

Use of hydrolysates in the treatment of cow's milk allergy

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Background: Hydrolyzed formulas (HFs) are in general well tolerated by children with cow's milk allergy (CMA), although cases of allergic reactions have been reported and residual allergenicity of HF has been demonstrated

Objective: To review the most relevant studies of the HFs for residual allergenicity, tolerance, and safety in the past 20 years.

Data Sources and Methods: MEDLINE searches for the years 1970 to 2001 using the following algorithm (hydrolysate and allergy; food intolerance/allergy; protein hydrolysate).

Results: The literature confirmed that although some antigenicity remains, HFs are well tolerated by children with CMA. Rice HF has proven safe when tested by double-blind, placebo-controlled food challenge in a study population of 18 children allergic both to cow's milk (CM) and soy protein.

Conclusions: Absolute avoidance of CM proteins means substitution by soy-, rice-, or amino acid-based formulas. As 8 to 14% of infants allergic to CM react to soy and amino acid-based formulas are expensive, scientific societies recommend the use of formulas based on extensively hydrolyzed CM proteins as first alternatives in children with CMA. Although both soy- and rice-based HFs have now been shown to be safe for these children, further nutritional and clinical studies are needed.

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INTRODUCTION

Cow's milk allergy (CMA) has been known since Hippocrates described an adverse reaction to milk,¹ but it was only with the classical studies of Praustnitz and Kustner in the 1920s that food allergy was demonstrated. Cow's milk proteins (CMPs) are among the most common food allergens and the prevalence of CMA ranges from 0.3 to 7.5% in the general population.^{2,3} The prevalence of CMA among food-allergic infants and children is age-dependent, ranging from 39% in the first year of life to 27% at 3 years and 8% at 6.⁴

When children are diagnosed with CMA, dietary substitution of CMP becomes essential. The diet must be nutritionally adequate, palatable, and safe.⁵ However, there is no international consensus as to the cow's milk (CM) substitute of first choice. The European Society of Pediatric Allergy and Clinical Immunology/European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPACI/ESPGHAN) statement⁶ on the treatment and prevention of food allergy recommends the use of extensively hydrolyzed formulas (EHFs) as first-choice treatment.

The main features of this document are 1) that goat's or ewe's milk are not recommended because of a high degree of cross-reactivity with CMPs; 2) partially hydrolyzed products with moderately reduced allergenicity are not recommended; 3) formulas based on intact soy protein isolates are not generally recommended for the initial treatment of infants; and 4) products with greatly reduced allergenicity based on

extensively hydrolyzed proteins, or amino acid mixtures, are recommended for the treatment of CMA.

More recently, the Committee on Nutrition of the American Academy of Pediatrics (AAP) has made similar recommendations,⁷ with one striking difference: intact soy milk is recommended for the initial treatment for immunoglobulin (Ig)E-mediated CMA.

"Formulas based on intact soy protein isolates are recommended for the initial treatment of food allergy in infants, particularly after the age of six months [...]; [however,] formulas based on intact soy proteins are not recommended for the treatment of infants with proctocolitis and enterocolitis not IgE-associated."⁷

METHODS

This review discusses milk substitutes based on hydrolyzed proteins. All these formulas are obtained from proteins that undergo a preliminary heat treatment that modifies the conformation of proteins and facilitates their subsequent enzymatic hydrolysis. Pepsin, trypsin, or pancreatic extracts are then used to hydrolyze sequential epitopes with the objective of obtaining the least allergenic and antigenic formula.⁸ Currently available hydrolysates are derived from CM, soy, or rice. In this paper, the literature was classified on the basis of the protein source.

CMP Hydrolyzed Formulas (HFs)

These formulas are derived from casein or from the whey fraction. After purification of the protein fraction, allergenicity is reduced by heat treatment to denature conformational epitopes, together with extensive enzymatic hydrolysis and ultrafiltration (mesh size between 5 and 10 kDa).^{9,10} Pasteurization alone has little effect on the allergenic activity of

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CMPs.¹¹ However, some residual antigenic activity has been found in all types of extensive hydrolysates both in vitro¹²⁻¹⁸ and in vivo.^{12,13,18-22} Sampson et al¹⁸ studied 25 children allergic to CM diagnosed by double-blind, placebo-controlled food challenge (DBPCFC) and found that all subjects tolerated both Nutramigen (Mead Johnson, Evansville, IN) and Alimentum (Abbott Labs, North Chicago, IL; both casein EHF) in blinded food challenges. Alimentum and Nutramigen analyzed by enzyme-linked inhibition assay were found to contain similar amounts of CMPs and 5 of the 10 children had a positive prick test to Alimentum and 6 of 10 to Nutramigen, however.

Wahn et al¹² compared EHF and partially HF (PHF) in vivo (prick tests in 20 patients and challenges in 8) and in vitro (radioallergosorbent test [RAST] and RAST inhibition). All EHF showed allergenic activity by prick tests, whereas RAST and RAST inhibition showed lower residual antigenic activity with casein hydrolysates than with whey-derived formulas. Challenge tests in eight patients positive to a challenge with whole CM gave no reactions to casein EHF and 2 of 8 positive reactions to whey EHF. PHFs gave positive reactions in 5 of 8 patients.

Ragno et al¹³ studied 20 children allergic to CM and fed soy formula. Prick tests, RAST, and DBPCFC were performed with casein EHF (Alimentum), whey EHF (Profylac, ALK-Abelló, Hørsholm, Denmark), and a PHF (Nidina HA, Nestlé Research Laboratories, Vevey, Switzerland). All formulas tested showed residual antigenic activity in at least some subjects: prick tests were positive in 3 of 20 with Alimentum and Profylac and 9 of 20 with Nidina HA, whereas RAST was positive in 3 of 20 for Alimentum, 4 of 20 for Profylac, and 13 of 20 for Nidina HA. DBPCFC was positive in 2 of 20 with Alimentum, 2 of 15, with Profylac, and 9 of 20 with Nidina HA.

Tables 1 and 2 list the commercially available CM hydrolysates tested in the studies described. Tables show that any formula may provoke a reaction among infants who are allergic to CM.^{12,13,19-21} The antigenicity and allergenicity of hydrolysate formulas is partially dependent on the molecular weight of their constituent proteins,²³ but even small frag-

ments of 700 to 1,400 Da can be immunogenic.²⁴ In 1998 it was shown²⁵ that the minimal molecular mass for IgE binding in vitro is between 1,400 and 970 Da, whereas peptides with a molecular mass >1,400 Da are needed for skin reactivity. If this is so, all commercially available HFs will contain peptide fragments that can induce clinical reactions in allergic infants and children, and it is therefore not surprising that clinical reactions have been reported with every type of CM hydrolysate currently available.

Halken et al²¹ investigated 66 children with CMP allergy or intolerance diagnosed by controlled elimination and challenge procedures. All 66 children underwent open, controlled challenges and tolerated Profylac; this group was compared with a group of 16 infants matched for age and challenged with and fed Nutramigen. All infants in these two groups tolerated both Profylac and Nutramigen, and no side effects were recorded. Among 35 children with IgE-mediated reactions, 2 of 35 had positive skin prick tests (SPTs) and 3 of 28 had specific IgE class 2 against Profylac.

In 1995, Hill et al¹⁹ found 18 children with a history of adverse reactions after ingestion of casein or whey hydrolysate formulas from parent reports and medical records. Seven had positive SPTs and RAST reaction to milk proteins and 11 had non-IgE-associated symptoms (irritability, vomiting, diarrhea, failure to thrive). All children were fed an amino acid-based formula (Neocate, Nutricia, Zoetermeer, the Netherlands) with remission of symptoms. After 2 months, DBPCFC was performed and 12 of 18 patients developed adverse reactions during challenges: 4 of 5 patients reacted to Nutramigen and 2 of 4 to Alfaré (Nestlé).

Martin-Esteban et al²² evaluated the clinical tolerance and safety of extensively hydrolyzed casein-whey protein formula in 33 patients with immediate CM hypersensitivity. The hydrolysate was tolerated by 31 of 33 patients (or 94%). Recently, Giampietro et al²⁰ investigated 32 children allergic to CMPs, performing SPTs and DBPCFC with two EHF, Nutrilon Pepti and Profylac. SPTs were positive in 6 of 31 for Nutrilon Pepti and 4 of 28 for Profylac; at DBPCFC only 1 of 31 reacted adversely to Nutrilon Pepti and 2 of 28 to Profylac.

Table 1. In Vitro Studies on Residual Allergenicity of Commercially Available Hydrolysates of CMPs

Hydrolysate	Authors							
	Sampson ¹⁸	Wahn ¹²	Ragno ¹³	Dean ¹⁷	Makinen ¹⁶	Restani ³³	Restani ¹⁵	Van Hoeyveld ²⁵
Nutramigen	P	P	—	P	B	ND	ND	—
Pregestimil	—	P	—	P	—	C	ND	—
Alimentum	P	—	P	—	—	ND	—	—
Profylac	—	—	P	—	B	ND	—	—
Pepti Junior	—	—	—	P	B	C,B	C	—
Nutrilon Pepti	—	—	—	—	—	C	C	—
Alfaré	—	P	—	ND*	B	C	—	P
Nidina HA	—	—	P	—	—	C	C	—
Vivena HA	—	—	—	—	—	C	C	—
Aptamil HA	—	—	—	—	—	C	C	—

P, positive detection of residual allergenicity; C, casein detected; B, β -lactoglobulin detected; ND, not detected; —, not tested.

Table 2. In Vivo (Skin Prick Test and/or Challenge) Studies on Residual Allergenicity of CMP Hydrolysates

Hydrolysate	Authors				
	Sampson ¹⁸	Wahn ¹²	Ragno ¹³	Hill ¹⁹	Giampietro ²⁰
Nutramigen	P	P	—	C	—
Pregestimil	—	P	—	N	—
Alimentum	P	—	P/C	—	—
Profylac	—	—	P/C	—	P/C
Nutrilon Pepti	—	—	—	—	P/C
Alfarè	—	P/C	—	C	—
Nidina HA	—	—	P/C	—	—
Vivena HA	—	—	—	—	—
Aptamil Ha	—	—	—	—	—

P, skin prick-positive; C, challenge-positive; N, negative; —, not tested.

Although residual antigenicity was demonstrated, more than 90% of allergic infants and children tolerate EHF.^{12,13,18,20,21} Table 3 summarizes the results of 5 of the 6 studies reported above evaluating clinical tolerance to CM hydrolysates, and shows that more than 90% of allergic children tolerate EHF. This is the basis of the recommendations of the AAP⁷ and the ESPACI/ESPGHAN.⁶ The AAP document requires that hypoallergenic formulas should be tested carefully, and

“[T]hese tests should at least ensure at 95% confidence intervals that 90% of infants with documented cow’s milk allergy do not react with defined symptoms to the formula under double-blind, placebo-controlled conditions.”

According to the AAP document,

“extensively hydrolyzed proteins derived from cow’s milk [...] have been subjected to extensive clinical testing and meet the standard for hypoallergenicity.”

Two double-blind, parallel randomized, clinical trials have shown that CM EHF, and both whey- and casein-derived HFs are efficacious and safe for infants with colic.^{29,30} As the cause of infant colic is still not understood, such demonstrations of efficacy should not lead to a diagnosis of CMA in infants on the basis of successful treatment by elimination of CMP.

Soy Hydrolysate Formula

There is only one soy hydrolysate formula, and it is derived from soy and pork (cattle until 1995) collagen proteins. Soy hydrolysate has so far received limited attention³¹; some studies^{12,16,17} show a residual antigenic activity, probably explained by cross-reactivity of CMP epitopes with some peptides present in soy collagen hydrolysate^{12,32} (Table 4). In most cases this cross-reaction has no clinical importance, and it has been shown that sera from CMA children rarely react with Pregomin (Milupa, Friedrichsdorf, Germany).³³ Controlled clinical studies have not been conducted to evaluate the clinical tolerance and nutritional properties of soy collagen hydrolysate, which remains an interesting but not thoroughly evaluated alternative for children with CMA.

Rice Hydrolysate Formula

Recently, a new HF obtained by hydrolysis of rice proteins has become available. Rice was formerly considered to trigger allergic symptoms,^{34,35} but the only paper³⁶ specifically addressing the issue has estimated frequency in allergic children as <1%. We investigated the clinical tolerance to rice HF (RHF) in a group of 18 patients with IgE-mediated CMA who developed immediate or delayed clinical symptoms of soy allergy after 2 to 18 months of treatment with a soy protein formula. In this group, CMA was diagnosed by either

Table 3. Studies on Tolerance of CM Hydrolysates: Percentage of Tolerant Children

Hydrolysate	Author				
	Sampson ¹⁹	Wahn ¹²	Ragno ¹³	Halken ²¹	Giampietro ²⁰
Nutramigen	100	100	—	100	—
Pregestimil	—	100	—	—	—
Alimentum	100	—	90	—	—
Profylac	—	—	87	100	94
Pepti Junior	—	—	—	—	—
Nutrilon Pepti	—	—	—	—	97
Alfarè	—	75	—	—	—
Nidina HA	—	—	55	—	—

—, not tested.

Table 4. Residual Allergenicity for CM Proteins in Soy-Collagen Formula: in Vitro and in Vivo Studies

Hydrolysate	Authors			
	Wahn ¹² 1992	Dean ¹⁶ 1993	Makinen ¹⁷ 1993	Niggemann ³² 1999
Pregomin	P/R/C*	R	B	P/R

P, prick test-positive; R, RAST inhibition; C, challenge-positive; B, β -lactoglobulin detected.

* Tolerance with DBPCFC was 87.5%.

history or challenge (13 children) and soy allergy was confirmed by DBPCFC with soy. With a cut-off point for SPT positivity set by a wheal diameter >3 mm, all patients were found to be sensitized by the CM and soy reagents, fresh CM, and soy formula. Thirteen of 18 patients were SPT-positive to casein, 10 of 18 to lactalbumin, 8 of 18 to rice, and 2 of 18 to RHF. Serology was consistent with these findings: 2 children had positive SPT for RHF and 8 had detectable IgE for rice (SPT and/or RAST). As all children normally consumed rice in their diet, a rice challenge was not considered necessary. A DBPCFC with RHF 24 g, corresponding to 180 mL of reconstituted formula, was administered. All children accepted it without difficulty, and it tested negative in all cases.³⁷ Although cross-sensitivity between legumes and cereals has been shown by Lehrer et al,³⁸ such cross-reactivity is not so clinically important as to warrant the elimination of RHF from the diet of children allergic to soy. Thus, when allergy to both CM and soy is diagnosed, RHF constitutes a safe alternative to CM hydrolysates and offers a safe and nutritionally adequate alternative food with which to avoid unnecessary dietary restrictions.

CONCLUSION

There is a wide choice of safe and nutritionally adequate formulas to use as CM substitutes in children with CMA. From the recommendations of both ESPACI/ESPGHAN and AAP, there emerges a consensus that, although EHF's retain a detectable and clinically significant amount of antigenicity, they can be used as generally safe for most children with CMA. In this context these patients should undergo evaluation (including the specific formula) before an EHF is adopted as a substitute.

If we want to bypass bovine proteins absolutely, which should ideally be part of the treatment of CMA, the use of soy, rice, or an amino acid-based formula is necessary. However, from 8 to 14% of infants with CMA react to soy,³⁹ and amino acid-based formulas are both expensive and unpalatable. This can lead to growth delay through low caloric intake. Table 5 shows an inverse correlation between the degree of hydrolysis and the palatability of formulas.⁴⁰ There are no adverse reactions reported with the use of amino acid formulas.^{19,26-28} Should symptoms persist with EHF use, such products may be used. Soy collagen hydrolysate is an interesting, albeit not thoroughly evaluated, alternative formula

Table 5. Main Clinical Features of HF's According to the Degree of Hydrolysis and Protein Source⁴⁰

Feature	Casein EHF	Whey EHF	PHF
Low allergenicity	3+	3+	2+
Nutritional value	2+	2+	3+
Low osmolarity	2+	2+	2+
Good taste	1+	2+	3+
Low price	1+	1+	2+

for children with CMA. The recently available RHF has been shown to be safe for patients allergic to both CMPs and soy. Their palatability and patients' acceptance are good.

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