

Allergenicity of milk protein hydrolysate formulae in children with cow's milk allergy

V. Ragno, P. G. Giampietro, G. Bruno, L. Businco

Allergy and Immunology Division, Department of Paediatrics, University of Rome "La Sapienza", Viale Regina Elena 324, I-00161 Rome, Italy

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Abstract. Cow's milk protein hydrolysate formulae have been developed to lower or eliminate the allergenicity of cow's milk proteins, and to reduce the antigenic load and the risk of sensitization. Cross-reactivity between different hydrolysate formulae and cow's milk proteins has been demonstrated. We have studied 20 children (median age 31 months, range 15–76 months) with a history of IgE-mediated cow's milk allergy. All the children had immediate allergic respiratory and/or cutaneous and/or gastro-intestinal reactions to cow's milk ingestion. In addition, the children had positive prick skin tests and positive RAST to cow's milk. Prick skin test, RAST, and double-blind placebo controlled food challenges were performed with three different hydrolysate formulae: a casein hydrolysate formula and two whey formulae, one partially and one extensively hydrolyzed. All 20 children had immediate allergic reactions after the challenge test with cow's milk. Only 2/20 children had a positive challenge test with a casein hydrolysate formula (Alimentum): one developed asthma and one urticaria. Two of the 15 children challenged with an extensively hydrolysed whey formula (Profylac) developed perioral erythema. Nine out of 20 children had a positive challenge test with a partially hydrolysed whey formula (Nidina H.A.): four developed asthma, three urticaria and two lip oedema. All children had positive prick skin tests to cow's milk proteins (casein and/or lactalbumin); 9 to Nidina H.A.; 3 to Profylac, and 3 to Alimentum. Specific IgE antibodies to cow's milk were present in all children; in 13 to Nidina H.A., in 4 to Profylac, and in 3 to Alimentum.

Key words: Allergenicity – Cow's milk allergy – Cow's milk protein hydrolysate formulae

Introduction

Cow's milk (CM) protein hydrolysate formulae (HF) have been developed with the aim of decreasing or eliminating the allergenicity of CM proteins and of reducing the antigenic load and the risk of sensitization. The use of these products is based on the premise that predigested protein, i.e. a mixture of amino acids and peptides, provides nutrients in a non-allergenic form. Thus, HF have been classified as "hypo-allergenic" and have been used for feeding babies with cow's milk allergy (CMA) or cow's milk intolerance [2, 15] and/or for the prevention of atopic disease [5, 9, 22, 23]. Depending on protein source, bovine casein and bovine whey HF are available on the market. More recently a 50% casein and 50% whey HF has been developed in Europe. Depending on processing techniques HF differ in the extent of hydrolysis. The reduction of the molecular weight is associated with a reduction of the palatability caused by the formation of bitter-tasting peptides during proteolysis of casein [8]. Several investigators have demonstrated the cross-reactivity between different HF and CM proteins [13, 14, 17, 20]. We first reported anaphylactic reactions in five infants with IgE-mediated CMA fed a whey HF [4], thus indicating that this formula has immunoreactive epitopes, subsequently confirmed by others [6, 10, 19]. The aim of this study was to investigate the allergenicity of three different HF in children with IgE-mediated CMA. Our study indicates that the degree of hydrolysis strongly influences the allergenicity of these products: extensively HF appear to be the least allergenic in children with CMA.

Methods

Twenty children (median age 31 months, range 15–76 months) with a history of IgE-mediated CMA attending our clinic from June 1990 to February 1991 were included in the present study. All the children had immediate allergic respiratory (asthma) and/or

Correspondence to: L. Businco

Abbreviations: CM = cow's milk; CMA = cow's milk allergy; DBPCFC = double-blind placebo controlled food challenge; HF = hydrolysate formula

cutaneous (contact urticaria and/or generalized urticaria or angioedema) and/or gastro-intestinal (diarrhoea) reactions to CM ingestion. In addition, the children had positive prick skin tests and positive RAST to CM.

Skin testing was done by the prick method on the volar surface of the forearm. The prick tests were read at 20 min and considered positive as follows: + when the wheal was half of the histamine wheal; ++ when the wheal was equal to the histamine wheal; +++ when the wheal was twice the histamine wheal; ++++ when the wheal was more than twice the histamine wheal.

Children were tested with diluent control, histamine control, whole CM protein, casein and lactalbumin (Lofarma, Milano, Italy). In addition to CM, a casein HF (Alimentum, Ross), and two whey HFs a highly hydrolyzed (Profylac, ALK) and a partially hydrolyzed (Nidina HA, Nestlé) formula were tested. These formulae were freshly prepared according to the instructions on the can; a drop was placed on the volar surface of the forearm and a skin test by prick was done. A new pin was used for each prick test.

Specific IgE to the following were evaluated: CM, casein, α -lactalbumin, β -lactoglobulin, Alimentum, Profylac and Nidina HA (Phadezim RAST, Pharmacia, Uppsala, Sweden). RAST discs for the HF were kindly supplied by Prof. G. Johansson. The limit for positivity was set to >0.35 PRU/ml (Phadebas RAST Units/ml) (1° class).

Double-blind placebo controlled food challenge (DBPCFC) were performed in a randomized order in 20/20 children up to 100 ml of CM, Alimentum, Nidina HA, and placebo and in 15/20 with Profylac. A soy-based formula (Isomil, Abbott) was used as placebo, because all the children were fed this product without reaction. Randomization was performed by one of us (L.B.) who was not aware of the follow up of the child. CM, HF, or placebo were administered as follows: a drop was put upon the inner border of the lower lip, and a further 5 ml were given after 5 min. If no symptoms, 100 ml were given after 30 min. After the last administration of the tested formula the children were kept under observation for at least 4 h and then discharged. The next challenge test was done 1 week after the previous challenge.

Profylac, Isomil, Nidina H.A., powder and Alimentum ready to feed were used for the challenge test and for the prick test. The challenge test with Profylac was not done in five children due to no compliance.

Results

All the 20 children had immediate allergic reactions after the challenge test with CM; the main symptoms were: urticaria in 7 children, asthma in 5, angioedema in 3,

Table 1. Main symptoms occurring after DBPCFC with CM and three hydrolysate formulae in 20 children with CMA

	Cow's milk	Nidina H.A.	Profylac	Alimentum
Children tested	20	20	15	20
Symptoms:				
Respiratory (nasal or wheezing)	7	4	—	1
Urticaria	7	3	—	1
Angioedema	3	—	—	—
Lip oedema	—	2	—	—
Perioral erythema	—	—	2	—
Vomiting and/or diarrhoea	3	—	—	—
Total	20	9	2	2

Table 2. Positive response to prick by prick, RAST and challenge with three hydrolysate formulae in 20 children with CMA

	Prick <i>n</i>	RAST <i>n</i>	Challenge <i>n</i>
Alimentum (casein)	3/20	3/20	2/20
Profylac ^a (whey protein)	3/20	4/20	2/15
Nidina H.A. ^b (whey protein)	9/20	13/20	9/20

^a Highly hydrolysed

^b Partially hydrolysed

angioedema and asthma in 2, vomiting in 1, diarrhoea in 1, vomiting and diarrhoea in 1.

Two of the 20 children had a positive challenge test with Alimentum, 2/15 with Profylac and 9/20 with Nidina H.A. (Table 1). The positive response to the challenge test occurred within 45 min (2–45' min) in all the children.

All the children had positive prick skin tests to CM proteins (casein and/or lactalbumin); 9/20 to Nidina H.A.; 3/20 to Profylac, and 3/20 to Alimentum. Specific IgE to CM were present in 20/20 children; in 13/20 to Nidina H.A., in 4/20 to Profylac, and in 3/20 to Alimentum (Table 2).

Discussion

In this study we have utilized in vivo (DBPCFC and prick test) and in vitro (RAST) methods to test the allergenicity of three different HF in a population of highly sensitive children with documented IgE-mediated CMA. Our data show that positive challenge tests to the partially hydrolysed whey formula occurred in 9/20 of the children. IgE antibodies to this product were present in the skin in 9/20 of the children and in 13/20 in the serum.

This strongly indicates that, despite the definition of "hypo-allergenic", this HF contains a large number of reactive epitopes. Approximately 30% of the peptides of this formula have a molecular weight of more than 5000 kDa. In addition, it has been shown that significant amounts of native casein and β -lactoglobulin are still present compared with extensively hydrolysed formula [3, 16, 18].

The immune response to a foreign protein includes antibodies directed against determinants on the surface of the protein (conformational epitopes) as well as determinants hidden within the native protein (sequential epitopes) [1]. The residual antigenicity and potential allergenicity is dependent on the food-processing technologies. The heat treatment can eliminate conformational epitopes and enzymatic cleavage can eliminate sequential determinants. Although the proteins of HF contain peptides of lower molecular weight than the native protein source, the peptides still have allergenic potency and can be recognized by the cell-bound IgE of children allergic to CM [4, 13, 14, 17, 20, 21]. The present study shows that even the two extensively hydrolysed formulae still have immune reactive epitopes, being the challenge tests positive to the extensively hydrolysed whey formula in 13%

of the children, and to the casein in 10%. This data is in agreement with the results of our previous study, which showed that an extensively hydrolysed whey formula triggered anaphylactic reactions in children with IgE-mediated CMA [4].

Casein HF have been available for over 40 years and there have been few reports of adverse reactions to these formulae [19]. It has been recently confirmed the safety of a casein HF (Alimentum) in 20 children with CMA: no child reacted to this product [18]. However in our study 2/20 children had a positive DBPCFC to Alimentum. The median age of the children of our present study was 3 years, while that of the patients of Sampson et al. was 5 years. It is known that usually the younger the child is, the more severe the reactions to food will be. In addition, the symptoms presented by our patients were immediate and severe: angioedema, urticaria, respiratory symptoms, while the main presenting symptoms in the patients of Sampson et al. were atopic dermatitis and gastro-intestinal manifestations, but several had life-threatening reactions and much greater levels of milk specific antibodies [18].

Schwartz et al. [20] described children with different forms of CMA. The group who had the most severe reactions (urticaria, angioedema, wheezing) with CM, also had positive skin test to the casein HF (Alimentum, Nutramigen), while other patients with less severe form of CMA did not have positive skin test to these products but had positive prick test to the partially hydrolysed whey formula [20]. In conclusion, HF still have allergenic potency and their peptides can be recognized by IgE antibodies of some children allergic to CM, thus triggering allergic reactions, which may even be life-threatening in certain circumstances. In addition, the present study strongly confirms that partially hydrolysed whey formulae are not safe in children with IgE-mediated CMA and we would like to stress that, in order to eliminate the potential confusion regarding the meaning of "hypo-allergenic", this definition should be removed from the front of the package of these products, as recently recommended [12] and approved in the US.

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