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%.[1,2] 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.[3] However, recent studies suggest that the natural history of CMA is changing, with an increasing persistence until later ages,[4,5] and increasing severity of illness.[1,3]

Recent guidelines addressing the optimal therapeutic approach for children with CMA recommend the use of substitutive formulas.[6,7] 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.[8] 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	*Lactobacillus rhamnosus* 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.[9] Briefly, 1 drop (50 μL) 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 dieticians 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.