

Comparison of the residual allergenic activity of six different hydrolyzed protein formulas

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The residual allergenic activity of protein hydrolysates contained in marketed "hypoallergenic" infant formulas can be determined both by in vivo methods (skin prick tests, provocation tests) and by in vitro methods (radioallergosorbent test, radioallergosorbent inhibition, crossed radioimmunoelectrophoresis, basophil histamine release). In general, whey hydrolysates containing a high percentage of larger peptides have the highest capacity to induce positive skin test and provocation test results, and to bind to human serum IgE antibodies of cow milk-sensitive children. Casein hydrolysates appear to have very little residual allergenic activity. Before hypoallergenic formulas are prescribed for cow milk-sensitive children, individual testing (e.g., skin test, challenge tests) is recommended. (J PEDIATR 1992;121:S80-S84)

The most frequent adverse reactions to food in infancy and early childhood are IgE-mediated hypersensitivity reactions to proteins from cow milk and chicken egg. According to several studies, the cumulative prevalence of cow milk allergy ranges from 0.3% to 7.5%. The differences reported are probably due to differences in study design, definitions, and diagnostic methods.¹⁻³

Food allergens leading to specific sensitization have been demonstrated to be proteins or glycoproteins.⁴ More than 40 different proteins from cow milk that produce IgE antibodies in atopic subjects have been identified.^{5, 6} Major allergens are found in both the casein and whey fractions of cow milk; the most important allergens are α -lactalbumin and β -lactoglobulin.

In the past, other protein sources, such as soy protein, have been widely used as a dietary alternative in children with cow milk allergy. However, approximately 10% to 20% of children with immediate hypersensitivity to cow milk are allergic to soy products. Thus the possibility of reducing the allergenic activity by physicochemical methods has offered

new therapeutic alternatives. Heat treatment of cow milk allergens leads to denaturation and loss of conformational epitopes, and enzymatic cleavage may destroy sequential epitopes.⁷⁻⁹ Pasteurization has little effect on the allergenic activity of cow milk allergens.¹⁰

Commercially available, extensively hydrolyzed proteins used in formulas marketed to treat cow milk allergy differ considerably in their molecular weight profile and hence in their residual allergenic activity. In highly sensitized infants and children, hydrolysate formulas designated as "hypoallergenic" may induce anaphylactic reactions.^{11, 12} The

RAST Radioallergosorbent test

aim of our study was to compare the residual allergenic activity of different hydrolysate formulas in cow milk-sensitized children.

Several methods have been applied to assess the residual allergenic activity in protein hydrolysates. These include tests, such as the radioallergosorbent test, RAST inhibition, or crossed radioimmunoelectrophoresis, that assess the capacity of dietary peptides and proteins to bind to serum IgE of sensitized individuals.⁵ Allergenic proteins induce IgE-mediated histamine release from actively sensitized basophils of cow milk-sensitive individuals. However, histamine release from leukocytes of atopic patients may occur spon-

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taneously by nonspecific mechanisms⁶; therefore this assay using protein hydrolysates has limitations. Skin prick tests are used to assess the allergenic activity of hydrolysates.^{13, 14} None of the tests is completely predictive of potential allergic reactions; titrated oral challenge tests are still the "gold standard" for diagnostic procedures.

METHODS

Patients. Twenty infants and children (5 months to 9½ years of age) with a history of immediate hypersensitivity to cow milk (urticaria, asthma, gastrointestinal symptoms) and elevated serum IgE antibody concentrations (RAST class ≥ 2) to cow milk were included in the study.

Hydrolysates. Six different hydrolysates were investigated:

1. Extensive casein hydrolysate I (Nutramigen; Bristol-Myers, Evansville, Ind.)
2. Extensive casein hydrolysate II (Pregestimil; Bristol-Myers, Evansville, Ind.)
3. Extensive whey hydrolysate I (Alfaré; Nestlé, Munich, Germany)
4. Partial soy-collagen hydrolysate (Pregomin; Milupa, Friedrichsdorf, Germany)
5. Partial whey hydrolysate II (Beba H.A. and Good Start H.A.; Nestlé, Munich, Germany)
6. Partial whey hydrolysate III (experimental and ultrafiltrated hydrolysate; gift of Nestlé, Vevey, Switzerland).

Skin test. Each protein hydrolysate powder (14 gm) was dissolved in 100 ml of distilled water. Skin prick tests were performed with both hydrolysate solutions and whole cow milk with the use of a standard lancet (tip length 1 mm) on the volar surface of the forearm. Mean wheal diameters were determined after 10 minutes. Histamine (1 mg/ml) and isotonic saline solution served as positive and negative controls, respectively.

Specific serum IgE. Serum specific-IgE antibody levels of the patients were determined by Phadebas-RAST (Pharmacia, Freiburg, Germany) with the use of cow milk allergen disks and allergen disks prepared from the different protein hydrolysates (Allergopharma J. Ganzer KG, Reinbek, Germany). The different allergen disks were produced according to the methods of Ceska et al.¹⁵ and Maasch et al.¹⁶ The extracts were coupled to cyanogen-bromide-activated paper disks as follows.

For preparation of the allergen disks, 2 µg protein¹⁷ was used for coupling to each disk and then measured. To determine the RAST classes, we used a reference serum (Pharmacia). For nonspecific binding the allergen disks were measured with a nonallergic control pool serum ($n = 154$). As tracer for specific IgE, antihuman IgE labeled with iodine 125 (Pharmacia) was used.

RAST inhibition. The IgE-binding activity of all hydrolysates

was determined by RAST inhibition^{18, 19} using the sera of the patients, cow milk allergen disks, and, as inhibition extracts, disks prepared from the different hydrolysates and the cow milk extract used for the preparation of the cow milk allergen disks. As tracer for specific IgE, ¹²⁵I-antihuman IgE (Pharmacia) was used.

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

RESULTS

All tested hydrolysates induced significantly smaller ($p < 0.01$) mean wheal reactions compared with those induced by whole cow milk in sensitized children. On the basis of skin tests, both extensively hydrolyzed casein formulas showed the least allergenic activity. Whey hydrolysates containing a significant percentage of higher molecular weight peptides retained relatively high residual allergenic activity. Ultrafiltration of such a whey hydrolysate resulted in a further reduction of allergenic activity (Fig. 1).

Ten serum samples with high concentrations of specific IgE antibody to cow milk were used to detect IgE-binding proteins or peptides in the hydrolysates. Compared with the IgE-binding capacity of whole cow milk, that of all six hydrolysates was significantly lower. For each hydrolysate tested, there was a wide range of antibody-binding capacity in sera from different patients. The highest IgE binding capacity was observed in the soy-collagen hydrolysate, followed by both partial and extensive whey hydrolysates. Both extensively hydrolyzed casein formulas had the lowest IgE-binding capacity in the RAST test (Fig. 2).

Control tests using pooled serum from nonallergic donors showed no detectable IgE (RAST class 0) with any hydrolysate disks, indicating that positive values could not be attributed to nonspecific binding. There was a positive correlation between the skin test results and serum concentrations of specific IgE antibody to whole cow milk (Spearman rank correlation coefficient: $r_s = 0.74$; $p < 0.01$). Because of the limited number of patients with reactions, correlation coefficients could not be calculated for each separate formula. All hydrolysates were able to inhibit IgE binding to cow milk in the RAST inhibition test, but to a lower degree than cow milk itself.

The soy-collagen hydrolysate and the partial whey hydrolysate II both had relatively high residual allergenic activity, with a total RAST inhibition of 4% to 7% and 43%,

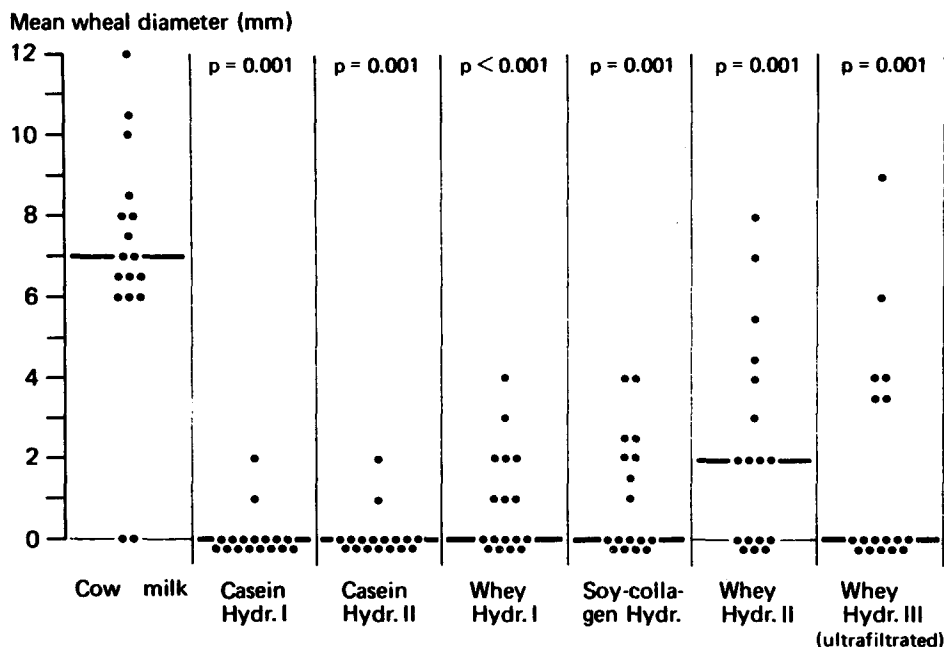


Fig. 1. Results of skin puncture tests with whole cow milk and six different hydrolysates (*Hydr.*) in 17 cow milk-sensitive children (p = level of significance compared with whole cow milk). (From Rugo E, Wahl R, Wahn U. Clin Exp Allergy 1992;22:635-9.)

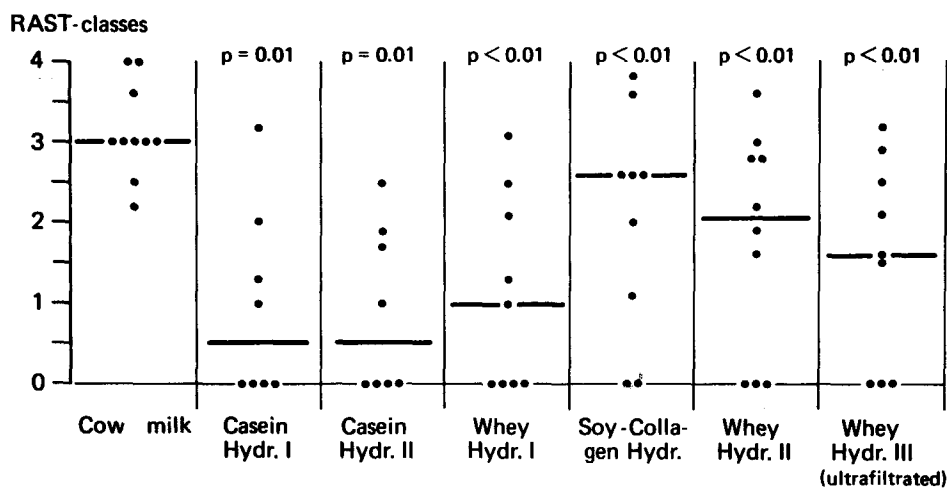


Fig. 2. Specific serum IgE antibodies binding to cow milk and six different hydrolysates (*Hydr.*) in 10 cow milk-sensitive children. (From Rugo E, Wahl R, Wahn U. Clin Exp Allergy 1992;22:635-9.)

respectively. Extensively hydrolyzed whey (I) and both casein formulas showed little IgE-binding activity (Fig. 3). Ultrafiltration of partial 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 results on challenge tests with whole cow milk at the concentrations tested (Table),

challenge tests with all six formulas could be performed in a single-blind, titrated manner. Five of eight children responded with symptoms similar to those induced by whole cow milk when challenged with partial whey hydrolysate II; four also reacted to the ultrafiltrated partial whey hydrolysate II. Extensive whey hydrolysate I induced an immediate reaction in two patients; perioral urticaria was observed

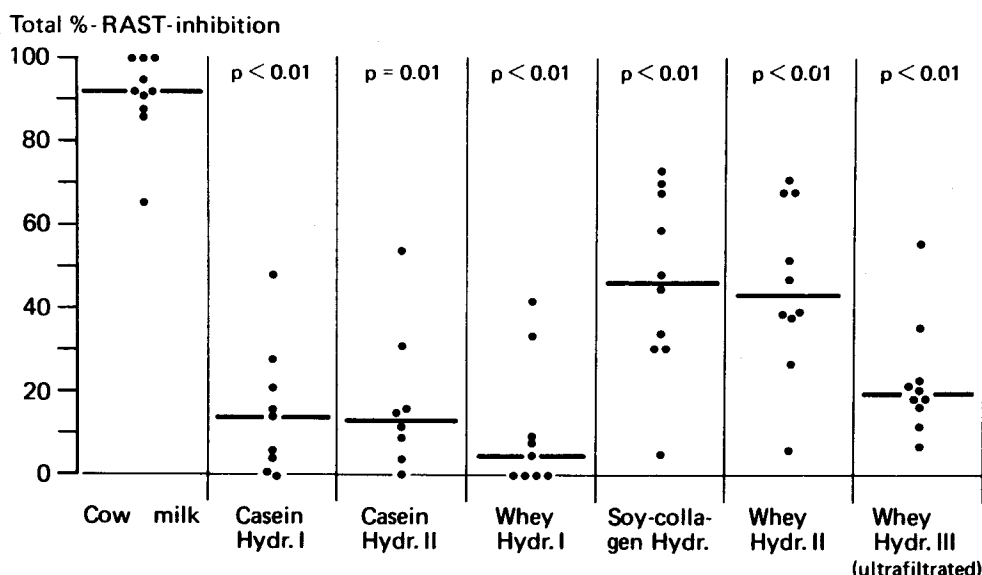


Fig. 3. Total percentage of RAST inhibition measured with cow milk and six different protein hydrolysate (*Hydr.*) extracts, with the use of sera from cow milk-sensitive patients ($n = 10$) and cow milk allergen disks. (From Rugo E, Wahl R, Wahn U. Clin Exp Allergy 1992;22:635-9.)

Table. Results of titrated oral challenge tests in children with cow milk allergy

Test solution	Positive reactions to test formula (ml*)						Negative reactions
	0.2	1	2	5	10	20	
Native cow milk	3	1	2	1	0	1	0
Casein hydrolysate I formula	0	0	0	0	0	8	0
Casein hydrolysate II formula	0	0	0	0	0	0	8
Whey hydrolysate I formula	0	1	0	1	0	0	6
Soy-collagen hydrolysate formula	0	0	0	0	0	1	7
Whey hydrolysate II formula	0	2	1	0	0	2	3
Ultrafiltrated whey hydrolysate III formula	0	2	0	0	0	2	4

*Volume of test formula given orally on separate days to eight children known to be sensitive to cow milk. Positive reactions included urticaria, asthma, gastrointestinal symptoms.

only once after challenge with the soy-collagen hydrolysate. Both extensive casein hydrolysates were tolerated by all children without any adverse reactions.

DISCUSSION

Commercially available infant formulas based on hydrolyzed protein use heat treatment or enzymatic cleavage or both to produce peptides of minor allergenic potential. As our data indicate, hypoallergenic means "containing fewer allergens" and not necessarily "no detectable allergen." For dietary use in patients with cow milk allergy, it is crucial that the allergenic activity of the formulas be low enough to give no clinical reaction. Therefore Sampson et al.²⁰ recently recommended that all infant formulas promoted as hypoallergenic be tested in milk-allergic patients to assess their allergenic potential.

In our study, by means of in vivo and in vitro methods, we could demonstrate that especially whey hydrolysate II, containing a high percentage of peptides of more than 1500 daltons molecular weight (up to 20,000 daltons), has considerable residual allergenic activity. Whey hydrolysate II may be responsible for anaphylactic reactions in highly sensitized children.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 milk-sensitive patients also had 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 cross-reactivity of epitopes on cow milk proteins and the peptides present in this hydrolysate must be assumed. Our

data agree with the results reported by Strobel and Fairclough,²¹ indicating that antibodies raised against a cow milk formula recognize epitopes displayed by peptides of hydrolysates, including the soy-collagen hydrolysate. In our study, whey hydrolysate I was the whey formula with peptides of the lowest molecular weight range. In RAST inhibition the residual allergenic activity was very low, whereas the two other whey preparations with a higher molecular weight had significantly higher allergenic activity.

Both casein hydrolysates studied showed very little residual allergenic activity either in vivo or in vitro. IgE-mediated allergic reactions to casein hydrolysates have occasionally been observed.¹² We recently studied a patient in whom generalized urticaria had developed 10 minutes after a challenge with 100 ml of a casein hydrolysate. However, in general, we can confirm the observations of other authors²⁰ that these products are well tolerated by cow milk-sensitive children.

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 despite 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 directed only to the allergenic activity of the hydrolysates as demonstrated in sensitized individuals.

CONCLUSION

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 among different hypoallergenic formulas. The challenge for the food industry is to develop hydrolysates with low molecular weight, low residual allergenic activity, complete nutritional value, and acceptable taste. We recommend that before a hydrolysate is introduced into the diet of a sensitized infant or child, its safety should be tested by in vivo or in vitro methods.

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