

## Formula Selection for Management of Children with Cow's Milk Allergy Influences the Rate of Acquisition of Tolerance: A Prospective Multicenter Study

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**Objectives** To prospectively evaluate the effect of different dietary management strategies on the rate of acquisition of tolerance in children with cow's milk allergy (CMA).

**Study design** Otherwise healthy children (aged 1-12 months) diagnosed with CMA were prospectively evaluated. The study population was divided into 5 groups based upon the formula used for management: (1) extensively hydrolyzed casein formula ([EHCF], n = 55); (2) EHCF + *Lactobacillus rhamnosus* GG [LGG], n = 71); (3) hydrolyzed rice formula (RHF, n = 46); (4) soy formula (n = 55); and (5) amino acid based formula (n = 33). A food challenge was performed after 12 months to assess acquisition of tolerance.

**Results** Two hundred sixty children were evaluated (167 male, 64.2%; age 5.92 months, 95% CI 5.48-6.37; body weight 6.66 kg, 95% CI 6.41-6.91; IgE-mediated CMA 111, 42.7%). The rate of children acquiring oral tolerance after 12 months was significantly higher ( $P < .05$ ) in the groups receiving EHCF (43.6%) or EHCF + LGG (78.9%) compared with the other groups: RHF (32.6%), soy formula (23.6%), and amino acid based formula (18.2%). Binary regression analysis coefficient (B) revealed that the rate of patients acquiring tolerance at the end of the study was influenced by 2 factors: (1) IgE-mediated mechanism (B -2.05, OR 0.12, 95% CI 0.06-0.26;  $P < .001$ ); and (2) formula choice, such that those receiving either EHCF (B 1.48, OR 4.41, 95% CI 1.44-13.48;  $P = .009$ ) or EHCF + LGG (B 3.35, OR 28.62, 95% CI 8.72-93.93;  $P < .001$ ).

**Conclusions** EHCF accelerates tolerance acquisition in children with CMA if compared with other dietetic choices. This effect is augmented by LGG. (*J Pediatr* 2013;163:771-7).

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Cow's milk allergy (CMA) is the most common food allergy in early childhood, with an estimated incidence ranging between 2% and 3%.<sup>1,2</sup> The long-term prognosis for the majority of affected infants generally is good, with 80%-90% naturally acquiring tolerance to cow milk proteins (CMP) by the age of 5 years.<sup>3</sup> However, recent studies suggest that the natural history of CMA is changing, with an increasing persistence until later ages,<sup>4,5</sup> and increasing severity of illness.<sup>1,3</sup>

Recent guidelines addressing the optimal therapeutic approach for children with CMA recommend the use of substitutive formulas.<sup>6,7</sup> However, these recommendations are based largely on the safety, nutritional value, and relative costs of these formulas. The potential impact of different hypoallergenic formulas on disease duration in children with CMA are not considered due to a lack of comparative data.

We have demonstrated that the addition of the probiotic *Lactobacillus rhamnosus* GG, (LGG) to an extensively hydrolyzed casein formula (EHCF) accelerates acquisition of tolerance in infants with CMA compared with patients receiving EHCF alone.<sup>8</sup> To investigate whether a similar benefit is observed comparing EHCF containing LGG with other formulas, we designed a study to prospectively evaluate the effect of various dietetic choices on acquisition of tolerance after 12 months in children with CMA.

AAF	Amino acid based formula	DBPCFC	Double-blind placebo-controlled food challenge
APT	Atopy patch testing		
B	Binary regression analysis coefficient	EHCF	Extensively hydrolyzed casein formula
CM	Cow milk	LGG	<i>Lactobacillus rhamnosus</i> GG
CMA	Cow's milk allergy	RHF	Hydrolyzed rice formula
CMP	Cow milk proteins	SF	Soy formula
		SPT	Skin prick testing

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## Methods

This open nonrandomized trial was conducted from July 1, 2010-June 30, 2012. We prospectively evaluated otherwise healthy infants (1-12 months of age at the diagnosis) referred to 3 tertiary care pediatric allergy centers for a diagnostic oral food challenge for suspected CMA. All subjects were in stable clinical condition without symptoms of CMA, and already treated for a period of 15-30 days prior to recruitment with a formula that was selected and prescribed by a family pediatrician or physician when the symptoms appeared. Management following study entry did not vary depending upon formula type. Patients who used pre-probiotic products in the last 4 weeks, and patients with CMP-induced anaphylaxis, eosinophilic disorders of the gastrointestinal tract, food protein-induced enterocolitic syndrome, concomitant chronic systemic diseases, congenital cardiac defects, active tuberculosis, autoimmune diseases, immunodeficiency, chronic inflammatory bowel diseases, celiac disease, cystic fibrosis, metabolic diseases, lactose intolerance, malignancy, chronic pulmonary diseases, and malformations of the gastrointestinal tract were excluded.

At the first visit (visit 1), we performed: (1) full anamnestic and clinical evaluation; (2) skin prick testing (SPT) and atopy patch testing (APT); and (3) oral food challenge to confirm the diagnosis of CMA. Patients with a certain diagnosis of food allergy based upon the result of oral food challenge were enrolled and continued on an exclusion diet using the same formula prescribed by the referring physician for the treatment of CMA. We planned a new full clinical evaluation after 6 months (visit 2), and again after 12 months (visit 3), including all of the tests performed at visit 1 to evaluate whether the subjects had achieved oral tolerance to CMP. Demographic and clinical characteristics were also obtained in each subject. The study was approved by the Ethics Committee of the University of Naples, Federico II. The clinical evaluation and study protocols were identical in each study center.

SPT was performed using fresh cow milk (CM) containing 3.5% fat applied to the patient's volar forearm, and a 1-mm single peak lancet (ALK, Copenhagen, Denmark), with histamine dihydrochloride (10 mg/mL) and isotonic saline solution (sodium chloride 0.9%) as positive and negative control, respectively. Reactions were recorded on the basis of the largest diameter (in mm) of the wheal and flare at 15 minutes. The SPT result was considered "positive" if the wheal was 3 mm or larger, without reaction of the negative control.

APT was performed as previously described.<sup>9</sup> Briefly, 1 drop (50  $\mu$ L) of fresh CM containing 3.5% fat was placed on filter paper and applied with adhesive tape to the unaffected skin of the child's back, using 12-mm aluminium cups (Finn Chambers On-Scan Pore; Epitest Ltd Oy, Tuusula, Finland). Isotonic saline solution was the negative control. The occlusion time was 48 hours and results were read 20 minutes and 24 hours after removal of the cups. To exclude false positive reactions, we also tested allergens in

a 1:10 solution. Seventy-two hours after the start of the test, reactions were classified as follows: – negative; +/- doubtful: erythema only; + weakly positive: erythema and slight infiltration; ++ strongly positive: erythema, infiltration, papules; +++ very strongly positive: erythema, infiltration, papules, vesicles. Infants and their families were requested to report any delayed skin reaction that was noticed after this time. Irritant or doubtful reactions, including sharply demarcated confluent erythema, or reactions confined to margins without infiltration, were deemed negative.

All food challenges were performed in a double-blind placebo-controlled food challenge (DBPCFC) manner, and took place in the outpatient clinic of the centers involved in the study, on 2 separate days with a 1-week interval. Parents of infants taking antihistamine were advised to withhold these medications for 72 hours before and during the challenge. Randomization and preparation of the challenges were performed by experienced food allergy dietitians not directly involved in the procedures. Briefly, every 20 minutes, successive doses (0.1, 0.3, 1, 3, 10, 30, and 100 mL) of fresh pasteurized CM containing 3.5% fat or an amino acid-based formula (AAF) were administered. Full emergency equipment and medications (epinephrine, antihistamines, and steroids) were available. In each center, the results were assessed simultaneously by 3 experienced pediatric allergists. Study subjects were scored for 9 items divided into 4 main categories: (1) general (lowered blood pressure plus tachycardia); (2) skin (rash, urticaria/angioedema); (3) gastrointestinal (nausea/repeated vomiting, crampy-like abdominal pain, diarrhea); and (4) respiratory (sneezing/itching, nasal congestion/rhinorrhea, stridor deriving from upper airway obstruction or wheezing) on a 0- to 3-point scale (0, none; 1, light; 2, moderate; and 3, severe). If at least 2 of the 3 physicians independently scored any item at level 3, or 2 (or more) items at level 2, the test result was considered positive. Clinical symptoms occurring within 2 hours of administering the highest dose were defined as "immediate reactions," and those occurring more than 2 hours after the highest dose were defined as "delayed reactions." The infants were observed for 2 hours after the final dose, and then discharged. In the case of a positive DBPCFC, at any testing dose, the patient remained under observation until symptom resolution. If the patient did not show any symptoms within the first 24 hours, parents were advised to give one single feed of 100 mL of the tested formula (pasteurized CM with 3.5% fat vs placebo) every day at home for 7 days. If any symptoms occurred during this period, the patients returned to the outpatient clinic on the same day. After 7 days of administration, the patients were examined and the parents interviewed at the center. To rule out false-negative challenge result, parents were asked to contact the center if any symptoms occurred in the following 7 days after the DBPCFC procedures. The challenge was considered negative if the patient tolerated the entire challenge, including the observation period. Clinical acquisition of tolerance was defined by the presence of a negative DBPCFC. Children with negative DBPCFC were reevaluated after 6 months to check the persistence of acquisition of tolerance.

**Table.** Baseline main demographic and clinical characteristics of the study population

	Group 1 EHCF	Group 2 EHCF + LGG	Group 3 RHF	Group 4 SF	Group 5 AAF
N	55	71	46	55	33
Male, n (%)	41 (74.5)	38 (53.5)	28 (60.9)	37 (67.3)	23 (69.7)
Age, m (95% CI)	5.03 (4.20-5.86)	5.73 (4.83-6.62)	6.65 (5.53-7.76)	6.45 (5.45-7.45)	5.93 (4.57-7.30)
Weight, kg (95% CI)	6.47 (6.00-6.95)	6.66 (6.14-7.18)	6.97 (6.36-7.58)	6.96 (6.41-7.51)	6.04 (5.31-6.78)
Breastfeeding $\geq$ 2 months, n (%)	41 (74.5)	54 (76.1)	38 (82.6)	38 (69.1)	24 (72.7)
IgE-mediated CMA, n (%)	24 (43.6)	27 (38)	23 (50)	23 (41.8)	14 (42.4)
Gastrointestinal symptoms, n (%)	35 (63.6)	51 (71.8)	30 (65.2)	31 (56.4)	25 (75.8)
Vomiting, n (%)	23 (41.8)	27 (38)	17 (37)	18 (32.7)	10 (30.3)
Diarrhea, n (%)	18 (32.7)	24 (33.8)	13 (28.3)	13 (23.6)	19 (57.6)
Cutaneous symptoms, n (%)	25 (45.5)	29 (40.8)	17 (37)	27 (49.1)	11 (33.3)
Atopic dermatitis, n (%)	21 (38.2)	26 (36.6)	15 (32.6)	21 (38.2)	9 (27.3)
Urticaria, n (%)	6 (10.9)	6 (8.5)	5 (10.9)	8 (14.5)	5 (15.2)
Respiratory symptoms, n (%)	6 (10.9)	7 (9.9)	6 (13)	7 (12.7)	3 (9.1)

The primary end point was the rate of patients acquiring clinical tolerance to CMP after 12 months of exclusion diet with different formulas.

### Statistical Analysis

The Kolmogorov–Smirnov test was used to determine whether variables were normally distributed. For continuous variables, groups were compared using the *t* test and the Mann–Whitney U test. The  $\chi^2$  test and Fisher exact test were used for categorical variables. Binary logistic regression analysis was conducted to assess the possible influence of the following variables on the primary outcome: sex, age at randomization, breast-feeding, symptoms, IgE-mediated mechanism, and type of formula. The level of significance for all statistical tests was 2-sided,  $P < .05$ . All analyses were conducted on an intention-to-treat basis by a statistician blinded to patient group assignment, using SPSS, v. 16.0 for Windows (SPSS Inc, Chicago, Illinois).

## Results

A total of 329 infants (aged <12 months) were referred to the study centers for suspected CMA (Figure 1; available at [www.jpeds.com](http://www.jpeds.com)). Fourteen were excluded because of the presence of at least 1 exclusion criteria and 55 were excluded because of a negative DBPCFC. All of the subjects diagnosed with CMA consented to participate in the study.

A total of 260 infants with CMA were subdivided in 5 groups depending on the formula they were receiving at study entry: group 1 (EHCF, Nutramigen [Mead Johnson, Rome, Italy],  $n = 52$  and Nutribén hydrolyzed [Nutribén, Milan, Italy],  $n = 3$ ); group 2 (EHCF + LGG, Nutramigen LGG [Mead Johnson],  $n = 71$ ); group 3, (hydrolyzed rice formula [RHF], Risolac [Plasmon, Milan, Italy],  $n = 46$ ); group 4, (soy formula [SF, Isomil [Abbott, Milan, Italy]],  $n = 23$ ; Sinelac [Humana, Milan, Italy],  $n = 18$  and Nutrilon Soya [Nutricia, Milan, Italy],  $n = 14$ ); group 5, (AAF, Neocate [Nutricia],  $n = 16$ ; Nutramigen AA [Mead Johnson],  $n = 9$ ; and Sineall [Humana],  $n = 8$ ). Seven patients were lost to follow-up (group 1,  $n = 2$ ; group 2,  $n = 3$ ; group 3 = 0; group 4,  $n = 1$ ; group 5,

$n = 1$ ). Demographic and clinical characteristics of all 5 groups at baseline were similar (Table). The rate of patients with CMA-related enterocolitis was similar in the 5 study groups.

### SPT and APT Results

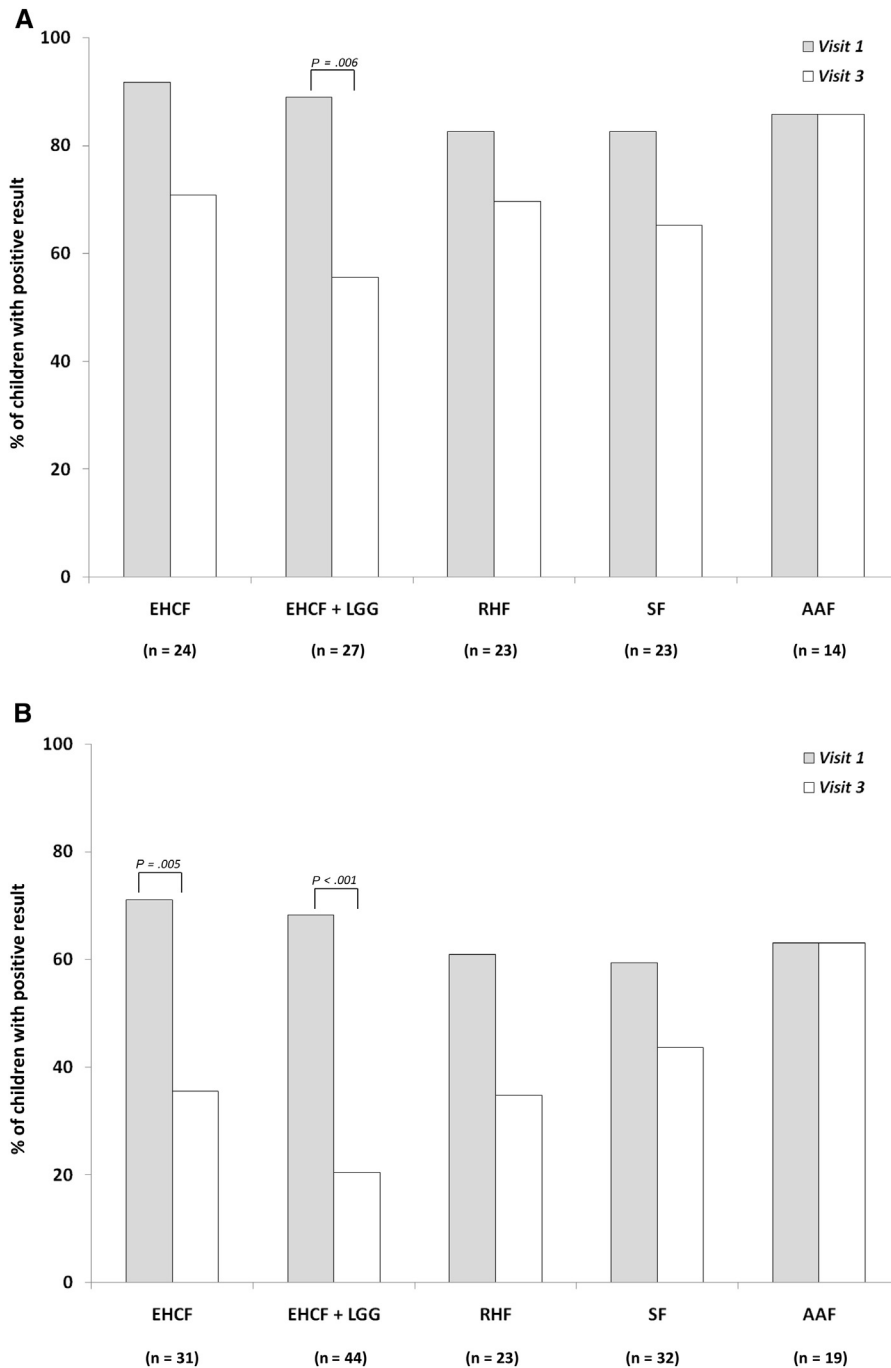
Skin prick tests were performed in all study subjects at baseline (visit 1), and were repeated after 12 months (visit 2) in infants presenting with IgE-mediated CMA. At visit 1, 96 subjects showed positive SPT. The median wheal size (IQR) was similar in the 5 groups: group 1, 8.5 mm (3.0); group 2, 7 mm (4.7); group 3, 6 mm (4.0); group 4, 6 mm (4.0); group 5, 7 mm (3.0). At visit 2, the total number of patients with positive SPT tended to decrease in all groups except in group 5. The difference was significant only in group 2 (Figure 2, A). Similarly, APTs were performed in all patients at baseline (visit 1) and after 12 months at visit 3 in infants with non-IgE-mediated CMA. At visit 1, 97 subjects showed positive APT and no differences were observed among groups regarding the number of patients with positive APT and severity of skin signs. At visit 2, the number of children with positive APT decreased in all groups except in group 5, but the difference was significant only in group 1 and group 2 (Figure 2, B).

### Development of Tolerance

Figure 3 shows the rate of acquisition of full tolerance after 12 months of an exclusion diet in each group. At 12 months, group 1 and group 2 demonstrated higher rates of CM tolerance compared with other groups. Patients with negative DBPCFC at 12 months were able to consume at least 1 full cup daily of CM without signs and symptoms related to CMA in the following 6 months.

All reactions induced by the DBPCFC were assessed by the physicians involved in the study. No placebo reactions were observed among all challenges. The vast majority of patients with IgE-mediated CMA reacted to the first 4 doses without differences among groups. No adverse events were observed during the study.

All patients with non-IgE-mediated CMA who presented with a positive challenge reacted within 48 hours after the procedure, without differences among groups. Figure 4 shows



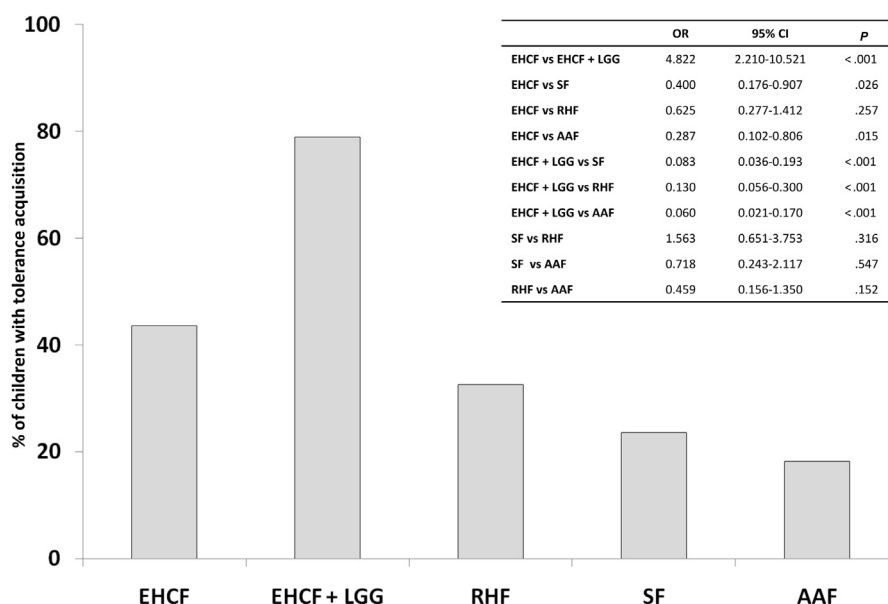
**Figure 2.** Results of allergy screening test during the study. **A**, SPT in children with IgE-mediated CMA. **B**, APT in children with non-IgE-mediated CMA.

the rate of patients acquiring tolerance at 12 months subdivided according to the CMA mechanism. Binary regression analysis coefficient (B) revealed that the rate of acquisition of tolerance at the end of the study was influenced by the mechanism of CMA (ie, being lower in subjects with an IgE-mediated mechanism [B -2.05, OR 0.12, 95% CI 0.06-0.26;  $P < .001$ ]) and by formula type, being increased with use of EHCF (B 1.48, OR 4.41, 95% CI

1.44-13.48;  $P .009$ ) and EHCF + LGG (B 3.35, OR 28.62, 95% CI 8.72-93.93;  $P < .001$ ).

## Discussion

Our results show that the selection of a particular type of formula influences the rate of acquisition of tolerance in children with CMA. Our main finding is that children affected



**Figure 3.** Rate of patients acquiring tolerance to oral food challenge after 12 months of exclusion diet with different formulas.

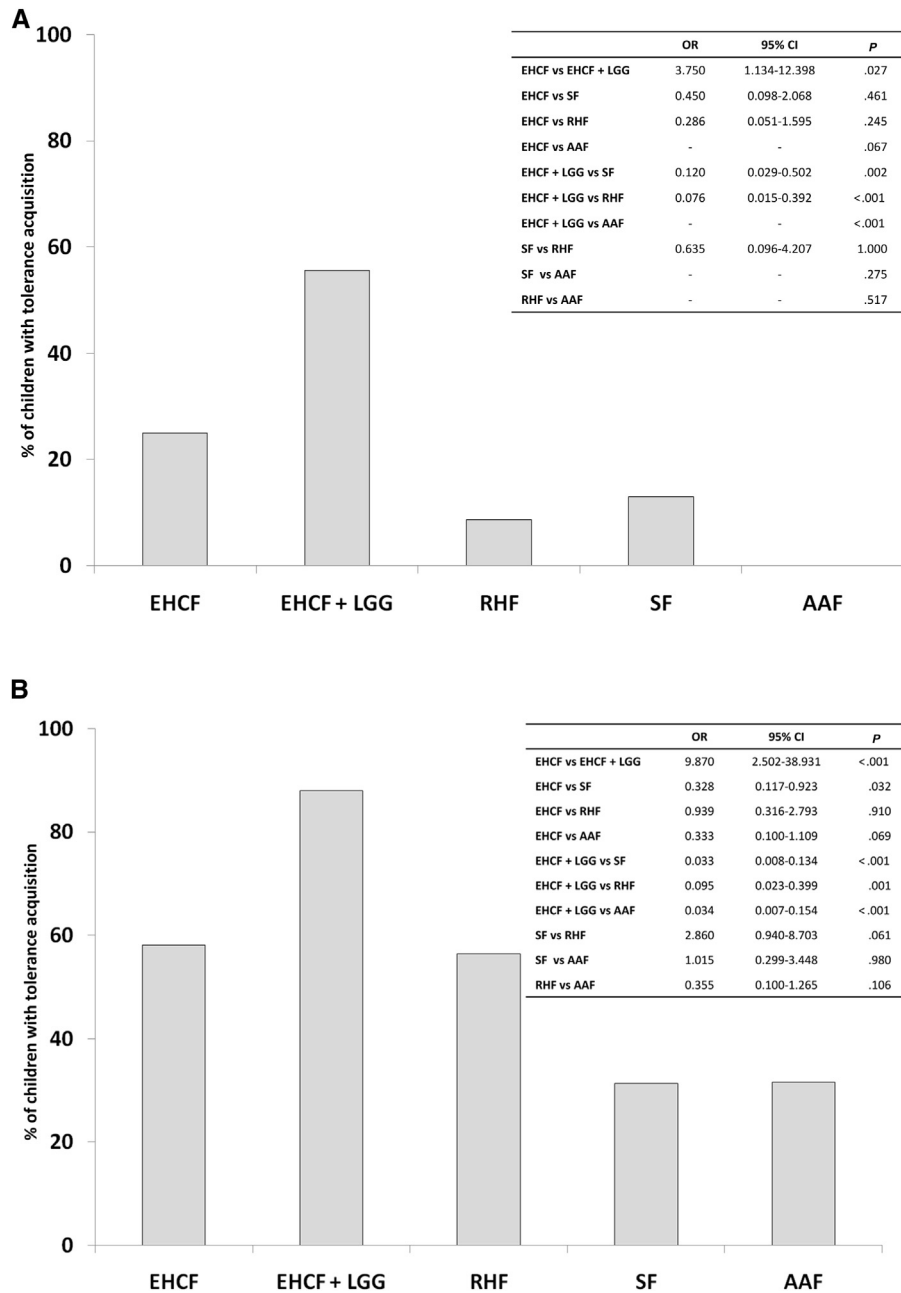
by CMA who receive EHCF alone or in combination with LGG, for the dietary management of their condition, achieve tolerance at 12 months significantly more frequently than their peers who receive RHF, SF, or AAF.

Subgroup analysis showed that among subjects with IgE-mediated or with non-IgE-mediated CMA, only those receiving EHCF containing LGG achieved tolerance more frequently than those receiving other formulas. A positive trend in the rate of patients acquiring tolerance at the end of the study period was observed for children receiving EHCF alone compared with children receiving RHF, SF, or AAF. The results could be explained at least in part by the number of patients enrolled in the different groups resulting in a lower power to detect differences. However, these results are consistent with our previous data showing that in both IgE- and non-IgE-mediated CMA, the addition of LGG to EHCF resulted in a higher rate of acquisition of tolerance after 12 months of treatment.<sup>8</sup>

Two other studies have addressed the possible influence of the choice of different formulas on the rate of acquisition of tolerance in patients with CMA. The first study showed no differences in acquisition of tolerance at 12 months in children with IgE-mediated CMA receiving a partially hydrolyzed rice protein formula supplemented with lysine and threonine compared with those receiving an extensively hydrolyzed CMP formula (containing casein, 40%; and whey proteins, 60%).<sup>10</sup> The second study compared SF, extensively hydrolyzed CMP (casein or whey proteins) formulas, and RHF in children with IgE-mediated CMA.<sup>11</sup> This study included subjects treated with both an EHCF and an extensively hydrolyzed whey proteins formula in the same group and had small numbers of patients in each group. Despite these limitations, this study also found no differences between formula

groups in the rate of acquisition of tolerance to CMP after 12 months. These data are in agreement with our findings that in infants with IgE-mediated CMA, management with EHCF does not result in a significantly higher rate of acquisition of tolerance rate at 12 months compared with a RHF, but all studies to date are relatively small and a difference may be detectable with a larger sample size.

Patients exposed to CM residues achieve oral tolerance earlier than patients who follow different dietary regimens; this may be due to a specific immunomodulatory effect induced by hydrolyzed casein peptides, as suggested by animal model.<sup>12,13</sup> These small peptides are absent in the other formulas evaluated in our study. There are also clinical data indicating that EHCF is able to prevent allergic manifestations in at-risk children.<sup>14</sup> LGG compared with EHCF alone has been shown to more effectively attenuate the increased intestinal permeability observed in infants with food allergy, and to decrease fecal calprotectin and the persistence of occult fecal blood losses at 1 month in infants with CMA.<sup>15,16</sup> Administration of LGG is associated with a complex response in intestinal mucosa, reflected by the up- and down-regulation of several genes involved in the immune response, inflammation, cell-cell signaling, signal transcription, and transduction.<sup>17</sup> LGG is known to modulate immune functions via various pathways, including those involving enterocytes, monocytes, mast cells, dendritic cells, and regulatory T lymphocytes.<sup>18-21</sup> LGG alters the generation of cytokines that may be involved in IgE- or non-IgE-mediated CMA (ie, interleukin-4, interleukin-5, interleukin-10, interferon- $\gamma$ , transforming growth factor- $\beta$ , tumor necrosis factor- $\alpha$ ) and, thereby, can positively modulate the major pathways involved in CMA pathogenesis.<sup>18-22</sup> It is important to recognize that these results cannot be generalized to other



**Figure 4.** Rate of patients acquiring tolerance after 12 months of exclusion diet with different formulas in the 5 study groups subdivided according to the CMA mechanism. **A**, IgE-mediated CMA. **B**, Non-IgE-mediated CMA.

probiotics or other *Lactobacillus* strains. Other *Lactobacillus* strains have different modes of action and varied effectiveness in model immune cell systems.<sup>22</sup> The differences between *Lactobacillus* strains is further demonstrated by comparative genomics studies that reveal that strain GG contains 331 strain-specific proteins.<sup>23</sup> Finally, it has been recently demonstrated that daily supplements of LGG resulted in a dramatic shift in the composition of the intestinal microbial community with a large increase in the number of taxa previously associated with a decreased risk for the development of allergy and atopy.<sup>24</sup>

The main limitations of our study are related to the lack of randomization and the lack of patient groups treated with other potentially available dietary strategies for the management of CMA (ie, extensively hydrolyzed whey formula, or extensively hydrolyzed SF). This was necessary because of the difficulties in recruitment of patients with CMA prior to treatment initiated by the primary care physician. Patients were first evaluated when referred for food challenge testing. Randomization at this stage of management would further complicate analysis. Therefore, formula selection was determined by the initial managing physician and was

presumably based upon issues such as availability, taste, different prices, and specific practice patterns that impact choices of physicians.

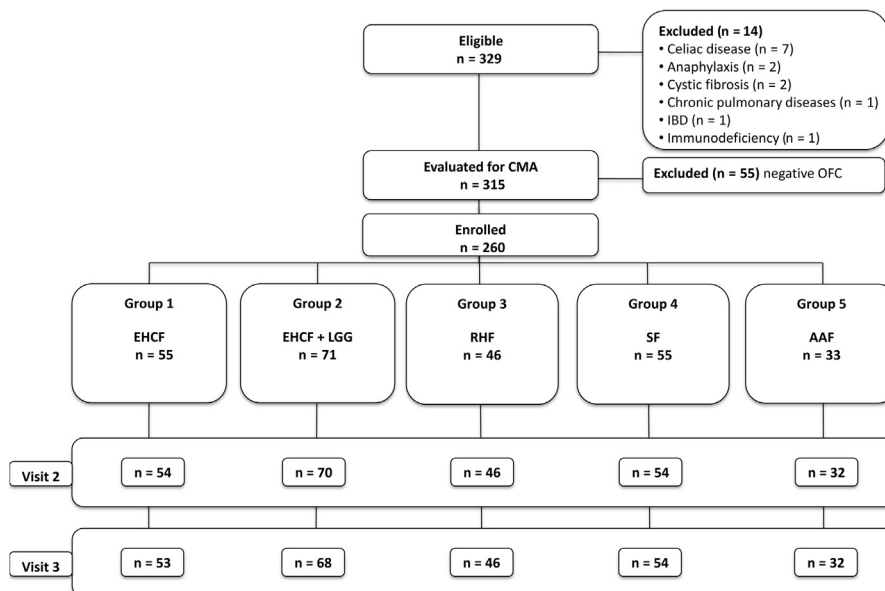
Recent data suggest that the number of patients with persistent CMA beyond infancy until later ages is increasing.<sup>4,25,26</sup> It is possible that this trend towards persistence of CMA beyond infancy can be explained by changing patterns of formula selection in infants with CMA with an increased use of RHF, SF, or AAF. The most recent European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) Clinical Guideline recommends the initial administration of an extensively hydrolyzed CMP formula in most infants with CMA because it is well tolerated and less expensive.<sup>6</sup> Our findings add further data to support this recommendation because we observed the earlier acquisition of tolerance to CMP. Furthermore, our data suggest that administration of EHCF with added LGG is more likely to lead to the development of tolerance at an earlier age than other formulas. If this finding is confirmed in other populations, the use of EHCF with LGG may allow earlier normalization of the child's diet with resultant reduced impacts on their quality of life and lower medical costs. ■

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## References

- Sicherer SH. Epidemiology of food allergy. *J Allergy Clin Immunol* 2011; 127:594-602.
- Boyce JA, Assa'ad A, Burks AW, Jones SM, Sampson HA, Wood RA, et al. Guidelines for the diagnosis and management of food allergy in the United States: summary of the NIAID-Sponsored expert panel report. *J Allergy Clin Immunol* 2010;126:1105-18.
- Høst A, Halken S, Jacobsen HP, Christensen AE, Herskind AM, Plesner K. Clinical course of cow's milk protein allergy/intolerance and atopic diseases in childhood. *Pediatr Allergy Immunol* 2002; 13(Suppl 15):23-8.
- Skripak JM, Matsui EC, Mudd K, Wood RA. The natural history of IgE-mediated cow's milk allergy. *J Allergy Clin Immunol* 2007;120:1172-7.
- Levy Y, Segal N, Garty B, Danon YL. Lessons from the clinical course of IgE-mediated cow milk allergy in Israel. *Pediatr Allergy Immunol* 2007; 18:589-93.
- Koletzko S, Niggemann B, Arato A, Dias JA, Heuschkel R, Husby S, et al. Diagnostic approach and management of cow's milk protein allergy in infants and children: a practical guideline of the GI-committee of ESPGHAN. *J Pediatr Gastroenterol Nutr* 2012;55:221-9.
- Venter C, Arshad SH. Guideline fever: an overview of DRACMA, US NIAID, and UK NICE guidelines. *Curr Opin Allergy Clin Immunol* 2012;12:302-15.
- Berni Canani R, Nocerino R, Terrin G, Coruzzo A, Cosenza L, Leone L, et al. Effect of *Lactobacillus* GG on tolerance acquisition in infants with cow's milk allergy: a randomized trial. *J Allergy Clin Immunol* 2012;129: 580-2. 582.e1-5.
- Berni Canani R, Buongiovanni A, Nocerino R, Cosenza L, Troncone R. Toward a standardized reading of the atopy patch test in children with suspected cow's milk allergy-related gastrointestinal symptoms. *Allergy* 2011;66:1499-500.
- Reche M, Pascual C, Fiandor A, Polanco I, Rivero-Urgell M, Chifre R, et al. The effect of a partially hydrolyzed formula based on rice protein in the management of infants with cow's milk protein allergy. *Pediatr Allergy Immunol* 2010;21(4 Pt 1):577-85.
- Terracciano L, Bouygue GR, Sarratut T, Veglia F, Martelli A, Fiocchi A. Impact of dietary regimen on the duration of cow's milk allergy: a random allocation study. *Clin Exp Allergy* 2010;40:637-42.
- Gill HS, Doull F, Rutherford KJ, Cross ML. Immunoregulatory peptides in bovine milk. *Br J Nutr* 2000;84(Suppl 1):S111-7.
- Sandré C, Gleizes A, Forestier F, Gorges-Kergot R, Chilmoneczyk S, Léonil J, et al. A peptide derived from bovine beta-casein modulates functional properties of bone marrow-derived macrophages from germfree and human flora-associated mice. *J Nutr* 2001;131: 2936-42.
- Von Berg A, Filipiak-Pittroff B, Krämer U, Link E, Bollrath C, Brockow I, et al. GINIplus study group. Preventive effect of hydrolyzed infant formulas persists until age 6 years: long-term results from the German Infant Nutritional Intervention Study (GINI). *J Allergy Clin Immunol* 2008;121:1442-7.
- Baldassarre ME, Laforgia N, Fanelli M, Laneve A, Grosso R, Lifschitz C. *Lactobacillus* GG improves recovery in infants with blood in the stools and presumptive allergic colitis compared with extensively hydrolyzed formula alone. *J Pediatr* 2010;156:397-401.
- Pohjavuori E, Viljanen M, Korpela R, Kuitunen M, Tiittanen M, Vaarala O, et al. *Lactobacillus* GG effect in increasing IFN-gamma production in infants with cow's milk allergy. *J Allergy Clin Immunol* 2004;114:131-6.
- Di Caro S, Tao H, Grillo A, Elia C, Gasbarrini G, Sepulveda AR, et al. Effects of *Lactobacillus* GG on genes expression pattern in small bowel mucosa. *Dig Liver Dis* 2005;37:320-9.
- Pan SJ, Kuo CH, Lam KP, Chu YT, Wang WL, Hung CH. Probiotics and allergy in infants-an update review. *Pediatr Allergy Immunol* 2010;21: e659-66.
- Ghadimi D, Fölster-Holst R, de Vrese M, Winkler P, Heller KJ, Schrezenmeier J. Effects of probiotic bacteria and their genomic DNA on TH1/TH2-cytokine production by peripheral blood mononuclear cells (PBMCs) of healthy and allergic subjects. *Immunobiology* 2008; 213:677-92.
- Donato KA, Gareau MG, Wang YJ, Sherman PM. *Lactobacillus rhamnosus* GG attenuates interferon- $\gamma$  and tumor necrosis factor- $\alpha$ -induced barrier dysfunction and pro-inflammatory signaling. *Microbiology* 2010;156:3288-97.
- Mileti E, Matteoli G, Iliev ID, Rescigno M. Comparison of the immunomodulatory properties of three probiotic strains of *Lactobacilli* using complex culture systems: prediction for in vivo efficacy. *PLoS One* 2009;4:e7056.
- Cai S, Bay BH, Lee YK, Lu J, Mahendran R. Live and lyophilized *Lactobacillus* species elicit differential immunomodulatory effects on immune cells. *FEMS Microbiol Lett* 2010;302:189-96.
- Kankainen M, Paulin L, Tynkkynen S, von Ossowski I, Reunanen J, Partanen P, et al. Comparative genomic analysis of *Lactobacillus rhamnosus* GG reveals pili containing a human-mucus binding protein. *Proc Natl Acad Sci USA* 2009;106:17193-8.
- Cox MJ, Huang YJ, Fujimura KE, Liu JT, McKean M, Boushey HA, et al. *Lactobacillus casei* abundance is associated with profound shifts in the infant gut microbiome. *PLoS One* 2010;5:e8745.
- Santos A, Dias A, Pinheiro JA. Predictive factors for the persistence of cow's milk allergy. *Pediatr Allergy Immunol* 2010;21:1127-34.
- Prescott S, Allen KJ. Food allergy: riding the second wave of the allergy epidemic. *Pediatr Allergy Immunol* 2011;22:155-60.



**Figure 1.** Numbers of participants throughout the study. *IBD*, inflammatory bowel disease; *OFC*, oral food challenge.