

Serum antibodies against native, processed and digested cow's milk proteins in children with cow's milk protein intolerance

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Summary

Children with or without cow's milk protein intolerance were investigated for serum antibodies to native cow's milk proteins, processed cow's milk proteins and native- and pepsin-digested β -lactoglobulin. Antibodies of IgG and IgA isotypes were determined with ELISA and those of IgE isotype with RAST.

Children who exhibited slowly appearing untoward reactions to cow's milk feeding had significantly higher titres of IgG antibodies against both native and digested β -lactoglobulin than children with an immediate type of reaction or no intolerance. The IgG antibodies to pepsin-digested β -lactoglobulin were efficiently inhibited by native β -lactoglobulin even at low concentrations, which suggests that there were no antibodies specific for the degraded proteins. The children with slow reactions to cow's milk also tended to have higher antibody levels of the IgG and IgA isotypes to both native and processed cow's milk. Antibodies to this mix of antigens discriminated less well, however, than the antibodies to isolated β -lactoglobulin between the groups of children with slowly appearing reactions, with immediate reactions and the controls. Seven out of nine children with an immediate type of reaction to cow's milk protein had IgE antibodies against cow's milk protein determined with the RAST, while this type of antibodies could not be demonstrated in any child in the two other groups. Using processed or digested protein as antigen did not increase the sensitivity of the antibody determinations.

Introduction

Intolerance to cow's milk can manifest itself in various ways. Many of the reactions have immunological pathogenesis or are accompanied by immunological phenomena. Cow's milk can cause allergic disease often seen as immediate reactions. In such patients it is usually possible to demonstrate serum IgE antibodies to cow's milk

proteins and/or a positive skin test [1–4]. Such antibodies have also been shown in very young infants [4]. Other patients may react with symptoms which appear more insidious. In these cases with slow reactions, IgE antibodies are less frequently found. Instead, high titres of IgG and IgA antibodies to cow's milk protein are present [2, 5]. Such antibodies, albeit at lower levels, are regularly found in healthy children and adults [5, 6], which limits their diagnostic value.

In industrialized countries young infants are rarely presented with cow's milk in its native form; the milk is usually processed to a suitable formula. Digestion in the gut may also alter the antigen composition of the milk proteins.

In this study we have investigated the occurrence of antibodies against processed cow's milk proteins and digested β -lactoglobulin in infants with proven cow's milk protein intolerance and in infants with suspected but not proven intolerance.

Materials and methods

Patients

Infants with proven immediate reaction to cow's milk protein. This group consisted of nine children, 2 to 24-months-old, seven less than 12 months, with acute reactions to cow's milk feeding, repeated on three or more challenges. All but one had reactions from the skin appearing within minutes after cow's milk protein had been given. One infant had acute and delayed reactions from the gastrointestinal tract. Eight of the children had a positive skin test to cow's milk, the remaining one had eosinophilia and increased total IgE. At the time of sampling, most of the patients had been given cow's milk only on single occasions. Two infants had been fed cow's milk formula over a period of 4 and 5 days, respectively.

Infants with proven slow reaction to cow's milk protein. This group consisted of six infants, 3 to 10-months-old, with long-standing gastrointestinal symptoms such as diarrhoea and failure to thrive related to the introduction of cow's milk feeding. All improved on cow's milk elimination and symptoms returned at renewed cow's milk feeding at least once. Four of the infants were investigated with regard to the presence of malabsorption and enteropathy (villous atrophy) which could be demonstrated in all. Samples for cow's milk protein antibody analysis were drawn after 1.5–9 months of exposure to cow's milk.

Infants with no proven cow's milk protein intolerance. This group consisted of eight infants, 1.5 to 13-months-old, with gastrointestinal symptoms, loose stools, and five of these also had failed to thrive after the introduction of cow's milk. Three infants improved spontaneously, four improved on a cow's milk protein free diet, but in none of these cases did symptoms reappear when cow's milk was given again after a few weeks. One infant who failed to thrive, and who did not improve after elimination of cow's milk, lived in an unfavourable socio-economic environment and later improved in a foster home. Six of the infants were investigated with regard to malabsorption and enteropathy with normal findings. Serum samples were drawn after 1–11 months exposure to cow's milk.

Antigens

Defatted native cow's milk, defatted humanized formula consisting of spray-dried cow's milk (Baby Semp I, Semper AB, Stockholm, Sweden), β -lactoglobulin A + B (Miles Labs Inc., Kankakee, Ill. U.S.A.), and β -lactoglobulin after pepsin-digestion (for 8, 24 and 48 min) were used as antigens, respectively.

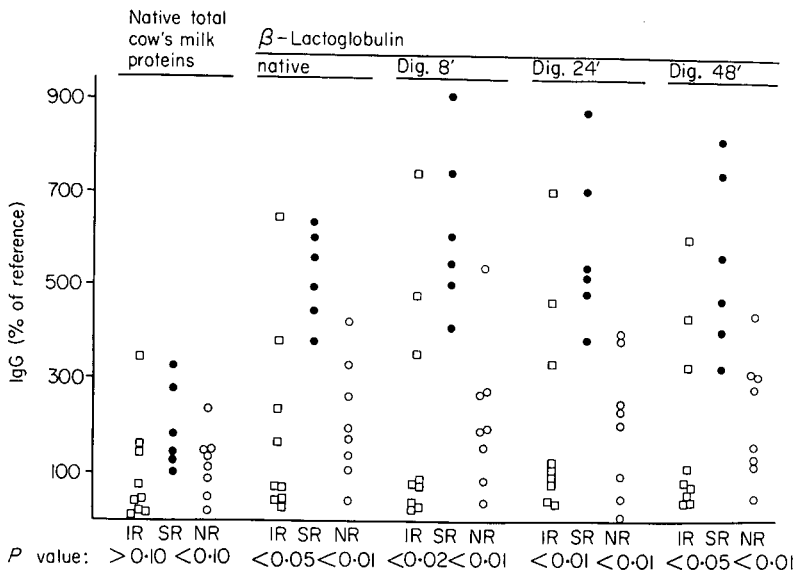


Fig. 1. IgG antibodies to native and pepsin-digested β -lactoglobulin in children with immediate type of reaction (IR), with slow reaction (SR) and without intolerance (NR).

Antibody determinations

Serum antibodies of the IgG and IgA class were determined utilizing enzyme-linked immunosorbent assay (ELISA) as described earlier [5].

ELISA values were expressed as a percentage of a reference serum in the dilution 1/100. IgE values were given as the ratio between sample counts and the background.

For inhibition experiments in ELISA, tubes were coated with native and pepsin-digested β -lactoglobulin (48 min digestion), respectively. Serum samples in dilution 1/500 were pre-incubated for 30 min at 37°C with native total cow's milk proteins and native β -lactoglobulin, respectively, before the mixture was added to the test tubes. The concentrations of the antigens in the inhibition experiments ranged from 10^{-3} to 10 times the concentration used for coating in the ELISA antibody determinations.

IgE antibodies against cow's milk proteins were determined with a radioallergosorbent test (RAST). Defatted native cow's milk and Baby Semp I were coupled to CNBr-activated paper discs [7] and a mixture of the three pepsin-digested fractions of β -lactoglobulin were coupled to CNBr-activated Sepharose (Pharmacia AB, Uppsala, Sweden) according to the manufacturer's instructions. Serum (50 μ l) was incubated with either one paper disc or Sepharose gel (200 μ l) for 4 hr at room temperature. After three washings rabbit anti-human IgE ^{125} I (Phadebas RAST isotope reagents, Pharmacia AB, Uppsala, Sweden) was added and the tubes incubated over night. Following repeated washings the tubes were counted for 2 min in a gamma counter.

Results

Compared with the adult reference serum IgG antibodies to native as well as pepsin-digested β -lactoglobulin were found in considerably higher titres than to the other proteins, especially in infants with slow reaction to cow's milk (Fig. 1). The difference between these patients and the other two groups was statistically significant and was more pronounced for digested than for native β -lactoglobulin. The antibody levels

discriminated well between infants with a slow reaction to cow's milk and those without a proven reaction, while the values showed some overlapping in infants with immediate and slow reactions to cow's milk (Fig. 1).

Antibodies of the IgG and IgA isotypes against native and processed total cow's milk proteins were found in almost all infants in the three groups studied (Fig. 2). In all infants with a slow reaction to cow's milk the IgG titres exceeded the level of the adult reference serum (100% in Fig. 2). Such high antibody levels were also seen in several infants who belonged to the other groups. The range of values showed considerable overlap for the groups and the discriminating capacity was low. The IgA antibodies showed a similar pattern as the IgG antibodies, and the differences between the groups were less pronounced.

The IgG antibodies against digested β -lactoglobulin were inhibited efficiently by native β -lactoglobulin, even at low concentrations (Fig. 3). The inhibition was particularly efficient for antibodies at high titres in sera from infants with proven intolerance. The IgG antibodies against native β -lactoglobulin were likewise efficiently inhibited by native total cow's milk proteins in low concentrations (Fig. 4).

Antibodies of the IgE isotype were found against native cow's milk protein, processed protein and/or β -lactoglobulin in seven out of nine patients with an immediate type of reaction to cow's milk (Fig. 5). Native and processed total cow's milk proteins revealed IgE antibodies in six of the nine patients each, whereas β -lactoglobulin did so in four of eight investigated infants. The IgE levels against the different protein preparations did not differ significantly. IgE antibodies could not be detected in any patient in the other two groups.

Discussion

In the present study, high serum antibody levels to cow's milk proteins were regularly found in children with cow's milk protein allergy of both immediate and slow type. With ELISA technique, the highest IgG antibody titres were obtained against native and pepsin-digested β -lactoglobulin. The efficient inhibition of the antibodies to pepsin-digested β -lactoglobulin by the native protein did not indicate an immunization to any new antigenic determinants uncovered by the digestion. This finding is in agreement with the results of investigations in rabbits of the effect of enzymatic

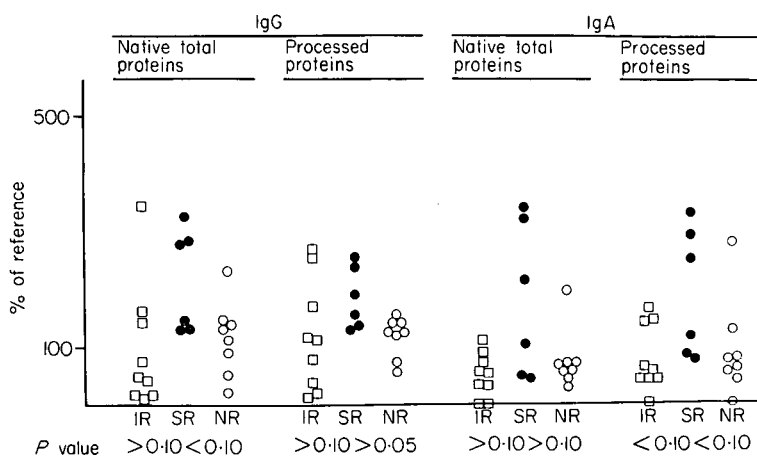


Fig. 2. IgG and IgA antibodies to native and processed cow's milk protein. (Same symbols as in Fig. 1.)

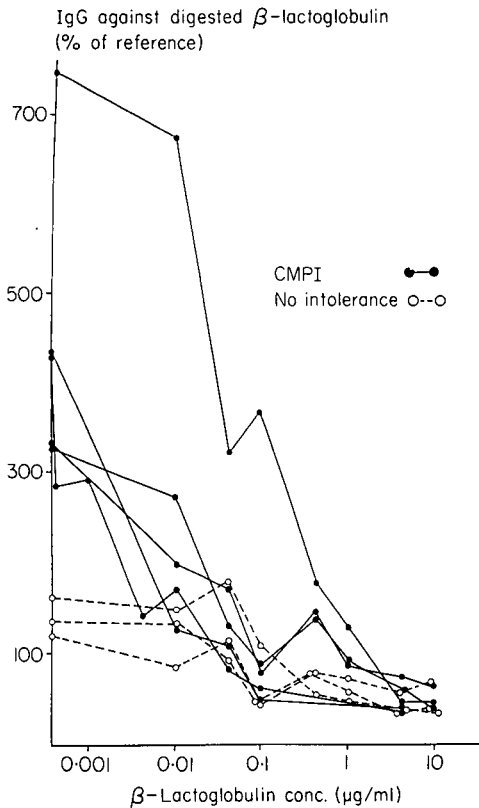


Fig. 3. Inhibition of IgG antibodies against digested β -lactoglobulin by various amounts of added β -lactoglobulin. Titres are expressed as ELISA value in per cent of reference. Children with reactions to cow's milk ($\bullet$), children with no proven reaction ($\circ$).

digestion on the antigenicity of bovine serum albumin [8]. The efficient inhibition of anti- β -lactoglobulin antibodies by total cow's milk proteins indicated that most of the antibodies were bound to the β -lactoglobulin in the mixture. The difference between the antibody levels to β -lactoglobulin and to total cow's milk proteins could be explained by a less efficient antibody-binding of the mixture than of the purified β -lactoglobulin in the ELISA system. An alternative explanation is that the adult reference serum contained low levels of anti- β -lactoglobulin antibodies.

Compared with whole cow's milk, the use of β -lactoglobulin as antigen in ELISA discriminated better between infants with late onset reactions to cow's milk protein and those without intolerance to cow's milk. This is in contrast with our previous results [5]. The discrepancy may be explained by the use of a better defined antigen stored only for a short period of time in the present study compared with our previous one.

Antibodies of IgE type were found in seven of nine individuals with immediate onset of reactions. Using processed or digested proteins did not increase the sensitivity of the determination. This is in contrast with Haddad *et al.* [9] who reported more readily detected IgE antibodies against digested than to native cow's milk proteins. Kletter *et al.* [1] did not detect any IgE antibodies to non-processed β -lactoglobulin in the sera from infants below 1-year-old with suspected cow's milk allergy, while in the present study, IgE antibodies to cow's milk were found even in young infants.

IgG Antibodies against native β -lactoglobulin
(% of reference)

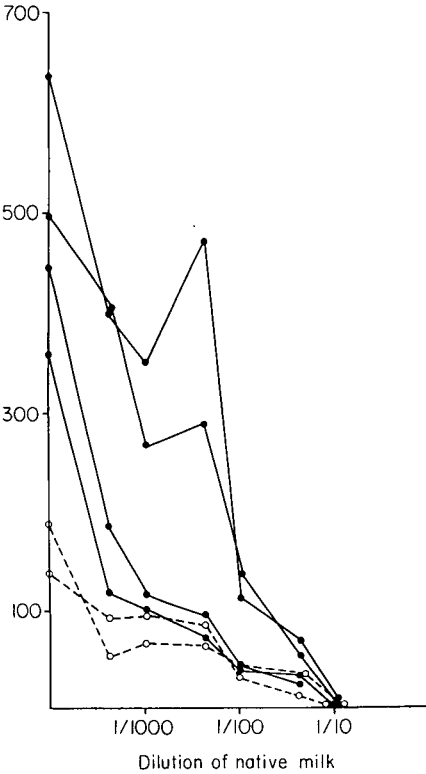


Fig. 4. Inhibition of IgG antibodies against native β -lactoglobulin by various amounts of added defatted native cow's milk. Titres are expressed as ELISA value in per cent of reference. (Same symbols as in Fig. 3.)

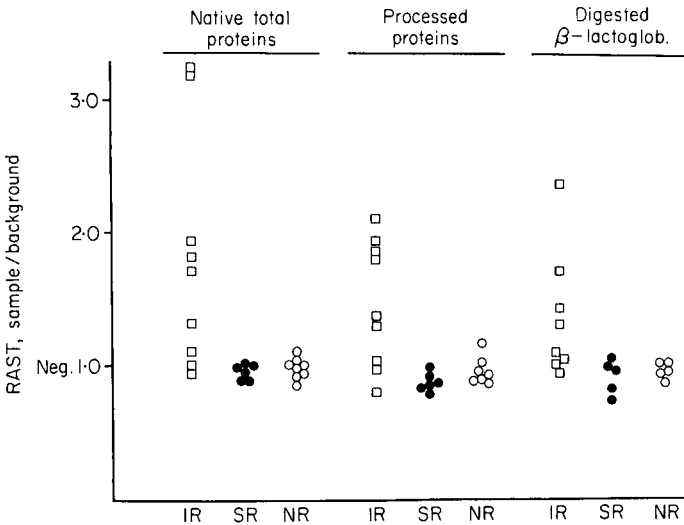


Fig. 5. IgE antibodies to native cow's milk protein, processed cow's milk protein and pepsin-digested β -lactoglobulin.

The findings of high levels of IgG antibodies against cow's milk proteins, especially β -lactoglobulin in slowly appearing intolerance to cow's milk, suggest that these reactions to cow's milk may be mediated by immune complexes. This may correspond with the findings of Paganelli *et al.* [10], who could demonstrate immune complexes with different composition in patients with cow's milk allergy than in healthy persons after ingestion of milk. It is more likely, however, that the increased levels of antibodies indicate a prolonged exposure to the antigens in a gut with increased permeability due to mucosal damage seen in these patients [11]. Thus, in previous studies, increased levels of antibodies to gliadin have been shown in patients with late reactions to cow's milk protein [11,13] and to cow's milk proteins in patients with coeliac disease [5].

In conclusion, the present study demonstrates that the immunological phenomena which are associated with cow's milk protein intolerance vary with the type of clinical reaction. In slowly appearing reactions to cow's milk proteins, high levels of specific IgG antibodies, both to native and digested β -lactoglobulin, are found. These antibodies might be useful in the diagnosis of the disease. In the immediate type of cow's milk allergy, IgE antibodies are common also in young infants. These antibodies are directed against antigen determinants similarly expressed on native, processed and digested cow's milk proteins.

Acknowledgments

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