

Macromolecular absorption and cows' milk allergy

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Abstract

The absorption of macromolecules was analysed by measuring serum concentrations of human α -lactalbumin after feeding human milk, using a competitive radioimmunoassay. The control group consisted of 78 children fed by cows' milk formula; concentrations of α -lactalbumin in their serum were low. The median concentrations in the different age groups varied between 7-13 μg α -lactalbumin/l serum/l human milk/kg body weight. Twenty eight children with cows' milk allergy were studied before introduction of a diet free of cows' milk. Nineteen had gastrointestinal and nine skin symptoms. High serum α -lactalbumin concentrations were found. Only two children had α -lactalbumin concentrations below 100 μg α -lactalbumin/l serum/l human milk/kg body weight. Altogether 76 children on a diet free of cows' milk were studied directly after a cows' milk challenge; 26 developed symptoms. They had significantly higher serum α -lactalbumin values than the 50 children with no symptoms upon challenge. Eighteen of the 26 children with symptoms had serum α -lactalbumin concentrations of more than 100 μg /l serum/l human milk/kg body weight in contrast with two of the 50 with no symptoms. Total serum IgE antibodies were analysed in 43 children. No correlation was found between concentrations of serum IgE and serum α -lactalbumin in the children with cows' milk allergy.

It is generally accepted that antigenic macromolecules can penetrate the small intestinal mucosal membranes in quantities that may be of immunological importance.^{1,2} This transfer is more pronounced at an early age and even more pronounced in preterm infants.³ It is tempting to speculate that the increased absorption of macromolecules in neonates is a natural phenomenon leading to a state of tolerance to common food antigens. Studies in animals indicate that it is easier to induce immune tolerance in younger animals given dietary antigen from birth.^{4,5}

It has been reported that patients with allergic diseases have an increased permeability to macromolecules through the small intestinal mucosa.^{1,6,7} It is possible that an increased absorption of macromolecules may be a part of the atopic constitution. It is also possible that an allergic reaction to food antigens may itself lead to an increased macromolecular absorption.

Methods of studying the permeability of the human intestine are based on determinations of marker molecules either in blood or urine. A

number of marker proteins have been used such as bovine serum albumin, ovalbumin, cows' milk proteins, and horseradish peroxidase. Inert molecules like polyethylene glycols or polyvinyl pyrrolidone have also been used as well as different carbohydrates. Synthetic polymers like polyethylene glycol molecules have a low molecular weight range from 200-2000, which is considerably smaller than most biologically important macromolecules. Besides, the transfer of these substances through the gut does not parallel the transfer of food proteins.⁸

When studying macromolecular absorption in humans with food allergy it seems most logical to use a protein molecule as a marker. When heterologous proteins, like cows' milk proteins, are used as markers local intestinal and systemic immune responses must be considered. This can be avoided by using a human protein as a marker of macromolecular absorption thereby achieving a more reliable measure of gut protein transfer.

In our studies we chose human α -lactalbumin (molecular weight 14 000), which is the dominant whey protein of human milk, as a marker of macromolecular absorption.⁹

The aim of this study was to determine whether absorption of human α -lactalbumin was increased in children with suspected cows' milk allergy, before elimination of cows' milk, and after a cows' milk challenge.

Patients and methods

A total of 78 children fed on cows' milk formula acted as controls; there were 38 girls and 40 boys. They were either healthy children at the child health unit or patients at outpatient clinics who did not have an allergy or gastrointestinal problems.

There were two groups of patients. The first group consisted of 28 children with cows' milk allergy who were studied before introduction of a cows' milk free diet (13 girls and 15 boys). The children were divided in two groups according to their symptoms. Nineteen children had gastrointestinal symptoms (diarrhoea, vomiting, abdominal pain, failure to gain weight) and nine had skin symptoms (urticaria, eczema). All became symptom free and gained weight when treated with a strict diet free of cows' milk; 15 children received soya formula (SojaSemp, Semper), and 13 casein hydrolysate formula (Nutramigen, Bristol-Myers). In all these children the symptoms vanished on a diet free of cows' milk and returned after challenge with cows' milk.

The second group consisted of 76 children

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Accepted 31 August 1990

(40 girls and 36 boys) being treated with a cows' milk free diet: 44 children received SojaSemp and 32 Nutramigen. They were studied immediately after a challenge with cows' milk. The children were divided in two groups according to their symptoms. Fifty two had gastrointestinal symptoms and 24 had skin symptoms. Increasing amounts of cows' milk (1 ml-100 ml) were given during one day. Twenty six children developed symptoms on the challenge and 50 children did not.

All the children were divided into four groups according to age: 2-6 months, 6-12 months, 12-24 months, and older than 24 months.

Capillary blood samples were taken 30 and 60 minutes after intake of preheated (63°C, 30 minutes) human milk from our milk bank. Infants 2-6 months of age received 30 ml/kg body weight and infants above 6 months of age received 20 ml/kg body weight with a maximum of 200 ml.

Total serum IgE was analysed according to Kjellman *et al*¹⁰ in 43 children and specific cows' milk IgE in serum from eight children.¹⁰

A competitive radioimmunoassay was used to measure concentrations of α -lactalbumin in serum.⁹ No interspecies antigenic cross reactivity of human α -lactalbumin against bovine α -lactalbumin has been shown with the Ouchterlony technique, and infants fed cows' milk had no measurable amounts of human α -lactalbumin in serum.⁹ Results were expressed as μg α -lactalbumin/l serum/l given human milk/kg body weight. The detection limit for the radioimmunoassay was 10 $\mu\text{g/l}$ serum. Peak values (at 30 or 60 minutes) were used.

The study was approved by the ethical committee, University of Lund.

Data were analysed by the Mann-Whitney U test.

Results

The serum α -lactalbumin concentrations in the control children were low, and median concentrations in the different age groups were between 7-13 μg α -lactalbumin/l serum/l human milk/kg body weight (fig 1).

In 28 children allergic to cows' milk before the introduction of a cows' milk free diet high

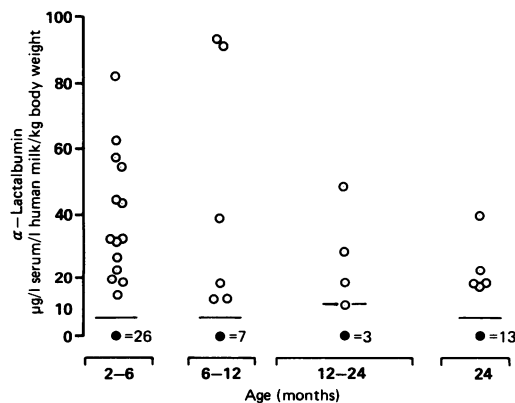


Figure 1 Serum concentrations of human α -lactalbumin after a human milk feed in 78 control children of different ages fed on cows' milk formula. Solid circles denote children with undetectable concentrations of α -lactalbumin; the solid line shows median concentrations.

serum concentrations of α -lactalbumin were found (fig 2). Only two children in this group had α -lactalbumin below 100 $\mu\text{g/l}$ serum/l human milk/kg body weight. The total serum IgE values were analysed in 21 children. The children with high total IgE ($n=10$) had a median serum α -lactalbumin concentration of 146 $\mu\text{g/l}$. The children with normal serum IgE values ($n=11$) had a median concentration of 150 $\mu\text{g/l}$. Cows' milk IgE antibodies were analysed in serum from three children.¹⁰ In two of these children cows' milk IgE antibodies were found in serum. In both the serum α -lactalbumin concentrations were high (197 and 926 $\mu\text{g/l}$). The third child, however, who had no detectable cows' milk IgE antibodies, also had a high serum α -lactalbumin (625 $\mu\text{g/l}$).

Figure 3 shows the results from the 76 children who were studied immediately after a challenge with cows' milk. All were on a cows' milk free diet. The children who had a clinical reaction to the cows' milk challenge ($n=26$) had higher serum concentrations of α -lactalbumin than those who did not react to the challenge ($n=50$). Eighteen out of 26 children with symptoms had serum α -lactalbumin concentrations above 100 $\mu\text{g/l}$ serum/l human milk/kg body

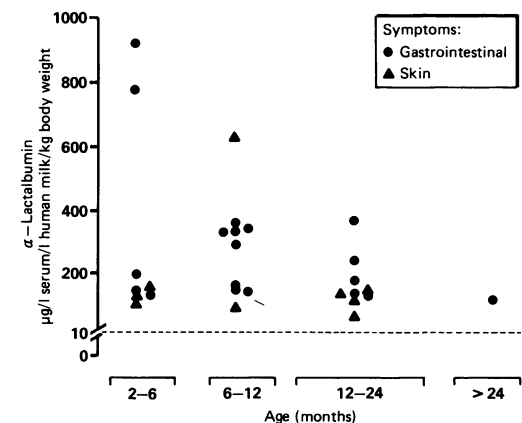


Figure 2 Serum concentrations of human α -lactalbumin after a human milk feed in 28 children with cows' milk allergy before introduction of cows' milk free diet.

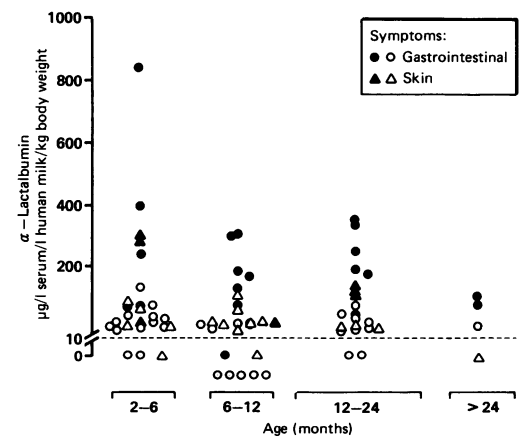


Figure 3 Serum concentration of human α -lactalbumin after a human milk feed directly after a challenge with cows' milk in 76 children suspected of cows' milk allergy. All the children were on a cows' milk free diet. The open symbols denote the symptoms in the children who did not react to the challenge ($n=50$); the solid symbols denote the symptoms of those who did ($n=26$).

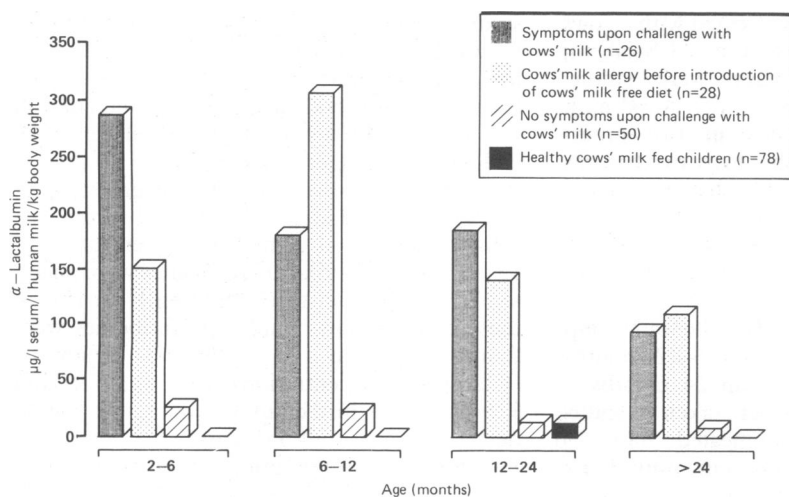


Figure 4 Serum content of human α -lactalbumin (median values) after a human milk feed in all four study groups.

weight in contrast to two out of 50 with no symptoms. One 8 month old boy who did react to the challenge had a serum α -lactalbumin concentration of $<10 \mu\text{g } \alpha\text{-lactalbumin/l serum/l human milk/kg body weight}$. He had previously developed diarrhoea in response to cows' milk. When challenged he developed diarrhoea after the second dose of cows' milk. The diarrhoea disappeared after 12 hours. The boy was said to have cows' milk allergy but was fed with cows' milk again two weeks later without any problems.

The total serum IgE was analysed in 22 children who had a reaction on challenge. Six of these children had high total serum IgE values and a median serum α -lactalbumin of $310 \mu\text{g/l}$. Sixteen children with normal total serum IgE values had a significantly ($p=0.02$) lower median serum α -lactalbumin ($155 \mu\text{g/l}$). The total serum IgE was normal in the 26 children tested who had no reaction upon challenge. Only one of eight children tested who reacted upon the challenge had measurable cows' milk serum IgE antibodies (a boy 8 months of age with a total serum IgE of 80 kU/l and a serum α -lactalbumin of $315 \mu\text{g/l}$).

Figure 4 shows the median serum α -lactalbumin concentrations in all the groups of children. The serum α -lactalbumin in children with cows' milk allergy and children with symptoms upon cows' milk challenge are more than 10 times higher than in the control group and children with no symptoms upon challenge with cows' milk.

Discussion

This study has shown that children with cows' milk allergy, before starting treatment with a cows' milk free diet, have serum concentrations of α -lactalbumin that was more than 10 times higher than those in age matched controls. The concentrations were increased whether the children presented with intestinal or skin symptoms. The highest serum values of α -lactalbumin were found in children with gas-

trointestinal symptoms. Small intestinal mucosal damage caused by the allergic reaction could have been one reason for the increased absorption of α -lactalbumin.

Alternatively increased gut absorption of macromolecules may be present in all individuals predisposed to allergy. We were unable to find any correlation between serum IgE and serum α -lactalbumin concentrations in the children with cows' milk allergy, however, do not have an IgE mediated allergy but a cell mediated immune reaction giving rise to an increase in gut intraepithelial lymphocyte counts and to various degrees of villous atrophy.^{11 12}

There are, as far as we know, no earlier studies on the macromolecular absorption of homologous protein in food allergy. Results from studies using heterologous proteins are difficult to compare with ours as they are influenced by the local and systemic immunological reactions in the patients. Dannaeus *et al* studied 42 children with coeliac disease on food to determine the uptake of ovalbumin in serum after a test meal.¹³ The concentrations of ovalbumin were lower in the children with high antibody values than in those with low values. The antigen concentrations were reported to be significantly lowered after treatment with sodium cromoglycate in six patients. Paganelli and coworkers demonstrated the presence of β -lactoglobulin as well as ovalbumin in a complexed form after a test meal in patients with atopic eczema as well as in normals.¹⁴ Children allergic to cows' milk were reported to have IgA, IgG, IgE, and C_{1q} containing complexes as well as ovalbumin and β -lactoglobulin containing complexes after a cows' milk challenge, whereas healthy controls mostly had IgA and β -lactoglobulin containing complexes after a similar challenge.¹⁵

Our results suggesting increased absorption of protein molecules in the gut are in contrast to findings from studies using low molecular weight markers.^{7 16} Du Mont *et al* studied gastrointestinal permeability in 18 atopic eczematous children compared with 10 control children using sugar mixtures of L-rhamnose and lactulose.¹⁷ A significant difference in gastrointestinal permeability was not demonstrated between atopic and control children. Färlth-Magnusson *et al* studied gastrointestinal permeability in allergic and healthy children after treatment with sodium cromoglycate using polyethylene glycol molecules (molecular weight 400–1000).⁷ There were no significant differences in the first test using polyethylene glycol between healthy and allergic children. However, after treatment with sodium cromoglycate the uptake was significantly lower in allergic children. Jackson *et al* using polyethylene glycols with higher molecular weights found that the absorption of large molecules (molecular weight 4000) was increased in all patients with eczema regardless of the presence of food allergy.¹⁸ This was consistent with increased antigen absorption in atopic disease.

The present study has shown an increased absorption of human α -lactalbumin in children reacting to the cows' milk challenge. This could

be explained in two ways: firstly, the jejunal mucosa in infants with cows' milk allergy could be damaged after exposure to cows' milk, allowing absorption of relatively large amounts of macromolecules. Secondly, an increased macromolecular absorption may be seen in these children even when free of symptoms during a cows' milk free diet.

Savilahti studied the intestinal mucosa after a milk challenge.¹⁹ He showed that at the time of clinical relapse there was a two to four times increase in the numbers of IgA and IgM containing cells in the jejunal mucosa. He suggested that cows' milk was the agent responsible for this local intestinal reaction, which coincided with the development of clinical symptoms. Further longitudinal prospective studies are needed including the repeated analyses of human α -lactalbumin absorption, to elucidate whether increased macromolecular absorption is part of an allergic constitution. Further studies are also necessary to determine whether absorption of human α -lactalbumin can be used as an objective method to measure the reaction to cows' milk after a cows' milk challenge.

In conclusion it has been found that increased absorption of proteins as measured by serum content of human α -lactalbumin occurs in children with cows' milk allergy. Whether the increased macromolecular absorption is a part of an allergic constitution or results from mucosal damage remains to be elucidated.

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