

How allergenic are hypoallergenic infant formulae?

E. RUGO*, R. WAHL† and U. WAHN*‡

*University Children's Hospital, Free University Berlin and †Allergopharma J. Ganzer KG, Research and Development Department, Reinbek, Germany

Summary

In a comparative study six different protein hydrolysates, marketed as 'hypoallergenic' infant formulae were investigated by skin prick tests, RAST, RAST inhibition and titrated provocation tests. Whey hydrolysates containing a high percentage of larger peptides were found to have the highest capacity to induce positive skin tests, provocation tests and to bind to human serum IgE antibodies of cow's milk allergic children. Casein hydrolysates appeared to have the least residual allergenic activity. We recommend that 'hypoallergenic' formulae should be tested in each case, before being prescribed to cow's milk sensitive children.

Clinical and Experimental Allergy, Vol. 22, pp. 635–639. Submitted 29 August 1991; revised 3 December 1991; accepted 13 December 1991.

Introduction

IgE-mediated hypersensitivity reactions to proteins from cow's milk together with hen's egg allergy represent the most frequent adverse reactions to food in infancy and early childhood. According to several studies the cumulative prevalence of cow's milk allergy ranges from 0.3 to 7.5%. The differences reported are probably due to differences in study design, definitions and the diagnostic methods used [1–3].

Food allergens leading to specific sensitization have been demonstrated to be proteins or glycoproteins [4]. More than 40 different proteins from cow's milk giving rise to the production of IgE-antibodies in atopic subjects have been identified [5,6]. Major allergens are found in both the casein- and whey-fraction of cow's milk, where the most important allergens have been demonstrated to be α -lactalbumin and β -lactoglobulin.

In the past other protein sources like soy-protein have been widely used as a dietary alternative in cow's milk allergy. Since, however, approximately 10–20% of children with immediate hypersensitivity to cow's milk have been found to be allergic to soy products, the possibility to reduce the allergenic activity by physico-chemical methods has offered new therapeutic alternatives. Heat treatment of cow's milk allergens leads to denaturation and loss of conformational epitopes, while enzymatic cleavage may destroy sequential epitopes [7–9]. Pasteuri-

zation has little effect on the allergenic activity of cow's milk allergens [10].

The commercially available protein hydrolysates, which are marketed for a dietary therapy of cow's milk allergy differ considerably with regard to the molecular weight of their proteins and peptides and consequently to their residual allergenic activity. There is evidence that in highly sensitized infants and children hydrolysates marketed as 'hypoallergenic formulae' induce anaphylactic reactions [11,12]. Therefore the aim of the following study was to determine and compare the residual allergenic activity of different hydrolysate formulae with regard to cow's milk sensitized children.

Patients and methods

Patients

Twenty infants and children (5 months–9.5 years) with a history of immediate reactions to cow's milk (urticaria, asthma, gastrointestinal symptoms) and elevated serum IgE antibody concentrations (RAST ≥ 2) to cow's milk were included in the study.

Hydrolysates

Six different hydrolysates were investigated:

- 1 Casein Hydrolysate I (Nutramigen, Bristol-Myers)
- 2 Casein Hydrolysate II (Pregestimil, Bristol-Myers)
- 3 Whey Hydrolysate I (Alfaré, Nestlé)
- 4 Soy-Collagen Hydrolysate (Pregomin, Milupa)

Correspondence: Professor Dr U. Wahn, Kinderklinik, Universitätsklinikum Rudolf Virchow, Heubnerweg 6, 1000 Berlin 19, Germany.

‡ Supported by the Deutsche Forschungsgemeinschaft (SFB 174, A 11)

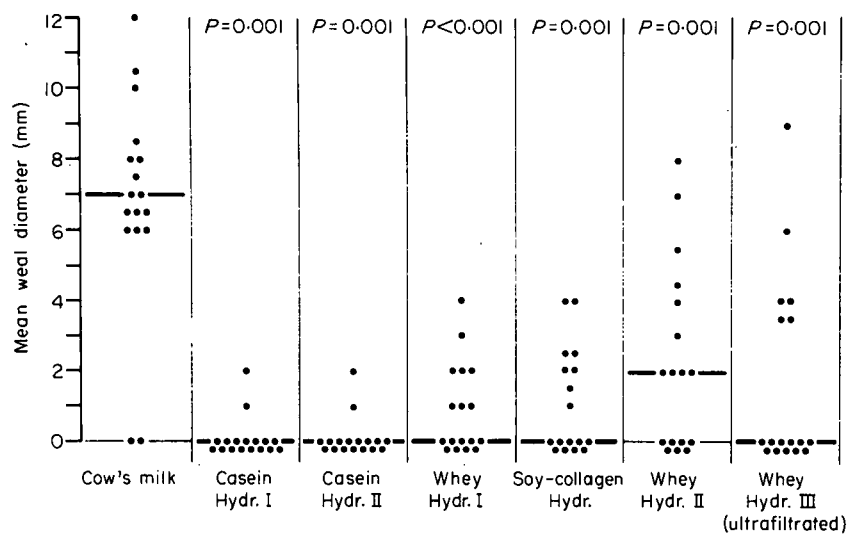


Fig. 1. Results of skin puncture tests with whole cow's milk and different hydrolysates in 17 cow's milk sensitive children (P =level of significance in comparison to whole cow's milk).

- 5 Whey Hydrolysate II (Beba H.A., Good Start H.A., Nestlé)
- 6 Whey Hydrolysate III (experimental, ultrafiltrated, kind gift of Nestlé company).

Skin test

Each hydrolysate (14 g) was dissolved in 100 ml of distilled water. Skin prick-tests were performed with hydrolysate solution as well as whole cow's milk with a standard lancet (tip length 1 mm) at the volar side of the forearm. Mean weal diameters were determined after 10 min. Histamine (1 mg/ml) and isotonic saline served as positive and negative controls.

Specific Serum IgE

Serum specific IgE-antibody levels of the patients were determined by Phadebas-RAST (Pharmacia, Freiburg, Germany) using cow's milk allergen disks and allergen disks prepared from the different protein hydrolysates (Allergopharma). The different allergen disks were produced according to the prescription of Ceska *et al.* [13] and Maasch *et al.* [14]. The extracts were coupled to CNBr-activated paper disks.

For the preparation of the allergen disks 2 μ g protein [15] were used for coupling on each disk. After the coupling the amount of the protein coupled on each disk was measured. For the determination of the RAST-classes a reference serum (Pharmacia) was used. For non-specific binding the allergen disks were measured with a non-allergic control pool serum ($n=154$). 125 J-antihuman IgE (Pharmacia) was used as tracer for specific IgE.

RAST inhibition

The IgE-binding activity of all hydrolysates was determined by RAST-inhibition [16,17] using the sera of the

patients, cow's milk allergen disks and as inhibition extracts the ones which were prepared from the different hydrolysates and the cow's milk extract which was used for the preparation of the cow's milk allergen disks. As tracer for specific IgE 125-J-antihuman-IgE (Pharmacia) was used.

Challenge tests

Single blind challenge tests were performed in a titrated manner after elimination of cow's milk from the diet for at least one week. One challenge was performed per day. Patients received increasing amounts of the hydrolysates 1, 2, 5, 10, 20 ml. Challenge with cow's milk was started at 0.2 ml and continued with 0.5, 1, 2, 5, 10 and 20 ml. The challenge procedure was stopped after clinical symptoms were observed.

Results

All hydrolysates tested induced significantly smaller ($P<0.01$) mean weal reactions in sensitized children compared to whole cow's milk. By means of skin test, both casein hydrolysates showed the least allergenic activity. Whey hydrolysates containing a significant percentage of higher molecular weight peptides were shown to retain a relatively high residual allergenic activity. Ultrafiltration of such a whey product results in a further reduction of allergenic activity (Fig. 1).

Ten serum samples with high specific IgE-antibody concentrations to cow's milk were used for the detection of IgE-binding proteins or peptides in the hydrolysates. Compared to whole cow's milk the IgE-binding capacity of all six hydrolysates was significantly lower. Within each hydrolysate there was a wide range of antibody

Fig. 2. Specific serum IgE antibodies binding to cow's milk and different hydrolysates in 10 cow's milk sensitive children.

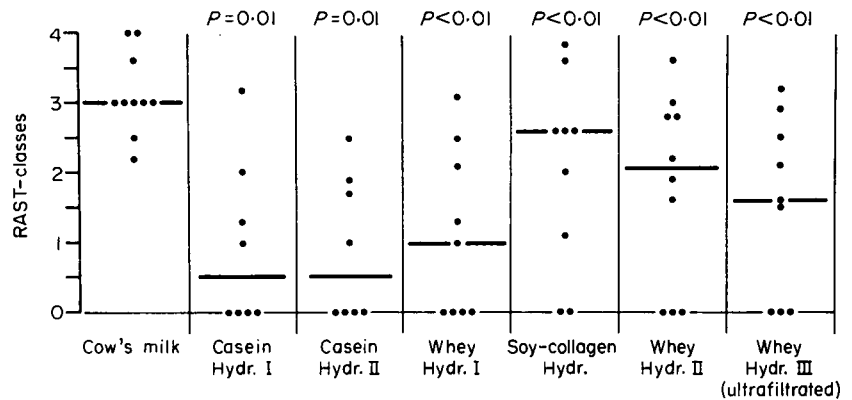
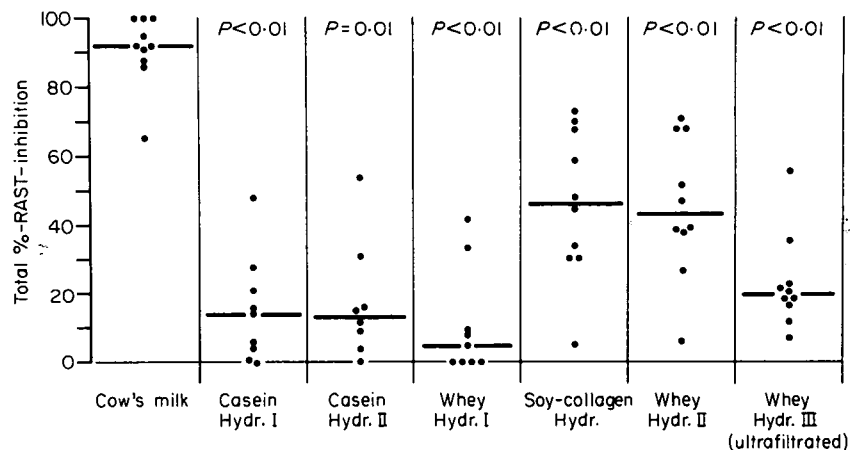


Fig. 3. Total %-RAST-inhibition measured with cow's milk and different protein hydrolysates-extracts, using sera of cow's milk sensitive patients ($n=10$) and cow's milk allergen disks.



binding capacity between different patient's sera. The highest IgE-binding was observed in the soy-collagen hydrolysate followed by whey hydrolysates, while both casein hydrolysates were demonstrated to have the lowest IgE-binding capacity in RAST-test (Fig. 2).

Control experiments with a pool serum from non-allergic donors and all hydrolysate disks showed no detectable IgE (RAST-class 0), indicating that positive values could not be attributed to non-specific binding.

There was a positive correlation between the skin test results and specific serum IgE concentrations to whole cow's milk (Spearman rank correlation coefficient $r_s=0.74$, $P<0.01$). Due to the limited number of patients reacting to the hydrolysates, correlation coefficients could not be calculated for each hydrolysed formula.

All hydrolysates were able to inhibit IgE-binding to cow's milk in the RAST-inhibition test, however, to a lower degree than cow's milk itself. Similar to the preceding test the soy-collagen and the non ultrafiltrated whey hydrolysate II showed a relatively high residual allergenic activity with a total RAST-inhibition of 47% and 43% respectively, while whey hydrolysate I and the two casein formulae were found to retain rather little IgE-

binding activity (Fig. 3). Ultrafiltration of whey hydrolysate II resulted in a significant decrease of the capacity to inhibit IgE-binding in the RAST-inhibition test.

In eight children with positive challenge tests to whole cow's milk at the concentrations tested challenge tests with all six hydrolysates could be performed in a single blind, titrated manner. Five out of eight children responded with similar symptoms as induced by whole cow's milk on challenge with whey hydrolysate II, four of them also developed symptoms to the ultrafiltrated hydrolysate. Whey hydrolysate I induced an immediate reaction in two patients, while only once a perioral urticaria was observed upon challenge with the soy-collagen hydrolysate. Both casein hydrolysates were tolerated by all children without adverse reactions.

Discussion

The commercially available protein hydrolysates, often labelled 'hypoallergenic', are the results of heat treatment and/or enzymatic cleavage in order to produce peptides of minor allergenic activity. As our data indicate, 'hypoallergenic' means containing less allergens and not neces-

sarily 'no detectable allergen'. For dietary use in patients with cow's milk allergy it is crucial that the allergenic activity of the formulae is sufficiently low as to give no clinical reaction. Therefore Sampson [18] recently recommended that all infant formulae promoted as 'hypoallergenic' be tested in milk allergic patients to assess their allergenic potential.

In our study we could demonstrate by means of *in vivo* and *in vitro* methods that especially in whey hydrolysate II, containing a high percentage of peptides greater than 1500 Da molecular weight (up to 20 000 Da), there is a considerable residual allergenic activity, which may be responsible for anaphylactic reactions in highly sensitized children as reported by several authors [11].

The allergenic activity observed in the soy-collagen hydrolysate could theoretically be explained by a coexisting soy-allergy in some children. Four of our cow's milk sensitive patients had also elevated serum IgE to soy proteins as demonstrated by RAST. However, the inhibition of IgE-binding by this formula in RAST-inhibition tests cannot be explained by hypersensitivity to soy proteins, therefore crossreactivity of epitopes on cow's milk proteins and the peptides present in this hydrolysate has to be assumed. Our data agree with the results reported by Strobel *et al.* [19], indicating that antibodies raised against a cow's milk formula recognized epitopes displayed by peptides of hydrolysates including the soy-collagen hydrolysate.

In our study, whey hydrolysate I is the whey formula with the peptides of the lowest molecular weight range. In RAST inhibition the residual allergenic activity was demonstrated to be very low, while the two other whey preparations with a higher molecular weight showed a significantly higher allergenic activity.

Both casein hydrolysates studied in our investigation showed only very little residual allergenic activity both *in vivo* and *in vitro*. Although IgE-mediated allergic reactions to casein hydrolysates have been occasionally observed [12], in general, these products are well tolerated. Our data are in agreement with the studies of other authors [18].

Certain protein hydrolysates (whey hydrolysate II) are marketed as products for the prevention of both food allergy and atopic symptoms. Recent data from animal experiments and clinical studies suggest that specific tolerance might be induced by early feeding with whey hydrolysates in spite of the presence of relatively large peptide fragments in this formula. Our study did not address the question of preventive efficacy of hydrolysate feeding and was only directed to the allergenic activity of the hydrolysates as demonstrated in sensitized individuals.

From our data we conclude that depending on the

protein source, the degree of hydrolysis, heat treatment and the application of ultrafiltration there may be considerable differences in allergenic activities between different 'hypoallergenic' formulae. It will be a challenge for the food industry in the future to develop hydrolysates with low molecular weight, low residual allergenic activity, complete nutritional value and acceptable taste in the future. We recommend that before a hydrolysate is introduced in the diet of a sensitized infant or child, its safety should be carefully tested by either *in vivo* or *in vitro* methods.

References

- 1 Bahna SL, Gandhi MD. Reliability of skin testing and RAST in diagnosis of food allergy. In: Chandra RK, ed. *Food allergy*. St John's Newfoundland: Nutrition Research Education Foundation, 1987:139-47.
- 2 Bahna SL, Gandhi MD. Milk hypersensitivity. II. Practical aspects of diagnosis, treatment and prevention. *Ann Allergy* 1983; 50:295-301.
- 3 Bahna SL. Diagnostic tests for food allergy. *Clin Rev Allergy* 1988; 6:259-80.
- 4 Taylor SL, Lemanske RF, Bush RK, Busse WW. Chemistry of food allergens. In: Chandra RK, ed. *Food allergy*. St John's, Newfoundland: Canada, 1987:21-44.
- 5 Gjesing B, Osterballe O, Schwartz B, Wahn U, Lowenstein H. Allergen-specific IgE antibodies against antigenic components in cow milk and milk substitutes. *Allergy* 1986; 41:51-6.
- 6 Wahn U. Antigens in cow's milk and hen's egg allergy. In: Schmidt E, ed. *Food allergy*. New York: Nestlé Nutrition Workshop Series, 1988; 17:81-8.
- 7 Görtler I, Urbanek R. Untersuchungen zur Antigenität und Allergenität der hypoallergenen Hydrolysate zur Säuglingsernährung. *Monatsschr Kinderheilk* 1990; 138:605-10.
- 8 Jost R, Monti JC, Pahud JJ. Whey protein allergenicity and its reduction by technological means. *Food Technol* 1987; 41:118-21.
- 9 Pahud JJ, Schwarz K, Granato D. Control of hypoallergenicity by animal models. In: Schmidt E, ed. *Nestlé Nutrition Workshop Series*, New York 1988; 17:199-207.
- 10 Høst A, Samuelsson EG. Allergic reactions to raw, pasteurized, and homogenized/pasteurized cow milk: a comparison. *Allergy* 1988; 43:113-18.
- 11 Buscino L, Cantani A, Longhi MA, Giampietro PG. Anaphylactic reactions to a cow's milk whey protein hydrolysate (Alfa-Ré, Nestlé) in infants with cow's milk allergy. *Ann Allergy* 1989; 62:333-5.
- 12 Saylor JD, Bahna SL. Anaphylaxis to casein hydrolysate formula. *J Pediatr* 1991; 118:71-4.
- 13 Ceska M, Eriksson R, Varga JM. Radioimmunosorbent assay of allergens. *J Allergy Clin Immunol* 1972; 49:1-9.
- 14 Maasch HJ, Wihl JA, Schultze-Werninghaus G, Geissler W, Wahl R. A manufacturer's criteria for in-house reference preparations for RAST-inhibition. *Ann Allergy* 1987; 58:29-33.

- 15 Wahl R, Geissler W, Maasch HJ. Comparison of classical Lowry, modified Lowry and a dyc-binding assay for the estimation of protein in allergen extracts and influence of different parameters on the modified Lowry assay. *Biol Chem Hoppe-Seyler* 1985; 366:979–84.
- 16 Wahl R, Maasch HJ, Geissler W. Comparison of three variations of the radioallergosorbent test-inhibition assay for measuring allergenic activity of grass pollen extracts. *Anal Biochem* 1983;134–89.
- 17 Yman L, Ponterius G, Brand R. RAST-based allergen assay methods. *Develop Biol Standard Basel*: Karger, 1975:151.
- 18 Sampson HA, Bernhisel-Broadbent J, Yang E, Scanlon SM. Safety of casein hydrolysate formula in children with cow milk allergy. *J Pediat* 1991; 118:520–5.
- 19 Strobel S, Fairclough LM. Whole cow's milk and hydrolyzed infant formulae: analysis of systemic immune responses and antigenic crossreactivity. In: Harms HK, Wahn U, eds. *Food allergy in infancy and childhood*. Berlin: Springer-Verlag, 1989:158–65.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.