IgE and IgG cross reactivity between cow and buffalo milk proteins in Swiss albino mice model

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Cow's milk allergy is quite frequent in the first years of human life. When breast-feeding is not possible, a cow's milk substitute must be provided for allergic subjects. Different alternatives to cow's milk have been suggested as protein sources (soy, hydrolyzed proteins, goat's milk, etc.), but all these dietetic solutions are not without risks for polyallergic or more sensitive subjects. To obtain new information on the suitability of other mammalian milks for allergic children, we evaluated the cross-reactivity between milk proteins from cow and buffalo. The cross-reactivity of cow whey proteins with IgG and IgE anti buffalo whey proteins and cross-reactivity of cow caseins with IgG and IgE anti buffalo caseins, obtained by immunization of Swiss albino mice, was carried out by ELISA. IgEs and IgGs from mice allergic to cow's milk are capable of cross-reacting with buffalo milk proteins. Homologies in amino acidic composition could justify the cross-reactivity observed between caseins and whey proteins from cow's and buffalo's milk.

IgG- und IgE-Kreuzreaktivität zwischen Kuh- und Büffelmilchproteinen im Modell mit Schweizer Albino-Mäusen

Im ersten menschlichen Lebensjahr tritt die Kuhmilchallergie vergleichsweise häufig auf. Wenn Stillen nicht möglich ist, muss für allergische Kinder ein Kuhmilchsubstitut verfügbar sein. Verschiedene Alternativen zur Kuhmilch wurden in Hinblick auf die Eiweißquelle empfohlen (Soja, hydrolysierte Proteine, Ziegenmilch etc.). Alle diese diätetischen Lösungen sind nicht ohne Risiko für polyallergische oder besonders empfindliche Personen. Um neue Informationen über die Eignung anderer Säugetiermilchen für allergische Kinder zu erhalten, wurde die Kreuzreaktivität zwischen Milchproteinen von Kühen und Büffeln ermittelt. Die Kreuzreaktivität von Molkenproteinen der Kuhmilch mit IgG- und IgE-Antibüffel-Molkenproteinen und die Kreuzreaktivität von Kuhcaseinen mit IgG- und IgE-Antibüffel-Caseinen (erhalten durch Immunisierung von Schweizer Albino-Mäusen) wurden mithilfe eines ELISA analysiert. IgGs und IgEs von Mäusen mit einer Allergie gegenüber Kuhmilch zeigen eine Kreuzreaktion mit Büffelmilchproteinen. Homologien in der Aminosäurenzusammensetzung dürften die Kreuzreaktivität zwischen den Caseinen und Molkenproteinen von Kuh- und Büffelmilch erklären.

11 Milk allergy (cross reactivity cow and buffalo milk)

11 Milchallergie (Kreuzreaktivität von Kuh- und Büffelmilchproteinen)

1. Introduction

Homology between milk proteins of different animal species can be the cause of cross reactivity. This homology corresponds to immunochemical properties and three-dimensional conformation similarity between allergens of species which are taxonomically near or distant (1). Cross-reaction occurs when two food proteins share part of the amino acid sequence (at least, that containing the epitopic area) or when the three-dimensional on formation makes two molecules similar in their capacity to bind specific antibodies. In general, cross-reactivity reflects the phylogenetic relations between animal species: homologous proteins from vertebrates are often cross-reactive.

Bovine milk contains several proteins which can be generally classified in two major groups: caseins and whey proteins. Because of the absence of β-lactoglobulin in human milk, this protein has been considered the most important cow's milk allergen for a long time (2-4). Several independent studies have demonstrated that the casein fraction also has an important antigenic potential (5-7). Moreover, while antigenicity of β-lactoglobulin can be partially eliminated by technological treatments (8), caseins maintain the capability of binding IgEs even after strong denaturating processes (6, 9).

For children sensitized to cow's milk proteins whose mothers cannot breast-feed their babies, a milk substitute must be provided. Available substitutes include heat-treated cow's milk, goat's milk, soy milk, hydrolysed cow's milk proteins and elemental diet. At present, the most usual alternatives are soy and milk protein hydrolysed formulas but there is evidence that 10–20% of children with cow's milk allergy do not tolerate soy derivatives (10-13) and some cases of anaphylactic reaction to extensively hydrolysed formulas have been described (14-16). On these bases, the identification of a more suitable protein source for cow's milk-allergic children represents an important goal for paediatricians and nutritionists. Although its use is rare, goat's milk can be introduced in the diet of allergic children, this is because some children allergic to cow's milk can tolerate goat derivatives (17, 18). However, there is evidence of cross-reactivity between cow's milk and other mammalian milk proteins (goat and sheep) (19-21). To obtain new information on the suitability of other mammalian milks for allergic children, we evaluated the cross-reactivity between milk proteins from Sahiwal cow and Murrah buffalo.

2. Materials and methods

2.1 Mice

Three to four-week-old male Swiss albino mice were maintained in the Small Animal House of the National Dairy Research Institute. The animals were housed in sterilized plastic polypropylene cages and kept at room temperature in a sterilized condition and were placed on a special milk-free diet. All proce-

dures were approved by the Institutional Animal Ethical Committee.

2.2 Milk samples

Fresh raw milk samples were collected from the Murrah breed buffalo and Sahiwal breed cattle from the Cattle Yard of National Dairy Research Institute, Karnal, India.

2.3 Separation of milk fractions (caseins and whey proteins)

Different milk samples were filtrated with gauze in order to remove some impurities. Then the milk was centrifuged at 3000 rpm for 30 min at 4ºC and the top fat layer was removed by a spatula. The skimmed milk was acidified to pH 4.6 by adding 3 M HCl slowly (22, 23). The precipitated casein was washed with distilled water. Furthermore, the solution was incubated for 30 min at 40ºC and caseins were removed by centrifugation at 8000 rpm for 15 min at 4ºC. The supernatant containing whey proteins was dialyzed using 10 kDa cut-off membrane. The total concentration of whey proteins was determined by Lowry method (24) and aliquots were stored at -20ºC until used.

2.4 Preparation of antibodies, anti-buffalo caseins/ whey proteins and anti cow caseins/ whey proteins

Four groups of mice (n=7) were immunized by intra-peritoneal injection of 20µg whole casein or whey proteins of cow and buffalo milk adsorbed on to 2% of alum $Al(OH)_3$ in 200µl of PBS, while control mice was given PBS placebo. The mice were boosted 2 times at weekly intervals. One week after the last injection, mice were bled and each mouse's antiserum was separated by centrifugation at 5000 rpm for 10 min. Sera were collected and stored at -20℃ until analysis.

2.5 Anti-milk protein IgE antibody analysis

The cross-reactivity of cow whey proteins with anti-buffalo whey proteins IgG and IgE was carried out by ELISA. Similarly, the cross-reactivity of cow caseins with anti-buffalo casein IgG and IgE obtained by immunization of mice was carried out.

Caseins and whey proteins-specific IgE antibodies are detected using enzyme-linked immunosorbent assay (ELISA). Plastic microtitre plates (Nunc, Copenhagen, Denmark) are coated with 100 µg/ml of protein (caseins or whey proteins) in PBS by overnight incubation at 37℃. The plates are blocked by incubation for a further 1hr at 37℃ with 1% BSA (Sigma Chemical Co., St. Louis, MO) in PBS (for plates containing caseins) or 1% fish gelatine (for the plates containing whey proteins). Mouse serum samples diluted (1:5) in 1% BSA in PBS were added to wells and incubated for 1h at 37℃. There follows a further incubation for 1 h at 37℃ with peroxidase-labelled goat anti-mouse IgE diluted 1:10,000 in 1% BSA in PBS. Enzyme substrate TMB (3, 3', 5, 5' tertamethyldiamine benzidine containing 0.03% H_2O_2) was added to each well and the reaction stopped after 15min by the addition of 100 µl of 2N H_2SO_4. Between the incubations, the plate was washed with PBS containing 0.05% Tween 20. Substrate conversion is measured as optical density at 450nm using an automated ELISA reader (Microscan, EC Co., India Pvt Ltd., Hyderabad, India).

2.6 Statistical analysis

The results were expressed as Mean ± SEM. The statistical analysis of data was done using one way analysis of variance (ANOVA) and the differences between the groups tested using Tukey–Kramer post-hoc test using PRISM 3 statistical analysis software.

3. Results and discussion

Casein was prepared by reducing pH of skim milk with 3N HCl to isoelectric point called isoelectric casein because casein become insoluble at this pH. Cow and buffalo caseins were prepared by lowering pH to 4.6. Casein constitutes approximately 80% of total milk proteins in bovine, caprine and buffalo milk (25, 26). Our results represent 21.2±0.2 and19.6±0.2g casein from one liter of buffalo and cow milk, respectively.

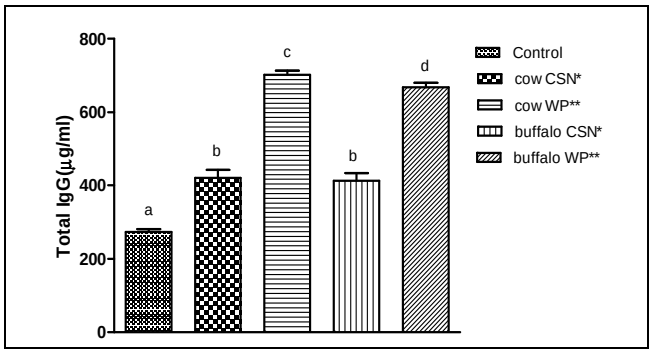

Fig. 1: Levels of total IgG for the immunized mice with caseins and whey proteins from cow and buffalo milk (n= 7) compared to control (n= 7) measured by sandwich ELISA. Results are expressed as mean ± standard error of means. CSN, caseins; WP, whey proteins; ^{a,b,c,d}Values with different letters are significantly different ($p < 0.001$).

After 21 days of sensitizing, which corresponds to the day of sacrifice, we detected in the serum of immunized mice a high quantity of total IgG and IgE. The level of total IgG in cow-WP sensitized mice was 2.5 folds higher than in control group as shown in Fig. 1. Total IgG levels in negative control mice were found 273.31µg/ml. However, immunization with caseins of cow and buffalo increased the IgG levels by 54.03 and 50.94% respectively. In case of cow and buffalo whey proteins immunized mice, the IgG levels were significantly increased and reached 412.55 and 667.19 µg/ml respectively. Thus our observations support the previous studies reported caseins, β-LG and α-lactoalbumin are major immunogenic proteins in cow milk that generate IgG response (27-29).

Total serum IgE levels in normal control mice at basal level were 124.36 ng/ml and increased significantly and reached 416.43 and 363.44 ng/ml after sensitization with caseins of cow and buffalo milk, respectively (Fig. 2). However, with whey proteins of cow and buffalo milk, the levels of serum IgE were reached 429.97 and 387.26 ng/ml respectively. These

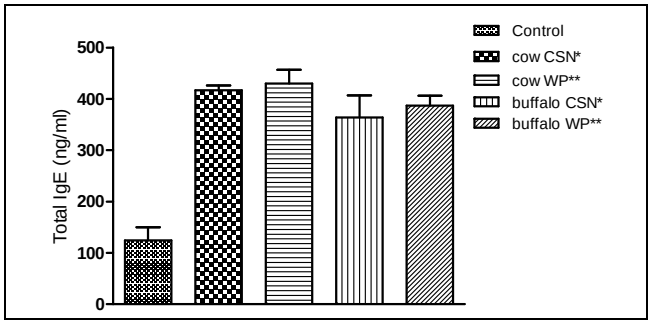

Fig. 2: Levels of total IgE for the immunized mice with caseins and whey proteins from cow and buffalo milk (n = 7) compared to control (n = 7) measured by sandwich ELISA. Results are expressed as mean ± standard error of means. CSN, caseins; WP, whey proteins.

results showed the good power of the milk proteins used to cause an immunological response.

Cross-reactivity of cow whey proteins carried out to combine these proteins with IgE and IgG anti buffalo whey proteins showed that there was a very high cross reaction i.e. 91.86 and 96.27% (Figs. 3 & 4). Similarly the cross-reactivity of cow caseins with IgE and IgG anti buffalo caseins was 86.1 and 90.66% (Figs. 3 & 4).

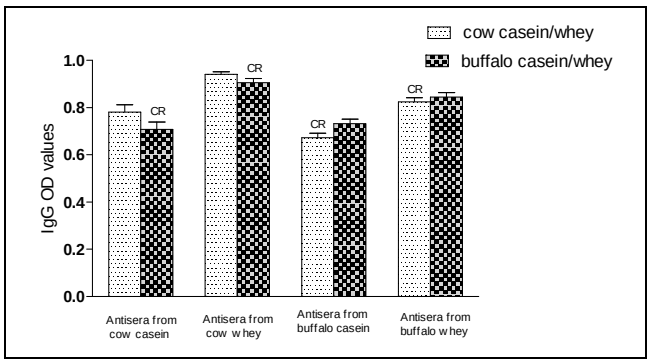

Fig.3: The IgG cross-reactivity of cow caseins / whey proteins and buffalo caseins / whey proteins by indirect ELISA; CR, cross reactivity.

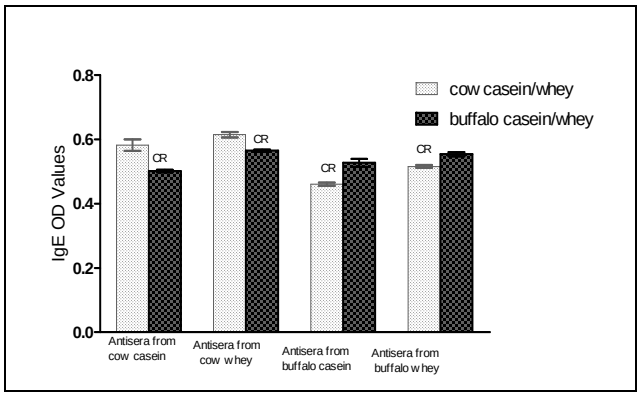

Fig. 4: The IgE cross-reactivity of cow caseins / whey proteins and buffalo caseins / whey proteins by indirect ELISA; CR, cross reactivity.

Cross-reactivity is associated with similarity of three-dimensional protein conformations (conformational epitopes) or similarity of critical amino acid sequences (linear/ sequential epitopes). Cross-reactivity between proteins from different species (both animals and vegetables) depends in general on phylogenetic relationships. Vertebrate homologous proteins are often cross-reactive (30). Our results also indicated that whey proteins show a high cross reactivity as compare to the caseins of cow and buffalo milk. This is may be due to more homology between whey proteins of cow's and buffalo's milk as compare to that of caseins. It could be explained by the fact that these two proteins are similar in the composition of their structures and can contain similar sequences.

Indeed this researcher showed that the proteins of buffalo milk seem to be recognized by serum antibodies of IgE type of children allergic to proteins in cow's milk. This can be explained by the fact that the cow and the buffalo are two species taxonomically close. There is no significant difference between the whey proteins of cow's and buffalo's milk. In addition to that and according to RESTANI *et al*. the cross reactions were observed between proteins of milk and meats, such as caseins and albumin, of different species like the cow, goat, ewe and mare and it was explained by the probable resemblance in the sequence of their proteins (31, 27).

4. Conclusion

Heterogeneity of cow's and buffalo's milk proteins seems not to be sufficient to avoid cross reactivity between both species' milk. The cow caseins and whey proteins have a sufficient antigenic potential to cross react with IgE and IgG anti buffalo caseins and whey proteins.

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