Hydrolyzed Proteins in Infant Feeding

Nestlé Nutr Inst Workshop Ser, vol 86, pp 11–27, (DOI: 10.1159/000442699)

Abstract
Hydrolyzed proteins are used worldwide in the therapeutic management of infants with allergic manifestations and have long been proposed as a dietetic measure to prevent allergy in at risk infants. The degree and method of hydrolysis, protein source and non-nitrogen components characterize different hydrolyzed formulas (HFs) and may determine clinical efficacy, tolerance and nutritional effects. Cow’s milk (CM)-based HFs are classified as extensively (eHF) or partially HF (pHF) based on the percentage of small peptides. One whey pHF has been shown to reduce atopic dermatitis in high-risk infants who are not exclusively breastfed. More studies are needed to determine the benefit of these formulas in the prevention of CM allergy (CMA) and in the general population. eHFs represent up to now the treatment of choice for most infants with CMA. However, new developments, such as an extensively hydrolyzed rice protein-based formula, could become alternative options if safety and nutritional and therapeutic efficacy are confirmed as this type of formula is less expensive. In some countries, an extensive soy hydrolysate is available.

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Introduction

Atopic diseases and related health costs have dramatically increased in the last decades, and identification of the optimal approach for both prevention and treatment of allergy represents an important health priority.

Prevention of allergy and/or atopic disease is complex because different pre- and postnatal factors interfere with its expression, and are difficult to be fully identified and controlled. For the treatment of food allergies, guidelines recommend elimination of the culprit food allergen(s) [1–3] and, in cow’s milk (CM) allergy (CMA), the choice of an extensively hydrolyzed formula (eHF; with still
small protein peptides) or an amino-acid-based formula is determined by the severity of the allergic manifestations, adverse reactions, acceptance, costs and availability of the formula. Whether a small amount of tolerated proteins in the diet facilitate acquisition of tolerance is still a matter of research. A recent study has shown that early introduction of peanuts modulated specific immune responses and significantly decreased the frequency of the development of peanut allergy among high-risk children [4].

Cow’s Milk Allergy

CMA is, together with egg allergy, the most frequent food allergy in early childhood and affects 0.5–6% of infants [1, 5]. The incidence is determined by the type of feeding (breast- or formula fed) and the criteria of diagnosis (self-report or challenge proven) [1]. Symptoms of CMA are nonspecific, range from mild to severe and often involve different organs (e.g. the skin, respiratory and/or gastrointestinal tract and the systemic circulation), induce general manifestations (irritability, sleeping problems, poor growth or shock), and overlap with other disorders (i.e. gastroesophageal reflux disease) or functional conditions. A correct diagnosis may be challenging even for expert physicians, particularly in patients with negative IgE tests, but it is pivotal for an adequate management. Moreover, in the last years, an increased number of children is reported to be sensitized at the same time to different CM proteins (CMPs) and to other food allergens even during exclusive breastfeeding; with allergic manifestations occurring early in life due to an impaired development of oral tolerance [6].

Cow’s Milk Proteins

CM presents two different fractions of proteins: casein (Cas) and whey proteins, both with allergenic properties. Whole Cas consists of four major proteins (αs1-, αs2-, β- and κ-Cas; Bos d 8) plus three γ-Cas deriving from the hydrolysis of β-Cas, namely γ1, γ2 and γ3. In whey, there are α-lactalbumin (α-Lac; Bos d 4) and β-lactoglobulin (β-Lg; Bos d 5), bovine serum albumin (BSA; Bos d 6), lactoferrin and immunoglobulins (Bos d 7) [7].

Cas, β-Lg and α-Lac are considered major allergens, i.e. more than 50% of the individuals with CMA are sensitized to those proteins [7]. Some patients are only sensitized to minor proteins, which are present at very low concentrations in milk, such as BSA and lactoferrin [7]. Immunoreactive epitopes and peptide fragments of both β-Lg and Cas have been well characterized. Due to the relative
resistance of β-Lg to acid hydrolysis and proteases, part of the protein remains intact and is absorbed as such.

The region of the major site of phosphorylation in bovine α- and β-Cas is strongly immunoreactive, resistant to digestive degradation and well conserved in other ruminant species (degree of sequence homology >80%), which explains cross-reactivity and allergic reactions in patients with CMA in case they consume ewe’s or goat’s milk as a substitute [8]. Milk allergenicity can be reduced by various processing methods but mainly by hydrolysis. Emerging knowledge of the immunogenicity of CMPs and peptides is of fundamental relevance for both patients and infant formula manufacturers to select and offer effective formulas for CMA.

Hydrolyzed Formulas

Many food allergens are stable and resistant to digestion by gastrointestinal enzymes or are digested into high molecular weight (MW) peptides which retain the IgE binding and T-cell-stimulating properties [7]. The molecular basis of alterations in allergenicity is the inactivation or destruction of epitopes. However, methods of CM processing should be carefully selected because they may even produce new epitopes (neotopes) or access hidden epitopes by denaturation of the native allergen (cryptotopes) [7].

Hydrolyzed formulas (HFs) differ according to the method, the timing and the degree of hydrolysis (i.e. intact protein molecules are broken down into peptides of various MW which have less allergenicity), the protein source (Cas, whey, rice or soy) and other nutritional components. The method of hydrolysis is critical for different allergens as heating denatures conformational epitopes (such as the ones of Cas and whey proteins), whilst glycation (nonenzymatic glycosylation) and lactic acid fermentation reduce allergenicity of α-Lac and β-Lg, specific enzymes act on sequential epitopes (from β-Lg), and ultrafiltration eliminates large proteins (such as β-Lg; table 1) [7]. Milk proteins in HFs are hydrolyzed using both specific industrial proteases, such as alcalase, pronase and papain, and gastrointestinal digestive enzymes, such as pepsin, trypsin and chymotrypsin, resulting in allergenic or bioactive peptides which can act differently from the intact native ones. The heating of proteins in the presence of reducing sugars produces the so-called Maillard reaction or glycation, which modifies milk proteins, their structure, aggregation and allergenic effect in different ways, which depend on the specific combination of proteins, sugars and temperature [7]. Cas and α-Lac are more heat stable than the whey proteins β-Lg and BSA. Heating milk at 120°C for 15 min did not alter the antigenicity of bovine Cas, but
recognition of β-Lg by IgE was reduced by nearly 70% at 75°C whilst BSA and immunoglobulins lost their antigenicity at 70–100°C [9]. This could explain, at least partially, why some CMA patients are more tolerant to boiled milk than to raw milk. Processing at high temperatures (baking) further reduces the allergenicity of many food proteins, most likely by altering the conformation structure of heat-labile proteins and consequently destroying their allergenic epitopes [10]. Microwave treatment (at 200 W for 3 min) increases hydrolysis of β-Lg and bovine whey proteins in comparison with conventional heating and the same proteolytic treatment [11]. High pressure also induces structural changes in (whey) milk proteins, such as denaturation, and enhances accessibility of potentially immunogenic hydrophobic regions to the enzymes and formation of aggregates, which may affect and reduce the allergenic potential of CMPs [12].

Proteolytic enzymes are even produced during fermentation by certain lactic acid bacteria that fragment epitopes and reduce allergenicity of both β-Lg and Cas [13–15].

**Partially and Extensively Hydrolyzed Formulas**

There is no general agreement on unique standards to specifically define partially hydrolyzed formula (pHF) and eHF, but the MW and percentage of the peptide fragments are generally used to classify the formulas into pHF or eHF. Both pHF and eHF consist of a wide range of peptide sizes. A pHF contains

<table>
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<tr>
<td>Heating</td>
<td>Denatures conformational epitopes</td>
<td>Cas and whey</td>
<td>Cas and α-Lac are more heat stable than β-Lg and BSA</td>
<td>7, 9, 10</td>
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<td>Enzymes</td>
<td>Destroy linear epitopes</td>
<td>Whey</td>
<td>Dependent on the specific enzymes used</td>
<td>7</td>
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<td>(Ultra)-filtration</td>
<td>Blocks large proteins</td>
<td>Cas and whey</td>
<td>Dependent on the size of the pores; immunoglobulins and lactoferrin have large MW</td>
<td>7</td>
</tr>
<tr>
<td>High pressure</td>
<td>Denaturation, enhanced accessibility to enzymes</td>
<td>Whey</td>
<td>Often combined with enzymatic treatment</td>
<td>12</td>
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<tr>
<td>Glycation or Maillard</td>
<td>Modifies the structure and aggregation of proteins</td>
<td>α-Lac and β-Lg</td>
<td>Dependent on heating in the presence of reducing sugars</td>
<td>7</td>
</tr>
<tr>
<td>Lactic acid fermentation</td>
<td>Produces proteolytic enzymes</td>
<td>Cas and β-Lg</td>
<td>Property of specific strains of bacteria</td>
<td>13–15</td>
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peptides with a MW generally <5 kDa, ranging from 3 to 10 kDa, with some commercial pHF containing 18% of peptides over 6 kDa [16]; an eHF should contain only peptides that have an MW <3 kDa [17] but usually has more than 90% of peptides <3 kDa, with 1–5% of peptides >3.5 kDa [16]. In contrast, the MW of whole CMP ranges from 14 kDa (α-Lac) to 24 kDa (Cas) and up to 67 kDa (BSA) [7]. The weight of peptides has immune and clinical relevance because the ‘bigger’ the peptides the ‘more allergenic’ they can be. Peptides >6 kDa and predominantly >10 kDa frequently act as allergens [18]. However, allergenic peptides of 3–5 kDa MW have been described more than 20 years ago [19]. For peptides <3 kDa, there is no agreement about the lowest MW cutoff for allergenicity [20]. In vitro assays showed reaction of sera of allergic patients to peptides of estimated MWs of 500–600 Da [21, 22]. Disagreement found in the literature about this issue may be due to the hydrolysis process of proteins used and the sensitivity of patients against the allergen [20].

pHFs are developed with the aim of minimizing the number of sensitizing epitopes within milk proteins, while at the same time retaining peptides with sufficient size and immunogenicity to possibly stimulate the induction of oral tolerance. Because pHF contains large CM peptides that can cause severe reactions in CMA patients, pHF is not recommended for the treatment of CMA [1–3]. eHFs are extensively hydrolyzed in order to destroy allergenic epitopes, maintaining the nitrogen in the form of free amino-acid formulas (AAFs) or very small peptides which are indicated in treatment but can as well be used in prevention. Which type induces the best oral tolerance in infants, pHF or eHF, is still debated. The MW profile of proteins only enables to differentiate protein characteristics of formulas, but does not clearly determine the allergenic properties and clinical response [18].

Because of the different responses to whey or Cas allergens, children who do not tolerate whey-based eHF (eHF-W) may be able to tolerate Cas-based eHF (eHF-C) and the other way round [23].

Commercial HFs vary in protein source (e.g. Cas, whey, soy or rice), method and percentage of hydrolysis, content of β-Lg or other proteins, content of non-protein components (such as lactose, DHA, nucleotides or probiotics) and osmolarity implying that the immune and clinical ‘results’ of one formula cannot be transferred to another.

Prevention of Allergy

The prevention of allergic diseases is a public health priority in many developed countries due to high morbidity, impaired quality of life, and impressive social and medical costs related to allergy. Tolerance development and allergy risk are
influenced by a complex array of factors, including genetics, epigenetic regulation of gene expression, birth and feeding mode, microbial environment, and exposure to environmental toxins or pollutants [24].

Allergy prevention can be directed at three potential stages: primary prevention, which inhibits immune sensitization; secondary prevention, which avoids disease expression subsequent to sensitization, and tertiary prevention, which suppresses symptoms after disease expression [25]. However, tertiary prevention can be considered as early treatment.

Prenatal prevention is complex and multifactorial, and dietetic intervention during pregnancy is not currently substantiated by scientific evidence [2]. Postnatally, dietetic prevention is based on the promotion of breastfeeding and HF with a documented preventive effect for the first months in formula-fed high-risk infants [2, 26].

For infants >4–6 months of age, there are insufficient data to support a protective effect of any dietary intervention regarding the development of atopic disease [2, 17]. A recent study showed that the early introduction of peanuts modulated immune-specific responses and significantly decreased the frequency of the development of peanut allergy among children at high risk for this allergy [4].

**Target Population: Who Is the High-Risk Infant?**

The risk of atopy is increased to about 1:3 if the parent or sibling is atopic, and 70% if both parents are atopic [26]. Hence, the presence of a positive family history of atopic disease represents the condition to define the newborn baby as at-risk infant. In the literature, this definition varied from infants/children having two allergic parents or relatives [25] to (a more recent one) at least one parent and/or sibling [17] with a documented (history of) allergic disease sometimes also supplemented with an increased cord blood IgE [2]. However, familiar allergy should be doctor diagnosed and not based on self-reported symptoms, as the perceived prevalence of food allergy is far greater than the real occurrence, determining an overproportion of subjects considered at high risk. Conversely, as many atopic subjects do not have a family history of atopy [26], the cost-benefit ratio of prevention in the overall population should be considered, on condition that the preventive recommendations have no adverse effects.

Extrapolating the data from the 15-year follow-up of the largest German Infant Nutritional Intervention (GINI) and LISA birth cohort studies, it has recently been reported that parental allergic diseases with first onsets before and after the birth of a child both increase the risk of childhood allergic diseases, especially for asthma. Furthermore, it suggests that a reasonable proportion of participants (6.6% in that report) in longitudinal studies are misclassified with
respect to their family history of allergic disease when this history is only assessed at baseline [27].

Clinical trials have demonstrated that the risk of developing atopic dermatitis is reduced when using HF to feed infants with a documented risk of atopy when breastfeeding is not practiced [1, 2, 16, 26–30]. In 2006, a Cochrane review, based on 14 randomized controlled trials (RCTs) or quasi-RCTs, concluded that there is no evidence to support feeding with HF for the prevention of allergy compared to exclusive breastfeeding. In high-risk infants who are not breastfed, there is limited evidence that prolonged feeding with HF compared to CM standard formula (SF) reduces infant allergy and infant CMA [26]. As the potential benefit of these formulas has only been documented in infants at genetic risk of developing atopic disease, additional studies are needed particularly among unselected infants or infants at low risk [2, 31].

**Partially Hydrolyzed Formulas**

Compared to eHFs, pHFs offer economical and taste advantages, and a theoretical benefit in inducing oral tolerance to the CMP as they still have a higher residual allergenicity.

A meta-analysis showed that a specific whey-based pHF (pHF-W) significantly reduces the incidence of atopic dermatitis approximately 44% [11 trials; summary relative risk (RR) estimate 0.56, 95% CI 0.4–0.77] up to 3 years of life in at-risk infants compared to CM-SF [28]. Another meta-analysis based on the use of the same pHF involving 3,284 participants (1,027 in pHF and 2,257 in control groups) reported reductions in all allergic diseases of 52% (5 RCTs: RR 0.48, 95% CI 0.23–1.00) at 3 and 6 months of age, 38% at 12 months (4 RCTs: RR 0.62, 95% CI 0.45–0.85; number needed to treat 12) and 58% at 30–36 months (1 RCT: RR 0.42, 95% CI 0.19–0.90) compared to SF [29]. For atopic dermatitis or atopic eczema (8 RCT), using a random effect model, the use of pHF compared with SF statistically significantly reduced the incidence of eczema at 1 year (4 RCTs: RR 0.68, 95% CI 0.48–0.98), but not at 4–6 months (5 RCTs), 2 years (3 RCTs) or 30–36 months (2 RCTs) [29]. However, many trials included in the analysis presented a lack of methodological rigor in sequence generation, allocation, blindness, definition of atopic manifestations, outcome data, intention-to-treat analysis, calculation of the sample size, selective reporting and other biases [29, 31].

A single-blind RCT, published after these meta-analyses, enrolling 620 high-risk infants before birth, found a higher incidence of allergic symptoms at 2 years in the ones fed with soy formula (44%) compared to a similar rate (37.7 vs. 37.3%) in pHF- and CM formula-fed participants. There was no difference in skin prick test reactivity or, at 6 and 7 years, in the rates of eczema,
asthma or allergic rhinitis among the three groups [32]. However, different methodological issues have been raised that call for caution, including the unclear reason for publishing the results 15 years after collecting the data, outcome assessment through telephone interviews with parents and changing definitions of outcome indicators compared with previous publications on this cohort [33]. Moreover, the formulas were introduced after discontinuation of breastfeeding, determining a possibly delayed intervention and reduced clinical efficacy.

**Partially versus Extensively Hydrolyzed Formulas**

Only few studies compared the effect of pHFs and eHFs [29, 31]. Differences in the study design, definition of atopic manifestations, selection of formulas (not all commercially available), presence of concomitant intervention and small sample sizes limited the comparative analysis [31].

In a prospective comparative Danish study, 595 high-risk infants were randomized at birth to one of three different blinded formulas. Of 478 infants who completed the study, 232 were exclusively breastfed, 79 received eHF-C, 82 eHF-W and 85 pHF-W during the first 4 months of life. The three formula groups were identical in regard to the atopic predisposition, cord blood IgE, birthplace, tobacco smoke exposure, gender, duration of breastfeeding, and age at introduction of the formula and solid foods. No significant differences were found in the three groups regarding the cumulative incidence of atopic dermatitis or respiratory symptoms. pHF was found to be less effective than eHF in preventing CMA (0.6 vs. 4.7%; p = 0.05) [34].

GINI, the largest German study, prospectively analyzed 2,252 at-risk infants, of which 945 represented the intervention arm and were randomly assigned to receive one of three HFs (n = 689) or CM-SF (n = 256). The 3 HFs were pHF-W, eHF-W and eHF-C [35]. The authors reported a significant reduction in eczema at all study points (1, 3, 6 and 10 years) when using the pHF (odds ratio, OR, 0.56; 95% CI 0.32–0.99) or the eHF-C (OR 0.42, 95% CI 0.22–0.79) but not with eHF-W or CM-SF. The RRs for the cumulative incidence of atopic dermatitis in the intention-to-treat analysis (n = 2,252) were 0.82 (95% CI 0.68–1.00) for pHF-W, 0.91 (95% CI 0.76–1.10) for eHF-W and 0.72 (95% CI 0.58–0.88) for eHF-C compared with the infant SF. The cumulative incidence of atopic manifestations was better reduced with the eHF-C compared to the other HFs. No effect was found regarding rhinitis, asthma and urticaria [30, 35]. Thus, this study highlights that different hydrolysates may have different effects on atopic disease [17].

Economic analyses in 5 European countries (Denmark, France, Germany, Spain and Switzerland) have evaluated the costs and cost-effectiveness of a spe-
cific brand of 100% pHF-W (NAN-HA®) compared with a CM-SF in the prevention of atopic dermatitis in at-risk children [36, 37]. Outcomes were considered from the perspective of the public health care system (e.g. the Ministry of Health; MOH), family and society. The cost-effective analysis per avoided case of atopic dermatitis showed the following results: pHF-W versus SF EUR 982–1,343 (MOH perspective), EUR −2,202 to −624 (family perspective) indicating savings, and EUR −1,220 to 719 from the societal perspective. The main costs are related to formula (MOH and society) and time loss (family). In the cost-minimization analysis, pHF-W yielded savings of EUR 4.3–120 million compared with eHF-W when the latter was used in disease prevention. In conclusion, pHF-W was cost-effective versus SF in the prevention of atopic dermatitis and cost savings compared with eHF when used in disease prevention [36, 37]. However, it should be noted that Nestlé employees are involved as coauthors in these papers, what may have induced a bias.

Other Formulas
The use of AAFs and rice hydrolysates for the prevention of atopic disease has not been studied. Soy formulas, on the other hand, have a long history of use for atopic disease in infants. In a Cochrane review based on 5 RCTs or quasi-RCTs, the authors concluded that feeding with soy formula should not be recommended for the prevention of atopy in infants at high risk of developing allergy [38].

Treatment of Allergy

The basic treatment of CMA is avoidance of intact CMPs. In early childhood, a milk substitute is needed and, if the diagnosis of CMA is confirmed, the elimination diet in the nonbreastfed infant using an eHF with documented efficacy are recommended by all allergy guidelines, and the therapeutic formula should be maintained for 6 months or until 9–12 months of age [1].

pHF should not be used because of a high degree of antigenicity and allergenicity. Although lower than pHF, residual allergenicity is present even in eHF whilst the only anallergic formulas are the elemental ones based on AAFs that cannot determine an immune stimulation [1–3]. AAFs are peptide-free formulas that contain mixtures of essential and nonessential amino acids [17]. AAFs are only indicated in treatment. AAFs are recommended for infants who refuse or do not tolerate eHF or in the most severe cases of CMA [1–3]. Compared to eHFs, costs of AAFs are higher in most countries, and they have a different taste and, possibly, a different long-term nutritional effect [2, 39].
Soy protein is as allergenic as CMP [2], although there are also reviews that conclude that soy allergy is less frequent [40]. According to ESPGHAN, soy formulas are not recommended for infants <6 months of age [1], although the American Academy of Pediatrics does not make this difference according to age [17]. Alternative milk substitutes such as sheep’s and goat’s milk should not be used because of a high degree of cross-reactivity with CMP [2]. Milk from other mammals such as mares and donkeys may be tolerated by some children with CMP allergy [2]; however, to the best of our knowledge, there is no infant formula on the market derived from mares or donkeys, and thus these milk formulas cannot be recommended for infants and young children because of nutritional inadequacy.

Recent treatment modalities such as oral immunotherapy involving the ingestion of increasing amounts of milk allergen on a regular basis to desensitize and potentially make patients permanently tolerant have been developed [41]. Currently, this strategy cannot be applied in young children [41].

**Partially Hydrolyzed Formulas**

pHF is tolerated by approximately 60% of milk-allergic individuals [42]. Because it contains potentially allergic CM peptides and can cause severe reactions, it is not recommended for the treatment of CMA but only for its prevention [1, 2, 17, 42].

**Extensively Hydrolyzed Formulas**

CM-derived eHF is the preferred treatment option in infants with CMA who are not breastfed except in the ones who refuse or do not tolerate eHF or in the most severe cases, in which AAF should be started. The majority of infants and children with CMA tolerate an eHF with whey or Cas as a nitrogen source [1]. According to the literature, a few (severe) allergic infants (2–10%) might still react to eHF-C and eHF-W [1–3, 17]. The choice of the eHF should be based on the efficacy demonstrated by scientific studies [1].

Two recent trials with a new eHF-C [43] and a new eHF-W [44] in infants with mild-to-moderate CMA showed that all confirmed CMA cases tolerated the formulas tested [1]. A symptom-based score decreased significantly in all infants within 1 month [43, 44], and the highest reduction occurred in those with challenge-confirmed CMA [43]. The thickened version of the eHF-C produced also a reduction in regurgitation in infants with positive or negative challenge [43].

HFs may indeed improve reflux-related and digestive symptoms not only by an immune mechanism but also by acting on gastric emptying time. A double-blind, randomized, crossover study compared gastric emptying in 20 healthy
newborns fed CM-SF, pHF and eHF containing 50 ml $^{13}$C-octanoic acid. eHF resulted in significantly faster gastric emptying than SF and pHF (medians 46 vs. 55 and 53 min, respectively) [45].

There is limited evidence that the addition of pre- or probiotics (e.g. *Lactobacillus rhamnosus* GG, LGG, or *Bifidobacterium lactis*) to eHF offer an additional benefit [46, 47].

A cohort study in 412 infants with CMA with data extracted from the Truven Health MarketScan® Commercial Claims Database in the US showed that 56% of infants fed eHF-C enriched with the probiotic LGG were estimated to have been successfully managed by 9 months compared to 38% of eHF-C-fed infants and 35% of AAF-fed infants (p < 0.05 and p = 0.003, respectively) [48]. Infants in the AAF group used significantly more health care resources and prescribed drugs than infants in the other two groups. The estimated costs of managing a CMA infant over the first 12 months following the start of feeding were USD 3,577, 3,781 and 6,255 for an infant fed eHF-C plus LGG, eHF-C only and AAF, respectively [48].

Rice-Based Extensively Hydrolyzed Formulas
One prospective open, randomized clinical study compared the clinical tolerance of a hydrolyzed rice protein formula (HRPF) with a CMP eHF in 92 infants (mean age 4.3 months, range 1.1–10.1 months) with IgE-mediated CMA. The HRPF was well tolerated in all infants tested, and measurement of IgE levels towards CMP during the study showed no significant differences between the two formula groups. During the follow-up (at 3, 6, 12 and 24 months), children receiving HRPF showed similar growth and development of clinical tolerance to those receiving an eHF [49].

A new extensively HRPF (eHRPF) for infants was tested in 40 infants with CMA confirmed by food challenge. All infants tolerated the eHRPF (according to a symptom-based score and growth parameters) and symptoms significantly decreased in the first month of the eHRPF intervention [50, 51].

However, the content of arsenic in rice infant formula needs to be controlled [52], and further studies with short- and long-term data on allergic reactions, nutritional adequacy and safety are needed.

Other Formulas
Adverse reactions in CMA individuals consuming soy milk have been attributed to a 30-kDa, glycinin-like protein from soybean that cross-reacts with Cas CM. The European and American guidelines do not recommend soy in infants with CMA as first option [1, 2, 53]. Tolerance to soy protein has been reported in 83–92% of the infants with CMP allergy [40, 53]. Nutritional and safety concerns
are mostly related to absorption of micronutrients and phytate and isoflavone contents. More recently, a review on soy infant formula concluded it to be safe [54].

Other protein sources have been assessed in the treatment of CMA. In some countries, goat’s milk exists as commercialized infant formula and is adapted to the nutritional needs of infants. However, the cross-reactivity with CMP is about 80–90% [8]. Milk from other mammalians or chicken-based formulas cannot be recommended for the treatment of CMP allergy for limited data on tolerance, safety and nutritional adequacy [1–3].

**Acquisition of Tolerance**

Oral tolerance, defined as hyporesponsiveness to innocuous antigens, may be explained by T-cell anergy, clonal deletion by apoptosis and active (contact-dependent) or cytokine-mediated (immune deviation) suppression exerted by subsets of regulatory T cells [55]. Important variables with regard to oral tolerance induction include genetics, age, dose and timing of postnatal oral antigen administration, antigenic structure and composition, gut epithelial barrier integrity and the degree of concurrent local immune activation (reflected by local cytokine profiles and expression of costimulatory molecules on antigen-presenting cells; fig. 1) [55].

Most children have outgrown CMA by 3 years of age, but in a minority it can become persistent. Non-IgE-mediated allergy typically resolves earlier than IgE-mediated allergy. Conversely, high specific IgE and low IgA levels to β-Lg at diagnosis and low CM-specific IgG4 during follow-up are associated with persistent CMA [56].

In an open prospective comparative study, 260 infants diagnosed with CMA (IgE-mediated CMA in 43%) were evaluated for acquisition of tolerance. The rate of children acquiring oral tolerance after 12 months of treatment was significantly higher (p < 0.05) in the groups receiving eHF-C (43.6%) or eHF-C plus LGG (78.9%) compared with the other groups: rice HF (32.6%), soy formula (23.6%) and AAF (18.2%). Binary regression analysis (coefficient B) revealed that the rate of patients acquiring tolerance at the end of the study was influenced by two factors, the IgE-mediated mechanism (B –2.05, OR 0.12, 95% CI 0.06–0.26; p < 0.001) and the formula chosen, i.e. those receiving either eHF-C (B 1.48, OR 4.41, 95% CI 1.44–13.48; p = 0.009) or, even better, eHF-C plus LGG (B 3.35, OR 28.62, 95% CI 8.72–93.93; p < 0.001) [46].
Impaired growth in infants with food allergy may be related both to the disease itself, causing inadequate intake or compromised absorption, and/or to inappropriate nutrient content of the dietetic regimen. The maintenance of a nutritionally adequate diet is not easy, especially in more severe cases, but is mandatory [39]. In the first 4–6 months of life, formulas represent all and in the second semester of life about half of the nutrient source, and hence nutritional appropriateness is obviously mandatory to avoid short- and long-term health consequences [39]. For optimal utilization, the hydrolyzed protein source should respond to a precise pattern of indispensable amino acids with the branched-chain amino acids and valine representing around 50% of the essential amino acid quote [57]. AAFs are also diversified within the different types of formulas, with taurine practically being the only amino acid in SFs, while branched-chain amino acids and glutamate are the major amino acids in eHF [39]. In the GINI study, the longest prospective, randomized, double-blind trial of full-term at-risk neonates, eHF-C-fed infants showed a significantly lower gain in weight and BMI than infants fed the other formulas in the first year of life. No significant differences in weight and BMI were found among the other formula groups (pHF-W, eHF-W and CM-based formula) or the breastfed group over the entire period up to 10 years of life [58].

**Fig. 1.** Tolerance and allergy. GALT = Gut-associated lymphoid tissue.

**Nutritional Value**
According to adult taste, there is an inverse relation between peptide size and palatability that can influence the amount of intake compared to non- or less-hydrolyzed peptides. In infancy, eHF-C determined a significantly more savory, bitter and sour-tasting preference as long as the infants were not weaned [39]. According to adult taste, rice hydrolysates taste better than CM-based eHFs.

**Conclusion**

pHF and eHF represent a valid substitute of CM-SFs in infants at risk for or with CMA. The degree and method of hydrolysis, and nonnitrogen and additional components determine the efficacy, tolerance and nutritional effect of different HF. pHF may offer a beneficial preventive effect on eczema in formula-fed newborns with a family risk of atopy. eHF is tolerated by 90% of infants with CMA and is the treatment of choice in all except severe cases of CMA. Promising results were recently obtained with eHRPF. Nutritional adequacy of the HF should be carefully evaluated to ensure appropriate growth in allergic infants. A proper diagnostic approach and follow-up are necessary to avoid unnecessary or excessively prolonged use of HF.

**Disclosure Statement**

Y.V. is consultant for ASPEN, Biocodex and United Pharmaceuticals. S.S. has participated as a consultant and/or speaker for Arla Foods, Danone/Nutricia, IMS Health, Menarini, Milte and Nestlé.

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