in spite of the elimination of dairy products. This flour contained 11.5% of "food-quality" lactose. The inhibition of RAST with the three milk proteins by CFP confirmed the presence of α -lactalbumin in this flour. Consecutively, RAST inhibition experiments confirmed that "food-quality" lactose is the vector for α -lactalbumin.

Specific IgE to casein and β -lactoglobulin was low and RAST with these proteins was not inhibited

Table 1. Prick tests with foods carried out on A.G., aged 22 months (diameters of wheals [mm])

Control	Negative	β -Lactoglobulin	0
Codeine 9%	4	α -Lactalbumin	2
Mustard	8	Casein	1
Ovalbumin	2	Bovine gammaglobulins	0
Ovomucoid	3.5	Bovine serum albumin	0
Ovotransferrin	0	Beef	0
Lysozyme	2	Wheat flour	2
Egg yolk	0	Peanut	3
		Cod	0

Negative prick tests: maize, rice, beef, veal, pork, lamb, chicken, soy, peas, kidney beans, celery, banana, and apple.

by CFP, indicating that these proteins are probably not present in the flour. The presence of α -lactalbumin was demonstrated, but other proteins, such as serum albumin or bovine immunoglobulins, were suspected. Indeed, the presence of specific IgE to bovine gammaglobulins was confirmed. One evidence of this was the strong RAST inhibition for milk (70%) obtained by 23 mg/ml of lactose (corresponding to 200 mg/ml of CFP). The amount of α -lactalbumin present in this quantity of lactose was far from being enough to account for this inhibition.

The child ingested daily 8.5–14 g of CFP; that is, 8–70 μ g α -lactalbumin. This extremely low reactogenic quantity is comparable to that constituted by the traces of food protein present in breast milk (17–20). It is also comparable to the amount of protein present in vegetable oils (such as sunflower and peanut) of about 200–300 μ g/l. A tiny quantity (7–10 μ g) may trigger atopic dermatitis in the young infant (7, 19, 21). A volume of 0.5 ml of milk has been shown to induce an allergic reaction to a "nondairy" product (5).

The observed hyperpermeability of the small intestine could explain the exquisite sensitivity by its favoring the access of milk allergens to the blood. This hyperpermeability is probably the consequence of food allergy (15) and giardiasis.

Finally, the tiny amounts of α -lactalbumin in CFP were considered to cause the daily symptoms.

A strict avoidance of CFP (in addition to other dairy products, mustard, peanut, and egg) was advised. The child was free of all chronic symptoms when she was readmitted 1 year later as a control. Only rare episodes of angioedema of the lips were observed in the following year.

Since then, the firm producing this preparation of CFP has recently substituted pharmaceutical quality lactose no. 2 for the "food-quality" lactose previously employed. Thus, the study of a peculiar case of food allergy can modify food-processing

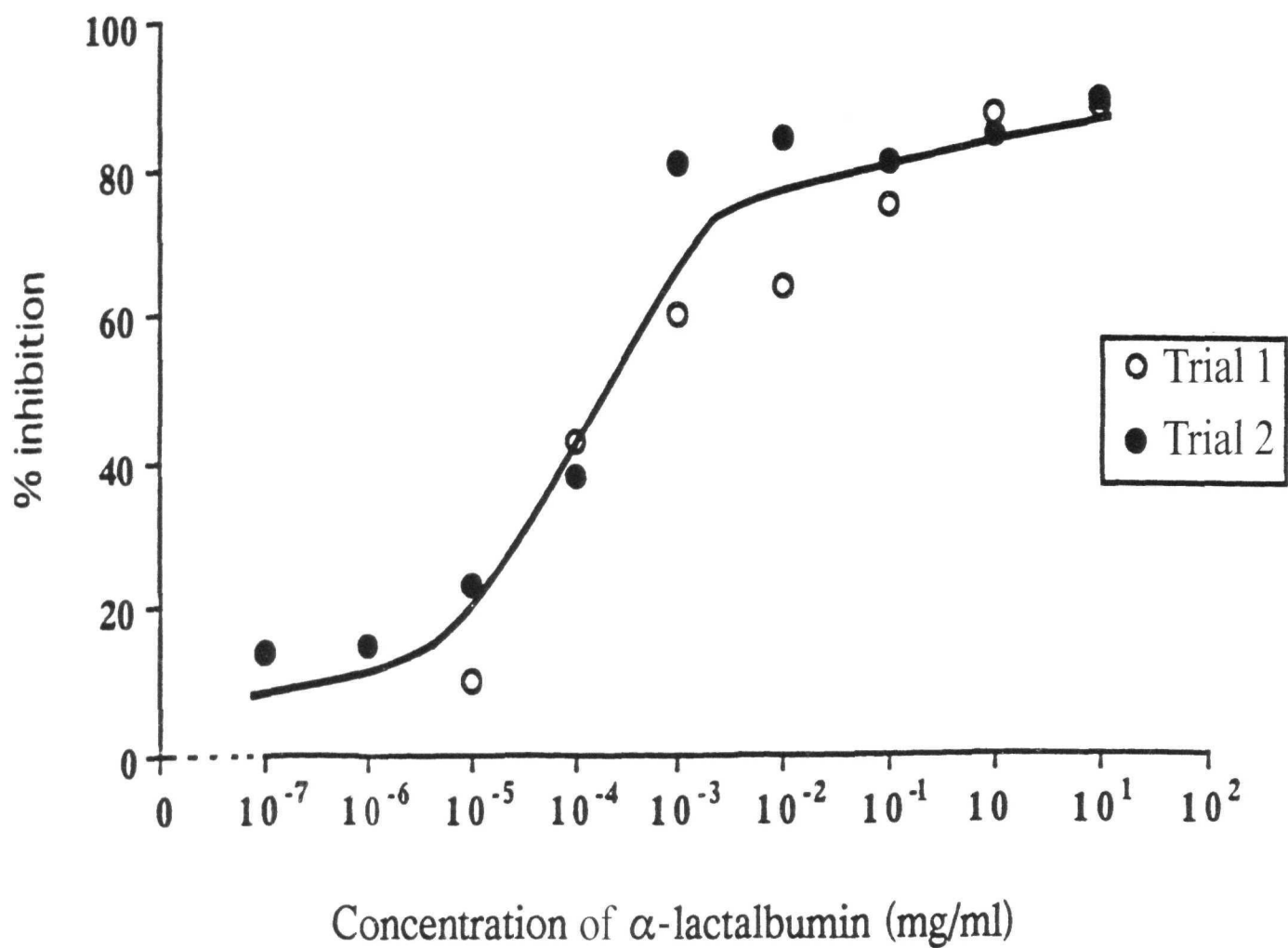


Fig. 1. Inhibition percentage of α -lactalbumin RAST by different concentrations of α -lactalbumin. Inhibition of RAST with α -lactalbumin is linear and proportionate to α -lactalbumin dose for concentration of 10^{-5} – 10^{-2} mg/ml.

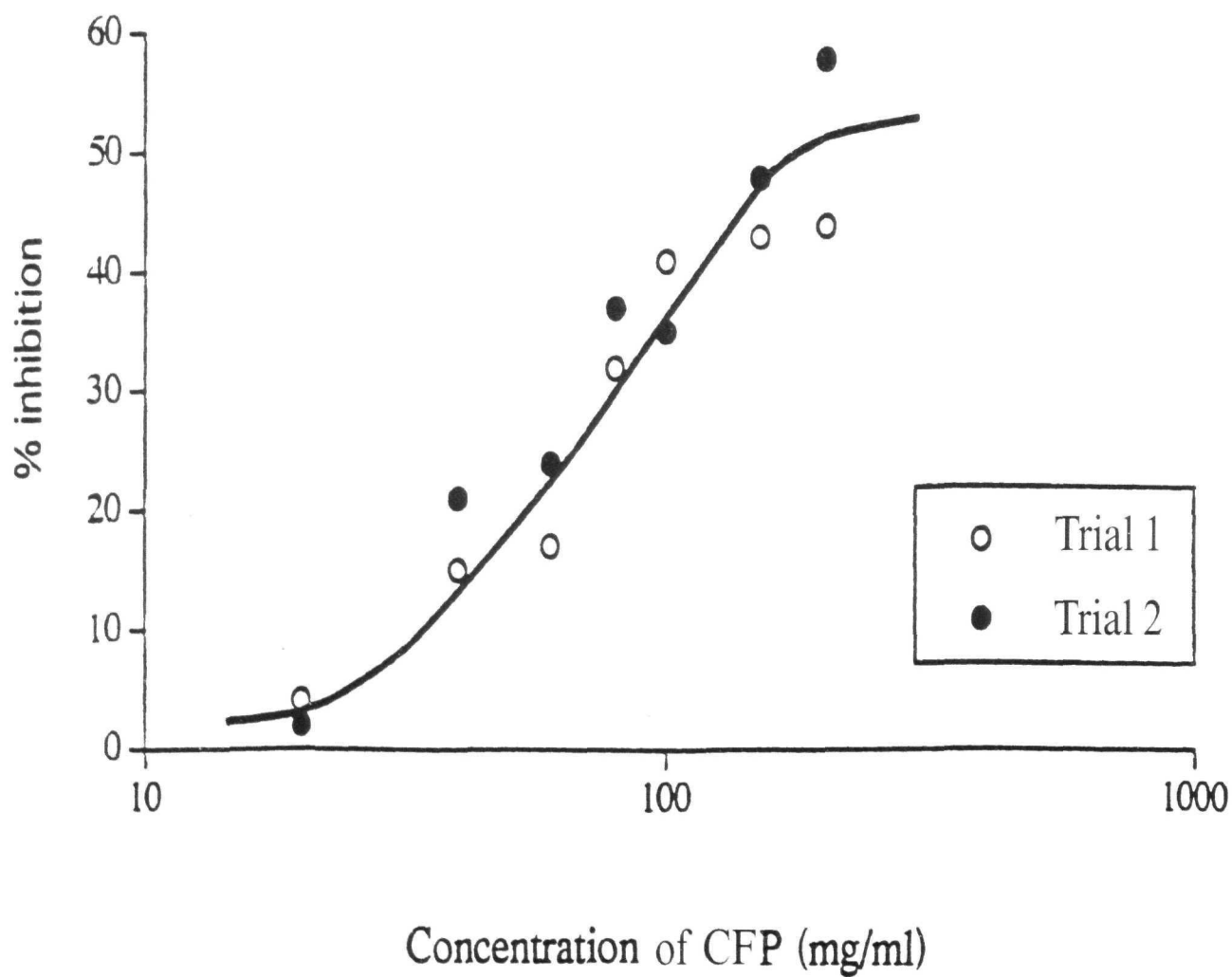


Fig. 2. Inhibition percentage of α -lactalbumin RAST by different concentrations of CFP. Curve like that in Fig. 1 is observed.

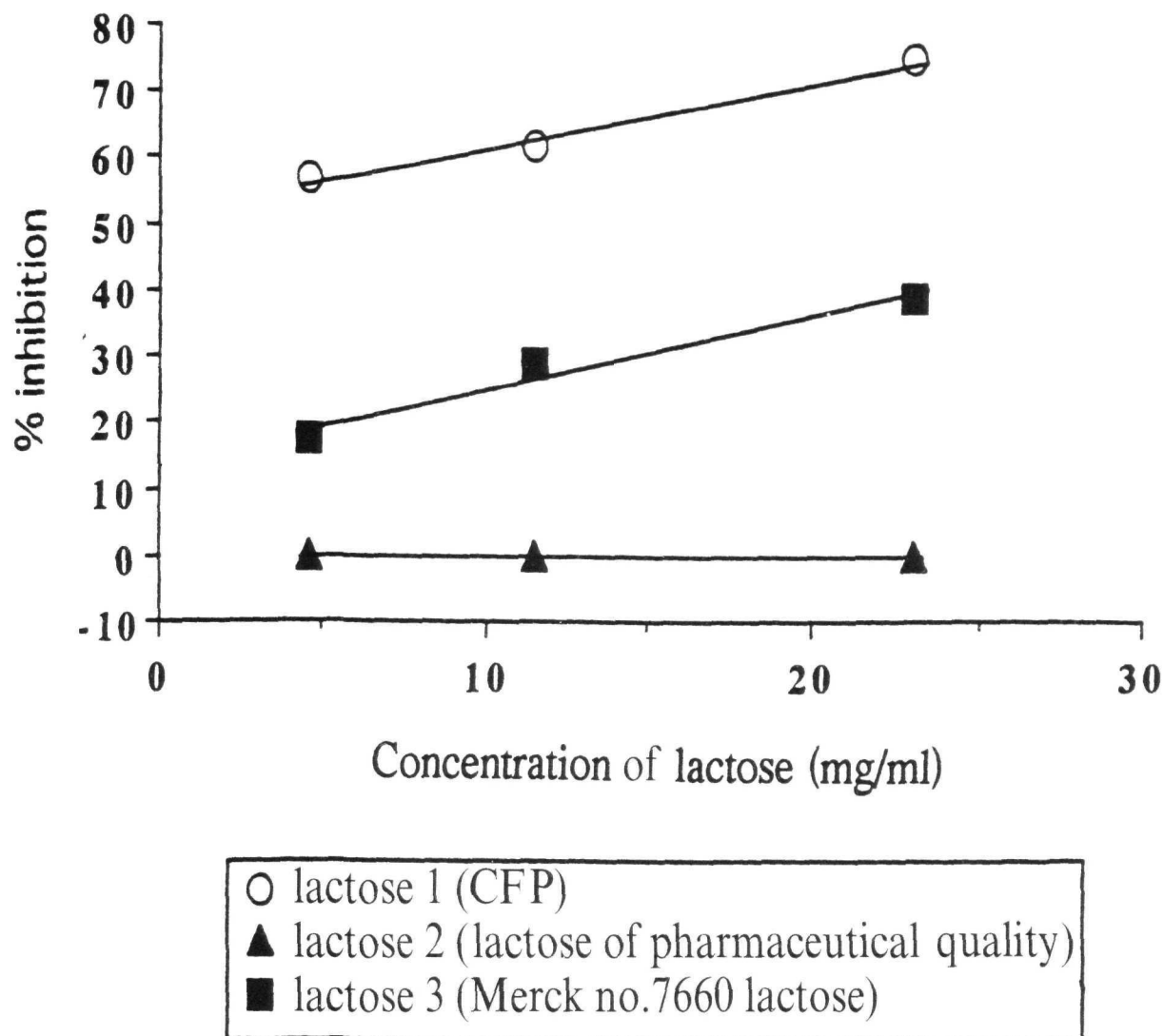


Fig. 3. Inhibition of RAST with milk by three kinds of lactose. Maximal inhibition was observed with lactose included in CFP (lactose 1.96% pure). Pharmaceutical degree of purity was controlled by absence of inhibition for lactose 2 (99% pure), which has since been used by manufacturer. Intermediate inhibition using lactose 3 (<98% pure) demonstrates some contamination by α -lactalbumin. Previously, lactose 3 was used to detect intolerance of lactose, thus excluding allergy to milk proteins.

procedures, contributing to the improvement of public health.

In conclusion, foodstuffs guaranteed free of cow's milk proteins, such as kosher pareve labeled "dairy free" (6), should not contain "food-quality" lactose, especially when the consumer must trust the labeling of so-called hypoallergenic foodstuffs.

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