

# *In vitro* allergenicity of cows' milk substitutes

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## Summary

There are numerous alternatives to cows' milk formula for allergic children. We have investigated the allergenicity of several of these using RAST and RAST inhibition on serum from 16 patients with a known history of cows' milk protein intolerance (CMPI) and 16 atopic controls. A RAST grade of  $\geq 3$  for cows' milk was present in all those with CMPI, whilst all the controls gave RAST of  $\leq 1$ . Modified cows' milk formula, goats' infant formula, sheep and goats' milk produced similar results to cows' milk. Only two patients had RAST  $\geq 3$  for soya milk and the soy/beef hydrolysate gave positive results in only three patients. One had positive RAST to Nutramigen and two to Pregestimil. Of the whey hydrolysates investigated, Pepti-junior gave seven positive RASTs whilst we were unable to bind Alfara to the sepharose in sufficient quantities to interpret the results which were negative in all cases. RAST inhibition data on pooled sera from the same patients agreed with the RAST results. The inhibition curves showed high inhibition with goats', sheep, modified cows' milk formula and the casein formula, AL110 (50%). Soy and soy/beef hydrolysate showed a much lower inhibition pattern. Casein hydrolysates showed low inhibition while the whey hydrolysate produced higher inhibition. We have shown that despite claims of low allergenicity, some of these alternative formulae are antigenically recognized *in vitro* by some cows' milk intolerant patients.

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## Introduction

Cows' milk protein intolerance (CMPI) much of which is IgE mediated [1] is relatively common in infancy. Many alternative formulae feeds have been developed for milk allergic infants which at the same time fulfil the infants' nutritional requirements. Such formulae have also been recommended as prophylaxis against the development of allergy. However, there has been no systematic comparison of their allergenicity (capacity to stimulate an allergic response).

The immuno-chemical characteristics of these so-called 'hypoallergenic' formulae vary considerably and are dependent on the protein source, extent of hydrolysis, post-hydrolysis processing and removal of the peptidase used during the manufacturing process.

In this study we have examined the *in vitro* allergenicity and IgE-binding capacity of various infant formulae and cows' milk substitutes by employing both radioallergo-

sorbent test (RAST) on sera from CMPI patients and RAST-inhibition methods.

## Materials and methods

### Patients

We obtained sera from 15 children and one adult with known histories of CMPI. The children were aged from 7.5 months to 11.36 years (mean 6 years) with 10 males and five females and the adult patient was a female aged 24. Negative control sera came from 16 atopic children without history of reaction to cows' milk protein but positive skin reactions to number of aeroallergens. The controls were aged from 1.11 to 13.89 years (mean 7.5 years) with 11 males to four females. These children attended the asthma and allergy clinics and had a venepuncture for other purposes.

All the case patients had a history of an immediate, type-I reaction to cows' milk (cows' milk RAST result  $\geq 3$ , Pharmacia Commercial RAST) and a positive skin-prick test (weal  $\geq 3$  mm) to 10% whole cows' milk extract (Bencard). The clinical features of the patients are shown

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problems [11]. Therefore, whilst soy may be an appropriate alternative feed for CMPI with skin and lung symptoms, a less antigenic formula is required with gut CMPI.

Our own and other studies clearly show that goats' and sheeps' milk are the least suitable alternatives [8] and the RAST data suggests that there is considerable crossreactivity between these milks as many who reacted had never received goats' or sheep milk. Over 90% of our CMPI patients had positive RASTs to both goats' and sheep milk. Gjessing *et al.* [12] have shown that anti-cows' milk IgE produced by CMPI children reacted with goats' milk proteins. McLaughlan *et al.* [13] showed that 70% of guinea pigs orally sensitized to cows' milk had anaphylactic reactions to oral challenges with milk protein. In addition to the immunological crossreactivity, the composition of goats' and sheep milk is unsuitable for infant feeding.

Numerous 'hypo-allergenic' formulae are now available for CMPI patients. Allergenicity of these formulae is claimed to be reduced by decreasing the molecular size of the proteins. Hydrolysates have been manufactured for over 40 years using two methods: heat denaturation [14] and/or enzymatic (tryptic) methods [5]. Both procedures are used to yield an optimal balance between palatability and residual antigenicity. In general, there appears to be a limit for antigenicity for peptides with molecular weight at around 1200 to 1600. However, the peptide molecular weight distribution data of a 'hypo-allergenic' formula provides only an approximate estimate of the allergenic characteristics and does not necessarily guarantee non- or hypo-allergenicity. The residual antigenicity and thus potential allergenicity is dependent on the processing applied and these are often a well kept secret of the manufacturer.

Our *in vitro* study as well as those reported by other investigators [15,16] demonstrated that there was cross-reacting IgE production against some of the formulae in some patients. Obviously our *in vitro* studies should be followed by *in vivo* challenge in order to establish the clinical implications. However, this is difficult to justify if there is potential to produce repeated severe reactions. Thus the *in vitro* results may be used as a guideline for conducting clinical trials with the most hypoallergenic formula.

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